AUSTRALASIAN PIG SCIENCE ASSOCIATION

MANIPULATING PIG PRODUCTION

XIV

EDITORS

J.R. PLUSKE AND J.M. PLUSKE

NOVEMBER 2013

14th APSA 2013 conference melbourne australia

Manipulating Pig Production XIV

Proceedings of the Fourteenth Biennial Conference of the
Australasian Pig Science Association (APSA)

Melbourne, Australia

24th - 27th November, 2013

Edited by Professor John Pluske and Dr. Jo Pluske

AUSTRALASIAN PIG SCIENCE ASSOCIATION (INC.)
Werribee, Victoria, Australia

Australasian Pig Science Association (Inc.)
Conference 14th; 2013: Melbourne, Australia.
Manipulating Pig Production XIV

Includes bibliographies and index
ISBN: 978-0-9806880-3-0
ISSN: 1324 9177

Printed by UniPrint, The University of Western Australia, Crawley, Western Australia.

Contents

List of Contributors

Ader P.	BASF SE, Ludwigshafen, Germany
Agyekum A.K.	University of Manitoba, Winnipeg, MB R3T 2N2, Canada
Amdi C.	Department of Large Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark
Amerah A.M.	Dupont Industrial Biosciences-Danisco Animal Nutrition, Marlborough, Wiltshire, UK, SN8 1AA
Andersen I.L.	Norwegian University of Life Science, 1432 Ås, Norway
Anderson S.T.	School of Biomedical Sciences, The University of Queensland, Brisbane, St Lucia, QLD 4072
Athorn R.Z.	Rivalea Australia Pty Ltd, Corowa, NSW 2646
Auckland C.H.	Murdoch University, Murdoch, WA 6150
Awati A.	Dupont Industrial Biosciences-Danisco Animal Nutrition, Marlborough, Wiltshire, UK, SN8 1AA
Baidoo S.K.	Southern Research and Outreach Centre, Waseca, MN, USA 56093
Ball R.O.	Swine Research and Technology Centre, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada
Banhazi T.M.	University of Southern Queensland, Toowoomba QLD 4350
Barchia I.M.	Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568
Barnes A.L.	Murdoch University, Murdoch, WA, 6051
Barnsley J.A.	The University of Sydney, Camden, NSW 2570
Barugh I.W.	Massey University, Palmerston North 4442, New Zealand
Batstone D.J.	Advanced Water Management Centre, The University of Queensland, Australia, 4072
Baumgard L.H.	Iowa State University, Ames, IA 50011, USA
Baxter E.M.	Scottish Agricultural College, Edinburgh, EH9 3JG, UK
Beausoleil N.J.	Massey University, Palmerston North 4442, New Zealand
Bell K.	Department of Agriculture, Fisheries and Forestry (Qld), Toowoomba, QLD 4350
Bergmann A.	Institute of Farm Animal Genetics (FLI), 31535 Neustadt am Rübenberge, Germany
Bergstra T.J.	Wageningen University, Chair Group Business Economics, 6707 KN No.1, Wageningen
Billman-Jacobe H.	The University of Melbourne, Parkville, VIC 3010
Bindhu L.V.	Kemin Industries (Asia) Pte Ltd, Singapore 758200
Birchall S.	AgSystems Design, Shepparton, VIC 3631
Black J.L.	John L Black Consulting, Warrimoo, NSW 2774
Blackall P.	The University of Queensland, Brisbane, St Lucia, QLD 4072
Blank R.	Christian-Albrechts-Universität zu Kiel, Germany
Blavi L.	Universitat Autònoma de Barcelona, Bellaterra, Barcelona 08193, Spain.
Boddington M.P.	Asian Agribusiness Consulting, Suite B209 Bai Jia Zhuang Commercial Centre, Beijing China
Bøe K.E.	Norwegian University of Life Science, 1432 Ås, Norway
Bonnicci J.F.	The University of Melbourne, Parkville Vic 3010
Bouwman E.G.	South Australian Research and Development Institute, Roseworthy, SA 5371
Brangenberg N.	Ministry for Primary Industries, Wallaceville, New Zealand
Brewster C.J.	Rivalea Australia Pty Ltd, Corowa, NSW 2646
Brookes V.J.	The University of Sydney, Camden, NSW 2570
Bryden W.L	The University of Queensland, St Lucia, QLD 4072
Cadogan D.J.	Feedworks, Lancefield, VIC 3435
Cakebread P.L.	The University of Melbourne, Parkville Vic 3010
Campbell R.G.	CRC for High Integrity Australian Pork, Roseworthy, SA 5118
Capozzalo M.M.	Murdoch University, Murdoch, WA 6150
Chambers J.P.	Massey University, Palmerston North 4442, New Zealand
Channon H.A.	Australian Pork Limited, Barton, ACT 2600; The University of Melbourne, Parkville, VIC 3010
Chen T.Y.	South Australian Research and Development Institute, Roseworthy, SA 5371
Chenoweth P.J.	Chenovet Pty Ltd, Wagga Wagga, NSW 2650
Chevaux E.	Lallemand Animal Nutrition, Blagnac, France
Chidgey K.L.	Massey University, Palmerston North 4442, New Zealand
Clark C.	AusPac Ingredients Pty Ltd, Tamworth, NSW 2340
Clarke T.	Murdoch University, Murdoch, WA, 6051
Collins A.M.	Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568
Collins C.L.	Rivalea Australia Pty Ltd, Corowa, NSW 2646
Collins T.	Murdoch University, Murdoch, WA, 6051
Condous P.C.	The University of Adelaide, Roseworthy, SA 5371
Cowieson A. J.	The University of Sydney, Camden, NSW 2570

Cowled B.	AusVet Animal Health Services, Conjola Park, NSW 2539
Cronin G.M.	The University of Sydney, Camden, NSW 2570
D'Souza D.N.	Australian Pork Limited, Barton, ACT 2600
de Jager N.	The University of Queensland, Brisbane, St Lucia, QLD 4072
de Lange C.F.M.	University of Guelph, Guelph, ON N1G 2W1, Canada
Dekkers J.C.M.	Iowa State University, Ames, IA 50010, USA
Devreese A.M.	University of Copenhagen, Denmark.
Diffey S.	University of Wollongong, Wollongong, NSW 2522
Dimovski K.	The University of Melbourne, Parkville, VIC 3010
Dobbinson S.	FreshPork, Christchurch, New Zealand
Downing J.A.	University of Sydney, Camden, NSW 2570
Doyle R. E.	Charles Sturt University, Wagga Wagga, NSW 2678
Doyle R.E.	The University of Sydney, Camden, NSW 2570
Dron N.M.	Charles Sturt University, Wagga Wagga, NSW 2678
Dunlop H.	Chris Richards and Associates Pty Ltd, Bendigo VIC, 3550
Dunshea F.R.	The University of Melbourne, Parkville, VIC 3010
Dusel G.	University of Applied Sciences, FB 1- Life Sciences D-55411 Bingen am Rhein, Germany
Dyck M.K.	The University of Alberta, AB, T6G 2P5, Canada
Edwards A.C.	ACE Livestock Consulting Pty Ltd, Cockatoo Valley, SA 5351
Emery D.	The University of Sydney, Camden, NSW 2570
Farish M.	Scottish Agricultural College, Edinburgh, EH9 3JG, UK
Feng Y.	Asiapac Biotechnology, Dongguan City, Guangdong, 523808 PRC
Ferguson N.	Westpork Pty Ltd, Maylands, WA 6051
Ferguson N.S.	Nutreco Canada Agresearch, Guelph, N1G 4T2 Canada
Figueroa J.	Universitat Autònoma de Barcelona, Bellaterra, Barcelona 08193, Spain
Fiorentini L.	Università Cattolica Sacro Cuore, 29122 Piacenza, Italy
Fitt L.R.	ACE Laboratory Services, Bendigo, VIC 3550
Fleming P.A.	Murdoch University, Murdoch, WA, 6051
Flinn P.C.	Kelspec Services, Dunkeld, VIC 3294
Foxcroft G.R.	The University of Alberta, AB, T6G 2P5, Canada
Frio A.J.L	Alltech Philippines Corp, Muntinlupa City, Manila, Philippines
Frouel S.	Dupont Industrial Biosciences-Danisco Animal Nutrition, Marlborough, Wiltshire, UK, SN8 1AA
Gabler N.K.	Iowa State University, Ames, IA 50011, USA
Gadella B.M.	Utrecht University, The Netherlands
Gallo A.	Università Cattolica Sacro Cuore, 29122 Piacenza, Italy
Gidley M.J.	The University of Queensland, Brisbane, St Lucia, QLD 4072
Giuberti G.	Università Cattolica Sacro Cuore, 29122 Piacenza, Italy
Goh L-B.	Kemin Industries (Asia) Pte Ltd, Singapore 758200
Gopalan P.	Advanced Water Management Centre, The University of Queensland, Australia, 4072
Greenwood E.C.	The University of Adelaide, Roseworthy, SA 5371
Grubbs J.K.	Iowa State University, Ames, IA 50011, USA
Guillou D.	Lallemand Animal Nutrition, Blagnac, France
Gurman P.M.	South Australian Research and Development Institute, Urrbrae, SA 5064; University of Tasmania, Hobart, TAS 7001
Guzmán-Pino S.A.	Universitat Autònoma de Barcelona, Bellaterra, Barcelona 08193, Spain
Hales J.	University of Copenhagen, Denmark.
Hamilton A.J.	The University of Melbourne, Parkville, VIC 3010
Hamilton D.H.	South Australian Research and Development Institute, Urrbrae, SA 5064
Hampson D.J.	Murdoch University, Murdoch, WA 6150
Hansen C.F.	Department of Large Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark
Hansen L.U.	University of Copenhagen, Denmark
Harris A.J.	Iowa State University, Ames, IA 50011, USA
Hemsworth P.H.	The University of Melbourne, Parkville, VIC 3010
Henman D.J.	Rivalea Australia Pty Ltd, Corowa, NSW 2646
Heo J.M.	University of Manitoba, Winnipeg, MB R3T 2N2, Canada; Chungnam National University, Daejon 305-764, South Korea
Herde P.	South Australian Research and Development Institute, Roseworthy, SA 5371
Hermesch S.	Animal Genetics and Breeding Unit, University of New England, Armidale, NSW 2351
Hernandez A.	Murdoch University, Murdoch, WA 6150.

Hernandez-Jover M.	Charles Sturt University, Wagga Wagga, NSW 2678
Hesse R.	Kansas State University, Manhattan, KS, 66045 USA
Hewitt R.J.E.	CHM Alliance Pty Ltd, Millmerran, QLD 4350
Hogeveen H.	Wageningen University, Chair Group Business Economics, 6707 KN No.1, Wageningen
Hogg G.G.	The University of Melbourne, Parkville, VIC 3010
Holyoake P.K.	Department of Environment and Primary Industries, Bendigo, VIC 3551
Hosking B.J.	Asiapac Biotechnology, Dongguan City, Guangdong, 523808 PRC
Htoo J.K.	Evonik Industries AG, Hanau, Germany
Hugenholtz P.	The University of Queensland, Brisbane, QLD 4072
Hughes P.E.	South Australian Research and Development Institute, Roseworthy, SA 5371
Hung A.T.	Asiapac Biotechnology, Dongguan City, Guangdong, 523808 PRC
Isaac D.	BEC Feed Solutions Pty Ltd, Carole Park, QLD 4600
Jagger S.	ABN, Peterborough, PE2 6FL, UK
Jarman T.	Alltech Thailand Corp, Ltd., Khlongtoey Nua, Bangkok, Thailand
Jenkins S. N.	Institute of Agriculture, The University of Western Australia, Crawley WA 6009, Australia
Jensen P.D.	Advanced Water Management Centre, The University of Queensland, Australia, 4072
Johnson C.B.	Massey University, Private Bag 11-222 Palmerston North, New Zealand
Johnston L.J.	West Central Research and Outreach Centre, Morris, MN, USA 56267
Jose C.G.	Department of Agriculture and Food WA, South Perth, WA 6151
Kang L.	Asiapac Biotechnology, Dongguan City, Guangdong, 523808 PRC
Kanitz E.	Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany
Kells N.J.	Massey University, Private Bag 11-222 Palmerston North, New Zealand
Kelly J.M.	South Australian Research and Development Institute, Rosedale, SA 5350
Kennaway D.J.	The University of Adelaide, Adelaide, SA 5005
Kennett T.E.	Australian Pork Farms Group, Stirling, SA 5152
Kiarie E.	Dupont Industrial Biosciences-Danisco Animal Nutrition, Marlborough, SN8 1AA
Kiermeier A.	South Australian Research and Development Institute, Urrbrae, SA 5064
Kim I.H.	Dankook University, Cheonan, Choongnam 330-714, South Korea
Kim J.C.	Department of Agriculture and Food WA, South Perth, WA 6151
Kim Y.K.	Dankook University, Cheonan, Choongnam 330-714, South Korea
Kind K.L.	The University of Adelaide, Roseworthy, SA 5371
Kirkwood R.N.	The University of Adelaide, Roseworthy, SA 5371
Knapp S.A.	CHM Alliance Pty Ltd, Millmerran, QLD 4350
Knight A.L.	The University of Adelaide, Roseworthy, SA 5371
Knight D.R.	The University of Western Australia, Crawley, WA 6009
Krogh U.	Aarhus University, Tjele, Denmark
Langendijk P.	South Australian Research and Development Institute, Roseworthy, SA 5371
Langridge M.D.	Department of Agriculture and Food Western Australia, South Perth, WA 6151
Lay D.C.	US Department of Agriculture-ARS, West Lafayette, IN 47907, USA
Le Treut Y.	Lallemand Animal Nutrition, Blagnac, France
Lee G.S.H.	The University of Western Australia, Crawley, WA 6009
Lei Y.	Dankook University, Cheonan, Choongnam 330-714, South Korea
LeMoine A.	University of Leeds, School of Biology, LS2 9JT, UK
Li H.L.	Dankook University, Cheonan, Choongnam 330-714, South Korea
Li L.	Animal Genetics and Breeding Unit, University of New England, Armidale, NSW 2351
Li X.	University of Minnesota, St. Paul, MN, USA 55108
Li Y.Z.	West Central Research and Outreach Centre, Morris, MN, USA 56267
Lievaart J.J.	Charles Sturt University, Wagga Wagga, NSW 2650
Limm T.M.	ACE Laboratory Services, Bendigo, VIC 3550
Lines D.S.	Australian Pork Farms Group, Stirling, SA 5152
Liu F.	The University of Melbourne, Melbourne, VIC 3010
Lonergan M.S.	Iowa State University, Ames, IA 50011, USA
Lu Y.	The University of Queensland, Brisbane, QLD 4072
Luxford B.G.	Rivalea Australia Pty Ltd, Corowa, NSW 2646
Macnamara B.L.F.	The University of Sydney, Camden, NSW 2570
Macnamara G.F.	The University of Sydney, Camden, NSW 2570
Mallo J.J.	Norel, Madrid, Spain
Mancera Gracia J.C.	Nutreco R&D, Boxmeer, 5830 AE The Netherlands

Mansfield J.	Murdoch University, Murdoch, WA 6150
Marchant-Forde J.N.	US Department of Agriculture-ARS, West Lafayette, IN 47907, USA
Martyniuk A.	The University of Adelaide, SA 5371
Masoero F.	Università Cattolica Sacro Cuore, 29122 Piacenza, Italy
Matthews R.L.	The University of Sydney, Camden, NSW 2570
McDonald E.J.	University of Sydney, Camden, NSW 2570
McDonald T.N.	Rivalea Australia Pty Ltd, Corowa, NSW 2646
McGahan E.J.	FSA Consulting, Toowoomba, QLD 4350
McKenzie P.	Scolexia Pty Ltd, Moonee Ponds, VIC 3039
McLeod B.E.	Rivalea Australia, Corowa, 2646, NSW
McMunn K.A.	US Department of Agriculture-ARS, West Lafayette, IN 47907, USA
McPherson F.J.	Charles Sturt University, Wagga Wagga, NSW 2650
Mesas L.	Norel, Madrid, Spain
Miller D.W.	Murdoch University, Murdoch, WA, 6051
Miller H.M.	University of Leeds, School of Biology, LS2 9JT, UK
Moehn S.	Swine Research and Technology Centre, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada
Moeser A.J.	Center for Comparative Medicine and Translational Research, North Carolina State University, College of Veterinary Medicine, Raleigh, NC, USA
Moore K.	Department of Environment and Primary Industries Victoria, Bendigo, VIC 3551
Moore K.L.	Department of Agriculture and Food Western Australia, South Perth, WA 6151
Morel P.C.H.	Massey University, Palmerston North 4442, New Zealand
Morrison R.S.	Rivalea Australia Pty Ltd, Corowa, NSW 2646
Moschini M.	Università Cattolica Sacro Cuore, 29122 Piacenza, Italy
Moustsen V.A.	Housing and Environment, Pig Research Centre, Denmark
Mullan B.P.	Department of Agriculture and Food Western Australia, South Perth, WA 6151
Müller R.	University of Copenhagen, Denmark
Muller T.L.	CHM Alliance Pty Ltd., Loganholme QLD 4129
Naatjes M.	Christian-Albrechts-Universität zu Kiel, Germany
Neslo R.	University Medical Center Utrecht, The Netherlands
Neumann E.	Massey University, Palmerston North, New Zealand
Nguyen A.T	Department of Large Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark
Nguyen G.T.	The University of Queensland, St Lucia, QLD 4072
Nogueira M.G.	The University of Sydney, Camden, NSW 2570
Nyachoti C.M.	University of Manitoba, Winnipeg, MB R3T 2N2, Canada
O'Meara R.L.	ACE Laboratory Services, Bendigo, VIC 3550
O'Keefe M.	FSA Consulting, Horsham, VIC 3401
Oksbjerg N.	Aarhus University, Tjele, Denmark
Omaleki L.	The University of Queensland, Brisbane, St Lucia, QLD 4072
Ortiz A.	Norel, Madrid, Spain
Owusu-Asiedu A.	Dupont Industrial Biosciences-Danisco Animal Nutrition, Marlborough, Wiltshire, UK, SN8 1AA
Palou R.	Centre for Nutrition and Food Science, The University of Queensland, Brisbane, St Lucia, QLD 4072
Partridge G.G.	Danisco Animal Nutrition, Marlborough, UK
Patience J.F.	Iowa State University, Ames, IA 50011, USA
Patterson J.	University of Alberta, Edmonton, Alberta, T6G 2P5, Canada
Payne H.G.	Department of Agriculture and Food WA, South Perth, WA 6151
Pearce S.C.	Iowa State University, Ames, IA 50011, USA
Pedersen M.	Department of Food Science, University of Copenhagen, Frederiksberg C, Denmark
Pérez J.F.	Universitat Autònoma de Barcelona, Bellaterra, Barcelona 08193, Spain
Péron A.	Danisco Singapore Pte Ltd, 61 Science Park Road, Singapore 117525
Pethick D.W.	Murdoch University, Murdoch, WA 6150
Peucker S.K.J.	CHM Alliance Pty Ltd, Millmerran
Plush K.	The University of Adelaide, SA 5371
Pluske J.M.	SciEcons Consulting, Subiaco, WA 6008
Pluske J.R.	Murdoch University, Murdoch, WA 6150
Pope G.	Graeme Pope Consulting, Nuriootpa, SA 5355
Preston M.	The University of Queensland, Brisbane, St Lucia, QLD 4072
Rajapakse U.A.	The University of Adelaide, Roseworthy, SA 5371
Rath D.	Institute of Farm Animal Genetics (FLI), 31535 Neustadt am Rübenberge, Germany

Rault J-L.	The University of Melbourne, Parkville, VIC 3010
Resink J.W.	Nutreco R&D, Boxmeer, 5830 AE The Netherlands
Rice M.	The University of Melbourne, Parkville VIC 3010
Richer-Lanciault M.	Nutreco Canada Agresearch, Guelph, N1G 4T2 Canada
Riley J.	JCR Associates International, Pittsworth, QLD 4356
Riley T.V.	The University of Western Australia, Crawley, WA 6009; PathWest Laboratory Medicine, Crawley, WA 6009
Rischewski J.	Christian-Albrechts-Universität zu Kiel, Germany
Ross T.	University of Tasmania, Hobart, TAS 7001
Roubos P.J.	Nutreco R&D, Boxmeer, 5830 AE The Netherlands
Roura E.	The University of Queensland, Brisbane, St Lucia, QLD 4072
Ru Y.	Danisco Singapore Pte Ltd, 61 Science Park Road, Singapore 117525
Rzepus M.	The University of Queensland, Brisbane, QLD 4072; Università Cattolica del Sacro Cuore, Italy
Sacy A.	Lallemand Animal Nutrition, Blagnac, France
Sanz-Fernandez M.V.	Iowa State University, Ames, IA 50011, USA
Sawyer K. S.	Rivalea Australia Pty Ltd, Corowa, NSW 2646
Schild S-L.	Pig Research Centre, Copenhagen, Denmark
Scott P.C.	Scolexia Pty Ltd, Moonee Ponds, VIC 3039
Shrestha B.	The University of Queensland, Brisbane, St Lucia, QLD 4072
Shurson G.C.	University of Minnesota, St. Paul, MN, USA 55108
Singh C.	The University of Melbourne, Parkville, VIC 3010
Singh R.	The University of Queensland, Brisbane, St Lucia, QLD 4072
Skerman A.G.	Department of Agriculture, Fisheries and Forestry, Toowoomba, QLD 4350
Smit M.	University of Alberta, Edmonton, Alberta, T6G 2P5, Canada
Smith G.M.	Department of Agriculture and Food Western Australia, South Perth, WA 6151
Solà-Oriol D.	Universitat Autònoma de Barcelona, Bellaterra, Barcelona 08193, Spain
Sopade P.A.	The University of Queensland, St Lucia, QLD 4072
Sørensen G.	Pig Research Centre, 1609 Copenhagen, Denmark
Squire M.	The University of Western Australia, Crawley, WA 6009
Stafford K.J.	Massey University, Palmerston North 4442, New Zealand
Stanislawek W.	Ministry for Primary Industries, Wallaceville, New Zealand
Stassen E.N.	Wageningen University, Chair Group Animals in Society, 6708 WD No.1, Wageningen
Stumbles P.A.	Murdoch University, Murdoch, WA 6150
Suckling A.	Murdoch University, Murdoch, WA 6150; Linley Valley Pork, Wooroloo, WA 6558
Suddith A.	Kansas State University, Manhattan, KS, 66045 USA
Sugumar C.	Kemin Industries (Asia) Pte Ltd, Singapore 758200
Susenbeth A.	Christian-Albrechts-Universität zu Kiel, Germany
Sutherland M.A.	AgResearch Ruakura, Private Bag 3115, Hamilton, New Zealand
Sweeny J.P.A.	Department of Agriculture and Food WA, South Perth, WA 6151
Swinbourne A.M.	The University of Adelaide, Roseworthy, SA 5371
Tait S.	The University of Queensland, St Lucia, QLD 4072
Tay G.K.	The University of Western Australia, Crawley, WA 6009
Taylor A.E.	University of Leeds, Leeds, LS2 9JT, UK
Taylor U.	Institute of Farm Animal Genetics (FLI), 31535 Neustadt am Rübenberge, Germany
Terry R.	The University of Adelaide, Roseworthy, SA 5371
Tham K.	Ministry for Primary Industries, Wallaceville, New Zealand
Theil P.K.	Aarhus University, Tjele, Denmark
Thorup F.	Pig Research Centre, Copenhagen, Denmark
Tickle K.M.	Rivalea Australia Pty Ltd, Corowa, NSW 2646
Tredrea A.M.	The University of Sydney, Narrabri, NSW 2390
Trezona M.	Department of Agriculture and Food Western Australia, South Perth, WA 6151
Tsoi S.	The University of Alberta, AB, T6G 2P5, Canada
Tuchscherer M.	Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany
Tung L.Y.J.	The University of Western Australia, Crawley, WA 6009
Turni C.	The University of Queensland, Brisbane, St Lucia, QLD 4072
Turpin D.	Murdoch University, Murdoch, WA 6150
Valcanis M.	The University of Melbourne, Parkville, VIC 3010
Valientes R.A.	DSM Nutritional Products Philippines, Inc., Bonifacio Global City, Taguig, 1634, Philippines

van Barneveld R.J.	Barneveld Nutrition Pty Ltd, Loganholme, QLD 4129
van Burgel A.J.	Department of Agriculture and Food Western Australia, South Perth, WA 6151
van der Heijden M.	Selko Feed Additives, Tilburg, The Netherlands
van Hees H.	Nutreco R&D, Boxmeer, 5830 AE The Netherlands
van Sambeek D.M.	Iowa State University, Ames, IA 50011, USA
van Wettere W.H.E.J.	The University of Adelaide, Roseworthy, SA 5371
Vanderfeen A.M.	ACE Laboratory Services, Bendigo, VIC 3550
Verdon M.	The University of Melbourne, Parkville VIC 3010
Walk C.L.	AB Vista Feed Ingredients, Marlborough, UK
Ward M.P.	The University of Sydney, Camden, NSW 2570
Weaver A.C.	The University of Adelaide, Roseworthy, SA 5371
Weaver T.R.D.	The University of Melbourne, Parkville, VIC 3010; South Australian Research and Development Institute, Urrbrae, SA 5064
Weber P.M.	Pig Research Centre, Copenhagen, Denmark; University of Copenhagen, Denmark
Whyte M.C.	Rivalea Australia, Corowa, 2646, NSW
Wilkinson J.I.	University of Sydney, Camden, NSW 2570
Wilkinson S.J.	The University of Sydney, Camden, NSW 2570
Williams B.A.	The University of Queensland, Brisbane, St Lucia, QLD 4072
Williams C.E.	The University of Sydney, Camden, NSW 2570
Willis S.	Queensland Department of Agriculture, Fisheries and Forestry, Toowoomba, QLD 4350
Wilson M.E.	Zinpro Corporation, Eden Prairie, MN, USA 55344
Wilson R.H.	Rob Wilson Consulting, Perth, WA 6012
Wilson R.L.	Charles Sturt University and E.H. Graham Centre, Wagga Wagga, NSW 2678
Wiseman J.	University of Nottingham, Loughborough, LE12 5RD, UK
Zhan M.	The University of Queensland, Brisbane, St Lucia, QLD 4072
Zhang Z.Z.	Asiapac Biotechnology, Dongguan City, Guangdong, 523808 PRC
Zheng D.W.	Asiapac Biotechnology, Dongguan City, Guangdong, 523808 PRC
Zimmerman J.	Iowa State University, Ames, IA 50011, USA

APSA Awards

The APSA Fellow Award

The APSA Fellow Award was first presented in 2007. This prestigious award is offered in recognition of past and present members who have made an outstanding contribution to APSA as well as their contribution and commitment to pig science.

Previous recipients:

Dr Ray King (2007)

Dr David Hennessy (2007)

Dr Michael Taverner (2009)

Dr Ian Williams (2011)

The Batterham Memorial Award

The Batterham Memorial Award is a prestigious award conferred by APSA in memory of the late Dr Ted Batterham. Ted Batterham's love of pigs began at the NSW Agriculture, Wollongbar Research Station in the mid 1960's when he began work with Dr John Holder to solve the problem of variability in the growth of pigs fed meat meals. At that time abattoirs in NSW produced meat meals that were variable because there was little control on either the raw materials used or cooking times and temperatures. Ted soon realized that part of the variability was explained by the content of bone but, something much more fundamental that would keep Ted focused and fascinated for the rest of his professional life, was the variability of available lysine in these meals. Ted knew that if proteins were heated in the presence of carbohydrates and fats, lysine would become unavailable to the pigs own enzymes.

Ted went to Melbourne University to commence a PhD with Tony Dunkin to develop an *in vivo* assay in rats and pigs to quantify the available lysine not just in meat meals but in a range of other protein sources and cereals. He returned to Wollongbar and became a world leader in the availability of amino acids in feedstuffs for pigs and poultry. Not content just to solve a problem, Ted wanted to find solutions and reasoned that, if the availability of lysine was known, any shortfall could be remedied by supplementation with synthetic lysine. That idea stimulated research that delved into ways that the biological value of proteins could be enhanced by supplementation with synthetic amino acids.

Ted's research career was always focused on industry issues and driven by a desire to find suitable solutions. He knew that progress was best made by teams of people stimulating and supporting each other, and that investment in young people was essential.

The Batterham Memorial Award is made to a young scientist within 10 years of graduation. Its aim is to "stimulate and develop innovation in the pig industry". It is anticipated that the cash award will enable the recipient to broaden his or her exposure to national or international pig science.

Previous winners of the Batterham Memorial Award:

Robert van Barneveld (1995)

John Pluske (1997)

Kaye Coates (1999)

Darryl D'Souza (2001)

Patricia Mitchell (2003)

Eva Ostrowska (2005)

David Cadogan (2007)

Rebecca Morrison (2009)

Cherie Collins (2011)

APSA – Behind the Scenes

APSA has remained a successful and relevant Association through the dedication and commitment of the elected Committees since 1987. The following contributions are gratefully acknowledged by the Australasian pig science community.

1987	***President***	***Secretary***	***Committee***	***Editor(s)***
	Ray King	David Hennessy	John Barnett	APSA Committee
	Vice President	***Treasurer***	Ted Batterham	
	Paul Hemsworth	Greg Cronin	Chris Hansen	
			Paul Hughes	
			Noel Johnston	
1989	***President***	***Secretary***	***Committee***	***Editor(s)***
	Ray King	David Hennessy	John Barnett	John Barnett
	Vice President	***Treasurer***	Ted Batterham	David Hennessy
	Paul Hemsworth	Greg Cronin	Chris Hansen	
			Paul Hughes	
			Noel Johnston	
1991	***President***	***Secretary***	***Committee***	***Editor(s)***
	Paul Hemsworth	David Hennessy	Ross Cutler	Ted Batterham
	Vice President	***Treasurer***	Ted Batterham	
	Mike Taverner	Greg Cronin	Bruce Mullan	
	Past President		Roger Campbell	
	Ray King		Peter Cranwell	
			Chris Hansen	
1993	***President***	***Secretary***	***Committee***	***Editor(s)***
	Mike Taverner	David Hennessy	Robert Love	Ted Batterham
	Vice President	***Treasurer***	Ross Cutler	
	Bruce Mullan	Greg Cronin	Peter Cranwell	
	Past President		Ted Batterham	
	Paul Hemsworth			
1995	***President***	***Secretary***	***Committee***	***Editor(s)***
	Bruce Mullan	Dean Revell	Paul Hartmann	David Hennessy
	Vice President	***Treasurer***	David Hampson	Peter Cranwell
	Andy Paterson	Rob Smits	Sue Skirrow	
	Past President		Frank Dunshea	
	Mike Taverner			
1997	***President***	***Secretary***	***Committee***	***Editor(s)***
	Frank Dunshea	Neil Gannon	Robert Love	Peter Cranwell
	Vice President	***Treasurer***	Tony Peacock	
	Robert van Barneveld	Rob Smits	Robin Warner	
	Past President		Ian Williams	
	Bruce Mullan			
1999	***President***	***Secretary***	***Committee***	***Editor(s)***
	Robert van Barneveld	Colin Cargill	John Pluske	Peter Cranwell
	Vice President	***Treasurer***	Susanne Hermesch	
	Neil Gannon	Heather Bray	Ian Williams	
	Past President		John Hargraves	
	Frank Dunshea			

2001	***President***	***Secretary***	***Committee***	***Editor(s)***
	Ian Williams	Sally Tritton	Susanne Hermesch	Peter Cranwell
	Vice President	***Treasurer***	Mingan Choct	
	John Pluske	Rob Smits	Darryl D'Souza	
	Past President		Ray King	
	Robert van Barneveld		Mike Taverner	
2003	***President***	***Secretary***	***Committee***	***Editor(s)***
	John Pluske	Hugh Payne	Ian Barugh	Janet Paterson
	Vice President	***Treasurer***	Frank Dunshea	
	Pat Mitchell	Darryl D'Souza	Susanne Hermesch	
	Past President		Ray King	
	Ian Williams		Geogy Philip	
2005	***President***	***Secretary***	***Committee***	***Editor(s)***
	Pat Mitchell	Ian Barugh	Frank Dunshea	Janet Paterson
	Vice President	***Treasurer***	Geogy Philip	
	Bruce Mullan	Graham Pearson	Patrick Morel	
	Past President		Rob Smits	
	John Pluske			
2007	***President***	***Secretary***	***Committee***	***Editor(s)***
	Bruce Mullan	Hugh Payne	Frank Dunshea	Janet Patterson
	Vice President	Karen Moore	Heather Channon	Jenny Barker
	Neil Gannon	***Treasurer***	Rob Smits	
	Past President	Megan Trezona	Darryl D'Souza	
	Pat Mitchell			
2009	***President***	***Secretary***	***Committee***	***Editor(s)***
	Neil Gannon	Karen Moore	Frank Dunshea	Robert van Barneveld
	Vice President	***Treasurer***	Heather Channon	
	Darryl D'Souza	Megan Trezona	Brian Luxford	
	Past President		Trish Holyoake	
	Bruce Mullan			
2011	***President***	***Secretary***	***Committee***	***Editor(s)***
	Darryl D'Souza	Karen Moore	John Pluske	Robert van Barneveld
	Vice President	***Treasurer***	Cherie Collins	
	David Cadogan	Megan Trezona	Heather Channon	
	Past President		Trish Holyoake	
	Neil Gannon			
2013	***President***	***Secretary***	***Committee***	***Editor(s)***
	David Cadogan	Amy Lealiifano	John Pluske	John Pluske
	Vice President	***Treasurer***	Alison Collins	Jo Pluske
	Cherie Collins	Megan Trezona	Jae Kim	
	Past President		Emalyn Loudon	
	Darryl D'Souza			

Acknowledgements

The Australasian Pig Science Association (APSA) biennial conference is now widely regarded as one of the most important international gatherings to present and publish recent advances in pig science. From its humble beginnings in Albury, New South Wales in 1987, APSA now represents one of the must attend pig science conferences, attracting presenters and delegates from all over the globe. This has come about as a consequence of the hard work and dedication of the current ASPA committee and many others over the past 25 years.

The APSA organisation would like to thank everyone that was involved in the preparation and the management of the 2013 conference. The considerable efforts by a motivated group of scientists, who mostly volunteer their time, have once again produced the very same high standards now expected of APSA.

The continued support of APSA members and others associated through pig and related science through the submission of high quality papers to these proceedings is greatly acknowledged. To all the delegates who have attended, presented and contributed to the discussions, the 2013 committee thank you. Many thanks also to the many international delegates for taking the time and effort to travel and attend the meeting, many which have also presented papers and/or posters.

The scientific program, for the Biennial Conference, has always been based on the symposia opinions and reviews provided by invited speakers. Firstly, a huge appreciation to the invited international speakers, Ron Ball, George Foxcroft, Jeff Zimmerman and Adam Moeser. Also many thanks to: Paul Hemsworth who presented the A.D. Dunkin Memorial Lecture; the opinion and review authors Peter Scott, Damien Batstone, Sasha Jenkins, David Pethick, and Robert van Barneveld; the symposia leaders Jae Cheol Kim and Cherie Collins. APSA also thanks the various chairpersons who have the difficult job of ensuring that the program runs to schedule, and also a gratitude to the judges of the APSA Medal, APSA Poster and the Batterham Memorial Award.

There are very few conferences now held where the proceedings are produced prior to the conference and to such a high editorial and scientific content. A high appreciation to APSA editors, John and Jo Pluske for the excellent job in generating a world-class proceedings. To all the reviewers, who once again volunteered their time to provide guidance and to further strengthen the invited and submitted papers, thank you.

I have been overwhelmed with the dedication and tiresome work by the organising committee over the past two years. Many thanks goes to Cherie Collins (Vice President), Darryl D'Souza (Immediate Past President), Amy Lealiifano (Secretary), Megan Trezona (Treasurer), John Pluske, Alison Collins, Jae Cheol Kim and Emalyn Loudon. A special acknowledgement to Amy Lealiifano, other than doing a very professional job keeping the committee on track and to task, who has brought the APSA membership into the 21st century, organising and helping with the design of a very functional website making the promotion and function of APSA much more efficient, including online purchases of current and past proceedings, to renew or join the APSA membership, online submission of papers and more.

APSA also greatly acknowledges all of the conference sponsors, who provide generous support to allow the Conference to exist and function. APSA has always had a strong relationship with Australian Pork Limited and the Pork CRC Limited, and it is most pleasing to see both combine their support as Principal Sponsors again for the 2013 meeting. We encourage those in the APSA community to support those sponsors that help support our great conference.

Lastly, the XIV Biennial Conference was organised in conjunction with YRD who has acted as secretariat for the last four conferences, and it was an absolute pleasure to work with Kate Murphy and Angeline Deo to produce another outstanding meeting.

Dr David Cadogan, President

Sponsors of the 14th Biennial APSA Conference: 2013

Principal

Gold Welcome Reception

Meat supplied by

Gold Conference Dinner

Gold Networking Dinner

Conference Applications

Sponsors of the 14th Biennial APSA Conference: 2013

Silver

Bronze

Coffee Cart Sponsor

Conference Prizes

Acknowledgements to the Referees

The proceedings of the fourteenth biennial conference of the Australasian Pig Science Association, 'Manipulating Pig Production XIV', contains 126 one-page papers, two opinion papers, four review papers, four symposia papers and the paper presented as the Dunkin Memorial Lecture. As is the policy of the Association, all papers were reviewed by external referees. The APSA committee and Editors gratefully acknowledge the assistance generously given during 2013 by the following referees:

Amdi C.
Athorn R.
Blache D.
Black J.
Brewster C.
Bunce M.
Bunter K.
Cadogan D.
Cake M.
Campbell R
Capozzalo M.
Carter R.
Channon H.
Chen T.
Chenoweth P.
Chidgey K.
Clarke T.
Close W.
Collins A.
Collins C.
Cronin G.
Cutler R.
de Jong J.
Diffey S.
Downing J.
D'Souza D.
Dunshea F.
Edwards A.
Edwards M.
Emery D.
Fahy T.
Finlaison D.
Fleming P.
Foxcroft G.
Frey B.
Gabler N.
Gannon K.
Gannon N.
Gidley M.
Goodband R.
Guillou D.
Guzmán-Pino S.
Hampson D.
Hansen C.
Hemsworth P.
Henman D.
Heo J.M.
Hermesch S.
Hewitt R.
Hughes P.
Johnston L.
Jose C.
Kim J.
King R.
Kirkwood R.
Kruger I.
Langendijk P
Leury B.
Loudon E.
Luxford B.
Marenda M.
McGilchrist P
McKenzie P.
McPherson F.
Miller H.
Mitchell P.
Moeser A.
Montoya C.
Morrison R.
Mullan B.
Muller T.
Nottle M.
O'Leary S.
Owusu-Asiedu A.
Payne H.
Pérez J.
Plush K.
Pluske J.M.
Pluske J.R.
Rault J-L.
Resink J-W.
Roura E.
Smits R.
Solà Oriol D.
Suckling A.
Thornton E.
Tokach M.
Trezona M.
Turni C.
Turpin D.
van Barneveld R.
van Wettere W.
Ward M.
Wilkinson S.
Williams B.
Willis S.
Wilson M.
Wilson R.H.
Wynn P.
Zijlstra R.

Preface

The importance of research and innovation for the national and international pork industry has never been so crucial. The volatility in global cereal and oilseed protein meal prices has continued, and the business environment is still stagnating from the global financial crisis. However, the latest major driver for change in pork production, and hence the demands in research and innovation, is coming from the large corporate supermarkets, animal rights groups and general consumers of pig meat. This has given a new focus on the requirement of knowledge, particularly on low environment impact systems, antibiotic-free diets, pig welfare and sow-stall-free production systems. With this in mind, the A.C. Duncan Memorial Lecture will provide an overview of the roles and functions of science in establishing animal welfare recommendations and standards while trying to balance the expectations of consumers and animal rights groups.

There is still a large amount of research and new information being generated in pig science and pork production, but reductions in funding have created a reduction in extension services to producers. In this context, the committee of the Australasian Pig Science Association (APSA) thought it important to highlight during the conference, the importance of delivering science for maximum industry benefit, outlining current problems and issues and potential solutions from an end-user and an academic point of view. The remaining invited papers and symposia present recent research and relevant information on nutrition and management of the prolific sow, monitoring herd health and immunity, gastrointestinal tract barrier function and systemic response to nutrition and management, and genomic approaches for quantifying microbial communities to the benefit of the pig industry; an environmental perspective. Additionally, the 126 refereed one-page papers in *Manipulating Pig Production XIV* guarantee that the conference program will have something for everyone associated with the pork industry.

Since its inception, the APSA conference has provided an excellent forum to present new findings, foster in-depth discussion, and hopefully provide solutions to some of the industry problems. The 14th APSA meeting upholds these aims and provides a real opportunity for researchers to present their findings to their global peers, and interact with key industry figures and organisations.

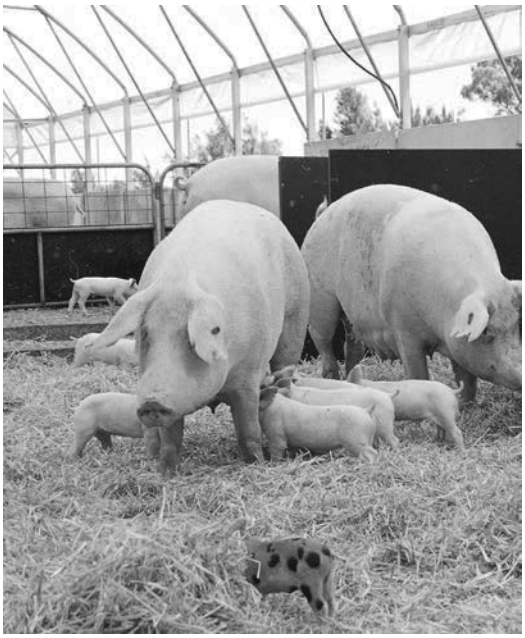
The Cooperative Research Centre (CRC) for High Integrity Australian Pork and Australian Pork Limited (APL) have been integral in funding the majority of the research and development in Australia, as well as training undergraduate and postgraduate students and generating junior scientists. APSA outwardly promotes the involvement of students and early-career scientists, both privately and publically funded, at the meeting, and again the conference has a considerable number of young scientists attending and presenting their work.

It has been a great honour to preside over APSA for the last two years and to have contributed in facilitating the networking of many of those involved in pork production. Thank you for making the meeting a great success and I hope you will continue to be part of the future success of the Association.

Dr David Cadogan, President

CHAPTER 1

The A.C. Dunkin Memorial Lecture



The role of science in improving animal welfare

P.H. Hemsworth

Animal Welfare Science Centre, The University of Melbourne, Parkville, VIC 3010.

Introduction

The animal welfare movement is increasingly influencing views on animal use and the acceptability of various animal management options (Hemsworth *et al*., 2007; Fraser, 2008). The movement has its origins in an array of experiences and ethical views, which can arouse strong sentiments and disparate individual targets for attention. Stakeholders in the animal welfare domain include the public (generally as consumers), owners or concerned observers; special interest groups such as animal welfare and animal rights groups; businesses based on the commercial supply of animals and animal products; and those developing, implementing or auditing compliance with relevant policy at government, industry or community level. Consumer and public attitudes to animal welfare have the potential to affect the use of animals in society, influencing for better or worse the operations of livestock industries, medical research, the management of feral and wild animals, and the care and use of recreational and companion animals.

While consumer and public attitudes to animal welfare have the potential to affect society's use of animals, science has a critical role in underpinning governments' decisions, on behalf of the community, on animal use, and the attendant conditions and compromises (Mellor and Bayvel, 2004; Hemsworth *et al*., 2007; Matthews and Hemsworth, 2012). However, many people in the general community are ignorant of the conditions under which domestic animals live, how they are treated and their species-specific requirements. Failure to assure these stakeholders that the welfare standards for domestic animals are underpinned by sound science will not only hinder the adoption of welfare-friendly new technology in the animal industries, but also has the potential to adversely influence the profitability and viability of these industries by affecting specific animal uses.

A substantial body of research over many decades utilising the behavioural, nutritional, physiological, genetic, veterinary and other animal-based sciences has provided solutions to serious nutritional, environmental and health problems for livestock that not only substantially improved animal productivity and health but also animal welfare (Mellor and Bayvel, 2008; Mellor *et al*., 2009; Fraser *et al*., 2013). In the last two decades or so, scientific attention has turned to addressing the behavioural and emotional requirements of animals, aspects that have been demonstrated to also have animal welfare implications (Mellor *et al*., 2009).

These improvements in animal productivity, health and welfare driven by science have been associated with major changes in how animals are perceived, especially with regard to what their needs are, how management practices affect them and how they should be treated (Mellor *et al*., 2009). These changes in our perception of animals are part of the continuing development in how society thinks about the ways that animals may be ethically used for human purposes.

There are five key areas of endeavour necessary to continue to rationally address genuine welfare risks to livestock:

i. Undertake animal welfare science;

ii. Understand public and consumers attitudes to animal welfare;

iii. Provide public and industry education;

iv. Set animal welfare recommendations and standards through broad stakeholder consultation;

v. Design welfare assessment schemes that provide assurance to industry, the community, markets, and regulatory authorities that agreed standards are verified in practice (Hemsworth *et al*., 2007).

In relation to the welfare of commercial pigs, Australia's research, development and extension (RD&E) capability and funding opportunities place it in an excellent position to continue to improve pig welfare through understanding both the genuine welfare risks to pigs and the public's attitudes to animal welfare and its interests in engaging in this topic. However, there are some key challenges that will require targeted research, education and consultation amongst the major stakeholder groups. While the five key areas of endeavour are considered, this paper focuses firstly on the scientific assessment of animal welfare and its challenges and secondly on Australia's scientific contribution to pig welfare.

Decisions on animal use

Moral responsibility to society's animal

The relationships that develop between people and their animals in our society are inevitably unequal. The care of these animals is basically governed by two principles that apply to a range of animal uses from individual pets to livestock production. These principles are on the one hand, compliance with the objectives of human profit, benefits or pleasure and on the other hand, a duty of humane care towards animals (Matthews and Hemsworth, 2012). The latter is based on the widely-held view in our community that the use of animals by humans is acceptable provided that such use is humane (Mellor and Littin, 2004).

Most people accept that humans have a moral obligation towards farm, companion and laboratory animals. In addition to the undeniable benefits that these animals provide to humans, the domestication of these animals has increased their dependence on humans and thus reinforces this obligation. However, what is at question for most people is the extent of this obligation, particularly in relation to the standards of welfare that society should provide to these animals (Hemsworth and Coleman, 2011).

Considerations in decisions on animal use

An individual's decision on the acceptability of a specific animal use can be a difficult and complex choice. Decisions on specific animal use are affected by a number of considerations (Hemsworth *et al*., 2007; Hemsworth and Coleman, 2011; Matthews and Hemsworth, 2012), including scientific information on the harms and benefits to the animal caused by its use. While attitudes to animals at the individual level influence how people behave towards them, attitudes to animals at the community level can also influence the development of animal-related policy and legislation. Attitudes to animals appear to be particularly affected by people's affective or emotional responses, as well as their perception of the utility or instrumental value of the animal (Serpell, 2004). Individuals may also judge a decision or choice of an animal use on the basis of its adherence to reasons or moral principles such as: enhancement of personal character (virtue ethics); support for self-interest (egoism); consequences of the choice for everyone, not just themselves (equal consideration or utilitarianism); or that good results do not justify using evil means to violate an animal's rights (rights-based justifications of equal consideration). Animal ethics embraces not only our duties and obligations to animals, but also our duties and obligations to animal users and society in general (Levy, 2004). Thus, the impact of the animal use on the animal owner, the environment and the economy may also affect these decisions.

Therefore, ultimately the question of the acceptability of a specific animal use is an ethical one in which science can provide factual evidence that needs to be utilised to provide objectivity to the answer (Matthews and Hemsworth, 2012): when combined with other beliefs and principles, facts can provide guidance on animal use (Levy, 2004). In setting animal welfare recommendations and standards, governments and others acknowledge and to varying degrees take account of the many and varied viewpoints through broad stakeholder consultation.

The development of a clear consensus on an ethically and scientifically defensible philosophy on animal welfare, however, is obviously difficult. Society's attitudes to the use of and obligations to farm, companion and laboratory animals are disparate, influenced by demographic factors, religion and culture and vary over time with economic and ideological changes (Hemsworth and Coleman, 2011). As Teutsch (1987) reported, there are clear within- and between-country variations in the attitudes of people to their obligations towards animals. Notwithstanding these variations, Coleman (2008) concluded that most countries in the Western world show similar patterns of attitudes to farm animal welfare. The Eurobarometer surveys (European Commission, 2007), which provide a snapshot of attitudes to animal welfare across the European community, and Australian research (Coleman, 2008) indicate that the public appear to be more concerned about the welfare of poultry and pigs than other farm animals. This consistent concern for the welfare of poultry and pigs presumably relates to restrictions on space, social contact and choice of stimuli for interaction associated with their close confinement (Barnett *et al*., 2001).

Polarisation of views

The animal welfare debate is often polarised. For example as Coleman (2010) has commented, stakeholders such as animal industry groups and animal rights activists, who are highly involved in a controversial issue, will find media coverage of an issue to be relatively disagreeable. This tendency for stakeholders to perceive bias in media coverage arises in two ways (Coleman, 2010). The first of these is the 'hostile media' effect: this describes the tendency for people who are highly involved in an issue to see news coverage of that issue as biased, particularly biased against their own point of view merely by virtue of the fact that it comes from the media. The second process is the 'persuasive press inference' hypothesis: this asserts that one reason people are concerned about media bias is that they believe that what is reported

in the mass media will substantially influence public opinion. Thus stakeholders often will see public opinion on such issues as contrary to, or at least incompatible with, their own opinions and this tends to polarise those individuals who are most actively involved, for example, practitioners or opponents of livestock production systems (Coleman, 2010). Furthermore, these stakeholders may use over generalisations, over simplifications and biased selectivity regarding the scientific support advanced for their cause.

The community and other stakeholders

The drivers of change in animal welfare occur within both the public and private domains (Matthews and Hemsworth, 2012). Public attitudes about animal welfare, although often based on limited knowledge, have a significant role in determining how people behave both as consumers and as citizens (Coleman, 2008). At the societal level, changes in people's attitudes are usually the driving force behind improvements in animal-related legislation and public policy (Coleman, 2008; Fraser, 2008; Serpell, 2008). Similarly, targeting of retailers and consumers is an effective strategy used by welfare lobby groups to effect change in livestock production practices and, indeed, there are examples in many countries of retailers responding to this kind of pressure by requiring suppliers to adhere to specific private standards of welfare in the raising and slaughter of their livestock (see Matthews and Hemsworth, 2012). While changes driven from the private domain may be more responsive to community or consumer concerns, Matthews and Hemsworth (2012) concluded that these changes may not achieve the desired improvements in animal welfare to the extent that they are not grounded in science or formal risk assessment. Evidence-based policy and legislation together with credible monitoring of animal welfare standards and/or enforcement to provide assurance for the community, market, industry and regulatory authorities should be the cornerstones for progressing demonstrable improvements in animal welfare. Furthermore, an essential ingredient in utilising evidence-based policy and legislation to achieve animal welfare improvements is that governments and others engage in broad stakeholder consultation in this process.

It is therefore clear that the community is a key driver of change: community views affect decision makers at the political, regulatory and retail levels. However, public attitudes about animal welfare are often based on limited knowledge, and the public's beliefs are largely acquired from the mass media, perhaps filtered by opinion leaders (Coleman, 2010). Therefore, there is a need to ensure that those in the community who are interested in engaging in this topic be well informed, and the review by Coleman (2010) on how information on animal welfare should be disseminated, by whom and to whom, is summarised here. The target groups should include farmers and post-farm gate participants including transport drivers and abattoir workers, legislators and regulators, retailers, and the general community. These target groups may not be homogeneous, but each has identifiable needs for knowledge and skills relevant to farm animal welfare. The approach that is likely to be most effective is to provide appropriately targeted dispassionate and factual information to the community. In this way, when debates about animal welfare occur, all the stakeholders involved, such as animal-rights groups, retailers, farmers, legislators, and regulators, are more likely to have better outcomes if discussion is based on a shared understanding of what current practices are and what science can reveal about welfare. Given that the mass media are the preferred source of information, the use of science-based media coverage and informed ethical debate is likely to have the best effect, albeit over a fairly long time frame. Since the role of opinion leaders has been well recognised as playing an important mediating role in information transfer to the community, further research to establish whether such opinion leaders in animal welfare do exist in the community and whether they have influence on public attitudes is required.

Furthermore, since schoolchildren are most likely the only target group that can be educated in the sense of knowledge transfer, Coleman (2010) suggests that schoolchildren, particularly those living in urban areas where there is little exposure to farm animals, need to be provided with the basic facts about the origin of their food. This knowledge should make children less susceptible to extreme views about the welfare of farm animals because the knowledge they have acquired will tend to make them less resistant to inappropriate persuasion.

The scientific assessment of animal welfare

Animal welfare

Animal welfare is a state within an animal. It is not management procedures applied to the animal, nor features of the animal's environment, which may affect its welfare (Mellor *et al*., 2009). Duncan (2004, 2005) and many others have argued that animal welfare ultimately concerns animal feelings or emotions. Emotions are classically described through a behavioural component (a posture or an activity), an autonomic component (visceral and endocrine responses), and a subjective component (emotional experience or feeling) (Dantzer, 1988). Given the very nature of emotional self-experience, there is no way

to know if animals experience emotions similar to humans (Boissy *et al*., 2007). It is obviously a major challenge to study and understand emotions in animals, but there have been some promising recent developments in the comparative study of emotions that show that there are many homologous neural systems involved in similar emotional functions in both humans and other mammals, and perhaps other vertebrates (see review by Panksepp, 2005).

Since animal welfare relates to experienced sensations, the animal must be sentient and conscious (Mellor, 2012). These emotional experiences can be negative or positive and they arise as the integrated outcomes of sensory and other neural inputs from within the animal's body and from its environment. These experiences are all subjective, having varying degrees of emotional contents and, based on human experience, are likely to include negatives affects such as fear, thirst, hunger, nausea and pain, and positives affects such as satiety, contentment and playfulness (Mellor, 2012). Emotions, when they reach thresholds of both a specific sensation and a specific intention, motivate animals to engage in behaviours for which there are some strong evolutionary benefit (Denton *et al*., 2009). As subjective states they cannot be measured directly, but there are informative indirect indices of such experiences such as the physiological and behavioural responses of animals (Mellor, 2012). The assessment of animal welfare will be considered in more detail in the next section.

There has been and still remains a clear priority to avoid animal suffering. Suffering is a term in common use to denote negative or noxious subjective or emotional mental experiences (Mellor *et al*., 2009). The term usually refers to strongly negative experiences. Suffering is not a demonstrable entity in itself, despite the common use of the term in that way. It is a generic term representing negative or noxious mental experiences such as anxiety, fear and pain. Thus an animal or person is said to be suffering when anxiety, fear, pain and/or other forms of distress become more intense and approach their maxima (derived from Mellor *et al*., 2009).

The mandate to avoid suffering is prescribed in the prevention of cruelty legislation, or what is often now titled animal welfare legislation, in many Western countries which specifically refers to cruelty in terms of "unreasonable pain or suffering" [e.g. Victoria, Australia (Anonymous, 2007)] or "unnecessary suffering" [the United Kingdom (Anonymous, 1911)]. It should also be recognised though that the legislation in many of these countries refers to its purpose as not only "to prevent cruelty to animals" but also "to encourage the considerate treatment of animals" [e.g. Victoria, Australia (Anonymous, 2007)]. Indeed there is an emerging shift in community values towards not merely minimising suffering in domesticated animals, but also enhancing pleasure in these animals (Tannenbaum, 2001). For many a consideration of animal welfare includes not only the avoidance of suffering, but also the presence of positive emotional experiences (Duncan, 2004; Green and Mellor, 2011). Indeed some have suggested that it is widely accepted that "good welfare is not simply the absence of negative experiences, but rather is primarily the presence of positive experiences such as pleasure" (Boissy *et al*., 2007).

Three concepts of animal welfare used by scientists to assess animal welfare

Although science can provide the factual basis of the impact of a husbandry or housing practice on the biology of the animal, there is some uncertainty amongst scientists on the concept of animal welfare (Fraser, 2003a, 2008; Sandøe *et al.*, 2004). For many scientists, animal welfare has been assessed on the basis of how well the animal is performing from a biological functioning perspective. For others, animal welfare concerns affective states, such as anxiety, fear, pain, hunger and other negative feelings or emotions, as well as positive emotions, such as euphoria, satiety and playfulness. Another concept in the literature, albeit not well enunciated, is predicated on the view that the welfare of animals is improved in environments or situations in which the animals display normal or "natural" behaviour. The so-called 'five freedoms', that is freedom from hunger and thirst, from discomfort, from pain, injury and disease, to express normal behaviour, and from fear and distress (FAWC, 1993), include aspects of all of these three concepts. While most would accept that these freedoms are necessary to avoid suffering, in terms of a consensus on animal welfare assessment, there has been little attempt to define the levels of freedom that are desirable together with the adverse consequences of not providing such freedoms. Moreover, some animal welfare scientists have argued that use of the term 'freedom' is misleading, as none of the 'five freedoms' is in fact achievable in the life of an animal, and have developed alternatives which are aligned with these three concepts and provide a more useful basis for animal welfare assessment (see Green and Mellor, 2011). The three concepts of animal welfare can lead to the different methodologies or a combination of methodologies to assess an animal's welfare: biological functioning; affective states; and normal or natural behaviour are discussed in the following sections.

Biological functioning

The rationale underpinning this concept is that difficult or inadequate adaptation will generate welfare problems for animals. Broom (1986, 1996) defined the welfare of an animal as "its state as regards its

attempts to cope with its environment". The "state as regards attempts to cope" refers first, to how much has to be done in order to cope with the environment and includes biological responses such as the functioning of body repair systems, immunological defences, physiological stress responses and a variety of behavioural responses, and second, to the extent to which these coping attempts are succeeding. These behavioural and physiological responses include abnormal behaviours, such as stereotypies and redirected behaviours, and the stress response, respectively, while the success of the coping attempts are measured in terms of lack of biological costs, such as adverse effects on the animal's ability to grow, reproduce and remain healthy and injury-free (i.e., fitness effects).

The responses to stress are integral to the ability of an animal to cope and, in turn, to the welfare of the animal. Behavioural and physiological adaptive responses are utilised by individuals to cope with challenges (Broom, 1986, 1996; Broom and Johnson, 1993; Moberg, 2000; Barnett, 2003), but marked challenges may overwhelm an individual's capacity to adapt and lead to its death. However, although less severe challenges would not be fatal, they can still have significant biological costs, leading for example to impaired growth, reproduction and health, which in turn may result in welfare problems for the animal. In other words, it is the biological cost of stress that is the key to understanding the associated welfare implications (Moberg, 2000; Barnett, 2003). How well an animal is coping with physical challenges and emotional states will be reflected in the normality of its biological functioning, and severe risks to welfare will be associated with the most extreme coping attempts.

Early attempts in the 1960s and 1970s to study animal welfare focused on measuring animal stress. These attempts have been used to criticize this biological functioning-based concept of animal welfare (Duncan, 2004) without recognizing that this concept considers not only the magnitude of the stress responses but more importantly the consequences of these stress responses. While animals utilise the stress response to try to deal with challenges to homeostasis, in terms of animal welfare it is the consequences for the animal that are important. Failure to adapt to stressors can adversely affect an animal's fitness because of adverse effects on nitrogen balance, reproduction, injury and health (Barnett, 2003). The effects on health include ulcers, hypertension, arteriosclerosis and a suppression of the immune system and some effects may be permanent even if the stressor is subsequently removed (Moberg, 2000). Furthermore, in addition to a broad range of physiological responses including those of the nervous, immune, circulatory and endocrine systems, particularly those of the hypothalamic-pituitary-adrenal axis, animals also utilize behavioural responses to try to deal with these challenges. Long-term behavioural responses, such as stereotypies demonstrated by bar-biting in sows and pacing, weaving and wind-sucking in stabled horses, are 'harmful' behavioural responses that have clear biological costs for animals, such as physical injury, digestive complications and the loss of production. Thus the focus of this assessment of animal welfare using this concept of biological functioning extends to the consequences of the stress response rather than the response *per se*. The development of the stress response and consequently the manner in which animals deal with difficulties in their lives have been well reviewed by Broom and Johnson (1993) and Moberg (2000).

Some have also criticised this concept of animal welfare on the basis that it does not adequately include emotions. However, this would only be valid if emotions are independent of other biological processes, but this is unlikely since the mental state of an animal is an integral component of its biological state (Dantzer and Mormede, 1983). Emotional responses are produced in the limbic system, which projects to several parts of the brain, including those involved in the initiation and maintenance of the stress response, thus explaining why emotional insults activate a stress response (Kaltas and Chrousos, 2007).

This concept of animal welfare has been used by scientists to assess the effects of housing, husbandry and handling practices. For example, a broad examination of the behavioural, physiological and fitness responses in handling studies, particularly in pigs and poultry, have generally shown that negative or aversive handling, imposed briefly but regularly, will increase fear of humans and correspondingly reduce growth, feed conversion efficiency, reproduction and health of these animals (see Waiblinger *et al*., 2006; Hemsworth and Coleman, 2011). A chronic stress response has been implicated in these effects on productivity since in many of the pig handling studies (see Hemsworth and Coleman, 2011), handling treatments which resulted in high fear levels also produced either a sustained elevation in the basal free cortisol concentrations or an enlargement of the adrenal glands. Studies examining surgical husbandry procedures have also used a broad examination of the behavioural, physiological and fitness responses to assess animal welfare (Mellor *et al*., 2000; Hemsworth *et al*., 2009; Colditz *et al*., 2010).

In conclusion, how well an animal is coping with physical challenges and emotional states will be reflected in the normality of its biological functioning, and difficult or inadequate adaptation will adversely affect the fitness of the animal through a range of long-lasting behavioural and neuroendocrine responses. These behavioural and physiological responses include abnormal behaviours, such as stereotypies and redirected and displacement behaviours, and the stress responses including those involving both the

sympathetic-adrenal-medullary and the hypothalamic-pituitary-adrenal axes, respectively, while the biological cost includes adverse effects on the animal's ability to grow, reproduce and remain healthy and injury-free.

Affective states

The second concept, often called the affective state or feelings-based concept, defines animal welfare in terms of emotions and emphasises reductions in negative emotions, such as pain and fear, and increases in positive emotions, such as comfort and pleasure (Duncan and Fraser, 1997). Duncan (2004) has argued that animal welfare ultimately concerns animal feelings or emotions as follows. All living organisms have certain needs that have to be satisfied for the organism to survive, grow and reproduce and if these needs are not met, the organism will show symptoms of atrophy, ill-health and stress and may even die. Higher organisms (vertebrates and higher invertebrates) have evolved 'feelings' or subjective affective states that provide more flexible means for motivating behaviour to meet these needs. Thus the central argument is that although natural selection has shaped animals to maximize their reproductive success, this is achieved by proximate mechanisms involving affective states (pain, fear, separation distress, etc.) which motivate behaviour (Fraser, 2003b).

While emotions are poorly defined, impossible to measure directly, and difficult to measure indirectly (Duncan, 2005), there has been a substantial growth over the last two decades in the literature on this topic of emotions (Panksepp, 1998, 2005; Denton *et al*., 2009). There are numerous definitions of emotions in the literature but an emotion can be defined as an intense but short-lived affective response to an event, which is associated with specific body changes and thus is classically described through a behavioural component (a posture or an activity), an autonomic component (visceral and endocrine responses) and a subjective component (emotional experience or feeling) (Dantzer, 1988). Denton *et al*. (2009) considered two classes of emotions. Primordial emotions are viewed as the subjective element of the instincts which are the genetically programmed behaviour patterns which participate in maintaining homeostasis. They include thirst, hunger for air, hunger for food, pain and hunger for specific minerals etc. There are two constituents of a primordial emotion, the specific sensation which when severe may be dominant, and the compelling intention for gratification by a consummatory act. They may dominate the stream of consciousness, and can have plenipotentiary power over behaviour. These primordial emotions are predominantly driven by sensors detecting deviation from normal within the body (interoceptors), and this class of emotions contrasts with another class of emotions which are most often fired by the distance receptors (exteroceptors) within the eyes, ears and the nose. These distance receptor evoked emotions, like rage, fear, hate, envy, happiness, playfulness, affection, anxiety, depression and disgust, and are those to which the term emotion is most commonly applied and they are most often determined by situational perception. When emotions reach both a commanding specific sensation and a compelling specific intention, they motivate animals to engage in behaviours for which there is some strong evolutionary benefit (Denton *et al*., 2009).

Animal emotions have in the past been considered inaccessible to scientific investigation because they have been described as human subjective experiences or even as illusory concepts outside the realm of scientific inquiry (Panksepp, 1998). Given the very nature of emotional self-experience, there is no way to know if animals experience emotions similar to humans (Boissy *et al*., 2007). However, behaviour, structure, and brain chemistry are similar in humans and in a large number of animal species: other mammals are attracted to the same environmental rewards and drugs of abuse as humans; human emotions appear to be dependent on very similar sub-cortical brain systems situated in deep brain regions where evolutionarily homologous "instinctual" neural systems exist; and artificial activations of the deep brain systems that promote emotional actions are liked and disliked by animals, as measured by a host of approach and avoidance responses (see review by Panksepp, 2005).

Preferences can be measured as a means to determine what resources are important to an animal. Initial use of preference methodologies appeared in the literature in the 1970s (e.g., Hughes and Black, 1973; Dawkins, 1976). Preference testing using a Y maze apparatus that allows a choice between access to two different resources has been used to provide information about specific features in the animal environments such as flooring on raceways (Hutson, 1981), restraint methods (e.g., Pollard *et al.*, 1994), handling treatments (Rushen, 1986) and ramp design (Phillips *et al.*, 1988), with the overriding objective of optimising captive environments for animals. Essentially, these tests are designed to answer the question *"what is the relative importance of this feature for this animal?"*

Aversion learning techniques have been used to study the animal's motivation to avoid husbandry and handling treatments. For example, Rushen (1986) studied the avoidance of sheep to electro-immobilisation, a procedure in which a pulsed, low-voltage current can be used to immobilise the animal. Sheep were trained to associate a location with a specific treatment and avoidance was assessed based on the effort

required to move them repeatedly to the treatment location. It was found over repeated trials that sheep showed increasing avoidance of a location in which they were restrained with electro-immobilisation than to a location in which they were restrained without it.

While the consistent choice or preference of one resource over another or others indicates the animal's relative preference, some have argued that in addition to establishing what an animal prefers, it is important to understand the strength of the preference (Dawkins, 1983; Matthews and Ladewig, 1994). To address the question of the strength of an animal's preference, experiments have incorporated varying levels of cost (e.g., work effort, time and relinquishing a desirable resource) associated with gaining access to a resource or avoiding aversive stimulation. For example, Dawkins (1983) varied the price paid for access to litter by increasing the duration of feed withdrawal before the test. She found that although hens preferred litter to wire floors, their preference was not strong enough to outweigh the attraction of food and concluded that in both experiments there was no evidence that hens regarded litter as a necessity. Food can be considered as the "gold standard" in preference testing (Matthews and Ladewig, 1994), and therefore is generally expected to produce a maximal response or preference.

Furthermore, Dawkins (1983) suggested that quantitative measures of the importance of resources for animals can be derived from measures of demand elasticity. Consequently, 'behavioural demand' studies, using operant conditioning techniques in which the animal must learn to perform a response, such as pecking at a key or pushing through a weighted door, to gain access to a resource, have been used to study the animal's level of motivation to access or avoid the situation being tested. For example, Matthews and Ladewig (1994) studied the behavioural demand functions of pigs for the resources of food, social contact and a stimulus change (door opening). The amount of work, in the form of pushing a plate, required for access to each reinforcer (resource) was systematically varied. It was found that while the demand for opening the pen door was highly elastic (i.e. the willingness of the pigs to access the resource declined as the effort increased), the demand for food was inelastic and the demand for social contact was intermediate.

While it seems likely that animals will avoid aversive stimulation and choose positive stimulation, preference and motivation testing have generated considerable debate relating to conceptual and methodological difficulties (see Nicol *et al*., 2009; Fraser and Nicol, 2011). For example, familiarity with a resource may affect choice, a choice at a point in time may not reflect interactions of different motivational states over time, a positive resource may remind the animal of a resource that it may not otherwise miss, the choices may not be within the animal's cognitive capacity and vigilance behaviour may be misinterpreted as a choice.

Other approaches utilised in assessing affective states include measures of behaviour, cognitive bias and, particularly in assessing negative affective states, physiology (Boissy *et al.*, 2007; Mendl *et al*., 2009).

As with biological functioning, clarifying the conceptual link between animal preferences and animal welfare is difficult. Nevertheless, as argued by a number of authors (e.g., Fraser and Matthews, 1997; Widowski and Hemsworth, 2008), while studies of motivation can provide evidence that the performance of some behaviours (or preferences) may be important to the animal, additional evidence, particularly on the occurrence of abnormal behaviour, stress physiology and health, are necessary to provide a more comprehensive assessment of the impact of restriction on animal welfare.

Normal or natural behaviour concept

The third main concept of animal welfare, which is not often well-enunciated, promotes the principle that animals should be allowed to express their normal behaviour. For some this also implies that animals should be raised in 'natural' environments and allowed to behave in 'natural' ways.

The term "abnormal behaviour" in domestic animals invariably raises questions about what is normal (Mills, 2010), particularly when most behavioural differences between wild and domestic animals appear to be quantitative rather than qualitative in character, and best explained in differences in response thresholds (Price, 2002). Considered as an aspect of the behaviour of an animal, abnormal behaviour is frequently defined as behaviour that is either atypical for the species, outside the normal behavioural pattern that has evolved in the natural habitats of the species or outside the range usually observed in the species in non-captive situations (Keeling and Jensen, 2005).

The difficulty of deciding what constitutes the natural environment for domestic animals is illustrated when reviewing the history of the domestic hen, as described by Appleby *et al*. (1992). The progenitor of the domestic fowl was the Red Jungle Fowl (*Gallus gallus*), a tropical species confined to forested areas and thick vegetation. There are now two modern hybrids, the egg laying bird that reaches point of lay at 16-18 weeks of age at a body weight around 1.8-2.0 kg and that lays close to one egg a day, and the meat bird which reaches slaughter weight of about 2.5 kg as quickly as five weeks of age. What is the 'natural

environment' of a young bird selected for meat production or an adult hen selected for egg laying, both of which are the same species, with a history of about 8000 years of selection for fighting capabilities and 100 years of intense selection for production attributes? Is an outdoor area with relatively little structural diversity, except perhaps for some grass, a natural environment for a tropical species?

In the early literature, the view that animals should perform their full 'repertoire' of behaviour was very common, however there is broad agreement within science that it is often difficult to attribute actual suffering when the expression of certain behaviours is prevented or is absent when it would be expected to be present (Dawkins, 2003). Furthermore, as Fraser (2003a) noted, "Few scientists today would support the simple view that animal welfare depends on the animal carrying out all its natural behaviour in a natural environment because natural environments contain many hardships (harsh weather, predators), and natural behaviour includes many means of dealing with hardship (shivering, fleeing)."

Thus the concept of 'natural' would need to be more specific before it could give guidance in assessing animal welfare, since generalisations may lead us astray and achieve the opposite of what is intended. Similarly, the 'natural behaviours' that are desirable or undesirable in terms of animal welfare require definition together with the rationale for their inclusion or exclusion. More recently the emphasis has been on behavioural indicators of poor coping such as fearfulness, aggression and stereotypies (EFSA, 2005), responses that are also utilised in the biological functioning concept of animal welfare.

Related to this notion of the importance of displaying normal behaviour is that of 'behavioural (or ethological) need'. The term 'behavioural need' appears to have been introduced into the scientific literature without any scientific evidence (Duncan, 1998). Dawkins (1990) and Fraser and Duncan (1998) suggested that the term 'behavioural need' refers to situations that elicit intense negative emotions and likely evolved for those behaviours in which an immediate action is necessary to cope with a threat to survival (e.g., escape from a predator) or reproductive fitness (e.g., nesting). In contrast, other types of behaviour that can be performed when the opportunity arises (e.g., play, grooming) are more likely to be associated with positive emotional states. Duncan (1998) defined "behavioural needs" as behaviour patterns that are very strongly motivated, and, if they are not allowed expression, the animal's welfare may be jeopardised. However, any argument for impaired welfare due to restriction of these behaviours would be strengthened by supporting evidence of decreased health or increased physiological stress (Cooper and Albentosa, 2003; Duncan, 2005).

Australia's scientific contributions to pig welfare

Australian scientists over many decades have been significant contributors to the international RD&E effort addressing serious nutritional, environmental and health problems for livestock, and indeed its contribution to improving the production efficiency of the pig in terms of its nutrition, genetics and health is well recognised (Taverner, 1991). Australia has also made a significant contribution in understanding and addressing housing and handling impacts on pig welfare. Examples include the development of robust methodologies to study the stress physiology of pigs and consequently the inclusion of these methodologies to measure a broad range of behavioural, physiological and fitness responses that provided the foundation to study and understand the influence of housing and husbandry practices on gestating sow welfare (Barnett and Hemsworth, 1990; Barnett *et al*., 2001; Barnett, 2003); understanding the influence of housing and husbandry practices on the behaviour and welfare of parturient and lactating sow welfare (Barnett *et al*., 2001; Morrison *et al*., 2011); and understanding the major human characteristics, such as attitude and behaviour towards animals, that affect animal welfare and productivity and consequently the development training programs initially for pig stockpeople and subsequently for stockpeople in other livestock industries and at abattoirs (Hemsworth and Coleman, 2011). Some of these developments will be briefly considered in more detail now.

Australian research in the 1990s examined the effects of design features of group housing on sow welfare and this research still provides an important contribution to our knowledge as the pork industry moves away from stall housing to group housing during gestation. This research showed that the design of the group housing system, particularly space and the characteristics of feeding stalls for groups, is important in terms of aggression and stress in sows. Reducing floor space increases aggression and stress, based on plasma cortisol concentrations, in gestating gilts and sows (Hemsworth *et al*., 1986, Barnett *et al*., 1992, 1993; Barnett, 1997). More recent research indicates that reducing floor space within the range of 1.4 to 3.0 m^2/sow increases aggression and stress, based on plasma cortisol concentrations, and reduces farrowing rate in sows (Hemsworth *et al*., 2013). Barnett *et al*. (1992) and Barnett (1997) examined the effects of providing feeding stalls in pens for group-housed pregnant gilts and found that feeding stalls and particularly full body length feeding stalls reduced stress based on cortisol concentrations and improved immunological responsiveness assessed by a cell-mediated response to a mitogen injection. However, while aggression around feeding was reduced, feeding stalls did not affect skin lesions. This research also

indicated that while the provision of feeding stalls in pens reduced aggression, stress and improved immunological responsiveness, increased floor space, either total or outside the feeding stall, also reduced stress and improved immunological responsiveness. Barnett and colleagues also conducted a series of short-term experiments on methods to reduce aggression at mixing, such as modifying pen size and shape, masking odours, sedation using pharmacological agents, grouping after dark and *ad libitum* feeding and concluded that all or some of these methods may only be effective in postponing aggression rather than reducing it (see review by Barnett *et al*., 2001). Furthermore, these authors considered that aggression, injuries and stress arising from mixing was an impediment at the time to wider adoption of group housing and recommended further research on mixing strategies to provide producers with the requisite information to reduce the risks to animal welfare. Indeed the imperative to minimise animal welfare risks arising from mixing is still pertinent today (Bench *et al*., 2013a). This body of Australian research clearly indicates that protection of animals at feeding and floor space affect aggression and stress. This research group was one of the first to emphasise the importance of the design feature of the housing system rather than the housing system *per se* (Barnett *et al*., 2001).

The principle that management, including supervising and managing animals, affects farm animal welfare is widely recognised within the livestock industries (Hemsworth and Coleman, 2009). However, the manner in which management affects animal welfare, both directly and indirectly, is probably not fully appreciated. At the level of farm management, human resource management practices, including employee selection and training, and animal management practices, such as best practice in housing and husbandry, and implementation of welfare protocols and audits, all impact on farm animal welfare. At the stockperson level, together with the opportunity to perform their tasks well, stock people require a range of well developed husbandry skills and knowledge to effectively care for and manage farm animals.

Australian research on human-farm animal relationships over the last three decades, initially in the pork industry and subsequently in other livestock industries, has stimulated considerable RD&E (Hemsworth and Coleman, 2011). There are three main lines of evidence demonstrating the impact of stock people on the welfare of pigs and other farm animals: handling studies in controlled experimental conditions, observations in commercial settings, and intervention studies in commercial settings. Conditioning and habituation to humans, occurring both early and later in life, are the most influential factors affecting the behavioural responses of farm animals to humans. The results of handling studies in the laboratory and intervention studies on farms, using cognitive behavioural training of stock people, on the relationships between stockperson attitudes, stockperson behaviour, animal behaviour and stress physiology provide evidence of causal relationships between these variables. Furthermore, this research provides a strong case for introducing stockperson training courses in the livestock industries which target stockperson attitudes and behaviour.

The training or intervention procedure used as an experimental tool in this research in the pork industry has been commercialised for the pork industry, and is called “ProHand” ("Professional Handling Program"). The ProHand approach has been extended into a package for dairy stock people and, following recent research at abattoirs, into packages for pig and red meat abattoir stock people in Australia. Training packages based on the ProHand principles have been developed in Europe as part of the EU 6th Framework for stock people in the pig, poultry and cattle industries. Details of these training programs are described by Hemsworth and Coleman (2011). This body of research highlights the importance of the stockperson on animal welfare and provides the evidence for pronouncements such as that in British Codes of Recommendations for the Welfare of Farm Livestock (Ministry of Agriculture, Fisheries and Food, 1983) that “Stockmanship is a key factor because, no matter how otherwise acceptable a system may be in principle, without competent, diligent stockmanship, the welfare of animals cannot be adequately catered for”.

Addressing the scientific challenges

Multidisciplinary assessment of animal welfare

The assessment of animal welfare requires the use of multiple indicators from multiple disciplines but the quantification of the relative importance of these indicators is lacking (Barnett *et al*., 2001; Fraser, 2008; Nicol *et al*., 2011). Basically scientists have used two main concepts in studying animal welfare, biological functioning and affective states, and these studies require the disciplines of animal behaviour, immunology, neurophysiology, psychology, stress physiology and veterinary science.

Assessment of animal welfare on the basis of biological functioning involves examining how well the animal is performing from a biological functioning perspective, that is, how well the animal has adapted to its environment. Assessment on the basis of affective states (emotions) involves examining both negative states, such as fear, pain, hunger and distress, and positive ones, such as satiety, contentment and

playfulness (Mellor *et al.*, 2009). This scientific uncertainty in relation to animal welfare concepts does not necessarily diminish the robustness of rigorous research utilising criteria or methodologies promulgated by these different concepts (Barnett and Hemsworth, 2009).

While different concepts and consequently different methodologies may be used to assess animal welfare, the validity of the welfare criteria can be tested in several ways (Barnett and Hemsworth, 2009): first, with the finding that there are correlations between independent measures of different concepts of animal welfare; and second, with the finding that an intuitively aversive or rewarding condition reduces or improves animal welfare, respectively, on the basis of the measures of different concepts of animal welfare. Indeed, there is limited evidence of the relatedness of these concepts (Nicol *et al*., 2009, 2011; Stevens *et al*., 2009): that is, animals are motivated to choose those resources or behaviours that maintain normal biological functioning in terms of behaviour, physiology and health. Further research on the relatedness of these concepts is required. However, in the immediate term any argument for impaired welfare due to restriction of a resource or behaviour would be strengthened by evidence that animals are highly motivated to access the resource or perform the behaviour, respectively, as well as evidence of disruption to biological function, such as occurrence of abnormal behaviour, increased stress and poor health.

Two reviews, one by European reviewers (Borell *et al*., 1997) and the other by Australian reviewers (Barnett *et al*., 2001), which at the time influenced to varying degrees welfare policies on stall housing of pregnant sows in many countries, reached different conclusions on the effects of sow housing. Both reviews utilised to varying degrees the concepts of biological functioning and affective states in assessing risks to sow welfare and Borrell *et al*. (1997) also considered the opportunity to carry out natural behaviour in their assessment of animal welfare. Borell *et al*. (1997) concluded that "Since overall welfare appears to be better when sows are not confined throughout gestation, sows should preferably be kept in groups", whereas Barnett *et al*. (2001) concluded that "On balance, it would appear that both individual and group housing can meet the welfare requirements of pigs". Their differing conclusions appear to be due more to differences in the emphasis that they placed on different variables in assessing animal welfare and the importance of the design of the system. While recognising that the major disadvantage for sow welfare in group housing was injuries arising from fighting and slipping, Borell *et al*. (1997) considered that the major disadvantages for sow welfare in stall housing were high levels of stereotypies, unresolved aggression between neighbours and inactivity associated with unresponsiveness, weaker bones and muscles and various clinical conditions such as urinary tract infections and poorer cardiovascular fitness. In contrast, Barnett *et al*. (2001) considered that the design of the housing system was more important to welfare than the housing system *per se*. However, Barnett *et al*. (2001) suggested that the effects of duration of housing in stalls should be evaluated and Borell *et al*. (1997) concluded with reference to groups, that only housing systems resulting in minimal aggression or injury should be used.

Barnett *et al*. (2001) concluded that it is often difficult to determine the relative importance of different variables to welfare. In reviewing the development of hen welfare standards in the European Union, Savory (2004) concluded that the freedom to "perform normal behaviour" is often given more weight in interpreting welfare risks than freedom from discomfort, pain, injury and disease. Fraser (2003a) made the point that scientists and others often introduce value assumptions (usually unwittingly) in weighing different attributes. His question concerning which is more important for hen welfare, access to pasture with more freedom of movement or being housed in cages with more freedom from coccidiosis, puts into focus this uncertainty surrounding differences in the emphasis placed on different variables in assessing animal welfare. Thus as several authors (Fraser, 2003a; Sandoe *et al*., 2004) have recommended, in making such value assumptions more explicit, that scientists should ensure that limitations associated with value judgements are more visible.

Assessing positive emotions

Animal welfare is a state of the animal, which can vary on a continuum from suffering through to positive emotions. While the present mandate to minimise suffering in domesticated animals should remain a focus in the future, it is clear that there is growing community interest in also enhancing pleasure in these animals (Tannenbaum, 2001). For many a consideration of animal welfare includes not only the avoidance of suffering, but also the opportunity for positive emotional experiences. While recognising the inconsistencies in the literature, the review by Boissy *et al*. (2007) concludes that play, affiliative behaviours and some vocalizations appear to be the most promising indicators for assessing positive experiences in laboratory and farm animals.

The evidence provided by Boissy *et al*. (2007) for these indicators of positive emotions is summarised as follows. While vocalisations have long been used as markers of negative emotions in animals, some specific vocalisations are produced by rats, cats and sheep in intuitively "positive" contexts, such as sex,

winning fights, play, grooming and licking and nursing offspring, but inhibited by negative situations. There are several sources of evidence to suggest that play is a rewarding activity. For example, animals actively seek out play partners and solicit play behaviour; the opportunity to play can be used as a reward in place preference conditioning experiments; and thwarting of play often leads to a rebound when the opportunity arises. Allogrooming, which is seen in farm animals such as cattle, horses and pigs, and is associated with reinforcing social bonds and in reducing tension in groups of animals, appears to be rewarding in the short term. The solicitation of social licking demonstrates the rewarding function of the behaviour, at least for the receiver. Soothing effects of allogrooming in terms of a reduction in heart rate have been demonstrated in cattle, horses and primates, but not pigs.

Research on positive emotions in animals is clearly an ambitious task, but the implications are substantial. In addition to the growing interest in providing animals with opportunities to experience positive emotions, there is limited evidence that promoting positive emotions may improve animal welfare. The effect of close human proximity to piglets when nursing during the first day of life on their response to subsequent tail docking was recently examined (Muns Vila *et al*., 2012). The 'positively-conditioned' piglets displayed a behavioural response to tail docking and capture that was less intense and of shorter duration than those that did not receive human contact at suckling. Pedersen *et al*. (1998) examined the effects of handling on tether-housed sows and found that positive handling reduced cortisol concentrations in comparison to minimal and negative handling. The results of these limited studies suggest that rewarding or positive experiences may enhance the animal's ability to cope with stress that may occur during routine management of livestock, but clearly further research is required on this important topic.

Welfare effects may differ between individuals

As Fraser (2003a) noted, there are situations in which decisions on animal welfare need to balance different effects on different animals. An example of this is group housing of sows.

There is evidence that the social rank may affect how individual sows perform in groups from both welfare and productivity perspectives. Mendl *et al.* (1992) found that sows of low success in displacing other sows in an established group with an electronic feeding station had higher concentrations of salivary cortisol and were more responsive to an ACTH challenge, both indicative of a chronic stress response, than sows of high or no success. Furthermore, the low success sows had lighter piglets than the other two categories of sows. Similarly, Nicholson *et al*. (1993) reported that, compared to dominant and submissive sows in the same group, socially intermediate sows showed signs of stress such as elevated cortisol concentrations and reduced natural T killer-cell activity and had lower farrowing rates and smaller litter sizes. Recent research with sows in small groups with floor feeding has shown that individual sow aggressive behaviour, in terms of the ratio of aggression delivered to aggression delivered and received, early after mixing was related to subsequent total and fresh injuries, cortisol concentrations, weight gain during gestation and the number of piglets born alive (Verdon *et al*., 2013; Hemsworth *et al*., 2013). These results in general indicate that sows that engaged in aggression and gain dominance have less injuries and possibly less stress. Their increased weight gain and litter size may be due to increased feed intake through priority access to feed and/or less stress. Furthermore, these dominant sows have higher litter sizes.

Thus while factors such as increased floor space and protection at feeding reduce aggression and stress overall in group-housed sows (Arey and Edwards, 1998; Barnett *et al*., 2001; Bench *et al*., 2013a,b), it is clear that a better understanding of the effects of the composition of the group, particularly aggressive behaviour of individual sows, may have important implications for the welfare and reproductive performance of the group as a whole as well as the individual. Group housing of sows allows all more freedom of movement, exploration and socialisation, but a few may suffer from excessive aggression, stress and injuries. While it is obviously important to reduce aggression and stress at a group level, it is also important to appreciate the welfare implications for individuals. Where individuals are at risk, we need to decide what priority to attach to different classes of animals: the majority, the most vulnerable or the most productive.

Science and animal ethics

Science provides the means to understand the impact of animal use on the animal. Science thus has and should continue to have a prominent role in underpinning our decisions on animal use and the attendant conditions and compromises. The exclusion of science will result in emotive or self-interested arguments from sectional interests dominating community debate. This is not to say that people's emotional responses are not relevant to the debate. Indeed such responses reflect, in part, current community values; however, they should contribute to, not pre-empt, the debate (Hemsworth *et al*., 2007). For example, the publicly engaging concepts of 'free range' and 'capacity to express natural behaviour' among domesticated animals can lead to compromised welfare when implemented in circumstances which on the face of it suggest that welfare would be improved. Illustrating this, Weeks *et al*. (2012) reported in a study of 1,486 UK flocks

that mortality of hens in cages over a 52-week laying period was 5.4% and the mortality in free-range hens was 9.5%, a value 77% higher. The variability in flock mortality or standard deviation was also higher, with cages at 3.1% and free range at 7.4%, a 143% increase. Scott *et al.* (2007) studied some aspects of the health and welfare of finishing pigs housed in either fully-slatted or straw-based accommodation. The results highlight the relative health and welfare advantages and disadvantages of these two systems for finishing pigs. For example, lameness and tail-biting tended to be the more prevalent health conditions in the fully-slatted system, while in the straw-based system pigs showed more enteric and respiratory disease. Pigs with straw spent a large proportion of their time manipulating it, while pigs without straw were less active and spent more time manipulating the pen features. Pigs with straw had more severe toe erosions, while pigs without straw had more severe heel erosions. As with any housing or husbandry systems, further research is required in these systems to determine both the causes and changes necessary to improve animal welfare.

Therefore, ultimately the question of whether or not a specific animal use is acceptable is an ethical one in which science can provide facts that need to be utilised to provide a rational answer. When combined with other beliefs and principles, facts can provide behavioural guidance. Both science and views on our duties to animals are central to this question of how we ought to behave to our animals, but there are also other considerations that may influence our decision on animal use, such as the impact of the animal use on the animal owner, the environment and the economy. In setting animal welfare recommendations and standards, governments and others acknowledge and to varying degrees take account of the many and varied viewpoints through broad stakeholder consultation.

Conclusions

There are some key challenges that will require targeted research, education and consultation amongst the major stakeholder groups. The science of animal welfare is barely in its infancy and clearly a substantial increase in multidisciplinary studies, utilising the disciplines of animal behaviour, immunology, neurophysiology, psychology, stress physiology, and veterinary science, is necessary to inform sound and robust animal welfare policies. Animal welfare research is particularly important in the light of the community's increasing scrutiny of animal use practices, polarisation of views by practitioners and opponents of livestock production, and the on-going development of livestock production systems that are designed to address food safety, sustainability and environmental protection, in addition to animal welfare standards.

In relation to the welfare of commercial pigs, Australia's RD&E capability and funding opportunities through, for example, the government and industry funding provided by the Co-operative Research Centre for High Integrity Pork and the Australian Pork Limited, places it in an excellent position to continue to improve pig welfare through understanding both the genuine welfare risks to pigs and the public's attitude to animal welfare and its interests in engaging in this topic.

References

ANONYMOUS (1911). Protection of Animals Act, 1911. Government of the United Kingdom.

ANONYMOUS (2007). Prevention of Cruelty to Animals Act, 1986. Government of Victoria, Australia.

APPLEBY, M.C., SMITH, S.F. and HUGHES, B.O. (1992). *British Poultry Science.* **33**:227-238.

AREY, D.S. and EDWARDS, S.A. (1998). *Livestock Production Science.* **56**:61-70.

BARNETT, J.L. (1997). In "Livestock Environment V, Volume II", pp.613-618, eds. R.W. Bottcher and S.J. Hoff (Bloomingham, Michigan, USA).

BARNETT, J.L. (2003). In "Manipulating Pig Production IX", pp.107-120, ed. J.E. Paterson. (Australasian Pig Science Association: Werribee).

BARNETT, J.L. and HEMSWORTH, P.H. (1990). *Applied Animal Behaviour Science*. **25**:177-187.

BARNETT, J.L. and HEMSWORTH, P.H. (2009). *Journal of Applied Animal Welfare Science.* **12**:114–131.

BARNETT, J.L., HEMSWORTH, P.H., CRONIN, G.M., NEWMAN, E.A., MCCALLUM, T.H. and CHILTON, D. (1992). *Applied Animal Behaviour Science*. **34**:207-220.

BARNETT, J.L., CRONIN, G.M., MCCALLUM, T.M. and NEWMAN, E.A. (1993). *Applied Animal Behaviour Science*. **36**:111-122.

BARNETT, J.L., HEMSWORTH, P.H., CRONIN, G.M., JONGMAN, E.C. and HUTSON, G.D. (2001). *Australian Journal of Agricultural Research.* **52**:1-28.

BENCH, C.J., RIOJA-LANG, F.C., HAYNE, S.M. and GONYOU, H.W. (2013a). *Livestock Science.* **152**:208-217.

BENCH, C.J., RIOJA-LANG, F.C., HAYNE, S.M. and GONYOU, H.W. (2013b). *Livestock Science.* **152**:218-227.

BOISSY, A., MANTEUFFEL, G., JENSEN, M.B., OPPERMANN, M., SPRUIJT, B., KEELING, L.J., WINCKLER, C., FORKMAN, B., DIMITROV, I., LANGBEIN, J., BAKKEN, M., VEISSIER, I. and AUBERT, A. (2007). *Physiology and Behaviour.* **92**:375–397.

BORELL, E., VON BROOM, D.M., CSERMELY, D., DIJKHUIZEN, A.A., EDWARDS, S.A., JENSEN, P., MADEC, F. and STAMATATIS, C. (1997). The Welfare of Intensively Kept Pigs. Report of the Scientific Veterinary Committee (European Union, Brussels).

BROOM, D.M. (1986). *British Veterinary Journal*. **142**:524-526.

BROOM, D.M. (1996). *Acta Agriculturae Scandinavica, Section A - Animal Science.* **27:**22-28.

BROOM, D.M. and JOHNSON, K.G. (1993). *Stress and Animal Welfare*. Chapman and Hall, London, UK.

COLDITZ, I.G., PAULL, D.R., LLOYD, J.B. and FISHER, A.D. (2010). *Australian Veterinary Journal*. **88**:483–489.

COLEMAN, G.J. (2008). *OIE Technical Series (World Organisation for Animal Health).* **10**:26-37.

COLEMAN, G.J. (2010). *Journal of Veterinary Medical Education.* **37**:74-82.

COOPER, J.J. and ALBENTOSA, M.J. (2003). *Avian Poultry Biology Review*. **14:**127-149.

DANTZER, R. (1988). Les émotions. Presses Universitaires de France, Paris, pp.121.

DANTZER, R. and MORMÈDE, P. (1983). *Journal of Animal Science.* **57**:6-18.

DAWKINS, M. (1976). *Applied Animal Ethology*. **2:**245-254.

DAWKINS, M. (1983). *Animal Behaviour.* **31**:1195-1205.

DAWKINS, M. S. (1990). *Behavioral and Brain Sciences.* **13**:1–9.

DAWKINS, M.S. (2003). *Zoology*. **106**:383-387.

DENTON, D.A., MCKINLEY, M.J., FARRELL, M. and EGAN, G.F. (2009). *Consciousness and Cognition.* **18**:500–514.

DUNCAN, I.J. (1998). *Poultry Science*. **77**:1766–1772.

DUNCAN, I.J.H. (2004). In "The Well-being of Farm Animals: Challenges and Solutions", pp.95-101, eds. G.J. Benson and B.E. Rollin (Iowa, USA, Blackwell Publishing).

DUNCAN, I.J.H. (2005). *Revue scientifique et technique (International Office of Epizootics).* **24:**483-492.

DUNCAN, I.J.H. and FRASER, D. (1997). In: "Animal Welfare", pp.19-31, eds. M.C. Appleby and B.O. Hughes. (CAB International, Wallingford, Oxforshire, UK).

EFSA (European Food Safety Authority). (2005). *Welfare Aspects of Various Systems for Keeping Laying Hens.* Annex to the *EFSA Journal* **197**:1-23.

EUROPEAN COMMISSION (2007). *Attitudes of EU Citizens Towards Animal Welfare.* Special Eurobarometer 270/Wave 66.1.

FAWC (Farm Animal Welfare Council). (1993). Second Report on Priorities for Research and Development in Farm Animal Welfare. 1A Page Street, London.

FRASER, D. (2003a). *Animal Welfare.* **12**:433-443.

FRASER, D. (2003b). In "XI ISAH Congress in Animal Health", pp. 61-66 (23-27th February 2003, Mexico City, Mexico).

FRASER, D. (2008). Understanding Animal Welfare: The Science in its Cultural Context. Wiley-Blackwell, Chichester, West Sussex, UK.

FRASER, D. and DUNCAN, I.J.H. (1998). *Animal Welfare.* **7**:383-396.

FRASER, D. and MATTHEWS, L.R. (1997). In: "Animal Welfare", pp.159-174, eds. M.C. Appleby and B.O. Hughes. (CAB International, Wallingford, Oxforshire, UK).

FRASER, D., DUNCAN, I.J.H., EDWARDS, S.A., GRANDIN, T., GREGORY, N.G., GUYONNET, V., HEMSWORTH, P.H., HUERTAS, S.M., HUZZEY, J.M., MELLOR, D.J., MENCH, J.A., PARANHOS DA COSTA, M., SPINKA, M. and WHAY, H.R. (2013). *The Veterinary Journal.* (DOI information: 10.1016/j.tvjl.2013.06.028).

FRASER, D. and NICOL, C. J. (2011). In "Animal Welfare", pp.183-199, eds. M.C. Appleby, J.A. Mench, I.A.S. Olsson and B.O. Hughes. (CAB International, Oxon UK).

GREEN, T.C. and MELLOR, D.J. (2011). *New Zealand Veterinary Journal.* **59**:263–271.

HEMSWORTH, P.H. and COLEMAN, G.J. (2009). In "Food Safety Assurance and Veterinary Public Health. Volume 5, Welfare of Production Animals: Assessment and Management Risks", pp.133-147, eds. F.J.M. Smulders and B. Algers. (Wageningen Academic Publishers, The Netherlands).

HEMSWORTH, P.H. and COLEMAN, G.J. (2011). "Human-Livestock Interactions: The Stockperson and the Productivity and Welfare of Farmed Animals". (CAB International, Oxon UK).

HEMSWORTH P.H., BARNETT, J. L., HANSEN, C. and WINFIELD, C.G. (1986). *Applied Animal Behaviour Science*. **16**:259-267.

HEMSWORTH, P.H., BARNETT, J.L., RICKARD, M. and COLEMAN, G.J. (2007). *Farm Policy Journal.* **4**:23-31.

HEMSWORTH, P.H., BARNETT, J.L., KARLEN, G.M.A., FISHER, A.D., BUTLER, K.L. and ARNOLD, N.A. (2009). *Applied Animal Behaviour Science*. **117**:20-27.

HEMSWORTH, P.H., MORRISON, R.S., VERDON, M and RICE, M. (2013). Final Report to the Co-operative Research Centre for High Integrity Australian Pork (Project 1C-102), May 2013.

HUGHES, B.O. and BLACK, A.J. (1973). *British Poultry Science.* **14**:615-619.

HUTSON, G.D. (1981). *Australian Journal of Experimental Agriculture and Animal Husbandry.* **21**:474-479.

KALTAS, G.A. and CHROUSOS, G.P. (2007). In "Handbook of Psychophysiology", pp.303-318, eds. J.T. Cacioppo, L.G. Tassinary and G.G. Berntson. (Cambridge University Press, Cambridge).

KEELING, L. and JENSEN, P. (2005). In "The Ethology of Domestic Animals 2nd ed.", pp.85–101. ed. P. Jensen. (CABI Publishing: Wallingford, UK).

LEVY, N. (2004). What Makes Us Moral? Crossing the Boundaries of Biology. Oneworld Publications, Oxford, UK.

MATTHEWS, L.R. and LADEWIG, J. (1994). *Animal Behaviour*. **47**:713-719.

MATTHEWS, L.R. and HEMSWORTH, P.H. (2012). *Animal Frontiers.* **2**:40-45.

MELLOR, D.J. (2012). *New Zealand Veterinary Journal.* **60**:1-8.

MELLOR, D.J. and BAYVEL, A.C.D. (2004). Proceedings of an OIE Conference, February, Paris, France pp.249-259.

MELLOR, D.J. and BAYVEL, A.C.D. (2008). *Applied Animal Behaviour Science*. **113**:313-329.

MELLOR, D.J. and LITTIN, K.E. (2004). *Animal Welfare.* **13**:127–132.

MELLOR, D.J., COOK, C.J. and STAFFORD, K.J. (2000). In "Biology of Animal Stress", pp.171-198, eds. J.A. Mench and G. Moberg. (CAB International, Wallingford, Oxfordshire, UK).

MELLOR, D.J., PATTERSON-KANE, E. and STAFFORD, K.J. (2009). *The Sciences of Animal Welfare.* Wiley-Blackwell Publishing, Oxford, UK, pp1-212.

MENDL, M., ZANELLA, A.J. and BROOM, D.M. (1992). *Animal Behaviour*. **44**:1107–1121.

MENDL, M., BURMAN, O.H.P., PARKER, R.M.A., PAUL, E.S. (2009). *Applied Animal Behaviour Science.* **118**:161–18, 2009.

MILLS, D.M. (2010). In "The Encyclopaedia of Applied Animal Behaviour and Welfare", pp.2–3. eds, D.M. Mills, J.N. Marchant-Forde, D.B. Morton, C.J.C. Phillips, P.D. McGreevy, C.J. Nicol, P. Sandoe and R.R. Swaisgood. (CABI Publishing, Wallingford, UK).

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD. (1983). In "*British Codes of Recommendations for the Welfare of Livestock"*. (Her Majesty's Stationary Office, London, UK).

MOBERG, G.P. (2000). In "Biology of Animal Stress", pp.1-21, eds. J.A. Mench and G. Moberg. (CAB International, Wallingford, Oxfordshire, UK).

MORRISON, R.M., CRONIN, G.M. and HEMSWORTH, P.H. (2011). In "Manipulating Pig Production XIII", pp.219-238, ed. R.J. van Barneveld (Australasian Pig Science Association: Werribee).

MUNS VILA, R., FARISH, M., RAULT, J-L. and HEMSWORTH, P.H. (2012). In "Proceedings of the 46th Congress of the International Society of Applied Ethology", p.26. (Vienna, Austria).

NICOL, C.J., CAPLEN, G., EDGAR, J. and BROWNE, W.J. **(**2009). *Animal Behaviour*. **78**:413-424.

NICOL, C.J., CAPLEN, G., STATHAM, P. and BROWNE, W.J. (2011). *Animal Behaviour*. **82**:255-262.

NICHOLSON, R.I., MCGLONE, J.J. and REID, L.N. (1993). *Journal of Animal Science*. **71** (Suppl. 1):112.

PANKSEPP, J. (1998). Affective Neuroscience. (The Foundation of Human and Animal Emotions. Oxford, University Press, London, UK).

PANKSEPP, J. (2005). *Consciousness and Cognition.* **14:**30–80.

PEDERSEN, V., BARNETT, J.L., HEMSWORTH, P.H., NEWMAN, E.A. and SCHIRMER, B. (1998). *Animal Welfare.* **7:**137-150.

PHILLIPS, P.A., THOMPSON, B.K., and FRASER, D. (1988). *Canadian Journal of Animal Science.* **68**:41-48.

POLLARD, J.C., LITTLEJOHN, R.P. and SUTTIE. J.M. (1994). *Applied Animal Behaviour Science*. **39**:63-71.

PRICE, E.O. (2002). Animal Domestication and Behaviour. (CAB International, Wallingford, Oxfordshire, UK).

RUSHEN, J. (1986). *Applied Animal Behaviour Science*. **16**:363-370.

SANDØE, P., FORKMAN, F. and CHRISTIANSEN, S.B. (2004). *Animal Welfare.* **13**:S121-S126.

SAVORY, C.J. (2004). *Animal Welfare*. **13**:S153–S158.

SERPELL, J.P. (2004). *Animal Welfare*. **13**:S145–51.

SERPELL, J.A. (2008). On Measuring Progress In Animal Welfare. Report for the World Society for the Protection of Animals, October.

SCOTT, K., CHENNELLS, D.J., ARMSTRONG, D., TAYLOR, L., GILL, B.P. and EDWARDS, S.A. (2007). *Animal Welfare.* **16**:53-62.

STEVENS, B., BARNETT, J.L., TILBROOK, A. and HEMSWORTH, P.H. (2009). In: "Manipulating Pig Production XII", pp.28, ed. R.J. van Barneveld, (Australasian Pig Science Association: Werribee).

TANNENBAUM, J. (2001). In "Why Animal Experimentation Matters: The Use of Animals in Medical Research", pp.93-130, eds. E. F. Paul, and J. Paul (Transaction Publishers, Somerset, N.J.).

TAVERNER, M.R. (1991). In "Manipulating Pig Production III", pp.1-9, ed. E.S Batterham (Australasian Pig Science Association: Werribee).

TEUTSCH, G.M. (1987) In "Ethical, Ethological and Legal Aspects of Intensive Farm Animal Management", pp.9-40, eds. E. von Loeper, G. Martin, J. Muller, A. Nabholz and G. van Putten. (Tierhaltung, 18).

VERDON, M., MORRISON, R., RICE, M. and HEMSWORTH, P. H. (2013). In "Proceedings of the 47th Congress of the International Society of Applied Ethology", p.68. (June, Florianopolis, Brazil).

WAIBLINGER, S., BOIVIN, X., PEDERSEN, V., TOSI, M-V., JANCZAK, A.M., VISSER, E.K. and JONES, R.B. (2006). *Applied Animal Behaviour Science*. **101**:185–242.

WEEKS, C.A., BROWN, S.N., RICHARDS, G.J., WILKINS, L.J. and KNOWLES, T.G. (2012). *Veterinary Record, In Practice.* **170**: 647-651.

WIDOWSKI, T.M. and HEMSWORTH, P.H. (2008). In "Proceedings of the XXIII World's Poultry Congress". (Brisbane, Australia, June, CD ROM).

CHAPTER 2

Delivering Science for Industry Benefit

OPINION: Delivering science for maximum industry benefit: A commercial perspective on enhancing research outcomes for the pork industry

R.J. van Barneveld

Barneveld Nutrition Pty Ltd, Loganholme, QLD 4129.

Abstract

As an industry, pork production in Australia has a strong reputation of innovation and research adoption as a means of remaining internationally competitive. This paper aims to demonstrate that most stakeholders in the Australian pork industry could further enhance the role they play in industry research and development and if the roles are understood, there is an opportunity to improve the effectiveness of research and delivery of the outcomes for maximum industry benefit. Pork producers have demonstrated that they are proactive investors in research and development and that they are prepared to work together on critical research issues, and this provides a firm base on which to build. Researchers are somewhat compromised within most institutions in terms of resourcing and freedom to operate, and need to focus on the standard of science, an understanding of key aspects of pork production as a business, and delivery of outcomes to the end users. Governments and universities have significant room to improve the research environment in which their researchers operate, the incentives offered to researchers to deliver outcomes and the resourcing of industry-based research programs. Research investors and managers need to encourage researchers to focus on how their research could impact the decision making capacity of the end user, and this inherent value, rather than trying to apply superficial economic assessments that attempt to value the outcome. They also need to mentor the industry to develop a balanced portfolio of basic and applied research with long and short term outcomes. Industry partners could make better use of existing industry research resources to underpin their product development, rather than just for product assessments. All stakeholders need to consider more of the intangible benefits (e.g., professional development, new knowledge, industry cohesion) that arise through the support of an industry through research investment.

Introduction

"Delivering science for maximum industry benefit" is a broad proposition. To address this then the following need to occur: a) define "industry" (i.e., is it pork producers only or the entire value chain?), b) understand the form of the "benefit" (i.e., do the benefits have to be tangible and delivered on-farm?), and c) decide whether we deliver research outcomes or "science". My interpretation of the intent of this topic is to identify the key factors that ensure research outcomes are adequately extended to and utilised by target end-users in the pork industry so that economic, social and physical benefits are conferred at every stage of the research and adoption process. If case studies were used to address this topic, it is likely that in-house research programs within well-resourced multi-national companies would be the most successful model. In this situation the desired research outcomes are highly defined and specific in the form of a product or know-how as a result of experience and (or) market research. To achieve such outcomes, a) the scientist is high calibre and has an intimate understanding of the business and the key drivers of research success, b) the scientist can focus on the research with minimal diversions seeking additional research investment, c) there are critical research milestones that must be met with the performance (and potentially salary) of the scientist measured against these, d) the research program is well resourced physically and financially with a capacity to utilise specific external research resources as required, e) the path to market is understood and self-resourced, and f) there are resources dedicated to intellectual property protection, regulatory processes, market access, manufacturing, marketing and ultimately sales from the research outcomes. Most importantly, all facets of the business place value on the tangible and intangible benefit of investing in a comprehensive research program recognising that not all of the benefits will be able to be measured by financial returns alone.

Lindsay (2001) presented a good example of the inadequacy of financial analysis alone when he reviewed the research approach adopted by Dupont. Bankruptcy almost resulted in the early 1960s when one of the world's most successful innovators and manufacturers (gunpowder, explosives, nylon, neoprene) applied a narrow financial evaluation of return on investment in its research departments after a decision had been made on strictly financial grounds that the key to greater profits from research lay in emphasising the development rather than the research. Dupont quickly ran out of new things to develop and it only poorly understood the products that it was developing anyway, so the success rate dropped dramatically. Subsequently, Dupont adhered to a long term research investment policy that was based on a) the scientific

merit of the project, b) the accomplishments of the scientific investigator, and c) the relevance of the proposed work to Dupont. The only other stipulation that they put on the whole process was that the manager of the research, the person who made the decision about whether to fund a project or not, should be outstanding in both technical competence and business perspective.

The Australian pork industry research, development and adoption model is somewhat more diverse and complex than in-house corporate research. By comparison, the research focus is often diverse and poorly defined, a significant proportion of the scientists operate within less than well-resourced public-sector institutions, research costs are often inflated by institutional overheads, the scientists often do not understand the relative value of their research to a production system or the production system priorities, the end-users do not understand or appreciate the potential tangible and intangible benefits of the science, there is often no clear path to market, attempts to protect intellectual property (IP) become so constraining the research struggles to progress, a lack of attention to IP management over time ultimately makes commercialisation difficult, industry partners are often involved in the research process far too late, and if milestones or research outcomes are not achieved, there are very few consequences for the scientist and the research investor has limited recourse. In the short term it is unlikely that the industry will emulate a successful in-house corporate research program at industry level, so the industry is left with the challenge of how existing resources to enhance research outcomes for the pork industry can be utilised.

The aim of this paper is to demonstrate that most stakeholders in the Australian pork industry could enhance the role they play in industry research and development (Figure 1). If these roles are understood and fulfilled, an opportunity exists to significantly improve industry innovation, research adoption and utilisation, and long-term business sustainability as producers of food and other products from pigs, or put more succinctly "maximise industry benefit".

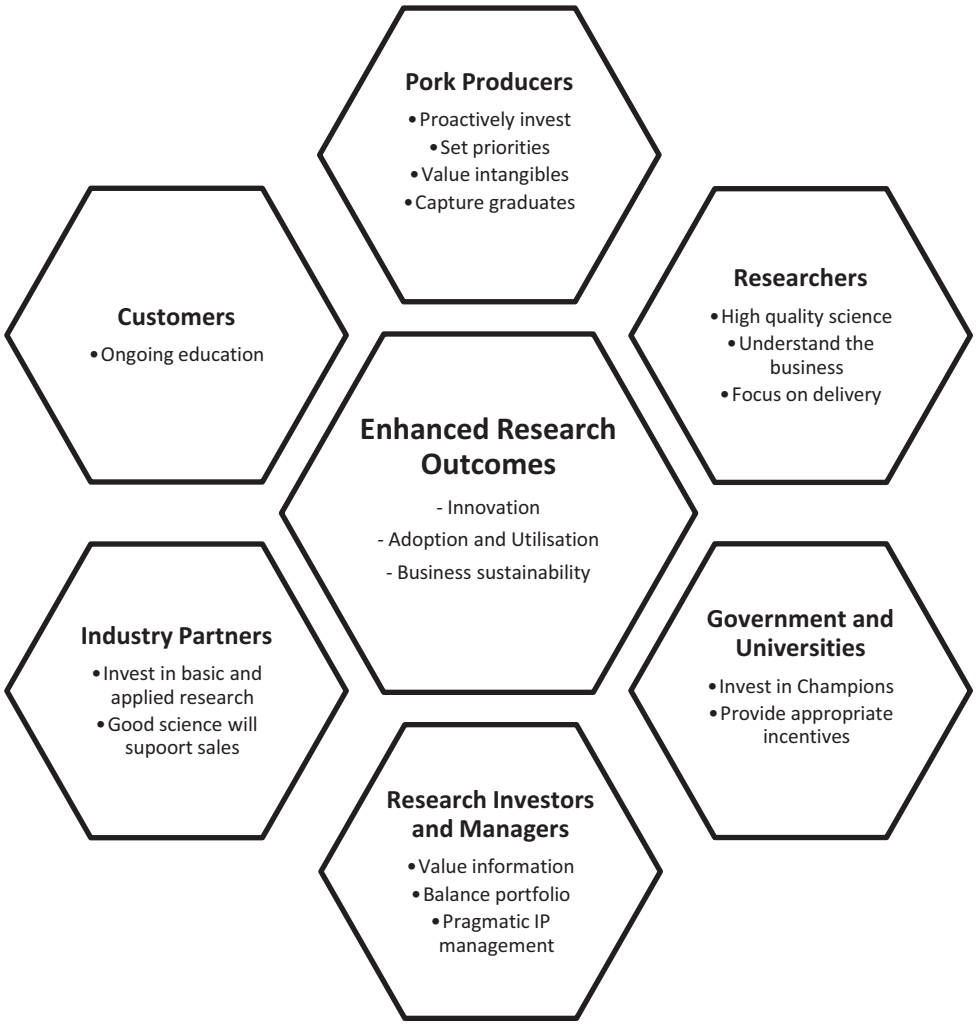


Figure 1. *Stakeholder contributions that could contribute to enhanced research outcomes for the Australian pork industry.*

Pork producers

In my opinion, pork producers in Australia are the stakeholders that are closest to fulfilling their role towards ensuring the industry benefits from investment in research. They are highly cohesive in their approach to industry challenges, they have demonstrated a willingness to invest in research, they have developed innovative ways for industry and research providers to work together on significant research initiatives, they are rapid adopters of new technology, and they are active in communicating their research priorities. In addition, the Australian pork industry has experienced firsthand the consequences of diminished investment in research and development, and it is a position the industry does not want to revisit. Following the consolidation of the Pork Council of Australia, the Australian Pork Corporation and the Pig Research and Development Corporation into a single entity in 2000 (i.e., Australian Pork Ltd.), it can be surmised that research and development priorities across the organisation did not feature highly in the first two strategic plans [2002-2005 (Australian Pork Ltd., 2002) and 2003-2006 (Australian Pork Ltd., 2005), respectively]. It was not until 2005 that a proactive set of industry priorities for research and development was published, but by that time the damage had been done. There had been a loss of facilities, world-class research expertise, innovation and momentum at a time when the industry needed it most.

The lull in established research processes between 2000 and 2005 was enough to motivate the Australian pork industry to develop alternative models for research and development investment. As end-users, the industry successfully developed a bid to the federal government to support the establishment of a Cooperative Research Centre (CRC) for an Internationally Competitive Pork Industry (Pork CRC). This bid secured Commonwealth investment of $25.7 million, further cash contributions of $10.7 million from participants, and in-kind contributions exceeding $43.2 million; a total investment of cash and in-kind of $79.6 million. This was followed in 2010 by another bid to establish a CRC for High Integrity Australian Pork valued at $132 million over 8 years with more than 40 national and international participants. Through these CRCs, the industry has effectively reinvigorated pork research, has developed new ways to tackle research issues together, new resources have been secured for the conduct of research (particularly access to facilities within commercial enterprises), a steady flow of highly trained post-graduates have emerged, the industry is effectively leveraging levy funds collected for research purposes to increase the critical mass available for research, and the research topics are highly relevant to the industry as a whole. As summarised by Keniry (2011), the outcomes from these CRCs exceeded participant and industry expectations and demonstrated the value of the CRC model for aligning industry needs with Australia's research capabilities. The challenge now facing the pork industry is the model that will be developed post-2018 when the investment in the CRC for High Integrity Pork is concluded.

In addition to the CRC program, the pork industry has further demonstrated its willingness to optimise investment in research and development. While already investing heavily in research through compulsory levy contributions, in 2009 the industry voted to increase the proportion of the statutory levy directed towards research and development from $0.70 per pig sold to $1.00 per pig sold, thus ensuring all available matching funds were secured from government under the Pig Industry Act 2001 up to 0.5% of the gross value of production (GVP).

One of the best examples of pork producers working together to solve an industry issue through research was the development and commercialisation of the "Porkscan" suite of technologies for measurement of P_2 backfat and carcase attributes (Figure 2). Five commercial producers and (or) processors with their own processing capacity or strong links to an abattoir, together with Australian Pork Ltd., successfully assembled a consortium in 2006 to develop an alternative to traditional pig carcase grading tools. This was motivated in part by existing suppliers of P_2 measurement equipment failing to respond to producer needs and uncertainty surrounding future costs with the major equipment supplier moving to a leasing arrangement based on a per carcase fee. The likely increase in grading cost had a significant impact on producer returns and profitability and the industry moved quickly to find an alternative. The consortium was successful in identifying existing technologies that could be assembled to meet their needs, and secured $400,000 in matching funding from the Australian Government "Industry Cooperative Innovation Program" to complete the project. Within 18 months, the consortium had developed advanced image analysis and enhancement software for use with proprietary ultrasound equipment with a modified handpiece and stand-off probe, all housed in a harsh environment cabinet. This technology could accurately measure P_2 and loin muscle depth to an AUS-MEAT accreditation standard. The project also investigated the use of light striping to measure carcase conformation, which when combined with the ultrasound measurements, gave an accurate prediction of lean meat yield. Finally, the project attempted to develop belly scanning technology for a full visual assessment of this most variable component of the carcase so that carcases could be directed towards the most appropriate market. Today, the P_2 measurement technology is commercialised via Porkscan Pty Ltd (which is fully self-funded) and is

operational in four of the five major pig-processing abattoirs in Australia. Further research is underway to make the carcase conformation technology more robust and functional.

Analysis of the reasons why Porkscan was successful and the key attributes that ensured science was delivered for maximum industry benefit can be highlighted as follows:

- Competitors in the market place worked together towards a well-articulated and common industry goal;
- There was a degree of urgency surrounding delivery of the outcome;
- The end-users of the technology were actively involved in the research program;
- End-users invested cash and in-kind in the research and actively leveraged their investment to ensure sufficient funds were available to complete the task;
- Scientists involved in the research were well versed with the commercial requirements of the end-users;
- Australian Pork Ltd. championed the process and facilitated agreements with the participants.

While an excellent example of a successful research and development program, delivery of the Porkscan technologies has not been without its challenges. Most significantly, reliance on third party technologies, such as the ultrasound equipment, does make the business vulnerable to corporate decisions by unrelated entities. Secondly, when the suite of technologies was being assembled, little thought was given to the challenges of maintaining and servicing this equipment on a commercial basis. Subsequently, Porkscan Pty Ltd has developed cheaper "plug and play" platforms that make the operation of the business far more viable and offer potential to expand the business beyond the foundation participants. Finally, there has been a tendency to focus on the difference between Porkscan measurements and older technologies, which has caused some delays in implementation, but to date the Porkscan measurements have been shown to be highly robust and repeatable, and given the scans can be recorded and reviewed at a later date they are difficult to discredit.

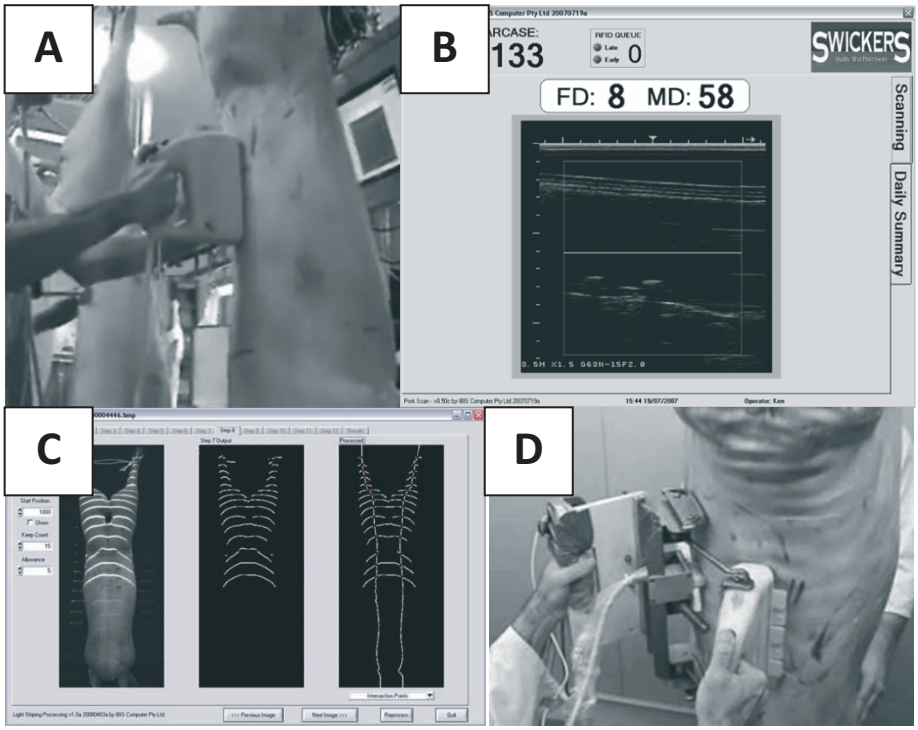


Figure 2. *"Porkscan" suite of technologies including: A) ultrasound measurement of P_2; B) enhanced image analysis and capture of P_2 backfat and loin muscle depth; C) light striping for carcase conformation measurements; and D) belly scanning equipment.*

Despite their existing efforts, pork producers can do more to ensure research is undertaken and outcomes delivered for maximum industry benefit. Areas where pork producers could enhance their involvement include:

- Producers need to be more active and equipped to capture graduates arising from the industry research program and involve them in their commercial production systems on a full-time basis. The industry risks training a range of high-calibre individuals who will be lost to other industries if the appropriate

mechanisms to employ them when they have finished their undergraduate or post-graduate studies are not available;

- Producers need to remain highly active in priority setting for research and the scrutiny of research projects during the planning phase. It is important that during this process, producers do not try and pre-empt potential market perceptions in relation to a production method or technology but first ascertain the value through research. A current example relates to the on-going use of farrowing crates in commercial production systems. While exclusion of farrowing crates from production systems may seem like a logical progression from the voluntary decision to cease use of sow stalls by 2017, the benefits are far less clear as is the motivation to change. As an industry, research into the use of farrowing crates in production systems should be encouraged but the core objectives of the research should not be to identify a suitable alternative on the off chance a competitive advantage could be conferred in the market place if implemented. Instead, research should be prioritised to first quantify if use of farrowing crates compromises sow welfare and, if it does not, then demonstrate that their application is in the best interests of both the sow and piglet compared to a wide range of alternatives. If, through this process, sow welfare was shown to be measurably compromised and a commercially viable alternative identified that protected piglets from overlay, then the industry should move quickly towards the alternative, but not before;
- At present, the pork industry in Australia has a highly applied research program with many short term projects. As an industry, the intangible benefits of research need to be recognised with some effort invested into ensuring that a good, long term, basic research program is adequately resourced. This will not be an easy task, but in the past this pipeline has maintained Australia at the forefront of pork industry research worldwide, despite the comparatively small size of the local industry.

Researchers

It would be easy to target researchers as the source of many of the issues that influence the suboptimal generation or utilisation of research outcomes by the Australian pork industry. In fact, when I started preparing this paper my initial thoughts were that the bulk of the content would be directed towards researchers and their associated failings and case studies that demonstrate how things should be done, but in execution it appears that most areas for improvement lie elsewhere. This is due to the fact that the systems in which many industry researchers operate are conducive to conduct of science rather than adoption, do not foster a healthy research environment, and stakeholders other than researchers have the greatest capacity to influence changes in this respect. Having said this, a scientist isolated in a laboratory working on a specific research interest at their leisure is an equally unhealthy environment in terms of ensuring delivery of outcomes for industry benefit.

Areas of focus within a researcher's control that could enhance delivery of research outcomes for maximum industry benefit include:

- Researchers should not underestimate the technical capacity and expertise that exists in modern pig production systems. In larger enterprises, many of the production managers and owners have the education and technical background to question the bulk of researchers on their science and the interpretation of their results. The days of "dumbing down" data so the pig farmer can understand it are behind us, and researchers need to focus more on the quality of the science they are undertaking;
- In terms of quality of science, I believe we have entered a vicious cycle. Reduced access to adequate research funds, inappropriate forms of recognition, and insufficient time and resources to complete the research properly often mean researchers efforts are directed towards tasks other than good science and the training of high quality undergraduate and post-graduate students. With each successive generation of scientists, the capacity is probably greater but the standard of research is lower. Basic experimental principles are often overlooked, and the amount of resources directed towards fundamentally flawed experimental designs is of concern. If nothing else, the pork research community needs to address this as a priority;
- While we want researchers to be researchers, it is critical that they understand the fundamentals of pork production as a business and how their contributions through science can make a difference. This understanding will only be gained through close association with commercial production systems and first hand exposure to their daily challenges. To this end, programs that foster internships for young scientists within commercial operations while they are developing their research career are invaluable. Researchers also need to recognise that undertaking research within a corporate enterprise as opposed to a university or government department in no way compromises their integrity as a scientist and in most cases enhances the quality of their research and their exposure to the industry;
- Inappropriate systems of recognition by universities and government are discussed in the subsequent section, but recognising the glacial pace of change in most institutions, researchers may be able to take a proactive response to ensuring research outcomes are realised by the end user. While a final report or

research paper may hold the highest institutional kudos, these are of less importance to end users. Instead, researchers should make extra effort to ensure their outcomes, or at least the value of the information they have generated, is conveyed directly to the target end users as a normal course of research conduct, rather than as a contractual obligation;

Government and universities

Unlike researchers, it is possible that universities and state government departments could make a greater contribution to the delivery of research outcomes to the pork industry. Increasing overhead and administration costs, a limited number of scientists dedicated to research and extension activities, heavy reliance on external public sector investment, and possibly misdirected professional incentives appear to hinder the effectiveness of these institutions. Ironically, one of the contributors to the demise of pork industry research within government institutions and universities coincided with the corporatisation of rural research and development organisations from 1990. Instead of complimenting institutional investment in industry research and ensuring the research was well aligned with industry needs and priorities, rural research and development corporation investment has effectively become the sole source of recurrent research investment within many institutions, if they have maintained an investment in pork industry research at all. While there have been some significant institutional capital investments that could be directed towards pork industry research, these resources are of little value if they are not adequately resourced with staff and base operational funds.

There was a time in recent history when Australian pork industry research undertaken within government institutions and universities was without parallel, and it is worth trying to revisit some of the key elements of success related to these programs. A cadetship scheme within the Victorian Department of Agriculture and a dedicated industry-savvy mentor such as Tony Dunkin in the 1970s and 1980s, combined with appropriate resources and facilities, led to the generation of some of the most significant pork industry research outcomes for multiple generations (e.g., Taverner *et al.*, 1981; Campbell *et al.*, 1984; Campbell and Taverner, 1988). In addition, many of the participants played, and in some cases continue to play, significant industry leadership roles (e.g., Mike Taverner, Roger Campbell, Ted Batterham, Paul Hemsworth, John Barnett, Greg Cronin, Ray King and Bruce Mullan). The resourcing of champions within Australian institutions also led to research outcomes that were well ahead of their time and even today, could be better utilised by industry. Two notable examples include the development of the AUSPIG simulation model by Dr John Black while he was with the CSIRO, and the development and subsequent successful commercialisation of Improvac® [Zoetis Australia, West Ryde, NSW (formerly Pfizer Animal Health)] by Dr David Hennessy within the then Victorian Department of Agriculture (Hennessy, 2009). Australian Research Council Federation Fellowships, which ceased in 2008, and subsequently Centres of Excellence, Future Fellowships and Australian Laureate Fellowships (Australian Research Council, 2013) provide a possible framework for the emulation of mentorship schemes of the past, but do not feature prominently in the agriculture sector.

Recognising that there is increasing pressure on government institutions and universities to provide resources for a wider range of competing disciplines, it is unlikely that there will be a return to days gone by. Having said that, steps taken by the then Federal Primary Industries Ministerial Council in 2009 to consolidate agricultural research across the country have increased resources directed towards industry research in key states with South Australia being the State of focus for government pork industry investment. Looking forward, universities investing in pork industry research may wish to consider how the following impacts on the successful uptake of research outcomes by the pork industry:

- Career progression within universities can be influenced by the number of publications in peer-reviewed journals and the journal in which the publication appears;
- Attainment of research investment by university researchers can be categorised and scrutinised. Investment quantum, as well as the source of the investment, may be used as a gauge for success. For example, investment in university research from CRCs, which has been shown to greatly enhance the delivery of research outcomes for industry benefit, is held in low regard by some universities in comparison to funding from sources such as the Australian Research Council (with often less demonstrable industry impacts);
- For good reason, many universities and government departments are wary of IP protection and go to great lengths to ensure any IP they help develop results in an equitable return to the host institution. However, in some cases these lengths are becoming almost untenable with significant frustration often ensuing when the potential IP in question cannot even be identified at the time the research contract is being prepared. For many of the reasons described in this paper, simple knowledge of a process in no way ensures it will have a positive commercial outcome and we need to have confidence that ideas and products can be developed without necessarily compromising our capacity to protect IP at a later date.

Conversely, what can be said is that many good ideas and research programs have failed to deliver commercial outcomes because the constraints around IP protection have become so great that the research cannot cost-effectively proceed. It can also be said that the imperative to publish in high quality journals represents a significant conflict when it comes to protection of IP generated in a university or government institution.

Like universities, governments may wish to consider the consequences of the following on effective delivery of science for industry benefit:

- Compared with countries like the United States of America, tax incentives offered for commercial investment in research in Australia make the proposition marginal at best and certainly does not encourage organisations to significantly increase their contributions;
- State Acts relating to veterinary science and federal organisations like the Agricultural Pesticides and Veterinary Medicine Authority are making the conduct of Animal Science as a research discipline difficult, and have an impact on our capacity to research and utilise valuable technologies in the pork industry. The industry is reaching the stage where one has to question whether these Acts and Authority's exist to protect and maintain animal welfare and food safety or to preserve the income and capacity of the veterinary profession. At some point, common sense needs to return to the debate. Note this is not a criticism of veterinarians *per se*.

Research investors and managers

Second only to pork producers, I think research investors and managers are highly cognisant of the elements that must be in place to ensure optimal utilisation of research outcomes for maximum industry benefit. From a pork industry perspective, one of the most significant contributions made by research investors and managers has been securing the endorsement from the Primary Industries Ministerial Council in 2010 for the pork industry research, development and extension strategy. This initiative, supported by Australian Pork Ltd. and the Pork CRC Ltd., ensures the industry has the base resources and facilities available for the conduct of basic and applied research in both commercial and institutional settings. This approach not only ensures enduring, high quality resources are available for research, but it is a far more efficient way of utilising resources (assuming there are sufficient relevant projects to keep a resource fully occupied) and has resulted in millions of dollars in savings compared to traditional ways of investing in research. Further initiatives that could be adopted by research investors and managers include:

- Encouraging researchers to focus on how their research could impact the decision making capacity of the end user, and this inherent value, rather than trying to apply superficial economic assessments that attempt to value the outcome. A survey of statements made by researchers in their applications to research investors would suggest very few have any idea of the many factors that will influence the value of the outcome. When asked to describe the benefits to industry through the conduct of their research, it is not uncommon to see superficial statements to the effect that the research will produce "an extra pig per litter" or a "saving in feed costs of $20/tonne" with linear dollar values then applied to quantify the massive gains to be made by industry. If these benefits were cumulative, the situation would have been reached long ago where Australian pork producers were producing 40 or more pigs per litter and stockfeed manufacturers were paying the pig producers to use the feed. Rarely would an outcome of "an extra pig per litter" take into account the available space for progeny grow-out on farm, the marginal benefit of extra pigs if contract grow out space is required or overstocking results, or the impact of additional pigs on the price paid by buyers in a market that is highly sensitive to oversupply. One solution to this may be to change the focus from the "value of the outcome" to the "value of the information" when assessing research investments. Howard (2012) summarised this approach by stating, "If researchers can demonstrate that their information gathering activity will generate a result that is observable, relevant to the uncertainty of interest, material in that it has the possibility to change the alternative selected, and finally economic in the sense that finding the result does not cost more than it could possibly be worth, producers will be much better placed to assess and utilise the outcomes";
- Placing more emphasis on the researcher directly demonstrating adoption of research outcomes by an end-user, or at least illuminating the path to use, than the final report for the research project. For reasons described above, there is a tendency for more researcher focus on the project approval, conduct and reporting process than the reason the research was conducted in the first place;
- Ensuring there is an adequate balance of basic and applied research and short- and long-term research. In addition, many research programs take many years to culminate in a commercial outcome, if at all, and it would be fair to say we are poorly equipped to monitor and manage IP and commercial imperatives associated with the research if it transitions across investment bodies, research providers, research managers and researchers during the course of its development.

Industry partners

Industry partners represent the many individuals and organisations that supply and service the pork industry without being directly involved in pig production. They are important stakeholders in the industry and have a significant role in research investment, commercial research programs and adoption of research outcomes. In the short term, industry partners could affect the most significant advances in the way science is approached for the optimum delivery of outcomes to the industry.

The involvement of industry partners in research generally involves the assessment of products they have for sale. Here, there is ample opportunity to improve not only the effectiveness of the research, but the level of innovation associated with the work that is undertaken. As a start, supply of free product and some financial incentives to "see if you can detect an improvement on farm" is counterproductive and does not qualify as "science". Taking the next step and investing in an on-farm experiment to repeat work conducted elsewhere under "local conditions" can sometimes pay dividends but often the experiment lacks replication, commercial imperatives often result in a change in the protocol mid-way through the experiment, and a positive outcome does little to improve the existing pool of knowledge. Unfortunately, design of many of these experiments often heavily favours the product being tested and the outcomes frequently fail peer review. The result is a loss in end-user confidence that impedes uptake of the results and utilisation of the technology. Industry partners need to facilitate and invest in research that expands their "solutions" portfolio so that end-users not only have confidence that the product has an efficacy and is based on robust science, but also has many potential applications in a wide variety of environments. Commercially relevant research is important in this respect, but this does not mean the research has to be completed on a fully commercial farm. Many research facilities now exist that have sufficient replication to detect small differences in performance, but without necessarily having a fully controlled environment and high health status stock, thus making them more relevant to most commercial situations.

While commercial assessment of products is an important part of the research adoption process, industry partners could benefit more from strategic investment with established pork industry research providers for product development and enhancement. They could become more involved in research at earlier stages of development and the level of investment could increase significantly to reach even 0.5% of revenues. Industry partners could also give more credence to the intangible assets derived from the conduct of high quality research within an industry, and develop a wider array of research and scholarship portfolios as part of an industry development initiative, rather than just for direct commercial gain.

Customers and consumers

The final dominant stakeholder in the pork industry research chain is the ultimate consumer of pork, and those that supply consumers (i.e., supermarkets, butchers, restaurants, food industry etc.). Unfortunately, I could make any number of recommendations relating to how customers and consumers could change their behaviour to improve the delivery of science for enhanced pork industry outcomes, but none of them would have any influence. Customers and consumers tend to set the rules, and in many cases, their credence values can set both the research agenda and the adoption pathway. For example, the industry response to customer and consumer pressure to eliminate sow stalls from production systems has necessitated a range of research projects aimed at managing gestating sows in groups or to study the potential for mating during lactation. Consumer perceptions have also restricted the use of a range of highly valuable research outcomes by pork producers including Improvac®, porcine somatotropin, and ractopamine. If customers and consumers are to change their behaviour to the benefit of the pork industry in terms of enhancing research outcomes, this will only occur if all other stakeholders continue with delivery of education about the industry, how pork contributes to food production and the ever increasing demand for food worldwide, and how technology can improve these production systems. Food production is one of the few industries where consumers tend to prefer historical production methods over modern options, with this preference largely driven by misplaced fear of consumption side effects (especially when we consider some of the food safety standards of old). The more consumers understand how information and technology can enhance food production, the better the chance we have of delivering science for maximum industry benefit.

Conclusions

Compared to other agricultural sectors in Australia and overseas, the pork industry has a strong reputation for innovation and rapid adoption of research outcomes for maximum industry benefit. Despite this, there is a role for all industry stakeholders to play in enhancing delivery of outcomes to better effect. Pork producers have demonstrated a strong willingness to invest in high quality research. This needs to be matched with commitment from researchers to undertake the highest quality science and focus on adoption of research outcomes as the ultimate goal, for governments and universities to ensure researchers are

adequately resourced and provided with appropriate and relevant incentives to deliver, and for research investors and industry partners to maintain their roles as important contributors to the research and development process.

References

AUSTRALIAN PORK LTD (2002). Australian Pork Ltd Strategic Plan 2002-2005. (Australian Pork Ltd: Canberra).

AUSTRALIAN PORK LTD (2005). Australian Pork Ltd Strategic Plan 2005-2010. (Australian Pork Ltd: Canberra).

AUSTRALIAN RESEARCH COUNCIL (2013). National Competitive Grants Program. http://www.arc.gov.au/ncgp/default.htm.

CAMPBELL, R.G., TAVERNER, M.R. and CURIC, D.M. (1984). *Animal Production.* **38:**233-240.

CAMPBELL, R.G. and TAVERNER, M.R. (1988). *Journal of Animal Science.* **66:**676-686.

HENNESSY, D.P. (2009). In "Manipulating Pig Production XII", pp. 204-210, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

HOWARD, R.A. (2012). In "The Flaw of Averages – Why we underestimate risk in the face of uncertainty", pp. 126, ed. S.L. Savage. (Wiley: New Jersey).

KENIRY, J.S. (2011). In "Manipulating Pig Production XIII", pp. 3-8, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

LINDSAY, D.R. (2001). In "Manipulating Pig Production VIII". pp.1-8, ed. P.D. Cranwell. (Australasian Pig Science Association: Werribee).

TAVERNER, M.R., HUME, I.D. and FARRELL, D.J. (1981). *British Journal of Nutrition.* **46:**149-158.

OPINION: Delivering science for maximum industry benefit: An academic's perspective

D.W. Pethick

Murdoch University, Murdoch, WA 6150.

Abstract

This paper discusses some of the factors that contribute to the ability of scientists in the discipline of Animal Science (including Agricultural and Veterinary Sciences) to make significant industry impact. Factors discussed include the need for sound undergraduate training in fundamental sciences taught in an agricultural context and better recognition of industry impact as a metric for scientific achievement and promotion. Funding of program over project-based research is a suggested means for ensuring better impact. Finally, the term 'scientific engineer' is used to describe the 'ideal' scientist that our industries are striving for.

Background

This paper discusses some of the factors that contribute to the ability of scientists in the discipline Animal Science (including Agricultural and Veterinary Sciences) to make significant industry impact.

Training

A strong theoretical base in the sciences is essential: Training of undergraduates in the applied disciplines of Agricultural, Animal or Veterinary Sciences is a crucial starting point for the development of scientists into the future. I began my Bachelor of Agricultural Science in 1971 and undertook essentially two years of biological sciences followed by two years of 'true' agricultural science where subjects such as biochemistry were taught in years three and four within a strong agricultural context (these subjects were called Agricultural Biochemistry I and II). The subjects were still strongly theoretical and included long and detailed practical sessions aimed at honing laboratory and written skills. At least for the author this was a most enjoyable learning period, because I was from a mixed farming background and had little interest in acquiring further practical skills like handling sheep/cattle or learning the history of machinery development for broad acre cropping. After the 4-year undergraduate degree, a 1-year full time research Honours program was undertaken studying the aetiology of pregnancy toxemia in ewes. This again involved a combination of theoretical biochemistry of ketosis combined with on-farm studies of clinical cases. Looking back, I was privileged to receive a strongly theoretical course in Agricultural Science that gave me a very strong scientific base that still benefits me to this day. In this context, professionals within agricultural supply chains are highly skilled and they want to deal with people who know and understand science.

Are today's degrees doing the job? Today's degrees are a little different than the one I completed. First, degrees are no longer 'free' and back in the 1970s new graduates didn't 'worry' as much about obtaining jobs, as this seemed almost automatic. Second, modern-day degrees are shorter (a number of universities have moved/are moving to 3-year science degrees having a Major in Agricultural Science or Animal Science, for example) and tend to be focused on a vocational stream compared to the more broad-based degrees in the 1970s, 1980s and 1990s. Rather than all students undertaking a range of subjects, which equipped them with a broad base, today's degrees tend to assemble subjects from specific disciplines often outside of Agriculture and Animal Science. For example, the subject 'biochemistry' might in fact be taught within a medical context. Such offerings are driven by the need for larger class sizes and the economies of scale given the ever-decreasing investment in the university sector by Government (which for undergraduate students is now essentially a subsidy, given that more than 50% - and sometimes up to 75% - of university revenue is obtained from non-government sources). Indeed the agricultural and animal sciences have gone through a difficult period with declining student enrolments further exacerbating the decline of many degrees in these areas. Moreover, the (generally) low student numbers in universities that have been omnipresent for many years means that specialist staff in the majority of animal science disciplines have become depleted, since undergraduate student teaching loads largely support tenured staff.

One approach to improve the specialist teaching in animal science is to align such a degree with Veterinary Science in some way, because there are obvious curriculum synergies to improve economies of scale. This now happens in a number of universities in Australia, and indeed has been commonplace in other countries (e.g., the USA, where Animal Science is generally regarded as "pre-vet") for many years, and facilitates a stronger flow of students, and hence staff, into the basic animal science disciplines.

An area of weakness at least for me, was poor training in the art of writing and obtaining grants. This was partly because I studied my PhD internationally and so I was initially naïve of the Australian research and development systems upon my return. Certainly my PhD students, and those of the majority of my colleagues, are closely imbedded within research grants where they are given some of the research (e.g., preparing animal ethics applications), reporting and financial management responsibilities. Such skills will place them in good stead if they decide to continue in research (or an allied field) as a career.

Universities and Animal Science

Universities, perhaps like all employees, are a combination of good and bad. A significant weakness is that they cover just so many disciplines, which makes decision making difficult as there is usually just one set of rules for all, from the arts and humanities to the sciences. Another weakness is the excessive emphasis placed on the Australian Research Council (ARC) grants system by university administrators when, in the area of the 'animal sciences', more and stronger focus should clearly be directed towards the relevant Rural Research and Development Corporations (RDCs). Ironically, and in terms of the money returned from Canberra to universities through the research block grant funds that universities receive to assist in research and research training, a Meat and Livestock Australia (MLA) "Strategic and Applied Research Funding" grant, or an Australian Pork Limited (APL) "Research and Innovation Open Tenders" grant, has exactly the same return as an ARC grant (see Category 1 research grants; http://www.innovation.gov.au/RESEARCH/RESEARCHBLOCKGRANTS/Pages/AustralianCompetitiveGrantsRegister.aspx).

Another weakness is the excessive focus on inputs such as the monetary values of grants obtained and an almost singular focus on publications as the major output for determining promotion, prestige and even university rankings within the discipline of 'Animal Science'. Indices like the *h* index and citation frequency are increasingly seen as defining metrics with some faculties/schools/departments even suggesting particular thresholds that should be achieved for probation of (new) early-career staff and promotion of existing staff. Clearly these metrics are important but they are just one of many factors that should be considered. An overwhelming weakness, however, is the lack of credence given to industry reports, industry engagement and industry "impact". The recent Excellence in Research Australia (ERA) exercises placed no emphasis on industry impact. Moreover, all universities now strongly promote themselves in the press as 'world class', 'global', 'international' and so on, but rarely do they promote themselves as delivering outcomes for societal change.

A further weakness in the university sector, at least in Australia, is the excessive competition between universities meaning that rationalisation of course offerings and concentration of expertise within States is spoken about but seldom achieved. In the latter stages of undergraduate degrees sharing of course material and even students between universities would be a better use of (increasingly) scarce resources. There are clearly too many Animal Science- and Veterinary Science-related degrees in Australia; in contrast, California (population about 38 million) has one Veterinary Science faculty and The Netherlands (population about 17 million) has one University teaching Agriculture. Rationalisation of course offerings will almost certainly require a political solution given State and university rivalries.

Of course the strengths of universities includes their diversity which when managed appropriately can bring new ideas and groups together to solve research agendas. Another great strength of universities is flexibility that academics have with respect to their field(s) of research. For instance, I have published works on eight species (sheep, cattle, pigs, horses, rats, chickens, dogs and humans), which is entirely related to being imbedded within a diverse faculty of Veterinary Science.

Project versus program research

Program research the way of the future: Australia has developed mechanisms for promoting research in 'animal science' that are generally seen as the 'envy of the world'. In particular these systems include the RDCs and more recently the Cooperative Research Centre (CRC) program. The RDC system is where producer and processor levies are collected and matched by Government, for the purpose of undertaking research across the relevant animal industry supply chains. Producer-owned companies such as MLA and APL then manage these funds. Traditionally projects were (and still are) called for and then funding of individual researchers has been undertaken to solve single-issue research agendas, with research projects generally spanning 12-24 months. However, research questions have increasingly become more complex and require the following: (1) a national focus; (2) teams with broader skills; (3) clear industry engagements to maximise the impact of the research; and (4) in most cases, funding for a greater length of time than 'one-off' projects. The CRC system encapsulates program research at its best. In the red meat sector, major CRC programs were initiated by collaborative planning involving scientific and industry

stakeholders. These are then managed and massaged into shape with the help of MLA. This early involvement of the entire supply chain assures all players have 'skin in the game', which is in the form of private, levy and government dollars plus significant in-kind contributions from research and collaborating industry organisations. The success of the Beef, Sheep, Pork and Poultry CRCs are well recognised and documented. Indeed they have been so well recognised for their industry outcomes that many industry players are worried about the future research 'landscape' when the current wave of animal science and production-based CRCs finish. However, a cut-down CRC-type approach can easily be managed by an individual RDC given industry support and the will to succeed. The development of MLA's Meat Standards Australia (MSA) program, for both beef and lamb and sheep meats, are very prominent examples.

Unfortunately program research can be challenging for many of the Federal and State government research agencies and even some universities. Such institutions are often unwilling to contribute research staff in-kind and instead are increasingly insisting that staff time be paid for from the research funds. This is a naïve approach and could be aligned to a commercial company asking for research and development without contributing to the costs. It is puzzling logic to suggest that scientists who are paid for by the taxpayer should then be paid again!

Publishing and industry outcomes

Research should be published to achieve industry credibility: Most levy-funded research can be classified as public good, especially in the sheep and cattle sectors. Only a small proportion of the outcomes have a significant intellectual property (IP) component meaning that focusing on patents and the like as outcomes is short-sighted, unhelpful, and in the vast majority of cases will never meet the transaction costs associated with the exercise. Nevertheless many research institutions and universities place a great emphasis on IP, which is usually misguided and only serves to delay the contracting and research processes. It is relatively simple to understand when IP is important, e.g., when dealing with a company product, and this should happen quickly and easily.

Given that IP is not typically a crucial issue it is important that research outcomes are published so as to underpin credibility and industry uptake. This is especially the case when the research is contributing to new industry structures. Classical examples are the development of the MSA program for beef and lamb and sheep meats. These programs have created new systems that did not exist 10 years ago, and many components of these systems have placed new requirements on livestock producers, sale yards, transporters, abattoirs, wholesalers and retailers. Industry-changing programs quite naturally are often challenged by stakeholders, e.g., why the need to do this and that? There is typically just one simple answer, namely "because of the science", and it's not science until it is published. Meat and Livestock Australia in combination with the Beef and Sheep CRCs should be congratulated on their instance to publish all outcomes relating to MSA (and in many other areas) typically as 'special editions' in national and international journals.

The role of RDC's

Close engagement with the RDC's is important: The RDCs are complex organisations with high-level expertise and connections across the supply chain. For example, MLA has subsections that cover on-farm research, off-farm research, industry systems, livestock producer engagement, economic analysis and marketing. Moreover they have a close relationship with the Australian Meat Processors' Corporation and the relevant peak councils. Given this wealth of expertise and systems it is important that researchers engage fully and professionally with 'their' appropriate RDC. This is not often the case and instead many researchers simply see the RDCs as a funding agency, which is a clear recipe for failure and final disgruntlement.

There are numerous ways to increase the engagement of researchers with the RDCs and industry. One example is program-based research, as discussed above, where a significant industry issue is addressed over a sustained period of time and is given sufficient funding and resources to do so. In my own area of research this would represent MLA's programs in genetic and eating quality improvement, which has seen sustained research programs for more than 20 years. A related example is the Lamb Supply Chain Group that has been a successful initiative undertaken by MLA and the Sheep CRC. This group meets quarterly to plan strategies for increasing lean meat yield and eating quality in prime lambs. The group has all subsections of MLA represented, i.e., the key peak council, plus scientists and extension people from universities and the State Departments of Agriculture/Primary Industries. The outcomes have seen the growth of MSA lamb, initiation of new research into carcase grading for lamb, new genetic systems for carcase yield and eating quality. Perhaps most importantly the group has managed the now close collaboration that exists with major stakeholders in the form of processors and retailers. In summary, a

sustained research program with transparent scientific and engagement strategies is the most powerful mechanism for ensuring high quality research and Industry outcomes.

Each RDC, therefore, should ask themselves how they have performed in program research? As an example, perhaps the Australian pork industry might self examine the adequacy or otherwise of sustained commitment to meat science?

End user engagement

Changing the practice of end users is a very powerful tool for adoption: Adoption of research within the sheep and cattle sectors can be difficult and indeed there are scientists who are experts in extension. There are all sorts of jargon in this field, e.g., 'early adopters', 'innovators', the 'middle majority', the 'laggards' and so on, to describe the propensity of livestock producers to adopt new research outcomes. A far more effective root to adoption is to change the practice of end users. The MSA systems are a case in point, namely that once lamb and beef processors/retailers provided financial incentives for beef and lamb carcasses that complied with grading requirements, adoption became rapid and extensive.

Conclusions

Research in the animal sciences is a demanding and sometimes risky business. One essential ingredient not mentioned so far is the need for a sustained approach from individuals, scientific organisations and the RDCs. Successful researchers don't see 'funding opportunities' but rather an opportunity to contribute to industry change, growth and success. The MSA schemes have taken some 15 years since their inception to finally become truly industry re-defining programs and research and adoption is still on-going. Indeed, I believe the French term 'Engineer' should be used in Australia. In the French scientific system, there are scientists, scientific engineers, technicians and extension experts. Scientific Engineer positions are highly sought after and high-quality scientists, who are also experts in translating outputs for industry, fill them. A case in point is the conversion of research in molecular biology and genetics into industry outcomes for the sheep and cattle industries. The molecular biologists made early inroads into a limited number of genes that might have relevant production effects, but essentially the area has under delivered given the investment. In fact it has been the quantitative geneticists (scientific engineers) who have converted molecular technologies into industry outcomes in the form of genomic selection. Training and systems, as discussed above, should encourage and reward scientific engineers, who to some extent can be trained. However engineers are also born, meaning they have the desire, passion, and background industry knowledge to reach this status. It is important that research administration within all organisations embraces the term 'scientific engineer' and has appropriate rewards.

Dr. Robert Banks (AGBU, University of New England) and Professor John Thompson (Emeritus Professor, University of New England) are thanked for their editorial help in crafting this manuscript.

Eating quality linked to ultimate pH and tenderness in Australian fresh pork

C.G. Jose[1], M. Trezona[1], H.A. Channon[2] and D.D'Souza[2]

[1]Department of Agriculture and Food WA, South Perth, WA 6150. [2]Australian Pork Limited, Barton, ACT 2600.

Based on recent consumer taste panel assessments (Channon and Warner, 2011), the eating quality of fresh Australian pork is of concern due to a high fail rate. Over the past decade, monitoring of ultimate pH (pH_u) of pork has shown a decline from the normal pH_u range (5.5-5.7) to below 5.5 (low). Lower pH_u can negatively impact meat quality measures such as drip loss and tenderness (Huff-Lonergan *et.al.*, 2010); however it is not known if consumers perceive a reduction in eating quality in meat with a lower pH_u .Due to the impact of low pH_u on meat quality, this study hypothesised that fresh Australian pork with a low pH_u will be rated lower for overall liking by consumers.

Entire male and female carcasses from two supply chains were assessed over a 3-month period. The pH of the *m. longissimus dorsi* (loin) were measured at 24 h (pH_{24}) in the chiller and again at 72 h (pH_u) after loins were removed. A total of 160 samples were selected for use balanced on supply chain, sex and pH_u (normal and low) (n=20). This allowed for a continuous pH_u range of 5.3-5.7 across sex and supply chain. Four steaks were cut from each loin and consumers were asked to score each cooked (to 70^o C) sample for overall liking and tenderness from 0-100 (poor to excellent). These scores were the adjusted for each consumer and presented as a mean for each loin. Samples were also scored for quality grade score and purchasing opinion from 1 (unsatisfactory) to 5 (excellent), with scores of 2 or less considered as a fail. The shear force of each loin was also assessed. Data were analysed using a linear mixed effects model (SAS®; USA) to determine interactions between sensory scores, pH and shear force; sex and supply chain were fixed effects and day of sampling was the random term.

Consumers considered the eating quality of loin to be unsatisfactory or below average for 21% of all samples tasted, and "would not" or "probably would not" purchase 29% of samples due to overall eating quality. Overall liking scores reduced ($P<0.05$) by 10% as pH_u declined (over the range of pH_u evaluated) (Figure 1a), accepting the initial hypothesis. Quality grade score and sensory tenderness were also reduced by lower pH_u but in females only ($P<0.05$), with sensory tenderness decreasing by 22% in females across the range of pH_u evaluated (data not shown). Shear force was negatively correlated with overall liking (r=-0.31), with scores decreasing by 15% as shear force increased ($P<0.05$; Figure 1b). No influence of supply chain or pH_{24} was observed on any sensory scores, however, pH_{24} was weakly correlated with shear force (r=0.23). These results suggest that the overall liking of pork is dependent on pH_u, while shear force is a factor of both pH_u and rate of pH decline (pH_{24}).

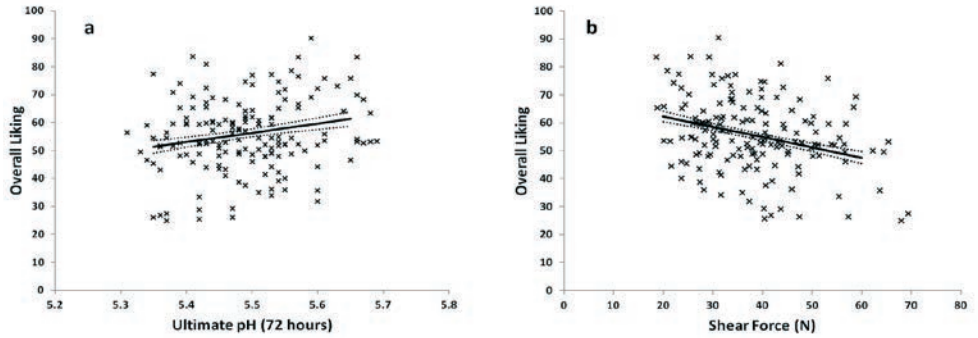


Figure 1. *The correlation between ultimate pH(a) and shear force (b) with overall consumer liking. Lines are predicted means and markers are consumer adjusted means data.(±SEM)*

Given the magnitude of the effect of shear force on overall liking in this study, product tenderness is a valuable indicator of eating quality. Nevertheless, pH_u plays an important role in the tenderisation process (Huff-Lonergan *et al.*, 2010) and impacts on overall liking. Optimising the consistency of both pH_u and pH decline is likely to decrease product variation and improve eating quality of fresh Australian pork.

CHANNON, H.A. and WARNER, R.D. (2011) In "Manipulating Pig Production XIII", p.262, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).
HUFF-LONERGAN, E., ZHANG, W. and LONERGAN, S.M. (2010). *Meat Science.* **86**:184-195.

Supported in part by Pork CRC Limited Australia.

Piglet mortality in the PigSAFE loose farrowing system compared to farrowing crates during autumn and winter

R.S. Morrison[1], M. Farish[2], M.C. Whyte[1] and E.M. Baxter[2]

[1]Rivalea Australia, Corowa, 2646 NSW. [2]Scottish Agricultural College, Edinburgh, EH9 3JG UK.

The PigSAFE (Piglet and Sow Alternative Farrowing Environment) loose farrowing pen has been developed in the United Kingdom. This system was designed to allow the sows to perform maternal behaviours such as isolation, nest building and bonding with piglets. The pen design incorporates a nest area, with solid flooring to allow provision of nesting material and sloped walls against which the sow can slide more slowly to ground level for suckling. A heated creep area is easily accessible for the piglets from the nest. A separate slatted dunging area is bounded by walls with barred panels to adjacent pens to discourage farrowing outside the nest and allow physical contact and social interaction between sows. A feeding crate for the sow is included at one side of the pen, where the sow can be locked in to allow safe inspection or treatment of the piglets (Baxter *et al.*, 2011). The aim of this experiment was to compare piglet mortality and growth performance in PigSAFE farrowing pens compared to farrowing crates over the autumn and winter seasons. The hypothesis tested was piglet mortality and growth performance would be similar in both housing treatments.

Two hundred and forty sows (Large White x Landrace-PrimeGro™ Genetics) were randomly selected prior to entry to their farrowing accommodation. One hundred and twenty sows were randomly allocated to each housing treatment [average parity =1; range 0 (gilt) to 4] over four time replicates. The housing treatments were located in Corowa, New South Wales, in two adjacent buildings. The buildings were similar in terms of ventilation and construction material. The buildings were open-sided with shutters and heating which enabled temperature control. In the farrowing crate shed drippers were located above each sow for cooling and in the PigSAFE shed misters and fans were located above the dunging area. The sheds were both managed by the same people. The experiment began in March and finished in August 2012. The average daily minimum and maximum temperatures inside the buildings were 18-22°C in the PigSAFE shed and 20-23°C for the farrowing crate shed. The total number of piglets born (born alive, still born and mummified piglets), number of piglets born alive and number of piglet deaths were recorded for each litter. Piglet mortality (%) (from birth to weaning) was calculated for each litter. Live weights of litters were recorded at birth and weaning (~ 24 d of age), and daily sow feed intake post-farrowing was recorded. Univariate GLM analysis (IBM SPSS, Version 21.0; USA) was undertaken using each sow/litter as the experimental unit with the sow as the blocking factor.

Table 1. *Average litter, piglet and sow characteristics in lactation and piglet survival in the farrowing crate and PigSAFE housing treatments.*

	Farrowing Crate	PigSAFE	SEM	Significance
Litters farrowed	112	116	-	-
Number piglets born piglets/litter	11.22	11.81	0.191	0.360
Piglets born alive/litter	10.76	11.22	0.191	0.432
Piglet mortality (%)	13.72	13.29	0.919	0.775
Piglet rate of gain (kg/day)	0.23	0.23	0.005	0.684
Sow feed intake (kg/day)	7.42	7.67	0.070	0.228

*Number piglets born alive used as covariate in analysis; SEM, standard error of mean.

There was no difference (P>0.05) in the total number of piglets born, piglets born alive, piglet mortality, piglet rate of gain and sow daily feed intake during lactation between the PigSAFE and farrowing crate systems. These data confirm the hypothesis that piglet survival and growth performance in the PigSAFE system were similar to a farrowing crate over autumn and winter months in Australia. Further research will investigate piglet survival and growth performance over Australian summer conditions.

BAXTER, E.M, ADELEYE, O.O., JACK, M., ISON, S. and EDWARDS, S.A. (2010). In. "Manipulating Pig Production XIII", p.239, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

The financial assistance of Rivalea Australia and Pork CRC Limited Australia and the technical support of the Rivalea Research and Innovation and Production management and staff is gratefully acknowledged. The intellectual input of Professor Sandra Edwards and Dr Greg Cronin is also gratefully appreciated and acknowledged.

Pig appeasing pheromones reduce duration of aggression but not cortisol levels in newly-mixed gestating sows

K.J. Plush[1], P. Herde[2] U.A. Rajapakse[1], P.E. Hughes [2] and W.H.E.J. van Wettere[1]

[1]The University of Adelaide, Roseworthy, SA 5371. [2]South Australian Research and Development Institute, Roseworthy, SA 5371.

Synthetic appeasing pheromones mimic maternal odours secreted by mammary glands and their application can reduce anxiety levels in cats, dogs and horses. In piglets, reduced aggression and increased post-weaning performance has been reported (McGlone and Anderson, 2002). Group housing of sows can increase stress, particularly during group formation and hierarchy establishment, resulting in compromised welfare and reproduction. The aim of this experiment was to determine if aggression and stress are reduced by pig appeasing pheromones in newly-mixed gestating sows.

Within five d of mating 48 sows were allocated to two treatments ensuring even parity distribution: a control group (CON) that received no pheromones, and a group receiving pig appeasing pheromones (PAP) housed with two pheromone diffusing blocks (IRSEA; Saint Saturnin, France). Sows were penned in closed rooms in groups of six with a space allowance of 2.2 m^2 and offered 2.2 kg/d of a dry sow diet. Total injury scores and saliva samples were collected at 13:00 on the d around mixing. Saliva was analysed for free cortisol by radioimmunoassay (DiaSorin, Minnesota, USA). Video footage was collected 6 h following floor feeding and analysed for aggression duration over the observation period using Observer XT v10.5 software (Noldus Inc., Wageningen, The Netherlands). Data were analysed using a linear mixed model in SPSS v19 (SPSS Inc., Chicago, USA) with sow as a random effect, and block, pen, parity, time and treatment as fixed effects. Conception rate was analysed by the Chi-Squared test statistic.

Cortisol levels were unaffected by d relative to mixing and treatment (Table 1). Total injury score increased ($P<0.001$) post mixing but did not differ across treatment. Duration of aggression was highest on the d of mixing when compared with subsequent d ($P<0.001$), and was higher in the CON sows (77.2±16.9 sec) relative to PAP (18.6±20.4 sec; $P<0.001$). No difference in conception rate was found between treatments (CON, 81.0% and PAP, 83.3%; Chi-square statistic = 0.04, $P>0.05$).

Table 1. *Mean ± SEM of salivary cortisol (nmol/L), total injury score, and duration of aggression (sec) for sows housed in the presence (PAP) or absence (CON) of PAP.*

	Day post mixing					Significance		
	-1	0	1	3	7	Day	Treatment	Day x Treatment
Cortisol (nmol/L)								
CON	14.9 ±4.3	13.2 ±4.6	9.8 ±4.6	14.5 ±4.6	14.1 ±4.5	NS	NS	NS
PAP	13.6 ±4.1	19.3 ±4.7	15.9 ±4.5	16.7 ±4.6	18.9 ±4.6			
Total injury score								
CON	30.3 ±4.9	-	36.9 ±4.9	40.7 ±4.9	40.7 ±4.9	<0.001	NS	NS
PAP	32.4 ±5.1	-	37.2 ±5.1	38.2 ±5.2	40.6 ±5.1			
Aggression (sec)								
CON	-	179.9 ±28.7	39.6 ±28.7	84.2 ±37.4	114 ±28.7	<0.001	<0.001	NS
PAP	-	65.5 ±36.1	14.9 ±35.1	13.2 ±60.4	17.9 ±35.1			

NS, not significant ($P>0.05$)

Whilst the duration of aggressive behaviour was reduced in the PAP sows, no consequences for injury, cortisol level or reproduction were observed and this is most likely explained by the small sample size. These initial results warrant further investigation into the effects of reduced aggression in the presence of PAP on the welfare and production of sows under a commercial setting.

MCGLONE, A. and ANDERSON, D. (2002). *Journal of Animal Science*. **80:**3179-3183.

The authors wish to thank the South Australian Pig Industry Fund and Pork CRC Limited Australia for funding this work.

CHAPTER 3

Feed Preference, Applied Nutrition and Grains

Umami-enhanced feed intake in weaned pigs increases plasma cholecystokinin and insulin

R. Palou[1], B. Shresta[1], S. T. Anderson[2] and E. Roura[1]

[1]Centre for Nutrition and Food Science, The University of Queensland, Brisbane, St Lucia, QLD 4072. [2]School of Biomedical Sciences, The University of Queensland, Brisbane, St Lucia, QLD 4072.

Weaning is associated with abrupt social, environmental and nutritional changes often resulting in low feed intake in pigs. Nursery diets are formulated with high quality and highly palatable ingredients and often supplemented with taste enhancers to stimulate feed intake. Data generated during preliminary research indicated that umami taste enhancers (e.g., glutamic acid) are highly preferred in pigs and may enhance post-weaning consumption. The aim of this study was to assess the efficacy of an umami taste supplement to increase feed intake after weaning and assess blood hormonal changes indicative of stress and appetite.

Forty-two piglets (21/21 females/males) weaned at 24 d of age (7.0±2.1 kg; mean±SD) were selected from the University of Queensland Gatton piggery. Thirty-six pigs were weaned, transported to the Herston Medical Research Centre (HMRC) and housed in pairs of the same gender (d 1). Pigs were randomly assigned to one of three dietary treatments: feed-deprived anorexia group (AG), a standard diet [14.93 MJ/kg digestible energy (DE); 185g/kg crude protein; 0.085 available lysine/DE) group (SD), and a group fed the SD supplemented with an umami additive based on glutamic acid (UD) at 1% of the diet. Both groups SD and UD, had *ad libitum* access to feed. The six remaining pigs were transported to the HMRC and euthanised the same day (pre-weaning group; PG). On d 3, six pigs of the AG, SD and UD groups were weighed and feed intake recorded prior to sacrifice. Once sedated, blood samples were collected from the jugular vein, and plasma stored at -80°C until tested using commercial RIA and ELISA kits for cortisol, haptoglobin, glucagon, ghrelin, leptin, insulin, peptide YY (PYY), cholecystokinin (CCK), gastric inhibitory peptide (GIP), and glucagon-like peptide 1 (GLP-1). Data were analysed using the ANOVA and Fisher's LSD in Minitab.

Table 1. *Blood hormone levels in pre-weaned piglets (PG), feed-deprived post-weaned pigs (AG), and post-weaned pigs offered an umami-supplemented (UD) or a normal diet (ND).*

Hormones	Pre-weaning (PG)	Anorexia (AG)	Umami (UD)	Standard (SD)	Significance
Cortisol (ng/ml)	48.5	81.6	46.4	63.6	0.13
Haptoglobin(mg/ml)	0.67^{a}	1.21^{b}	1.10^{ab}	1.19^{b}	0.04
Insulin (µIU/ml)	5.35^{ab}	3.23^{a}	9.42^{b}	7.13^{ab}	0.05
Glucagon(pmol/L)	73.5	81.0	61.1	76.0	0.33
Ghrelin (pg/ml)	242	163	187	188	0.30
Leptin (ng/ml)	0.63	0.65	0.77	0.76	0.89
PYY (pmol/L)	83.8	86.5	75.8	76.7	0.43
CCK (pmol/L)	7.20^{ab}	0.78^{a}	16.63^{b}	2.95^{a}	0.01
GIP (pg/ml)	464	328	485	391	0.59
GLP-1(pM)*	16.0^{a}	74.4^{b}	34.9^{ab}	34.5^{ab}	0.09

^{a,b}Means in a row not having the same superscript are significantly different ($P<0.05$); *($P<0.1$)

At the end of the 48 h experiment, AG pigs lost weight (-191±122.3 g/pig/d) while UD pigs gained significantly ($P<0.05$) more weight than the SD pigs (192±86.7 and 76±141.4 g/pig/d, respectively). Similarly food consumption was highest ($P<0.05$) for UD compared to SD groups (120±17.9 versus 82± 48.9 g/pig/d, respectively). Plasma hormonal profiles are presented in Table 1. CCK levels seem to relate to levels of hunger being lower ($P=0.01$) in AG and SD groups compared with the UD group. The increase in haptoglobin may reflect an immunologic stress related to the change of facilities at weaning (Kim *et al*., 2011). Compared to the pre-weaned group, SD and AG weaned pigs had higher ($P=0.04$) levels of haptoglobin which were partially prevented in the UD group. Insulin concentrations were lower ($P=0.05$) for the AG group compared with UD group reflecting a prolonged fasting state. In conclusion, an umami supplement in the post-weaning diet exhibited higher feed intake and growth in post-weaned pigs. Feed intake was associated with lower hunger and immune stress indicated by CCK, insulin and haptoglobin levels.

KIM, M., YANG, J., UPADHAYA, S., LEE, H., YUN, C. and HA, J. (2011). *Journal of Veterinary Sciences*. **12:**151-157.

Pig preference for flavours conditioned by post-ingestive consequences under protein deficiency after weaning

S.A. Guzmán-Pino, D. Solà-Oriol, J. Figueroa and J.F. Pérez

Universitat Autònoma de Barcelona, Bellaterra, Barcelona 08193, Spain.

Mammals develop strong conditioned flavour preferences based on positive oral and post-oral associations (Touzani *et al.*, 2010), and this learning might be a determinant for an appropriate diet selection pattern when a nutrient deficiency occurs. The present study tested the hypothesis that pigs in a protein deficient status may be able to prefer flavours previously associated to protein solutions based on learned associations.

A total of 240 entire male and female pigs (Pietrain × [Landrace × Large White]) were weaned at 28 d of age (initial body weight [BW] 7.2 ± 1.08 kg; mean±SEM) and distributed according to their BW into 24 pens (10 pigs/pen). Pens were randomly assigned to a high protein [HP, 204 g crude protein (CP)/kg as-fed] or a low protein diet (LP, 142 g CP/kg), with similar digestible energy (DE) contents (15 MJ/kg) and total lysine/DE ratios (0.98 g Lys/MJ DE). Methionine, methionine + cysteine, threonine and tryptophan were balanced to lysine in both diets. However, the contents of isoleucine (Ile), valine (Val) and other essential dietary amino acids were not balanced to lysine, and their contributions in the LP diet were lower (0.38 g Ile and 0.33 g Val/MJ DE) than in the HP diet and the requirements for weanling pigs (0.53 g Ile and 0.67 g Val/MJ DE; NRC, 2012). Diets were based on maize, barley, wheat, and extruded soybean and were offered *ad libitum* in mash form. From d 10 to 17 after weaning, pigs fed with the HP and LP diets were given eight alternate training sessions. Two equally preferred flavours (strawberry and creamy-cheese; conditioned stimulus, CS) were mixed with protein (animal plasma, 60 g/L) or carbohydrate (maltodextrin, 60 g/L) water-based solutions (unconditioned stimulus, US) and were offered to the animals in an extra container of 5L. After conditioning, in d 18 and 19 after weaning, pigs' preference for the CS was assessed by using a choice test of three minutes. Four random pigs of each pen were offered with two pans containing 800 mL of the two CS dissolved in water. Solution intake, feed disappearance and BW of pigs were monitored from weaning to d 18 and analysed with ANOVA. Preference values for the CS were compared to the neutral value of 50 % by using a Student's *t*-test, (SAS®; USA).

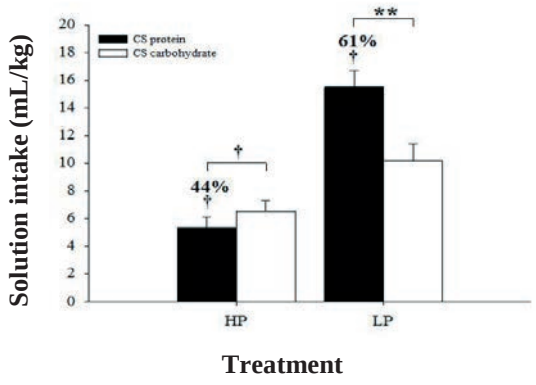


Figure 1. *Intake and preference of pigs fed a high protein (HP) or a low protein diet (LP) for conditioned stimulus (CS) related to protein or carbohydrate solutions. Clasps indicate different intakes between both solutions (†=P<0.1, **=P<0.01). Numbers on top of the bars represent the preference for this solution and its difference from the neutral value (†=P<0.1).*

Pigs fed the LP diet had a lower average daily feed intake (P<0.001), $BW_{Day\ 18}$ (9.5 versus 12.5 kg, P<0.001), average daily gain (P<0.001) and higher feed conversion ratio (P<0.001) than pigs fed with the HP diet. Pigs fed the HP diet showed a tendency to a higher intake of the CS related to carbohydrate, than those CS related to protein consequences (P=0.1; Figure 1). On the other hand, pigs fed the LP diet showed higher intake and a preference for the CS, related to protein consequences, as compared to the CS related to carbohydrate (P<0.01). Results show that pigs with protein deficiency may be able to show an appropriate diet selection pattern, likely based on the relationship between sensorial properties and post-ingestive consequences of the tested solutions.

NRC. (2012). "Nutrient requirements of swine", 11th ed. (The National Academy Press: Washington).
TOUZANI, K., BODNAR, R. and SCLAFANI, A. (2010). *Pharmacology, Biochemistry and Behavior*. **97:**55-62.

Supported by the Spanish Government and the Chilean research fellowships "Becas Chile".

Influence of dietary electrolyte balance on feed preference, appetence and performance in post-weaned pigs

S.A. Guzmán-Pino, D. Solà-Oriol and J.F. Pérez

Universitat Autònoma de Barcelona, Bellaterra, Barcelona 08193, Spain.

Dietary electrolyte balance (dEB), calculated as the net balance between fixed cations and anions ([Na + K] – [Cl]), represents, in part, the net acid or alkaline load contributed by the diet (Patience *et al.*, 1987). The mineral fortification of diets in the feed industry may modify the dEB. The hypothesis tested in the present study is that post-weaned pigs would be able to discriminate a preference for diets differing on the mineral content and the dEB, and they could choose those that optimise growth performance.

A total of 336 entire male and female pigs (Pietrain × [Landrace × Large White]) were used in two experiments. Two isoproteic and isoenergetic starter diets [190 g crude protein, 10.25 MJ net energy (NE)/kg as-fed] differing in dEB (Low and High dEB diets) were used in both experiments. In the Low dEB diet, calcium (Ca) chloride (12.2 g/kg) was provided as a Ca source to meet Ca requirements for pigs (7.5 g/kg), while calcium carbonate (8.6 g/kg) and sodium bicarbonate (20 g/kg) were incorporated in the High dEB diet. The estimated contribution of Na, K and Cl in the Low and High dEB diets was 1.9, 7.2 and 10.1 g/kg, and 7.3, 7.2 and 4.2 g/kg, respectively. Diets resulted in different dEB: -16 mEq/kg in the Low dEB diet and 388 mEq/kg in the High dEB diet. Experiment 1 used 96 pigs. A total of 48 pigs (initial body weight [BW] 13.3±0.9 kg; mean±SD) were distributed into 12 pens (four pigs/pen) at 21 d after weaning, and from d 21 to 35 after weaning, their preference for the Low or the High dEB diet was assessed by using a long-term choice test. Two feeders were simultaneously placed at the front of the pens, each containing one of the experimental diets. The remaining 48 naive pigs were allocated in 12 pens (four pigs/pen) at 35 d after weaning and their appetence for the diets was assessed by using a 2 h one-feeder test. In this case, just one diet was offered to the animals on alternate days. In Experiment 2, 240 pigs (initial BW 11.3±1.3 kg) were distributed at 21 d after weaning into 24 pens (10 pigs/pen), and randomly assigned to receive either the Low or the High dEB diet from d 21 to 33 after weaning (n=12 per treatment). After this period, pig preference for the diets was assessed following the same procedure described above for 30 min. Feed intake and BW of pigs were monitored and analysed with ANOVA by using the GLM procedure (SAS®; USA). Preference values were compared to the neutral value of 50 % by using a Student's *t*-test.

Table 1. *Feed intake and growth performance of pigs after weaning fed a low electrolytic balance (Low dEB) or a high electrolyte balance (High dEB) diet, and preference for the High dEB diet (Experiment 2).*

Item	Low dEB	High dEB	SEM	Significance
Initial body weight (kg)	11.27	11.34	0.387	0.901
Average daily feed intake (g)	905	859	18.2	0.089
Average daily gain (g)	504[a]	438[b]	12.7	0.001
Feed conversion ratio	1.80[a]	1.97[b]	0.031	0.001
Final body weight (kg)	17.3	16.6	0.468	0.292
Preference for High dEB diet (%)	70.9	70.8	-	-

[a,b]Means within a row not having the same superscript are significantly different (P<0.05).

When pigs had the opportunity to choose between the Low and the High dEB diet during the long-term (Experiment 1) or 30 min (Experiment 2) choice test, they showed a higher preference (78% in Experiment 1, P<0.001; and 71% in Experiment 2, P<0.001) for the High than for the Low dEB diet (data not shown). A greater appetence (P<0.01) for the High than for the Low dEB diet was also observed by naive pigs in the 2 h one-feeder test of Experiment 1. However, when offered as single diets in Experiment 2, pigs on the Low dEB diet showed a tendency (P=0.089) to a higher average daily feed intake, a higher average daily gain (P<0.01), and a lower feed conversion ratio (P<0.01) than did pigs fed the High dEB (Table 1). Results show that the mineral content and the dEB affected the preference, appetence and performance of weaned pigs; however, they did not show preference for that diet that optimised their performance.

PATIENCE, J.F., AUSTIC, R.E. and BOYD, R.D. (1987). *Journal of Animal Science.* **64:**457-466.

Supported by the Spanish Government and the Chilean research fellowships "Becas Chile".

Pigs show high sensory-motivated intake for low levels of dextrose but not for low levels of maltodextrin

E. Roura[1], B. Shrestha[1] and S. Diffey[2]

[1]The University of Queensland, Brisbane, St Lucia, QLD 4072. [2]University of Wollongong, Wollongong, NSW 2522.

Increasing feed intake after weaning is a priority for the pork industry and identifying feed ingredients with high preference will help develop novel feed formulations that stimulate appetite. Preferences for ethanol and saccharides including polysaccharides such as maltodextrin (MAL) and polycose (POL) (a commercial MAL) are higher than for sucrose in rodents (Treesukosol *et al*., 2011). It was hypothesised that pigs, similar to rodents, will also have a high preference for MAL/POL and ethanol. The aim of this experiment was to study preference thresholds (defined as the lowest dose with a preference significantly higher than 50%) and sensory-motivated intake at low doses of MAL, POL, dextrose, maltose and several alcohols (ethanol, xylitol, erythritol, and maltitol) in pigs.

A total of 96 pigs were selected by initial body weight (average 13.5±2.3 kg; (mean±SEM)) and housed in 48 pairs of males or females in two environmentally controlled rooms. Feed (14.75 MJ/kg digestible energy (DE); 204 g/kg crude protein; 0.079 g lysine/MJ DE) and water were offered *ad libitum*. Pigs were trained on a double choice (DC) procedure offering two stainless steel bowls containing either water or a 200 mM sugar solution as described previously (Roura *et al*., 2011). After training, experimental solutions were tested in 2-minute DC sessions twice daily. The DC consisted of offering water in one bowl and either sugar (at 200 mM), a positive reference, or: POL (at 1, 5, 10, 15, 20, 60, 80 and 100 g/L), MAL (at 1, 5, 10, 20, 30, 50 and 70 g/L), dextrose (at 10, 20, 30 and 40 mM), maltose (at 3, 15, 30 and 60 mM) or one alcohol (ethanol, xylitol, erythritol, and maltitol) at 5, 10, 15 and 20 mM. Each solution was tested in 11 pairs of pigs following an incomplete block design. Test solution intake (intake) and test solution preference, measured as a percentage ratio of test solution consumed over total (test + water), were analysed using a linear mixed model incorporating the positive (sugar 200 mM) and negative (water) controls.

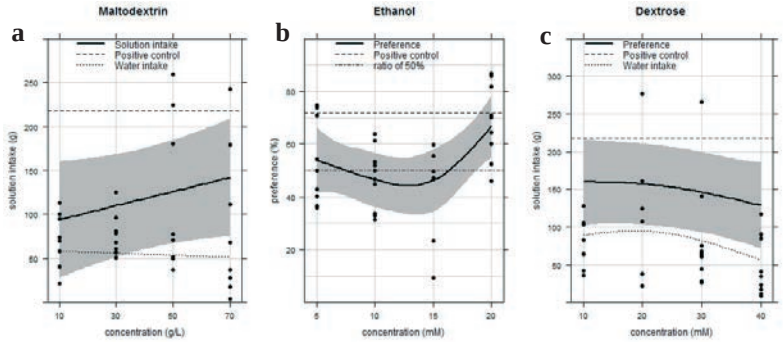


Figure 1. *Effect of concentration on sensory-motivated intake of maltodextrin or dextrose and on preference of ethanol in pigs. Shaded regions represent approximate 95% confidence bands.*

Pigs chose sugar at 200 mM over water with a significant preference of 72% and an average sensory-motivated intake of 218 g over the 2-minute test. Pigs did not show any preference or sensory-motivated intake for MAL or POL at inclusion levels below 2%. However, at the highest concentration tested (7%), MAL showed 64% preference and 142 g of intake, both higher ($P<0.01$) than 50% and water intake respectively (Figure 1a). In addition, several significant ($P<0.05$) preferences were found among the rest of the saccharides and alcohols including ethanol (20 mM) showing the highest value of 67% (Figure 1b) and maltose (10 mM) and xylitol (5 mM) both with 60%. No differences were found for erythritol and maltitol in preference or intake. In contrast, the sensory-motivated intake of the dextrose 10, 20, 30 and 40 mM solutions were 182, 180, 169 and 151 g respectively, all higher ($P<0.05$) than water consumption (average 78.5±10.5 g) and not different ($P>0.05$) to sugar consumption (Figure 1c). Intake of ethanol solutions was marginally above 100 g and not different ($P>0.05$) to water. Similarly the other treatment consumptions did not differ ($P>0.05$) from water. It is concluded that pigs do not respond to low levels of complex carbohydrates while the responses to dextrose solutions and high levels of POL and MAL might be worth further research.

ROURA, E, SHRESTHA, B., ZENG, Y., ZHANG, D. and DIFFEY, S. (2011). In "Manipulating Pig Production XIII", p. 97, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

TREESUKOSOL, Y., SMITH, K.R. and SPECTOR, A.C. (2011). *Physiology and Behaviour* **105:**14-26.

Supported in part by Pork CRC Limited Australia. Preliminary data partially presented at the XVI ISOT, Stockholm, Sweden.

Pigs show high preference and sensory-motivated intake for low levels of tartaric and phosphoric acids

E. Roura[1] B. Shrestha[1] and S. Diffey[2]

[1]The University of Queensland, Brisbane, St Lucia, QLD 4072. [2]University of Wollongong, Wollongong NSW 2522.

Common feed formulation practices in post-weaned pig diets include the use of acidifiers. Their mode of action has been related to their antimicrobial and acid-binding activities mainly based on *in vitro* tests. However, *in vivo* data regarding the efficacy of acids improving feed digestibility and gut microbiota are not consistent. The potential impact of acids on palatability and feed intake in pigs have been recently evaluated by Suarez *et al*. (2010), showing high preferences for tartaric and citric acids at 1% inclusion in complete feeds, but lower levels were not tested. It was hypothesised that commonly used dietary acids in solution at low concentration levels will be highly preferred in pigs. The objective of the trial was to assess preference thresholds and sensory-motivated intake of selected acids commonly used in pig feeds.

Ninety-six pigs were selected by initial body weight (average 9.7±2.3 kg; mean±SEM) and housed in 48 pairs of males or females in two environmentally controlled rooms. Feed (14.75 MJ/kg digestible energy (DE); 204 g/kg crude protein; 0.079 g lysine/MJ DE) and water were offered *ad libitum*. Pigs were trained on a double choice (DC) procedure offering two stainless steel bowls containing either water or a 200 mM sugar solution as described previously (Roura *et al*., 2011). After training, experimental solutions were tested in 2 minute DC sessions twice daily. The DC consisted of offering water in one bowl and either sugar (at 200 mM), a positive reference, or: citric acid (CA) at 12.5, 25 and 50 µM and 0.1, 0.2, 0.38, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 10, 12, 18 and 24 mM; tartaric acid (TA) at 1, 3, 6, and 9 mM; and formic (FA), lactic (LA) and phosphoric (PA) acids at 0.5, 1, 2 and 4 mM. Each solution was tested in 11 pairs of pigs following an incomplete block design. Test solution intake (intake) and test solution preference, measured as a percentage ratio of test solution consumed over total (test + water), were analysed using a linear mixed model incorporating cubic smoothing splines. Preference values were compared to the neutral value of 50% and intake values to the positive (sugar 200 mM) and negative (water) controls. Statistical significance was declared at P<0.05.

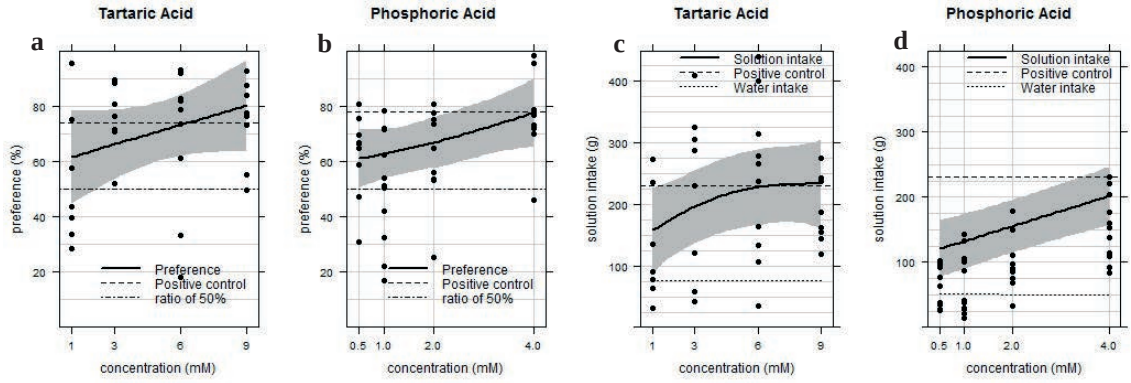


Figure 1. *Preference (%) and sensory-motivated intake (g/2 mins) for tartaric (TA) and phosphoric (PA) acids in pigs. Shaded regions in the figures represent approximate 95% confidence bands.*

Sugar (200 mM) was significantly (P<0.01) preferred (74%) and consumed (230 g) over water in the 2-minute tests. All doses tested for CA showed significant (P<0.05) preference over water with values ranging from 55% (doses below 0.4 mM) to 78% (3 mM). The CA sensory-motivated intake was significantly (P<0.05) higher than water for all the doses tested up to 12 mM. Intakes for 18 and 24 mM were not different to water. However, the highest preference (Figures 1a and 1b) and sensory-motivated intakes (Figures 1c and 1d) were found for TA and PA. A top of 80% preference and 233 g of intake and 77% preference and 203 g of intake were recorded for the 9 mM TA and 4mM PA solutions, respectively. Significant (P<0.05) preferences and intakes were also found for FA and LA only for the two highest doses tested (2 and 4 mM). However, FA did not show any sensory-motivated intake and LA a moderate increase (P<0.05) up to 99 g at 4 mM. In summary, significant preferences for CA are linked to a significant but low capacity to motivate intake while TA and PA appear to have a high potential to stimulate appetite in pigs.

ROURA, E, SHRESTHA, B., ZENG, Y., ZHANG and D. DIFFEY, S. (2011). In "Manipulating Pig Production XIII", pp. 106-117, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

SUAREZ, J., ROURA, E. and TORRALLARDONA, D. (2010). *Journal of Animal Science*. **88(E-Suppl. 2):**651.

Supported in part by Pork CRC Limited Australia.

Preference thresholds and sensory-motivated intake for four high intensity sweeteners in piglets

E. Roura[1] B. Shrestha[1] and S. Diffey[2]

[1]The University of Queensland, Brisbane, St Lucia, QLD 4072. [2]University of Wollongong, Wollongong, NSW 2522.

The feed industry has commonly relied on high intensity sweeteners (HIS) to stimulate feed intake in post-weaned piglets. The most popular HIS used in piglet feeds is Na-saccharine (SAC). However, compared to humans, pigs have a low sensitivity for SAC (Glaser *et al*., 2000), only 3 to 5 fold higher than sucrose. The same authors showed that acesulfame-K (ASK) and sucralose (SUC) were also preferred by pigs. In addition, plant derived HIS rebaudioside A (REBA) has gained interest as a sweetener in human food applications, but little is known on its efficacy in pigs. We hypothesise that pigs may have a higher preference for ASK, SUC and REBA than for SAC. The aim of this experiment was to determine preference thresholds (defined as the lowest dose with a preference significantly higher than 50%) and sensory-motivated intake a low doses of ASK, SAC, SUC and REBA in pigs.

Ninety-six piglets were selected by initial body weight (average 11.8±2.2 kg; mean±SEM) and housed in 48 pairs of males or females in two environmentally controlled rooms. Feed (14.75 MJ/kg digestible energy (DE); 204 g/kg crude protein; 0.079 g lysine/MJ DE) and water were offered *ad libitum*. Pigs were trained on a double choice (DC) procedure offering two stainless steel bowls containing either water or a 200 mM sugar solution as described previously (Roura *et al*., 2011). Experimental solutions were tested in 2-minute DC sessions consisting of offering water in one bowl and either sugar (at 200 mM), a positive reference, or: ASK (at 0.3, 0.6, 0.9 and 1.2 mM), SAC (at 0.5, 1.0, 1.5, 3.0, 6.0 and 9 mM), SUC (at 5, 10 38, 50, 75, 100, 150, 300, 450 and 600 µM) and REBA (at 0.12, 0.3, 0.6, 0.9 and 1.2 mM). Each solution was tested in 11 pairs of pigs using an incomplete block design. Test solution intake (intake) and test solution preference, measured as a percentage ratio of test solution consumed over total (test + water), were analysed using a linear mixed model incorporating cubic smoothing splines. Preference values were compared to the neutral value of 50% and intake values to the positive (sugar 200 mM) and negative (water) controls.

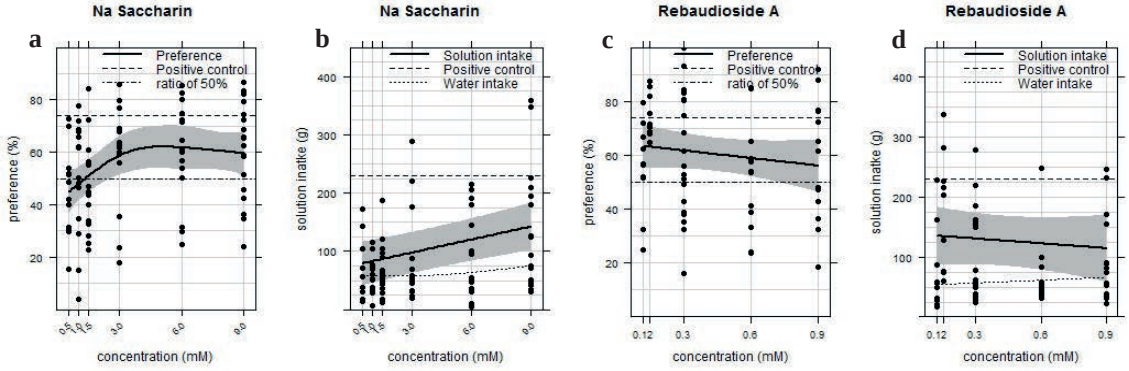


Figure 1. *Effect of concentration on preference and sensory-motivated intake of NAC and REBA in pigs. Shaded regions in the figures represent approximate 95% confidence bands.*

In a preliminary DC test sucrose preference threshold in pigs was 6 mM (*unpublished results*). In the current experiment sugar at 200 mM resulted in a significant preference of 74% and an average intake of 230 g. Preference for SUC was significant (P<0.05) at or above 300 µM, but with relatively low values (below 60%). Furthermore, the sensory-motivated consumption was not significant, being below 100 g at all tested levels. Pigs did not show any significant preference or appetite for ASK tested up to 1.2 mM. The preference threshold for SAC was around 3 mM (Figure 1a). The SAC preference reached the highest value of 62% with 6 mM and the highest intake of 142 g at the highest dose tested (9 mM) (Figure 1b). In contrast, the best preference and sensory-motivated intake values for REBA of 63% and 135 g, respectively, were found at the lowest concentration tested of 120 µM (Figures 1c and 1d). In conclusion, our results confirm previous findings related to SAC, a compound defined as a HIS in humans that is only around 3-fold more intense than sucrose in pigs. In contrast, the intensity found for REBA in pigs warrants further research.

GLASER, D., WANNER, M., TINTI, J.M. and NOFRE, C. (2000). *Food Chemistry*. **68:**375-385.

ROURA, E, SHRESTHA, B., ZENG, Y., ZHANG, D. and DIFFEY, S. (2011). In "Manipulating Pig Production XIII", pp. 106-117, ed. R.J. van Barneveld, (Australasian Pig Science Association: Werribee).

Supported in part by Pork CRC Limited Australia. Rebaudioside A was kindly donated by Pure Circle Inc.

Pigs show no sensory-motivated intake for several cereal and tuber starches except hydrolysed corn starch

E. Roura[1] B. Shrestha[1] and S. Diffey[2]

[1]The University of Queensland, Brisbane, St Lucia, QLD 4072. [2]University of Wollongong, Wollongong, NSW 2522.

Increasing feed intake after weaning remains an area for improvement in pig production. Starch is the main glucose reservoir in plant tissues and the main energy source in pig diets. The high preference of pigs for simple carbohydrates (i.e., sugars) is well known. However, pig preferences for different sources of starch have not been studied. Existing literature shows that pig preferences for cereals are correlated with the starch content (Torrallardona and Sola-Oriol, 2009). It was hypothesized that cereal preferences are due to the starch. The aim of this experiment was to study preference thresholds and sensory-motivated intake responses in pigs to two tubers and six cereal starches.

Ninety-six piglets were selected by initial body weight (average 13.5±2.3 kg; mean±SEM) and housed in 48 pairs of males or females in two environmentally controlled rooms. Feed (14.75 MJ/kg digestible energy (DE); 204 g/kg crude protein; 0.079 g lysine/MJ DE) and water were offered *ad libitum*. Pigs were trained on a double choice (DC) procedure offering two stainless steel bowls containing either water or a 200 mM sugar solution as described previously (Roura *et al*., 2011). After training, experimental solutions were tested in 2-minute DC sessions twice daily. The DC consisted of offering water in one bowl and either sugar (at 200 mM), a positive reference, or starch extracts of: tapioca (T) or potato (P) at 1, 2, 3 and 4%, wheat (W), rice (R), corn (C), waxy corn (CW), high amylose corn (CA) or hydrolysed corn (HC) at 0.1, 0.5, 1.0 and 2.0%. Each solution was tested in 11 pairs of pigs following an incomplete block design. Test solution intake (intake) and test solution preference, measured as a percentage ratio of test solution consumed over total (test + water), were analysed using a linear mixed model incorporating cubic smoothing splines. Preference values were compared to the neutral value of 50% and intake values to the positive (sugar 200 mM) and negative (water) controls.

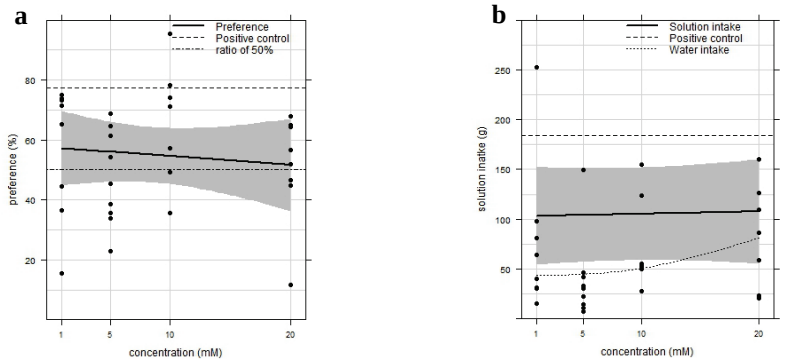


Figure 1. *Effects of concentration on preference (%) and sensory-motivated intake (g) for HC starch in pigs. Shaded regions in figures represent approximate 95% confidence bands.*

Sugar at 200 mM was significantly (P<0.05) preferred over water with a 78% ratio and an average motivational intake pooled across treatments of 253 g over the 2-minute tests. None of the solutions of T, P, W, R, C, CW or CA starches tested resulted in a preference significantly different to 50%. The preference values for HC starch showed the highest standard errors among treatments. Consequently, preference values were not statistically significant (Figure 1a). However, pigs showed a significant sensory-motivated increase in intake at 1, 5 and 10 g/L compared to water (Figure 1b). The intake values for HC starch across doses were marginally above 100 g, thus significantly lower (P<0.05) than the positive control sugar. None of the intakes of the other solutions tested were statistically different to water consumption. It is concluded that pigs do not show sensory-driven preference for non-hydrolysed cereal or tuber starches in the water solutions tested. However, a sensory-motivated increase in intake for HC starch warrants further investigation on potential effects of technological treatments on starch appetite in pigs.

ROURA, E., SHRESTHA, B., ZENG, Y., ZHANG D. and DIFFEY, S. (2011). In "Manipulating Pig Production XIII", p. 97, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

TORRALLARDONA, D. and SOLA-ORIOL, D. (2009). In "Voluntary feed intake in pigs", pp. 215-236, eds D. Torrallardona and E. Roura. (Wageningen Academic Publishers: The Netherlands).

Supported in part by Pork CRC Limited Australia. Preliminary data partially presented at the XVI ISOT, Stockholm, Sweden.

Isoenergetic diets differing in arabinoxylans or β glucans show similar taste receptor expression profile in pig tongue

M. Rzepus[1,2], N. de Jager[1], M. Preston[1], M.J. Gidley[1], B.A. Williams[1] and E. Roura[1]

[1]The University of Queensland, Brisbane, St Lucia, QLD 4072. [2]Università Cattolica del Sacro Cuore, Italy.

The taste receptor type 1 family (T1R) responds to dietary nutrients and consists of three proteins that form dimers for sweet (T1R2/T1R3) and umami (T1R1/T1R3) taste sensing. The sweet dimer responds to carbohydrates (i.e., mono- and disaccharides) whereas the umami dimer responds to amino acids (e.g. glutamic acid). Our current understanding is that inadequate dietary supply of these nutrients (i.e., energy or amino acids) in pigs may cause up regulation of the expression levels of the Tas1R genes (Roura, 2011). Dietary fibre has been reported to impact on the availability and digestibility of some essential nutrients, such as lysine (Lys) and threonine (Thr), or net energy (Dégen *et al*., 2007). Therefore, we hypothesised that fibre-rich diets will impact on the gene expression level of Tas1Rs. This research studied the effect of arabinoxylans (AX) and β glucans (BG) on the level of expression of Tas1Rs in porcine taste tissues.

In total, 18 male Large White pigs were included in this study (6 × 3 diets). The control (CTR) was a standard wheat-starch based diet consisting of 17.4 MJ/kg digestible energy (DE), 197 g/kg crude protein and 14.4 g/kg available lysine. The two treatments were isoenergetic compared to CTR, but contained 10% additional fibre through the replacement of starch in the CTR diet by AX and BG, respectively. Pigs (23.9±2.4 kg; mean±SEM) were blocked by litter and randomly assigned to one of the three diets, with a 1 week adaptation. The animals received two meals per day (morning and afternoon) for 2 weeks before being sacrificed. Water was offered *ad lib*., Tissue samples from tongue circumvallate-, fungiform- and foliate papillae were collected and immediately frozen in liquid nitrogen, then stored at -80 ^{o}C pending RNA extraction. Real-time PCR amplification was performed to estimate Tas1R1, Tas1R2 and Tas1R3 mRNA abundance. Data were analysed using the Tukey's Honestly Significant Difference (HSD) test in R.

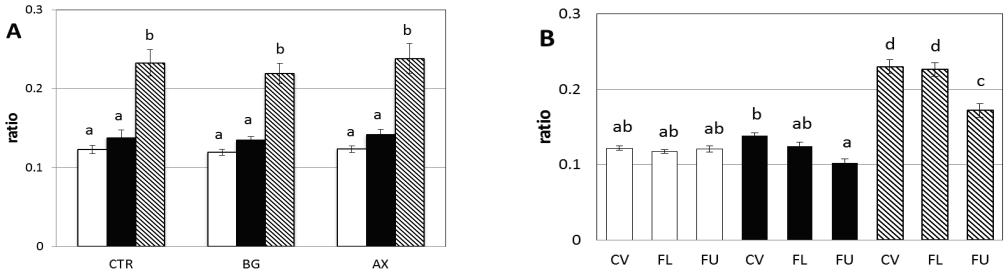


Figure 1. *Relative mRNA abundance (as a ratio to reference genes) of Tas1R genes in porcine tongue. Tas1R1 (solid white bars), Tas1R2 (solid black bars) and Tas1R3 (hatched bars);* ***A.*** *By dietary treatment in circumvallate (CV) papilla: control (CTR), β-glucans (BG) and arabinoxylans (AX).* ***B.*** *Across treatments by type of papillae: CV, fungiform (FU) and foliate (FL). Means with different letters differ significantly (P<0.05).*

The expression of Tas1R3 was higher (P<0.05) than the levels of Tas1R1 and Tas1R2 independent of dietary treatment and type of papillae. This is c onsistent with existing literature in other mammalian species. Figure 1A shows there were no differences (P>0.05) in the expression level of the three Tas1R genes across dietary treatments in circumvallate, fungiform or foliate papillae. Since the diets were isoenergetic and pigs had similar growth performance (data not shown), these results suggest that energy homeostasis is a principal driver in modulating Tas1R expression in the tongue. Across treatments, fungiform papillae had lower (P<0.05) expression of Tas1R2 and Tas1R3 when compared to the circumvallate papillae (Figure 1B). This may be consistent with the lower number of taste sensory cells in fungiform papillae. In conclusion, the level of expression of Tas1R3 in pig tongue is the highest among the Tas1R genes. High levels of AX and BG administered as part of isoenergetic diets did not affect Tas1R expression in pig tongue.

DÉGEN, L., HALAS, V. and BABINSZKY, L. (2007). *Acta Agriculturae Scandinavica A: Animal Sciences*. **57**:1–9.

ROURA, E. (2011). In "Manipulating Pig Production XIII", pp. 106-117, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

Research funded by the ARC Centre of Excellence in Plant Cell Walls. The contributions by Dr Dagong Zhang and Dr Walter Gerrits in experimental and diet design are thankfully acknowledged.

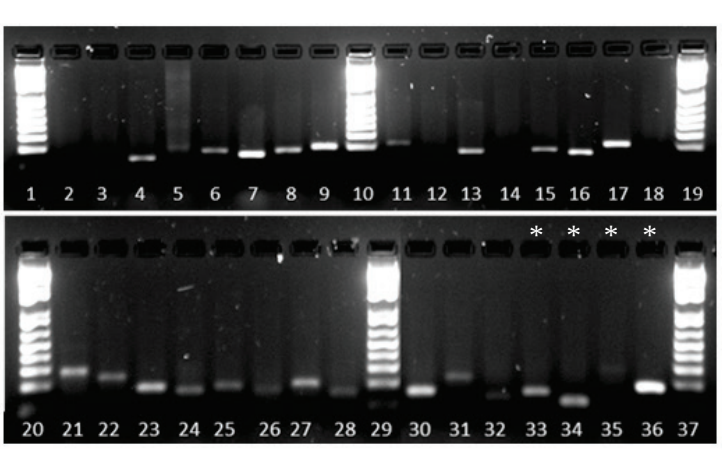
Towards defining the taste receptor repertoire in the pig

N. de Jager[1], M. Zhan[1], M. Rzepus[1, 2] and E. Roura[1]

[1]The University of Queensland, Brisbane, St Lucia, QLD 4072. [2]Università Cattolica del Sacro Cuore, Italy.

The advent of the pig genome has furnished pig scientists with novel tools to understand pig physiology. Current research is involved in understanding factors controlling feed intake and the chemosensing system seems to play a fundamental role in and outside the oral cavity. For example, it has become apparent from research mainly done in rodents that receptors responsible for detecting taste in the tongue are also present in the stomach, heart, brain and gastrointestinal tract (Roura, 2011). This is suggestive of a nutrient sensing role and could have wide ranging implications. However, to date, there has not yet been evidence of a systematic approach to catalogue the full set of taste receptors in the pig. We hypothesise that the taste receptor repertoire in pigs will be concordant with rodent and human literature.

Six Large White piglets (24±3 d of age; mean±SEM) were anaesthetised and sacrificed prior to weaning. Tissues including three sections of the tongue, circumvallate-, fungiform- and foliate papillae were collected and frozen in liquid nitrogen. Total RNA was extracted using a Qiagen RNeasy kit. Candidate genes included receptors for sweet and umami taste (Tas1r1, Tas1r2 and Tas1r3), bitter taste (16 Tas2Rs), fat or fatty acid receptors (GPR120, GPR40, GPR41, GPR43 and GPR84), glutamic/amino acid receptors (mGLUR1, mGLUR4) and an amino acid receptor (GPRC6A). Full length mRNA sequences for these genes were retrieved from the latest pig genome assembly Sscrofa 10.2 in NCBI. Primers were designed to amplify unique regions within each of these genes. *In silico* primer BLASTing confirmed the specificity of the primers to their target transcripts. In addition, real time PCR following the MIQE guidelines (Bustin *et al*., 2009) and the delta delta normalisation method (Pfaffl, 2001) on all genes for the three tissues was carried out on an ABI-7900HT using SYBR green and confirmed single disassociation peaks.



1	1kb ladder	20	1kb ladder
2	Tas1R1 (109)	21	Tas2R42 (300)
3	Tas1R2 (77)	22	Tas2R60 (248)
4	Tas1R3 (134)	23	Tas2R134 (184)
5	Tas2R1 (215)	24	GPR120 (170)
6	Tas2R3 (186)	25	GPR40 (187)
7	Tas2R4 (160)	26	GPR41 (169)
8	Tas2R7.1 (188)	27	GPR43 (201)
9	Tas2R7.2 (213)	28	GPRC6A (160)
10	1kb ladder	29	1kb ladder
11	Tas2R7.3 (236)	30	GPR84 (150)
12	Tas2R9 (116)	31	mGLUR1 (230)
13	Tas2R10 (173)	32	mGLUR4 (123)
14	Tas2R16 (138)	33	TBP* (153)
15	Tas2R20 (182)	34	GAPDH* (104)
16	Tas2R38 (155)	35	ACTB* (292)
17	Tas2R39 (224)	36	RPLP1* (181)
18	Tas2R41 (134)	37	1kb ladder
19	1kb ladder		

Figure 1. *Agarose gels (2%) following electrophoresis of PCR products from the newly designed primers for 27 taste receptor candidate genes showing the amplification of single products/bands of the predicted size, indicated as the number of base pairs in brackets following the gene name. The white * indicates 4 reference gene controls. Lanes 1, 10, 19, 20, 29 and 37 contain 1kb ladders.*

Conventional PCR results (Figure 1) confirm the amplification of a single product of the correct size for each candidate gene. The band for Tas2R16 (lane 14) is too faint to visualise in this format; however it is present when viewed under a gel visualiser. Additional evidence of its expression was confirmed by the presence of a single amplification peak in real time PCR. The expression of the remaining candidate genes was confirmed in the tongue by real time PCR, with no differences in abundance between the three types of papillae (data not shown). In conclusion, our research confirms the presence and abundance of taste receptor genes in the pig tongue, concordant with human and rodent literature. Our research provides tools necessary to investigate the role that taste receptor genes/nutrient sensors play in feed intake, nutrient specific appetite and nutrient absorption in pigs.

BUSTIN, S.A., BENES, V., GARSON, J.A., HELLEMANS, J., HUGGETT, J., KUBISTA, M., MUELLER, R., NOLAN, T., PFAFFL, M.W., SHIPLEY, G.L., VANDESOMPELE, J. and WITTWER, C.T. (2009). *Clinical Chemistry*. **55:**611-622.

PFAFFL, M.W. (2001). *Nucleic Acids Research.* **29:**e45.

ROURA, E. (2011). In "Manipulating Pig Production XIII", p.106, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

Pre- and post-natal flavour exposure through maternal diet modifies feed preference and productive performance in post-weaned pigs

L. Blavi[1], D. Solà-Oriol[1], J.J. Mallo[2], L. Mesas[2], A. Ortiz[2] and J.F. Pérez[1]

[1]Universitat Autònoma de Barcelona, Bellaterra, Barcelona 08193, Spain. [2]Norel, Madrid, Spain.

Early flavour exposure may modulate feed preferences and neophobia of young mammals. In particular the presence of specific volatile compounds in amniotic fluid and milk could be driven by flavour supplementation of maternal diets (Oostindjer *et al.,* 2009). In previous studies, we have observed a positive association between flavours and the hedonic reward of the uterine experience and familiarity effect in early-weaned pigs (Figueroa *et al*., 2013). This early learning process may be a strategy to reduce the stress associated to the sudden dietary change at weaning, allowing significant increases in feed consumption. The aim of the present work was to study the productive performance and feed preference of pigs either conditioned or not with a commercial flavour (Fluidarom 1003® (Fl); 375 ppm) through the maternal diet.

A total of 12 sows was distributed according to their body weight (BW) and body condition into two groups and fed a commercial gestation (28 d before the farrowing date) and lactation diet, either as a non-flavoured Control diet (C; n=6) or with Fl (F; n=6). A total of 123 entire male and female piglets (Pietrain × [Landrace × Large White]) were weaned into 12 pens (complete litters of 10/11 piglets/pen) at 28 d of age [initial BW 7.6±1.6 kg (mean±SEM) for piglets from C sows and 7.3±1.9 kg for F piglets]. Piglets from F sows were fed a pre-starter diet supplemented with flavour (FF) and piglets from C sows were fed a diet without flavour supplementation (CC). Feed intake and BW of piglets was monitored weekly to calculate BW; average daily feed intake (ADFI) and average daily gain (ADG). On d 2 post-weaning, a choice test (30 min) was conducted between two diets supplemented either with Fluidarom 1003® or Lacto-Vanilla (LV; 500 ppm) as a negative control to all 12 pens. Pig preference and performance data after weaning were analysed with ANOVA by using the MIXED and GLM procedures of SAS® taking into account pre- and post-weaning exposure to Fl. Preference values for the Fl were compared to the neutral value of 50 % using a Student's t-test.

Table 1. *Effects of including Fluidarom 1003® into the sows and pre-starter diet (FF) as compared to a non-inclusion (CC) on BW (kg) after weaning.*

Treat	BW d7	BW d14
FF	8.71	10.32
CC	8.60	10.01
Pooled SEM	0.165	
Day (Pr>F)	<0.001	
BW0 (Pr>F)	<0.001	
Treatment (Pr>F)	0.373	
Treatment x Day (Pr>F)	0.069	

BW d0 included as covariate; SEM, standard error of mean.

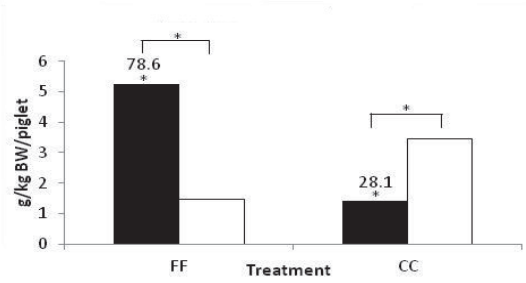


Figure 1. *Intake and preference for Fluidarom 1003® (■) and Lacto-Vanilla (□).*
Clasps indicate different intakes between flavored feeds (=P<0.05). Numbers on top of the bars represent intake percentage of Fluidarom 1003® and its difference from the neutral value (*=P<0.05).*

No different BW and ADG were observed between treatments. However, there was a tendency (P=0.069) in the interaction between treatment and day indicating that while no different BW was observed on d 7, higher BW for the FF animals was achieved at the end of the pre-starter period. Moreover, higher ADFI tended to be observed (P=0.10) for the first week after weaning for the FF piglets as compared to CC (239 versus 200 g/d); data not shown. Accordingly, pigs from the control group (CC) preferred (P<0.05) the LV diet but FF pigs strongly preferred (P<0.05) the Fl diet compared to LV (Figure 1). It is concluded that the inclusion of 375 ppm of Fluidarom 1003® in the sow diets for late gestation and lactation could be a good strategy to increase feed preference and post-weaning feed intake of pigs.

FIGUEROA, J., SOLÀ-ORIOL, D., VINOKUROVAS, L., MANTECA, X. and PÉREZ, J.F. (2013). *Animal Feed Science and Technology*. In press: doi:10.1016/j.anifeedsci.2013.04.023.
OOSTINDJER, M., BOLHUIS, J.E., VAN DEN BRAND, H. and KEMP, B. (2009). *Chemical Senses.* **34:**775-787.

Increasing valine:lysine but not isoleucine:lysine in diets improves growth of pigs after weaning

C.J. Brewster, D.J. Henman, C.L. Collins, K.S. Sawyer, T.N. McDonald and B.G. Luxford

Rivalea Australia Pty Ltd, Corowa, NSW 2646.

Valine (Val), isoleucine (Iso) and leucine (Leu) comprise the branched chain amino acids (BCAA) and are essential amino acids for pig growth. The BCAA undergo a complex catabolism in the pig. One of the enzymes involved, branched-chain alpha-keto acid dehydrogenase, is regulated by a catabolite of Leu meaning that in diets with excess Leu (common where blood meal is used at more than 40 kg/t), Iso and Val will be catabolised even if they are not in excess. This can lead to deficiencies of Val and Iso available for protein synthesis and subsequent reduced pig growth performance (Gloaguen *et al.*, 2011).

In wheat-based diets and after lysine (Lys), methionine (Met), threonine (Thr) and tryptophan (Trp), Val and Iso are considered next most limiting. With increased interest in the use of low crude protein feeds as a means of controlling post-weaning scours (Aumaitre *et al*., 1995), dietary deficiencies of amino acids such as Iso and Val becomes more likely. The aim of this experiment was to establish if weaner growth performance could be increased by using diets with high Iso:Lys and/or Val:Lys levels.

One thousand and eight piglets (PrimeGro Genetics™, Rivalea Australia) were weaned at an average of 25 d and housed within sex in commercial pens of 14 pigs/pen. All piglets were fed a common starter diet *ad libitum* for 10 d post weaning. At 11 d post-weaning (6.6 kg±0.42; mean±SEM) pens were randomly allocated to a 2 x 2 factorial design with the respective factors being the dietary Iso:Lys (0.52 and 0.57) and the dietary Val:Lys (0.63 and 0.86) All diets were formulated to contain 14.9 MJ digestible energy (DE)/kg and 0.88 g standardised ileal digestible lysine/MJ DE and were fed *ad libitum* for a 35 d test period. Dietary Leu:Lys levels were kept below 1.1, a level determined by Gloaguen *et al.* (2011) to not impact growth performance. Pen weights were obtained at d 0, 21 and 35, with feed intake and feed efficiency measured on a pen basis during this time. Data were analysed by ANOVA using Genstat 15th Edition (VSN International, Oxford UK).

Table 1. *Influence of Iso:Lys and Val:Lys on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion (FCR) of weaner pigs (d 0 was 11 d post-weaning).*

	Iso:Lys 0.52		Iso:Lys 0.57		SED[1]	Significance[2]		
	Val:Lys 0.63	Val:Lys 0.86	Val:Lys 0.63	Val:Lys 0.86		Iso:Lys	Val:Lys	Iso x Val
Days 0-21								
ADG (g/d)	376	423	362	412	19.2	0.36	<0.01	0.91
ADFI (g/d)	494	526	479	499	26.3	0.26	0.17	0.72
FCR	1.31	1.25	1.33	1.21	0.04	0.74	<0.01	0.22
Days 0-35								
ADG (g/d)	435	469	411	451	18.9	0.12	<0.01	0.85
ADI (g/d)	633	659	621	633	30.7	0.39	0.39	0.75
FCR	1.45	1.40	1.52	1.40	0.04	0.18	<0.01	0.18

[1] SED, Standard error of difference.

Increasing dietary Iso:Lys from 0.52 to 0.57 did not improve growth performance (Table 1). However, increasing dietary Val:Lys from 0.63 to 0.86 improved (P<0.01) ADG and FCR at both low and high dietary Iso:Lys levels between both 0-21 and 0-35 days. These results indicate that the Val:Lys requirement for weaner pigs is above 0.63 regardless of dietary Iso:Lys levels, and that there is no benefit in increasing weaner pig Iso:Lys levels above 0.52 where dietary leucine:lysine is kept below 1.1.

AUMAITRE, A., PEINIAU, J. and MADEC, F. (1995). *Pig News and Information.* **16:**73N-79N.

GLOAGUEN, M., LE FLOC'H, N., BROSSAED, L., BAREA, R., PRIMOT, Y., CORRENT, E. and VAN MILGEN, J. (2011). *Animal.* **5**:1734-1742.

Formic acid supplementation in phytase-containing pig diets improves phosphorus but not zinc utilisation

R. Blank[1], M. Naatjes[1], J. Rischewski[1], P. Ader[2] and A. Susenbeth[1]

[1]Christian-Albrechts-Universität zu Kiel, Germany. [2]BASF SE, Ludwigshafen, Germany.

Dietary acidification in combination with phytase (PHY) has been claimed to enhance the effectiveness of PHY due to higher solubility of phosphorus (P) and/or phytate and other minerals due to lower stomach pH, and subsequently improved absorption *(*Jongbloed *et al.,* 2000*).* The aim of this study was to investigate at what minimal supplementation level of formic acid (FA) to PHY containing wheat-barley-soybean meal-based diets the P and zinc (Zn) total tract apparent digestibility in pigs is improved.

For the experiment, 12 barrows (Pietrain × dbNaima) with an initial body weight of 32.4±1.5 kg (mean±SEM) were assigned to a triplicate 4 × 4 Latin Square. The basal diet was formulated to contain 13.8 MJ/kg metabolisable energy (ME), 187 g/kg crude protein (CP), 10.5 g/kg lysine, 3.5 g/kg P and 40 mg/kg Zn (no supplementation of inorganic P- or Zn-sources). Dietary treatments consisted of the basal PHY-(500 units/kg; NATUPHOS®, BASF SE, Ludwigshafen, Germany) containing diet supplemented with no or 1.0, 2.5, and 4.5 g/kg of FA (Amasil® 85, BASF SE, Ludwigshafen, Germany). Pigs were fed at 1.75 times the energy requirements for maintenance and were kept individually in metabolism crates. Faeces and urine were collected quantitatively. Feed and faeces were analysed for organic matter (OM), CP, energy, P and Zn, and urine was analysed for P and Zn, in order to calculate the coefficient of total tract apparent digestibility (CTTAD) for the according feed components as well as P and Zn retention. Data were analysed according to a 4 × 4 Latin square with pig, period, and treatment as factors using the GLM procedure of SAS. Least-square means and standard errors of the means (SEM) for each parameter were calculated and significant differences (P<0.05) between least-squares means were determined by the Tukey-Kramer-Test.

Table 1. *Diet component CTTAD and P and Zn retention in pigs fed PHY- containing diets supplemented with increasing levels of FA.*

	FA , g/kg					
	0	1.0	2.5	4.5	SEM	Significance
CTTAD						
Organic matter	0.894	0.898	0.899	0.900	0.0035	0.620
Crude protein	0.869^{b}	0.885^{ab}	0.887^{ab}	0.894^{a}	0.0055	0.022
Energy	0.878	0.883	0.884	0.884	0.0040	0.644
P	0.542^{b}	0.542^{b}	0.566^{ba}	0.608^{a}	0.0154	0.016
Zn	0.251	0.192	0.229	0.266	0.0328	0.431
Retention						
P retained/P intake	0.539^{b}	0.539^{b}	0.564^{ba}	0.605^{a}	0.0155	0.015
Zn retained/Zn intake	0.237	0.174	0.214	0.250	0.0337	0.416

^{a,b} Means in a row not having the same superscript are significantly different (P<0.05).

Formic acid supplementation had no effect (P>0.05) on the CTTAD of OM and energy, but increased (P<0.05) CTTAD of CP. Supplementation of 4.5 g FA per kg diet increased (P<0.05) the CTTAD of P and P retention by 12% in comparison to the control diet without FA supplementation. The CTTAD of Zn and Zn retention were unaffected (P>0.05) by FA supplementation (Table 1).

The effect could be explained by a lower pH of stomach digesta when feed is supplemented with FA. This would result on the one hand in higher phytate solubility and therewith an increased availability of phytate to be cleaved by PHY, and on the other hand an increased PHY activity itself by shifting the digesta pH to more optimal conditions of the enzyme. The results indicate that dietary acidification of PHY-containing pig diets by FA supplementation may be a suitable tool to further improve the efficiency of P digestion and to improve sustainability of pig production due to reduced P excretion into the environment.

JONGBLOED, A.W., MROZ, Z., VAN DER WEI-JONGNLOED, R. and KEMME, P.A. (2000). *Livestock Production Science.* **67**:113-122.

Performance and energy utilisation in weaner pigs supplemented with high levels of exogenous phytase

D.J. Cadogan[1], C.L. Collins[2], A. Péron[3] and G.G. Partridge[4]

[1]Feedworks, Lancefield, VIC 3435. [2]Rivalea Australia Pty Ltd, Corowa, NSW 2646. [3]Danisco Animal Nutrition, Singapore 117525. [4]Danisco Animal Nutrition, Marlborough, UK.

Phytase is commonly used in pig diets between 500 to 1000 units of activity (FTU) per kg of feed (Selle and Ravindran, 2008). There is evidence, however, that doses of phytase beyond 1000 FTU/kg will further promote animal performance, particularly when zinc oxide (ZnO) is supplemented at pharmacological levels in weaner diets (Augspurger *et al*., 2004). The hypothesis tested in this experiment was that young pig growth performance would be increased by levels more than 1000 FTU/kg feed of the exogenous *E. coli* 6-phytase (Phyzyme® XP, Danisco Animal Nutrition) when ZnO is added at 2,500 ppm.

Male and female pigs (PrimeGro™, Rivalea Australia), weaned at 24 d of age and housed separately in pens of seven, were fed a standard commercial starter diet for 6 d. At 31 d of age (8.4±0.78 kg; mean±SEM) the pigs were allocated to five dietary treatments, with 12 replicates per treatment, using a randomised design with sex, entry time and starting weight as blocking factors The control treatment was a wheat-based diet formulated to 14.7 MJ/kg of digestible energy (DE), 1.28% available lysine, 0.44% available P and 0.28% phytate P. The control diet was supplemented with phytase at 0, 500, 1000, 2000 and 4000 FTU/kg feed. Diets were steam-pelleted at 82˚ C and fed for 28 d. Feed and water were provided *ad libitum*. Bodyweight (BW) and feed disappearance were recorded at d 14 and d 28. Uncontaminated faecal samples (approximately 1 kg per pen) were collected between d 18 and 22, and DE was determined using acid insoluble ash as an indigestible marker. Data were analysed for linear and quadratic dose effects using SPSS PASW Statistics 18. Sex effects were not significant (P>0.05) and were excluded from the model.

Table 1. *Performance and the DE content of diets supplemented with increasing levels of phytase.*

	Phytase dose rate (FTU/kg of feed)						Significance	
	0	500	1000	2000	4000	SEM	Linear	Quadratic
1-14 days								
ADG	396^{a}	428^{ab}	447^{b}	464^{b}	463^{b}	7.4	0.005	0.001
FCR	1.29^{b}	1.24^{ab}	1.21^{a}	1.19^{a}	1.21^{a}	0.013	0.066	0.025
ADFI	508	531	539	552	557	8.6	0.075	0.084
BW at 14 d	13.9	14.5	14.6	14.8	14.9	0.23	0.121	0.196
1-28 days								
ADG	548^{a}	561^{ab}	599^{b}	616^{b}	608^{b}	9.7	0.002	<0.001
FCR	1.38^{b}	1.38^{b}	1.35^{ab}	1.33^{a}	1.36^{ab}	0.009	0.214	0.174
ADFI	757^{a}	771^{ab}	807^{bc}	821^{bc}	827^{c}	6.9	0.012	0.016
BW at 28 d	23.7^{b}	24.2^{ab}	25.1^{ab}	25.6^{a}	25.4^{a}	0.32	0.118	0.079
DE (MJ/kg)	14.3^{bc}	14.2^{c}	14.3^{bc}	14.5^{ab}	14.6^{a}	0.09	<0.001	<0.001

^{a,b,c} Means within a row not having the same superscript are significantly different (by LSD, P<0.05); SEM, Standard error of mean; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; BW, bodyweight.

Increasing levels of phytase produced a linear (P=0.05) and quadratic (P=0.001) response in ADG of weaner pigs over both recorded growth periods. Increasing phytase also caused linear (P=0.012) and quadratic (P=0.016) effects on ADFI during the 0 to 28 d period. The best 28-d growth performance response was observed for the diet containing 2,000 FTU/kg feed, producing the best weight gain and FCR (P<0.01). Faecal DE content improved both linearly (P<0.001) and quadratically (P<0.001) up to the highest phytase level of 4000 FTU/kg feed, causing a 0.34 MJ/kg increase over the control diet. In conclusion, the present study confirmed the hypothesis that high levels of phytase, beyond 1000 FTU/kg feed, have significant positive benefits on piglet performance and energy utilisation in the presence of high levels of zinc.

AUGSPURGER, N.R., SPENCER, J.D, WEBEL, D.M. and BARKER, D.H. (2004). *Journal of Animal Science*. **82:**1732-1739.
SELLE P.H. and RAVINDRAN V. (2008). *Livestock Science*. **113:**99–122.

Effects of a microencapsulated blend of organic acids and plant extracts on performance and total-tract apparent digestibility in finishing pigs

H.L. Li, Y. Lei, Y.K. Kim and I.H. Kim

Dankook University, Cheonan, Choongnam 330-714, South Korea.

Previous studies showed that 3,000 mg/kg of a microencapsulated mixture of organic acids and plant extracts improved growth performance of weanling pigs (Grilli *et al.*, 2010). Therefore, this study was conducted to test the following hypothesis: supplementation of a blend of microencapsulated organic acids and plant extracts (MOP) improves finishing pigs' growth. The aim of this study was to investigate the effects of MOP on growth performance and total tract digestibility in finishing pigs.

A total of 75 crossbred pigs [Yorkshire × Landrace × (Duroc)] aged 98 d [body weight (BW) 56.2±3.77 kg, mean±SEM] were randomly distributed across three dietary treatments (five replicate pens with five pigs per pen) according to BW and sex (two gilts and three barrows). The diets were CON, control diet (corn-soybean meal basal diet); MOP1, CON + 0.025% MOP; and MOP2, CON + 0.050% MOP. The product MOP (VetAgro SpA; Aviplus-S®, 42100 Reggio Emilia, Italy) contained citric acid (25%) and sorbic acid (16.7%), thymol (1.7%) and vanillin (1.0%). Individual pig BW was recorded at week 5 and week 10, and feed consumption was recorded on a pen basis during the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain:feed (G:F) ratio. The coefficient of apparent total tract digestibility (CATTD) of dry matter (DM), gross energy (GE) and nitrogen (N) was determined by using chromic oxide (0.2%) as an inert indicator in the diets during d 63-70. Fresh faecal grab samples randomly collected from two pigs (one gilt and one barrow) per pen on d 70 were mixed and pooled, and a representative sample was stored in a freezer at -20°C until analysed. Data were analysed using a randomized complete block design following GLM procedures (SAS®; USA), with each pen used as the experimental unit.

Table 1. *The effects of MOP supplementation on the growth performance and CATTD of finishing pigs.*

Treatment	CON	MOP1	MOP2	SEM	Significance
Initial BW	56.3	56.2	56.0	0.2	0.537
Final BW	110.3^{b}	112.5^{ab}	113.4^{a}	0.8	0.024
0-5 wk					
ADG (g)	726^{b}	757^{a}	771^{a}	7	0.012
ADFI (g)	1896	1911	1937	40	0.251
G/F ratio	0.382	0.396	0.398	0.008	0.094
Overall					
ADG (g)	771^{b}	804^{ab}	820^{a}	12	0.018
ADFI (g)	2111	2138	2176	23	0.110
G/F ratio	0.365	0.376	0.376	0.008	0.114
CATTD					
DM	0.72^{b}	0.75^{ab}	0.78^{a}	0.012	0.024
GE	0.68^{b}	0.72^{ab}	0.76^{a}	0.013	0.008

^{a,b}Means in a row not having the same superscript are significantly different (P<0.05); MOP, microencapsulated blend of organic acids and plants extracts; SEM, standard error of mean.

Pigs fed the MOP2 diet had a higher (P<0.05) BW than those fed the CON diet at the end of the fifth and tenth week of the study. During week 0-5, pigs fed the MOP1 and MOP2 diets exhibited greater (P<0.05) ADG than pigs fed the CON diet. During wk 6-10, there was no difference (P>0.05) among treatments. During week 0-10, ADG in the MOP2 treatment was greater (P<0.05) than in the CON treatment. Pigs fed the MOP2 diet had higher CATTD of DM and GE than those fed the CON diet (P<0.05). A higher CATTD may partly explain the improvement of growth performance. In conclusion, the present results indicate that dietary supplementation with 0.05% MOP improved the growth performance and CATTD of DM and GE in finishing pigs.

GRILLI, E., MESSINA, M.R., TEDESCHI, M. and PIVA, A. (2010). In "Livestock Science", p.173, eds. D. Torrallardona, J. Brufau, E. Esteve-Garcia, R. Lizardo, J. Gasa and J.F. Aguilera. (Elsevier B.V.)

Supported in part by Vetagro S. P. A. Inc.

Manipulating Pig Production XIV

The effect of dam parity on growth of pigs differs between herds

S. Hermesch and L. Li

Animal Genetics and Breeding Unit, a joint venture of NSW Department of Primary Industries and University of New England, University of New England, Armidale, NSW 2351.

Dam parity affects growth of` pigs (Standal, 1973). An Australian study examined the effects of dam parity on piglet performance and immunity in detail (Miller *et al*., 2012). However, information about the effects of dam parity on lifetime growth of progeny is surprisingly sparse. The effects of dam parity on growth of progeny are influenced by on-farm husbandry practices. Knowledge of differences between herds may be used to identify management strategies to alleviate the effects of dam parity on the performance of growing pigs. Further, information about the effects of dam parity on the performance of growing pigs is required to derive the economic value for sow longevity (Ludemann *et al*., 2013). The aim of this study was to quantify the effects of dam parity on growth of pigs for different herds.

Data included 261,761 Large White, Landrace and Duroc pigs recorded from 2000 to 2010 in nine herds of the National Pig Improvement Program in Australia (http://npip.une.edu.au). Herds had between 9,328 and 31,607 records. Average daily gain was measured at an average live weight of 92.8 kg. The model fitted sex, dam parity, breed and contemporary group defined as month of birth within herds as fixed effects using SAS software. Dam parities above the 8th parity were defined as one level in the analyses (results not shown). Analyses were run separately for each herd to obtain least squares means (LSM) of dam parity within each herd, which are illustrated as the difference in LSM from the third parity (Figure 1).

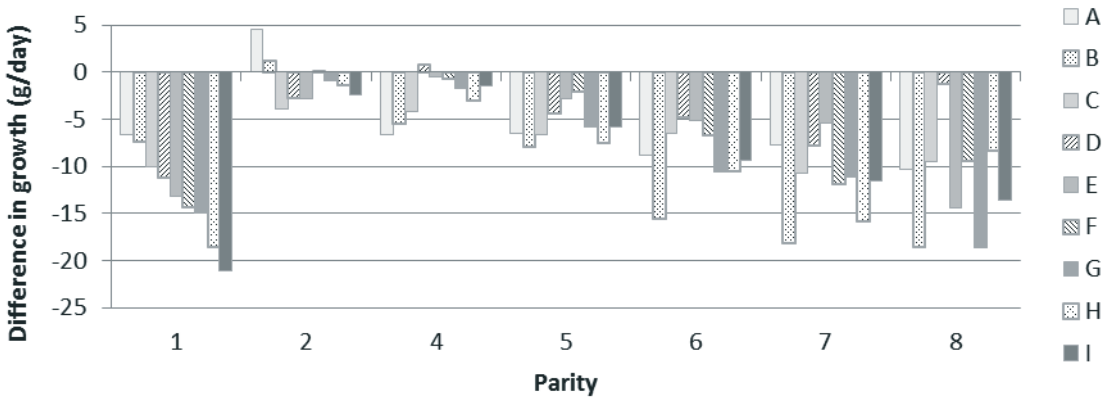


Figure 1. *Deviation of predicted growth of pigs originating from the nth dam parity (n=1,2,4,..8) from predicted growth of pigs originating from the third dam parity for nine herds (A, …, I).*

The predicted growth of pigs averaged across the nine herds was 634, 646, 647, 644, 641, 639, 636 and 635 g/day for the first to eighth dam parity, respectively. In comparison, Standal (1973) reported a 10-g reduction in growth rate of pigs from first-parity dams but no significant variation from an overall mean of 610 g/d for later parities of dams. In the present study, the magnitude of reduced growth in pigs from later-parity dams was similar to that for pigs from first-parity dams. The reduction in growth of pigs from first-parity dams varied between herds from -6.7 to -21.1 g/d. Variation in the effects of dam parity relative to the third parity of dams between herds were smallest for the second and fourth dam parity, possibly indicating that on-farm management fits these dams better. The change in patterns across parities demonstrates that some herds should re-evaluate husbandry practices for first-parity dams (e.g., herd F), older dams (e.g., herd B), or both groups of dams (e.g. herds G, H, I). Further, the implications of these dam-parity effects on growth of progeny for the economic value of sow longevity should be quantified.

LUDEMANN, C.I., AMER, P.R. and HERMESCH, S. (2013). *Proceedings of the Association for the Advancement of Animal Breeding and Genetics*, Napier, New Zealand, submitted.

MILLER, Y.J., COLLINS, A.M., EMERY, D., BEGG, D.J., SMITS, R.J. and HOLYOAKE, P.K. (2013). *Animal Production Science.* **53**:46-51.

STANDAL, N. (1973). *Acta Agriculturae Scandinavia.* **23**:225-231.

Supported in part by the Pork CRC Limited Australia.

An update on near infrared reflectance analysis of cereal grains to estimate digestible energy content for pigs

J.L. Black[1], P.C. Flinn[2], S. Diffey[3] and A.M. Tredrea[4]

[1]John L Black Consulting, Warrimoo, NSW 2774. [2]Kelspec Services, Dunkeld, VIC 3294. [3]University of Wollongong, NSW 2522. [4]The University of Sydney, Narrabri, NSW 2390.

The digestible energy (DE) content of cereal grains fed to pigs can vary by over 3 MJ/kg for wheat, barley and triticale and almost 2 MJ/kg for sorghum (Black *et al*., 2009). A difference of 1 MJ/kg in DE content is estimated to be worth up to $18/t when the price of wheat is $250/t (Black *et al*. 2009). Common methods used by grain buyers to assess the nutritional quality of grains, especially 'test weight' (kg/hl) and screenings percentage, are not related to the DE content of the grains (Black *et al*., 2009). Thus, a hypothesis was established that near infrared reflectance (NIR) technology can be used to develop calibrations to accurately predict the DE value of cereal grains for pigs. An initial calibration was derived from results obtained in the Premium Grains for Livestock Program (PGLP). Subsequently, five experiments have been conducted to validate and upgrade the calibration.

Cereal grains, including wheat, barley, triticale, sorghum, pearl millet, maize and rice, varying widely in chemical and physical characteristics, were collected from germplasm archives, plant breeders and farmers. Growing conditions ranged from irrigation to drought, frost damage and pre-harvest germination. Other grains were produced by desiccating crops at intervals after flowering, with portions being germinated for up to 48 h. Once initial NIR calibrations were established, grains identified by the NIR software as 'spectral outliers' were also selected. The faecal DE content of grains was determined in digestibility experiments with male pigs weighing approximately 35 kg and fed at 2.5 times maintenance (0.5 MJ DE/weight$^{0.75}$) a diet containing 94% grain, 2% acid insoluble ash as an internal marker, minerals and vitamins. Approximately 30% of grains used in each experiment had been fed in previous experiments for connectivity between experiments. Linear mixed model technology which accommodates non-grain sources of variation was used to determine statistically corrected DE values for each grain. Whole grain samples were scanned twice on a scanning NIR monochromator (FOSS model 6500 or XDS, Denmark) and mean spectra obtained. WinISI software (FOSS, Denmark) was used to derive calibrations from the spectra and DE values using modified partial least squares regression following optimum scatter correction and math treatment. After establishment of the first calibration, the accuracy of predicting experimental DE values (validation) was determined and the new results added to upgrade the calibration (Table 1).

Table 1. *NIR calibration statistics for predicting DE content (MJ/kg as fed) of cereal grains for pigs following each experiment.*

Calibration	N[1]	Mean	SD	RSQ	SEC	1-VR	SECV	RPD
PGLP - (1)	91	13.48	0.79	0.84	0.32	0.77	0.38	2.08
(1) + Exp 1 - (2)	121	13.68	0.73	0.85	0.29	0.82	0.31	2.35
(2) + Exp 2 - (3)	170	13.76	0.71	0.89	0.24	0.86	0.27	2.63
(3) + Exp 3 - (4)	219	13.71	0.71	0.90	0.23	0.87	0.26	2.76
(4) + Exp 4 - (5)	258	13.69	0.70	0.89	0.23	0.86	0.26	2.68
(5) + Exp 5 - (6)	288	13.68	0.69	0.89	0.23	086	0.26	2.65

[1] N, observations used in calibration; Mean of observations; SD, standard deviation of observations; RSQ, R^2 for predicted and observed relationship; SEC, standard error of calibrations; 1-VR, 1-Variance Ratio or fraction of variance accounted for when some observations are used for 'cross validation'; SECV, standard error of cross validation, RPD, ratio of Prediction to Deviation = SD/SECV an indication of the value of the calibration, < 1.5 calibration unsatisfactory; 1.5–2.0: calibration can distinguish between high & low values; 2.0-2.5: calibration is quantitative; 2.5-3.0: calibration predictions good; > 3.0: calibration predictions excellent.

Additional grains in the calibration increased the ability to reliably predict DE of unknown samples as shown by an increase in RPD from 2.08 to 2.65 and a decrease in SECV from 0.38 to 0.26 MJ/kg. Further improvement in accuracy of prediction (SECV) will be small because the standard error of the measurements is 0.13 MJ/kg. The DE content of grains for pigs can now be predicted within 0.52 MJ/kg with 95% confidence. The RPD declined when results from Experiments 4 and 5 were included because maize and coloured sorghum were added. These grains had markedly different spectra from those used previously. Adding these with normal grains should further enhance RPD values and the 'robustness' of the calibration and provide feed mill operators and producers with confidence in predicted values.

BLACK, J., NIELSEN, S., TREDREA, A. and FLINN, P. (2009). 14th Australian Barley Technical Symposium, Maroochydore, Queensland.

Supported by Pork CRC Limited Australia.

A survey of particle size and particle size variability of milled grains available for use in Australian pig feeds

G.T. Nguyen[1], W.L. Bryden[1], M.J. Gidley[1], A.C. Edwards[2], S.Willis[3], J.L. Black[4], R.H. Wilson[5] and P.A. Sopade[1]

[1]The University of Queensland, St Lucia, QLD 4072. [2]ACE Livestock Consulting Pty Ltd, Cockatoo Valley, SA 5351. [3]Queensland Department of Agriculture, Fisheries and Forestry, Toowoomba, QLD 4350. [4]John L Black Consulting, Warrimoo, NSW 2774. [5]Rob Wilson Consulting, Perth, WA 6012.

Various grains are used in manufacturing pig feeds to supply starch, proteins and other nutrients. These grains are commonly ground in disc-, hammer- and roller-mills (Sopade *et al*., 2011) for direct use (mash diets) or prior to pelleting (pellet diets). Nutrient digestibility in pigs is dependent, amongst others, on grain particle size (Montoya and Leterme, 2011). Maximising feed efficiency, therefore, requires grains to be milled to their optimum sizes. With special reference to Australia, we are not aware of any studies that have documented the particle size profiles of the major cereals and pulses that are used in pig feeds. The present study investigated the particle size distributions of milled grains to understand the variability, provide recommendations for grain milling and, consequently, quality control in feed mills. It was hypothesised that grains for pig feeds are milled to similar particle size parameters.

About 100 milled grains from 16 feed and research mills in Queensland, South Australia and Western Australia were sieved on-site using a recently-designed manual sieving device described in Sopade *et al*., (2011). These mills used disc-, hammer- and roller-mills. The volumes retained in the seven compartments of the device were recorded and used to calculate the geometric mean diameter (D_{gw}) and geometric standard deviation (S_{gw}) according to ASABE (2008). Data are presented as D_{gw} and S_{gw} without statistical analyses because grain varieties and planting seasons were not controlled.

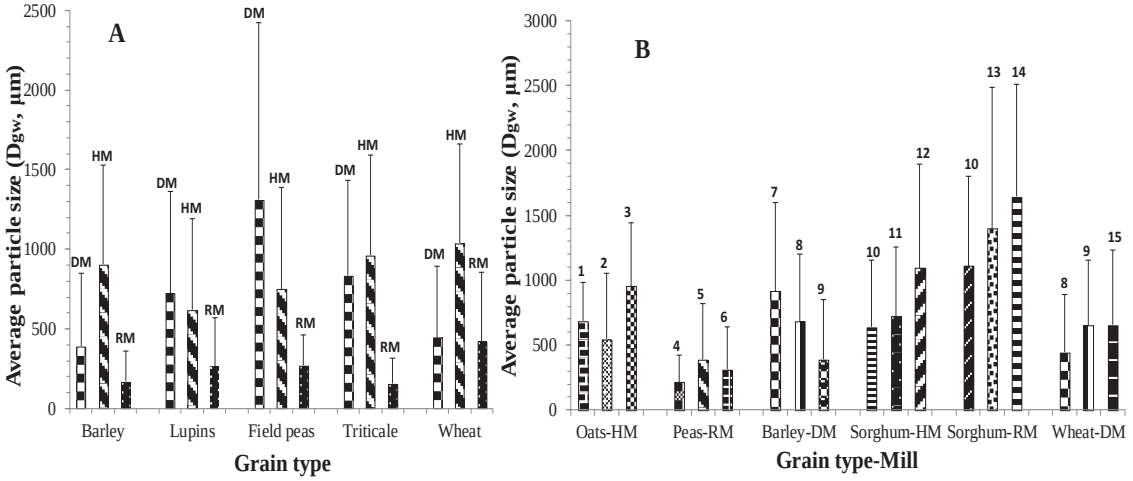


Figure 1. *Average particle size (D_{gw}), and particle size dispersion (S_{gw}) shown as error bars. Similar grains in different mills and mill types (A); Similar grains and mill types in different mills (B); DM, HM and RM = disc-, hammer- and roller-mills respectively; 1-15 are feed mill codes.*

Figure 1 represents the variations in the average particle size (D_{gw}) and particle size dispersion or span (S_{gw}) of the samples. While grain and mill differences accounted for these, the results show that particle sizes of the grains varied widely. However, one particle size is not proposed for all grains because grains differ in composition and morphology, which result in different digestion rates in pigs. Nevertheless, the wide particle size range observed requires an understanding of the optimum size for the major grains, and the need for particle size monitoring in feed mills. Detailed animal studies are required for the former task, while a simple, robust and affordable device is required for the latter.

ASABE (2008). 'Method of Determining and Expressing Fineness of Feed Materials by Sieving'. American Society of Agricultural and Biological Engineers. Niles Road, St. Joseph, MI 49085-9659, USA.

MONTOYA, C.A. and LETERME, P. (2011). *Animal Feed Science and Technology* **169**:113-120.

SOPADE, P., GIDLEY, M. and BLACK, J. (2011). 'Processing Methods for Grains with Special Reference to Particle Size'. Project Report. Cooperative Research Centre for High-Integrity Australian Pork, SA 5118.

Support in part by Pork CRC Limited Australia.

Design and evaluation of a manual sieving device for monitoring particle size in feed manufacture

G.T. Nguyen[1], W.L Bryden[1], M.J. Gidley[1], A.C. Edwards[2], S.Willis[3], J.L. Black[4], R.H. Wilson[5] and P.A. Sopade[1]

[1]The University of Queensland, St Lucia, QLD 4072. [2]ACE Livestock Consulting Pty Ltd, Cockatoo Valley, SA 5351. [3]Queensland Department of Agriculture, Fisheries and Forestry, Toowoomba, QLD 4350. [4]John L Black Consulting, Warrimoo, NSW 2774. [5]Rob Wilson Consulting, Perth, WA 6012.

Milled grains for pig diets have various particle size distributions depending on the grains and mills. Grain particle size influences animal performance (Wondra *et al*., 1995; Montoya and Leterme, 2011), and this demands particle size management to maximise feed efficiency. Particle size analysis can be done with a set of sieves on an electric shaker (ASABE, 2008) or the non-expensive and rapid Bygholm® particle size tester. However, the tester has only three sieves with unknown apertures, and gives empirical particle size parameters. This paper describes a manual sieving device with the simplicity of the Bygholm® tester, but with improved ergonomics and six sieves of known apertures to calculate particle size parameters that have engineering meanings for likely assessment of feed efficiency. The hypothesis was that the new device yields particle size parameters that are not different from those from sieves on an electric shaker.

The manual device (450 x 100 x 50 mm^3) was constructed of transparent polycarbonate, has graduated compartments (1 x 90 mm, 6 x 60 mm) separated by sieves 2.46–0.10 mm, a top slide lock, and a handle (Figure 1A). Milled grains are put into the first compartment with a cover on the first sieve. The cover is removed on filling the first compartment, prior to shaking for 5 min. The volume or weight of retained particles is recorded and entered into a smartphone- or tablet-compatible spreadsheet that is based on the procedure in ASABE (2008) to calculate the geometric mean diameter (D_{gw}) and standard deviation (S_{gw}). A prototype of the device was tested in Queensland, South Australia and Western Australia with up to 100 milled grains. A statistical evaluation of the data was done (Minitab®, Version 16.0; USA).

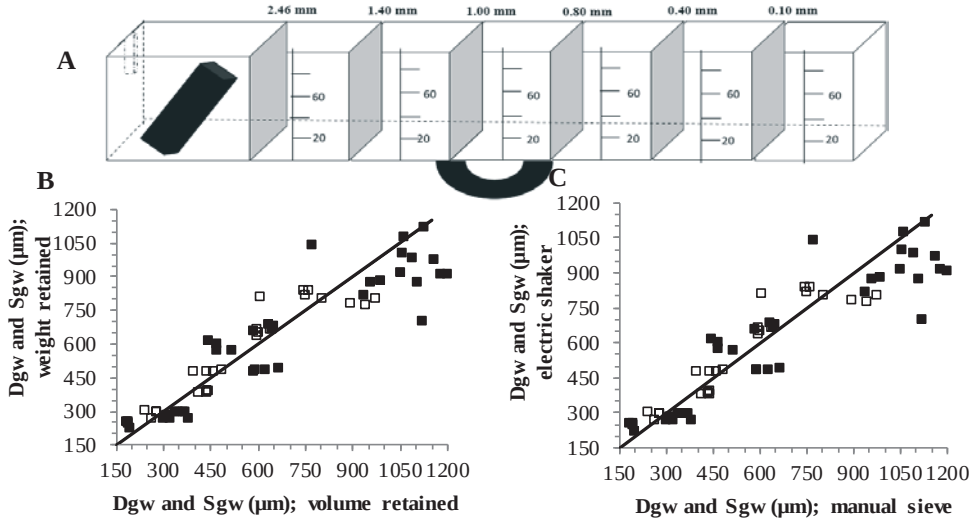


Figure 1. *The manual sieving device. (A), design; (B), relationships between D_{gw} (□) and S_{gw} (■) of milled grains calculated by percentage of volume and weight retained; (C), relationships between D_{gw} and S_{gw} obtained by the manual sieving device and electric shaker with sieves.*

The D_{gw} and S_{gw} from the volume retained were not different (P>0.05) from the values from the weight retained (Figure 1B). The volume retained is quicker to compute, and it is recommended for the device. The D_{gw} and S_{gw} from the device were also not different (P>0.05) from the values from a set of nine sieves (4.0-0.075 mm) on an electric shaker (Figure 1C). From the results, the device is valuable in monitoring particle size in feed mills. The volume marks on the device need to be improved, and the device made robust to withstand the conditions in feed mills and for prolonged use.

ASABE (2008). 'Method of Determining and Expressing Fineness of Feed Materials by Sieving'. American Society of Agricultural and Biological Engineers. Niles Road, St. Joseph, MI 49085-9659, USA.

MONTOYA, C.A. and LETERME, P. (2011). *Animal Feed Science and Technology* **169**:113-120.

WONDRA, K.J., HANCOCK, J.D., BEHNKE, K.C. and STARK, C.R. (1995). *Journal of Animal Science* **73**:2564-2573.

Support in part by Pork CRC Limited Australia.

CHAPTER 4

The Gastrointestinal Tract and Applied Nutrition

SYMPOSIUM: Barrier function and systemic response of the gastrointestinal tract to the aspects of management and nutrition: Introduction

J.R. Pluske[1] and J.C. Kim[2]

[1]Murdoch University, Murdoch, WA 6150. [2]Department of Agriculture and Food Western Australia, South Perth, WA 6151.

The epithelium that lines the gastrointestinal tract (GIT), in conjunction with the mucosa-associated lymphoid tissue, represents the first line of defence against potentially pathogenic microorganisms and antigens present in the intestinal lumen, and is a crucial site of regulation for innate and adaptive immune functions. In states of health, the epithelium forms a semi-permeable barrier that limits the translocation of bacteria, toxins and (or) antigens into the body, thereby minimising potentially chronic inflammatory responses and systemic disease. Sub-epithelial components such as the enteric nervous system and immune cells are intimately involved in the regulation of secretion and absorption under normal and pathophysiologic processes in the GIT, and thus also constitute a critical component of barrier function. Ultimately, the epithelium is also responsible for the efficient digestion and absorption of nutrients and fluid for maintenance and lean tissue growth.

Barrier function in the GIT is adaptable and is regulated in response to a range of internal and external stimuli such as nutrients, cytokines, stressors and (or) pathogenic microbes. The integrity of barrier function is, therefore, an important component of optimal GIT structure and function. However damage to the epithelial barrier, and hence the balance between activation of inflammatory cascades and immunoregulatory responses, can occur if there are exaggerated responses to pro-inflammatory cytokines, such as what can arise for example in the post-weaning period.

The first paper in this symposium, by Dr. Jae Kim and Dr. Bruce Mullan from the Department of Agriculture and Food WA and Professor John Pluske, from Murdoch University, summarises the literature in pigs where changes in barrier function have occurred and its subsequent effects on GIT structure and function. Their paper then examines the influence of a number of different nutritional strategies to minimise impacts of chronic subclinical infection and stressors that impact barrier function, and therefore improve the efficiency of pig production.

The second paper, by Dr Adam Moeser from North Carolina State University, focuses on a description of intestinal barrier function in the GIT with emphasis on the key structural and physiologic components of the mucosal barrier. The paper then discusses how the function of the GIT is impacted by management factors such as weaning and the age at which piglets are weaned; the impacts of post-weaning colibacillosis are also discussed.

SYMPOSIUM: Impact of the systemic response to stressors and subclinical and clinical infection on intestinal barrier function and growth in pigs

J.C. Kim[1], B.P. Mullan[1] and J.R. Pluske[2]

[1]Department of Agriculture and Food Western Australia, South Perth, WA 6151. [2]Murdoch University, Murdoch, WA 6150.

Abstract

Chronic exposure to clinical/subclinical infection and stressors compromises intestinal barrier function and activates a systemic response which reduces the rate of protein deposition and growth efficiency. The systemic response influences performance of pigs in two ways: alteration of nutrient partitioning and eicosanoid mediator-induced neurological responses to infection such as anorexia. The roles of nutrition to tackle both routs of the systemic response are reviewed. Evidence is provided that the systemic responses alter nutrient partitioning and increase requirements for tryptophan and sulphur amino acids. Nutrients that reduce either cyclooxygenase and 5-lipoxygenase activity or cyclooxygenase and lipoxygenase gene expressions, and hence minimise production of eicosanoid mediators such as prostaglandin E_2 and leukotriene $B_{4,}$ includes omega-3 fatty acids, boron, probiotics and antioxidants. The purpose of this review is to provide an overview of several nutritional strategies to minimise impacts of chronic subclinical infection and stressors on the efficiency of pork production.

Introduction

Physiological and immunological responses to subclinical/clinical infection and nutritional and psychological stressors significantly alter intestinal barrier functions (Kim *et al.*, 2012a) and the way pigs partition nutrients (Kim *et al.*, 2012b). Pigs in a commercial production system are continuously exposed to various pathogens and viruses that activate the immune system at subclinical or clinical levels. Growth efficiency of pigs is significantly compromised at even subclinical level activation of the immune system due to altered nutrient partitioning, compromised intestinal barrier functions and malnutrition caused by anorexia. The systemic response of pigs to immune system activation orchestrated by mast cells drains nutrients for production of immune molecules, such as cytokines, acute phase proteins and immunoglobulins, away from deposition as lean and lipid. Furthermore, stimulation of the eicosanoid pathways by immune system activation subsequently increases production of arachidonic acid derivatives such as prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), which are known to provoke anorexia and fever through stimulation of the neuroendocrine system (Rivest, 2010) and control the severity and duration of the immune response, respectively (Devchand *et al.*, 1996).

Understanding the systemic responses to stressors and subclinical infection on intestinal barrier function and growth, and subsequent nutritional strategies to suppress their impact on nutrition and health of pigs, is one of the least explored areas in pig nutrition yet they can have significant influences on health, welfare and growth efficiency of pigs. Therefore, this review will focus on the systemic response to stressors and subclinical infection on intestinal barrier function and growth, and will present possible nutritional strategies that can suppress or minimise the impact of the systemic response to stressors and subclinical infection.

Acute and systemic immune responses to subclinical and clinical intestinal infection

Development of mucosal immunity and immunophysiology of the gut-associated lymphoid tissue (GALT) was recently reviewed by Emery and Collins (2011). Briefly, pathogens and antigens are entering the system either through microfold (M) cells in the Peyer's patch or transcellular and paracellular pathways in the intestinal epithelium. The antigens and pathogens that enter through M cells are then transferred to dendritic cells (DC) where toll-like receptors (TLRs) are expressed. Induction of inflammation then stimulates maturation of DC and antigen/pathogen information is presented to T cells in the Peyer's patch. Alternatively, antigens or antigen loaded DC enter into mesenteric lymph nodes where subsequent T-cell recognition occurs. Antigens translocated through epithelial paracellular or transcellular pathways are disseminated either to the mesenteric lymph nodes via antigen presenting cells or the peripheral lymph nodes and interact with T cells (Mowat, 2003).

In the acute phase of pathogen invasion, locally existing sentinel cells like macrophages and mast cells are immediately activated and release pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and

tumor necropsy factor (TNF)-α and increase neutrophils, monocytes and lymphocytes to the site of inflammation (Goddeeris *et al*., 2002). One of the most important acute phase responses after inflammation is increased recruitment of phagocytes and subsequent specific immune response against the pathogens and antigens (Goddeeris *et al*., 2002). Phagocytes such as neutrophils, macrophages and mast cells release proteases and reactive oxygen species in the process of phagocytosis of antigens which causes irreversible tissue damage to the host (Wyman and Schneiter, 2008). Unlike lymphocytes where T-cell and B-cell receptors recognise antigens, neutrophil-driven phagocytosis and stimulation of mast cells are initiated by immunoglobulin (Ig) receptors (Wyman and Schneiter, 2008). Immunoglobulin receptors (IgG receptors in neutrophils and macrophages and IgE receptors in mast cells) then stimulate intracellular protein kinases which increase intracellular Ca^{2+} concentration. Increased intracellular Ca^{2+} concentration stimulates degranulation and production of eicosanoid mediators via activation of phospholipase 2 (Irvine, 2003; Wyman and Schneiter, 2008).

Among the many types of leukocytes, mast cells have a central role in acute- and systemic-immune response and have been studied extensively (Abraham and St John, 2010). Mast cells can directly recognise pathogens through recognition of common patterns of pathogens through pattern recognition receptors such as TLRs or binding of specific-pathogen associated antibodies (Supajatura *et al*., 2001; Abraham and St John, 2010). Upon recognition, mast cells have the ability to respond to the invasion in three different mechanisms. First, mast cells contain heterogeneous tryptases and chymases, which are degranulated when pathogens/antigens are recognised. For example, *in vitro* studies showed that co-incubation with commensal bacteria such as non-pathogenic *E. coli*, *Bifidobacteria* and *Lactobacillus* did not induce mast cell degranulation, while co-incubation with pathogenic *E. coli* induced mast cell degranulation through interaction between the *E. coli* adhesion protein and CD48 on mast cells (reviewed in Wesolowski and Paumet, 2011). Mast cells have the ability to modify their phenotype during the course of infection and can replenish their granules after degranulation to memorise the antigens/pathogens to control re-infection (Abraham and St John, 2010). Second, mast cells have the ability to serve as a phagocyte along with macrophages, dendritic cells and neutrophils. Mast cells internalise many types of pathogens through IgG receptors via direct interaction with bacterial adhesion, and the cytosolic lysosomes use reactive oxygen species and acidification process for phagocytosis (Wesolowski and Paumet, 2011). Thirdly, upon pathogen recognition, mast cells secret many cellular mediators including proinflammatory cytokines and regulate innate and acquired immune functions. Mast cell-derived proinflammatory cytokines such as histamine, IL-6 and TNF-α are secreted shortly after bacterial infection and are known to recruit neutrophils and DC to the infection sites and lymph nodes during *E. coli* infection (Weisolowski and Paumet, 2012). Mast-cell derived IL-4 and eicosanoids such as LTB_4 enhance recruitment of macrophages for intracellular phagocytosis and increase vascular permeability of the endothelial cells and oedema at the site of infection. Mast cells are also able to communicate with epithelial cells, T-cells and B-cells and increase mucus production, T cell chemotaxis and lymphocyte retention in draining lymph nodes to improve antibody production, respectively (Abraham and St John, 2010). Initiation of adaptive immunity is caused by mast-cell derived TNF-α which increases monocytes-derived antigen presenting DC in the draining lymph nodes and stimulate antibody production through T- and B-cells. Mast cells are then sensitised with the produced pathogen-specific antibodies (pathogen specific IgG or IgE) through Fc receptors, which are used for adaptive immune function (Abraham and St John, 2010).

One of the most important effects of mast cell stimulation is increased eicosanoid production, such as PGE_2 and LTB_4, that induce infection-associated anorexia (Wymann and Schneiter, 2008) and control the duration of inflammation, respectively (Devchand *et al*., 1996). Production of eicosanoid mediators in the cell and nuclear membranes is initiated through the action of phospholipase A_2 or C which respectively convert membrane-bound phospholipids and diacylglycerols to arachidonic acids. Arachidonic acids are then converted to either PGE_2 or LTB_4 by cyclooxygenase and lipoxygenase, respectively (Folco and Murphy, 2006; Wymann and Schneiter, 2008; Kalinski 2012). In human medicine, steroids and non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin are used to directly inhibit phospholipase and cyclooxygenase activities (Wymann and Schneiter, 2008). Another route that can modulate the impact of inflammation is by facilitating LTB_4 degradation since its clearance in the system is directly associated with the severity and duration of the inflammatory response (Devchand *et al*., 1996). In this context Devchand *et al*. (1996) used a mouse ear swelling test where LTB_4 was topically applied to the ear, and then measured ear thickness as an index of inflammatory response. Unlike normal mice, peroxisome proliferator-activated receptors-α (PPARα)–knockout mice significantly increased the severity and duration of the inflammatory response. The PPARα is a nuclear transcription factor that facilitates systemic catabolism of LTB_4 (Narala *et al*., 2010). Therefore, the PPARα knockout mice which lack the ability to catabolise LTB_4 extended the severity and duration of inflammation. Catabolism of LTB_4 is facilitated by PPARα that activates microsomal ω- and peroxisomal β-oxidation pathways and primarily

occurs in the immune cells in the inflammation site and also in the hepatocytes (Devchand *et al*., 1996; Yokomizo *et al*., 2001).

Acute and systemic immune responses to stressors

Pigs in commercial production systems encounter numerous socio-physiological and environmental stressors that trigger acute and systemic immune responses. Stress stimulates the hypothalamus-pituitary-adrenal axis and releases a 41-amino acid peptide, corticotrophin-releasing factor (CRF), from the paraventricular nucleus of the hypothalamus (Moeser *et al*., 2007; Salak-Johnson and McGlone, 2007; Teitelbaum *et al*., 2008). The CRF then binds to two G-protein-coupled receptors, CFR-r1 and CRF-r2, in the peripheral tissues and stimulates acute and systemic immune responses (Moeser *et al*., 2007; Teitelbaum *et al*., 2008). For example, the stress of weaning (Moeser *et al*., 2007), especially if done before 21 d of age (Smith *et al*., 2010), significantly increased jejunal and colonic expression of CRF and CRF receptors. Increased release of CRF also stimulates mast cells and Smith *et al*. (2010) demonstrated that pigs weaned at 18 d increased their CRF contents, mast cell numbers and mast cell tryptases' expression in the jejunum compared with pigs weaned at 23 d. Moreover, in an Ussing Chamber model, it was demonstrated that exposure to CRF increases TNF-α and tryptases' activity and the proportion of the degranulated mast cell in the ileum of 6-8-week old pigs (Overman *et al*., 2012). In a pig study, a four-hour transportation significantly increased natural killer (NK) cell cytotoxicity in pigs with intermediate and submissive social status compared with socially dominant pigs, and a significant positive correlation between NK cell cytotoxicity and plasma cortisol level was reported (McGlone *et al*., 1993). This particular study indicates that susceptibility to social stressors is mainly dependent on the social status of pigs, and pigs in low hierarchy are prone to the CRF-induced immune response.

Impact of acute and systemic immune response on intestinal barrier function

Subclinical or clinical inflammation and socio-physical and environmental stressors such as weaning, transportation, mixing, isolation, heat and cold stress stimulates mast cells either directly or through release of CRF and negatively affects epithelial barrier function (Berkes *et al*., 2003; Overmen *et al*., 2012).

Enteric pathogens and antigens in the intestinal lumen enter through transcellular or paracellular pathways, and the presence of enteric pathogens and antigens in the intestinal lumen significantly influence intestinal permeability. Although mechanisms behind the inflammation-induced decrease in transcellular permeability is not well understood, mechanisms for inflammation- and stress-induced reductions in paracellular permeability are well studied and are primarily regulated by the tight junction between enterocytes. The major tight junction proteins are transmembrane protein complexes occludins and claudins, and cytosolic proteins zonula occludins (ZO). At the apical-lateral membrane junction of the enterocytes, claudin-1 and occludin binds the two lateral membranes of the adjacent enterocytes and ZO-1, ZO-2 and ZO-3 connect the claudin-1 and occludin to cytoskeletal actins (Groschwitz and Hogan, 2009). It is known that enteric pathogens and their toxins increase paracellular permeability by altering the tight junction structure via actomyosine ring alteration or tight junction protein redistribution (Berkes *et al*., 2003). *Clostridium difficile*, enterotoxigenic *Escherichia coli* (ETEC) and *Bacteroides fragilis* alter actomysoin rings and increase paracellular permeability through depolymerising actin, myosin light chain phosphorylation and proteolysis of tight junction proteins, respectively. On the other hand *Clostridium difficile*, ETEC, *Vibrio cholera* and *Clostridum perfringens* redistribute tight junction proteins through mobilisation of occludin and ZO-1 away from the tight junction, occludin degradation by haemagglutinin protease, and redistribution of claudin (Berkes *et al*., 2003). Stress-induced release of CRF is also known to increase transcellular permeability (Teitelbaum *et al*., 2008) and paracellular permeability by redistribution of occludin (Overman *et al*., 2012). Especially in a weaner pig model, it was demonstrated that CRF increases paracellular permeability by stimulating release of TNF-α and protease from mast cells (Overman *et al*., 2012).

Intestinal barrier function is technically quantified by measuring the resistance to transcellular permeability (Transepithelial electrical resistance, TER; short circuit ion transport I_{SC}) and the permeability to a macromolecule such as horseradish peroxidase, 4-KDa FITC-Dextran, 180 kDa [^{3}H]mannitol, 5000 kDa [^{14}C]inulin or lactulose, that can only be translocated through paracellular pathway. In a mouse study, Roxas *et al*. (2010) demonstrated that ETEC infection decreased TER by 60% and increased 4-KDa FITC-Dextran flux four-fold in the colonic tissues at 8 d after infection. Increased CRF release observed in early weaned pigs (15 d versus 28 d) decreased TER and increased mucosal to serosal ^{3}H mannitol flux by 20% in the jejunal epithelium when measured at nine weeks of age (Smith *et al*., 2010). Pearce *et al*. (2012) demonstrated in a grower pig study (46 kg live weight) that acute exposure to heat stress (24 h at 35 °C, 24-43% humidity) increased paracellular permeability by two-fold and six-fold in the ileum and colon, respectively, compared with pigs in the thermo-neutral condition (21 °C, 35-

50% humidity). The study also showed that transcellular permeability was increased by 2-fold and 30% in the ileum and colon, respectively, in the pigs acutely exposed to the heat stress. Therefore, it is clearly demonstrated that either infection or stress-induced release of CRF significantly compromises intestinal barrier function in pigs.

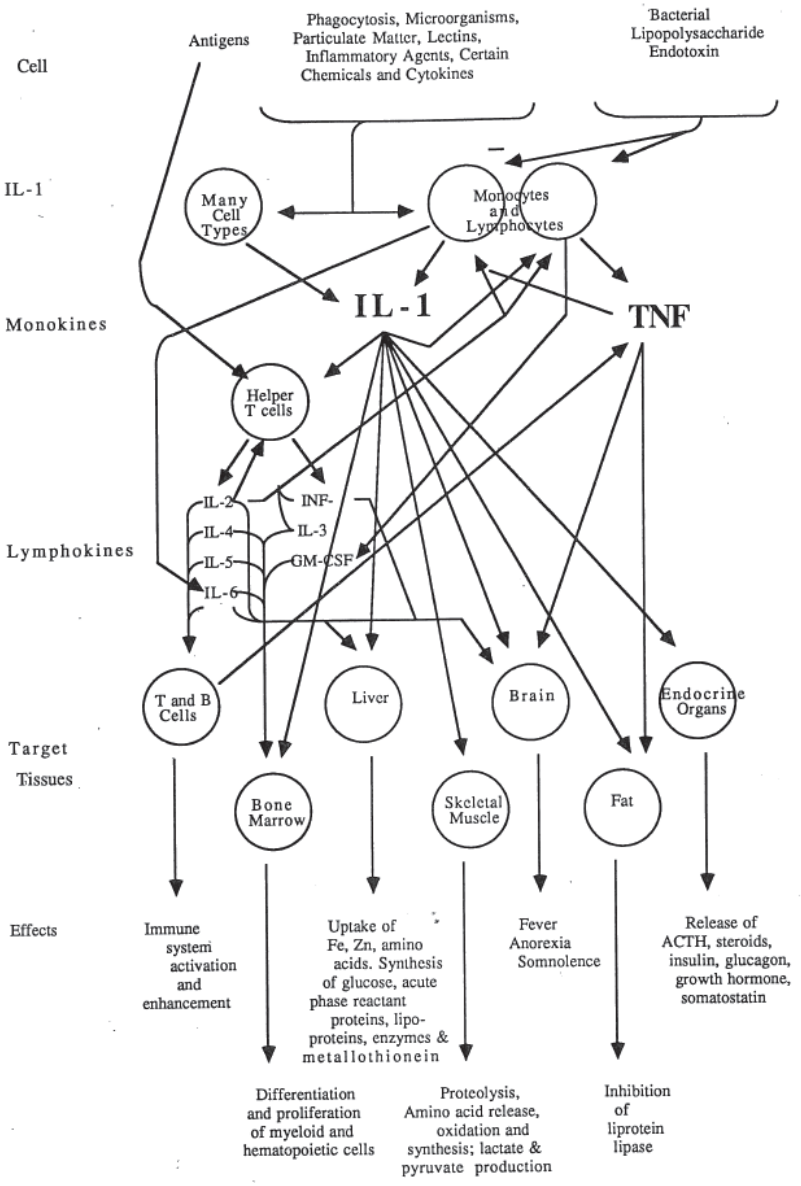


Figure 1. *Acute and systemic responses to infection (from Beisel, 1991).*

Impact of acute and systemic immune response on growth and protein deposition

Pigs kept under commercial conditions are continuously exposed to microorganisms, and typically respond to these immune system challenges by elevated release of acute-phase proteins, cytokines, increased metabolic use of protein (i.e., synthesis of immune molecules), reduced feed intake and hence decreased protein deposition (Kim *et al*., 2012b). Cytokines involved in the acute and systemic response to infection and their effects on nutrient partitioning and mobilisation are illustrated in Figure 1 (Beisel, 1991). Release of pro-inflammatory cytokines from monocytes, macrophages and mast cells stimulate the hepatic uptake of amino acids and increase production of acute phase proteins (APP) in the liver. Moreover, physical, psychological and environmental stresses such as weaning, isolation, transportation and a disorderly feeding pattern are known to increase APP production through stimulation of the sympatho-adrenal and hypothalamic-pituitary-adrenal axis (Pineiro *et al*., 2007; Soler *et al*., 2013). Muscle proteins are catabolised and lipolysis is increased in severe and chronic cases of inflammation or stress where circulating and hepatic amino acid and energy reserves are not meeting the increased requirements from the hepatocytes and immune cells (Johnson, 1997; Rakhshandeh and de Lange, 2011, Kim *et al*., 2012b). Increased muscle protein catabolism and lipolysis then eventually reduces protein and fat deposition

(Rakhshandeh and de Lange, 2011). Apart from the increased metabolic requirements, stimulation of the central nervous system is known to reduce feed intake which exacerbates malnutrition of the immune system-activated pigs (Rakhshandeh and de Lange, 2011; Pastorelli *et al*., 2012a).

The consequences of subclinical levels of immune response on performance of pigs was examined recently, where weaner pigs were housed either in a disinfected room or in an uncleaned room that contained pathogens from the previous batch of pigs. In a 42-d feeding experiment, average daily gain of the pigs exposed to an unclean environment was reduced by 11-12% due to reduced feed intake (5%) and feed efficiency (7%, Pastorelli *et al*., 2012b; 2012c). This finding suggests that even a mild subclinical activation of the immune response has significant impacts on growth of pigs, and that both altered nutrient partitioning (feed efficiency) and the eicosanoid mediators-induced loss of appetite have contributed to the reduced daily gain. Another weaner pig study experimentally created a spectrum of ileitis by inoculating varying dose of a mucosal homogenate containing *Lawsonia intracellularis* (LI) (10^8 – 10^4 LI per pig; Paradis *et al*., 2012). Pigs inoculated with 10^8 and 10^7 LI showed clinical signs of ileitis while pigs inoculated with 10^5 and 10^4 LI did not show clinical signs of ileitis. The study found a dose-response reduction in daily gain, feed intake and feed efficiency as dose rate of LI was increased. Interestingly, this study showed that even subclinical infection with LI (pigs inoculated with 10^5 and 10^4 LI) decreased daily gain by 37%, feed intake by 21% and feed efficiency by 21%, and pigs were 3.5 kg lighter than control pigs at 21 d after inoculation.

Consequences of clinical levels of the immune response on performance of pigs have been analysed (meta-analysis) in 122 publications, which examined the effects of pathogen and disease/sanitary environment challenges on feed intake and growth response (Pastorelli *et al*., 2012a). Digestive bacterial infections, poor housing conditions, mycotoxicoses, parasitic infections and respiratory disease challenges reduced average growth rate by 40%, 16%, 30%, 8% and 25%, respectively. Interestingly, it was concluded that the major mechanism responsible for the reduction in growth rate was different dependent on the type of disease challenge. For example, in the case of digestive bacterial infection, parasite infection and poor housing conditions, the changes in maintenance requirement explained approximately 70-75% of growth reduction while feed intake explained 25-30% of the growth reduction. In contrast, feed intake explained 70% of the growth reduction in pigs challenged with mycotoxicosis and respiratory disease, while the changes in maintenance requirement explained 30% of the growth reduction (Pastorelli *et al*., 2012a). Therefore, it seems that a disease that affects intestinal structure/function such as pathogens/toxins/parasites-driven intestinal damage reduces digestion/absorption of nutrients, and hence a greater proportion of body weight loss might be caused by the compromised digestion/absorption capacity and the metabolic needs for tissue repairs, while a lower proportion of body weight loss was caused by compromised feed intake (Klasing *et al*., 1991; Sandberg *et al*., 2007; Pastorelli *et al*., 2012a). In addition, increased endogenous protein loss through intestinal secretion such as mucus, loss of water in case of diarrhoea and increased nutrient requirements for immune response (Sandberg *et al*., 2007) also increases metabolic costs and represent reduced growth independently to reduced feed intake (Pastrorelli *et al*., 2012a). The linear-plateau and curvilinear-plateau equations developed in the meta-analysis indicate that unlike respiratory disease where pigs' feed intake and growth rate fully recovered back to the rates observed in the control pigs, growth rate of the pigs challenged with a digestive tract bacterial infection was not recovered (10% lower) back to the growth rate in the control pigs at 40 d after the infection, despite feed intake being fully recovered at around 35 d (Pastrorelli *et al*., 2012a). This finding suggests that after 40 d of digestive bacterial infection, feed intake-independent growth reduction was observed and represents a lasting metabolic cost for maintenance of the immune system.

Chronic immune system activation significantly compromises body protein deposition as demonstrated in early studies by Williams *et al*. (1997a, b, c). Pigs acquired from a herd possessing antibody titers for pathogens reduced their body protein deposition by 26-28% (Williams *et al*., 1997a, c) and whole body nitrogen retention rate by 20% (Williams *et al*., 1997b) compared with pigs possessing low antibody titers. In rat studies, muscle protein synthesis was reduced by 22% while protein syntheses in the liver and spleen were increased by 2-fold and 3-fold, respectively, in rats infected with an *E. coli* compared with healthy rats (Breuille *et al*., 1994; 1998). In an experimentally-induced chronic immune system activation model, where finisher pigs (52-91 kg) were repeatedly injected with *E. coli* lipopolysaccharides (LPS), the rate of whole body protein deposition was 12% lower and the plasma urea content was 12% higher in immune system-activated pigs at the currently recommended levels of sulphur amino acids (SAA) than healthy pigs injected with physiological saline (see Figure 2; Kim *et al*., 2012b).

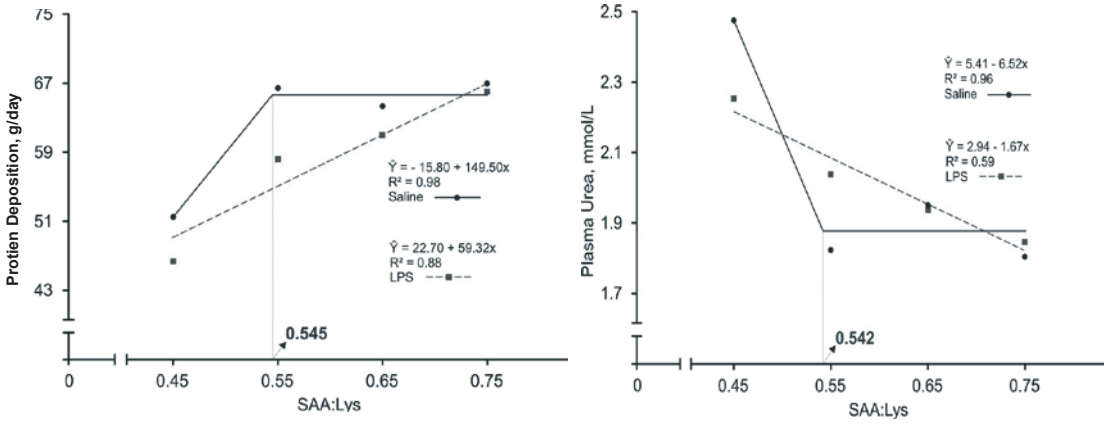


Figure 2. *Whole body protein deposition and plasma urea content in response to increasing standardised ileal digestible SAA:Lys ratios in either saline (—●—) or E. coli lipopolysaccharide (---■---) injected finisher pigs (Kim* et al.*, 2012b).*

Aspects of nutrition to reduce impacts of a systemic immune response

Subclinical and clinical levels of immune system activation decrease daily gain and feed intake through two major mechanisms: altered nutrient partitioning and decreased appetite. Therefore, searching for the responsible nutrients that either increase the requirements at immune system activation or have the ability to minimise production of immunosuppressive eicosanoid mediators may provide useful nutritional strategies to reduce the impact of immune system activation on the growth and health status of pigs.

Amino acids

Immune system activation decreases feed efficiency by altering nutrient partitioning. Release of pro- and anti-inflammatory cytokines stimulates the liver and increases hepatic uptake of amino acids to produce immune molecules (Breuille *et al*., 1994; 1998). Significant amino acid catabolism in the skeletal muscle is evident in immune system-activated pigs and rats and depends on severity and duration of the inflammation (Breuille *et al*., 1994; 1998; Williams *et al*., 1997a, b). A comprehensive review on roles of individual amino acids for whole body protein deposition in immune-system-activated pigs was presented in a previous meeting (Rakhshandeh and de Lange, 2011). Briefly, despite increased metabolic demand for glutamine, arginine, phenylalanine, tyrosine and branched-chain amino acids during mild immune system activation, their requirements do not increase due to sufficient *de novo* synthesis or sufficient amounts present in current diets. Although only limited information is available, it is believed that threonine requirements may be increased in cases where mucus production or threonine-rich immunoglobulin production is stimulated during immune system activation since threonine is the major amino acid in mucus and some immunoglobulins (Faure *et al*., 2007; Rakhshandeh and de Lange, 2011).

Tryptophan (Trp) is an important amino acid that is limiting the growth of pigs during states of immune system activation because it is a precursor for kynurenine, serotonin and melatonin that significantly increase during immune system activation (Rakhshandeh and de Lange, 2011). It is reported that the Trp requirement for protein deposition is increased when the immune system is activated while the Trp requirement for maintenance was not changed (de Ridder *et al*., 2012). Using intramuscular injection of an *E. coli* lipopolysaccharide (LPS), it was demonstrated that Trp requirement increased by 7% in immune system-activated growing pigs (de Ridder *et al*., 2012). In weaner pigs orally infected with an enterotoxigeninc strain of *E. coli*, increased Trp:lysine ratios from 0.18 to 0.22 (Trevisi *et al*., 2009a, b) and 0.26 (Capozzalo *et al*., 2012) improved daily gain and feed conversion efficiency, respectively.

The sulphur amino acids' (SAA), especially cysteine, requirement is known to increase in immune system-activated pigs. Immune system activation increased cysteine synthesis in humans (Yu *et al*., 1993) and rats (Malmezat *et al*., 2000), and upregulated hepatic gene expression for cystathionine β-synthase and cystathionine γ-lyase, the enzymes involved in cysteine biosynthesis in pigs (Rakhshandeh *et al*., 2010b). Cysteine is mainly used for production of glutathione and taurine that prevent tissue damage from reactive oxygen and nitrogen species generated from phagocytes and leukocytes that were recruited to the infection sites (Babior, 2000; Splettstoesser and Schuff-werner, 2002; Wyman and Schneiter, 2008; Rakhshandeh and de Lange, 2011). Furthermore, SAA are extensively used for acute phase protein synthesis and are used as a structural molecule for immune molecules such as immunoglobulins (Rakhshandeh and de Lange, 2011). Increased SAA requirement in immune system-activated pigs was well demonstrated in a growing pig study where immune system-activated pigs decreased urinary sulphur excretion while urinary nitrogen excretion was increased (Rakhshandeh *et al*., 2010a). This particular finding indicates that SAA are

limiting efficient utilisation of the other essential amino acids when the pigs' immune system is activated. Recently a grower finisher pig study was conducted with an immune system activation model where two groups of pigs were repeatedly injected either with an *E. coli* LPS or saline to mimic the level of immune system activation experienced in commercial herds, where chronic challenges from pathogens and viruses exist. The study was designed to determine SAA requirement for maximum protein deposition in either healthy or immune-system-activated pigs (See Figure 2, Kim *et al*., 2012b). This study demonstrated that unlike healthy pigs, the rate of whole body protein deposition in immune system activated pigs was decreased by 12% at currently recommended SAA levels (NRC, 2012) and maximum protein deposition was only reached when SAA were supplied at 20% higher levels than currently recommended by NRC (SAA:Lys ratio of 0.75; Kim *et al*., 2012b). These recent studies clearly showed that a higher SAA is required to attenuate the performance loss induced by immune system activation. Similarly, in a LPS-injected weaner pig model, dietary supplementation of 500 mg/kg N-acetylcysteine, a precursor of L-cysteine, attenuated LPS-induced intestinal damage and tight junction protein gene expression (Hou *et al*., 2012), production of TNF-α, IL-6 and $PGE_{2,}$ and TLR4 mRNA expression in the jejunal and ileal mucosae (Hou *et al*., 2012). These immunomodulative effects of SAA and N-acetylcysteine are most likely through the modulation of reactive oxygen and nitrogen species produced during inflammation and systemic response (Metayer *et al*., 2008; Hou *et al*., 2013).

Dietary n-3 and n-9 polyunsaturated fatty acids

Metabolism of omega-3 (n-3) fatty acids and impacts of their metabolites on health and systemic response was reviewed in depth by several authors (James *et al*., 2000; Palmquist, 2009; Lenihan-Geels *et al*., 2013). The anti-inflammatory effects of n-3 fatty acids are believed to be mediated by increased production of anti-inflammatory eicosanoids in relation to production of n-6 fatty acid-derived pro-inflammatory eicosanoids in the event of a systemic immune response. Presence of n-3 fatty acids in membrane-bound phospholipids compete for the enzymes that are involved in conversion of n-6 fatty acids to pro-inflammatory eicosanoid mediators, such as series 2 prostaglandins and series 4 leukotrienes, and therefore have the ability to reduce production of pro-inflammatory eicosanoids (Wall *et al*., 2010) (see Figure 3). Such competition and increased production of anti-inflammatory eicosanoids, such as series-3 prostaglandins and series-5 leukotrienes from the membrane-bound n-3 fatty acids in the event of a systemic response, is known to significantly reduce development, severity and duration of the immune response (James *et al*., 2000; Goddeeris *et al*., 2002; Palmquist, 2009; Wall *et al*., 2010; Lenihan-Geels *et al*., 2013). Membrane-bound n-3 fatty acids also act as substrates for cyclooxygenase and lipoxygenase enzymes that facilitate production of PGE_2 and LTB_4 from metabolism of arachidonic acid (Prescott *et al*., 2007; Wall *et al*., 2010). The n-3 fatty acid-driven competitive reduction in the quantity of pro-inflammatory eicosanoids eventually reduce expression of pro-inflammatory cytokines such as TNF-α and IL-1β (Caughey *et al*., 1996; Chytilova *et al*., 2013), which are mediated by down-regulation of transcription factors such as nuclear factor-kappaB (NF-*k*B) and PPARs via inhibition of reactive oxygen species (Anderle *et al*., 2004; Calder, 2006; Liu *et al*., 2012; van den Elsen *et al*., 2013).

There is evidence that supplementation of dietary n-9 fatty acid (oleic acid) also down-regulates production of pro-inflammatory eicosanoids in rats and humans (Stenson *et al*., 1984; Cleland *et al*., 1994; James *et al*., 2000). Eicosatrienoic acid acts as a substrate for 5-lipoxygenase and produces LTA_3 which is a strong inhibitor for the enzyme LTA_4 hydrolase, that is needed for LTB_4 production (Evans *et al*., 1985; Jakschik *et al*., 1983; James *et al*., 2000). The n-6:n-3 fatty acid ratio of 4:1 was suggested as a guideline for optimal immune function in human nutrition (Simopoulos, 2004), while pig diets typically contain a ratio greater than 10:1. Wilkinson *et al*. (2011) demonstrated that weaner pigs fed an n-6 fatty acid-rich diet were 7 kg lighter at 4 weeks after weaning compared with weaner pigs fed an n-3 fatty acid-rich diet. In a subsequent experiment conducted in a commercial farm, pigs fed an n-6-rich diet were 3 kg lighter and had significantly increased mortality (33% vs. 0.4%) compared with pigs fed an n-3-rich diet at 4 weeks after weaning. In a rat intestinal colitis model, feeding diets containing n-3 and n-9 fatty acids significantly reduced histological damage in the colon compared with rats fed a diet containing n-6 fatty acids (Jacobson *et al*., 2005). There is evidence that n-3 fatty acids significantly improve intestinal barrier function by increasing cytosolic tight junction protein ZO-1 mRNA expression and paracellular permeability in an *in vitro* epithelial monolayer model (Li *et al*., 2008), by increasing extracellular tight junction protein occludin mRNA gene expression and decreasing PPARγ gene expression in the rat ileal mucosa (Wang *et al*., 2012), and by attenuating IL-4 mediated trans- and para-cellular permeability in an *in vitro* model (Willemsen *et al*., 2008).

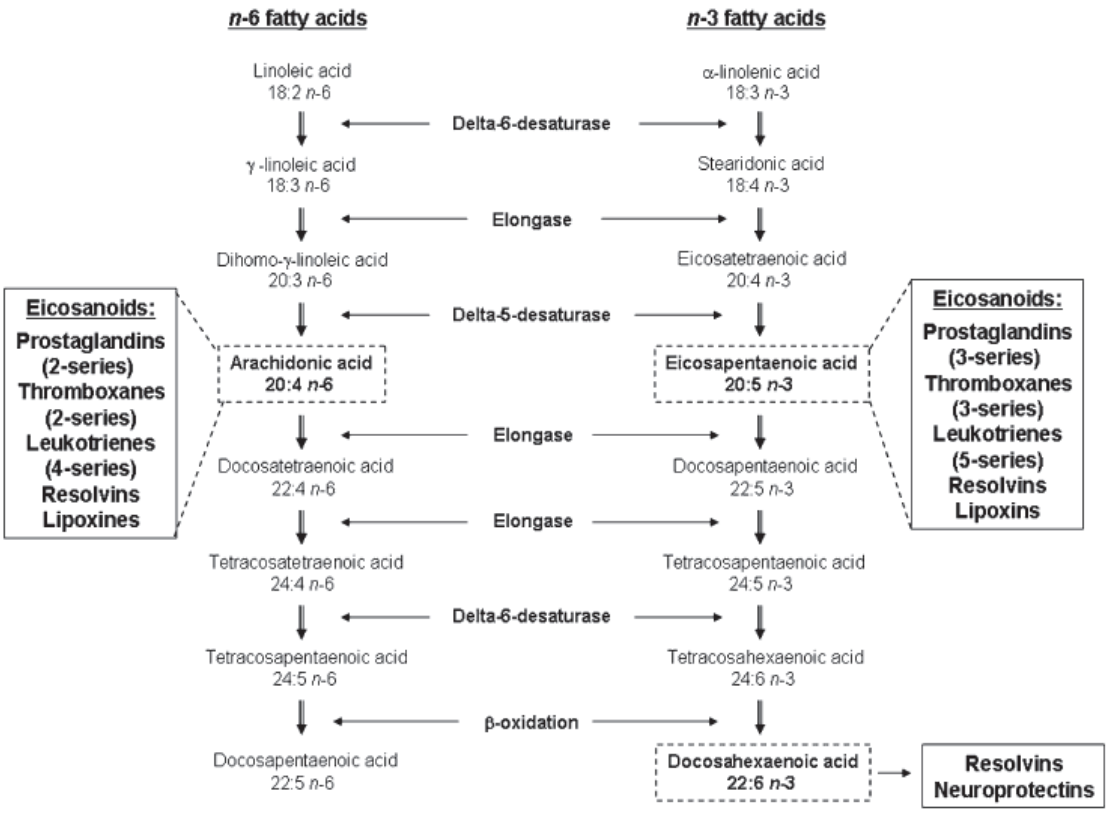


Figure 3. *Metabolism of n-3 and n-6 fatty acids and production of either anti-inflammatory or pro-inflammatory eicosanoids (from Wall* et al.*, 2010).*

Boron

Boron is a naturally occurring non-metallic mineral that does not accumulate in the tissue, and is widely used for medical purposes due to its antibacterial properties and ability to reduce heavy metal toxicity (Turkez *et al*., 2012). In the body, most boron (> 96%) exists as boric acid and reacts with molecules with hydroxyl groups to form boron ester. Therefore, boric acid promptly forms complexes with metabolically important sugars such as ribose, which is a component of adenosine (Nielsen, 2009; Nielsen and Meacham, 2011). This chemical characteristic allows boron to react with signaling molecules containing adenosine. For example, Nielsen (2009) demonstrated that boron-deprived rats (0 versus 3 mg boron/kg diet) showed significantly reduced plasma S-adenosyl-methionine and S-adenosyl-homocysteine, and increased circulating homocysteine. Reduced S-adenosyl-methionine is reported in many human diseases such as arthritis, cancer and impaired brain function because S-adenosyl-methionine has a central role for methylation of neurotransmitters, DNA, RNA, proteins phospholipids and hormones (Nielsen, 2009).

Apart from this, dietary boron is known to inhibit both PGE_2 and LTB_4 production by inhibiting cyclooxygenase and lipoxygenase activity in leukocytes in rats (Rajendran *et al.*, 1994, Hunt and Idso, 1999, Nielsen *et al*., 2007) through a NF-kB regulated pathway (Durick *et al*., 2004). Hunt and Idso (1999) suggested that dietary boron down-regulates leukocyte 6-phosphogluconate dehydrogenase and reduces reactive oxygen species produced by neutrophils for local phagocytosis, but exacerbates the inflammatory response in excess (Hunt and Idso, 1999). This notion was supported by a recent rat study (Ince *et al*., 2010) where supplementation of 100 mg boron/kg as either boric acid or borax significantly decreased an oxidative stress/lipid peroxidation bio-marker malondialdehyde and increased glutathione content in the blood. Boron is also known to inhibit the activity of serine proteases such as tryptase, the proteolytic enzymes released by activated mast cells and leukocytes which degrade structural proteins of pathogens but also degrade structural proteins of the host cells (Hunt, 2003). In addition, a recent *in vitro* study demonstrated that calcium fructoborate decreased IL-1β, IL-6 and nitric oxide production by LPS-stimulated murine macrophages (Scorei *et al*., 2010).

Only a few studies have been conducted in pigs and reported the beneficial effects of boron on performance and immune function, although no pig study has specifically measured the effects of boron on PGE_2 and LTB_4 production. Armstrong and Spears (2003) fed either a control diet or a diet supplemented with 5 mg boron/kg as sodium borate and found a significant increase in average daily gain due mainly to increased feed intake during 49 d after weaning (0.47 versus 0.36 kg) and also during the subsequent

grower phase (next 79 d, 1.00 versus 0.85 kg). More interestingly, this particular study tested a local inflammatory response by measuring skinfold swelling after intra-dermal injection of phytohemagglutin (150 μg) at 120 d of age and found that pigs fed a 5 mg boron diet significantly reduced skinfold swelling. Consistent improvements in daily gain, feed intake and local inflammation response were repeatedly reported in pigs fed a 5 mg boron diet (Armstrong *et al*., 2001; Armstrong and Spears, 2001; Armstrong *et al*., 2002). Given that the eicosanoid mediators (PGE_2 and LTB_4) induce anorexia which is partly responsible for decreased growth in subclinical and clinical infection, the consistent improvement in feed intake and daily gain in pigs fed a 5 mg boron diet may advocate that boron reduced *in vivo* production of eicosanoids. A dose-response study of boron on production of eicosanoid mediators in immune system compromised pigs is required to clarify the immunomodulatory role of boron in pigs.

Probiotics, prebiotics, synbiotics and role of non-starch polysaccharide-degrading enzymes

The presence of commensal bacteria in the intestinal tract improves intestinal barrier function through diverse mechanisms. The importance of commensal bacteria for intestinal barrier function was clearly demonstrated in many studies and one example is a mouse study where a 24-h water medication with streptomycin 2 d prior to oral infection with an enterohaemorrhagic strain of *E. coli* (O157:H7) significantly increased susceptibility to *E. coli* infection compared with mice without antibiotic pre-treatment (Roxas *et al*., 2010). In this study it was evident that mucosa-bound *E. coli* O157:H7 was significantly increased in the ileum, caecum and colon of the streptomycin (5g/L) pre-treated mice compared with the control mice. Moreover, mucosa-bound *E. coli* O157:H7 was detected at 10^3 CFU/g tissue in the streptomycin pre-treated mice at day 10 after infection, while no mucosa-bound *E. coli* was detected in the control mice. Therefore, both severity and duration of infection were increased by removing commensal bacteria (24 h water medication) in the intestinal tract prior to an event of infection. This result showed not only the importance of commensal bacteria for intestinal barrier function but also the danger of intermittent medication which is frequently used for veterinary intervention in the pig industry.

A healthier commensal microbial composition in the intestinal tract may be manipulated by supplementation of probiotics, prebiotics and (or) synbiotics. The mechanisms for how probiotics manipulate the intestinal barrier function are well summarized by several authors (e.g., Mennigen and Bruewer, 2009; Ng *et al*., 2009; Ohland and MacNaughton, 2010; Kenny *et al*., 2011). First, some prebiotics such as *Lactobacillus spp.* can directly manipulate intestinal barrier function by increasing mucus gene expression and secretion by goblet cells (Mack *et al*., 2003; Kim *et al*., 2008), by amplifying β-defensin expression in the enterocytes (Schlee *et al*., 2008), and by enhancing tight junction protein gene expression (Resta-Lenert and Barrett, 2003; Ulluwishewa, 2011). Second, probiotics such as *Bifidobacterium spp.* stimulate secretory IgA production in the lamina propria, which is secreted to the mucus layer and binds pathogens and antigens (Shu and Gill, 2001). Third, probiotics reduce pathogen proliferation and attachments by reducing intestinal pH (Ogawa *et al*., 2001) and by competitive exclusion (Sherman *et al*., 2005; Johnson-Henry *et al*., 2008), respectively. In addition, a recent transgenic mice study that impairs apolipoprotein E metabolism as a metabolic disease model, showed that supplementation of probiotics such as *L. rhamnosus GG* and *Propionibacterium freudenreichii spp. Shermanii JS* to the transgenic mice induced an inflammatory response by feeding a high-fat diet significantly decreased the number of mast cells in the colonic mucosa (see Figure 4). This report is consistent with previous findings in pigs that some probiotics have the ability to modulate systemic response such as suppressing pro-inflammatory cytokine production (Walsh *et al*., 2008; Zhang *et al*., 2010; Chytilova *et al*., 2013).

Prebiotics are non-digestible feed ingredients that can stimulate growth of beneficial bacteria, and synbiotics are combined supplementation of probiotics and prebiotics (Gibson and Roberfroid, 1995; Gourbeyre *et al*., 2011). Synbiotics are expected to synergistically build healthier commensal microbial composition compared with individual supplementation of probiotics and prebiotics, if the correct combination of prebiotics and probiotics are used (Schrezenmeir and de Vrese, 2001). Supplementation of oligofructose with a mixture of probiotics (*Lactobacillus spp., Bacillus subtilis, Saccharomyces cerevisiae*) to weaned pigs increased bifidobacteria in the ileum and colon and decreased coliform bacteria in the colon (Shim *et al*., 2005). More recently, Krause *et al*. (2010) supplemented diets with non-pathogenic *E. coli* and raw potato starch as synbiotics to weaned pigs experimentally infected with a pathogenic *E. coli* K88. Supplementation of synbiotics synergistically improved daily gain, feed intake and faecal score compared with individual supplementation of probiotics or prebiotics. Terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes showed that supplementation of synbiotics synergistically decreased abundance of *Clostridia* in the ileal digesta and synergistically increased microbial richness and diversity in the colon compared with individual supplementation of either probiotics or prebiotics.

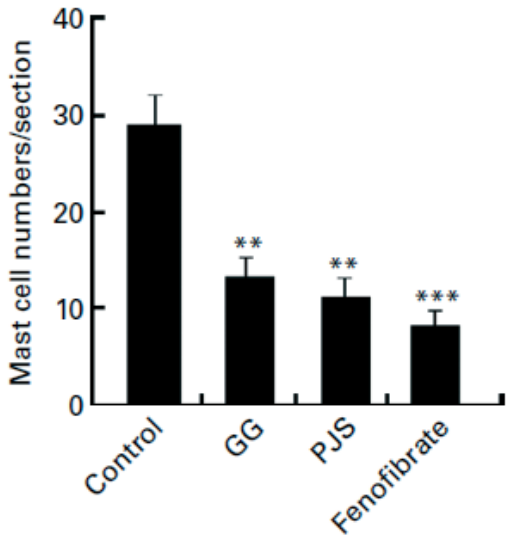


Figure 4. *Effect of probiotics on mast cell numbers in the proximal colon of transgenic mice that impairs apolipoprotein E metabolism as a metabolic disease model, which increased dietary fat-induced inflammatory responses. Mice were euthanised and tissue samples harvested at d 28 after feeding a high fat diet. GG: Lactobacillus rhamnosus GG. PJS: Propionibacterium freudenreichii spp. Shermanii JS. Fenofibrate is an activator of PPARα with established anti-inflammatory potency and also lipid-modulating capacity* (*from Oksaharju* et al., *2013*).

Given the synergistic effect of synbiotics, it is feasible that exogenous non-starch polysaccharide (NSP)-degrading enzymes can be strategically used to produce prebiotics (smaller molecular weight oligosaccharides) *in vivo* by reducing the molecular weight of the large NSP molecules originated from plant-based feed ingredients in pig diets. An *in vitro* study showed that addition of a NSP-degrading enzyme at a low dose solubilises insoluble NSP and increase soluble NSP content, while at higher dose rates the enzyme further reduced the solubilised NSP (Castanon *et al*., 1997). In a recent review, Kiarie *et al*. (2013) provided evidence that use of xylanase in a wheat-based broiler diet increased short-chain xylo-olgomers in the caecum and increased specific bacteria such as lactobacillus in pigs. Therefore, a low dose of arabinoxylanase in wheat/rye-based diets or β-glucanase in barley/oat-based diets in combination with suitable probiotics may need to be tested as synbiotics to improve intestinal health and hence eventually minimise systemic response in the commercial herds where pigs are consistently exposed to chronic subclinical infection. However, information as to the correct dosage and type of enzyme depends on major cereal grains and oilseed meals that maximise prebiotic production *in vivo* needs to be explored in detail.

Antioxidants

Other nutrients that have the ability to reduce PGE_2 and LTB_4 biosynthesis are the extracellular antioxidant selenium (Werz and Steinhilber, 1996; Hwang *et al*., 2006; Vunta *et al*., 2008) and the intracellular antioxidant vitamin E (Sakamoto *et al*., 1991; Wu *et al*., 1998; Venkatraman and Chu, 1999; Reiter *et al*., 2007; Jiang *et al*., 2011).

Selenium in the form of selenoproteins plays an important role as a major antioxidant to alleviate the cell damage from reactive oxygen species, especially in macrophages (Vunta *et al*., 2008). The 5-lipoxygenase enzyme is activated when lipid peroxidation is increased (Riendeau *et al*., 1989; Imai *et al*., 1998) and therefore the cellular redox status is an important factor for activation of lipoxygenase in the cell wall (Bryant *et al*., 1982). An *in vitro* study with rat granulocytes showed that selenium deficiency reduced fatty acid hydroperoxides and increased cellular 5-lipoxygenase activity (Weitzel and Wendel, 1993). In a human B-lymphocyte study, where cellular leukotriene synthesis was upregulated with transforming growth factor (TGF-β), selenium-dependent peroxidises suppressed cellular 5-lipoxygenase activity (Werz and Steinhilber, 1996). Moreover, selenium decreased COX-2 gene expression in the colon cancer cells (Hwang *et al*., 2006) and in macrophages, where subsequent reduction in TNF-α was reported (Vunta *et al*., 2008).

Vitamin E deficiency in pigs is known to decrease markers of cell-mediated immunity such as impaired lymphocyte proliferation (Lessard *et al*., 1991). Vitamin E supplementation is also known to increase humoral immune responses. For example, Ellis and Vorhies (1976) reported that, compared with a control diet (without vitamin E supplementation with approximately 20 IU/kg in the control diet),

additional vitamin E supplementation at 20 and 100 IU/kg increased two- and three-fold the levels of anti *E-coli* serum antibody in pigs challenged with an intramuscular injection of *E. coli* bacterium (0.5 mL of 10^9 cfu/mL, serotype O149:K88, 91:H19). In a broiler study, supplementation of 300 IU/kg vitamin E in an *E. coli*-challenged group (post-thoracic air sacs, 1.5 mL of 5 x 10^9/mL) reduced mortality from 40% to 5% (Tengerdy and Nockels, 1975). Therefore, a low vitamin E status at weaning can be a predisposing factor for viral (Beck, 2007) and bacterial diseases (Ellis and Vorhies, 1976), and bacterial infection such as *E. coli* further reduces body vitamin E reserves (Lauridsen *et al*., 2011). The protective effect of vitamin E has been reported to be associated with inhibition of the biosynthesis of PGE_2 by antagonising the lipid peroxidation of arachidonic acid and limiting the entry of precursor into PGE_2 production (Likoff *et al*., 1981).

Vitamin E acts as an intracellular antioxidant and is an important essential nutrient for maintenance of immune function in pigs. In an early mice study, it was demonstrated that the mice fed 500 ppm of synthetic vitamin E (dl-α-tochpheryl acetate) reduced the age-related PGE_2 increase in spleen homogenate (Meydani *et al*., 1986; Wu *et al*., 1998). In another rat study, α-tocopherol decreased PGE_2 production in the peritoneal macrophage stimulated with phorbol myristate acetate (Sakamoto *et al*., 1990). However, in a later study, Jiang *et al*. (2000) reported that γ-tocopherol rather than α-tocopherol is more potent in reducing PGE_2 synthesis by inhibiting COX-2 activity but not by affecting COX-2 protein gene expression in LPS-stimulated macrophages and epithelial cells. Subsequent studies showed that γ-tocopherol, δ-tocopherol and long-chain carboxychromanols (metabolites of vitamin E) were potent inhibitors of COX-2 and 5-lipoxygenase and decreased production of PGE_2 and LTB_4 (Jiang and Ames, 2003; Jiang *et al*., 2008; Jiang *et al*., 2011). Research to investigate the effect of different types of Vit E on production of eicosanoid mediators in immune system-activated pigs have not been conducted and warrant future study to elucidate the role of vitamin E on immune function of pigs.

Synergistic effects of vitamin E, selenium, n-3 fatty acids and NSAIDs such as aspirin as a COX inhibitor on reducing production of eicosanoid mediators have been reported. An early chicken study showed a synergistic reduction in mortality and PGE_2 production in the bursa when birds provided with both 300 mg/kg vitamin E and 50 mg aspirin/kg body weight in combination compared with individual supplementation of vitamin E or aspirin (Likoff *et al*., 1981). In a recent rat study the carrageenan-induced inflammation model was used, and Jiang *et al*. (2009) demonstrated that a combination of γ-tocopherol (33 mg/kg) and aspirin (150 mg/kg) inhibited PGE_2 production by 70% at the inflammation site while a combination of α-tocopherol and aspirin did not show any beneficial effect. Moreover, γ-tocopherol but not α-tocopherol reduced aspirin-induced side effects such as reductions in feed intake and gastric lesion score. Supplementation of vitamin E and selenium in combination synergistically increased antibody titres in antigen-challenged weaner pigs (Peplowski *et al*., 1981). In a rat oesophageal squamous cell carcinoma model, the combined supplementation of vitamin E and selenium decreased PGE_2 and LTB_4 production and gene expression of COX-2 and 5-lipoxygenase in the esophagus (Yang *et al*., 2011). In a rat study using the rheumatoid arthritis model, Venkatraman and Chu (1999) reported that combined supplementation of n-3 fatty acid and vitamin E synergistically decreased PGE_2 and LTB_4 contents in the serum.

Conclusions

Pigs in a commercial environment are consistently exposed to (sub) clinical infections and stressors, and systemic responses to such challenges significantly reduce the growth potential of modern pigs. The systemic immune response influences performance of pigs in two distinctive routes: alteration of nutrient partitioning and neurological response to infection such as anorexia. The role of nutrition for a multi-angle approach to tackle both routes of the systemic response was reviewed. Supplementation of selected amino acids that are extensively used during the systemic response to counteract the altered nutrient partitioning, and supplementation of nutrients that reduce production of eicosanoid mediators that in turn provokes a neurological infection response, were suggested as possible solutions. The majority of the evidence provided is largely based on rat infection models or *in vitro* studies, therefore further pig research for individual and synergistic effects of the suggested nutrients on production of eicosanoid mediators is required to establish robust and cost-effective nutritional strategies for efficient growth of pigs in a commercial production system.

References

ABRAHAM, S.N and ST. JOHN, A.L. (2010). *Nature Reviews Immunology*. **10**:440-452.

ANDERLE, P., FARMER, P., BERGER, A. and ROBERTS, M.A. (2004). *Lipid Metabolism.* **20**:103-108.

ARMSTRONG, T.A. and SPEARS, J.W. (2001). *Journal of Animal Science.* **79**:3120-3127.

ARMSTRONG, T.A., and SPEARS, J.W. (2003). *Journal of Animal Science.* **81**:2552-2561.

ARMSTRONG, T.A., FLOWERS, W.L., SPEARS, J.W. and NIELSEN, F.H. (2002). *Journal of Animal Science.* **80**:154-161.

ARMSTRONG, T.A., SPEARS, J.W. and LLOYD, K.E. (2001). *Journal of Animal Science.* **79**:1549-1556.

BABIOR, B.M. (2000). *The American Journal of Medicine.* **109**:33-44.

BECK, M.A. **(**2007**).** *Journal of Nutrition.* **137**:1338–1340.

BEISEL, W.R. (1991). In "Nutritional biochemistry and metabolism with clinical applications, 2nd edition". pp. 507-542, ed. M.C. Linder. (Appleton & Lange: Connecticut, USA).

BERKES, J., VISWANATHAN, V.K., SAVKOVIC, S.D. and HECHT, G. (2010). *Gut.* **52**:439-451.

BREUILLE, D., ARNAL, M. RAMBOURDIN, F., BAYLE, G., LEVIEUX, D. and OBLED, C. (1998). *Clinical Science.* **94**:413-424.

BREUILLE, D., ROSE, F., ARNAL, M., MELIN, C. and OBLED, C. (1994). *Clinical Science.* **86**:663-669.

BRYANT, R.W., SIMON, T.C. and BAILEY, J.M. (1982). *Journal of Biological Chemistry.* **257**:14937-14942.

CALDER, P.C. (2006). *American Journal of Clinical Nutrition.* **83**:1505-1519.

CAPOZZALO, M.M., KIM, J.C., HTOO, J.K., DE LANGE, C.F.M., MULLAN, B.P., HANSEN, C.F., RESINK, J.W., STUMBLES, P.A., HAMPSON, D.J. and PLUSKE, J.R. (2012). *Journal of Animal Science.* **90**:191-193.

CASTANON, J.I.R., FLORES, M.P. and PETTERSSON, D. (1997). *Animal Feed Science and Technology.* **68**:361-365.

CAUGHEY, G.E., MANTZIORIS, E., GIBSON, R.A., CLELAND, L.G. and JAMES, M.J. (1996). *American Journal of Clinical Nutrition.* **63**:116-122.

CHYTILOVA, M., MUDRONOVA, D., NEMCOVA, R., GANCARCIKOVA, S., BULECA, V., KOSCOVA, J. and TKACIKOVA, L. (2013). *Research in Veterinary Science.* **95**:103-109.

CLELAND, L.G., JAMES, M.J., PROUDMAN, S.M., NEUMANN, M.A. and GIBSON, R.A. (1994). *Lipids.* **29**:151-155.

DE RIDDER, K., LEVESQUE, C.L., HTOO, J.K. and DE LANGE, C.F.M. (2012). *Journal of Animal Science.* **90**:3485-3491.

DEVCHAND, P.R. KELLER H., PETERS, J.M., VAZQUEZ, M. GONZALEZ, F.J and WAHLI, W. (1996). *Nature.* **384**:39-43.

DURICK, K.A., TOMITA, M., HUNT, C. and BRADLEY, D. (2005). *The Federation of American Societies for Experimental Biology Journal.* **19**:A1705.

ELLIS, R. and VORHIES, M.W. (1976). *Journal of American Veterinary Medical Association.* **168**:231-232.

EMERY, D.L. and COLLINS, A.M. (2011). In "Manipulating Pig Production XIII". pp. 11-30, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

EVANS, J.F., NATHANIEL, D.J., ZAMBONI, R.J. and FORD-HUTCHINSON, A.W. (1985). *Journal of Biological Chemistry.* **260**:10966-10970.

FAURE, M., CHONE, F., METTRAUX, C., GODIN, C., BECHEREAU, J.P., VUICHOUD, J., PAPET, I., BREUILLE, D. and OBLED, C. (2007). *Journal of Nutrition.* **137**:1802-1807.

FOLCO, G. and MURPHY, R.C. (2006). *Pharmacological Reviews.* **58**:375-388.

GIBSON, G.R. and ROBERFROID, M.B. (1995). *Journal of Nutrition*. **125**:1401-1412.

GODDEERIS, B.M., BOERSMA, W.J.A., COX, E., VAN DER STEDE, Y., KOENEN, M.E., VANCAENEGHEM, S., MAST, J and VAN DEN BROECK, W. (2002). In "Nutrition and health of the gastrointestinal tract". pp. 97-134, eds. M.C. Blok, H.A. Vahl, L. de Lange, A.E. Van de Braak, G. Hemke, and M. Hessing. (Wageningen Academic Publisher: Wageningen, The Netherlands).

GOURBEYRE, P., DENERY, S. and BODINIER, M. (2011). *Journal of Leukocyte Biology.* **89**:685-695.

GRODCHWITZ, K.R. and HOGAN, S.P. (2009). *Journal of Allergy and Clinical Immunology.* **124**:3-20.

HOU, Y., WANG, L., YI, D., DING, YANG, Z., LI, J. CHEN, X., QUI, Y. and WU, G. (2013). *Amino Acids.* **45**:513-522.

HOU, Y., WANG, L., ZHANG, W., YANG, Z., DING, B., ZHU, H., LIU, Y., QUI, Y., YIN, Y. and WU, G. (2012). *Amino Acids.* **43**:1233-1242.

HUNT, C.D. (2003). *Journal of the Trace Element in Experimental Medicine*. **16**:291-306.

HUNT, C.D. and IDSO, J.P. (1999). *The Journal of Trace Elements in Experimental Medicine*. **12**:221-233.

HWANG, J-.T., KIM, Y.M., SURH, Y-.J., BAIK, H.W., LEE, S-.K., HA, J. and PARK, O.J. (2006). *Cancer Research.* **66**:10057-10063.

IMAI, H., NARASHIMA, K., ARAI, M., SAKAMOTO, H., CHIBA, N. and NAKAGAWA, Y. (1998). *Journal of Biological Chemistry*. **273**:1990-1997.

INCE, S., KUCUKKURT, I., CIGERCI, I.H., FIDAN, A.F. and ERYAVUZ, A. (2010). *Journal of Trace Elements in Medicine and Biology*. **24**:161-164.

IRVINE, R.F. (2003). *Nature Reviews Molecular Cell Biology*. **4**:1-12.

JACOBSON, K., MUNDRA, H. and INNIS, S.M. (2005). *American Journal of Physiology (Gastrointestinal Liver Physiology).* **289**:G13-G20.

JAKSCHIK, B.A., MORRISON, A.R. and SPRECHER, H. (1983). *Journal of Biological Chemistry.* **258**:12797-12800.

JAMES, M.J., GIBSON, R.A. and CLELAND, L.G. (2000). *American Journal of Clinical Nutrition.* **71**:343S-348S.

JIANG, Q. and AMES, B.N. (2003). *Federation of American Societies for Experimental Biology Journal.* **17**:816-822.

JIANG, Q., ELSON-SCHWAB, I., COURTEMANCHE, C. and AMES, B.N. (2000). *Proceedings of the National Academy of Science.* **97**:11494-11499.

JIANG, Q., MORELAND, M., AMES, B.N. and YIN, X. (2009). *Journal of Nutritional Biochemistry.* **20**:894-900.

JIANG, Q., YIN, X., LILL, M.A., DANIELSON, M.L., FREISER, H. and HUANG, J. (2008). *Proceedings of the National Academy of Science.* **105**:20464-20469.

JIANG, Z., YIN, X. and JIANG, Q. (2011). *The Journal of Immunology.* **186**:1173-1179.

JOHNSON, R.W. (1997). *Journal of Animal Science.* **75**:1244-1255.

JOHNSON-HENRY, K.C., DONATO, K.A., SHEN-TU, G., GORDANPOUR, M. and SHERMAN, P.M. (2008). *Infection and Immunity.* **76**:1340-1348.

KALINSKI, P. (2012). *The Journal of Immunology*. **188**:21-28.

KENNY, M., SMIDT, H., MENGHERI, E. and MILLER, B. (2011). *Animal.* **5**:462-470.

KIARIE, E., ROMERO, L.F. and NYACHOTI, C.M. (2013). *Nutrition Research Reviews.* **26**:71-88.

KIM, J.C., HANSEN, C.F., MULLAN, B.P. and PLUSKE, J.R. (2012a). *Animal Feed Science and Technology.* **173**:3-16.

KIM, J.C., MULLAN, B.P., FREY, B., PAYNE, H.G. and PLUSKE, J.R. (2012b). *Journal of Animal Science.* **90**:362-365.

KIM, Y., KIM, S.H., WHANG, K.Y., KIM, Y.S., OH, S. (2008). *Journal of Microbiology and Biotechnology.* **18**:1278-1285.

KLASING, K.C., JOHNSTONE, B.J. and BENSON, B.N. (1991). In "Recent advances in animal nutrition". pp. 135-146, eds. W. Haresign, and D.J.A. Cole. (Butterworth-Heinemann, Stoneham, MA).

KRAUSE, D.O., BHANDARI, S.K., HOUSE, J.D. and NYACHOTI, C.M. (2010). *Applied and Environmental Microbiology*. **76**:8192-8200.

LAURIDSEN, C., VERSTERGAARD, E-.M., HOJSGAARD, S., JENSEN, S.K. and SORENSEN, M.T. (2011). *Livestock Science*. **137**:161-167.

LENIHAN-GEELS, G., BISHOP, K.S. and FERGUSON, L.R. (2013). *Nutrients.* **5**:1301-1315.

LESSARD, M., YANG, W.C., ELLIOT, G.S., REBAR, A.H., VAN VLEET, J.F., DESLAURIERS, N., BRISSON, G.J. and SCHULTS, R.D. (1991). *Journal of Animal Science*. **69**:1575-1582.

LI, Q., ZHANG, Q., WANG, M., ZHAO, S., XU, G. and LI, J. (2008). *Molecular Immunology.* **45**:1356-1365.

LIKOFF, R.O., GUPTILL, D.R., LAWRENCE, L.M., MCKAY, C.C., MATHIAS, M.M., NOCKELS, C.F. and TENGERDY, R.P. (1981). *American Journal of Clinical Nutrition.* **34**:245-251.

LIU, Y., CHEN, F., ODEL, J., LIN, X., JACOBI, S.K., ZHU, H. and WU, Z. (2012). *Journal of Nutrition.* **142**:2017-2024.

MACK, D.R., SHRNE, S., HYDE, L., WEI, S. and HOLLINGSWORTH, M.A. (2003). *Gut.* **52**:827-833.

MALMEZAT, T. BREUILLE, D., POUYET, C., BUFFIERE, C., DENIS, P., MIRANDA, P.P. and OBLED, C. (2000). *American Journal of Physiology (Endocrinology and Metabolism).* **279**:E1391-1397.

MCGLONE, J.J., SALAK, J.L., LUMPKIN, E.A., NICHOLSON, R.I., GIBSON, M. and NORMAN, R.L. (1993). *Journal of Animal Science.* **71**:888-896.

MENNIGEN, R. and BRUEWER, M. (2009). *Annals of the New York Academy of Sciences.* **1165**:183-189.

METAYER, S., SEILIEZ, I., COLLIN, A., DUCHENE, S., MERCIER, Y., GERAERT, P.-A. and TESSERAUD, S. (2008). *Journal of Nutritional Biochemistry*. **19**:207-215.

MEYDANI, S.N., MEYDANI, M., VERDON, C.P., SHAPIRO, A.A., BLUMBERG, J.B. and HAYES, K.C. (1986). *Mechanisms of Ageing and Development.* **34**:191-201.

MOESER, A,J., KLOK, C.V., RYAN, K.A., WOOTEN, J.G., LITTLE, D., COOK, V.L. and BLIKSLAGER, A.T. (2007). *American Journal of Physiology (Gastrointestinal Liver Physiology).* **292**:G173-G181.

MOWAT, A.M. (2003). *Nature Reviews Immunology*. **3**:331-341.

NARALA, V.R., ADAPARA, R.K., SURESH, M.V., BROCK, T.G., PETERS-GOLDEN, M. and REDDY, R.J. (2010). *The Journal of Biological Chemistry*. **285**:22067-22070.

NG, S.C., HART, A.L., KAMM, M.A., STAGG, A.J. and KNIGHT, S.C. (2009). *Inflammatory Bowel Disease.* **15**:300-310.

NIELSEN, F.H. (2009). *Journal of Trace Elements in Medicine and Biology*. **23**:204-213.

NIELSEN, F.H. and MEACHAM, S.L. (2011). *Journal of Evidence-Based Complementary & Alternative Medicine.* **16**:169-180.

NIELSEN, F.H., STORCKER, B.J. and PENLAND, J.G. (2007). In: "Advances in plant and animal boron nutrition". pp. 277-290, eds. F. Xu, H.E. Goldbach, P.H. Brown, R.W. Bell, T. Fujiwara, C.D. Hunt, S. Goldberg and L. Shi. (Springer: The Netherlands).

NRC. (2012). "Nutrient requirements of swine", 11th ed. (The National Academy Press: Washington).

OGAWA, M., SHIMIZU, K., NOMOTO, K., TANAKA, R., HAMABATA, T., YAMASAKI, S., TAKEDA, T. and TAKEDA, Y. (2001). *International Journal of Food and Microbiology.* **68**:135-140**.**

OHLAND, C.L. and MACNAUGHTON, W.K. (2010). *American Journal of Physiology (Gastrointestinal Liver Physiology).* **298**:G807-G819.

OKSAHARJU, A., KOOISTRA, T., KLEEMANN, R., VAN DUYVENVOORDE, W., MIETTINEN, M., LAPPALAINEN, J., LINDSTEDT, K.A., KOVANEN, P.T., KORPELA, R. and KEKKONEN, R.A. (2013). *British Journal of Nutrition.* **110**:77-85.

OVERMAN, E.L., RIVIER, J.E. and MOESER, A.J. (2012). *PLoS ONE*. **7**:e39935.

PALMQUIST, D.L. (2009). *The Professional Animal Scientist.* **25**:207-249.

PARADIS, M.A., GEBHART, C.J., TOOLE, D., VESSIE, G., WINKELMAN, N.L., BAUER, S.A., WILSON, J.B. and MCCLURE, C.A. (2012). *Journal of Swine Health and Production.* **20**:137-141.

PASTORELLI, H., LE FLOC'H, N., MERLOT, E., MEUNIER-SALAUN, M.C., VAN MILGEN, J. and MONTAGNE, L. (2012c). *Animal.* **6**:1811-1820.

PASTORELLI, H., LE FLOC'H, N., MERLOT, E., MEUNIER-SALAUN, M.C., VAN MILGEN, J. and MONTAGNE, L. (2012b). *Journal of Animal Science.* **90**:4866-4875.

PASTORELLI, H., VAN MILGEN, J., LOVOTTO, P. and MONTAGNE, L. (2012a). *Animal.* **6**:952-961.

PEARCE, S.C., MANI, V., BODDICKER, R.L., JOHNSON, J.S., WEBER, T.E., ROSS, J.L., BAUMGARD, L.H. and GABLER, N.K. (2012). *Journal of Animal Science.* **90**:257-259.

PEPLOWSKI, M.A., MAHAN, D.C., MURRAY, F.A., MOXON, A.L., CANTOR, A.H. and EKSTROM, K.E. (1981). *Journal of Animal Science.* **51**:344-351.

PINEIRO, C., PINEIRO, M., MORALES, J., CARPINTERO, R., CAMPBELL, F.M., ECKERSALL, P.D., TOUSSAINT, M.J.M., ALAVA, M.A. and LAMPREAVE, F. (2007). *Animal*. **1**:133-139.

PRESCOTT, S.L., BARDEN, A.E., MORI, T.A. and DUNSTAN, J.A. (2007). *Clinical Science.* **113**:409-416.

RAJENDRAN, K.G., BURNHAM, B.S., SOOD, C.A., SPIELVOGEL, B.F., SHAW, B.R. and HALL, I.H. (1994). *Journal of Pharmaceutical Science.* **83**:1391-1395.

RAKHSHANDEH, A. and DE LANGE, C.F.M. (2011). In "Manipulating Pig Production XIII". pp. 31-46, ed. van Barneveld, R.J. (Australasian Pig Science Association: Werribee).

RAKHSHANDEH, A., HOLLIS, A., KARROW, N.A. and DE LANGE, C.F.M. (2010b). *Journal of Animal Science.* **88**:489.

RAKHSHANDEH, A., HTOO, J.K. and DE LANGE, C.F.M. (2010a). *Livestock Science.* **134**:21-23.

REITER, E. JIANG, Q. and CHRISTIN, S. (2007). *Molecular Aspects of Medicine.* **28**:668-691.

RESTA-LENERT, S. and BARRETT, K.E. (2003). *Gut.* **52**:988-997.

RIENDEAU, D., DENIS, D., CHOO, L.Y. and NATHANIEL, D.J. (1989). *Biochemistry Journal.* **263**:565-572.

RIVEST, S. (2010). *Progress in Brain Research*. **181**:43-53.

ROXAS, J.L., KOUTSOURIS, A., BELLMEYER, A., TESFAY, S., ROYAN, S., FALZARI, K., HARRIS, A., CHENG, H., RHEE, K.J. and HECHT, G. (2010). *Laboratory Investigation.* **90**:1152-1168

SAKAMOTO, W., FUJIE, K., HANDA, H., NISHIHIRA, J. and MINO, M. (1991). *Biochemica et Biophysica Acta.* **1074**:251-255.

SALAK-JOHNSON, J.L. and MCGLONE, J.J. (2007). *Journal of Animal Science* **85**:E81-E88.

SANDBERG, F.B., EMMANS, G.C. and KYRIAZAKIS, I. (2007). *Animal.* **1**:67-86.

SCHLEE, M., HARDER, J., KOTEN, B., STANGE, E.F., WEHKAMP, J. and FELLERMANN, K. (2008). *Clinical and Experimental Immunology.* **151**:528-535.

SCHREZENMEIR, J. and DE VRESE, M. (2001). *American Journal of Clinical Nutrition.* **73**:361s-364s.

SCOREI, R.I., CIOFRANGEANU, C., ION, R., CIMPEAN, A., GALATEANU, B., MITRAN, V. and IORDACHESCU, D. (2010). *Biological Trace Element Research.* **135**:334-344.

SHERMAN, P.M., JOHNSON-HENRY, K.C., YEUNG, H.P., NGO, P.S.C., GOULET, J. and TOMPKINS, T.A. (2005). *Infection and Immunity.* **73**:5183-5188.

SHIM, S.B., VERSTEGEN, M.W.A., KIM, I.H., KWON, O.S. and VERDONK, J.M.A.J. (2005). *Archives of Animal Nutrition.* **59**:419-427.

SHU, Q. and GILL, H.S. (2001). *Medical Microbiology and Immunology.* **189**:147-152.

SIMOPOULOS, A.P. (2004). *Food Reviews International*. **20**:77–90.

SMITH, F., CLARK, J.E., OVERMAN, B.L., TOZL, C.C., HUANG, J.H., RIVIER, J.E.F. BLISKLAGER, A.T. and MOESER, A.J. (2010). *American Journal of Physiology (Gastrointestinal Liver Physiology).* **298**:G352-G363.

SOLER, L., GUTIERREZ, A., ESCRIBANO, D., FUENTES, M. and CERON, J.J. (2013). *Research in Veterinary Science.* **95**:298-302.

SPLETTSTOESSER, W.D. and SCHUFF-WERNER, P. (2002). *Microscopy Research and Technique.* **57**:441-455.

STENSON, W.F., PRESCOTT, S.M. and SPRECHER, H. (1984). *Journal of Biological Chemistry.* **259**:11784-11789.

SUPAJATURA, V., USHIO, H., NAKAO, A., OKUMURA, K., RA, C. and OGAWA, H. (2001). *The Journal of Immunology.* **167**:2250-2256.

TEITELBAUM, A.A., GAREAU, M.G., JURY, J., YANG, P.C. and PERDUE, M.H. (2008). *American Journal of Physiology (Gastrointestinal Liver Physiology).* **295**:G452-G459.

TENGERDY, R.P. and NOCKELS, C.F. (1975). *Poultry Science*. **54**:1292-1296.

TREVISI, P., CORRENT, E., MESSORI, S., CASINI, L. and BOSI, P. (2009a). *Livestock Science.* **134**:236-238.

TREVISI, P., MELCHIOR, D., MAZZONI, M., CASINI, L., DE FILPPI, S., MINIERI, L., LALATTA-COSTERBOSA, G. and BOSI, P. (2009b). *Journal of Animal Science.* **87**:148-156.

TURKEZ, H., GEYIKOGLU, F., TATAR, A., KELES, M.S. and KAPLAN, I. (2012). *Experimental and Toxicological Pathology*. **64**:93-101.

ULLUWISHEWA, D., ANDERSON, R.C., MCNABB, W.C., MOUGHAN, P.J. WELLS, J.M. and ROY, N.C. (2011). *Journal of Nutrition.* **141**:769-776.

VAN DEN ELSEN, L.W.J., NUSSE, Y., BALVERS, M., REDEGELD, F.A., KNOL, E.F., GARSSEN, J. and WILLEMSEN, E.M. (2013). *British Journal of Nutrition.* **109**:1821-1831.

VENKATRAMAN, J.T. and CHU, W-.C. (1999). *Journal of the American College of Nutrition.* **18**:602-613.

VUNTA, H., BELDA, B.J., ARNER, R.Y., CHANNA REDDY, C., VANDEN HEUVEL, J.P. and SANDEEP PRABHU, K. (2008). *Molecular Nutrition and Food Research.* **52**:1316-1323.

WALL, R., ROSS, R.P., FITZGERALD, G.F. and STANTON, C. (2010). *Nutrition Reviews.* **68**:280-289.

WALSH, M.C., GARDINER, G.E., HART, O.M., LAWLOR, P.G., DALY, M., LYNCH, B., RICHERT, B.T., RADCLIFFE, S., GIBLIN, L., HILL, C., FITZGERALD, G.F., STANTON, C. and ROSS, P. (2008). *FEMS Microbiology Ecology.* **64**:317-327.

WANG, X., PAN, L., LU, J., LI, N. and LI, J. (2012). *Clinical Nutrition.* **31**:951-957.

WEITZEL, F. and WENDEL, A. (1993). *Journal of Biological Chemistry*. **268**:6288-6292.

WERZ, O. and STEINHILBER, D. (1996). *European Journal of Biochemistry*. **242:**90-97.

WESOLOWSKI, J. and PAUMET, F. (2011). *Immunologic Research*. **51**:215-226.

WIERZBICKI, M. and BRZEZINSKA-BLASZCZYK, E. (2009). *Microbiology and Immunology*. **53**:694-703.

WILKINSON, S.J., DOWNING, J.A., THOMSON, P.C. and NEWMAN, R.E. (2011). *Recent Advances in Animal Nutrition-Australia.* **18**:79-86.

WILLEMSEN, L.E.M., KOETSIER, M.A., BALVERS, M., BEERMANN, C., STAHL, B. and VAN TOL, E.A.F. (2008). *European Journal of Nutrition*. **14**:183-191.

WILLIAMS, N.H., STAHLY, T.S. and ZIMMERMAN, D.R. (1997a). *Journal of Animal Science.* **75**:2463-2471.

WILLIAMS, N.H., STAHLY, T.S. and ZIMMERMAN, D.R. (1997b). *Journal of Animal Science.* **75**:2472-2480.

WILLIAMS, N.H., STAHLY, T.S. and ZIMMERMAN, D.R. (1997c). *Journal of Animal Science.* **75**:2481-2496.

WU, D., MURA, C., BEHARKA, A.A., HAN, S.N., PAULSON, K.E., HWANG, D. and MEYDANI, S.N. (1998). *American Journal of Physiology Cell Physiology*. **275**:C661-C668.

WYMANN, M.P and SCHNEITER, R. (2008). *Nature Reviews Molecular Cell Biology*. **9**:162-176.

YANG, H., FANG, J., JIA, X., HAN, C., CHEN, X., YANG, C.Y. and LI, N. (2011). *Carcinogenesis.* **32**:381-388.

YOKOMIZO, T., IZUMI, T. and SHIMIZU, T. (2001). *Archives of Biochemistry and Biophysics.* **385**:231-241.

YU, Y.M., BURKE, J.F. and YOUNG, V.R. (1993). *The Journal of Trauma.* **35**:1-7.

ZHANG, L., XY, Y.Q., LIU, H.Y., LAI, T., MA, J.L., WANG, J.F. and ZHU, Y.H. (2010). *Veterinary Microbiology*. **141**:142-148.

SYMPOSIUM: Intestinal barrier function and systemic response of the gastrointestinal tract in pigs to aspects of management

A.J. Moeser

North Carolina State University, Raleigh, NC 27695, USA.

Abstract

The gastrointestinal tract is lined by a single layer of epithelial cells that play divergent roles in gastrointestinal health. On one hand, the epithelium must facilitate the digestion and absorption of vast amounts of nutrients and water on a continual basis. On the other hand, the epithelium must serve as a barrier that selectively prevents potentially deadly luminal contents, containing bacterial, viruses, antigens, and toxins from gaining access to the body. Thus, the integrity of the intestinal barrier is critical to both animal performance and disease resistance. This paper will focus on intestinal barrier function in the gut with emphasis on the key structural and physiologic components of the mucosal barrier and how its function is impacted by management factors.

Introduction

The surface area of the intestinal tract is greater than 300 m^2 representing the largest interface between the external environment and body. The intestinal epithelium that lines the mucosa is continuously exposed to harsh luminal conditions including pathogenic microorganisms, toxins, enzymes, and dietary antigens. The epithelial barrier is maintained by the single layer of epithelial cells which represent the first line of defence against the immense load of potentially pathogenic microorganisms and antigens present in the intestinal lumen. In health, the epithelium serves a divergent role in intestinal function: on one hand, the epithelium constitutes a semi-permeable barrier that limits the translocation of bacteria and toxins, antigens across the epithelium and into the body which is essential to prevent chronic inflammatory responses and systemic disease. Simultaneously, the epithelium is responsible for the facilitation of efficient digestive and absorptive properties for nutrients and fluid for maintenance and growth. When the epithelial barrier is damaged, the gut epithelium becomes leaky (increased permeability). This in turn allows the transmigration of luminal microorganisms, toxins, and antigenic substances into subepithelial tissues triggering inflammatory processes in the underlying lamina propria tissues. Furthermore, once the epithelial barrier has been breached, these pathogenic agents can freely enter the systemic circulation resulting in septicemia and multiple organ disease. Impairment in barrier function may predispose pigs to select enteric disorders. For example, the pathogenesis of F18 *E. coli* oedema disease requires a permeable intestinal epithelium to facilitate the absorption of shiga-like toxin 2e which is responsible for the clinical manifestations (Waddell and Gyles, 1995).

The intestinal mucosal barrier: basic functions and anatomical organization

To accomplish this divergent role, the intestinal epithelium is polarised, meaning that it is divided into an apical and basolateral region by tight junctions situated at the apical portion of the lateral membrane of adjacent epithelial cells (Figure 1). The apical membrane together with interepithelial tight junctions provides a continuous seal that serves as a barrier. This polarity allows the cell to compartmentalise ion transporters, to create a gradient across the cell that allows either absorption or secretion of electrolytes. For example, the Na^+-K^+ ATPase transporter is localised to the basolateral portion of the membrane of enterocytes, and generates an electrical potential across the cell that provides the energy to move other ions either into or out of the cell. The Na^+-K^+-ATPase maintains Na^+ at relatively low intracellular concentrations (15 mEq of Na^+ intracellular vs. 150 mEq of Na^+ in plasma), which preferentially allows Na^+ to enter the cell via a number of apical transporters on the apical membrane. Thus, these apical transporters (which include Na^+-coupled sugar and amino acid transporters) use the electrochemical gradient set up by Na^+-K^+ ATPase. It is this transport activity that the cellular energy to drive the absorption of nutrients.

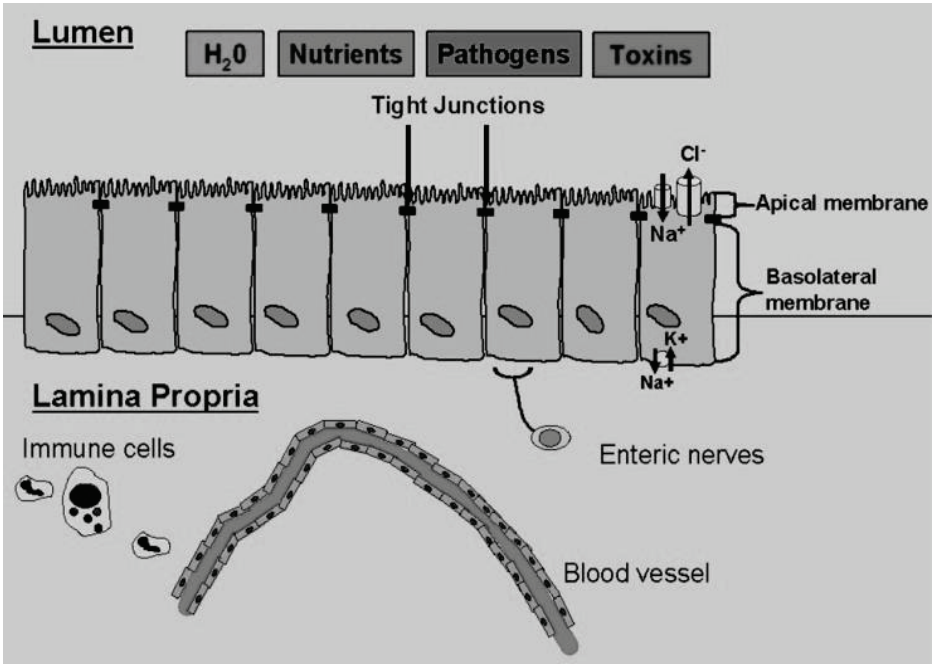


Figure 1. *Components of the intestinal mucosal barrier.*

A further anatomic consideration is the organisation of the mucosa into glandular crypts and villi (in the small intestine) or inter-crypt surface epithelium (in the colon). The crypt-villus axis divides the epithelium into secretory epithelium and absorptive epithelium. The epithelium that performs these opposing functions is derived from stem cells located within the crypts. Newly formed epithelial cells migrate along the crypt-villus axis, maturing and acquiring differing functions until they are ultimately sloughed from the tip of the villus approximately 5 d after their creation. Paneth cells remain at the base of the crypt, and therefore do not take part in the migration of cells toward the surface. Overall, immature epithelium within the crypts performs predominantly secretory functions, whereas the more mature epithelium on the surface is predominantly involved in absorption. This becomes critically important in a number of diarrhoeal diseases that preferentially injure the surface epithelium, disrupting electrolyte and fluid absorption, while leaving secretion in the crypts to continue, often at increased levels because of toxic or inflammatory stimuli.

The functional structure of the colon is similar, including the location of stem cells and immature epithelial cells within crypts, and migration of immature cells progressively out of the crypts. However, there are no villi in the colon. Instead, there is simply inter-crypt surface epithelium, which performs similar absorptive functions as the small intestine. In addition, the array of transporters in the colon differs. Because the colon is in most species the principal site of fluid and electrolyte absorption, this arrangement would seem to put the colon at a disadvantage because of the lack of surface area imparted by villi, yet the colon is capable of compensating for fluid loss in the crypts, suggesting that its ability to absorb fluid is not adversely affected by the lack of villi. This may be in part because the greatest contribution to increased absorptive capacity is the presence of epithelial microvilli, which are present throughout the gastrointestinal tract. In addition, the colonic epithelium is less 'leaky' than small intestinal epithelium as a result of close apposition of tight junctions, suggesting there is less back flow of fluid into the lumen in the colon following absorption.

The permeability properties are regulated predominantly by interepithelial tight junctions (TJs). The TJs are made up of a cluster of membrane [Claudins, Occludin, junctional adhesion molecule (JAM)] and cytosolic proteins (e.g., Zonulin Occludin 1-3) that regulate both permeability/barrier properties discussed above (fence function), and serve to polarise the epithelial cells which compartmentalises epithelial transporters to either the basolateral or apical membranes, which is critical for establishing electrochemical gradients that drive ion, fluid and nutrient transport. Therefore the make-up and integrity of the TJs serves a critical function in gut health and defence.

Sup-epithelial components such as the enteric nervous system (ENS) and immune cells are intimately involved in regulation of secretion and absorption under normal and pathophysiologic processes in the gastrointestinal tract and thus constitute a critical component of the gastrointestinal barrier. The ENS consists of an extensive network of nerve cells that play a critical role in intestinal ion transport. The ENS is composed of two major plexuses, the myenteric plexus (between the two muscle layers) and the submucosal plexus, which interconnect and regulate motor and sensory neural input, respectively. The

ENS can receive central input from the central nervous system via the parasympathetic and sympathetic branches of the autonomic network while also operating independently from the central nervous system. Chemical mediators of the ENS consist of a multitude of neurotransmitters; however, Ach and VIP are the major neurotransmitters released by enteric nerves that stimulate epithelial secretion. Norepinephrine is the predominant neurotransmitter released by nerves that have pro-absorptive effects by activating α-2 receptors on enterocytes and nerves. The neural effect of this mediator is principally inhibitory. The ENS is activated by numerous toxic, endocrine, and inflammatory mediators, resulting in intestinal secretion, and thus plays an important role in many diarrhoeal diseases. One important mechanism by which activation of the ENS stimulates secretory processes is via localised neural reflex arcs. The reflex arc consists of sensory nerves and interneurons that transmit to motor nerves that are mainly VIP and cholinergic. Sensory nerves in the intestinal mucosa are stimulated by bacterial toxins, products of infected epithelial cells, or inflammatory stimuli to regulate secretion by afferent-interneuron-secretomotor reflex arcs. Several bacterial and viral toxins (e.g., *Clostridium difficile toxin A*, Rotavirus viral enterotoxin NSP4) activate secretomotor reflex arcs contributing to fluid secretion and diarrheal disease (Moeser and Blikslager, 2007).

Another vital component of the intestinal mucosal barrier is immune cells capable of mounting a response to invading microorganisms or their toxins. In the event where the integrity of intestinal barrier is compromised (infection, stress, etc.), the intestinal barrier must be preserved to prevent excessive mucosal inflammation and systemic infection. This is accomplished by a rapid, robust innate immune response. The primary goal of the innate immune response is to rapidly clear or contain offending pathogens to prevent prolonged inflammation and sepsis (Medzhitov and Janeway, 1997). This immune response is initiated by recognition of bacterial ligands by epithelial and resident sub-epithelial immune cells, such as mast cells, macrophages and dendritic cells, resulting in a rapid burst of pro-inflammatory cytokines [e.g., interleukin (IL)-6, IL-8, and tumour necrosis factor (TNF)-α] and lipid-derived mediators (e.g., prostaglandins, leukotrienes) into the surrounding tissue and circulation (Marshall, 2004). Released pro-inflammatory mediators recruit effector cells such as neutrophils to the site of infection where, via multiple mechanisms, they aid in containing and eventually clearing the pathogen. The critical importance of this response has been demonstrated in infection and sepsis models in which animals lacking key innate immune functions exhibit decreased bacterial clearance and heightened mortality rates (Malaviya *et al.*, 1996; Belaaouaj *et al.*, 1998; Sutherland *et al.*, 2008). One of the major innate immune cells that are required for initiation of the innate immune cytokine response, neutrophil recruitment, and pathogen clearance, is the mast cell.

The adaptive immune response is carried out by the regulated presentation of antigens to lymphocyte populations that are also present within the lamina propria. Cells involved in the process are M cells located within the epithelium, which process and present antigen, and clustered populations of lymphocytes, particular in Peyer's patches located principally in the submucosa at the anti-mesenteric border of the ileum. This process results in the sub-acute response to specific antigens, typically from microorganisms or their toxins, and supersedes the innate immune response because subacute responses are far more targeted.

Regulation of intestinal barrier function

The intestinal barrier is dynamically regulated under physiological and pathophysiological conditions. An example of physiologic regulation of intestinal barrier is active glucose uptake mediated via the apically-expressed sodium glucose co-transporter 1 (SGLT-1) on intestinal epithelial cells. Activation of SGLT-1 induces a signalling pathway leading to phosphorylation of myosin light chain kinase (Turner *et al.*, 1997), which induces opening of the paracellular space (increased permeability) to facilitate water and Na^+ absorption. Under pathophysiologic conditions, such as inflammation and pathogen challenges, increased barrier-disrupting mediators (e.g., cytokines, proteases, and toxins) released by immune cells and (or) pathogens disrupt tight junction proteins resulting in large increases in intestinal permeability. In turn, increased intestinal permeability leads to antigen and bacterial translocation, inflammation, and clinical disease (e.g., diarrhoea, reduced growth rate). The pathophysiologic regulation of the intestinal barrier has been extensively reviewed by others.

Effects of management on intestinal barrier function

It has become evident that a number of management and (or) environmental stressors can significantly impact intestinal barrier integrity in pigs. Even in well-managed farms, stressors, including mixing, crowding, temperature, and pathogen stress, are major contributors to intestinal barrier disruption and thus reductions in performance and disease. One of the most stressful events a pig encounters during production is weaning. During weaning, the pig is faced with a multitude of concurrent stressors including maternal separation, diet changes, transport and commingling stress, and increased pathogen exposure (Madec *et al.*, 1998). As a result, weaned pigs are more susceptible to intestinal dysfunction resulting in impaired growth and feed efficiency and increased susceptibility to infectious enteric pathogens. There are profound changes in the structure and function of the gut at weaning including a marked impairment in

barrier function or increased permeability (Boudry *et al*., 2004; Moeser *et al*., 2007a), altered nutrient and electrolyte transport (Boudry *et al.*, 2004; Moeser *et al*., 2007a), villous atrophy, and inflammation (Kelly *et al*., 1990; McCracken *et al*., 1999). Although previous studies described above have focused on the short-term (1-2 weeks post-weaning) physiological and structural changes in the gut of the weaned pigs, we have accumulated extensive evidence that, depending on the degree of stress experienced at weaning, long-lasting alterations to intestinal function and pathogen defence are evident.

Impact of piglet weaning age on intestinal barrier function

In previous work, we demonstrated that weaning age has a significant impact on the severity of intestinal barrier injury induced by weaning. Specifically, we showed that weaning prior to 23 d of age induced a significant disruption in intestinal epithelial barrier function (increased intestinal permeability), increased electrogenic ion transport, and intestinal inflammation, compared with pigs weaned >23 d of age (Smith *et al.*, 2010). Further investigations revealed that the heightened intestinal injury in early weaned pigs was independent of feed intake and systemic neuroendocrine responses [measured as serum corticotropin-releasing factor (CRF) and cortisol] (Moeser *et al*., 2007b). Instead, early weaning-induced intestinal barrier injury was shown to be mediated via activation of CRF receptors (Moeser *et al*., 2007a).

Long-term impact of piglet weaning age on intestinal barrier function

There is growing evidence that early life stress can impact long-term intestinal health and disease susceptibility, however the biological mechanisms remain poorly understood. We tested whether early weaning stress (EWS) influenced long-term development of colonic mucosal barrier function. In this study, pigs were subjected to EWS or standard weaning (Control, weaned at 28 d of age) and on post-weaning days 0, 1, and 60, transepithelial electrical resistance (TER) (Figure 2A) and mucosal-to-serosal fluxes of ^{3}H-mannitol (Figure 2B) were measured across porcine colonic mucosal preparations in Ussing chambers. Compared with Control pigs, pigs subjected to EWS exhibited reduced TER ($P<0.01$) and elevated ^{3}H-mannitol flux ($P<0.05$) on 1 d post-weaning and persisted at 60 d post-weaning (Smith *et al*., 2010). Similar findings were observed in the ileum (data not shown). These data demonstrated that EWS injury has lasting influences on intestinal barrier function, which is likely to be a significant factor in increased disease susceptibility and reduced performance in early weaned pigs (Main *et al*., 2004; McLamb *et al*., 2013).

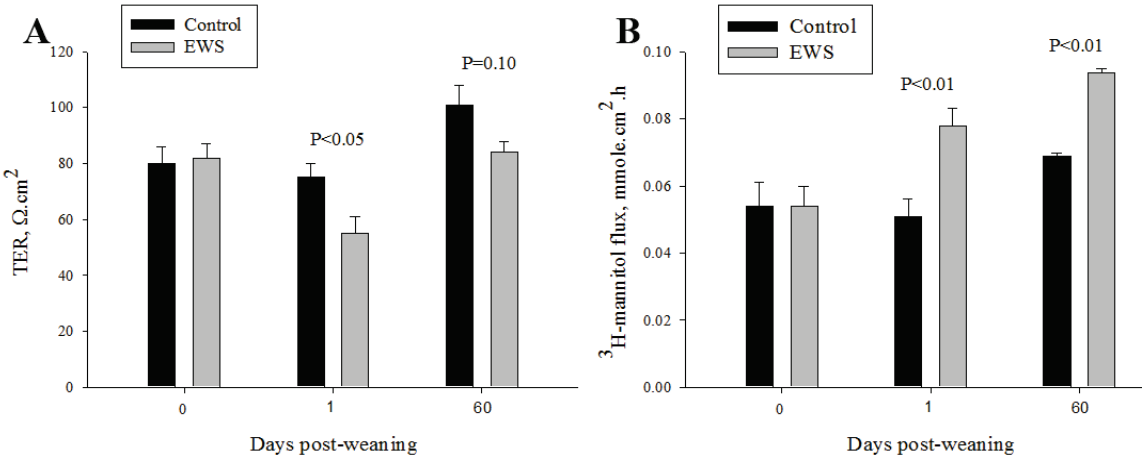


Figure 2. *Transepithelial electrical resistance (TER) and mucosa-to-serosal fluxes of ^{3}H-mannitol across colon from Control and EWS pigs. Colon from Control (28 d weaning age) and EWS (17 d weaning age) pigs were mounted on Ussing chambers on days 0, 1, and 60 post-weaning. Data represent means ±SE for n=6 pigs/group. Data were analysed using a Student's t-test within each post-weaning time point (from Smith* et al*., 2010).*

Long-term impact of early weaning stress on intestinal responses to subsequent production stress

To determine whether EWS had lasting effects on how pigs responded to stressors occurring later in production, we conducted a study with grower pigs (28 d post-weaning) that were previously subjected to EWS or weaning at 28 d of age (Control) and measured intestinal physiologic responses to a mild social stress (mixing stress). The rationale for this post-weaning time point was that this represented a common time point in which pigs are moved from the nursery to the grow-finish facilities. Following 6 h of mixing stress, EWS pigs exhibited significant elevations in colonic mucosal permeability, indicated by a reduction in TER (Figure 3A), and increased mucosal-to-serosal fluxes of ^{3}H-mannitol (Figure 3B) in colon mounted in Ussing chambers. In contrast, Control pigs subjected to the same stress did not display evidence of

mucosal barrier dysfunction. As a clinical index of intestinal dysfunction, faecal scores were recorded at the end of the 6 h mixing stress.

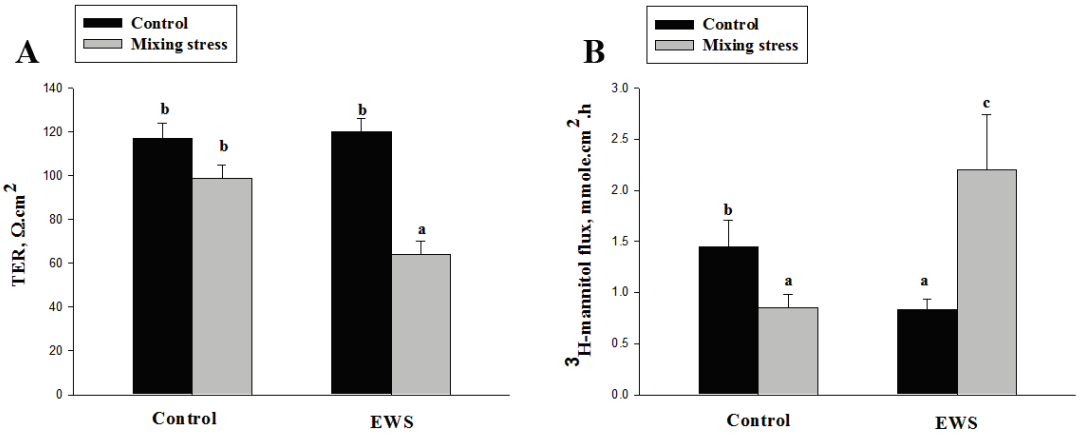


Figure 3. *Transepithelial electrical resistance (TER) and mucosa-to-serosal fluxes of ^{3}H-mannitol across colon from Control and EWS pigs subjected to mixing stress. Following a 6 h period of mixing stress, colon from Control (28 d weaning age) and EWS (17 d weaning age) pigs were harvested and mounted on Ussing chambers for measurements of intestinal permeability. Data represent means ±SE for n=6 pigs/group. Data were analysed using a 1-way ANOVA. A Tukey's test was performed to compare differences among treatment groups. Means lacking a common superscript differ (P<0.05) (Moeser,* unpublished data*).*

Early weaning stress pigs exhibited higher (P<0.01) faecal scores (mild diarrhoea) compared with non-stressed EWS pigs. In contrast, no significant changes in faecal scores were observed in Control pigs subjected to mixing stress (Figure 4).

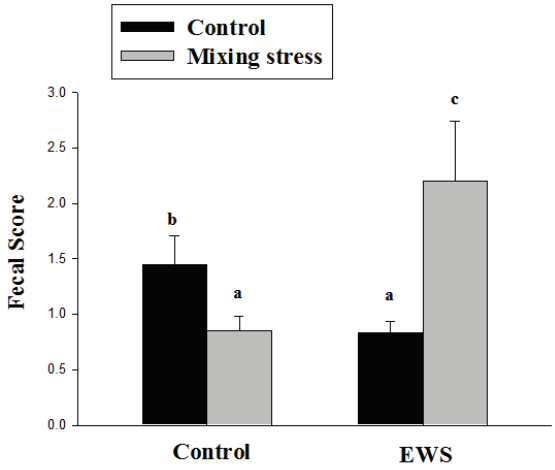


Figure 4. *Faecal scores in Control and EWS pigs in following mixing stress. Data represent means ±SE for n=6 pigs/group. Data were analysed using a 1-way ANOVA. A Tukey's test was performed to compare differences among treatment groups. Means lacking a common superscript differ (P<0.05) (Moeser,* unpublished data*).*

Impact of early weaning stress on subsequent intestinal responses to infectious challenge

Given that EWS induced lasting effect on intestinal barrier function and stress responsiveness, we investigated whether piglets subjected to EWS (16 d weaning age) influenced intestinal injury and clinical disease in response to a subsequent infectious challenge. In this study, we demonstrated that EWS pigs exhibited exacerbated clinical disease responses and more severe intestinal injury (impaired barrier function and histological damage) in response to subsequent F18 enterotoxigenic *E. coli* (ETEC) challenge, compared with pigs weaned at 22 d of age (McLamb *et al.*, 2013). Interestingly, we found that heightened disease in EWS pigs coincided with a markedly diminished ileal IL-6 response (by 70%), and IL-8 response (by 57%) compared with LW pigs. Reductions in the cytokine response correlated with reduced ileal neutrophil infiltration (by 85%). In line with the suppressed immune response against ETEC

challenge, significantly higher numbers of *E. coli* organisms were found adhered to the intestinal epithelium of EWS pigs. As mentioned above, a robust innate immune response (including pro-inflammatory cytokine release and neutrophil recruitment) is required for pathogen clearance and disease resolution; therefore, these data suggest that an important mechanism by which EWS predisposes pigs to subsequent infectious challenges may be due in part to lasting defects in innate immune function.

Pathophysiologic mechanisms of stress-induced intestinal barrier dysfunction

In previous studies, we showed that intestinal barrier injury induced by EWS was mediated by activation of stress signalling pathways in the gut mediated by CRF. Specifically we showed that administration of a CRF receptor antagonist drug (Astressin B), prior to weaning, prevented increases in intestinal permeability (Moeser *et al*., 2007a). Further investigations revealed that CRF pathways induce increases in permeability via activation of the immune system. Specifically, our laboratory has focused on the interactions between CRF and mast cells. Mast cells are innate immune cells that often reside at epithelial barriers and play important, but divergent roles, in intestinal defence and inflammation. Mast cells can modulate epithelial barrier by releasing a number of preformed granule mediators that can rapidly influence epithelial permeability, often within hours of their release (Abraham and Malaviya, 1997; Abraham and St John, 2010; Overman *et al*., 2012). Specific mast cell products that have been shown to increase intestinal permeability are mast cell proteases (tryptase, chymase), TNFα, and histamine. Our previous work demonstrated that exposure to porcine intestinal tissues to the stress mediator, CRF, induce mast cell degranulation and the specific release of mast cell proteases and TNF-α. Mast cell proteases and TNF-α were responsible for increases in intestinal permeability and disruption of the tight junction protein occludin (Overman *et al.*, 2012). It was also demonstrated that EWS in pigs induces persistent intestinal mast cell activation (Smith *et al*., 2010) and that administration of a mast cell stabilising agent, sodium cromoglycolate, prior to weaning, prevented weaning stress-induced intestinal permeability (Moeser *et al*., 2007b). The mechanism by which EWS induces persistent mast cell activation and contributes to intestinal barrier injury remains poorly understood. It is plausible that initial activation of mast cells during EWS induces intestinal barrier disturbances that, in turn, result in chronic antigen translocation from the lumen which further activates mast cells in a persistent cycle. However, the baseline intestinal inflammation in EWS intestinal tissues is relatively mild and not indicative of chronic relapsing inflammation as observed in severe inflammatory diseases such as the inflammatory bowel diseases in humans. It is also plausible that baseline intestinal permeability induced by persistent mast cell activation in EWS pigs could provide a low grade inflammatory response that "primes" the intestine to result in exacerbated intestinal responses to additional and(or) subsequent stressors (e.g., mixing stress, infection, etc.). Further understanding of these questions could lead to breakthroughs in treating intestinal disorders of pigs.

Although pig studies showed that aberrant mast cell activation is a key feature in EWS-induced intestinal disease in pigs, it should be emphasised that mast cells do play a critical defence and immunoregulatory role (Abraham and St John, 2010). Therefore, global blockade of mast cell function (e.g., sodium cromoglycolate) is likely not a viable long-term therapeutic strategy for stress-induced intestinal diseases. It is well known that intestinal mast cells play a central role in innate immune response to bacterial, parasitic, and viral infections by releasing pro-inflammatory cytokines (TNF, IL-6, LTB4) that mediate neutrophil recruitment into infected tissues and bacterial clearance (Abraham and St John, 2010; Bischoff, 2009) Specifically, it has been shown that mast-cell-derived IL-6 is a major mediator of survival from severe infections by enhancing intracellular killing of bacteria by neutrophils (Sutherland *et al*., 2008). As discussed above, our work indicated that while EWS induces chronic mast cell activation, mast cell responses to bacterial challenge were suppressed (McLamb *et al*., 2013). Specifically, we showed that pigs subjected to EWS and then later challenged with F18 enterotoxigenic *E. coli* exhibited a markedly diminished mast cell response indicated by reduced degranulation. Furthermore, suppressed ileal IL-6 and IL-8 responses were also observed. Impaired mast cell activation and cytokine release coincided with reduced ileal neutrophil recruitment, and enhanced intestinal injury and clinical disease severity. This may indicate that specific functions of the mast cell can be divergently regulated by stress.

Conclusion: Practical implications

Stress is an inherent aspect of animal production and is a major contributor to impaired performance and intestinal disorders. Despite the deleterious impact of stress on pig production and health, our understanding of the biology of stress-related intestinal disorders is limited. The research results presented here demonstrate that key early life events (weaning age) lead to not only short-term, but long-term developmental changes to the gut that can impact lifelong disease susceptibility. Specifically, these data show that early weaning (<21 d of age) leads to long-lasting stress hypersensitivity and impaired immune defenses that can markedly influence disease severity and performance. A greater understanding of the biological mechanisms by which stress impacts pig production and disease is likely to result in new

management and therapeutic strategies to enhance gut health and performance throughout the production lifespan of the pig.

References

ABRAHAM, S.N. and MALAVIYA, R. (1997). *Infection and Immunity.* **65**:3501-3508.

ABRAHAM, S.N. and ST JOHN, A.L. (2010). N*ature Reviews Immunology.* **10**:440-452.

BELAAOUAJ, A., MCCARTHY, R., BAUMANN, M., GAO, Z., LEY, T.J., ABRAHAM, S.N. and SHAPIRO, S.D. (1998). *Nature Medicine.* **4**:615-618.

BISCHOFF, S.C. (2009). *Seminars in Immunopathology.* **31**:185-205.

BOUDRY, G., PERON, V., LE HUEROU-LURON, I., LALLES, J.P. and SEVE, B. (2004). *Journal of Nutrition.* **134**:2256-2262.

KELLY, D., SMYTH, J.A. and McCRACKEN, K.J. (1990). *Research in Veterinary Science.* **48**:350-356.

MADEC, F., BRIDOUX, N., BOUNAIX, S. and JESTIN, A. (1998). *Preventative Veterinary Medicine.* **35**:53-72.

MAIN, R.G., DRITZ, S.S., TOKACH, M.D., GOODBAND, R.D. and NELSSEN, J.L. (2004). *Journal of Animal Science.* **82**:1499-1507.

MALAVIYA, R., IKEDA, T., ROSS, E. and ABRAHAM, S.N. (1996). *Nature.* **381**:77-80.

MARSHALL, J.S. (2004). *Nature Reviews Immunology.* **4**:787-799.

MCCRACKEN, B.A., ROOS, M.A., ZUCKERMANN, F.A. and GASKINS, H.R. (1999). *Journal of Nutrition.* **129**:613-619.

MCLAMB, B.L., GIBSON, A.J., OVERMAN, E.L., STAHL, C. and MOESER, A.J. (2013). *PLoS One.* **8**:e59838.

MEDZHITOV, R. and JANEWAY, C.A., Jr. (1997). *Current Opinion in Immunology.* **9**:4-9.

MOESER, A.J. and BLIKSLAGER, A.T. (2007). *Journal of the American Veterinary Medical Association.* **231**:56-67.

MOESER, A.J., KLOK, C.V., RYAN, K.A., WOOTEN, J.G., LITTLE, D., COOK, V.L. and BLIKSLAGER, A.T. (2007a). *American Journal of Physiology (Gastrointestinal Liver Physiology).* **292**:G173-181.

MOESER, A.J., RYAN, K.A., NIGHOT, P.K. and BLIKSLAGER, A.T. (2007b). *American Journal of Physiology (Gastrointestinal Liver Physiology).* **293**:G413-421.

OVERMAN, E.L., RIVIER, J.E., and MOESER, A.J. (2012). *PLoS One.* **7**:e39935.

SMITH, F., CLARK, J.E., OVERMAN, B.L., TOZEL, C.C., HUANG, J.H., RIVIER, J.E., BLIKSLAGER, A.T. and MOESER, A.J. (2010). *Am J Physiol Gastrointest Liver Physiol.* **298**:G352-363.

SUTHERLAND, R.E., OLSEN, J.S., MCKINSTRY, A., VILLALTA, S.A. and WOLTERS, P.J. (2008). *Journal of Immunology.* **181**:5598-5605.

TURNER, J.R., RILL, B.K., CARLSON, S.L., CARNES, D., KERNER, R., MRSNY, R.J. and MADARA, J.L. (1997). *American Journal of Physiology (Cell Physiology).* **273**:C1378-1385.

WADDELL, T.E. and GYLES, C.L. (1995). *Infection and Immunity.* **63**:4953-4956.

This work was funded by the United States Department of Agriculture (USDA), National Pork Board, and National Institutes of Health (grants K08 DK084313, R01 HD072968; to AJM).

SYMPOSIUM: Barrier function and systemic response of the gastrointestinal tract to the aspects of management and nutrition: Conclusions

J.R. Pluske[1] and J.C. Kim[2]

[1]Murdoch University, Murdoch, WA 6150. [2]Department of Agriculture and Food Western Australia, South Perth, WA 6151.

The paper by Dr. Kim and colleagues highlighted that the systemic immune response influences performance of pigs in two distinctive routes: alteration of nutrient partitioning, and neurological response to infection such as anorexia. The role of "nutrition" in tackling both routes of the systemic response initiated by compromised barrier function was reviewed, with attention being paid to the roles of selected amino acids and the supplementation of nutrients that reduce production of eicosanoid mediators as possible solutions to ameliorate immune system activation. Further research examining the individual and synergistic effects of putative nutrients, however, is required to establish widespread and cost-effective nutritional strategies to enhance the efficiency of growth of pigs under commercial conditions.

Pigs raised commercially are consistently exposed to (sub) clinical infections and (or) stressors, and the pigs' physiological responses to such challenges can significantly reduce growth potential and impact on disease outcomes. Research by Dr. Moeser and his team has shown that, at least in young pigs, progress has been made in understanding some of the processes by which physiological and pathophysiological stimuli, including cytokines and products released as a result of the stress response, regulate tight junction function in the gastrointestinal tract (GIT). In particular, it is evident that "stress" in the production cycle, as in the post-weaning period, leads to both short-term and long-term developmental changes to the GIT that can impact lifelong disease susceptibility.

Collectively, information presented in this symposium allows for a greater understanding of the biological mechanisms by which stress, disease and sub-optimal nutrition impacts barrier function and its subsequent effects on production and disease. It is hoped that discussion arising from this symposium will result in the investigation of new or revised management and therapeutic strategies to enhance GIT health and performance throughout the production lifespan of the pig, by focussing on either not compromising and (or) strengthening barrier function at vulnerable stages of the pig production cycle.

Betaine mitigates intestinal permeability in growing pigs induced by heat stress

N.K. Gabler[1], S. Frouel[2], A. Awati[2], A. Owusu-Asiedu[2], A.M. Amerah[2], G.G. Partridge[2] and F.R. Dunshea[3]

[1]Iowa State University, Ames, IA, USA 50011. [2] Dupont Industrial Biosciences-Danisco Animal Nutrition, Marlborough, Wiltshire, UK, SN8 1AA. [3]The University of Melbourne, Parkville, VIC 3010.

Pigs are highly heat susceptible because they lack functional sweat glands and produce a large amount of metabolic heat. As such, high ambient heat loads are an economic burden on the swine industry (St-Pierre *et al*., 2003). Heat stress (HS) causes hypoxia and endotoxaemia, reduced intestinal integrity and barrier function, which antagonises pig performance (Pearce *et al*., 2013). As severe weather fluctuations and excessively warmer than average climate conditions increase in frequency, dietary management strategies that alleviate the negative effects of heat stress are warranted. In biological systems, betaine can be used to regulate cellular homeostasis and the stress response. As explained by Eklund *et al.* (2005), betaine is a small naturally occurring trimethyl derivative of the amino acid glycine that serves as an osmolyte and methyl donor to protect cells against osmotic and temperature stresses. Our objective was to assess the ability of dietary betaine to perturb HS induced reductions in intestinal integrity in growing pigs.

Individually-penned PIC crossbred gilts (52±3.6 kg; mean±SE) were fed one of two experimental diets for 21 d: (1) Control corn-soybean-corn dried distillers grains with solubles diet (n=16; Con) or (2) Control diet containing 0.125% betaine (Bet; Betafin® natural betaine, n=8). Thereafter, eight Con-fed and eight Bet-fed gilts were moved to HS conditions for 6 h (36±2 °C, 30-50% humidity). The remaining eight Control gilts stayed in thermal neutral conditions (TN; 25 °C, 20-35% humidity). Rectal temperature (RT) and respiration rates (RR) were measured at 0, 2, 4 and 6 h. All pigs had *ad libitum* access to feed and water. After 6 h of TN or HS, all pigs were euthanised and freshly isolated ileum, caecum and colon segments were collected and mounted into modified Ussing chambers. Segment integrity was assessed via transepithelial electrical resistance (TER), and fluorescein isothiocyanate (FITC)-labelled 4.4 kDa dextran permeability (APP). All data were analysed using the PROC Mixed procedure of SAS (version 9.2).

Table 1. *Effects of betaine in mitigating heat stress (HS)-induced changes in intestinal integrity.*

Treatment	RT (°C)	RR (bpm)	Ileum TER (AU)	Cecum TER (AU)	Colon TER (AU)	Ileum APP (ug/min/cm)	Caecum APP (ug/min/cm)
TN-Control	39.2	46[a]	100[a]	100[a]	100	6.3[a]	7.0
HS-Control	40.5	129[c]	68[b]	80[b]	85	23.5[b]	13.0
HS-Betaine	40.3	114[b]	94[a]	109[a]	103	8.3[a]	8.0
SEM	0.19	6.5	6.9	7.2	13.1	4.70	3.35
Sign.	0.084	<0.001	0.005	<0.001	0.370	0.002	0.170

[a,b,c]Means in a column not having the same superscript are significantly different (P<0.05); RT, rectal temperature; RR, respiration rate (breaths per minute); TN, thermal neutral; TER, transepithelial electrical resistance; APP, 4.4 kDa FITC-Dextran apparent permeability coefficient; SEM, standard error of mean; Sign., significance.

No differences (P>0.05) in feed intake or growth rates were observed due to diet prior to stress load. Effects of the Con and Bet diets to attenuate HS-induced changes in RT, RR and intestinal integrity are reported in Table 1. Mild 6 h HS tended to increase the RT of both HS-Con and HS-Bet gilts compared to the TN-control gilts. However, HS-Bet pigs had lower (P<0.001) RR compared to the HS-Control treatment, but their RR was still elevated over the TN-Control pigs. *Ex vivo* measures of intestinal integrity showed acute heat exposure for 6 h decreased ileum (P<0.05) and caecum (P<0.05) TER in the HS-Control pigs, compared to both the HS-Bet and TN-Con pigs. The colon segment TER and APP (data not shown) were not altered due to diet or heat stress conditions. Ileum segments from the HS-Bet group also had reduced (P=0.015) macromolecule APP compared to the HS-Control pigs. The caecum and colon APP were similar (P>0.05) between the three treatments. These data indicate that natural betaine can mitigate HS induced intestinal permeability in swine.

EKLUND, M., BAUER, E., WAMATU J., and MOSENTHIN R. (2005). *Nutrition Research Reviews.* **18:**31-48.
PEARCE, S.C., MANI, V., BODDICKER, R.L., JOHNSON, J.S., WEBER, T.E., ROSS, J.W., BAUMGARD, L.H., and GABLER, N.K. (2012). *PLOS One.* 10.1371/journal.pone.0070215.
ST. PIERRE, N.R., COBANOV, B., and SCHNITKEY, G. (2003). *Journal of Dairy Science.* **86**:E52-E77.

This work was supported by DuPont Animal Nutrition.

Heat stress and feed restriction attenuates intestinal integrity in growing pigs

S.C. Pearce, D.M. van Sambeek, M.V. Sanz-Fernandez, L.H. Baumgard and N.K. Gabler

[1]Iowa State University, Ames, IA 50011, USA.

Swine are susceptible to high thermal loads that can cause acute and chronic illness and, if severe enough, mortality. It has been estimated that heat stress (HS) may cost the US swine industry over $300 million annually and global loses are in the billions (St-Pierre *et al*., 2003). Previously, we have reported that HS causes major reductions in pig performance (Pearce *et al*., 2013), alters intestinal function and integrity, and increases endotoxin load (Pearce *et al*., 2012). However, HS-induced feed intake reductions may partially explain some of the phenotype observed. Therefore, we hypothesised that short term feed intake induction is a major contributor to HS induced changes in growing pig intestinal integrity.

Individually penned PIC crossbred gilts [65±2.7 kg body weight (BW), mean ± SE] were allocated to one of three treatments: (1) thermal neutral (TN; 24 °C, 40% humidity, n=8); (2) heat stress conditions (HS; 37 °C, 40% humidity, n=8); or (3) pair-fed thermal neutral (PFTN; feed intake matched to HS counterparts and reared in TN conditions, n=8). All pigs had *ad libitum* access to water and were fed corn-soybean meal-DDGS diets [14.7 MJ/kg digestible energy (DE) and 0.82% standardised ileal digestible lysine]. After a week of acclimation under TN conditions, gilts were subjected to their environmental treatment for 12 h and then euthanised for tissue collection. Rectal temperature (RT), respiration rates (RR) and feed intake (FI) were measured at 0 and 12 h. Immediately prior to euthanasia, blood samples were collected into serum vacutainer tubes and freshly isolated ileum samples were mounted into modified Ussing chambers. Ileum intestinal integrity was assessed via transepithelial electrical resistance (TER) and fluorescein isothiocyanate (FITC)-labelled 4.4-kDa dextran permeability (APP). Blood gas variables (iSTAT®, hand held blood analyser) and concentrations of lipopolysaccharide (LPS) and LPS binding protein were also measured. All data were analysed using the PROC MIXED procedure (SAS®; USA).

Table 1. *Effects of rearing growing pigs under conditions of being thermal neutral (TN), heat stress (HS) or pair feeding (PFTN) on aspects of respiratory physiology and measures of intestinal integrity.*

Treatment	RT (°C)	RR (bpm)	PCO_2 (mm Hg)	Hct (%)	TER (AU)	APP (μg/min/cm)	LPS (AU)	LBP (μg/mL)
TN	39.2[a]	42.1[a]	52.1[a]	37.8	199.5[a]	2.4[a]	1.0[a]	14.1[a]
HS	41.8[b]	154.3[b]	31.3[b]	36.0	138.8[b]	10.5[b]	5.1[b]	7.2[b]
PFTN	39.1[a]	41.9[a]	47.3[a]	38.0	136.5[b]	10.2[b]	3.5[b]	10.5[ab]
SEM	0.1	2.5	1.1	1.3	17.9	3.0	0.68	2.61
Significance	***	***	***	NS	**	*	***	*

[a,b]Means in a column not having the same superscript are significantly different: *P<0.10; **, P< 0.05; ***, P<0.01; bpm, breaths per minute; PCO_2, partial pressure of carbon dioxide; Hct, hoematocrit; LBP, plasma LPS-binding protein, AU, arbitrary units; SEM, standard error of the mean; NS, not significant.

HS increased (P<0.01) RT, RR and blood PCO_2 compared to TN and PFTN gilts. Feed intake was reduced 90% (P<0.01; 1.06 versus 0.12 kg) in HS pigs and FI of PFTN pigs was matched to that of HS pigs. Intestinal integrity markers of TER (reduced, P<0.05) and APP (increased, P<0.10) were altered in HS and PFTN pigs compared to TN pigs. Many of the effects HS has on intestinal integrity parameters appear to be directly mediated by reduced feed intake. Interestingly, nutrient restriction alone (similar to the HS and PFTN pigs) can lead to alterations in intestinal function, transport, morphology, and may increase the risk of developing bacterial sepsis. As a result of this increased intestinal permeability, blood LPS was augmented and LBP attenuated due to HS and PFTN conditions. This may contribute to systemic inflammation and reduced growth potential in pigs. Altogether, these data indicate that high ambient heat loads and reduced energy intake negatively affect intestinal integrity and increase circulating LPS in pigs.

PEARCE, S.C., MANI, V., BODDICKER, R.L., JOHNSON, J.S., WEBER, T.E., ROSS, J.W., BAUMGARD, L.H., and GABLER, N.K. (2012). *Journal of Animal Science*. **90(Suppl. 4):**257-259.

PEARCE, S.C., GABLER, N.K., ROSS, J.W., ESCOBAR, J., PATIENCE, J.F., RHOADS, R.P., and BAUMGARD, L.H. (2013) *Journal of Animal Science*. **91(5):**2108-18.

St. PIERRE, N.R., COBANOV, B. and SCHNITKEY, G. (2003). *Journal of Dairy Science*. **86:**E52-E77.

This work was supported by USDA NIFA grant #2011-67003-30007.

Feeding a diet containing raw potato starch influences faecal consistency and digesta pH in weaner pigs

J.M. Heo[1,2], A.K. Agyekum[1] and C.M. Nyachoti[1]

[1]University of Manitoba, Winnipeg, MB R3T 2N2, Canada. [2]Chungnam National University, Daejon 305-764, South Korea.

Previous research has demonstrated that feeding nursery pigs a diet containing 7% raw potato starch (RPS) reduced post-weaning diarrhoea (PWD) without any adverse effects on growth performance, even though no effects were found on PWD in weaned pigs fed a diet containing 14% RPS (Bhandari *et al*., 2009). With this in mind, the overall purpose of this study was to determine the effects of dietary inclusion of lower amounts of RPS on characteristics of digestive function in weaner pigs. The study also examined a convenient alternative delivery system through the creation of RPS in capsules. In RPS capsule form, it is possible that there may be a time-release action through the large intestine that may be of further benefit. The hypothesis tested was that supplementation of RPS at low levels (i.e., 0.5 and 1.0%) in nursery pig diets would reduce the incidence of PWD with improvement in indicators consistent with gastrointestinal health. Those effects would be more desirable in pigs fed a diet containing RPS in capsules.

Sixty piglets (Yorkshire-Landrace × Duroc; 1:1 male and female gender ratio) weaned at 21±2 d (mean±SEM) with an initial body weight (BW) of 7.17±0.78 kg were assigned in a completely randomised design to one of five dietary treatments to give six observations per treatment and two pigs per pen. Dietary treatments consisted of a negative control corn-soybean meal-based diet (NC; no antimicrobial agents added) or the NC supplemented with RPS either as powder or in capsules, with each included at 0.5 or 1.0% as a top dressing on each day. Diets were formulated to meet nutrient requirement of pigs (14.8 MJ digestible energy (DE)/kg, 0.82g standardized ileal digestible lysine/MJ DE, 23% CP; NRC, 1998). Pigs were offered the experimental diets on an *ad libitum* basis for 28 d and drinking water was available at all times. Faecal consistency (FC) scoring was determined daily for the first 14 d after weaning (after Heo *et al*., 2008). At the conclusion of the study, six pigs per treatment (three of each gender) with a BW closest to the pen mean were euthanised to measure digesta pH at the ileum and caecum. As no gender effects (P>0.05) were detected, data were pooled and analysed for dietary treatment effects using the GLM procedure of SPSS (version 18.0, SPSS Inc., Chicago, Illinois, USA).

Table 1. *Faecal consistency and digesta pH in weaner pigs (n=6) fed a diet without or with raw potato starch for 14 d after weaning.*

Item	NC	0.5% RPS-P	0.5% RPS-C	1.0% RPS-P	1.0% RPS-C	SEM	Significance
Faecal consistency[1],%							
d 0-7	31.4	29.3	30.5	28.6	28.6	0.40	0.116
d 8-14	49.0^{a}	42.6^{b}	45.2^{ab}	35.7^{c}	32.0^{c}	0.66	<0.001
d 0-14	40.2^{a}	36.0^{b}	37.9^{ab}	32.1^{c}	30.3^{c}	0.45	<0.001
pH							
Ileum	7.7^{a}	7.4^{b}	7.3^{b}	7.3^{b}	7.2^{b}	0.03	0.002
Caecum	6.4^{a}	6.2^{b}	6.3^{b}	6.2^{b}	6.2^{b}	0.02	<0.001

^{a,b}Means in a row not having the same superscript are significantly different (P<0.05); NC, negative control without RPS or antimicrobial agents; 0.5% RPS-P, negative control + 0.5% RPS as powder; 0.5% RPS-C , negative control + 0.5% RPS in capsules; 1.0% RPS-P, negative control + 1.0% RPS as powder; 1.0% RPS-C, negative control + 1.0% RPS in capsules; SEM, standard error of mean; [1]Faecal consistency scoring [0 (0%), very hard; 1 (20%); well-formed faeces; 2 (40%), soft-formed; 3 (60%), sloppy; 4 (80%), pasty diarrhoea; 5 (100%), liquid diarrhoea.

Resistant potato starch supplementation improved (P<0.001) FC, and pigs fed the 1.0% dose of RPS had more solid faeces (P<0.05) compared with 0.5% RPS during the first 14 d after weaning, independent of the form of RPS. Furthermore, RPS supplementation decreased (P<0.05) ileal and caecal digesta pH (Table 1). The results of this experiment indicated that supplementing a weaner pig diet with at least 0.5% RPS independent of mode of delivery has the potential to enhance outcomes characteristic of a functional gut in weaned pigs without adverse effects on growth (data not shown).

BHANDARI, S.K., NYACHOTI, C.M. and KRAUSE, D.O. (2009). *Journal of Animal Science*. **87:**984-993.
HEO, J.M., KIM, J.C., MULLAN, B.P. HAMPSON, D.J. and PLUSKE, J.R. (2008). *Archives of Animal Nutrition*. **62:**343-358.
NRC. (1998). "Nutrient requirements of swine", 10th ed. (The National Academy Press: Washington).

This research was financially supported by Agri-Food Research and Development Initiative (ARDI) and Manitoba Starch Products in the Province of Manitoba, Canada.

Effect of diet type on post-weaning diarrhoea in ETEC-challenged pigs and susceptibility towards F4ab/ac fimbriae

J.W. Resink, J.C. Mancera Gracia, P.J. Roubos and H. van Hees

Nutreco R&D, Boxmeer, 5830 AE The Netherlands.

Post-weaning diarrhoea (PWD) caused by certain serotypes of enterotoxigenic *E. coli* (ETEC) is a major problem in weaner pigs. In the development of PWD, the stress pigs experience plays an important role (Pluske *et al*., 1997). Factors such as sub-optimal room temperatures, changes in the type of food and shifts in the gut microbiota could all increase the risk on PWD. The relative impact of these factors on faecal consistency was tested in weaner pigs with different susceptibilities for F4 fimbrial attachment.

A total of 120 pigs (Hypor x Top-Pie) were weaned at 25±1 d of age (8.3 kg±0.9 kg; mean±SEM). Pigs were equally divided across experimental treatments based on gender, litter and the presence of a genetic receptor for ETEC F4ab/ac fimbriae (Jensen *et al*., 2006). The experiment used a split plot design. The whole plot was ETEC challenge or no challenge both at normal room temperatures, and ETEC challenge with a suboptimal room temperature (27^0 C instead of 30^0 C in week one post-weaning and a steady decrease to 24^0 C instead of 27^0 C thereafter). Dietary treatments and antimicrobial pre-treatment were used as subplots arranged in a completely randomised 2×2 factorial design. The dietary treatments consisted of a low protein MW diet (complex Milkiwean Granito diet, Trouw Nutrition Belgium; 10.5 MJ net Energy (NE)/kg; 160 g crude protein (CP)/kg) or a high risk diet, HR (10.5 MJ NE/kg; 220 g CP/kg). The antimicrobial treatment consisted of an oral dose of 200,000 IU colistin sulphate/kg body weight on d 4 post-weaning or no oral dose. The pelleted weaner diets were fed directly after weaning until d 14. From d 15-28 all animals received the same pelleted diet (10.0 MJ NE/kg; 168 g CP/kg). Feed and water were provided *ad libitum.* For the challenge, *E. coli* type O149K88acK91 was cultured overnight in brain heart infusion medium. Pigs were challenged three times (on d 6, 7 and 8 post-weaning) with $5x10^8$ CFU ETEC per piglet per day. Faecal consistency was scored daily from 0 (firm, well-formed) to 3 (diarrhoea), and analysed with χ^2 homogeneity test of the Catmod procedure (SAS®; USA).

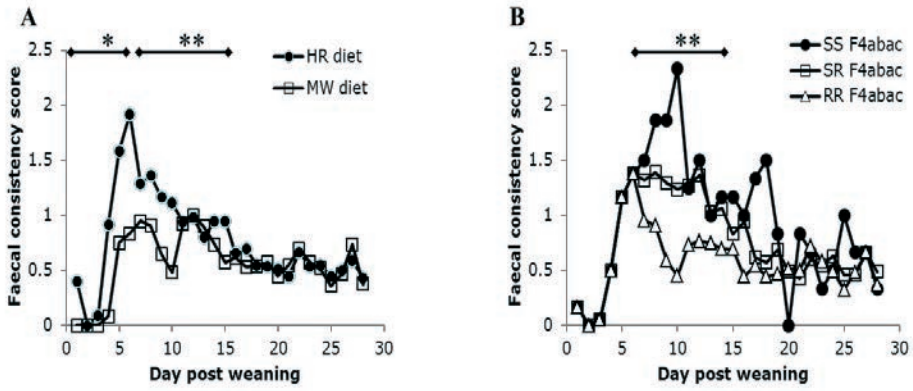


Figure 1. *The effect of diet type (A) and susceptibility for binding to* E. coli *F4ab/ac fimbriae (B) on average faecal consistency score. SS: homozygote susceptible; SR: heterozygote susceptible; RR: homozygote resistant; * P<0.05 in period from d 0-5; ** P<0.01 in period from d 6-14 (for HR diet > MW diet in A and SS>SR and SR>RR in B).*

The susceptibility of pigs for binding to *E. coli* F4ab/ac fimbriae as well as the diet composition had a significant effect on faecal consistency score (Figure 1) while room temperature and colistin pre-treatment did not have a significant effect. However, ETEC challenged pigs that were not pre-treated with colistin showed a lower (P<0.05) incidence of diarrhoea from d 21-28 post weaning (4% instead of 16-19% in the other groups; data not shown). These results indicate that preventive antibiotic treatment is not always beneficial. In addition, the presence of an intestinal receptor for adhesive ETEC F4ab/ac fimbriae as well as the complexity and/or the protein level of the weaner diet play crucial roles in the development of PWD.

JENSEN, G. M., FRYDENDAHL, K. SVENDSEN O., JØRGENSEN C.B, CIRERA, S. FREDHOLM M., NIELSEN, J.P. and MØLLER, K. (2006). *Veterinary Microbiology.* **115**:243-249.

PLUSKE, J. R., HAMPSON, D.J. and WILLIAMS, I.H. (1997). *Livestock Production Science.* **51**:215-236.

Evaluation of an in-feed acid to control *Salmonella* spp. in growing pigs

C.L. Collins[1], P. McKenzie[2], M. van der Heijden[3], K.S. Sawyer[1], C. Clark[4] and B.G. Luxford[1]

[1] Rivalea Australia Pty Ltd, Corowa NSW 2646. [2] Scolexia Pty Ltd, Moonee Ponds, VIC 3039. [3]Selko Feed Additives, Tilburg, The Netherlands. [4] AusPac Ingredients Pty Ltd, Tamworth, NSW 2340.

Salmonella spp. infection in growing pigs can be associated with reduced growth performance and a reduction in the number of full value pigs sold out at the end of a batch. Good hygiene practices and the use of all in all out (AIAO) housing will not eliminate the risk of S*almonella* spp. infection and other means to aid control need continual assessment. The use of formic acid in feed has shown potential to reduce *Salmonella* spp. serum antibodies and faecal excretion in finisher pigs (Creus *et al.*, 2007). A blended product containing formic acid (Fysal™ Nutreco, The Netherlands) has been used in the field at 2 kg/tonne by comparison across a large number of pigs (>100,000), with particular success in poor hygiene environments; 1 kg/tonne was ineffective (P. McKenzie, *pers. comm*.). As such, a dose-response study in a controlled environment was warranted to determine the magnitude of this response when pigs are housed in a conventional AIAO environment on concrete floors. The study tested the hypothesis that the inclusion of Fysal™ in feed will reduce *Salmonella* spp. prevalence and improve pig survival from 14 weeks of age through to sale.

A total of 3,258 pigs (1,641 females and 1,617 Improvac-vaccinated males, Large White x Landrace, PrimeGro™ Genetics) were housed in a commercial finisher facility in pens of approximately 38 pigs per pen (84 pens in total). At 14 weeks of age (average pig weight 43.5 kg ± 0.41; mean±SEM) pens were allocated within sex to one of four dietary treatments: A: control, no Fysal, B: 1.5 kg/t Fysal in feed, C: 3 kg/t Fysal in feed, D: Fysal step down program – Fysal in feed at 3 kg/t from d 0 to d 21, followed by 1.5 kg/t Fysal in feed from d 21 to d 56. All test diets were pelleted and fed *ad libitum* for the entire period. Body weights on a pen basis were recorded at d 0, 21 and 56, with feed intake and feed efficiency measured on a pen basis during this time. Faecal samples were collected from five pigs per pen at d 0, d 21 and again pre-slaughter. Faecal samples were analysed fresh for *Salmonella* spp. prevalence (culture method ISO 6579:2002/Amd 1:2007(E)) by the South Australian Research Development Institute. Pens with at least one positive sample were considered *Salmonella* spp. positive. Mortality and pen *Salmonella* spp. status were analysed for association with dietary treatment using a Chi-squared test. Growth performance and feed efficiency were analysed using ANOVA. All analyses were undertaken using Genstat (VSN International, Oxford UK).

Table 1. *Influence of dietary treatment on pig mortality and the prevalence of* Salmonella *spp. within pen.*

		Salmonella spp. d 0		*Salmonella* spp. d 21		*Salmonella* spp. d 56	
	Mortality (%)	- ve	+ ve	- ve	+ ve	- ve	+ ve
Control	2.7[a]	14	7	15	6	2[a]	19
Fysal 1.5 (kg/t)	1.1[b]	12	9	16	5	6[a,b]	15
Fysal 3 (kg/t*)	1.5[a,b]	11	10	13	6	8[b]	11
Step down	1.9[a,b]	13	8	16	5	8[b]	13

[ab]Values within a column not having the same superscript are significantly different (P<0.05); -ve number of *Salmonella* spp. negative pens; +ve number of *Salmonella* spp. positive pens.*Two pens omitted after d 0 due to a broken fence.

Prevalence of *Salmonella* spp. was similar across each of the dietary treatments at the start of the test period and again at d 21 (Table 1). In comparison, analysis of the faecal samples obtained at d 56 showed a marked increase in the overall number of *Salmonella* spp.-positive pens, with the inclusion of acid in feed maintaining a greater number of *Salmonella* spp. -negative pens compared to the controls. Average feed intake and rate of gain were similar across the treatment groups (data not shown). Mortality was reduced when pigs were offered Fysal at 1.5 kg/t for the entire test period compared to the control group (Table 1). Pen feed efficiency reflected the fewer deaths/removals in the Fysal treatment groups, although not significant [feed conversion ratio d 0-56: Control = 2.71, Fysal 1.5 kg/t = 2.59, Fysal 3kg/t = 2.61, step down = 2.63, P=0.12 (SED 0.055)]. The data presented herein support the use of Fysal™ to aid in the management of *Salmonella* spp. in growing pigs under the conditions of AIAO housing and concrete floors.

CREUS E,E., PEREZ, F.J., PERALTA, B., BAUCELLS, F. and MATEU, E. (2007). *Zoonoses Public Health*. **54**:314-319.

Addition of a xylanase enzyme complex to a corn-based diet influences gut morphology in newly weaned pigs

L. Kang[1], D.W. Zheng[1], Y. Feng[1], Z.Z. Zhang[1], A.T. Hung[1,2], J.C.Kim[3] and B.J. Hosking[1]

[1]Asiapac Biotechnology, Dongguan City, Guangdong 523808, PRC. [2]The University of Melbourne, Parkville, VIC 3010. [3]Department of Agriculture and Food Western Australia, South Perth, WA 6151.

Enzyme complexes are a common inclusion in feeds for young and newly weaned pigs to assist an immature digestive system. Multi-carbohydrases supplements have been reported to increase ileal dry matter (DM) and non-starch polysaccharide (NSP) digestibilities in young pigs (Kiarie *et al.,* 2007). Supplementation with multicarbohydrases increases the low molecular weight fibre that may serve as substrates for residual microorganisms, which then may influence the intestinal structure. This study examined features of gut morphology following the introduction of a xylanase-based enzyme formulation in a corn-soy based ration for young pigs. The hypothesis tested was that weaner pigs fed a diet with multi-carbohydrases will better maintain the intestinal structure than pigs fed a diet without multi-carbohydrases in the first 18 d after weaning

A total of 22 male pigs [Large White x Landrace x Duroc, 3-4 weeks old; initial live weight (LW) 7.4±0.09 kg; mean+SE] were obtained and six pigs were then euthanised at the start of the experiment to collect basal data. The remaining 16 pigs were housed in a climate-controlled room at a density of six pigs/pen and adjusted to three pigs/pen after d 9. Pigs were fed a commercial pig starter diet either without or with an enzyme complex providing a combination of xylanase, amylase, proteases and mannanase at 1.6, 0.6, 0.8 and 0.2 U/g, respectively. The diets [14.0 MJ digestible energy (DE)/kg, 1.4% digestible lysine] were pelleted at 50-60 ^{o}C and then crumbled. The diets were fed *ad libitum*. Gut morphology was examined via assessment of villous height and crypt depth in the duodenum (at 5% of SI), jejunum (at 50% of SI) and ileum (at 95% of SI) at slaughter on d 9 and 18 (n=4). Samples were preserved in formalin prior to sectioning and microscopy. The intestinal structure was expressed as villous height to crypt depth ratio (VH:CD). Statistical analysis was undertaken using a GLM procedure (Minitab v14) to test the effect of enzyme on VH:CD on d 9 and 18.

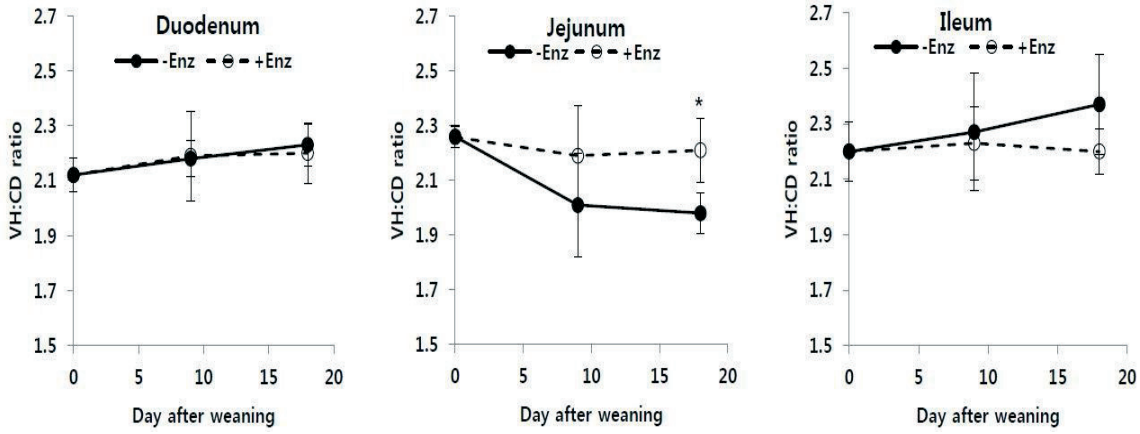


Figure 1. *Villous height to crypt depth ratio (VH:CD ratio, mean±SEM) as an indicator for intestinal structure measured with weaned male pigs after 9 and 18 d of feeding a corn-based diet with and without an enzyme complex containing xylanase.* (*Significance: * P<0.05).*

Pigs fed a corn-soybean meal-based diet with multi-carbohydrases' supplementation better maintained intestinal structure, measured as VH:CD ratio in the jejunum (P<0.05, Figure 1), but not in the duodenum and ileum. The higher VH:CD ratio in the jejunum from the pigs fed the diet with enzyme resulted from higher villi without changes in crypt depth. Since feed intake was not affected by the multi-carbohydrases supplementation (data not shown), the greater VH:CD ratio in the jejunum may not be confounded with the energy intake.

KIARIE, E., NYACHOTI, C.M., SLOMINSKI, B.A. and BLANK, G. (2007). *Journal of Animal Science.* **85**:2982-2993.

Acknowledgement is expressed to Dr NaShun Bayaer, South China Medical University, for assistance with dissections and imagery.

Pigs kept under commercial conditions respond to a higher dietary tryptophan:lysine ratio immediately after weaning

M.M. Capozzalo[1], J.C. Kim[2], J.K. Htoo[3], C.F.M. de Lange[4], B.P. Mullan[2], J.W. Resink[5], C.F. Hansen[6], P.A. Stumbles[1], D.J. Hampson[1], N. Ferguson[7] and J.R. Pluske[1]

[1]Murdoch University, Murdoch, WA 6150. [2]Department of Agriculture and Food WA, South Perth 6151. [3]Evonik Industries AG, Hanau, Germany. [4]University of Guelph, Guelph, ON N1G 2W1, Canada. [5]Nutreco R&D, Boxmeer, 5830 AE The Netherlands Boxmeer 5830, The Netherlands. [6]University of Copenhagen, Copenhagen, Denmark. [7] Westpork Pty Ltd, Maylands, WA 6051.

The recommended standardised ileal digestible (SID) ratio of tryptophan to lysine (Trp:Lys) is 0.16 for 7-11 kg pigs (NRC, 2012). However, Simongiovanni *et al.* (2012) showed the requirement for dietary Trp increases during inflammatory states, such as in the post-weaning period, suggesting that NRC (2012) recommendations might be insufficient. This study hypothesised that weaner pigs kept in commercial conditions will show a positive response to increased Trp:Lys ratios above the NRC (2012) recommendations.

Mixed-sex weaner pigs (Landrace x Large White; n=2,430; seven pens/treatment) were distributed to one of six treatments according to body weight (BW) and sex. Two wheat-based diets were formulated (15.0 MJ digestible energy/kg, 1.4% SID Lys) for SID Trp:Lys ratios (after Sauvant *et al*., 2004) of 0.16 and 0.26, with synthetic Trp added to the 0.26 diet. Diets were then blended to obtain intermediate SID Trp:Lys values of 0.18, 0.20, 0.22 and 0.24. The analysed Trp:Lys ratios were 0.18, 0.19, 0.21, 0.22, 0.24 and 0.26. Pigs were fed experimental diets for two weeks after weaning. Pig BW and feed intake were measured weekly and blood samples taken on d 4 and 11 (eight pigs/pen) for analysis of C-reactive protein (CRP), an inflammation marker. Data were analysed using GLM procedures in SAS (Ver 9.3), and linear and quadratic polynomial effects were examined.

Table 1. *Effect of Trp:Lys ratio on performance and CRP levels of pigs from weaning until 14 d after weaning. Values are expressed as least square means with pooled SEM.*

	Analysed Trp:Lys							Significance		
	0.18	0.19	0.21	0.22	0.24	0.26	SEM	Linear	Quad	ANOVA
ADG, g										
d 0-7	194	194	171	198	230	217	13.6	0.036	0.235	0.059
d 8-14	397	384	402	369	424	418	14.7	0.136	0.184	0.119
d 0-14	296^{ab}	289^{ab}	287^{a}	283^{a}	327^{c}	317^{bc}	9.81	0.012	0.073	0.014
ADFI, g										
d 0-7	282	299	289	282	288	328	12.9	0.091	0.144	0.151
d 8-14	548^{a}	542^{a}	532^{a}	498^{a}	523^{a}	646^{b}	23.1	0.047	0.001	0.003
d 0-14	415^{a}	420^{a}	411^{a}	390^{a}	406^{a}	487^{b}	12.7	0.010	<0.001	<0.001
FCR, g/g										
d 0-7	1.47	1.57	1.71	1.46	1.31	1.51	0.093	0.297	0.445	0.074
d 8-14	1.39^{ab}	1.43^{bc}	1.33^{ab}	1.37^{ab}	1.24^{a}	1.56^{bc}	0.064	0.495	0.036	0.034
d 0-14	1.42^{b}	1.47^{b}	1.44^{b}	1.39^{ab}	1.24^{a}	1.54^{b}	0.052	0.818	0.114	0.007
CRP, μg mL^{-1}										
d 4	16.2	15.8	19.6	14.1	19.5	18.4	2.12	0.364	0.882	0.382
d 11	14.7	15.8	14.2	10.4	17.3	11.6	2.00	0.384	0.982	0.161

[a, b, c, d]Means in the same row with different superscripts differ; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; CRP: C-reactive protein.

A linear effect was observed for BW at d 14 (P=0.034; data not shown) and ADG (P=0.012) in the first 14 d after weaning. The ADFI showed linear (P=0.047) and quadratic (P<0.001) effects for d 8-14 and the entire 14 d period. Pigs fed Trp:Lys ratios of 0.22 and 0.24 were most efficient at converting feed to body gain between d 8-14 (P=0.034) and in the entire 14 d period (P=0.007), respectively. The CRP levels suggested that a minimal inflammatory challenge occurred in this study. In agreement with Capozzalo *et al*. (2012), these data suggest a Trp:Lys ratio of 0.22-0.24 for optimal feed efficiency in commercially housed pigs immediately after weaning.

CAPOZZALO, M.M., KIM, J.C., HTOO, J.K., DE LANGE, C.F.M., MULLAN, B.P., HANSEN, C.F., RESINK, J.W., STUMBLES, P.A., HAMPSON, D.J. and PLUSKE**,** J.R. (2012). *Journal of Animal Science.* **90:**191-193.

NRC (2012). "Nutrient requirements of swine", 11th rev. ed. National Research Council (NAP, Washington, DC, USA).

SAUVANT, D., PEREZ J.-M. and TRAN, G. (2004). "Tables of composition and nutritional value of feed materials". (Wageningen Acad. Publ., Wageningen)

SIMONGIOVANNI, A., CORRENT, E., LE FLOC'H, N. and VAN MILGEN, J. (2012). *Animal.* **6:**594–602.

Supported through an Australian Research Council-Linkage grant (LP110100399) and Murdoch University.

Xylanase improves nutrient digestibility and growth performance in pigs fed a wheat- and barley-based diet containing cereal by-products

A. Owusu-Asiedu[1], A. Péron[2], Y. Ru[2] and G. Dusel[3]

[1]Dupont Industrial Biosciences-Danisco Animal Nutrition, Marlborough, Wiltshire, United Kingdom, SN8 1AA. [2]Danisco Singapore Pte Ltd, 61 Science Park Road, Singapore 117525. [3]University of Applied Sciences, FB 1- Life Sciences D-55411 Bingen am Rhein, Germany.

There is a strong economic need to replace nutrient rich conventional ingredients such as cereal grains and soybean meal (SBM) in swine feeds. By-products (BP) such as canola meal (CM), corn distillers dried grains with soluble (CDDGS) and wheat shorts are alternative substitutes, if available, but contain high levels of non-starch polysaccharides (NSP). Unfortunately, swine lack fibre degrading enzymes and are therefore not able to efficiently utilise these by-products (Bedford and Classen, 1992). It is therefore expected that diets containing these ingredients would respond positively to the addition of exogenous enzymes that act on the substrate, allowing an increase use of these by-products in pig diets, without negatively affecting animal performance. The current study evaluated efficacy of xylanase from *T. reesei* on performance and total tract digestibility in pigs fed wheat/barley-based diets containing high cereal BP.

The two experimental diets were; 1) a control containing wheat-barley, SBM, CM and 40% cereal BP (wheat bran/middlings; 20% and CDDGS; 20%), formulated to meet or exceed NRC (1998) requirements for pigs (25 to 110 kg body weight (BW) (CON), and 2) CON plus 2000 u/kg feed of xylanase (XYL). Xylanase was supplied by Danisco Animal Nutrition, Marlborough, UK). The formulated dietary metabolisable energy and digestible lysine contents were 12.7 and 12.5 MJ/kg and 0.95 and 0.85% in grower and finisher phase diets, respectively. Phytase (Phyzyme XP®, 500 FTU kg^{-1} feed) was added to all diets and titanium dioxide (0.3%) was added as a marker to help determine the coefficient of apparent total tract digestibility (CATTD) at the end of the grower phase. Thirty-six surgical castrates with initial mean BW of 29.0 kg were randomly assigned to pens to give nine pens/treatment. Pigs had free access to feed and water for the entire 86 d of the trial. Average daily feed intake (ADFI), average daily gain (ADG) and feed to gain ratio (F:G) were determined. Data were analysed using a mixed model (SAS®; USA).

Table 1. Effects of X*ylanase supplementation on performance and CATTD in pigs fed high fibre diets.*

	Control	Xylanase	SEM	Significance
CATTD				
Crude protein	0.68	0.74	0.007	0.100
Crude fibre	0.22[a]	0.30[b]	0.002	0.050
Energy	0.72[a]	0.75[b]	0.006	0.010
Growth performance (d 0-86)				
Final BW (kg)	100.2	104.7	1.26	0.070
Total weight gain (kg)	70.5[a]	75.1[b]	1.10	0.030
ADFI (kg/d)	2.40	2.41	0.06	0.970
ADG (g/d)	860[a]	915[b]	13.4	0.030
F:G	2.81	2.63	0.08	0.270

[a,b] Means within a row with not having the same superscript differ significantly (P < 0.05); SEM, standard error of mean.

Compared with the control diet, adding XYL increased (P<0.05) the CATTD of crude fibre and energy by 35.4 and 3.3%, respectively. Overall weight gain and ADG increased (P<0.05) by 6.5 and 6.3%, respectively, with XYL addition compared with the control diet. Xylanase addition had no effect (P>0.05) on ADFI and FCR (Table 1). In conclusion, addition of XYL increased total tract utilisation of diet components and performance in pigs fed a diet containing a high level of cereal by-products.

BEDFORD, M. R. and CLASSEN, H. L. (1992). *Journal of Nutrition.* **122**:560-569.
NRC. (1998). "Nutrient requirements of swine", 10th ed. (The National Academy Press: Washington).

Supported financially by Danisco Animal Nutrition UK Ltd.

The effects of dietary lysine concentration on growth performance of pigs from weaning to 15 weeks of age

A.E. Taylor[1], S. Jagger[2] and H.M. Miller[1]

[1]University of Leeds, Leeds, LS2 9JT, UK. [2]ABN, Peterborough, PE2 6FL, UK.

Compensatory growth is a phenomenon whereby an animal accelerates its growth after a period of suppressed growth, usually as a result of feed restriction (Hornick *et al.*, 2000). Compensatory growth may be a feeding strategy used to reduce feed costs and improve efficiency; however there are conflicting results in the literature on whether animals previously restricted subsequently exhibit compensatory gains. Previous studies in our group (Taylor *et al*., 2013) demonstrated full compensation wherein pigs fed a low lysine (Lys) [8.0 g/kg total Lys, 6.4 g/kg ileal digestible (ID) Lys] diet throughout the weaner stage demonstrated accelerated growth rates when fed a diet with Lys concentrations above that recommended by BSAS (2003). Control pigs received a high Lys weaner diet (17.5 g/kg total Lys, 15.2 g/kg ID Lys) followed by the same high Lys diet as pigs fed the low Lys diet. However were control pigs maximising growth at this Lys concentration? The hypothesises for this experiment were: 1) pigs previously fed the low Lys diet would show compensatory growth once moved onto a high Lys diet, and 2) growth performance of control pigs would not be enhanced by feeding higher dietary Lys levels.

A total of 196 mixed-sex pigs [Hampshire sire x (Large White x Landrace) dam] weighing 8.2±0.07 kg (mean±SEM) were weaned at 27.4±0.05 d of age and remained on trial for 11 weeks. Pigs were separated into seven pigs per pen creating seven replicates. Pigs were given *ad libitum* access to a high [17.5 g/kg total Lys, 15.2 g/kg ID Lys, 11.8 MJ/kg net energy (NE)] or a low (8.0 g/kg total Lys, 6.4 g/kg ID Lys, 11.8 MJ/kg NE) Lys diet throughout the weaner stage (4-7 weeks of age) followed by one of three diets during the grower (7-12 weeks of age) and finisher stage (12-15 weeks of age) creating four dietary treatments: 1) Control-Standard (CS); the high Lys diet followed by a diet containing 15.5 g/kg and 12.0 g/kg total Lys for the grower and finisher stage respectively; 2) Control-Higher (CH); the high Lys diet followed by a diet containing 18.0 g/kg and 14.0 g/kg total Lys; 3) Control-Lower (CL); the high Lys diet followed by a diet containing 13.0 g/kg and 10.0 g/kg total Lys; and 4) Weaner Restrict-Standard (WR-S); the low Lys diet followed by a diet containing 15.5 g/kg and 12.0 g/kg total Lys. Pigs were weighed at 4, 7, 12, and 15 weeks of age. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were recorded. Data were analysed using a GLM (Minitab 15.0) with pairwise comparisons.

Table 1. *Impact of feeding regime on growth performance from weaning through to 15 weeks of age.*

	Control			Weaner Restrict-Standard	SE	Significance
	Standard	Higher	Lower			
BW start (kg)	8.3	8.1	8.3	8.3	0.15	0.892
BW 7 weeks of age (kg)	15.2^{a}	15.3^{a}	15.5^{a}	12.0^{b}	0.29	<0.001
BW 12 weeks of age (kg)	42.6^{a}	42.2^{a}	42.9^{a}	38.1^{b}	0.51	<0.001
BW end (kg)	60.5^{a}	59.2^{ab}	57.7^{b}	54.3^{c}	0.66	<0.001
ADFI (kg)	1.183^{a}	1.151^{ab}	1.154^{ab}	1.062^{b}	0.0242	0.013
ADG (kg)	0.689^{a}	0.674^{a}	0.652^{a}	0.608^{b}	0.0081	<0.001

^{a,b,c}Means in a row not having the same superscript are significantly different ($P < 0.05$); BW, body weight; SE, standard error.

At 7 weeks of age the control pigs were heavier than WR pigs (P<0.001) due to a higher feed intake (P<0.005), a higher rate of gain (P<0.001) and a lower FCR (P<0.001). This weight difference was still apparent at 12 weeks of age (P<0.001). At the end of the trial the CS pigs were heavier than WR-S pigs (P<0.001). Increasing the dietary Lys concentration of control pigs after the weaner stage produced no further increase in growth rate; in contrast, reducing Lys concentration of the control pigs reduced growth rate indicating that the CS combination of diets provided sufficient Lys to maximise pig growth. In contrast to previous studies (Taylor *et al*., 2013), no evidence of compensatory growth was observed here. The WR-S pigs had the lowest feed intake which may have prevented these pigs from compensating.

BSAS. (2003). Nutrient Requirement Standards for Pigs. British Society of Animal Science, Midlothian, UK.

HORNICK, J.L., VANEENAEME, C., GERARD, O., DUFRASNE, I. and ISTASSE, L. (2000). *Domestic Animal Endocrinology.* **19**:121–132.

TAYLOR, A.E., JAGGER, S., TOPLIS, P., WELLOCK, I. and MILLER, H.M. (2013) . In "Proceedings of the British Society of Animal Science". p. 86. (The British Society of Animal Science: Nottingham, UK).

Supported by BBSRC and ABN.

Role of resistant starch from different sources on *in vitro* production of short-chain fatty acids in a pig model

G. Giuberti, A. Gallo, M. Moschini, L. Fiorentini and F. Masoero

Università Cattolica Sacro Cuore, 29122 Piacenza, Italy.

There is growing interest in incorporating nutrients that may act as potential prebiotic sources in pig diets, including resistant starch (RS). Pig colonic bacteria ferment RS to short-chain fatty acids (SCFA) that can exert several physiological effects related to energy supply and renewal of intestinal cells (Bach Knudsen *et al.*, 2012). The aim of this work was to evaluate whether the fermentation of RS obtained from different starch sources may influence SCFA fermentation patterns and related kinetics, in an effort to modulate fermentation end products by the pig microbial community.

An *in vitro* experiment based on enzymatic digestion followed by fermentation was conducted. Five native purified starches were tested (Table 1). Each ingredient was pre-treated with a pepsin-pancreatin hydrolysis (Boisen and Fernàndez, 1997). Then, 200 mg of each hydrolysed RS residue was incubated in triplicate in glass bottles in a buffered mineral solution inoculated with pig faeces (Giuberti *et al.*, 2013). Two fermentation runs were set up in two different days and bottles within runs were considered repetitions, with bottles between runs as replicates. Aliquots were carefully removed by a 2-ml plastic syringe at 0, 4, 8, 24, 48 and 72 h for SCFA (total and individual) determination by gas chromatography. Total SCFA profiles were fitted to a monophasic model and the maximum rate of production (R_{max}) and the time at which it occurs (T_{max}) were calculated (Giuberti *et al.*, 2013). Data were subjected to ANOVA using the mixed procedure (SAS®; USA) and the minimum significant difference (MSD) was generated from Tukey's test and used for multiple comparisons among means. The significance level was $P<0.05$.

Table 1. *Post-fermentative results and fitted kinetic parameters for the different hydrolysed residues.*

	SCFA[1]	MR Acetate[2]	MR Propionate[2]	MR Butyrate[2]	MR BCFA[2,3]	R_{max}[4]	T_{max}[5]
Resistant starch source							
Wheat	7.5	0.51	0.25	0.21[a]	0.03	1.19[a]	6.0[ab]
Normal amylose maize	6.0	0.56	0.27	0.15[b]	0.02	1.19[a]	5.3[b]
High amylose maize	6.5	0.54	0.27	0.17[b]	0.02	0.44[b]	7.8[a]
Low amylose maize	6.7	0.51	0.26	0.21[a]	0.02	0.91[ab]	5.9[b]
Rice	6.7	0.53	0.26	0.18[ab]	0.03	0.70[b]	6.4[ab]
√MSE	0.81	0.017	0.009	0.08	0.003	0.165	0.43
Significance	NS	NS	NS	<0.05	NS	<0.05	<0.05

NS: not significant; [1]SCFA, total short-chain fatty acid production (mmol/g dry matter incubated); [2] MR, molar ratio of the individual SCFA; [3]BCFA, branched-chain fatty acids (sum of iso-butyric and iso-valeric acids); [4]R_{max}, maximum rate of SCFA production (mmol/g dry matter incubated per hour); [5]T_{max}, time of occurrence of R_{max} (h). Means in the same column with different superscripts differ ([a,b];$P<0.05$); √MSE, mean square error.

The average SCFA productions, as well as the molar ratios (MR) of acetate, propionate and BCFA, were similar ($P>0.05$) between RS sources. On the contrary, the MR of butyrate was affected by the RS sources, showing that different types of RS may favour butyrate production. In particular, the highest MR of butyrate was produced by fermentation of RS from wheat and low amylose maize starches (on average 0.21; $P<0.05$), whereas RS from normal amylose maize yielded the lowest MR (0.15; $P<0.05$). Butyrate is the major fuel for colonocytes and can inhibit apoptosis of colon crypt cells *in vivo* (Bach Knudsen *et al.*, 2012). Differences were recorded for R_{max} and T_{max} values ($P<0.05$). Combined with information on the transit time, fermentation kinetics may give an indication about the site of fermentation in the gastrointestinal tract (Giuberti *et al.*, 2013). In conclusion, current data could facilitate the selection of ingredients to manipulate the fermentation in the intestine of pigs and to potentially enhance butyrate production.

BACH KNUDSEN, K.E., HEDEMANN, M.S. and LÆRKE, H.H. (2012). *Animal Feed Science and Technology*. **173**:41-53.
BOISEN, S. and FERNÀNDEZ, J.A. (1997). *Animal Feed Science and Technology*. **68**:277-286.
GIUBERTI, G., GALLO, A., MOSCHINI, M. and MASOERO, F. (2013). *Animal*. **20**:1-8.

Supported by AGROSCENARI project (MIPAAF, Italy).

CHAPTER 5

Nutrition, Feed Additives and Feed Grains, and Physiology

Xylanase improves growth performance in grower-finisher pigs fed hard and soft wheat-based diets

E. Kiarie[1], A. Péron[2], Y. Ru[2] and J. Wiseman[3]

[1]Dupont Industrial Biosciences-Danisco Animal Nutrition, Marlborough, SN8 1AA. [2]Danisco Singapore Pte Ltd, Singapore 117525. [3]University of Nottingham, Loughborough, LE12 5RD, UK.

The nutritive value of wheat for monogastric animals is known to vary due to, among other factors, the fibrous cell wall structure of the grain. For example, correlation of digestible energy (DE) content in 15 Canadian wheat samples with their chemical characteristics revealed that the non-starch polysaccharides' content, specifically the concentration of arabinose and xylose, explained more than 70% of the variation in DE content (Zijlstra *et al*., 1999). Previous studies in young piglets showed that supplemental xylanase reduced growth performance variability related to wheat varieties (Cadogan *et al*., 2003). Wheat endosperm hardness is a globally used classification of wheat varieties based on the chemical characteristics and ranks wheat as either 'soft' or 'hard', and was seen to affect nutrient digestibility in wheat fed to pigs (McCann *et al*., 2006). In this paper it is hypothesised that growth performance of pigs fed wheat varieties differing in endosperm hardness will be equally improved by supplemental xylanase. Therefore, the objective was to investigate the efficacy of xylanase on growth performance of growing-finishing pigs fed diets based on hard or soft wheat.

Animal use was approved by the institutional animal care. Forty-eight Rattlerow genetics male pigs were single-housed and, based on a 2 x 3 factorial arrangement, allotted to six diets in a completely randomized design to give eight pens per diet. The diets were based on either hard or soft wheat and fed without or with 1,000 or 4,000 U of xylanase/kg of feed. The wheat varieties were procured from local commercial suppliers and xylanase (Danisco Xylanase) was provided by Danisco Animal Nutrition, Marlborough. Diets were formulated for two phases: grower (30-55 kg body weight; BW) and finisher (55-90 kg BW), and wheat varieties were included at 50% in each phase. Barley, wheat feed and soybean meal were respectively included at 10, 24 and 12% in the grower phase and 3, 40 and 3% in the finisher phase. Diets were also fortified with additional amino acids and mineral supplements to meet nutrient requirements (NRC, 1998). The digestible energy (DE) content was 11.1 MJ/kg and 10.6 MJ/kg and total lysine was 0.91 and 0.70% in the grower and finisher phases, respectively. Pigs had free access to feed and water throughout the study. Feed intake and BW were measured weekly and the number of days that pigs took in each phase was recorded to determine average daily feed intake (ADFI), average daily gain (ADG) and gain to feed conversion ratio (FCR). Data were statistically analysed (SAS®; USA).

Table 1. *Effects of wheat varieties and xylanase on growth performance of grower-finishing pigs (30-90 kg BW) fed wheat-based diets.*

	ADG (kg/day)	ADFI (kg/day)	FCR (kg/kg)
Wheat effects			
Hard wheat	1.00	2.52	2.56
Soft wheat	0.99	2.43	2.47
SEM	0.032	0.034	0.060
Xylanase effects			
0 U/kg	0.94	2.48	2.64
1,000 U/kg	0.99	2.49	2.56
4,000 U/kg	1.06	2.47	2.35
Linear contrast	0.037	0.187	0.030
Quadratic contrast	0.975	0.237	0.327
SEM	0.030	0.041	0.073

SEM, standard error of the mean.

There was no interaction (P>0.05) between wheat variety and xylanase (data not shown) or the main effects of wheat on growth performance (Table 1). Xylanase linearly improved ADG and FCR (P<0.05; Table 1). Pigs fed the highest xylanase inclusion had 13% greater (P=0.03) ADG and -11% (P=0.04) better FCR than the control-fed pigs. In conclusion, wheat type had no effect on measured parameters and xylanase dose-dependently improved growth performance independent of wheat endosperm hardness.

CADOGAN, D.J., CHOCT, M. and CAMPBELL, R.G. (2003). *Canadian Journal of Animal Science*. **83**:105–112.

ZIJLSTRA, R.T., DE LANGE, C.F.M., and PATIENCE, J.F. (1999). *Canadian Journal of Animal Science* .**79**:187-194.

MCCANN, M.E.E., MCEVOY, J.D.G., MCCRACKEN K.J., and SIMMINS, P.H. (2006). *Irish Journal of Agricultural and Food Research*. **45**:173-185.

NRC. (1998). "Nutrient requirements of swine", 10th ed. (The National Academy Press: Washington).

Supported financially by Danisco Animal Nutrition UK Ltd.

Effect of substrate type on *in vitro* dry matter and energy disappearance of pig grower rations formulated with and without the addition of a xylanase-based enzyme complex

Y. Feng, Z.Z. Zhang, D.W. Zheng, L. Kang, A.T. Hung and B.J. Hosking

Asiapac Biotechnology, Dongguan City, Guangdong, 523808 PRC.

The synergistic effects of particular enzyme mixtures can have markedly different effects on nutrient release depending on substrate type and level of inclusion (Zhang *et al.*, 2013). The implications of this work and that of other studies using a semi-automated simulated digestion system (SDS) *in vitro* (Fang *et al.*, 2012) is that the response to enzyme addition depends greatly on the specific composition of the target substrate and the mix of enzymes used. This study reports on the *in vitro* assessment of rations used in a related feeding study (Kang, *unpublished*) on responses to the addition of a xylanase-based enzyme mixture to corn- and wheat-soybean meal (SBM) rations for growing pigs.

The *in vitro* procedure (Fang *et al.*, 2012) used the SDS in a two-stage simulation of the gastric and intestinal phases of digestion. The gastric phase of the incubation was undertaken over 4 h in buffered pepsin solution (pepsin 1550 U/ml) at pH 2.8. The intestinal phase involved 16 h incubation in phosphate buffer solution (pH 6.4) containing amylase (220 U/ml), trypsin (69 U/ml) and chymotrypsin (8.7 U/ml). Solutions were maintained at 39 °C and recirculated through the digestion vessels throughout the incubation. Samples were agitated continually throughout each phase of digestion. The test enzyme combination (Asiapac Biotechnology, Dongguan City, PRC) was added during manufacture and provided xylanase (EC 3.2.18; 8 U/g), mannanase (EC 3.2.1.78; 4 U/g) and protease (EC 3.4.23.6; 4U/g). Substrates were sub-sampled from rations used in a related feeding study and contained either corn alone as the sole grain component (Corn:SBM) or a mixture of corn and wheat providing 350 g/kg wheat (Wheat:SBM) in the ration. The rations were formulated to provide 13.8 MJ digestible energy (DE)/kg and 0.7% digestible lysine. Incubations were undertaken in replicate (n=5). Data were analysed (Minitab®, Version 14.0; USA) according to a 2 x 2 factorial design for main effects of substrate type and enzyme addition.

Table 1. *Effect of substrate type on SDS* in vitro *dry matter (DM) and energy disappearance (mg/g) of Corn:SBM and Wheat:SBM rations with and without (Nil) the addition of an enzyme complex containing xylanase.*

		Enzyme		SEM[1]	Significance		
Response	Substrate	Nil	Xylanase		Enzyme	Substrate	ExS
DM disappearance	Corn:SBM	599	606				
	Wheat:SBM	590	596	2.3	0.018	0.001	NS
Energy disappearance	Corn:SBM	556	565				
	Wheat:SBM	554	563	2.6	0.004	NS	NS

[1] SEM, standard error of the mean.

Dry matter disappearance was higher on the Corn:SBM diet (P<0.01) and was increased in both diets by the addition of xylanase (P=0.018). Energy disappearance was similar on both diets (554 and 556 g/kg; P=0.42) but was increased (555 and 564 g/kg; P<0.004) in both diets by enzyme addition.

The results were consistent with previous studies, using a reducing sugar technique, that showed responses in Corn:SBM mixtures to enzyme mixtures containing xylanase were greater than those where xylanase was the sole enzyme (Zhang *et al.*, 2013). Responses to xylanase on substrates expected to be low in soluble non-starch polysaccharides is further evidence that there is more to be understood about the specific nature of individual substrates and the effects of individual product inclusions on substrate release.

FANG, L., HONGFU, Z., FENG, Z, JUNJUN, W. and DEFA, L. (2012). *Journal of Animal and Veterinary Advances.* **11**:1819-1826.
ZHANG, Z.Z., FENG,Y., HUANG, C.S., HUNG, Z.Y. and HOSKING, B.J. (2013) In "24th Annual Australian Poultry Science Symposium", p 130, ed. J. Roberts. (The Poultry Research Foundation: Sydney).

A direct-fed microbial product increases production efficiency and reduces faecal ammonia levels in pigs

H.L. Li[1], Y. Lei[1], R.A. Valientes[2] and I.H. Kim[1]

[1]Dankook University, Cheonan, Choongnam 330-714 South Korea.[2]DSM Nutritional Products Philippines, Inc., Bonifacio Global City, Taguig, 1634, Philippines.

Exposure to high levels of noxious gases in livestock facilities not only adversely affects the health of animals and workers, but can also cause environmental problems such as the nitrification and acidification of rain (Le *et al*., 2005). Previous authors have reported improvements in growth performance and reductions of faecal noxious gas emission (Hong *et al*., 2006), by the dietary supplementation of probiotics or direct-fed microbials. Therefore, it was hypothesised that direct-fed microbial product (MicroSource® S) may benefit growth and total tract digestibility and decrease noxious gas emission. The purpose of this work was to evaluate the effects a direct-fed microbial product (MicroSource® S) on growth performance, total tract digestibility, and faecal characteristics in growing-finishing pigs.

A total of 120 growing [Yorkshire × Landrace × (Duroc)] pigs [BW=23.2±3.26 kg; mean±SEM] were used in this 16-week feeding trial (grower phase, 0-6 week; finisher phase, 6-16 week). Pigs were randomly distributed into one of two treatments on the basis of BW and sex. There were 12 pens/treatment, 5 pigs/pen (three barrows and two gilts). Dietary treatments were: CON, basal diet; T1, CON + 62.5 ppm direct-fed microbial (MicroSource® S). MicroSource® S contains bacteria of *Bacillus subtilis* and *Bacillus licheniformis* with a total microbial count of 1.18×10^9 CFU/g. Individual pig BW was checked at the beginning, at the end of 6, 12 and 16 weeks, and feed consumption was recorded on a pen basis during the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain:feed (G:F) ratio. The coefficient of apparent total tract digestibility (CATTD) of dry matter (DM), gross energy (GE) and nitrogen (N) were determined by using chromic oxide (0.2%) as an inert indicator in the diets during d 36-42 and d 106-112. NH_3 was measured using a Kjeltec 2300 Analyzer (Foss Tecator AB, Hoeganaes, Sweden). *Lactobacillus* concentration was determined by serial dilution in anaerobic diluent before inoculation onto Petri dishes of sterile agar. All data were subjected to statistical analysis as a randomised complete block design using the GLM procedures of SAS, and the pen was used as the experimental unit. The initial BW was used as a covariate for ADFI and ADG. Before carrying out statistical analyses of the microbial counts, logarithmic conversion of the data was performed. Differences among treatment means were determined using the Duncan's multiple range test with $P<0.05$ indicating statistical significance.

Table 1. *Effects of a direct-fed microbial product (MicroSource® S) on performance, CATTD, faecal gas emission and faecal microbial content in growing-finishing pigs.*

Treatment	Grower ADG (g)	Finisher, ADG (g)	Grower CATTD of N	Finisher CATTD of N	Grower NH3 emission at d 7 (ppm)	Finisher NH3 emission at d 7 (ppm)	*Lactobacillus spp.* at 6 weeks (log10 cfu/g)
CON	675	785	0.79	0.74	43.0	46.2	7.39[b]
T1	710	817	0.81	0.76	37.9	37.9	7.49[a]
SEM	11	8	0.005	0.005	1.2	1.9	0.03
Significance	*	*	*	*	*	*	*

CATTD, coefficient of total tract apparent digestibility; SEM, standard error of mean; * $P<0.05$.

Between weeks 0 to 6 and 0 to 16, ADG in T1 was higher ($P<0.05$) than in the CON diet, while there were no differences ($P>0.05$) in ADFI and G:F ratio throughout the experiment. The CATTD of DM and N was increased ($P<0.05$) in T1 compared with CON at 6 and 16 weeks. Faecal NH_3 emission was decreased ($P<0.05$) in T1 compared with CON at the end of 6 and 16 weeks. Pigs fed the diet with MicroSource® S had higher ($P<0.05$) faecal *Lactobacillus* spp. counts at 6 weeks, indicative of a healthier gut condition (de Lange *et al*., 2010). In conclusion, dietary supplementation with 62.5 ppm direct-fed microbial (MicroSource® S) improved growth performance, increased the faecal *Lactobacillus* spp. concentration, and decreased faecal NH_3 content of growing-finishing pigs.

DE LANGE, C.F.M., PLUSKE, J., GONG, J. and NYACHOTI, C.M. (2010). *Livestock Science*. **134:**124-134.

HONG, J.W., KIM, I.H., KWON, O.S., KIM, J.H., MIN, B.J. and LEE, W.B. (2006). *Asian-Australian Journal of Animal Science*. **19:**587-592.

LE, P.D., AARMINK, A.J.A., OGINK, N.W.M., BECKER, P.M. and VERSTEGEN, M.W.A. (2005). *Nutrition Research Reviews.* **18:**3–30.

Support in part by the DSM Nutritional Products Philippines, Inc.

Monosodium glutamate as a source of glutamic acid enhances weaner pig performance

R.J. van Barneveld[1], S.A. Knapp[2] and R.J.E. Hewitt[2]

[1]Barneveld Nutrition Pty Ltd, Loganholme, QLD 4129. [2]CHM Alliance Pty Ltd, Millmerran, QLD 4350.

L-glutamine (GLN), and the important role it plays in pig nutrition, has recently been highlighted (Wu *et al*., 2011). In routine diet formulations GLN is often overlooked but its abundance in physiological fluids and tissues and the important regulatory role it plays in functions as diverse as immune response, gene expression and nutrient metabolism suggests more attention is warranted. Certainly, Wu *et al.* (2011) found the role of GLN to be so significant that they recommended that creep, late gestation and lactating sow diets be supplemented with 10 g/kg GLN. An impediment to GLN use in commercial diet formulations is cost. However, GLN and the related amino acid, glutamic acid, form part of a large intra- and inter-cellular and inter-organ GLN-glutamate cycle (Curi *et al.*, 2005). Glutamic acid can substitute for GLN in pathways such as ATP production and the synthesis of amino acids like arginine and proline. It can also inhibit GLN degradation and thus has a sparing effect on the use of GLN as a metabolic fuel (Wu *et al.*, 2011). Monosodium glutamate (MSG) is a cost-effective, widely available feed ingredient comprising ≈ 780 g/kg glutamic acid, and may be a viable precursor to GLN in commercial pig diet formulations. The hypothesis tested was that inclusion of MSG in weaner diets would improve performance post-weaning.

Pens of weaned pigs [23 d, 5.5±0.1 kg (mean±SEM), 14 pigs per pen] were allocated to one of three treatments (10 pens/treatment) over a 3-week period using a randomised block design with sex, weight and entry time as blocking factors. Pens were weighed weekly, for 4 weeks, with feed disappearance recorded to correspond with weighing events. Pigs had access to feed on an *ad libitum* basis from a three-space stainless steel feeder, and *ad libitum* access to water via nipple drinkers. All pigs received a standard medication program of 0.25 ml intramuscularly of Draxxin (Tulathromycin 100 mg/ml, Zoetis, West Ryde NSW) upon entry, 65.7 g/1,000 kg liveweight (LW) of Sol-u-Mox (Amoxycillin trihydrate 870 mg/g; Bayer, Pymble NSW) and 42.9 g/1,000 kg LW of Linco-Spectin (Lincomycin hydrochloride 222 mg/g, Spectinomycin sulphate 445 mg/g; Zoetis, West Ryde NSW) in water for 28 and 21 d, respectively. Wheat-based diets were formulated to contain 15.2 MJ digestible energy (DE)/kg and 0.9 g available lysine/MJ DE. The control diet contained 0.2% NaCl but no MSG, Treatment 1 (MSG1) contained 6.75 g MSG/kg but no added NaCl (to contain overall dietary sodium content), and Treatment 2 (MSG2) contained 13.5 g MSG/kg and no added NaCl. These inclusion rates were used to deliver approximately 5 and 10 g/kg glutamic acid in finished diets. Data were analysed via a GLM ANOVA with time as a blocking factor, with differences determined by LSD ($P<0.05$). Sex effects were not significant ($P>0.05$).

Table 1. *Growth performance of pigs weaned at 23 d and receiving 6.75 g/kg (MSG1) or 13.5 g/kg (MSG2) monosodium glutamate (MSG) in diets for 28 d post-weaning.*

	Control	MSG1	MSG2	SED	Significance
Entry weight (kg)	5.5	5.4	5.7	0.29	0.824
Exit weight (kg)	13.9[a]	14.3[a]	15.5[b]	0.49	0.018
ADG (g/d)	297[a]	312[a]	344[b]	11	0.002
ADFI (kg/d)	0.42	0.41	0.44	0.015	0.335
FCR (kg/kg)	1.41[a]	1.33[b]	1.27[c]	0.025	<0.001

[abc]Means in a row not having the same superscript are significantly different ($P<0.05$); ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SED, standard error of difference.

Inclusion of MSG in diets at 13.5 g/kg improved ($P<0.05$) ADG, exit weight (by 1.6 kg more than the control) and FCR in the 28 d after weaning, with FCR improvement also evident at the 6.75 g/kg inclusion level (Table 1). The higher sodium content of the diets that resulted with the inclusion of MSG (MSG2, 0.36%; MSG1, 0.28%; Control, 0.27%) had no adverse effects on feed intake or pig performance. There was no impact of treatment on survivability. The "umami" flavour properties often associated with MSG did not have a positive effect on feed intake suggesting the primary mechanism for improved pig performance might be the supply of cellular energy, although this was not evaluated in this experiment. These results would suggest that MSG should be included in post-weaning diets, at a level equivalent to 10 g/kg glutamic acid.

CURI, R., LAGANHA, C.J., DOI, S.Q., SELLITTI, D.F., PROCOPIO, J., PITHON-CURI, T.C., CORLESS, M. and NEWSHOLME, P. (2005). *Journal of Cellular Physiology.* **204:**392-401.

WU, G., BAZER, F.W., JOHNSON, G.A., KNABE, D.A., BURGHARDT, R.C., SPENCER, T.E., LI, X.L. and WANG, J.J. (2011). *Journal of Animal Science.* **89:**2017-2030.

A blend of organic acids and medium-chain fatty acids improves feed intake of lactating sows and weaning weight of piglets

N.S. Ferguson[1], P.J. Roubos[2], M Richer-Lanciault[1] and J.W. Resink[2]

[1]Nutreco Canada Agresearch, Guelph, N1G 4T2 Canada. [2]Nutreco R&D, Boxmeer, 5830 AE The Netherlands.

Blends of organic and medium-chain fatty acids (MCFA) have the potential to stabilise the gastrointestinal tract (GIT) microbiota in pigs, thereby improving post-weaning gain and reducing the incidence of diarrhoea (Awati *et al*., 2012). For sows and especially during parturition, a healthy GIT with a stable microbiota is key to the prevention of dysbiosis (Sekirov *et al.*, 2010). Improved GIT health may improve the feed intake during lactation, which can increase milk production and weaning weight of pigs. This study was conducted to investigate sow and litter performance responses to the inclusion of a blend of organic acids (OA) and MCFA.

A total of 271 gilts and sows (parity 1-6; Danbred genetics) were divided over two dietary treatments. The treatments were a standard lactation diet (control) containing 13.7 MJ metabolisable energy (ME)/kg and 1.03% lysine, with and without a commercial blend of OA and MCFA with controlled release properties (inclusion level of 0.2%; Selko Feed Additives, Tilburg, The Netherlands). Treatment diets were fed 7 d prior to and during lactation. Litters were standardised to 12 piglets per gilt or sow within 24 h of birth. Piglets were weighed directly after birth at 1 d and at weaning. The average weaning age was 19.6±1.5 (mean±SEM) d. Individual sow data were used as the experimental unit. As the design was unbalanced (non-orthogonal, unequal replications per block), data were analysed using the Analysis of an Unbalanced design (GLM) in Genstat Ver 15.0 (2012) to determine significant treatment differences. Proportional data were analysed using binomial logistic regression analysis.

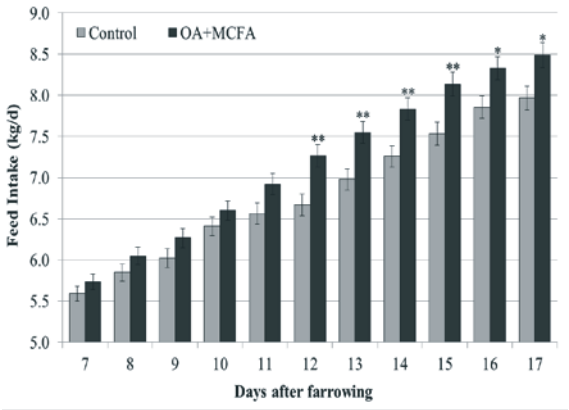


Figure 1. *Feed intake of sows after farrowing; *P<0.05; **P<0.01; Bars represent standard error of mean*

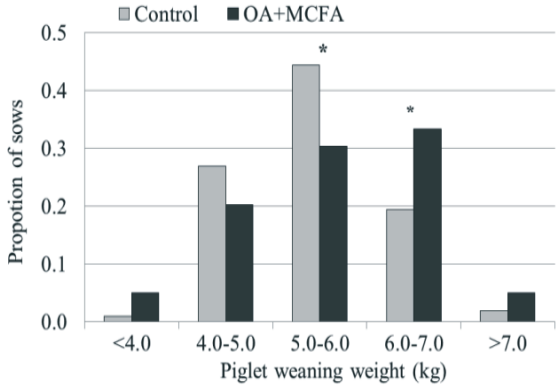


Figure 2. *Proportion of sows in different average piglet weaning weight categories; *P<0.05.*

Feeding OA+MCFA a week before farrowing and throughout the lactation period increased feed intake by 4.5% (272 g/day) (P=0.09) (Figure 1). The average birth weights were not different (P>0.05) between control and treated animals (1.356 versus 1.385), but the proportions of sows with heavier piglets at birth (1.6-1.8kg category) was higher (257%; P=0.023). Similarly at weaning there were no difference (P>0.05) in weight between control and treatment (5.39 versus 5.46 kg), but the proportion of sows producing heavier piglet weaning weights (6.0-7.0 kg category) increased by 173% (P<0.05) (Figure 2). Feeding OA+MCFA did not reduce pre-weaning mortalities (P>0.05).

In conclusion, this trial indicated an increase in sow lactation feed intakes and piglet weaning weights with the feeding of OA+MCFA. Further investigations are warranted to study changes in the microbiota of sows and piglets, which may be associated with the observed effects on feed intake of sows and pre-weaning piglet growth.

AWATI, A., SMITS, C. H. M. and TIMMERMANS, H.M. (2012). In "Proceedings XII International Symposium on Digestive Physiology of Pigs", p. 46. (Keystone, CO, USA).

SEKIROV, I., RUSSELL, S.L, ANTUNES, L.C.M and FINLAY, B. (2010). *Physiological Reviews*. **90:**859-904.

The use of an enzyme mixture to enhance growth and improve total tract digestibility in pigs fed diets with alternative ingredients

L.V. Bindhu, C. Sugumar and L-B. Goh

Kemin Industries (Asia) Pte Ltd, Singapore 758200.

There has been a tremendous increase in the use of dietary enzymes in pig diets to improve nutrient and energy digestibility and growth performance in recent years. Dietary enzymes can be used to supplement the pig's own enzyme production, including amylases to improve starch digestibility (Officer, 1995), proteases to improve protein digestibility (Hartman *et al*., 1961), and enzymes to target the non-starch polysaccharide (NSP) constituents in feed.

An enzyme mixture containing different enzymes (470 U/g amylase, 1650 U/g protease, 7450 U/g cellulase, 18,000 U/g xylanase and 800 U/g β glucanase) was developed to maximise digestibility of starch, protein and NSP in pig diets containing alternative feed ingredients. The present study evaluated the effect of this multienzyme supplementation on the total tract digestibility and growth performance of starter and grower pigs. Dietary treatments consisted of (1) Positive control diet formulated to meet NRC specifications (NRC 1998); (2) Negative control diet [metabolisable energy (ME) reduced by 0.42 MJ/kg compared to positive control diet) formulated by partial replacement of corn and soybean using alternative ingredients such as rice bran, cassava and DDGS (dried distillers grains and solubles); and (3) Negative control diet supplemented with multienzyme (250 g/t). Male pigs [Duroc × (Large White/Landrace)] were housed individually in metabolism crates and were fed test diets in mash form. After a 7 d adaptation period, faeces and urine were collected quantitatively over a 5 d collection period and coefficients of total tract apparent digestibility (CTTAD) were then calculated. For the growth trial, a total of 240 pigs (30 d of age) were assigned to three treatments following a completely randomised design. There were four replicates per treatment and 20 pigs/replicate. Feed and water were provided *ad libitum*. Body weight and feed consumed were measured at 30, 60 and 90 d of age. All data were subjected to ANOVA (Statgraphics, 2012; StatPoint Technologies Inc.).

Table 1. *Effect of enzyme supplementation on the coefficient of total tract apparent digestibility (CTTAD) of crude protein (CP) and dry matter (DM), and the DE content.*

	Positive control	Negative control	Negative control + Multienzyme	SEM	Significance
CTTAD, CP	0.855[a]	0.852[a]	0.872[b]	0.0036	0.04
CTTAD, DM	0.842[ab]	0.840[a]	0.860[b]	0.0004	0.07
DE (MJ/kg)	14.34[b]	13.96[a]	14.22[b]	0.075	0.001

[a,b]Means in a column not having the same superscript are significantly different (P<0.05).

Table 2. *Effect of enzyme supplementation on growth performance of pigs (30-90 d).*

Growth Parameters	Positive control	Negative control	Negative control + Multienzyme (250g/t)	SEM	Significance
Initial body weight (kg/pig)	7.53 [a]	7.47 [a]	7.51 [a]	0.15	0.225
Average daily gain(g/pig/day)	414[b]	406[a]	428[c]	3.1	<0.001
Average daily feed intake (g/pig)/day)	773[a]	776[a]	786[a]	6.9	0.093
Feed conversion ratio(FCR)	1.87[b]	1.91[c]	1.83[a]	0.01	<0.001

[a,b]Means in a row not having the same superscript are significantly different (P<0.05).

Enzyme supplementation produced a significant effect on the digestibility of diet components (Table 1), which translated to a consistent improvement in growth performance (Table 2). The present study therefore demonstrated the potential of the multienzyme mixture in improving nutrient quality of pig diets formulated with alternate feed ingredients.

HARTMAN, P.A., HAYS, V.W., BAKER, R.O., NEAGLE, H. and CATRON, D.V. (1961). *Journal of Animal Science*. **20**:114-123.
NRC. (1998). "Nutrient requirements of swine", 10th ed. (The National Academy Press: Washington).
OFFICER, D. I. (1995). *Animal Feed Science Technology.* **56**:55–65.

High feeding in late gestation subtly improves litter characteristics at birth and day 3 post-partum

W.H.E.J. van Wettere[1], T. E. Kennett[2], D.S. Lines[2], P. Herde[3] and P.E. Hughes[3]

[1]The University of Adelaide, SA 5005. [2]Australian Pork Farms Group, SA 5152. [3]Pig South Australian Research and Development Institute, Roseworthy, SA 5371.

Pre-natal nutrition can affect fetal growth, viability at birth and both survival and growth during the immediate post-natal period (Campos *et al*., 2012). However, it remains controversial whether increasing feed intake during the last three to four weeks of gestation improves, or does not affect, piglet and litter characteristics at birth. This study determined the effects of feeding level during the last four weeks of gestation and litter size gestated on piglet characteristics at birth and day three post-partum.

A total of 418 sows (parity 1.3±0.07; mean±SEM, range 0-4) were used in this study. The experimental design was a 2 x 2 factorial, incorporating two litter size groups (LS; Small, < 12 versus Large, ≥ 12) and two feeding levels from d 85 of gestation until farrowing (FL; High versus Low). Litter size groups were determined retrospectively based on the median value (12) for the herd. The feeding levels for the High and Low treatments were 2.8 and 2.2 kg per d for first gestation sows [14.0 MJ digestible energy (DE)/kg and 0.70% available lysine] and 2.3 and 2.9 kg per d for all other sows (13.1 MJ/DE/kg and 0.5% available lysine). Sows were fed via electronic sow feeders, and housed in straw-filled ecoshelters in groups of approximately 100. The total number of piglets born (TB), born alive (BA), born dead (BD) and mummified (Mm) were recorded at farrowing. Cross fostering occurred only during the first 24 h, when deemed essential for piglet health. Individual piglet birthweight (BW) and liveweight (LW) on d 3 post-partum were recorded. Treatment effects on all variables measured were analysed using an ANOVA model, unbalanced design (Genstat, 10th Edition, Rothamsted Experimental Station, Harpenden, UK).

As expected, litter size significantly affected most litter characteristics (Table 1). High feeding tended to decrease the incidence of stillborn (P=0.09) and low (<1kg) BW piglets (P=0.08), as well as the within-litter variation in BW (P=0.04; Table 1). Interestingly, High feeding increased the incidence of mummified fetuses in Small litters (Table 1). On d 3, minimum piglet LW and mean LW were heavier and the within-litter variation in LW was lower for High compared to Low litters (Table 1).

Table 1. *Effect of litter size and gestation feeding level on litter size and piglet characteristics on day 3 post-partum.*

Litter size (LS)	Small (< 12)		Large (≥12)		Pooled SEM[A]	Significance[B]		
Feeding level (FL)	Low	High	Low	High		LS	FL	LS.FL
Number of sows	94	118	100	106				
Total born	8.72	8.89	14.10	13.75	0.16	<0.01	0.06	0.17
Born alive	8.27	8.31	12.83	12.83	0.15	<0.01	0.33	0.90
Stillborn	0.45	0.42	1.08	0.77	0.06	<0.01	0.09	0.23
Mummified foetuses	0.01	0.19	0.18	0.15	0.02	0.11	0.18	0.02
Mean piglet BW (kg)	1.59	1.57	1.34	1.37	0.01	<0.01	0.34	0.20
Number of piglets < 1 kg BW	0.61	0.59	2.33	1.92	0.09	<0.01	0.08	0.24
Lightest piglet BW (kg)	1.14	1.15	0.80	0.85	0.02	<0.01	0.10	0.47
CV[C] BW (%)	16.94	16.09	21.54	20.23	0.34	<0.01	0.04	0.72
Mean piglet LW day 3 (kg)	1.98	2.09	1.79	1.84	0.02	<0.01	0.03	0.44
Lightest piglet LW day 3 (kg)	1.44	1.64	1.15	1.21	0.02	<0.01	<0.01	0.09
CV litter weight day 3 (%)	16.42	14.13	20.80	19.31	0.42	<0.01	<0.01	0.60

[A]SEM for litter size x gestation feeding level; [B]Litter size (LS) and gestation feeding level (FL); [C]CV, coefficient of variation.

The increased LW of the smallest piglets in the litter combined with the reduced within litter variation in BW and d 3 LW suggest the High feeding level promoted growth, and possibly maturation of the smaller foetuses. These data support previous evidence that higher feeding levels in late gestation can be beneficial.

CAMPOS, P.H.R.F., SILVA, B.A.N., DONZELE, J.L., OLIVIERA, R.F.M. and KNOL, E.F. (2012). *Animal* **6:**797-806.

Project funded by Pork CRC Limited Australia.

Performance of piglets suckling sows fed ractopamine-supplemented diets

W.H.E.J. van Wettere[1], T. E. Kennett[2], P. Herde[3] and P.E. Hughes[3]

[1]The University of Adelaide, SA 5005. [2]Australian Pork Farms Group, SA 5152. [3]South Australian Research and Development Institute, Roseworthy, SA 5371.

During the first lactation, ractopamine (RAC; Paylean®) reduced mobilisation of maternal reserves and impaired piglet growth (Pain *et al*., 2007). Although subsequent reproductive performance of RAC-supplemented sows was improved, adoption of this technology is limited by the lower weaning weights observed. Consequently, two studies were conducted to test the hypothesis that altering RAC inclusion rate and the period of inclusion rate relative to parturition, in the diet of first lactation sows would result in differential effects on milk composition, piglet growth rate and weaning weight.

In study one, 116 first parity sows were allocated to one of four treatment groups during lactation (25th Dec 2009 to 2nd Feb 2010; n = 29 sows), with RAC added to the diet at a dose of zero parts per million (ppm) (0R), 10 ppm (10R) and 20 ppm (20R) throughout lactation, or 10ppm from d 1-13 of lactation and 20 ppm from d 14 to weaning (10/20R). In study two, 119 sows were allocated to one of four treatment groups during lactation (4th March 2010 to 13th April 2010); zero ppm (0R; n = 30), 10 ppm throughout lactation (10R; n = 30), and 10 ppm (0/10R; n = 30) or 20 ppm (0/20R; n = 29) from d 10 of lactation to weaning. In both studies, sows received the same lactation diet [14.2 MJ digestible energy (DE)/kg and 10.2 g lysine/kg], and experimental measures were similar. Within 24 h of farrowing, litter size was standardised to 10-12 piglets in study one, and 11 piglets in study 2. Litters were weighed on d 1, 14 and 21 of lactation in study one, and d 1, 10 and 21 of lactation in study two. Milk samples were collected on d 4, 14 and 21 in study one, and d 3, 10 and 21 in study two. Data were analysed using ANOVA (Genstat, 10th Edition, Harpenden, UK).

The effects of maternal RAC treatment on litter weights for both studies are presented in Table 1. In study one, there were no treatment effects on litter size on d 1 (10.7±0.04; mean±SEM) or 21 (9.6±0.11) of lactation or litter weight (Table 1). In study one, milk protein was unaffected by treatment on d 4 of lactation (4.57±0.06 %), but had a tendency to be reduced by maternal RAC on d 14 (P=0.07) and d 21 (P<0.05) of lactation (Table 1). In study two, litter size was similar for all treatments on d 21 of lactation (9.7±0.15), and there was no effect of treatment on milk protein or litter weight at any stage of lactation.

Table 1. *Effect of supplementing sow lactation diets with RAC on milk protein and piglet growth.*

Study One						Study Two					
RAC Treat	Milk Protein (%)		Litter Weight (kg)			RAC Treat	Milk Protein (%)		Litter Weight (kg)		
	d 14	d 21	d 1	d 14	d 21		d 10	d 21	d 1	d 10	d 21
0R	4.4	4.6^{b}	15.7	40.2	53.2	0R	4.5	4.5	15.9	30.5	49.3
10R	4.3	4.3^{a}	15.7	38.3	52.0	10R	4.3	4.5	16.2	32.4	52.9
20R	4.2	4.3^{a}	15.9	39.5	52.8	0/10R	4.3	4.6	16.1	32.0	49.1
10/20R	4.3	4.4^{a}	15.7	37.5	51.5	0/20R	4.5	4.5	16.0	31.2	53.5
SEM	0.03	0.03	0.23	0.67	0.90	SEM	0.04	0.04	0.23	0.52	1.01

[a,b]Means in a column not having the same superscript are significantly different (P<0.05); SEM, standard error of mean.

Overall, RAC supplementation did not adversely affect litter weight during lactation and at weaning, which contradicts our previous findings (Pain *et al*., 2007), and does not support our hypothesis. The reduced concentration of protein in milk from RAC-supplemented sows in study one is consistent with our previous findings (Pain *et al*., 2007); however, no difference was observed in study two. These data combined with the observed reduction in mobilisation of body reserves in sows supplemented with 20 ppm RAC in the latter half of lactation (van Wettere *et al*., 2013) demonstrate that RAC could be used commercially to maintain body reserves and potentially improve subsequent reproduction of first litter sows, without adversely affecting piglet performance.

PAIN, S.J., HUGHES, P. E. and VAN WETTERE, W.H.E.J. (2007). In "Manipulation Pig Production XI", p.140, eds. J.E. Paterson and J. A. Barker. (Australasian Pig Science Association; Werribee).

VAN WETTERE, W.H.E.J., KENNETT T.E., HERDE, P., and HUGHES, P.E. (2013). In "Manipulation Pig Production XIII", p.105 eds. J.R. Pluske and J.M. Pluske. (Australasian Pig Science Association; Werribee).

This project was supported by Pork CRC Limited Australia.

Effect of ractopamine feeding strategies during the first lactation on sow performance

W.H.E.J. van Wettere[1], T. E. Kennett[2], P. Herde[3] and P.E. Hughes[3]

[1]The University of Adelaide, SA 5005. [2]Australian Pork Farms Group, SA 5152. [3]South Australian Research and Development Institute, Roseworthy, SA 5371.

During the first lactation, excessive mobilisation of sow body reserves, particularly protein, significantly impairs subsequent reproduction. Dietary ractopamine (RAC; Paylean®) increased lean growth in finisher pigs (Apple *et al.*, 2007), with lactation live weight loss also reduced in primiparous sows receiving a RAC-supplemented diet (van Wettere *et al*., 2009). In finisher pigs, the growth response to RAC is affected by ractopamine inclusion rate, with some studies also reporting further improvements when RAC concentrations are stepped up after 14 days (Apple *et al*., 2007). Two studies were conducted to test the hypothesis that altering RAC inclusion rate, and the period of inclusion relative to farrowing, in the diet of first lactation sows would differentially affect the mobilisation of body reserves and subsequent reproduction.

In study one, 116 first parity sows were allocated to one of four treatment groups during lactation (25th Dec to 2nd Feb 2010; n = 29 sows in each treatment), with RAC added to the diet at a dose of zero parts per million (ppm) (0R), 10 ppm (10R) and 20 ppm (20R) throughout lactation, or 10 ppm from d 1 to 13 of lactation and 20 ppm from d 14 to weaning (10/20R). In study two, 119 first lactation sows were allocated to one of four treatment groups during lactation (4th March to 13th April 2010); zero ppm (0R; n = 30), 10 ppm throughout lactation (10R; n = 30), and 10 ppm (0/10R; n = 30) or 20 ppm (0/20R; n = 29) from d 11 of lactation to weaning. In both studies, sows received the same lactation diet [9.6 MJ net energy (NE)/kg and 1.02% lysine], and experimental measures were similar. Daily feed intake was recorded, and sow liveweight (LW) and P2 backfat were measured on d 1 and 21 of lactation, as well d 14 (study one) and d 10 (study two). Weaning to oestrus interval (WO), farrowing rate and the second litter size (total born, TB) were recorded. Proportions were analysed using a χ^2 test, with all other data analysed using ANOVA (Genstat, 10th Edition, Harpenden, UK).

Lactation feed intake was unaffected by treatment (106±2.0 kg and 124±2.6 kg, mean±SEM; studies one and two, respectively). There was no effect of treatment on LW and P2 on d1 or 21 of lactation in either study. Sow LW and P2 were 196±1.6 kg and 19±0.4 mm on d 1 and 194±1.8 kg and 16±0.4 mm on d 21 in study one. In study two, sow LW and P2 were 192±1.8 kg and 17±0.4 mm on d 1 and 189±1.8 kg and 14±0.3 mm on d 21. In studies one and two, the proportion of sows expressing oestrus within 14 d of weaning was 0.85 and 0.91 and the proportion of mated sows farrowing for the second time was 0.78 and 0.88, with neither affected by treatment. In study one, RAC treatment did not alter sow LW change ($P>0.05$); however, in study two the introduction of 20 ppm of RAC to the diet on d 11 of lactation reduced ($P<0.05$) LW loss between d 10 and 21 of lactation (Table 1).

Table 1. *Effect of RAC inclusion rate and timing of inclusion on sow LW change, WO and TB.*

Study One						Study Two					
RAC Treat	LW change (kg)			WO (d)	TB	RAC treat	LW change (kg)			WO (d)	TB
	d1-14	d14-21	d1-21				d1-10	d10-21	d1-21		
0R	-2.29	-1.2	-3.49	8.0	10.9	0R	-3.22	-1.20[a]	-4.42	4.9	11.6
10R	-1.53	-0.73	-2.26	7.3	10.5	10R	-2.73	-1.27[a]	-4.00	4.8	11.9
20R	-3.24	1.63	-1.60	6.3	10.4	0/10R	-1.63	0.25[ab]	-1.28	5.0	11.6
10/20R	-0.57	-0.27	-0.84	6.8	10.9	0/20R	-3.75	3.58[b]	-0.17	5.5	12.7
SEM	0.73	0.58	0.96	0.71	0.34	SEM	0.69	0.68	0.90	0.20	0.32

[a,b]Means in a column not having the same superscript are significantly different ($P<0.05$); LW, liveweight; WO, weaning to oestrus interval; TB, total born; SEM, standard error of mean.

Overall, the current data support previous evidence that RAC supplementation during lactation can reduce mobilisation of body reserves. It also appears that RAC supplementation prior to d 10 or 14 exerts little effect on changes in LW.

APPLE, J.K., RINCKER, P.J., McKEITH, F.K., CARR, S.N., ARMSTRONG, T.A., MATZAT, P.A.S. and MATZAT, P.D. (2007) *The Professional Animal Scientist*. **23**:179–196.

VAN WETTERE, W.H.E.J., PAIN, S.J. HUGHES, P.E. (2009). In "Manipulation Pig Production XI", p.46, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

Project funded by Pork CRC Limited Australia.

Inclusion of spray-dried fish protein isolate in weaner pig diets improves feed intake

R.J. van Barneveld[1], R.J.E. Hewitt[2], S.A. Knapp[2] and D. Isaac[3]

[1]Barneveld Nutrition Pty Ltd, Loganholme, QLD 4129. [2]CHM Alliance Pty Ltd, Millmerran, QLD 4350. [3]BEC Feed Solutions Pty Ltd, Carole Park, QLD 4600.

The sensitivity for the umami taste in pigs is ten-fold that of sweet, with a much larger range of amino acids eliciting the umami taste receptors in pigs (alanine, arginine, asparagine, aspartate, glutamine, glutamate, proline and threonine) compared with three in humans (Roura and Tedo, 2009). This sensitivity and other data indicate that protein sources have the highest impact on feed preference. Ingredients such as fish meal, whey and spray-dried porcine plasma (SDPP) have been reported as having the highest impacts on feed preference, particularly immediately post-weaning, where the pig responds to malnutrition by increasing the expression of taste receptors for protein rich ingredients (Roura, 2011). A relatively new ingredient that appears to fit this liking for high-quality protein is a membrane-purified spray-dried fish protein isolate (FPI), purported to be comprised of peptides with high bioactivity. It is hypothesised that FPI will enhance performance post-weaning in line with responses to SDPP, as in Pierce *et al.* (2005).

Pens of weaned pigs [23 d, 5.6±0.1 kg (mean±SEM), 14 pigs per pen] were allocated to one of three treatments (10 pens/treatment) over a 3-week period using a randomised block design with sex, weight and entry time as blocking factors. Pens were weighed weekly, for 4 weeks, with feed disappearance recorded to correspond with weighings. Pigs had *ad libitum* access to feed from a three-space stainless steel feeder, and *ad libitum* access to water via nipple drinkers. All pigs received a standard medication program of 0.25 ml intramuscularly of Draxxin (Tulathromycin 100 mg/ml, Zoetis, West Ryde NSW) upon entry, 65.7 g/1,000 kg liveweight (LW) of Sol-u-Mox (Amoxycillin trihydrate 870 mg/g; Bayer, Pymble NSW) and 42.9 g/1,000 kg LW of Linco-Spectin (Lincomycin hydrochloride 222 mg/g, Spectinomycin sulphate 445 mg/g; Zoetis, West Ryde NSW) in water for 28 and 21 d, respectively. Wheat and maize-based diets were formulated to contain 15.2 MJ digestible energy (DE)/kg and 0.9 g available lysine/MJ DE. Diets used the same base ingredients. Protein and amino acid sources included soybean meal and soy protein isolates, blood meal, meat and bone meal, fish meal and milk powder. Treatment 1 diets included 30 g/kg SDPP, while Treatment 2 contained 30 g/kg of a membrane-purified fish protein isolate (FPI; PerfectDigest™ FPI, Bluewave Marine Ingredients, Lima, Peru). Data were analysed via a GLM ANOVA with time as a blocking factor. Differences were determined by LSD ($P<0.05$). Sex effects were not significant, ($P>0.05$).

Table 1. *Growth performance of weaned pigs receiving 30 g/kg spray dried porcine plasma (SDPP 3%) and 30 g/kg membrane-purified fish protein isolate (FPI 3%).*

	Control	SDPP 3%	FPI 3%	SED	Significance
Entry weight (kg)	5.7	5.5	5.7	0.25	0.658
Exit weight (kg)	14.1	14.4	14.3	0.53	0.969
Whole ADG (g/d)	300	320	309	14	0.610
Whole ADFI (kg/d)	0.41	0.43	0.42	0.014	0.573
Whole FCR (kg/kg)	1.37	1.34	1.37	0.042	0.710
ADG 0-7 days (g/d)	135[a]	171[b]	164[b]	12	0.035
ADFI 0-7 days (kg/d)	0.15[a]	0.16[ab]	0.18[b]	0.010	0.005
FCR 0-7 days (kg/kg)[1]	1.11	0.95	1.12	0.076	0.168

[ab]Means in a row not having the same superscript are significantly different ($P<0.05$); ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SED, standard error of difference; Whole, across the 4 week experimental period. [1]Reported FCR in the first week is likely lower than actual across all treatments due to dehydration after transport to research facility.

Inclusion of FPI improved ($P<0.05$) the feed intake of pigs in the first 7 d after weaning (Table 1). Inclusion of both SDPP and FPI resulted in superior ADG relative to the control in the first 7 d. However, it is likely the mechanism for improved performance differed between treatments (presence of IgG in the SDPP treatment and improved intake in the FPI treatment). Beyond 7 d, the benefits of both SDPP and FPI diminished, possibly due to reduced expression of umami receptors and/or the medication regime in place for all treatments suppressing effects previously observed with products like SDPP (see Pierce *et al.* 2005).

PIERCE, J.L., CROMWELL, G.L., LINDEMANN, M.D., RUSSELL, L.E. and WEAVER, E.M. (2005). *Journal of Animal Science.* **83:**2876-2885.

ROURA, E. (2011). In "Manipulating Pig Production XIII", p. 106, ed R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

ROURA, E. and TEDÓ, G. (2009). In "Voluntary feed intake in pigs", pp. 105-140, eds D. Torrallardona and E. Roura. (Wageningen Academic Publishers: The Netherlands).

Inclusion of spray-dried porcine plasma in non-medicated weaner diets maintains performance

R.J.E. Hewitt[1], D.J. Cadogan[2], S.A. Knapp[1] and R.J. van Barneveld[3]

[1]CHM Alliance Pty Ltd, Millmerran, QLD 4350. [2]Feedworks, Romsey, VIC 3434. [3]Barneveld Nutrition Pty Ltd, Loganholme, QLD 4129.

Diarrhoea has been shown to represent around 11% of all post-weaning losses with enterotoxigenic *E. coli* being the most common cause of disease in weaner pigs (Alexander, 1994). A consistent challenge for the pig industry is to identify means for controlling diarrhoea in young pigs that are both cost-effective and suitable for sustainable pork production. Spray-dried porcine plasma (SDPP) has been widely used in North and South America for its functional proteins, which appear to offer protection against bacteria, including *E. coli* (Owusu-Awiedu *et al.*, 2002), but its use in Australia has been limited by the availability of a local product. The presence of functional proteins such as immunoglobulins in SDPP lead us to hypothesise that inclusion at low levels of SDPP (20 g/kg and 30 g/kg) in non-medicated diets will support equivalent levels of performance to medicated diets.

Newly weaned pigs at 23 d of age [5.7±0.5 kg, (mean±SEM); 14 pigs per pen] were allocated to one of three treatments (10 pens/treatment) over a 3-week period using a randomised block design with sex, weight and entry time as blocking factors. Pens were weighed weekly, for 4 weeks, with feed disappearance recorded to correspond with weighing events. Pigs had access to feed on an *ad libitum* basis from a three-space stainless steel feeder, and *ad libitum* access to water via nipple drinkers. All pigs received 0.25 ml intramuscularly of Draxxin (Tulathromycin 100 mg/ml, Zoetis, West Ryde NSW) upon entry. The control treatment also received 65.7 g/1,000 kg liveweight (LW) of Sol-u-Mox (Amoxycillin trihydrate 870 mg/g; Bayer, Pymble NSW) and 42.9 g/1,000 kg LW of Linco-Spectin (Lincomycin hydrochloride 222 mg/g, Spectinomycin sulphate 445 mg/g; Zoetis, West Ryde NSW) in water for 28 and 21 d, respectively, while the remaining treatments were non-medicated. Wheat-based diets were formulated to contain 15.2 MJ digestible energy (DE)/kg and 0.9 g available lysine/MJ DE. Diets used the same base ingredients, and a common organic acid (3 g/kg). Protein and amino acid sources included soybean meal and soy protein isolates, blood meal, meat and bone meal, fish meal and milk powder. Treatment diets included the addition of 20 or 30 g/kg SDPP. Data were analysed via a GLM ANOVA with time as a blocking factor, with differences determined by LSD ($P<0.05$). Sex effects were not significant ($P>0.05$).

Table 1. *Growth performance of weaned pigs receiving 20 g/kg (SDPP 2%) and 30 g/kg spray-dried porcine plasma (SDPP 3%) without medication, compared with a medicated control.*

	Control	SDPP 2%	SDPP 3%	SED	Significance
Entry weight (kg)	5.7	5.8	5.7	0.25	0.980
Exit weight (kg)	12.8[a]	13.7[b]	13.8[b]	0.41	0.024
Whole ADG (kg/d)	0.253[a]	0.282[b]	0.290[b]	0.010	0.001
Whole ADFI (kg/d)	0.37	0.37	0.36	0.016	0.993
Whole FCR (kg/kg)	1.46[a]	1.30[b]	1.26[b]	0.064	0.004
Week 4 ADG (kg/d)	0.359[a]	0.462[b]	0.459[b]	0.027	0.001
Week 4 ADFI (kg/d)	0.57	0.56	0.56	0.029	0.810
Week 4 FCR (kg/kg)	1.61[a]	1.22[b]	1.25[b]	0.119	0.005

[a,b]Means in a row not having the same superscript are significantly different ($P<0.05$); ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SED, standard error of difference; Whole, across the 4 week experimental period.

Inclusion of SDPP at 20 g/kg and 30 g/kg increased ($P<0.05$) overall weight gain of pigs by 0.9 and 1.0 kg, respectively, in the 28 days after weaning, with most of this advantage achieved from 21 to 28 d when the control pigs medication regime changed. Pigs fed SDPP had equivalent performance to the medicated control from 0 to 21 d post-weaning. There was no significant advantage to using diets containing 30 g/kg SDPP compared with 20 g/kg SDPP. Whilst the lack of a negative control limits our interpretation of results, when medication was withdrawn from the control (week 4) pig performance reduced relative to SDPP treatments, suggesting an external challenge to the pigs, responsive to Linco-Spectin, that was overcome in pigs fed SDPP. These results suggest that SDPP inclusion in diets at 20 g/kg may be a cost-effective way of promoting pig performance post-weaning under reduced medication regimes.

ALEXANDER, T.J.L. (1994). In "Escherichia coli in Domestic Animals and Humans", pp.151-170, ed. C.L. Gyles. (CAB International: Wallingford, UK).

OWUSU-AWIEDU, A., BAIDOO, S.K., NYACHOTI, C.M. and MARQUARDT, R.R. (2002). *Journal of Animal Science.* **11:**2895-2903.

A case study of algal meal as an energy and protein source in weaner pig diets

D.J. Henman

Rivalea Australia Pty Ltd, Corowa, NSW 2646.

The production of algae coupled with for example, wastewater treatment, has potential in terms of benefits for the environment (Clarens *et al.*, 2010) and as a feed ingredient. Algae (as a meal) generated as a by-product has potential to be used as a niche feed ingredient as it can have a high protein, vitamin and mineral content (Kay, 1991). In this study, canola meal was substituted for algal meal in a weaner pig diet as a first step to determine any negative side effects of this substitution.

A total of 80 male weaner pigs (PrimeGro™ genetics) were weaned at an average age of 26 d (average weight 7.2 kg±0.89 kg; mean±SEM) and transferred into individual weaner pens. Pigs were offered a commercial starter diet for an initial 5-d period to acclimatise to solid feed and the new environment. After this acclimatisation period, all pigs were individually weighed and allocated to one of the test diets: a standard weaner diet containing of 14.5 MJ of digestible energy (DE) and 0.9 g of standardised ileal digestible lysine per MJ of DE; or a diet with 10% of the canola meal replaced with the equivalent weight of an oven-dried algal meal from a multi-strain culture selected from natural algae strains (Tarong, QLD). Differences in growth performance due to treatment were analysed by ANOVA for a randomised design. The experimental unit was the individual animal. Feed intake and feed efficiency data for pigs observed as wasting more than 15% of feed were removed from the statistical analyses. Differences in mortalities and removals due to the main effect of diet were analysed using Chi-squared analyses (IBM SPSS, Version 19.0; USA).

Table 1. *Over a 21-d period, the performance of individually-housed pigs fed a standard weaner diet or a diet in which 10% (by weight) of the canola meal was substituted with an algal meal.*

	Control	Algal Meal	SEM	Significance
Rate of Gain (g/day)	461	414	0.017	0.007
Feed Efficiency (g:g)	1.23	1.29	0.028	0.024
Daily Feed Intake (g/day)	567	530	0.020	0.071

SEM, standard error of the mean.

The inclusion of 10% algal meal into a weaner diet as a direct replacement for canola meal reduced growth rate by 10.2% and feed efficiency by 4.8% over a 21-d growth experiment (Table 1). The reduced performance was consistent with (post-experiment) amino acid and chemical analyses of the diets and algal meal. The algal meal contained lower lysine and methionine levels than the canola meal and the diet with algal meal was 12% lower in these amino acids than the control, and marginal in respect to requirement for both amino acids. The DE level was also subsequently found to be higher by 3% in the algal meal diet. The results suggest that algal meal could be a useful ingredient in weaner pig diets if the different composition and digestibility of the product is taken into account during diet formulation. The feed intake of the pigs on the algal meal diets was not affected until the third week of the experiment, suggesting that diet acceptance of algal meal when added at 10% is not a major issue. Field staff did notice that pigs on the algal meal diets had a scour, although not severe, and only a few pigs were lost to the experiment. The scour was very noticeable due to the green colour, which may indicate that the green chloroplasts in the algal meal do pass through the animal.

This case study indicated that inclusion of this specific source of algal meal, once formulated correctly into the diet, may not have any major negative effects on pig performance after weaning, although the higher level of scouring would need to be examined further in a commercial environment. However, and as suggested by Harun *et al.* (2010), the production process for microalgae products is only moderately economically viable and requires more development in genetic and metabolic engineering. If such development occurs, further research in the use of algal meal in diets for pigs, similar to that of Grinstead *et al*. (2000), may be warranted.

CLARENS, A.F., RESURRECCION, E.P., WHITE, M.A. and COLOSI, L.M. (2010). *Environmental Science and Technology* **44**:1813-1819.

GRINSTEAD, G.S., TOKACH, M.D., DRITZ, S.S., GOODBAND, R.D. and NELSSEN, J.L. (2000). *Animal Feed Science and Technology*. **83**:237-247.

HARUN, R., SINGH, M., FORDE, G.M. and DANQUAH, M.K. (2010). *Renewable and Sustainable Energy Reviews.* **14**:1037-1047.

KAY, R.A., (1991). *Food Science and Nutrition*. **30**:555-573.

Supported by Pork CRC Limited Australia.

A survey of heavy metal contaminants in common trace mineral sources in the Asia Pacific region

T. Jarman[1] and A.J.L Frio[2]

[1]Alltech Thailand Corp, Ltd., Khlongtoey Nua, Bangkok, Thailand. [2]Alltech Philippines Corp, Muntinlupa City, Manila, Philippines.

Heavy metals such as lead (Pb), arsenic (As) and cadmium (Cd) have been found to contaminate inorganic minerals, inorganic mineral premixes and pig feeds beyond the limits set by the European Union (EU) (Jarman *et al.*, 2010). These heavy metals can accumulate in animal tissues, organs and bones and render edible animal products as not fit for human consumption (Sharma *et al.*, 1982). Thus, the objective of this study was to check the incidence of heavy metal contamination (Pb, As and Cd) in different trace mineral sources for pig production.

A total of 233 samples weighing approximately 100 g each of pig compound feeds, inorganic mineral premixes and individual inorganic minerals was collected from 12 countries in the Asia Pacific region. Each sample was prepared using the method described in AOAC (1995). Samples were tested for Pb, As and Cd using an inductively coupled plasma emission spectrometer (ICP-OES). The EU limit levels for the compound feed (Pb 5, As 2, Cd 0.5 ppm), inorganic mineral premix (Pb 200, As 30, Cd 10 ppm) and inorganic zinc, copper and iron (Pb 100, As 30, Cd 10 ppm) were used as standards in determining if the samples were within limits or beyond (contaminated).

Inorganic mineral premixes, inorganic zinc (Zn), copper (Cu) and iron (Fe) had 12, 3, 14 and 7% contamination, respectively (Figure 1). Thirty-eight percent of the compound feeds were found to be contaminated as well.

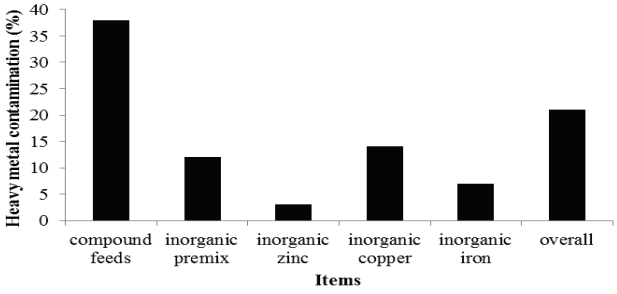


Figure 1. *Heavy metal contamination levels in pig compound feeds and different trace mineral sources.*

Complete feeds had high As (mean 7.82 ppm versus 2 ppm maximum) and Cd (mean 0.94 ppm versus 0.5 ppm maximum) (Table 1). Pb was high in inorganic Zn and inorganic Cu (mean 101.80 and 164.70 ppm respectively, versus 100 ppm maximum). The average levels of As in inorganic Zn, Cu and Fe were within limits although there are some samples which were above the limit (>30 ppm).

Table 1. *Level of heavy metal contamination in common sources of trace minerals for pig production**

Sample type	Total samples	Lead (ppm)		Arsenic (ppm)		Cadmium (ppm)	
		Mean	Range	Mean	Range	Mean	Range
Pig compound feeds	98	3.77	0-162	7.82	0-71	0.94	0-50
Inorganic mineral premix	57	82.17	0-3,530	18.91	0-284	3.51	0-112
Inorganic zinc	35	101.80	0.02-2,666	5.34	0.14-109	3.32	0.04-65
Inorganic copper	29	164.70	1.5-1,357	5.51	0.27-26	10.36	0-143
Inorganic iron	14	8.36	1.7-20	4.84	0.14-39	2.17	0.52-3

*EU maximum limits for pig feeds are Pb 5, As 2, Cd 0.5 ppm; Inorganic mineral premix – Pb 200, As 30, Cd 10 ppm; Inorganic Zn, Cu and Fe – Pb 100, As 30, Cd 10 ppm, respectively.

This survey shows 21% of samples collected from the Asia Pacific region had at least one heavy metal exceeding the limits set by the EU for heavy metal contamination. Risk assessment and continuous analysis for heavy metals must form the basis of selecting and using trace mineral sources, to perhaps prevent contaminating the feed supply.

AOAC (1995). AOAC INTERNATIONAL: Method 968.08 (16th edition).

JARMAN, T., FRIO, A.J.L., LEARY, A., KOCHER, A., FIKE S. and TIMMONS, B. (2010). In "Proceedings of the 21st Annual Australian Poultry Science Symposium", pp.174-177, ed. P. Selle. (Poultry Research Foundation: Camden).

SHARMA, R.P., STREET, J.C. and SHUPE, J.L. (1982). *Journal of Food Safety.* **4**:151-163.

Implications of the changing structure of the Chinese pig industry for feed additive manufacturers

M.P. Boddington

Asian Agribusiness Consulting, Suite B209 Bai Jia Zhuang Commercial Centre, Beijing China.

China is the world's largest pork producer and consumer (OECD-FAO, 2013) with an estimated production of 698 million pigs in 2012 (FAS, 2013). Feed is the largest expense in commercial pig production in China, accounting for about 60 % of the total cost (Gale *et al.,* 2012). As explained by Gale *et al.* (2012), an increasing number of farmers use commercial formula feeds that often includes corn, soymeal, other protein meals and feed additives. The objective of this paper is to provide an indication of the relationship between the changing Chinese pig industry structure and the use of different feed additives between 2008 and 2012.

According to Gale *et al.* (2012), since 2008 China's pig industry has seen volatile farm gate prices that have resulted in higher returns for farmers. Pork is the preferred meat in China with annual consumption estimated at 38 kg per capita (McOrist *et al.* 2011). Further, the Chinese pig industry has started to consolidate with smaller farms withdrawing from the industry and an increasing percentage of production coming from larger commercial farms. For example, over the four year period, 2008 to 2012, the number of backyard farms has decreased from 72 to 58 million farms (Table 1).

Table 1. *Changes in the Chinese Pig Industry between 2008 and 2012.*

Items	2008	2012	Percentage change
Pork production ('000 T)	46,200	53,400	16
Carcass weight (kg/pig)	75.5	78.0	3
Average live pig price (CNY/kg)	14.9	15.1	1
Total farms ('000)	72,022	58,081	-19
Backyard farms (%)	99.97	99.86	-19
Commercial farms (%)	0.026	0.133	45
Large scale pig farms (%)	0.004	0.007	64

Source: Gale *et al*. (2012); China Livestock Yearbooks, 2008 to 2012.

Asian Agribusiness Consulting (AAC) collects data, through on-farm face-to-face interviews and telephone interviews with pig farmers and industry key opinion leaders. This data pertains to husbandry practices and the use of different feed additives by the Chinese pig industry. A selection of such data is presented in Table 2.

Table 2. *AAC survey data showing changes in the use of non-medicated feed additives between 2008 and 2012.*

	Total (T)		
Feed additive	2008	2012	Percentage change
Acidifier	33,000	36,000	9
Mycotoxin binder	10,000	12,000	20
Organic trace minerals	35,000	43,000	23

Commercial-scale farms purchase commercial feeds, whilst small-scale farms use inexpensive crop stalks, bran and hulls from grains, food scraps, and forages (Gale *et al.,* 2012). Due to the increase in number of commercial farms over the period 2008 to 2012 (Table 1), it would be expected that there would be a higher usage of non-medicated feed additives. AAC data indicated that there were varying levels of increased use of acidifier, mycotoxin binder and organic trace mineral (Table 2). However, this increased use was lower relative to the percentage increase in number of farms that may be interested in using these products. The use of non-medicated feed additives to improve performance and manage animal health is likely to be a contributing factor to the increase in pork production shown in Table 1. However, even though the live pig price increased (Table 1), as explained by FAS (2013), the cost of feed also increased over this time period. The challenge for feed additive providers will be to convince producers that positive net returns can be made from using such products.

FAS (2013). Livestock and poultry: World markets and trade. Report of the Foreign Agricultural Service, USDA. 25pp.
GALE, F., MARTI, D., HU, D. (2012). China's Volatile Pork Industry. A report from the ERS, USDA, LDP-M-211-01.
McORIST, S., KHAMPEE, K., and GUO A. (2011). *Revue Scientifique et Technique*. **30**:961-968.
OECD-FAO (2013). Agricultural Outlook 2013-2022: Highlights. 119pp.

The influence of Danish versus Dutch feeding of lactating sows on piglet growth and milk composition: A pilot study

C. Amdi[1], A.T. Nguyen[1], M. Pedersen[2] and C.F. Hansen[1]

[1]Department of Large Animal Sciences, University of Copenhagen, Denmark. [2]Department of Food Science, Dairy Technology, University of Copenhagen, Denmark.

Piglet mortality in The Netherlands is lower (12.9%) than in Denmark (13.8%) (Christiansen, 2010) despite both countries having hyperprolific sows. Dutch and Danish sow feed recommendations differ, with the Dutch recommendations generally containing higher dietary fibre. It is therefore relevant to consider how suckling piglets are affected by these differences in sow feed recommendations during pregnancy and lactation (Danish versus Dutch). Inclusion of fibre in sow lactation diet is likely to improve growth rate of piglets during the suckling period (Quesnel *et al.*, 2009) due to increased production of short chain fatty acids, thus leading to an increased milk production and fat percentage in the sow's milk. We hypothesised that offspring born to sows fed a Dutch feeding regime would have greater weaning weights due to improved nutritional conditions namely the quality of milk produced during lactation.

This study was conducted as part of a larger project in a Danish production herd (660 sows) (St. Ladegaard, Sorø, Denmark) investigating Danish versus Dutch feeding regimes on feed efficiency and piglet mortality of Danish Landrace x Danish Yorkshire sows. During gestation, multiparous sows fed according to Danish recommendations were placed on different feeding levels depending on visual body condition scoring. During lactation they were fed according to appetite. Sows fed according to Dutch recommendations received different feeding levels depending on P2 back fat depth during gestation and lactation. During lactation both diets contained the same amount of potential physiological energy (PPE) of 7.70 MJ/feed unit for sows (FUsow), with a crude fibre content of 3.3% and 4.7% per FUsow for the Danish and Dutch feeding regime, respectively. Birth weights were recorded for 711 piglets from 50 sows and of these, 392 piglets that remained with their birth mother were ear-tagged at birth and followed through to 3 weeks of age. The ear-tagged piglets were weighed weekly and growth rates (average daily gain, ADG) calculated. Milk samples (about 30 ml) were collected at d 10 of lactation and analysed for dry matter, fat, protein and lactose concentrations using an infrared analyser (MilkoScan^{TM} FT2). Data were analysed using the MIXED procedure in SAS. Birth weight was included as a covariate for analysis of ADG, and sow included as a random effect in the model.

Sows fed the Dutch feeding regime had a higher ($P<0.05$) milk fat percentage than Danish-fed sows [7.49 % (SEM 0.25) versus 6.79 % (SEM 0.24)]. No differences ($P>0.05$) were found in dry matter, protein and lactose in the milk of Danish- and Dutch-fed sows.

Table 1. *Birth weight and ADG of piglets born to sows on a Danish or Dutch feeding regime.*

	Sow Feeding Treatments		SEM	Significance
	Danish	Dutch		
Number	315	396		
Birth weight (kg)	1.37	1.37	0.020	0.890
Number	156	236		
ADG d 0-7 (g/d)	186	163	22.8	0.005
ADG d 7-14 (g/d)	238	202	6.0	0.001
ADG d 14-21 (g/d)	234	201	6.7	0.001
Total ADG d 0-21 (g/d)	223	195	4.6	0.001

ADG, average daily gain; SEM, standard error of the mean.

Sows fed the Dutch feeding regime had more fat in the milk at d 10, most likely due to the higher fibre level in the feed. However, in this subsample of piglets this did not directly translate to increased growth rate in the piglets born to Dutch sows. On the contrary, the piglets from Danish-fed sows had a higher ADG than Dutch piglets up to 21 d of age. In this experiment it was not possible to measure total milk yield, however it cannot be ruled out that although Dutch sows had a higher milk fat percentage, Danish sows might have had a higher total yield, due to *semi ad libitum* feeding during lactation versus the more restrictive feeding regime of the Dutch sows. This could in turn have positively influenced growth rates.

CHRISTIANSEN, M.G. (2010). Notat nr. 1005. Danish Pig Research Centre.

QUESNEL, H., MEUNIER-SALAUN, M.C., HAMARD, A., GUILLEMET, R., ETIENNE, M., FARMER, C., DOURMAD, I.Y. and PERE, M.C. (2009). *Journal of Animal Science.* **87:**532-543.

The Villum Foundation and the Danish Pig Research Centre are acknowledged for financial support.

Dietary vitamin E and aspirin supplementation influence the performance and incidence of post-weaning colibacillosis in pigs experimentally infected with an enterotoxigenic strain of *Escherichia coli*

J.C. Kim[1], H.G. Payne[1], M.D. Langridge[1], J.P.A. Sweeny[1], B.P. Mullan[1] and J.R. Pluske[2]

[1]Department of Agriculture and Food, South Perth, WA 6151. [2]Murdoch University, Murdoch, WA 6150.

Increased biosynthesis of prostaglandin E_2 (PGE_2) from arachidonic acid, caused by immune system activation, negatively affects the performance and health of pigs (Wright *et al*., 2000). Xu *et al*. (1990) reported that supplementation of 125 ppm aspirin, an anti-inflammatory agent, improved the daily gain and feed conversion ratio of weaner pigs. Furthermore, in chickens, a combined supplementation of aspirin and vitamin E (Vit E) synergistically depressed PGE_2 biosynthesis and reduced mortalities after an *E. coli* infection (Likoff *et al*., 1981). The current experiment tested the hypothesis that Vit E and aspirin supplementation would have a synergistic effect on reducing post-weaning colibacillosis (PWC) and improving performance in pigs experimentally infected with an enterotoxigenic strain of *E. coli* (ETEC).

A total of 192 individually-housed male weaner pigs (Landrace x Large White) weighing 6.6 ± 0.04 kg (mean±SEM) were allocated to a 2 x 3 factorial experiment with the respective factors being without and with 125 ppm aspirin (acetylsalicylic acid; Bayer) and three levels of Vit E supplementations (50, 100, and 200 IU, *dl*-α-tocopheryl acetate; DSM). A wheat, soybean meal and skim milk powder-based basal diet was formulated to contain 15.3 MJ digestible energy (DE)/kg (10.7 MJ net energy (NE)/kg) and 0.9 g standardised ileal digestible lysine/MJ DE. Pigs were fed experimental diets *ad libitum* and fresh water was supplied through a bowl drinker. All pigs were challenged with *E. coli* serotype O149:K91:K88 at 7, 8 and 9 d after weaning. Pigs were weighed and feed intake was recorded weekly to calculate performance indices. Expression of diarrhoea as a faecal consistency score and the number of antibiotic treatments were recorded daily for 14 d after weaning. Faecal shedding of β-haemolytic *E. coli* was scored on a six-point scale after culturing faecal swabs on 5% horse blood agar plates and incubating overnight at 37 °C. Data were analysed by two-way analysis of variance (GenStat, 15th Edition; UK).

Table 1. *Interaction means for the effects of aspirin and vitamin E on performance and indices of PWC measured for 14 days after weaning in* E. coli*-infected pigs.*

Aspirin (A) (ppm)	0			125				*Significance*		
Vit E (E) (IU)	50	100	200	50	100	200	SEM	Aspirin	Vit E	A x E
ADG (g[1])	176	153	140	177	178	169	10.6	0.034	0.119	0.375
VFI (g[1])	195	174	167	183	185	167	10.0	0.998	0.090	0.503
FCR (g/g[1])	1.39	1.31	1.47	1.17	1.14	1.08	0.14	0.022	0.914	0.700
DI (%[2])	6.7^{ab}	6.5^{ab}	11.4^{bc}	7.6^{ab}	6.2^{ab}	4.2^{a}	1.72	0.126	0.701	0.043
Antibiotic treatments[3]	1.5^{ab}	1.7^{ab}	2.3^{b}	2.2^{b}	1.9^{ab}	1.1^{a}	0.36	0.724	0.910	0.023
E. coli score[4]	3.5^{b}	2.2^{ab}	4.2^{b}	3.0^{b}	1.5^{a}	2.5^{ab}	0.45	0.009	0.002	0.391

[1]ADG: average daily gain; VFI: voluntary feed intake; FCR: feed conversion ratio; [2]DI: Diarrhoea index (%); mean proportion of days with diarrhoea with respect to 14 d after weaning; [3]Mean numbers of antibiotic treatments; [4]Mean cumulative *E. coli* score per diet in the 14 d after weaning; [a,b,c] Means in a row not having the same superscript are significantly different (P<0.05).

Supplementation of aspirin alone improved (P<0.05) ADG and FCR. Significant interactions (P<0.05) occurred between aspirin and Vit E for indices of PWC, namely the DI and the number of antibiotic treatments. Aspirin and 100 ppm Vit E supplementation independently decreased (P<0.05) the β-haemolytic *E. coli* score. The results indicate differential effects of aspirin and Vit E supplementation on PWC, while aspirin supplementation independently improved ADG and FCR. These data support the proposition that a reduction in the ETEC-induced anti-inflammatory status after weaning supports enhanced production, possibly associated with a reduction in PGE_2 synthesis.

LIKOFF, R.O., GUPTILL, M.S.D.R., LAWRENCE, M.S.L.M., MCKAY, C.C., MATHIAS, M.S.M.M., NOCKELS, C.F. and TENGERDY, R.P. (1981). *American Journal of Clinical Nutrition.* **34**:245-251.

WRIGHT, K.J., BALAJI., R., HILL, C.M., DRITZ, S.S., KNOPPEL, E.L. and MINTON, J.E. (2000). *Journal of Animal Science.* **78**:1892-1899.

XU, Z.R., KORNEGAY, E.T., SWEET, L.A., LINDEMANN, M.D., VEIT, H.P. and WATKINS, B.A. (1990). *Journal of Animal Science.* **68**:1639-1647.

Supported in part by Pork CRC Limited; Vitamin E was kindly donated by DSM Nutritional Products Australia Pty Ltd.

Dietary effects of β-glucan yeast on vaccine immune response and performance of pigs

M.G. Nogueira[1], D. Emery[1], A.M. Collins[2], C.L. Walk[3], S.J Wilkinson[1] and A.J. Cowieson[1]

[1]The University of Sydney, Camden, NSW 2570. [2]Department of Primary Industries, Menangle, NSW 2567. [3]AB Vista Feed Ingredients, Marlborough, UK.

Vaccination against *Lawsonia intracellularis* has been successfully used to reduce lesions of proliferative enteritis (PE) and bacterial shedding in faeces, with induction of systemic and local immune responses (Nogueira *et al*., 2013). Beta-glucans (β-glucans), polymers present in many fungi, yeast and bacterial cell walls, are also known to enhance innate immune responses. The synergistic effect of dietary β-glucans and specific immune responses has been previously correlated with increased growth performance and resistance to *Streptococcus suis* in weaner pigs (Dritz *et al*., 1995). Such studies have not been conducted with *L. intracellularis*. In this experiment, we hypothesised that addition of β-glucan yeast to a weaner pig diet would enhance immune responses to oral vaccination with *L. intracellularis*.

A total of 49 entire male Landrace x Large White weaner pigs were selected from a commercial herd clinically free of PE and transferred to a controlled environment research facility. Pigs at 3 to 4 weeks (6.9±1.7kg; mean± SEM) were randomly allocated into four treatment groups: β-glucan supplemented and vaccinated (G1, n=12), β-glucan and unvaccinated (G2, n=12), no β-glucan and vaccinated (G3, n=12), and no β-glucan and unvaccinated (G4, n=13). A weaner pig diet with no antibiotic was supplemented with 1g/kg of β-glucan yeast (AB Vista) and given to G1 and G2. A week after feed acclimatisation, G1 and G3 pigs were orally vaccinated with 2.0 mL of Enterisol® Ileitis vaccine (Boehringer Ingelheim, USA) containing $10^{5.9}$ $TCID_{50}$ (50% tissue culture infective dose) organisms (d 0). Blood was collected from each pig at d 0, 7 and 25 post-vaccination and *L. intracellularis*-specific IgG levels were determined as percent inhibition (PI) with the Bioscreen Ileitis ELISA (GmbH, Germany). Ileal mucosa were collected at necropsy (d 28 post vaccination) to determine IgG (PI), IgA titres (Nogueira *et al*., 2013) and cytokine levels: IFN-γ, TNF-α, IL-6, IL-10, TGF-β1 (Quantikine®ELISA; R&D Systems, US). Pigs and feed were weighed weekly to determine average daily gain (ADG) and feed intake (FI). Data were analysed using a restricted maximum likelihood test (GenStat, 13th Edition; UK).

Table 1. *Means for* L. intracellularis*-specific IgG, IgA and cytokines in ileal mucosa from pigs given diets with (+G) or without (-G) β-glucan in vaccinated (+V) or unvaccinated (-V) groups.*

Treatment	IgG (PI value)	IgA (Titre)	IFN-γ (pg/mL)	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)	TGF-β1 (pg/mL)
G1 (+G+V)	24.2[a]	22.9[a]	121.7[a]	26.7[a]	19.7	84.2	78.1
G2 (+G -V)	18.3[b]	17.2[ab]	144.5[a]	14.5[b]	15.3	90.6	79.2
G3 (-G+V)	16.7[b]	11.8[b]	97.0[b]	15.6[b]	16.7	63.8	55.4
G4 (-G -V)	6.7[c]	9.2[c]	66.1[c]	3.4[c]	12.3	70.5	65.1
SEM	2.2	7.2	20.5	4.9	6.3	14.1	17.6
Significance of main effects and interactions							
β-Glucan	<0.05	<0.05	<0.05	<0.05	NS	NS	NS
Vaccination	<0.05	NS	NS	<0.05	NS	<0.05	<0.05
β-Glucan*Vaccination	NS	NS	NS	<0.05	NS	NS	NS

[a-c] Means within columns not having the same superscript are significantly different (P<0.05); NS, not significant (P>0.05); IFN-γ: Interferon-gamma, TNF-α: Tumor necrosis factor alpha; TGF-β1: Transforming growth factor beta 1; IL: Interleukin. SEM: Standard error of the mean.

Pigs fed β-glucan exhibited increased mucosal levels of *L. intracellularis*-specific IgG compared with non-supplemented pigs (Table 1). In addition, *L.intracellularis*-specific IgG responses in serum were also higher (P<0.05) in group G1 (PI: 33.4%), followed by G3 (PI: 22.8%), while G2 and G4 had similar responses (PI: 16.2% and 6.8%, respectively; data not shown). Pigs fed β-glucan had lower FI (P=0.005) at 2 weeks (657 g/pig/d) compared with pigs fed diets without β-glucan (717 g/pig/d); however no statistical differences in ADG were observed between treatments over 5 weeks. Both effects are likely mediated through stimulation of innate immunity with production of the pro-inflammatory cytokines, IFN-γ and TNF-α, to enhance acquire immunity and decrease appetite (Gallois *et al*., 2009).

DRITZ, S., SHI, J., KIELIAN, T., SMITH, J. and BLECHA, F. (1995). *Journal of Animal Science.* **11**:3341-3350.
GALLOIS, M., ROTHKÖTTER, H., BAILEY, M., STOKES, C. and OSWALD, P. (2009). *Animal.* **3**:1644-1661.
NOGUEIRA, M., COLLINS, A., DONAHOO, M. and EMERY, D. (2013). *Veterinary Microbiology.* **164**:131-138.

Supported by AB Vista Feed Ingredients, UK.

The impact of dietary phytase on immune responses and performance of pigs

M.G. Nogueira[1], D. Emery[1], A.M. Collins[2], C.L. Walk[3], S.J. Wilkinson[1] and A.J. Cowieson[1]

[1]The University of Sydney, Camden, NSW 2570. [2]Department of Primary Industries, Menangle, NSW 2567. [3]AB Vista Feed Ingredients, Marlborough, UK.

Proliferative enteropathy (PE), caused by *Lawsonia intracellularis*, reduces absorption of amino acids by impairing brush border enzyme activity in the intestinal mucosa (Collins *et al*., 2009). Control of PE via vaccination is therefore likely to be critical when pig diets are less digestible. The attenuated Enterisol® Ileitis vaccine protects pigs from PE and induces systemic and local immune responses (Nogueira *et al*., 2013). The use of phytase enzymes in pig diets improves phytate-P digestibility with absorption of cations and amino acids (Cowieson *et al*., 2009). Previously, *E. coli*-derived phytase has been related to increases in peripheral blood lymphocyte in broilers (Liu *et al*., 2008). Therefore, we hypothesised that dietary phytase would enhance immune responses to oral vaccination with *L. intracellularis* in pigs.

Forty-nine entire male Landrace x Large White pigs (6.9±1.7 kg; mean±SEM) aged 3 to 4 weeks were randomly allocated into four groups: no phytase and vaccinated (G1, n=12); no phytase and unvaccinated (G2, n=13); phytase and vaccinated (G3, n=12); and phytase and unvaccinated (G4, n=12). All diets were formulated to be nutritionally adequate (Ca: 9.3% and P: 0.72%), and *E. coli*-derived phytase (Quantum Blue, AB Vista) was added at 2000 FTU/kg in diets G3 and G4. After one week on these diets, 24 pigs in G1 and G3 were orally vaccinated with 2.0 mL of the Enterisol® Ileitis vaccine (Boehringer Ingelheim, USA) containing $10^{5.9}$ $TCID_{50}$ (50% tissue culture infective dose) *L. intracellularis* organisms. Blood was collected from each pig at d 0, 7 and 25 post-vaccination (p.v.) and *L. intracellularis*-specific IgG antibody was determined as percent inhibition (PI; Bioscreen Ileitis ELISA). Ileal mucosa were collected at necropsy (d 28 p.v.) to determine IgG (PI) and IgA titres (Nogueira *et al*., 2013), and IFN-γ, TNF-α, IL-6, IL-10, TGF-β1 (Quantikine® ELISA; R&D Systems) levels. Pigs and feed were weighed weekly to determine average daily gain (ADG) and feed intake (FI). Data were analysed by the restricted maximum likelihood test (GenStat, 13th Edition; UK).

Table 1. *Effect of phytase (P) and* L. intracellularis *vaccination (V) on specific antibodies and cytokines in ileal mucosa of pigs measured 28 d post-vaccination.*

Groups	IgG	IgA	IFN-γ	TNF-α	IL-6	IL-10	TGF-β1
	(PI value)	(Titre)	(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)
G1 (-P+V)	12.1[b]	16.8	66.0[b]	14.6	16.7	63.8	55.4
G2 (-P -V)	6.7[c]	17.2	41.0[b]	5.4	12.3	70.5	65.1
G3 (+P+V)	26.3[a]	17.7	105.7[a]	17.2	18.2	60.3	99.1
G4 (+P -V)	8.7[c]	15.3	97.1[a]	7.0	13.7	66.9	60.5
SEM	3.38	3.6	25.1	9.6	3.6	14.3	31.7
Main effects							
Phytase	<0.05	NS	<0.05	NS	NS	NS	NS
Vaccination	<0.05	NS	NS	<0.05	NS	<0.05	<0.05
Phytase*Vaccination	<0.05	NS	NS	NS	NS	NS	NS

[a-c] Means within columns not having the same superscript are significantly different (P<0.05); NS, not significant (P>0.05); IFN-γ: Interferon-gamma, TNF-α: Tumor necrosis factor alpha; TGF-β1: Transforming growth factor beta 1; IL: Interleukin. SEM: Standard error of the mean.

Vaccinated groups had higher (P<0.05) *L. intracellularis* IgG antibody levels in serum (G1: 26.2 and G3: 35.6) compared with unvaccinated pigs (G2: 7.4 and G4: 8.2), but no effect of diet was found (data not shown). No statistical differences in ADG and FI occurred between treatments over the five-week experiment. Pigs supplemented with phytase showed increased (P<0.05) levels of IFN-γ in ileal mucosa (Table 1). Vaccinating pigs increased specific IgG concentrations compared to non-vaccinated pigs, and this was further increased by feeding pigs phytase supplemented diets, leading to a phytase-vaccination interaction (P<0.05). However the mechanistic basis for the observed effects is yet to be elucidated.

COLLINS, A.M., BOLSIUS, N., VAN STRAATEN, J. and FELL, S. (2009). In. "Manipulating Pig Production XII", p.130, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).
COWIESON, A.J., BEDFORD, M.R., SELLE, P.H. and RAVINDRAN, V. (2009). *World's Poultry Science Journal*. **65**:401-418.
LIU, N., RU, Y., COWIESON, A., LI, F. and CHENG, X. (2008). *Poultry Science*. **87**:1105-1111.
NOGUEIRA, M., COLLINS, A., DONAHOO, M. and EMERY, D. (2013). *Veterinary Microbiology*. **164**:131-138.

Supported by AB Vista Feed Ingredients, UK.

Different fibre sources fed to weaner pigs influence production performance and acute phase protein levels

J.R. Pluske[1], A. Hernandez[1], J. Mansfield[1] and J.C. Kim[2]

[1]Murdoch University, Murdoch, WA 6150. [2]Department of Agriculture and Food WA, South Perth, WA 6151.

Dietary fibre is fermented by microbiota in the distal gastrointestinal tract (GIT) to short-chain fatty acids (SCFA). Previous studies (e.g., Pluske *et al*., 2002) have shown differential effects of SCFA on growth performance and the incidence of disease such as post-weaning diarrhoea (PWD), however more recently the SCFA have become recognised as potential mediators in inflammatory and immune functions in the GIT (Vinolo *et al*., 2011). This experiment examined the effects of infection with an enterotoxigenic strain of *E. coli* on pig performance, SCFA production, and biomarkers of inflammation after weaning.

A total of 80 Large White x Landrace pigs obtained at weaning (21 d of age; 6.78±0.121 kg, mean±SEM) were fed five experimental diets as follows: (1) rice-based diet (R), (2) rice diet with inulin (R+I); (3) rice diet with lupin hulls (R+LH), (4) rice diet with inulin and lupin hulls (R+I+LH), and (5) commercial diet based on wheat and barley (C). None of the diets contained antimicrobial compounds, and were balanced for the digestible energy (DE) (14.9 MJ DE/kg) and available lysine (1.25%) contents. Each treatment had four replicate pens with four pigs per pen. Pigs were fed *ad libitum* for 4 weeks. All pigs were challenged with an enterotoxigenic strain of *Escherichia coli* (*E. coli*) serotype O149:K91:K88 at 72, 96 and 120 h after weaning. The diarrhoea index and faecal shedding of *E. coli* were assessed according to Heo *et al*. (2009). Two medium-weight pigs per pen were bled on d 7, 14 and 21 to measure circulating levels of haptoglobin (Hp) and C-reactive peptide (CRP), biomarkers of an inflammatory response in the GIT (Petersen *et al*., 2004). On d 21 of the experiment one pig, of median weight, from each pen of the R, R+I+LH and C diets (four pigs per treatment) was humanely euthanised, and digesta samples from the mid colon were collected and appropriately stored until analysed for SCFA concentration using gas liquid chromatography. Data were subjected to one-way ANOVA using SPSS Statistics (version 21; IBM).

Table 1. *Performance (d 1-28 after weaning) and fermentation characteristics of digesta in weaner pigs fed different fibre sources.*

	Diet						
	R[6]	R+I	R+LH	R+I+LH	C	SEM[7]	Significance
Number[1]	4	4	4	4	4		
ADG[2] (g/d)	351^{ac}	287^{b}	339^{ab}	384^{a}	326^{bc}	17.4	0.025
ADFI[3] (g/d)	502^{ac}	434^{b}	476^{ab}	527^{c}	453^{b}	14.1	0.003
FCR[4] (kg/kg)	1.42	1.50	1.41	1.40	1.40	0.035	0.858
Total SCFA[5] Colon (mM/organ)	11^{a}	-	-	14^{a}	42^{b}	5.70	0.007

^{a,b,c}Means in a row not having the same superscript are significantly different (P<0.05); [1]Number, number of replicates; [2]ADG, average daily gain; [3]ADFI, average daily feed intake; [4]FCR, feed conversion ratio; [5]SCFA, short-chain fatty acids; [6]See text for details of diets; [7]SEM: standard error of mean.

Pigs fed diets R and R+I+LH grew faster than pigs fed diet R+I (P=0.025), with pigs fed other diets being intermediate in performance. Pigs fed diet R+I+LH consumed more feed than pigs fed diets R+I, R+LH and C (P=0.003), but the same as pigs fed diet R (Table 1). There were no effects (P>0.05) of treatment on FCR, diarrhoea index (d 0-14), *E. coli* score (from faecal swabs) or the number of pigs treated therapeutically with antibiotics. Levels of Hp and CRP were higher (P<0.05) only in C-fed pigs on d 14 and on d 14 and 21, respectively. The SCFA concentration in the mid colon was highest (P=0.007) in pigs fed diet C, but similar for those fed diets R and R+I+LH. These data suggest diet-specific changes, most likely attributable to production and (or) production patterns of SCFA, in the production of acute phase proteins in the pig after weaning. There were no effects of the different diets on PWD in this experiment.

HEO, J.M., KIM, J.C., HANSEN, C.F., MULLAN, B.P., HAMPSON, D.J. and PLUSKE, J.R. (2009). *Journal of Animal Science.* **87:**2833-2843.

PETERSEN, H.H., NIELSEN, J.P. and HEEGAARD, P.M.H. (2004). *Veterinary Research*. **35:**163-187.

PLUSKE, J.R., PETHICK, D.W., HOPWOOD, D.E. and HAMPSON, D.J. (2002). *Nutrition Research Reviews.* **15:**333-371.

VINOLO, M.A.R., RODRIGUES, H.G., NACHBAR, R.T. and CURI, R. (2011). *Nutrients.* **3:**858-876.

Supported by Murdoch University.

Relationships between fibre intake and the expression of genes linked to incretin secretion in the gastrointestinal tract of weaner pigs

J.R. Pluske[1], A. Hernandez[1], J. Mansfield[1], C.H. Auckland[1] and J.C. Kim[2]

[1]Murdoch University, Murdoch, WA 6150. [2] Department of Agriculture and Food WA, South Perth WA 6151.

Dietary fibre [predominately non-starch polysaccharides (NSP) and resistant starch] is fermented to short chain fatty acids (SCFA) by the microbiota in the distal gastrointestinal tract (GIT). The activation of G-protein coupled receptors (GPRs), such as GPR41 and GPR43, by SCFA in the epithelium activates the proglucagon (PG) gene in the L cells of the GIT to secrete incretins such as glucagon-like peptides (GLP-1, GLP-2) and peptide YY (PYY), that in turn are implicated in the control of GIT structure and function (Black *et al*., 2009). We are unaware of any work conducted in weaner pigs in this area. The hypothesis tested was that the expression of genes coding for GPRs and PG is influenced by the intake of NSP.

Details of the larger experiment from which this study is derived can be found in Pluske *et al*. (2013). Briefly, 48 pigs were fed either a rice-based diet (R), diet R with added inulin and lupin hulls (R+I+LH), or a commercial diet based on wheat and barley (C). Diets differed in the levels of soluble, insoluble and total NSP, ranging from 3.8-21.4 g/kg for soluble NSP, 9.6-93.1 g/kg for insoluble NSP, and 13.4-114.6 g/kg for total NSP. On d 21 of the experiment one pig of median weight from each pen (four pigs per treatment) was euthanised, and mucosa was collected from a 20-cm segment of the ileum and mid-colon. The mucosa was placed in RNAlater® for 24 h and subsequently stored at -20 °C. Standard procedures for RNA extraction, reverse transcription, and quantitative real-time polymerase chain reaction for GPR41, GPR43 and PG were conducted. Data were then analysed using the method of Pfaffl (2001), which determines the efficacy of the primers (relative to housekeeping genes) and then factors this into the determination of the quantity of RNA that is expressed. Multiple regression ANOVA (SPSS Statistics, version 21; IBM) relating NSP intake per pen and gene expression per pig was conducted.

Table 1. *Correlation coefficients for the relationships between NSP intake (g/pig/d) and the expression of GPR43 (ng/µl) and PG (ng/µl) in the mid-colon[1].*

	sNSP[2] intake	iNSP[3] intake	tNSP[4] intake	R^2 of model	Significance
GPR43	0.682	0.567	0.599	0.469	0.109
PG	0.352	0.204	0.237	0.160	0.457

[1]Four samples per treatment, except GPR43 where in treatments R+I+LH and C, n=3; [2]sNSP: soluble non-starch polysaccharides; [3]iNSP: insoluble non-starch polysaccharides. [4]tNSP, total non-starch polysaccharides.

There was a lack of expression of GPR41 in the mid-colon and ileum. The model used explained 46.9% of the variance in the expression of GPR43 in the mid-colon (P=0.109), with the strongest unique contribution made by the intake of soluble NSP. A similar model explained only 16% of the variation in the expression of PG in the same organ (P>0.05). In the ileum, intake of NSP did not correlate with GPR43 or PG expression (P>0.05). Results also showed a strong trend for a correlation between total concentration of SCFA and expression of GPR43 in the mid-colon (R^2=0.393, P=0.052), but no correlation between SCFA and expression of PG in the mid-colon (R^2=0.006, P>0.05) (data not shown). These data suggest that expression of GPR43 in the mid-colon is influenced by the intake of NSP, especially the soluble fraction as well as the concentration of SCFA in the same organ. There was no evidence of a strong correlation between intake of NSP and PG expression, however measurement of the concentration of GLP-1, GLP-2 and PYY in the plasma and their expression in the GIT of the same pigs might link the secretion of these hormones to the intake of NSP. Furthermore, the use of more replicates would strengthen the relationships.

BLACK, J.L., WILLIAMS, B.A. and GIDLEY, M.J. (2009). In "Voluntary Feed Intake in Pigs", pp. 189-214, eds. D. Torrallardona and E. Roura. (Wageningen Academic Publishers: The Netherlands).

PFAFFL, M.W. (2001). *Nucleic Acids Research.* **29:**2002-2007.

PLUSKE, J.R., HERNANDEZ, A., MANSFIELD, J. and KIM, J.C. (2013). In 'Manipulating Pig Production' XIV, p.115, eds. J.R. Pluske and J.M. Pluske. (Australasian Pig Science Association: Werribee).

Supported by Murdoch University.

Influence of nutrient asynchrony on whole body protein retention rate in growing pigs

J.C. Kim[1], H.G. Payne[1], M.D. Langridge[1], B.P. Mullan[1], J.R. Pluske[2], A.C. Edwards[3], C.L. Collins[4] and D.J. Henman[4]

[1]Department of Agriculture and Food WA, South Perth, WA 6151. [2]Murdoch University, Murdoch, WA 6150. [3]ACE Livestock Consulting Pty Ltd, Cockatoo Valley, SA 5351. [4]Rivalea Australia Pty Ltd, Corowa, NSW 2646.

The concept of nutrient synchrony depends on supplying dietary glucose and amino acids (AA) in a synchronised manner to increase nitrogen (N) utilisation efficiency by minimising the energy-dependent conversion of certain AA to glucose or glucose to AA (van den Borne *et al*., 2007). The hypothesis tested was that the whole body N retention rate would be limited in pigs fed a nutrient asynchronised diet compared to pigs fed a synchronous diet.

A duplicate 6x6 Latin Square experiment with 12 individually-housed entire male Large white x Landrace pigs (39.5±0.36 kg; mean±SEM) was conducted with six combinations of nutrient synchronised and asynchronous diets to determine N balance and N utilisation efficiency, using the total collection method. Selection of starch and protein sources was based on the results of *in vitro* starch and protein digestion studies (Sopade and Gidley, *unpublished*). Based on these results, combinations of three starch sources [barley (fast digestible starch, FDS), wheat (moderately digestible starch, MDS), sorghum (slowly digestible starch, SDS)] and two AA sources [casein (rapidly digestible AA, RDAA), soybean meal and canola meal (slowly digestible AA, SDAA)] were used to create a range of synchrony in the availability of glucose and AA. The experimental diets were FDS+RDAA, FDS+SDAA, MDS+RDAA, MDS+SDAA, SDS+RDAA, SDS+SDAA. The six dietary treatments were randomly assigned to two pigs per 10-d metabolism study (5-d adaptation plus a 5-d total collection), which were repeated three times to collect six faecal and urine samples per diet. Pigs fed at 3 times maintenance energy level (0900 and 1600 h). All diets were formulated to contain 13.8 MJ digestible energy (DE)/kg and 0.53 g available lysine/MJ DE whilst maintaining the ideal pattern of AA, but the timing of nutrient availability differed due to the differing ingredient compositions. Data were analysed by one-way ANOVA (Genstat 15; VSN International Ltd), because the purpose of the experiment was to examine the effect of individual combinations rather than interaction effects. As body weight and N intake are known to influence N retention rate, N output and N retention data were calculated as g/kg body weight $(BW)^{0.75}$/day, and N intake was used as a covariate.

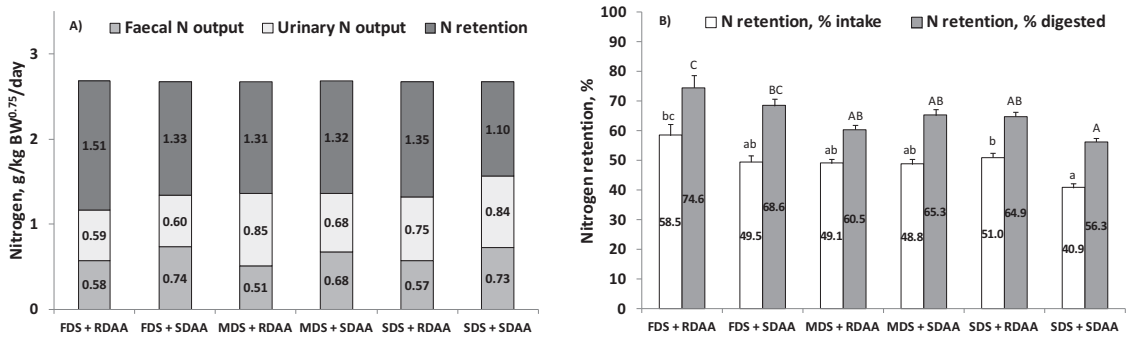


Figure 1. *Whole body N balance (A) and proportion of N retention (B) determined in growing pigs. Values are N intake-adjusted. Means with different letters differ significantly (P<0.05). N, nitrogen; FDS, fast digestible starch; MDS, moderately digestible starch; SDS, slowly digestible starch; AA, amino acids; RDAA rapidly digestible AA; SDAA, slowly digestible AA.*

Feeding RDAA decreased faecal N output (P<0.01); however, when glucose is not readily available (MDS or SDS with RDAA), feeding RDAA increased urinary N excretion (P<0.05) compared with FDS+RDAA. In conclusion, a combination of FDS and RDAA improved N retention rate (P<0.05) compared with a combination of SDS and SDAA. Based on these data, it is recommended that diet formulations for growing/finishing pigs should avoid a combination of SDS and SDAA to minimise metabolic N loss associated with nutrient asynchrony.

VAN DEN BORNE, J.J.G.C., SCHRAMA, J.W., HEETKAMP, M.J.W., VERSTEGEN, M.W.A. and GERRITS, W.J.J. (2007). *Animal*. **1:**666-674.

Supported in part by Pork CRC Limited Australia.

Digestible energy content for Berkshire triticale varies depending on season and site

J.M. Pluske[1], J.R. Pluske[2] and J.C. Kim[3]

[1]SciEcons Consulting, Subiaco, WA 6008. [2]Murdoch University, Murdoch, WA 6150. [3]Department of Agriculture and Food Western Australia, South Perth, WA 6151.

A major aim associated with the development of Berkshire, a triticale variety propagated with funding from the Pork CRC, was to produce a feed grain with a high level of digestible energy (DE) and hence an ingredient that offered cost efficiencies in diet formulations. Berkshire has now been registered as a Plant Breeders' Rights (PBR) variety and has been grown in New South Wales, Victoria, South Australia and Western Australia. However, little has been documented in regard to the variation in DE content of Berkshire across these growing regions.

Kim *et al*. (2005) concluded that the DE content of Western Australian wheats fed to weaner pigs varied according to the variety and growing region. It could therefore be hypothesised that the DE content of Berkshire would also vary depending on growing conditions. As a corollary to a larger plant-breeding project (see Kim *et al*., 2011), and using NIR AusScan, data pertaining to the faecal DE content of Berkshire were analysed from 239 samples grown at 35 different sites in New South Wales, South Australia, Victoria and Western Australia during the 2009/10 season.

The DE value ranged from 13.22 MJ/kg to 14.34 MJ/kg. A single factor analysis of variance (ANOVA) indicated a significant difference in the DE values between each of the States ($P<0.01$), except for Victoria and Western Australia (Figure 1). Removing the three outliers from the Victorian data set did not alter this result. There was also a difference ($P<0.01$) in the DE recorded between sites within each State. For example, in Western Australia the mean DE content for the most northern trial site, Northampton, was 13.84 MJ/kg whilst that of the most southern site, Coomalbidgup, was 13.64 MJ/kg.

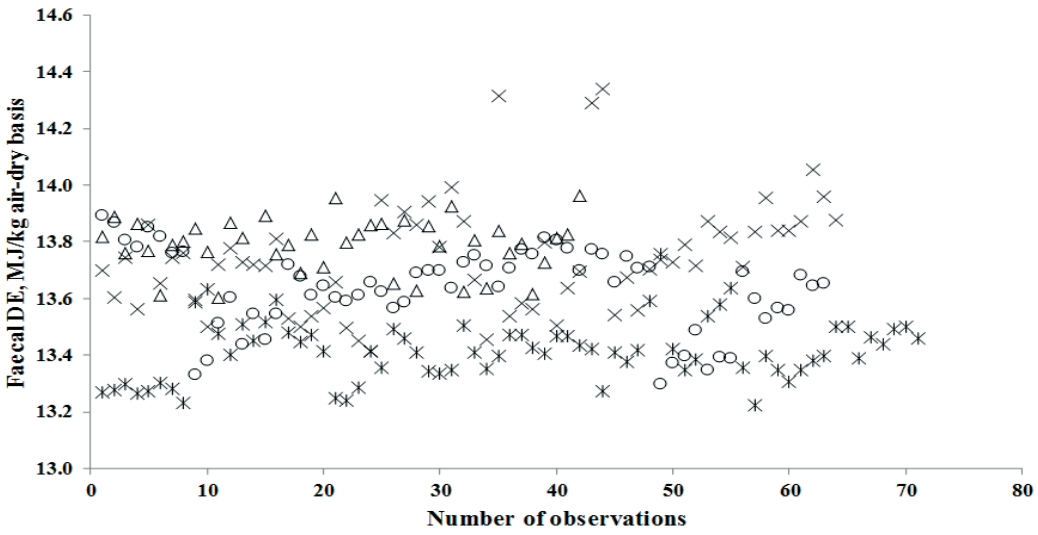


Figure 1. *The faecal DE content (MJ/kg) for Berkshire grown in the 2009/10 season at various trial sites in: New South Wales (○); South Australia (∗); Victoria (×); and Western Australia (△).*

In Australia, these data are the first known to demonstrate that the DE content of triticale is variable according to site. Season and subsequently yield may also be important factors in determining DE content. Data taken from samples grown at Dandaragan in WA, for example, indicated that the mean DE content for the 2009/10 season was 13.43 MJ/kg whilst that for the 2010/11 season was 13.73 MJ/kg. Further research would be required to explain this variation. This paper reinforces the need for objective energy content testing to maximise efficiencies in feed formulation and production.

KIM, J.C., PLUSKE, J.R., MULLAN, B.P., KING, R., WILSON, R.H., MULLAN, D., WALMSLEY, T. and PLUSKE, J.M. (2011). In "Manipulating Pig Production XIII", p.68, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).
KIM, J.C., SIMMINS, P.H., MULLAN, B.P. and PLUSKE, J.R. (2005). *Animal Feed Science and Technology*. **122:**257-287.

Supported in part by Pork CRC Limited Australia.

Why pork producers should consider the value of triticale

J.M. Pluske[1] and J.R. Pluske[2]

[1]SciEcons Consulting, Subiaco, WA 6008. [2]Murdoch University, Murdoch, WA 6150.

In Australia, the triticale price is generally established through negotiation in a specific market as opposed to other grains where price is less obscure, for example, published as daily and forecast prices. Mostly, grain growers are price takers so may consider market price history and forecasts when deciding to grow a particular crop. In the case of triticale where such information can be difficult to obtain, uncertainty associated with price and a reliable market can result in grain growers selecting alternative crops. One way to reduce price risk associated with triticale is to link its price to wheat. There is evidence in the literature (e.g., Beltranean *et al*., 2008) that suggests that when triticale is substituted for wheat in a diet, pigs do not reduce their feed intake or weight gain, and feed efficiency may improve. There may be reason therefore for the triticale price to be equivalent to the price of wheat (whatever grade is available to pork producers) because pork producers may not be worse off if they buy either. However, most often in Australia, pork producers expect to pay a lower price for triticale than wheat.

The effect of a lower price is relevant for grain growers in terms of gross margin. Typical variable costs for growing wheat and triticale in wheat belt regions of Western Australia were incorporated into a desktop experiment. By taking a May 2013 daily price for ASW wheat, $306/t, and reducing it by $20/t and $5/t (to simulate representative triticale prices), an estimation of the percentage change in gross margin from growing triticale instead of wheat can be made for ten different yield levels. A scenario involving reducing the wheat price to a level whereby growing triticale becomes economically unviable was also investigated in this study.

Under this arrangement and given the price of wheat was $306/t, growing triticale, priced at $286/t, instead of wheat would result in a percentage drop in gross margin for the grain grower from around 10% to 18% if yield for each crop was the same and below 2t/ha (Figure 1). If the yield was instead above 2t/ha, or if the price of triticale was instead reduced to $301/t, the percentage fall in gross margin from growing triticale instead of wheat would be minimal. When a lower base price of $220/t for wheat was considered, triticale priced at $200/t combined with yields below 0.9t/ha would result in an economically unviable crop. If yield was greater than 0.9t/ha, the gross margin would be between 15% and 70% lower than that of wheat at the same yield level. If triticale was instead priced at $215/t, growing it instead of wheat in low yielding regions would again be questionable whilst in higher yielding areas a grain grower may consider it if there were sufficient agronomic benefits from growing triticale instead of wheat.

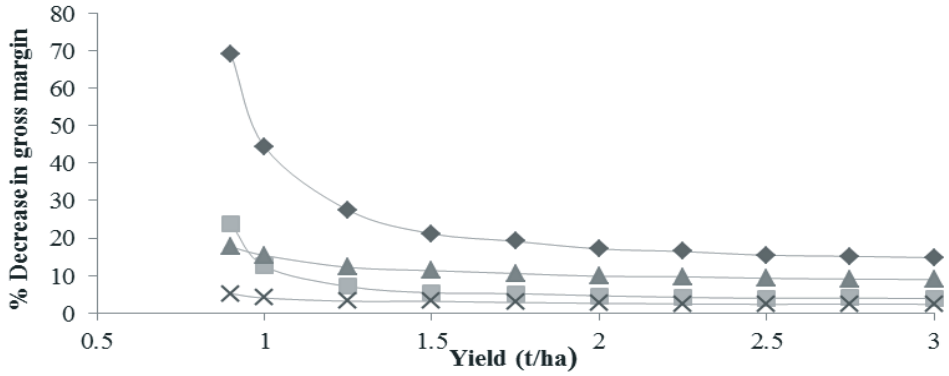


Figure 1. *The percentage decrease in the gross margin for each yield level when there is a reduction in the price of triticale from: $306/t to $286/t (▲); $306/t to $301/t (×); $220/t to $200/t (◆); and $220/t to $215/t (■).*

Agronomic benefits such as, a 2% yield advantage of triticale over wheat could induce grain growers to select triticale if the wheat price was high ($306/t) and the discount for triticale only $5/t. However, the 12% yield advantage necessary to compensate for a discount of $20/tonne when wheat price was low ($220/t) would likely be unobtainable for grain growers. The results from this paper suggested that it would only be economically rational for grain growers to grow triticale when the price relative to wheat is similar, and/or triticale yields, are relatively high. To ensure future supply of triticale, its value in pig rations should be considered if pork producers wish to be proactive in negotiating a price with grain growers that will induce them to grow it.

BELTRANEAN, E., SALMON, D.F., GOONEWARDENE, L.A. and ZIJLSTRA, R.T. (2008). *Canadian Journal of Animal Science*. **88**:631-635.

Particle size and particle size dispersion drive hydration of grains: Field peas (*Pisum sativum* L.) as a case study

G.T. Nguyen, W.L. Bryden, M.J. Gidley and P.A. Sopade

The University of Queensland, St Lucia, QLD 4072.

The water absorption index (WAI) and water solubility index (WSI) are used in assessing hydration and, therefore, functional properties of grains. The WAI or swelling indicates the ability to absorb or bind water and swell, while WSI measures the amount of water-soluble components (Mahasukhonthachat *et al*., 2010). Grains are processed to make pig diets, and the digestibility of the nutrients, notably starch and proteins, are affected. Characteristics affecting digestibility are how feeds (i) bind water during feeding, (ii) hydrate in the gastrointestinal tract (GIT) liquid, and (iii) disperse in the GIT for enzyme-substrate interactions. The WAI and WSI have been shown to essentially depend on mean particle size (D_{gw}) of milled grains (Mahasukhonthachat *et al*., 2010; Al-Rabadi *et al*., 2012). However, milling machines can produce different particle size distributions, which are described by both particle size dispersion or span (S_{gw}) and D_{gw}. We are not aware of any studies that have investigated how both D_{gw} and S_{gw} determine grain hydration. Using field peas, the present study examined the dependence of grain hydration on D_{gw} and S_{gw}, and hypothesised that grains hydrate independently of these parameters.

Two field peas varieties (*Maki* and *Walana*) were ground in disc-, hammer- and roller-mill at five, four and one setting(s) respectively. The particle size distributions were analysed with sieves (mm) 4.0, 2.8, 1.4, 1.0, 0.71, 0.5, 0.25, 0.125, 0.075, and pan (ASABE 2008), which also contains the calculations for geometric mean diameter (D_{gw}) and geometric standard deviation (S_{gw}) of the distributions. The WAI and WSI were measured in duplicate as in Mahasukhonthachat *et al*. (2010), and the milled grains were randomised prior to analysis. ANOVA and statistical evaluations were done (Minitab®, Version 16.0; USA).

Table 1. *Typical mean values of particle size parameters and hydration properties of field peas.*

Mill type	Sample*	Particle size parameter (mm)		Hydration properties	
		D_{gw}	S_{gw}	WAI (g/g solids)	WSI (g/100g solids)
Roller	*Maki*	0.28[a]	0.24[a]	3.4[a]	32.7[a]
	Walana	0.30[a]	0.25[a]	3.3[a]	31.8[b]
Disc	*Maki* (finest)	0.45[a]	0.30[a]	2.9[a]	17.8[a]
	Maki (coarsest)	1.76[b]	0.94[b]	2.5[a]	10.6[b]
Hammer	*Walana* (finest)	0.39[a]	0.27[a]	6.0[a]	28.4[a]
	Walana (coarsest)	1.86[b]	1.03[b]	2.5[b]	17.1[b]

[ab] Within a mill type, means in a column not having the same superscript are significantly different ($P<0.05$).

The field peas hydrated to different extents depending on the mill type and setting. There were no ($P>0.05$) varietal differences. Typical values of the particle size parameters and hydration properties are shown in Table 1 from the 20 means. Although there would be mill differences (e.g., thermo-mechanical effects), fine particles hydrated more ($P<0.05$) than coarse particles, with the hammer-milled peas hydrating the most. These observations agree with the results of Mahasukhonthachat *et al*. (2010) on sorghum, as fine particles with higher surface area bind more water. The hydration could also be due to the differences in the proportions of the coarse, medium and fine particles in the distribution. While D_{gw} averages these, S_{gw} reflects these proportions as a measure of the dispersion or span. Multiple regression analysis (Equations 1 and 2) revealed significant linear relationships between D_{gw}, S_{gw}, WAI, and WSI. The coefficients of these particle size parameters show opposite effects. Average particle size and particle size dispersion of milled grains control water hydration in field peas, and are important in evaluating milling of grains for pig diets.

$$WAI = -3.7\ D_{gw} + 10.2\ S_{gw} \quad (R^2 = 0.680;\ P<0.001) \qquad \text{(Equation 1)}$$

$$WSI = -19.4\ D_{gw} + 52.8\ S_{gw} \quad (R^2 = 0.600;\ P<0.001) \qquad \text{(Equation 2)}$$

AL-RABADI, G.J., TORLEY, P.J, WILLIAMS, B.A., BRYDEN, W.L. and GIDLEY, M.J. (2012). *Journal of Cereal Science.* **56**:396-403.

ASABE (2008). 'Method of Determining and Expressing Fineness of Feed Materials by Sieving'. American Society of Agricultural and Biological Engineers. Niles Road, St. Joseph, MI 49085-9659, USA.

MAHASUKHONTHACHAT, K., SOPADE, P. A. and GIDLEY, M. J. (2010). *Journal of Food Engineering*. **96**:18-28.

Support in part by Pork CRC Limited Australia.

In-vitro starch and protein digestion in field peas (*Pisum sativum* L.) reveal particle size dependence

G.T. Nguyen, W.L. Bryden, M.J. Gidley and P.A. Sopade

The University of Queensland, St Lucia, QLD 4072.

Starch and proteins are important nutrients in pig feeds, and are digested into smaller molecular compounds prior to absorption. Field peas contain these nutrients, are low in anti-nutritional factors compared with other pulses, and are used in cereal-based diets as protein supplements with acceptable animal performance (Stein *et al*., 2010). Grains are milled prior to use in pig diets. Hammer-, roller- and disc-milling are commonly used, with differences in particle size distribution (Sopade *et al*., 2011). Although the dependence of starch digestion on particle size has been well studied, limited studies exist as to how particle size affects starch and protein digestion in the same or different materials (Tinus *et al*., 2012). Knowledge of starch and protein digestion is also valuable to understanding nutrient asynchrony in feeds. Therefore, the present study investigated the relationship between particle size and starch and protein digestion in field peas. The hypotheses were that starch and protein digest at the same rate in field peas, and either digestion is independent of particle size.

Maki and *Walana* varieties of field peas were disc-, and hammer-milled using five nip- and four sieve-settings respectively. Roller-milled (one gap setting) samples were also studied. Particle size distributions were analyzed with sieves (mm) 4.0, 2.8, 1.4, 1.0, 0.71, 0.5, 0.25, 0.125, 0.075 and pan (Sopade *et al*., 2011), and the geometric mean particle size calculated. *In-vitro* digestion was measured as in Tinus *et al.* (2012), which discusses the modified first-order kinetic model used to describe the resulting digestograms. Analyses were randomised and duplicated, and an ANOVA was done (Minitab®, Version 16.0; USA).

Starch and protein digestion in the field peas exhibited monophasic digestograms irrespective of the variety, mill and mill settings. Hammer-milled peas digested more because of pronounced mechanico-frictional effects. The rate of protein digestion (K_{PR}) for the disc-milled peas was dependent ($P<0.05$) on the mean particle size, unlike the rate of protein digestion of the hammer-milled peas (Figure 1A). With starch, the rate of digestion (K_{ST}) of the hammer-milled peas was dependent ($P<0.05$) on the mean particle size, and there were no particle size effects ($P>0.05$) with the disc-milled peas (Figure 1B).

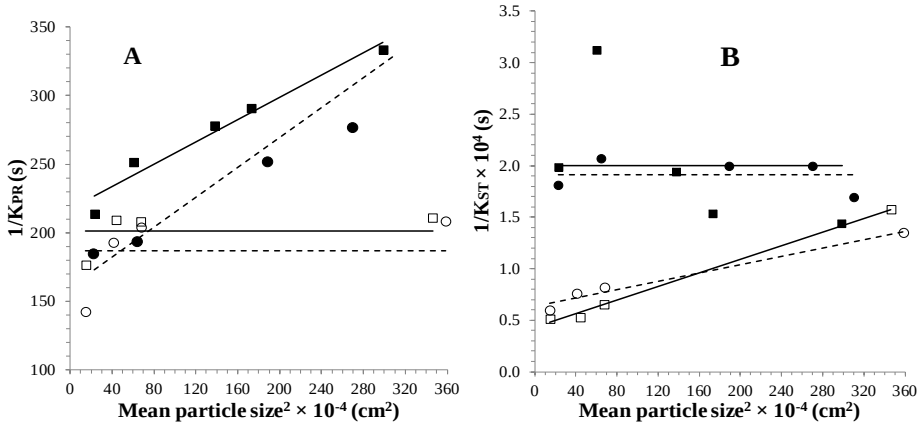


Figure 1. *The relationship between the square of the particle size of the field peas and the reciprocal of the rate of protein (A) and starch digestion (B); disc-milled Maki (●); hammer-milled Maki (○); disc-milled Walana (■); hammer-milled Walana (□); best fit lines (——, Walana;----, Maki).*

Protein digestion proceeded at a much faster rate (65 times) than starch digestion in the milled peas with possible implications for nutrient asynchrony, as reported before for another pulse (Tinus *et al*., 2012). For starch or protein digestion, a two-fold increase in particle size of field peas will essentially decrease the rate of digestion four-fold, irrespective of the mill type. Particle size dispersion or span (narrow, broad or skewed), which varies with mills, can contribute to the effects of mean particle size.

SOPADE, P., GIDLEY, M. and BLACK, J. (2011). 'Processing Methods for Grains with Special Reference to Particle Size'. Project Report. Cooperative Research Centre for High-Integrity Australian Pork, SA 5118.

STEIN, H.H., PETERS, N. and KIM, B.G. (2010). *Journal of the Science of Food and Agriculture.* **90**:1429-1436.

TINUS, T., DAMOUR, M., VAN RIEL, V. and SOPADE, P.A. (2012). *Journal of Food Engineering.* **113**:254-264.

Support in part by Pork CRC Limited Australia.

CHAPTER 6

Piglet Growth and Welfare, and Reproduction

Less brain sparing occurs in severe intrauterine growth-restricted piglets born to sows fed palm fatty acid distillate

C. Amdi[1], C.F. Hansen[1], U. Krogh[2], N. Oksbjerg[2], and P.K. Theil[2]

[1]University of Copenhagen, Frederiksberg C, Denmark. [2]Aarhus University, Tjele, Denmark.

In intrauterine growth-restricted piglets (IUGR), relatively more nutrients are redirected towards the brain (brain sparing) compared to a normal piglet, as part of a foetal adaptive reaction to placental insufficiency, which is intended to maintain as much oxygen supply to the brain as possible (Roza *et al*., 2008). The piglet brain is still growing between d 100 and d 110 of gestation (Nielsen, 1973), and it is hypothesised that the sows' diet might influence this final brain development. The objective of this study was to investigate the relative brain and liver weight and also brain to liver ratios of piglets suffering from IUGR, when sows were fed three different fat and fibre sources in a transition diet.

This study was conducted as part of a larger project investigating transition feeding on sow performance and piglet survival. Thirty-six second parity sows (Landrace x Yorkshire) mated with Duroc semen were transferred to a farrowing house at d 105 of gestation. Sows were fed one of nine diets in accordance with a 3 x 3 factorial design (three fibre diets x three fat sources). The three dietary fibre (DF) sources were a control (already in diet, from the wheat, barley and soy content), added sugar beet pulp and added alfalfa, and the three dietary fat sources were soy oil (S), palm fatty acid distillate (P) and trioctanoate (T). A total of 2.9, 3.0 and 3.1% dietary fat was included in the control, sugar beet pulp and alfalfa diets, respectively. Piglets were classified at birth based on head morphology and given a visual IUGR score from normal, mildly IUGR and severe IUGR, recognising 1) the IUGR piglet displaying the phenotype of a steep dolphin-like forehead, 2) bulging eyes, and 3) wrinkles perpendicular to the mouth (modified after Hales *et al*., 2013). Twenty-four h after birth of the first piglet in a litter, the median piglet within birth order in each litter was sacrificed, as were piglets born less than 900 g. In total, 80 piglets were sacrificed and weights of the liver, brain and total body weight were recorded. Data were analysed using the MIXED procedure in (SAS®; USA).

Brain sparing was related to severity of IUGR (Table 1), but was not affected by DF source (not shown). However, severe IUGR piglets had a smaller relative brain weight percentage of total body weight when the sow had been fed P oil compared to severe IUGR piglets that were born to sows fed S and T (Table 1). There was an influence of DF source on relative liver weight percentage of total body weight, with values being 2.5%, 2.5% and 2.3% for low fibre (control), sugar beet pulp and alfalfa, respectively (P<0.026, SEM 0.06). The alfalfa diet produced the lowest relative liver weights (P<0.05).

Table 1. *The interaction between fat in the sow' diet (S, P and T) on brain (B), liver (L) and organ ratios (B/L) of normal, mildly-IUGR and severe-IUGR piglets.*

	IUGR score										Significance		
	Normal			Mildly IUGR			Severe IUGR						
	S	P	T	S	P	T	S	P	T	SEM	IUGR	FAT	IUGR × FAT
Number	9	8	8	10	8	12	10	6	9				
B (%)	2.4^{a}	2.8^{a}	2.3^{a}	4.0^{b}	4.0^{b}	4.2^{b}	5.6^{c}	4.1^{b}	5.4^{c}	0.38	0.001	0.288	0.042
L (%*)	2.7^{c}	2.5^{bc}	2.8^{c}	2.5^{bc}	2.5^{bc}	2.0^{a}	2.2^{ab}	2.6^{c}	2.0^{a}	0.13	0.001	0.006	0.002
B/L	0.9^{a}	1.1^{ab}	0.9^{a}	1.7^{c}	1.6^{bc}	2.1^{c}	2.7^{d}	1.5^{bc}	2.8^{d}	0.23	0.001	0.015	0.004

^{a,b,c,d}Means in a row not having the same superscript are significantly different (P<0.05); No fibre×IUGR interactions were observed; *There was a main effect of DF on relative liver weight percentage of total body weight.

The present study suggests that nutrition of the sow in the last week of gestation affects both development of the foetal liver and brain development. Indeed, DF originating from sugar beet pulp increased the relative liver weight compared to dietary inclusion of alfalfa. Moreover, dietary inclusion of palm fatty acid distillate increased the relative weight of liver and brain in severe-IUGR piglets compared to severe-IUGR piglets born to sows fed soy oil or trioctanoate.

HALES, J., MOUSTEN, V.A., NIELSEN, M.B.F and HANSEN, C.F. (2013). *Journal of Animal Science.* Accepted for publication

NIELSEN, H.E. (1973). "Growth and development in pigs in the pre- and postnatal period with special reference to later growth and carcass composition". Landhusholdningsselskabets forlag.

ROZA, S., STEEGERS, E., VERBURG, B., JADDOE, V., MOLL, H., HOFMAN, A., VERHULST, F. and TIEMEIER, H. (2008). *American Journal of Epidemiology.* **168:**1145- 1152.

The Villum Foundation and the Ministry of Food, Agriculture and Fisheries of Denmark are acknowledged for support.

Intermittent suckling influences the performance of pigs before and after weaning

D.L. Turpin[1], P. Langendijk[2], T-Y. Chen[2] and J.R. Pluske[1]

[1]Murdoch University, Murdoch, WA 6150. [2]South Australian Research and Development Institute, Roseworthy, SA 5371.

The simultaneous and abrupt changes at weaning cause a growth check characterised by sub-optimal performance, marked changes to the gastrointestinal tract, and increased susceptibility to enteric diseases (Pluske *et al*., 1997). Intermittent suckling (IS) is a regimen in which the sow is removed from her piglets for a period of time each day before weaning. Previous studies have shown that IS reduces the post-weaning growth check by stimulating feed intake in lactation (Kuller *et al*., 2004), but the impacts of duration and length of separation have not been fully evaluated. The hypothesis tested in this study was that piglets separated from their dam for eight hours per day for seven d in the week before weaning would perform better after weaning compared to piglets in conventional weaning regimens.

Gilt litters (n=41) were allocated to one of three weaning regimens: conventional weaning (CW28) (n=14), where piglets had continuous access to the sow until weaning at d 28 (d 28); IS starting at d 21 (IS21) (n=14); and IS starting at d 28 (IS28) (n=13). In the IS treatments, litters were separated from the sow for eight h per d (0800 to 1600) in the week before weaning (d 28 and d 35 for IS21 and IS28, respectively). Creep feed was provided *ad libitum* from d 14 of lactation. At weaning, the middle five piglets from each litter were selected and housed in pens in a heated weaner facility. Average daily gain (ADG) and average daily feed intake (ADFI) were compared on different days between treatments using the GLM procedure of SAS (SAS Institute, USA), with the litter as the experimental unit.

Table 1. *Pre- and post-weaning performance for the control treatment and different IS regimens.*

	Treatment				
	CW28[1] (n=14)	IS21 (n=14)	IS28 (n=13)	SEM	Significance
ADG (g)					
Day 5 - d 1 pre-weaning	261^{a}	201^{b}	247^{ab}	10.1	0.038
First 2 d post-weaning	200^{a}	214^{a}	455^{b}	29.5	<0.001
Days 2-6 post-weaning	209^{a}	283^{a}	421^{b}	20.2	<0.001
Weaning weight, kg	7.4^{ax}	6.8^{ax}	8.3^{by}	0.16	0.833
ADFI (g)					
Day 5- d 1 pre-weaning	46^{a}	37^{a}	98^{b}	6.7	<0.001
Days 2-6 post-weaning	243^{a}	285^{a}	429^{b}	18.1	<0.001

^{a,b}Means within a row not having the same superscript are significantly different (P<0.05); [1]See text for treatment details; SEM, standard error of the mean; ^{xy} Means within a row not having the same superscript are a trend (P<0.1).

Intermittent suckling reduced pre-weaning growth rate only in IS21 piglets (P=0.038). The IS28 pigs grew fastest (P<0.001) in the first week after weaning. At d 46 of age, body weights were similar (P>0.05) across treatments (14.2 ± 2.4 kg; mean±SEM). Feed disappearance before and after weaning was highest (P<0.001) only in the IS28 pigs (Table 1). The clear effect of age on performance after weaning in the IS treatments suggests that extending weaning age to 35 d and IS allowed newly-weaned pigs to deal with the post-weaning stressors better, possibly by increased familiarisation of feed during lactation causing increased intake in the immediate post-weaning period (Pluske *et al.*, 2007).

KULLER, W.I., SOEDE, N.M., VAN BEERS-SCHREURS, H.M.G., LANGENDIJK, P., TAVERNE, A.M., VERHEIJDEN, J.H.M. and KEMP, B. (2004). *Journal of Animal Science.* **42:**405-413.

PLUSKE, J.R., HAMPSON, D.J. and WILLIAMS, I.H. (1997). *Livestock Production Science.* **51:**215-236.

PLUSKE, J.R., KIM, J.C., HANSEN, C.F., MULLAN, B.P., PAYNE, H.G., HAMPSON, D.J., CALLESEN, J. and WILSON, R.H. (2007). *Archives of Animal Nutrition.* **61:**469-480.

Supported by Pork CRC Limited Australia.

Voluntary feed intake by sows and weight gain by piglets in farrowing crates compared to UMB farrowing pens

G.M. Cronin[1], G.F. Macnamara[1], R.L. Matthews[1], J.A. Barnsley[1], C.E. Williams[1], B.L.F. Macnamara[1], K.E. Bøe[2] and I.L. Andersen[2]

[1]The University of Sydney, Camden, NSW 2570. [2]Norwegian University of Life Science, 1432 Ås, Norway.

Before industry is likely to adopt non-confinement farrowing pens for sows and litters, it is important that key performance variables are evaluated and compared with those of farrowing crates. In addition to piglet mortality and number of piglets weaned per sow, it is relevant to measure voluntary feed intake (VFI) by sows and growth of piglets to weaning. Cronin *et al.* (2000) reported higher VFI in week 3 of lactation for sows in Werribee farrowing pens compared to conventional crates. A benefit of higher sow feed intake during lactation may be heavier piglet weaning weights in gilt litters (Eissen *et al*., 2003) and reduced loss of body protein in thin sows (de Lange *et al*., 1980). Addressing these factors may also reduce the risk that sows are culled due to failure to re-breed after weaning. This experiment tested the hypothesis that lactating sows had higher VFI in UMB farrowing pens than crates between d 7 and 21 of lactation.

A total of 72 sows and litters, 36 in prototype Norwegian UMB (Universitetet for Miljø- og Biovitenskap) farrowing pens and 36 in conventional farrowing crates (Vereijken; 1.65 x 2.25 m), were studied at the University of Sydney piggery, Camden NSW. Each UMB pen contained a "nest" area (2.4 x 1.7 m) and a "non-nest" area (2.4 x 1.6 m), separated by a 270 mm-high "step-over" metal barrier with inward curled top. The nest area had solid walls on three sides; a straw dispenser was attached to one side wall, and sloped panels were attached to the opposite side wall and the rear wall. The floor was covered by a 30 mm-thick rubber mat. Heat pads (1.2 x 0.6 m) were embedded under the floor, positioned adjacent to each sloped panel. Production data were recorded over nine farrowing batches between April and September in three consecutive years. Each batch involved four sows in pens and four sows in crates and the experimental unit was the sow and litter. Data were analysed using ANOVA and analysis of covariance (GenStat v. 14.1), blocked on farrowing batch. The two farrowing systems were located in adjacent rooms of the same shed. Crates were located in an insulated, heated room (thermostat set at 21ºC) and each crate had a piglet heater above, and a heated floor mat in, the creep zone. In contrast, the UMB pens were located in a non-heated room that was partially insulated, originally housing dry sows. Thus there were unavoidable differences in the minimum ambient temperature between the rooms. After farrowing, sows were fed to appetite with a commercial lactation ration (16.8% crude protein and 12.8 MJ digestible energy/kg, and between d 7 and 21 of lactation, the amount of sow feed less residuals was weighed. Litters were weighed on d 1, 7, 14 and 21 of lactation and at weaning (mean d 26; range, d 22 to 30). Creep feed was not provided.

Piglets tended (P=0.103) to be heavier in farrowing crates than pens on d 1 of lactation (Table 1), perhaps reflecting larger litter size (11.8 and 12.6 piglets total born/litter in crates and pens, respectively; P=0.40) and (possibly) cooler UMB pen environment. Although mean piglet weight in crates and pens did not differ on d 7 or 14 (there was a strong trend for d 14), by d 21 piglets were about 0.5 kg heavier (P<0.05) in the crates. Sows had higher VFI in UMB pens than crates (P<0.05), supporting the hypothesis (Table 1). It is not clear if the higher VFI was related to lower ambient temperatures in the pens than crates.

Table 1. *Production variables comparing sows and litters in farrowing crates and prototype UMB pens.*

Variable	Crate	UMB pen	SED	Significance
Average piglet live weight adjusted for TB (kg)				
d 1 of lactation	1.44	1.38	0.040	0.103
d 7	2.58	2.47	0.138	0.400
d 14	4.42	4.14	0.148	0.064
d 21	6.32	5.83	0.224	0.034
Sow daily feed intake from d 7 to 21 (kg)	7.76	8.51	0.359	0.042

TB, total born; SED, standard error of difference.

Further investigation is required to separate the effects of ambient temperature and space/exercise. Nevertheless, the additional feed intake by UMB pen sows did not result in superior piglet growth. It is likely that piglet thermal requirements were not met in the UMB-pen room (e.g. lowest recorded minima were 15 and 9ºC, respectively for the crate and pen rooms).

CRONIN, G.M., LEFEBURE, B. and MCCLINTOCK, S. (2000). *Australian Journal of Experimental Agriculture*. **40**:17-23.
DE LANGE, P.G.B., VAN KEMPEN, G.J., KLAVER, J. and VERSTEGEN, M.W. (1980). *Journal of Animal Science*. **50**:886-891.
EISSEN, J., APELDOORN, E., KANIS, E, VERSTEGEN, M.W. and DE GREEF, K. (2003). *Journal of Animal Science*. **81**:594-603.

Supported in part by Pork CRC Limited Australia. Prototype UMB pens were donated by the Norwegian University of Life Sciences.

Stress responses of two-day-old piglets to tail docking

R.S. Morrison[1], K.S.B. Sawyer[1], N.J. Kells[2], C. B. Johnson[2] and P.H. Hemsworth[3]

[1]Rivalea Australia, Corowa, NSW 2646. [2]Massey University, Private Bag 11-222 Palmerston North, New Zealand. [3]The University of Melbourne, Parkville, VIC 3010.

Tail docking is a common practice in pork production to prevent tail biting in piglets. The aim of this experiment was to assess the stress responses of piglets to tail docking by either the clipper or cauterisation method. The hypothesis tested was that tail docking using clippers and cauterisation would cause an acute cortisol response and increase pain-related behaviours compared to the control treatment.

Seventy-two sows (Large White x Landrace-PrimeGro^{TM} Genetics) and their litters were selected over 6 weeks. Four entire male piglets were selected per litter at 2 d post-farrowing, with a total of 288 piglets used in the study. The piglets were randomly allocated to the following treatments: (1) Sham treatment-tail not docked; (2) Surgical castration; (3) Tail docked using sanitised clippers; and (4) Tail docked using a cauterising iron (Stericut® Tail Docker). The piglets in all treatments were handled in the same manner, for the same duration by the same two technicians. The tail was cut approximately 2 cm from the base of the tail in the two tail-docked treatments. Blood samples were collected via jugular veni-puncture at 15 min, 30 min and 24 hr post-treatment and analysed for total plasma cortisol using an extracted radioimmunoassay and validated for pig plasma using hydrocortisone H-4001 (Sigma Chemical Co., St Louis, MO) as a standard. Pain-related behaviour was assessed by measuring the frequency of escape attempts (Marchant-Forde *et al.,* 2009) and vocalisations (a bout criterion interval of 1 sec) during treatment. After treatment, behaviour was videotaped using mounted cameras (Signet Model QV-3063). The behaviour (postures and states) and pain-related behaviours (Hay *et al*., 2003) were measured by continuously observing each piglet for 60 sec every 5 min for 60 min post-treatment and at 23 h post-treatment.

Statistical analyses were performed by ANOVA using GenStat (Version 10;VSN International, Oxford UK) with each piglet as the experimental unit and the sow as a blocking factor. Data were tested for normality and square root transformation conducted where appropriate. Least Significant Difference (LSD) tests were performed. Chi-square analysis was used to analyse number of piglets that died or were removed.

There were no treatment effects (χ^2=0.70; P=0.951) on number of piglet deaths and removals. Cortisol concentrations at 15 and 30 min post-treatment were higher (P<0.001) in both tail docking treatments and the surgical castration treatment compared to the sham treatment. Cortisol concentrations were lower (P<0.05) in the cauterisation treatment compared to the clipper treatment 30 min after treatment. There were more (P<0.001) vocalisations and escape attempts in the surgical castration treatment compared to the sham and tail docking treatments. The pigs in the tail docked and surgical castration treatments spent more time (P<0.001) standing with their heads lowered in the 60 min post-treatment period compared to the sham treatment. There was no difference (P>0.05) between treatments in cortisol concentrations and behaviour 24 h post-treatment.

Table 1. *Mean total plasma cortisol (ng/ml) concentrations at 15 and 30 min post-treatment.*

	Sham	Surgical castration	Tail docked using clippers	Tail docked using cauteriser	SEM	LSD	Significance
Cortisol (15 min)	91^{a}	129^{c}	111^{b}	106^{b}	2.2	9.6	<0.001
Cortisol (30 min)	115^{a}	146^{c}	126^{b}	122^{ab}	1.9	8.2	<0.001

$^{a\,b,\,c}$Within rows values with different superscripts are significantly different (P<0.05); SEM, standard error of mean.

The hypothesis was accepted and tail docking piglets using clippers and cauterisation caused an acute stress response at 15 min post-treatment. Piglets in both tail docking and surgical castration treatments exhibited more pain-related behaviour in the 60 min post-treatment compared to the sham treatment. Piglets in the cauterisation treatment had a lower stress response at 30 min post-treatment compared to those in the clipper treatment, which may indicate welfare advantages of using this method. There were no significant stress responses between treatments at 24 h post-treatment.

HAY, M., VULIN, A., GENIN, S., SALES, P. and PRUNIER, A. (2003). *Applied Animal Behaviour Science.* **82:**201-218.
MARCHANT-FORDE, J.N., LAY, D.C., MCMUNN, K.A., CHENG, H.W., PAJOR, E.A. and MARCHANT-FORDE, R.M. (2009). *Journal of Animal Science.* **87**:1479-1492.

The financial assistance of Australian Pork Limited and the technical support of the Rivalea Research and Innovation group is gratefully acknowledged.

Electroencephalographic assessment of acute pain in piglets during tail docking

N.J. Kells[1], N.J. Beausoleil[1], J.P. Chambers[1], M.A. Sutherland[2], R.S. Morrison[3] and C.B. Johnson[1]

[1]Massey University, Palmerston North 4442, New Zealand. [2]AgResearch Ruakura, Private Bag 3115, Hamilton, New Zealand. [3]Rivalea Australia, Corowa, NSW 2646.

Tail docking of piglets is a common management practice carried out to prevent tail biting. Whilst tail docking is typically carried out within 48 h of birth, electroencephalographic (EEG) responses to pain have not been examined in 2-d-old piglets, whereas EEG responses typical of nociception have been reported in 20-d-old piglets (Haga and Ranheim, 2005). The aim of the present study was to assess changes in the EEG of piglets undergoing tail docking using either the clippers or cautery method.

Forty male Large White x Landrace piglets were randomly assigned to one of four treatments as follows: A: Tail docked at 20-d-old using clippers (20 CLIP), B: Tail docked at 20 d old using cautery (Stericut® Tail Docker) (20 CAUT), C: Tail docked at 2-d-old using clippers (2 CLIP), or D: Tail docked at 2-d-old using cautery (2 CAUT). Piglets were lightly anaesthetised (1% halothane in O_2) and EEG continuously recorded throughout treatment. Raw EEG data were subjected to Fast Fourier Transformation, yielding the summary variables median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{TOT}). All data were standardised to a percentage of pre-treatment values and mean F50, F95 and P_{TOT} for consecutive 15 second blocks following treatment subjected to repeated measures, ANOVA (SAS®; USA), with time as a repeated measure and treatment, age and litter as fixed effects.

Table 1. *The effects of method, age and time on the median frequency (F50), spectral edge frequency (F95) and total power (P_{TOT}) of the pig EEG following tail docking.*

Effect	F50		F95		P_{TOT}	
	F	Pr > F	F	Pr > F	F	Pr > F
Treatment x Time	1.70	0.0652	2.23	0.0097	0.64	0.8043
Time x Age	3.53	< 0.0001	4.96	< 0.0001	6.70	< 0.0001

Noxious stimulation typically elicits increases in F50 and F95 and a reduction in P_{TOT} of the mammalian EEG (Murrell and Johnson 2006). In the present study, responses to docking varied between methods over time, irrespective of age (Table 1). Docking by CLIP resulted in an increase in F95 from 15–60 sec after docking, whereas CAUT elicited no change in F95. Median frequency increased following docking in both treatments, however the duration was less with CAUT (45–60 seconds) than CLIP (45–90 seconds). Means did not differ between treatments (P>0.05) at any time point. Although P_{TOT} decreased after docking, the response did not vary between methods.

Responses to docking also varied by age over time, irrespective of method (Table 1). Docking at 2 d resulted in transient decreases in F50 and P_{TOT} and no change in F95. In contrast, docking at 20 d resulted in sustained increases in F50 and F95 and a sustained decrease in P_{TOT}. Mean F50 and F95 were significantly lower in 2 d-olds than 20 d-olds from 15–90 and 30–75 sec, respectively, after docking. Mean P_{TOT} was lower in 20 d-olds from 30–60 sec after docking. The differences in the magnitude and duration of EEG responses to docking between two and 20 d-old piglets suggests that docking at 2 d is less acutely painful than docking at 20 d. This may be due to maturational effects, or to the lingering effects of in-utero pain-suppression mechanisms (Diesch *et al*., 2009), or a combination of both. A decrease in F50 in response to noxious stimulation has previously been reported in very young mammals and was thought to relate to differences in the maturity of nociceptive pathways (Diesch *et al*., 2009).

These data suggest that docking with clippers is more acutely painful to piglets than docking with cautery and that docking within the first d of birth may result in less acute pain than docking at a later age. However, the potential for hyperalgesia following surgery at a very young age (McCracken *et al*., 2010) needs to be investigated before recommendations regarding age can be made.

DIESCH, T., MELLOR, D. J., JOHNSON, C. B. and LENTLE, R. (2009). *Laboratory Animals* **43**:224-231.
HAGA, H. and RANHEIM, B. (2005). *Veterinary Anaesthesia and Analgesia.* **32**:1-9.
MCCRACKEN, L., WARAN, N., MITCHINSON, S.L., and JOHNSON, C.B. (2010). *Veterinary Anaesthesia and Analgesia* **37**:375-381.
MURRELL, J.C. and JOHNSON, C.B. (2006). *Journal of Veterinary Pharmacology and Therapy.* **29**:325-335.

Supported in part by Australian Pork Limited, with the cooperation of Wairaka Farms and technical support from Santosh Kumar.

Gas alternatives to carbon dioxide for euthanasia: A piglet perspective

J-L. Rault[1], K.A. McMunn[2], J.N. Marchant-Forde[2] and D.C. Lay[2]

[1]The University of Melbourne, Parkville, VIC 3010. [2]US Department of Agriculture-ARS, West Lafayette, IN 47907, USA.

The neonatal stage is a critical time in the life of a pig, when they are prone to become sick or weak. This is the stage at which most euthanasia procedures are required. Any euthanasia method should be humane, practical, economical and socially acceptable. Scientific evidence supports that blunt force trauma is humane when carried out correctly (AVMA, 2007), but most people find it visually difficult to accept. The use of carbon dioxide (CO_2) is often recommended, however it is criticised as being aversive to pigs (Raj and Gregory, 1995). This research sought to: 1) identify a method of scientifically determining if piglets find a gas aversive, using an approach-avoidance test which relies on the piglet's perspective; and 2) test different gas mixtures to determine if they are effective and humane for neonatal piglet euthanasia.

Piglets were allowed to walk freely between one chamber filled with air and another chamber either gradually filled with gas mixtures (Experiment 1) or pre-filled with gas mixtures (Experiment 2). Experiment 3 tested the effectiveness of the euthanasia procedure. All piglets were the progeny of Yorkshire × Landrace dams bred to Duroc × Hampshire sires.

In Experiment 1, we tested CO_2 (90%) and air (10%); nitrous oxide (N_2O; 60%) and CO_2 (30%); argon, (Ar; 60%) and CO_2 (30%); and nitrogen (N_2; 60%) and CO_2 (30%), on 28, 2-week-old female piglets. The test was conducted for 10 min during which the piglet was free to move between chambers. However, for ethical reasons for Experiments 1 and 2, piglets were removed when they started to flail, which was defined as erratic, uncontrolled movements, uncoordinated jumps and thrashing, and considered signs of aversiveness. Hence, the test was shortest ($P<0.01$) for the piglets in the CO_2 treatment compared to piglets in the N_2O/CO_2, Ar/CO_2, and N_2/CO_2 treatments (means ± SEM of 3.1 ± 0.2, 8.5 ± 0.6, 9.6 ± 0.4, and 9.9 ± 0.1 min, respectively), supporting that CO_2 was more aversive than other gas mixtures. Nonetheless, all gas mixtures adversely affected the piglets, causing them to leave the test chamber.

In Experiment 2, 13 piglets were allowed to enter a chamber pre-filled with N_2/CO_2 or N_2O/CO_2 (both 60%/30%). Piglets exposed to the pre-fill chambers with either treatment started to flail in less than 20 s, much faster in comparison to the gradual fill method, which supports that this method was more aversive.

In Experiment 3, 28 sick, injured, starving, or splay leg piglets (4.7 ± 1.6 d of age) were euthanised using a two-step procedure. Piglets were first placed in a gradual fill chamber with one of four gas mixtures: 90% CO_2, N_2/CO_2, N_2O/CO_2 and a new treatment of N_2O/O_2 (the last three mixtures at 60%/30%), followed by placement into a 90% CO_2 pre-fill chamber when they started to flail or were anaesthetised.

Table 1. *Duration (min ± SEM) to reach the different stages in Experiment 3.*

Gas treatment	CO_2	N_2/CO_2	N_2O/CO_2	N_2O/O_2	Significance
Time to transfer from treatment to CO_2 chamber	2.9 ± 0.3^a	6.4 ± 0.6^b	6.7 ± 1.0^b	14.7 ± 2.1^c	0.001
Time to death after transfer	7.8 ± 1.3	7.6 ± 1.0	5.6 ± 0.3	9.3 ± 1.2	0.20
Total procedure duration	10.8 ± 1.3^a	13.9 ± 1.2^a	11.3 ± 1.4^a	24.0 ± 3.0^b	0.005

[a,b] Means in a row not having the same superscript are significantly different ($P<0.05$).

All three gas treatments that contained CO_2 killed piglets more quickly than N_2O/O_2 ($P = 0.005$; Table 1). However, N_2O/O_2 was the only treatment that anaesthetised the piglets. This observation was in sharp contrast to the squeals and flail behaviour exhibited by piglets which were exposed to the other treatment gases and then placed into CO_2. Although requiring about 12 min longer, a two-step procedure in which piglets are anaesthetised with a mixture of N_2O and O_2 prior to being euthanised by immersion in CO_2 may prove to be more humane than CO_2 alone. Development of its use into an automated procedure could make this method affordable and more humane than other alternatives, although legal requirements should be fulfilled.

AVMA. (2007). American Veterinary Medical Association Guidelines on Euthanasia. p. 6-10. Retrieved 22nd May 2010 from: http://www.avma.org/issues/animal_welfare/euthanasia.pdf

RAJ, A.B.M. and GREGORY, N.G. (1995). *Animal Welfare.* **4**:273-280.

Supported in part by the US National Pork Board.

Increasing feed intake in early gestation improves farrowing rate in first and second parity sows

K.S. Sawyer, R.Z. Athorn, C.L. Collins and B.G. Luxford

Rivalea Australia Pty Ltd, Corowa, NSW 2646.

Increasing feeding allowance during early pregnancy in first litter sows has been shown to positively affect sow growth rate, with higher growth rates linked to improved farrowing rates (Athorn *et al.* 2011; Langendijk *et al.*, 2011). In addition, Hoving *et al.* (2011) demonstrated that gilts and parity 1 (P1) sows who received 30% more feed intake during early gestation (3.25 versus 2.50 kg/d) displayed improved sow body weight recovery and an increased litter size, although the effect of feeding level on farrowing rate was not clear. Therefore, this study was designed to investigate the effects of feeding level during the first 30 d of gestation on farrowing rate of young sows. The study tested the hypothesis that increased daily feed intake during early gestation improves farrowing rate of P1 and parity two (P2) sows.

A total of 2,883 mated sows (1,625 P1 and 1,258 P2) (Large White x Landrace, PrimeGro^{TM} Genetics, Corowa, NSW) was housed in commercial group pens with animals of a similar mating date, numbering 45 or 90 sows per pen with a space allowance of 2.0-2.1 m^2/sow. Pens contained one electronic feeder for every 45 sows. The study observed sows gestating between July and December 2012. Sows were assigned to a standard feed curve, feeding a gestation diet containing 13.25 MJ digestible energy (DE)/kg. A ration of 2.7 kg/d was offered from mating through until d 30 of gestation. The ration was then reduced to 2.4 kg/d from d 31 for the remainder of gestation. The data used in this study was derived retrospectively from electronic records of actual feed intake on a daily basis. Each sow was included only once in the data set. Differences in farrowing rate due to early gestation feed intake were analysed within parity using Chi-squared analysis. All analyses were undertaken using GenStat, 13th Edition; UK.

Table 1. *Farrowing rate (FR) of P1and P2 sows at different feed intake levels from 1 to 30 d of gestation.*

	P1 (%)			P2 (%)		
	Day 1-10	Day 11-20	Day 21-30	Day 1-10	Day 11-20	Day 21-30
Number	249	47	40	101	21	30
1.6-2.0 (kg/d)	88.0	78.7^{a}	50.0^{a}	76.2^{a}	52.4^{a}	43.3^{a}
Number	582	489	387	437	306	276
2.1-2.5 (kg/d)	86.9	82.6^{a}	82.7^{b}	90.2^{b}	86.6^{bc}	88.8^{b}
Number	576	1065	1104	619	917	899
>2.5 (kg/d)	86.8	90.0^{b}	92.4^{c}	89.5^{b}	90.4^{c}	92.9^{c}
Significance	0.898	<0.001	<0.001	<0.001	<0.001	<0.001

$^{a\ b,\ c}$ Means within a column not having the same superscript are significantly different (P<0.05).

During the first 10 d farrowing rate of P1 sows was not affected by feeding level (Table 1). In comparison, P2 sows showed an improvement in farrowing rate when intake was increased above 2.0 kg/d. Days 11 to 20 and 21 to 30 of gestation were found to be the most critical periods for the impact of feed intake on farrowing rate. During these periods, both P1 and P2 sows showed an increase in farrowing rate with higher feed intake, which may suggest a greater nutrient supply is required during these periods to cover the demands of both pregnancy and body growth (including body weight recovery from the previous lactation) of these young parity sows. This study supports the hypothesis that increasing feed intake during early gestation has a positive effect on the farrowing rate of P1 and P2 sows. The authors recognise that as this data was collected retrospectively there may be other confounding factors affecting reproductive performance within each feeding level. Further research is required to confirm the impact of feed intake during early gestation on the reproductive performance of young parity sows (Athorn *et al*. 2013).

ATHORN, R.Z., SAWYER, K.S., COLLINS, C.L. and LUXFORD, B,G. (2013). In "Manipulating Pig Production XIV", p. 212, ed. J.R. and J.M. Pluske. (Australian Pig Science Association: Werribee, Australia).

ATHORN, R.Z., STOTT, P., SMITS, R.J. and LANGENDIJK, P. (2011). In "Manipulating Pig Production XIII", p.81. ed. R.J. van Barneveld. (Australian Pig Science Association: Werribee: Australia).

HOVING, L.L., SOEDE, N.M., VAN DER PEET-SCHWERING, C.M.C., GRAAT, E.A.M., FEITSMA, H. and KEMP, B. (2011). *Journal of Animal Science.* **89:**3542-3550.

LANGENDIJK, P., ATHORN, R.Z., STOTT, P. and SMITS, R.J. (2011). In "Manipulating Pig Production XIII", p.162. ed. R.J. van Barneveld. (Australian Pig Science Association: Werribee, Australia).

Ovulation rate and embryo survival are still major limiters of litter size

P. Langendijk, R.Z. Athorn, E.G. Bouwman, and T-Y. Chen

South Australian Research and Development Institute, Roseworthy, SA 5371.

In multiparous sows, embryo mortality ranges between 30 to 50% (Gerritsen *et al*., 2009; Wientjes, 2013). In recent literature, however, there is more emphasis on post-implantation losses (d 25 to d 40), when uterine space is said to become limiting (Foxcroft *et al*., 2007). However, there is limited data on the timing of embryo loss. In this study our aim was to assess the extent and the timing of embryo loss in multiparous sows from an Australian herd using commercial genetics.

Forty-two multiparous sows, Large White or Large White x Landrace, ranging from parity 2-12 (mean 4.9±0.4, mean±SEM; selected to represent the variation in ovulation rate), and mean body weight at weaning of 270±6 kg, were mated everyday of oestrus with $3x10^9$ pooled semen (SABOR, South Australia) and then slaughtered at d 9 (n=10), d 21 (n=15), or d 35 (n=17) after ovulation (as assessed by ultrasound; Aquila Vet, Esaote Pie Medical, Maastricht, Netherlands) to recover the reproductive tracts. Embryos on d 9 were recovered by flushing the uterine horns with saline, and d 21 and d 35 embryos were recovered by dissecting the uterine horns. Ovulation rate (number of corpora lutea) and number of embryos were recorded. Differences between days of gestation were compared using the GLM procedure (SAS®; USA).

Ovulation rate averaged 21.5±0.7, and varied from 12 to 32. At d 9 of gestation, 92±3% of the ovulations were represented by an embryo, with unrecovered embryos presumably accounted for by the efficiency of the recovery procedure, because fertilisation rate was expected to be 95-100% with the current insemination protocol. At d 21, 78±4% of ovulations was represented by an embryo, and by d 35, 64±4% of ovulations was represented by an embryo ($P<0.05$). Two-thirds of embryo mortality (22% out of 36% overall) had already occurred by d 21. The number of embryos was correlated to the number of ovulations ($r = 0.6$; Figure 1), and each extra ovulation resulted in 0.7 extra embryos at d 21 and 0.6 embryos at d 35 of gestation ($P < 0.05$). Parity effect could not be tested due to the low numbers used in the study.

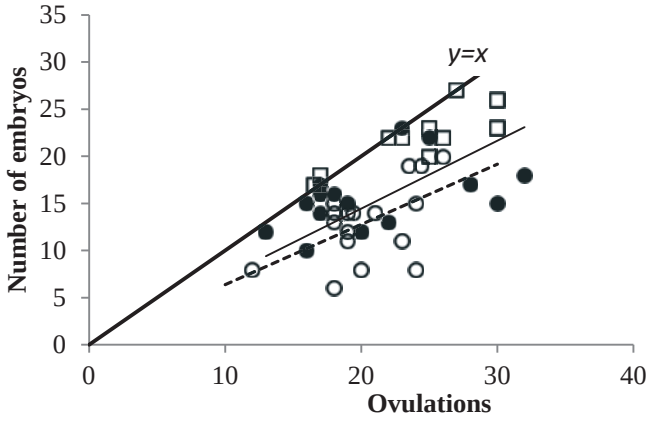


Figure 1. *Number of embryos at d 9 (□), d 21 (● and solid line), or d 35 (○ and dotted line) after fertilisation. For d 2; y = 0.7x (P < 0.05); for d 35; y = 0.6x (P < 0.05).*

In conclusion, ovulation is a strong determinant of embryo number and ultimately, litter size. This finding underlines the importance of management measures to increase ovulation rate in commercial operations and genetic strategies to increase the overall potential of ovulation rate in the breeding herd. Interestingly, two-thirds of embryo losses already occur between d 9 and d 21, presumably before or around implantation, emphasising the equal importance to address embryo survival to increase litter size.

FOXCROFT, G.R., BEE, G., DIXON, W., HAHN, M., HARDING, J., PATTERSON, J., PUTMAN, T., SARMENTO, S., SMIT, M., TSE, W. and TOWN, S. (2007). In "Paradigms in Pig Science". p.207-229, eds J. Wiseman, M.A. Varley, S. McOrist, B. Kemp. (Nottingham).

GERRITSEN, R., SOEDE, N.M., HAZELEGER, W., LANGENDIJK, P., DIELEMAN, S.J., TAVERNE, M.A.M. and KEMP, B. (2009). *Theriogenology.* **71**:432-440.

WIENTJES, J.G.M. (2013). Piglet birth weight and uniformity. PhD Thesis. Wageningen University, The Netherlands.

Supported by Pork CRC Limited Australia.

Split weaning increases subsequent embryo survival of sows mated in lactation

A.C. Weaver[1], K.L. Kind[1], G.R. Foxcroft[2], M.K. Dyck[2], S. Tsoi[2] and W.H.E.J. van Wettere[1]

[1]The University of Adelaide, Roseworthy, SA 5371. [2]The University of Alberta, AB T6G 2P5 Canada.

Sow ovarian follicle growth and ovulation are normally prevented by suckling during lactation. Terry *et al.* (2011) demonstrated that provision of boar contact during late lactation causes high incidences of lactation oestrus in sows suckling three, five, seven and 10 piglets (parity 2.9±0.17; mean±SEM). However, sows suckling 10 piglets had a reduced subsequent litter size (8.9±1.1) compared to sows suckling seven and five piglets (13.1±1.1 and 12.5±1.0, respectively). We hypothesised that removing a portion of the litter will improve subsequent embryo survival and alter gene expression of subsequent foetuses from sows mated in lactation.

A total of 47 multiparous (parity 3.3±0.2) sows were studied over five replicates. On d 3 of lactation, litter size was standardised to 11 piglets per sow and maintained at this level until d 18. Sows were allocated to one of three treatments: control (litter size maintained at 11; n=15), split-wean 7 (SW7; litter size reduced to seven on d 18; n=16); and oestrus split-wean 7 (OESSW 7; litter size reduced to seven at oestrus; n=16). From d 18 until weaning, sows received 15 min of full physical boar contact daily. Sows were artificially inseminated at first detection of oestrus and daily until last detection of oestrus. The interval from d 18 to expression of oestrus, duration of oestrus, and proportion of sows expressing oestrus was recorded. On d 30 post-mating, sows were slaughtered and ovulation rate, foetal number and foetal weight were recorded. Embryo survival was calculated as the number of foetuses expressed as a proportion of corpora lutea. The data were analysed using a general linear model with block and parity as fixed effects (SPSS Science Inc., Chicago, IL, USA), or a Chi-squared test (oestrous expression and pregnancy rate). Total RNA was extracted from foetuses from four control sows and four SW7 sows across four replicates by Trizol extraction to look at foetal quality. RNA was pooled per sow and compared in a custom designed porcine embryo-specific microarray (EMPV1: EmbryoGENE Porcine Array Version 1) (Tsoi *et al*., 2012), then analysed with the Flexarray 1.6.1 software. The EMPV1 has 43,795 probes which targets more than 20,000 unique genes.

Table 1. *Effect of split weaning on pregnancy rate, embryo survival, fetal number and weight, and placental weight at d 30 of gestation of sows mated in lactation.*

	Control	SW7	OESSW7
Pregnancy rate (%)[1]	60^{a}	94^{b}	$75^{a,b}$
Fetal number	11.9 ± 1.0^{a}	16.4 ± 0.8^{b}	11.4 ± 0.8^{a}
Embryo survival (%)	49.1 ± 3.7^{x}	74.6 ± 3.6^{y}	53.9 ± 4.5^{y}
Fetal weight (g)	1.3 ± 0.07^{x}	1.6 ± 0.06^{y}	1.6 ± 0.07^{y}

Means within a row not having the same superscript are significantly different; ^{a,b,c} P<0.05; ^{x,y}P<0.01; [1]Chi-squared analysis.

Sow live weight on d 18 was similar for all treatment groups (277±4.2 kg). There was no difference (P>0.05) in the percentage of sows expressing oestrus during lactation (control and OESSW7 combined, 73%; SW7, 76%) or days to oestrus (3.8±0.3 d). However, OESSW7 sows experienced a longer oestrus than control and SW7 sows (3.2±0.3, 2.8±0.3 and 2.8±0.3 d respectively; P=0.03). Pregnancy rates were lower (P<0.05) in control sows compared to SW7 and OESSW7. Ovulation rate was not different (P>0.05) between treatments (23.0±0.7). However, at d 30 post-mating the number of viable foetuses (P<0.05) and embryo survival (P<0.1)was higher in SW7 sows compared to control and OESSW7. Table 1. Analysis of microarray data indicated no differentially expressed genes between control and SW7 sows, which may be due to the small sample size and analysis of whole foetuses instead of one tissue. This data indicates that suckled litter size plays an important role in the subsequent litter size of sows mated in lactation and split weaning may be a viable strategy to maintain current production standards whilst mating in lactation. Further study is required to identify factors such as maternal environment that may be involved in mediating the effects of suckled litter size on subsequent reproductive outcomes.

TERRY, R., KIND, K.L., HUGHES, P.E. and VAN WETTERE, W.H.E.J. (2011). In "Manipulating Pig Production" p.210, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

TSOI, S., ZHOU, C., GRANT, J., PASTERNAK, J., RIGAULT, P., NIEMINEN, J., SIRARD, M-A., ROBERT, C. and FOXCROFT, G. (2012). *BMC Genomics.* **13**:370.

Supported in part by Pork CRC Limited Australia.

CHAPTER 7

Sow Reproductive Performance and Nutrition

SYMPOSIUM: Maximising productivity in the modern sow: Constraints to realising the genetic potential of the breeding herd and targeting nutrition for optimal productivity: Introduction

C.L. Collins

Rivalea (Australia), Corowa, NSW, 2646

Maximising the reproductive performance of the breeding herd is a critical driver in commercial pork production. Genetic selection has driven significant advances in sow productivity over time, with commercial genotypes currently available in other parts of the world displaying exceptional reproductive performance. Although access to these genetics is limited in some markets, ensuring that producers are able to take full advantage of their current breeding herd is vitally important.

Fully exploiting the genetic potential of the modern sow requires consideration to sow and boar components of reproductive efficiency as well as a clear understanding of the sow's nutritional requirements to support the demands of pregnancy and lactation throughout multiple parities.

The first paper of this symposium (Foxcroft *et al.* 2013) will consider factors currently limiting the breeding herd from achieving its full genetic potential. The authors will outline areas for improvement in both sow and boar breeding programs and will focus on strategies to close the gap between the genetic potential of current breeding stock and the realised value in the progeny being produced. The importance of measuring key traits linked to litter quality will be discussed as will the importance of reducing inefficiencies associated with current artificial insemination techniques.

The subsequent paper (Ball and Moehn 2013) will describe recent research on the energy and amino acid requirements of the modern or "prolific" sow, highlighting the need for targeted nutritional programs in order to achieve maximal reproductive performance. The authors will propose a revised feeding strategy for pregnant sows to optimise lifetime performance of both the sow and her offspring and will also discuss practical options for implementation on commercial farms.

SYMPOSIUM: Constraints to realising the genetic potential of the breeding herd

G. Foxcroft, M. Smit, M. Dyck and J. Patterson

University of Alberta, Edmonton, Alberta, T6G 2P5, Canada.

Abstract

Genetic selection has increased both prolificacy and productivity of the breeding sow and the post-natal growth performance of commercial progeny. However, increasing variation in litter birth weight and foetal development may be indirect consequences of interactions among multiple genes controlling prolificacy and prenatal development. Phenotypic plasticity in the litter phenotype also results from effects of sow metabolic state on the developing embryo. New genomic tools may provide the opportunity to better balance the selection of genes controlling the component traits affecting the size and quality of litters born, particularly in multiparous sows. In the shorter term, management strategies that recognise the implications of a repeatable litter phenotype merit consideration. In the case of terminal line boars, the use of artificial insemination (AI) in the pig industry has had a major impact on realising the genetic potential of select boars over the last 40 years. However, the effectiveness of standard semen analysis in commercial boar studs in identifying the relative productivity of sires is low compared to other food-animal species. Optimal use of superior boars is limited by a number of factors: (1) Employing the use of sub-fertile boars and low quality ejaculates reduces production efficiency; (2) The use of pooled semen from poorly defined males breaks the link between known genetic value of individual boars and the paternity of progeny produced; and (3) The excessive number of sperm used per litter born (probably over 9 billion sperm using current practices), and hence the high numbers of boars needed for semen production, reduces the genetic impact of the best boars compared to the limited number of superior sires used for meat and milk production in other food-animal species. Collectively, these inefficiencies in AI use in the pork industry require attention and can be addressed with innovative breeding programs to increase the genetic impact of AI boars.

Introduction

Genetic selection programs for economically important traits in commercial swine populations have largely resulted in parallel improvements in lean tissue deposition and feed conversion efficiency in the terminal-line offspring, and increased prolificacy of the production female in terms of pigs born per sow per year. At sow level, the total weight of the litter weaned has also increased, reflecting adequate voluntary feed intake and tissue mobilisation to drive increased milk production in lactation, without an obvious decrease in sow fertility after weaning. The overall implications of these changes for the physiology and management of modern commercial sow populations were the topic of previous reviews (Foxcroft, 2012; Kemp and Soede, 2012). There may, however, be less obvious effects on litter quality resulting from years of genetic selection in commercial pig populations that reflect the inadequacy of existing selection techniques for complex, multi-gene, traits like reproductive performance and disease susceptibility (see Knol *et al*., 2010). A new era of genomic selection should be able to address the increasing divergence between traits presently selected in nucleus sow populations (lean growth performance, feed utilisation efficiency, total pigs born or weaned) and the 'phenotypic plasticity' that is evident in the litters of mature sows at the commercial level of production. Implications of continued selection for increased litter size for critical quality traits (perinatal survivability and between-litter variability in litter average birth weight) in higher parity sows, and the long term metabolic response to increased productivity of the sow, will be discussed in the first part of this review.

The ultimate measures of boar performance at the production level are generally farrowing rate and litter size born. However, these are retrospective measures of boar fertility and can be highly influenced by breeding management and the quality of the gilts and sows bred (Colenbrander *et al*., 2003). Boar stud managers generally rely on a combination of thorough physical examinations of the boar and conventional semen evaluation (concentration, morphology, motility) as an alternative to actual fertility data (Gibson, 1989). Although these evaluations can establish that an animal is either sub-fertile or infertile, they cannot identify the relative fertility of boars that meet accepted industry standards for sperm and ejaculate quality (Ruiz-Sanchez, 2006). Our contention is that these "assurances of fertility" currently applied in most commercial AI centers provide a very conservative estimate of the relative fertility of individual boars. Furthermore, the high sperm numbers used in commercial AI practice (usually more than 3 billion total sperm per dose of extended semen), and the pooling of semen from multiple boars, masks the limited fertility of some of these boars. These differences in fertility become evident when lower numbers of sperm are used for AI and boars are evaluated on an individual basis. In a competitive pork industry, effective

prediction of relative boar fertility is essential and will allow for the removal of less efficient boars from commercial studs. The first benefit of adopting such strategies should be an apparent improvement in sow productivity, which is at present limited by using many boars in AI programs that have inferior performance on an individual basis, or do not "compete" effectively in hetero-spermic pools of semen. These strategies will also optimise the impact of genetically high-indexed boars at lower sperm numbers per AI dose. At the nucleus level this will allow for increased selection pressure by increasing the number of offspring bred per collection from high-ranking boars. At the level of terminal line production, this would allow for considerable improvements in production efficiency to be realised, by capitalising on boars with a high index for traits such as growth rate, feed conversion efficiency and the carcass characteristics of their progeny. If these changes in production strategy are to be realised, it is critical to identify boars of relatively low fertility that will not perform well when used in the more challenging situations of reduced sperm numbers per AI dose. The second section of this review will discuss the background physiology behind effective semen evaluation and production strategies that link the use of proven high fertility and high indexing boars with AI techniques that maximise the number of sows bred per boar in service.

Collective attention is needed to realise the full genetic potential of the boars and sows presently available to the pork industry. In part there is a need to capture the advantages of genomic selection by filling the "phenomic gap", which in the case of the sow requires a commitment to measure the component phenotypic traits that drive litter size and litter quality. In the case of the boar, extensive application of single-sire matings will provide the necessary phenotypic traits to allow evaluation of potential genetic markers of acceptable boar fertility. The creation of this new information, the application of improved selection strategies, and the application of production practices that increase the impact of the excellent genetic material available, will improve the overall efficiency of the pork production industry.

Aspects of sow performance

Responses to selection for overall productivity in contemporary sows

Given the number of quantitative trait loci (QTLs) for ovulation rate and the size of these effects, Knol (2003) suggested that a rapid increase in ovulation rate should be possible. However, he cautioned that this might lead to further populations of extremely hyper-prolific sows with a very high ovulation rate and a very high rate of prenatal mortality. Although it might be assumed that a trend towards extreme ovulation rates has been avoided as total numbers of pigs born has steadily increased over the last two decades, there is a paucity of information about the component traits affecting numbers born in nucleus selection programs. In general, selection for only increased numbers of total pigs born tends to produce negative associations for stillbirth rates and post-natal survival (reviewed by Foxcroft *et al.*, 2007a). These negative associations can be partly offset by selecting for the number of pigs born alive, the number of pigs surviving the immediate post-farrowing period, or the number of pigs weaned. Although heritability of these alternative traits is lower than heritability for litter size born, these selection strategies place greater emphasis on the birth of viable offspring. At a physiological level, they are indirectly biasing selection in favour of greater uterine capacity to support prenatal development.

Recent phenotypic data collected from both nucleus and commercial populations of mature sows indicated more variability among litters in litter average birth weight, than among pigs within these litters (Foxcroft *et al.*, 2007a, Smit *et al.*, 2013b). It appears that there is an overriding effect of the maternal environment on the litter phenotype expressed, irrespective of any within-litter variation in birth weight (see Knol *et al.*, 2010). Evidence for a largely maternal effect on litter birth weight phenotype provided the rationale for a series of studies to describe the biological origins of this phenotypic plasticity in contemporary commercial sow populations. The dilemma is how to continue with effective selection for important economic traits in commercial offspring, whilst avoiding parallel selection for negatively associated fertility traits in commercial dam-line females. Although co-selection and other strategies avoids some of these problems (Knap *et al.*, 2001; Knol, 2003; Dekkers *et al.*, 2011), selection pressures on pigs born live, and on post-natal survivability, only partly address the lack of phenotypic information on the key biological traits (ovulation rate, embryonic survival rates and uterine capacity in its broadest sense) that ultimately determine litter size and quality. In discussing the possibilities of the new genomic era in the context of improved selection for reproductive performance, Bidanel (2011) commented, "Genomic selection tools will undoubtedly result in more efficient genetic improvement programmes ... provided that more accurate phenotypes are available to understand the complex relationships between genotype and phenotype". The complexities of evolving sow and litter phenotypes need to be explored to fill the existing 'phenomic gap'.

Metabolic-reproduction interactions in the lactating and weaned sow

Changing relationships between lactational catabolism, the inhibitory effects of suckling, and reproductive performance of sows after weaning are becoming increasingly apparent (Foxcroft *et al.*, 2005, 2007b; Soede *et al.*, 2009; Foxcroft, 2012; Kemp and Soede, 2012). In the past, weaning-to-oestrous intervals (WEI) were reported to be affected by lactation length, litter size, season, nutrition and management practices. The literature suggested that metabolic state of the sow or manipulations in litter size during lactation would have clearly measurable effects on subsequent reproductive performance, especially in first parity sows. When considering WEI, Foxcroft *et al.* (2005) noted that in earlier studies, feed restriction at any time during lactation generally increased WEI. However, in more recent literature, WEI and other reproductive traits showed less response to lactational catabolism (Foxcroft *et al.*, 2010). Results from our most recent studies involving imposed feed restriction of first parity sows in a controlled research environment (Patterson *et al.*, 2011; Table 1) show that 85% or more sows return to oestrus within a 3–5-d interval in the first week after weaning and that the overall fertility of sows bred is high.

Embryonic survival was also affected by previous lactational catabolism in earlier studies (Zak *et al.*, 1997), and the timing of the period of feed restriction seemed to be a critical aspect of this response. Again, in more recent studies involving feed restriction in late lactation, the effects on embryonic survival and development were subtler. In the absence of treatment effects on WEI and ovulation rate, Vinsky *et al.* (2006) reported a gender-specific loss of female embryos and that, regardless of their sex, surviving embryos from previously catabolic sows were developmentally delayed. Subsequently, using an almost identical experimental model of feed restriction in primiparous sows, Patterson *et al.* (2011) reported no effect on embryonic survival but a significant impact on embryonic weight at day 30 of gestation (Table 1).

Table 1. *Least square means (± SEM) for sow body weight changes in lactation, litter weight weaned, sow reproductive performance after weaning and subsequent litter characteristics for Control primiparous sows fed at 90%, and Restrict sows fed at 60% of anticipated feed intake, in the last week of a 20-day lactation (after Patterson* et al.*, 2011).*

	Control (n = 49)	Restrict (n = 48)
Sow weight at farrowing (kg)	187.9 ± 1.8	192.3 ± 1.8
Sow weight at weaning (kg)	180.8 ± 2.0	172.0 ± 2.0[a]
Total litter weight weaned (kg)	65.1 ± 1.1	62.1 ± 1.1[b]
WEI (days)	5.0 ± 0.2	5.3 ± 0.2
Breeding rate (% of sows weaned)	90.0	88.3
Pregnancy rate (% of sows bred)	90.7	90.6
Day of gestation when euthanised	28.9 ± 0.1	28.6 ± 0.1
Ovulation rate	19.7 ± 0.4	20.2 ± 0.4
Number of live embryos	13.8 ± 0.4	14.2 ± 0.4
Embryonic survival (%)	71.2 ± 1.4	70.3 ± 1.4
Embryonic weight (g)	1.56 ± 0.03	1.46 ± 0.03[a]
Embryonic crown–rump length (mm)	25.2 ± 0.2	24.9 ± 0.2
Allantochorionic fluid volume (ml)	222.6 ± 4.8	216.7 ± 1.8

[a]$P < 0.001$; [b]$P < 0.005$, compared to the Control sows.

Collectively, these studies suggest that in situations of limited lactation feed, contemporary primiparous sows are able to consistently return to oestrus after weaning and the number of ovulatory follicles (ovulation rate) is not affected. However, the emerging pre-ovulatory follicles and their oocytes are of poorer quality (Soede *et al.*, 2009) and this may be reflected in gender-specific effects on embryonic survival and overall effects on embryonic development (Foxcroft *et al.*, 2009). The retrospective analysis of interactions between the metabolic state of individual restrict-fed sows and embryonic development of the subsequent litter reported by Patterson *et al.* (2011) suggested that sows responded differently to feed restriction in terms of their inclination to mobilise body tissues to maintain milk production. Furthermore, sows that protected the weight of the litter weaned by excessive mobilisation of body tissues (more than 40 MJ metabolisable energy (ME) per day of energy derived from sow tissues) to support milk production (Figure 1) were most 'at Risk' of compromising the subsequent development of the litters they conceived immediately after weaning (Figure 2). In summary, selection strategies applied by breeding companies over the last two decades has favoured increased milk production in lactation. In some dam-lines, the demands of increased milk production can be met by increased voluntary feed intake in lactation and a subpopulation of sows may even spontaneously ovulate before weaning (Langendijk *et al.*, 2007; Gerritsen *et al.*, 2008a,b, c; Kemp and Soede, 2012). In other dam-lines, extra milk production may come at the cost of increased tissue catabolism in at least some sows (see Control data in Figure 1). However, negative associations between good milk production and litter weight weaned (even at the expense of sow tissue catabolism), and subsequent fertility (WEI, breeding rate, ovulation rate, and even embryonic survival), appear to have decreased. The economic value of this sustained sow fertility should, however, be off-set

against apparent negative effects on embryonic quality in some dam-lines, which may translate into poorer quality litters being born to these sows (Patterson *et al.*, 2011). Continued studies of the complex interactions between sow metabolic state, subsequent fertility, and resulting litter quality will continue to underpin the development of optimal breeding strategies in the pork industry.

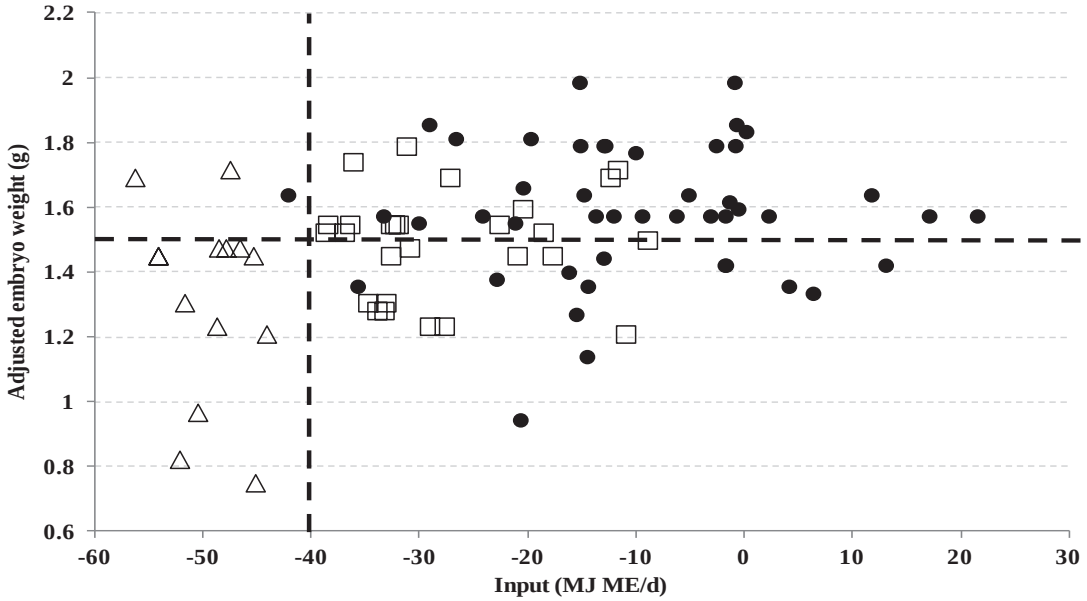


Figure 1. *Lack of an overall relationship between sow energy input from body tissue mobilisation and adjusted (day of gestation) embryo weight at day 30 of gestation for Control sows (●) fed to appetite in the last week of lactation compared to sows restricted to 50% of Control intakes [□ Restrict (Non-Risk), △ Restrict (Risk)] (after Patterson* et al.*, 2011).*

Among Restrict sows and litters, there appears to be a threshold of –40 MJ ME per day (9.1% bodyweight loss), at which there is a risk of compromised embryonic development in the subsequent litter (below mean population embryo weight of 1.51 g) (Figure 1).

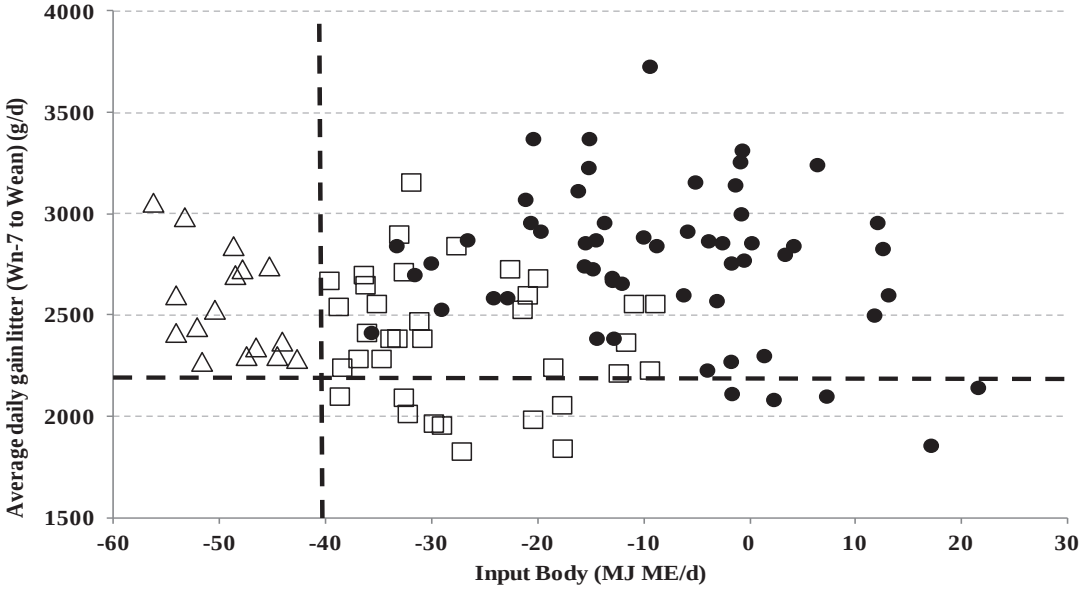


Figure 2. *Lack of an overall relationship between energy input from sow body tissue mobilisation and average litter growth rate for Control sows (●) fed to appetite in the last week of lactation (WeaN - 7 to Wean) compared to sows restricted to 50% of Control intakes [□ Restrict (Non-Risk), △ Restrict (Risk)] (after Patterson* et al.*, 2011).*

The thresholds of 40 MJ ME per day (9.1% bodyweight loss) and approximately 2.2 kg of litter average daily gain appear to define a subpopulation of Restrict (Risk) sows that maintain higher litter growth rates through excessive body tissue mobilisation compared with Restrict (Non-Risk) sows that were less catabolic at the expense of reduced litter weight gain (Figure 2).

From a practical perspective, available literature indicates considerable variation among commercially available dam-lines with respect to the interaction between sow milk producing capacity, breeding performance after weaning, and the growth potential of terminal-line progeny. Furthermore, as discussed further below, within each dam-line there will be changes in sow performance based on parity and management. Depending on the management practices adopted in the future, attention to the dam-line used will be an important consideration. "Robustness" of the sow in loose housing systems and in areas of high disease prevalence may be more important to the best economic returns to the producer than for example attributes for high sow prolificacy, which will require intensive and specialised management in the farrowing house and after weaning.

Pre-natal programming of litter quality

The accumulated experimental evidence supporting the hypothesis that "a low litter birth weight phenotype in a sub-population of mature sows is a consequence of indirect selection for high ovulation rates and a number of embryos at day 30 of gestation that exceeds normal uterine capacity", was recently reviewed by Foxcroft *et al.* (2009). The resulting intra-uterine crowding (IUC) primarily affects early placental development, which then triggers later effects on foetal development. Consequently, at farrowing, litters born to sows in which early intra-uterine crowding of embryos occurred, show benchmarks of intra-uterine growth restriction (IUGR). Marked variability in litter birth weight phenotype is believed to have resulted from many years of direct selection for total pigs born, and from the use of litter phenotypic information that tends to be limited to lower parity females in nucleus populations. Earlier research on the response to direct selection for increased ovulation rate *per se* (one of the key component traits determining litter size born), suggested that indirect selection against early embryonic survival in this population resulted in little long-term improvement in total born. However, recent data collected from mature sow populations suggest that extreme intrauterine crowding can occur when high ovulation rates are associated with reasonable, to good, embryonic survival to day 30 of gestation. The extent of the increasing imbalance between ovulation rate and early embryonic survival on the one hand, and uterine capacity to support normal placental development on the other, and of the impact this might have on pre-natal development, can be illustrated as follows.

In controlled experiments in our own research program, in which the number of developing embryos was manipulated using the technique of unilateral oviductal ligation, the number of embryos at day 30 of gestation in Control (unmodified sows) and in sows with tubal ligations (relatively un-crowded), was 15 versus 9, respectively. Nevertheless, even with 15 embryos surviving to day 30, placental development in Control sows was significantly limited at day 30 and also at day 90 of gestation, and by day 90, the classic markers of IUGR (reduced foetal weight and brain-sparing effects on organ development) were evident (Town *et al.*, 2004). Based on data from subsequent studies in John Harding's research group in Saskatoon using the same experimental model, a 1 kg reduction in birth weight was predicted to increase the odds of piglet death prior to weaning by some 20 times and to decrease lifetime ADG by 71 g/day (Dhakal, 2011). In contrast to the levels of relative IUC established in these experimental studies, in several populations of higher parity commercial sows we have records of between 25 and 30 ovulations as the norm (e.g., Patterson *et al.*, 2008), but more critically, in some of these sows as many as 25 to 29 viable embryos were present in the uterus at day 30 of gestation. In some sows, therefore, high ovulation rates are associated with very little early embryonic loss, and placental development is even more severely limited by the extreme intrauterine crowding present.

Based on our most recent studies in commercial sow populations (Silva, 2012; Smit *et al.*, 2013b), our working hypothesis continues to be that early crowding of embryos in the uterus in a proportion of mature sows pre-programs a low birth weight phenotype, irrespective of litter size born. Considering the data in Figure 3, we can identify at least two factors that contribute to variation in litter average birth weight in commercial sows. In Figure 3a, both the mean and maximum litter average birth weight decreases in very large litters, reflecting the trend towards lower birth weights in hyper-prolific sows (Foxcroft *et al.*, 2009). In litters with >16 piglets born, therefore, the absence of heavier birth weight litters seen in less prolific sows (up to 2 kg) is one component of a limited uterine capacity. There also appears to be a lower limit of litter average birth weight of around 900 g, which is more or less independent of litter size born. Equally, as shown in Figure 3b, total numbers born explains less than 5% of the variation in litter average birth weight in litters between 9 and 16 total born (Smit *et al.*, 2103b) and is predicted to reflect different patterns of prenatal survival in higher parity commercial sows. Overall, a low litter average birth weight has been associated with significantly fewer pigs born alive, more pigs born dead, and fewer pigs surviving to weaning, compared to high average birth weight litters (Smit, 2007; Smit *et al.*, 2013a,b). Additionally, necropsy data from stillborn pigs that fell within the mid-weight range for their respective litters, confirmed that low average birth weight litters carried all the negative phenotypic characteristics of IUGR (Smit, 2013; Smit *et al.*, 2013b).

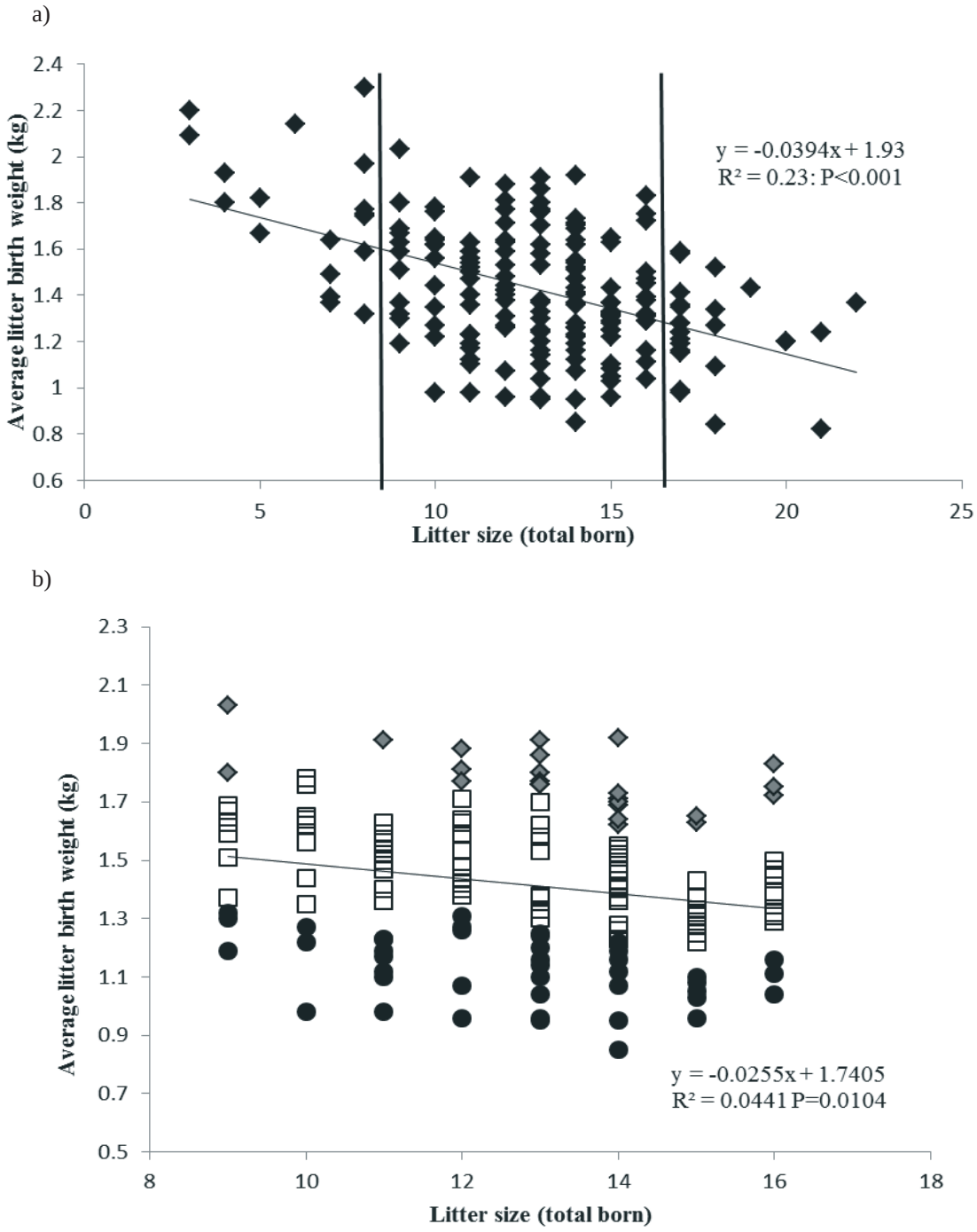


Figure 3. *Relationship between litter size (total pigs born) versus average litter birth weight for (a) all litter sizes (n=192), and (b) litters between 9 and 16 total born (n=148). The litters between 9 and 16 total born were classified as having a low (●), medium (□) or high (△) average birth weight (from Smit* et al., *2013b).*

Finally, accumulated results indicate that litter birth weight phenotype is fairly repeatable (Smit, 2103; Smit *et al.*, 2013b), and differences in post-natal growth performance of the lower compared to the higher birth weight litters can be tracked to the late finisher stage of production (Smit *et al.*, 2013b). Correlation analysis between litter average birth weight of the current litters and the three preceding litters within sows (Smit, 2013) established correlations (P<0.001) between litter average birth weight of the current litter and the previous litter (r=0.49), between the current litter and the previous two litters (r=0.49), and between the current litter and the previous three litters (r=0.50). The percentage of sows in the different birth weight categories in two consecutive farrowings is shown in Figure 4, and indicates that none of the sows switched between the LBW and HBW categories in consecutive farrowings.

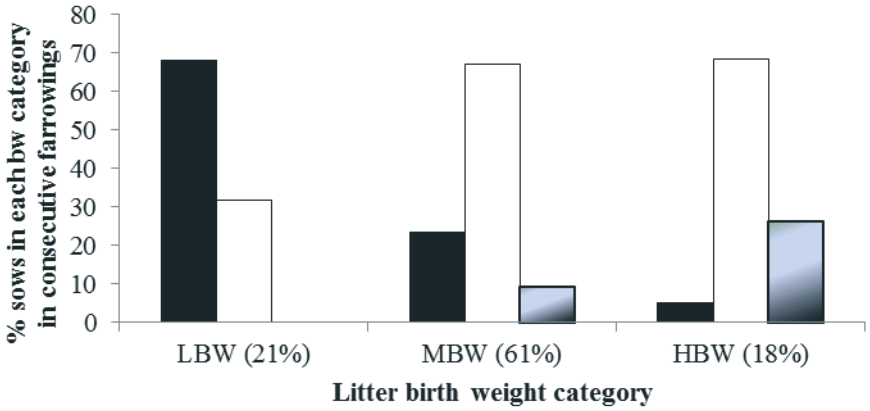


Figure 4. *Classification of sows (n = 105) into different birth weight (bw) categories in two consecutive farrowings, showing the repeatability of litter average birth weight. Numbers in parentheses indicate the proportion of sows classified as having a low (LBW: solid black bars), medium (MBW: open bars) or high (HBW: shaded bars) litter average bw, defined as being 1 sd below, within 1 sd, or 1 sd above the population mean average litter bw, respectively at the first recorded farrowing (from Smit et al., 2013b).*

Figure 4 shows the distribution of litter bodyweight categories at the next farrowing within each of the original classifications (i.e. of the 21% of sows initially classified as having a LBW litter, nearly 70% had a LBW litter and some 30% had a MBW litter at the next farrowing). Overall, virtually no sows switched between the low and high birth weight categories.

In ongoing studies we have confirmed similar repeatability in litter birth weight phenotype over successive parities in another commercial sow population. We have used this information to identify sows at the extremes of low and high litter birth weight for detailed study of the component reproductive traits involved (Table 2), and gene expression in reproductive tissues recovered in early gestation (Silva, 2012).

Table 2. *Reproductive data from sows (n=5 per category) with repeatable high and low birth weight (BW) phenotypes (from Silva, 2012).*

Parameter	High	Low	Significance
Number of ovulations	21.0 ± 3.1	26.0 ± 2.8	0.06
Average litter BW (kg)	1.55 ± 0.98	1.05 ± 0.89	<0.001
Total pigs born	13.0 ± 1.0	13.5 ± 1.1	0.46

Data show the mean ± s.e.m. number of ovulations at the time of slaughter and total pigs born litter average birth weight litter over the previous three parities.

The trend towards a higher ovulation rate in the sows with a low birth weight phenotype confirms unpublished data from sows recruited from the studies reported by Smit (2013), and is consistent with previous data suggesting that direct selection for litter size has inadvertently exaggerated selection for increased ovulation rates in a subpopulation of mature sows (Patterson *et al.*, 2008).

In summarising her doctoral studies, Smit (2013) discussed the practical and economic implications of these differences in litter birth weight phenotype. Segregation of higher parity sows based on predicted birth weight phenotype would provide the opportunity to direct the attention of farrowing room staff to the litters most at risk of perinatal mortality. Based on accumulated evidence for beneficial effects of nutritional interventions on litter quality and weaning weight, Smit *et al.* (2013a) firstly used supplementation of gilts from day 60 of gestation with a fish-oil based n-3 long chain polyunsaturated fatty acid supplement (m-PUFA) to increase the weaning weight of litters from treated gilts. However, similar m-PUFA supplementation of sows with a repeatable low birth weight phenotype provided no advantages to the low birth weight litters. Nevertheless, further studies of nutritional (nutrigenomic) approaches to improving prenatal development of "at risk" litters seem justified. The differences in growth performance of low compared to high birth weight litters (e.g., 9 days difference to reach a fixed market weight; Smit *et al.*, 2013b) also suggested that segregated housing and specific nutritional programs for progeny with differing post-natal growth potential would be beneficial, but even in relatively controlled conditions it has been difficult to capture these benefits.

Aspects of boar performance

The use of artificial insemination (AI) in the pig has had a major impact on genetic improvement in the swine industry over the last 40 years. However, the overall production efficiency of the breeding herd is highly dependent on the reproductive capacity of the boars used for breeding and the genetic merit of the boars for the performance of terminal line offspring. Given the widespread use of AI in swine production, poor quality boars will affect the reproductive outcome of numerous females. We instinctively know that not all boars are of equal quality, and all ejaculates collected for use in AI are subjected to standard semen analysis in commercial boar studs. However, the effectiveness of these evaluations is low compared to other food-animal species. This section of the paper reviews some limitations to the optimal use of superior boars in commercial pork production, namely: (1) Effects of using sub-fertile boars and low quality ejaculates on production efficiency; (2) The break in the link between known genetic value of individual boars and the paternity of progeny produced created by use of pooled semen from poorly defined males; and (3) The fact that excessive number of sperm used per litter born (probably over 9 billion sperm using current practices) reduces the genetic impact of our best boars compared to the limited number of superior sires used for meat and milk production in other food-animal species. Collectively, these inefficiencies in AI use in the pork industry represent a major disadvantage to pork producers in a global food-animal marketplace and can be addressed with innovative breeding programs to increase the genetic impact of AI boars.

Approaches to assessing boar fertility

There is a long history behind the search to find a single or combination of tests that can accurately predict male fertility from a semen sample (Amann, 1989). Unfortunately, there appears to be no simple answer to this very complex question (Rodriguez-Martinez, 2003). Laboratory assays often examine all of the sperm present in a sample for fertility, yet only 1-30 or so sperm are necessary to fertilise all available oocytes. Braundmeier and Miller (2001) suggested that the sperm that fertilize the oocytes *in vivo* might be a small, highly selected, sub-population that is not representative of the average sperm evaluated in the sample. They also suggest that, because sperm must meet many requirements for successful fertilisation, testing a single attribute is unlikely to be a true measure of ultimate fertility. Using similar reasoning, Rodriguez-Martinez (2003) suggested that to accurately predict semen quality it is necessary to test all key sperm attributes within large and heterogeneous sperm populations that potentially affect fertilisation and embryonic development. Nevertheless, the markers of relative fertility finally selected must ultimately predict the relative fertility of boars when using low sperm doses of extended semen for AI (Rodriguez-Martinez *et al.,* 2009). Braundmeier and Miller (2001) described two different sperm traits that affect fertility: (1) *Compensable* traits, which are those that can be overcome by introducing large numbers of sperm during insemination. Problems with motility and morphology will reduce the number of sperm that are able to reach the oocyte, but by introducing large numbers of sperm the reduction in fertility can be minimized; and (2) *Uncompensable* traits, which are those that cannot be overcome by introducing larger numbers of sperm. Therefore, to effectively predict fertility, it is essential to discriminate between compensable and uncompensable traits in an ejaculate. Conversely, evaluation of relative boar fertility *in vivo* using high sperm numbers per dose (e.g., three or more billion sperm) will mask differences in compensable traits and will not allow the industry to identify boars that will perform well in more demanding applications of AI.

Conventional semen evaluation generally includes a measure of seminal volume, sperm concentration, and the percentage of progressively motile and morphologically normal sperm (Amann *et al.*, 1995). Although some of these parameters are correlated with fertility in the boar (Flowers, 1997; Xu *et al.*, 1998), several authors suggest that in the bull this information, while important, does not accurately predict whether a male is truly fertile (Correa *et al*., 1997; Brahmkshtri *et al.*, 1999). Existing analyses are also usually inadequate for predicting relative fertility in healthy boars with ejaculate quality that meets normal industry standards (>70% motility and <30% abnormal sperm) (Flowers, 1997; Alm *et al.,* 2006), even though the reproductive efficiency of these boars may still be substantially different (Flowers, 1997; Tardif *et al.,* 1999; Popwell and Flowers, 2004; Ruiz-Sanchez, 2006). Differences in relative fertility become increasingly evident when low sperm doses (<2.0 billion sperm) are used for AI (Tardif *et al.,* 1999; Watson and Behan, 2002; Ardon *et al.,* 2003; Ruiz-Sanchez, 2006). Because this approach likely avoids the compensatory effect of using excessive sperm numbers per AI dose (Saacke *et al.,* 2000; Alm *et al.,* 2006), important fertility differences among boars become increasingly apparent.

Evidence for differences in relative boar fertility in commercial studs

The almost universal use of pooled semen doses in commercial boar studs severely limits the collection of data on relative boar fertility at production level, but the limited data available suggest a substantial range of fertility in contemporary populations of boars. Indeed, in the absence of routine procedures for

identifying relative boar fertility, a normal distribution of fertility traits should be expected. In recent discussions of overall breeding herd performance (Billy Flowers; *pers. comm*.), the point has also been made that limitations in AI technology may lead the industry to continually underestimate the existing productivity of contemporary commercial dam-lines. Available data obtained from a minimum of 50 single-sire matings per boar at the multiplication level (Tony Chandaruk; *pers. comm*.) indicated that the productivity of the top two thirds of these boars was very high, and at an average of over 13 pigs total born, would be consistent with good breeding herd performance. However, when the productivity of the lower one third of these boars was included, overall productivity falls by over one pig per litter born. This relatively inferior performance of 20 to 30% of boars evaluated is consistent with the more extensive data available in the literature. If the genetic merit of the more fertile boars at stud is high, the application of more efficient AI technologies would allow the merits of these "elite" boars to spread across a larger proportion of sows bred. However, with current AI practices, the substantial differences in boar productivity and the link to known progeny produced by individual boars are confounded by (1) the use of pooled semen, and 2) high sperm numbers per AI dose. The application of advanced AI techniques, such as post cervical AI (PCAI), single fixed time AI (sFT-AI), or these two techniques in combination, can dramatically increase utilization of the most desirable sires (Willenburg *et al*., 2012).

Breeding programs to increase the genetic impact of AI boars

Based on the above information, it is clear that future developments in AI technology should involve: (1) A move to single-sire inseminations with the lowest possible doses of semen; and (2) Use of ejaculates from boars with high genetic value and proven fertility in a "low semen dose" environment. In response to the need to improve the impact of elite boars on production efficiency and profitability, our research group developed a strategy for implementing an AI program using lower numbers of sperm per dose and single-sire breedings that was modeled on a 10,000 sow system using an internal 100-boar stud. Before any intervention occurred, the following assumptions were assumed to apply:

1. All sows were bred by AI using standard catheters and multiple inseminations (average 2.2) dependent on the duration of estrus and using existing breeding protocols;
2. Semen was pooled from multiple boars, with 3 billion sperm per AI dose;
3. Average genetic index (Estimated Breeding Values indexed on a relative 100 point system) was 115.8 (range from 90-150);
4. Pigs marketed per sow per year (PSY) after grade-outs averaged 24.5;
5. Wean to farrow loss was 7% and therefore, total pigs sold/year was 227,850;
6. The number of pigs produced per boar per year was 2,279.

A multiple-stage approach was proposed to improve the impact of superior boars within the system, but at each stage typically only one aspect of AI management was altered.

Stage 1. All sows are bred by AI using standard catheters multiple times (average 2.2 times) dependent on the duration of oestrus and standard farm protocol, but for all ***new boars entering the stud***, semen is processed as ***single-sire doses of 2 billion sperm cells per dose.*** A minimum of 50 single-sire matings per boar is used to identify the top 66% of boars in the stud (33% reduction in needs due to the change from 3 to 2 billion sperm per dose and hence 33% more doses created from the 66% best boars retained). The lowest performing boars are removed from service and overall responses in productivity were estimated as follows:

1. Average genetic index increases to 122.5 (range 110-150);
2. Improved fertility of retained boars produces a predicted increase in PSY to 26.5;
3. Wean to farrow loss remained at 7% but total pigs sold/year increased to 246,450;
4. Pigs produced per boar per year increases to 3,734.

Stage 2. All sows are bred by the ***post-cervical AI (PC-AI) technique*** multiple times (average 2.2 times) dependent on the duration of estrus and existing farm protocols, but semen is now used ***as single-sire doses of either 1 or 1.5 billion sperm cells per dose from boars with proven fertility at 2 billion sperm/dose using conventional in AI Stage 1.*** A minimum of 50 matings per boar continues to be used to identify the top 33% of boar in stud and the lower performing boars are again removed, with the following predicted outcomes:

1. Average genetic index increases to 129 (range 122-150);
2. Pigs per sow per year (PSY) after grade-outs still averages 26.5;
3. Wean to farrow loss is still 7% and total pigs sold/year remains at 246,450;
4. Pigs produced per boar per year is now 7,468.

Stage 3. All sows are bred ***using a single, fixed-time, AI protocol (sFT-AI) and the PC-AI technique using one single-sire dose of 1 or 1.5 billion sperm cells.*** A minimum of 50 matings per boar is still used

to identify top 15% of boar in stud and the lower performing boars are again removed from service, with the following predicted outcomes:

1. Average genetic index moves to 134 (range 127-150);
2. The number of pigs per sow per year (PSY) after grade-outs still averages 26.5;
3. Wean to farrow loss is still 7% and total pigs sold/year remains at 246,450;
4. The number of pigs produced per boar per year is now 16,430.

Potential economic gains that were predicted from adopting these advanced AI strategies are summarised in Table 3.

Table 3. *Cost benefit analysis of improved swine AI procedures, based on a 10,000 sow system and an integrated 100-boar commercial AI stud. Phases 1, 2 and 3 are those outlined in stages 1 to 3 above, and arrows and equal signs show the direction of change in specific items by Phase of the project.*

	Start		Phase 1		Phase 2		Phase 3
A.I.method[1]	Standard		Standard		PC-AI		SFT-AI
A.I. dose (billions)	3	↓	2	↓	1	=	1
Number of inseminations	2.2	=	2.2	=	2.2	↓	1
Numbers of boars needed	100	↓	66	↓	33	↓	15
Average index[2]	115.8	↑	122.5	↑	129	↑	134
Sows in production	10,000	=	10,000	=	10,000	=	10,000
PSY (after grade outs)[3]	24.5	↑	26.5	=	26.5	=	26.5
Wean-to-finish losses	7%	=	7%	=	7%	=	7%
Total pigs sold per year	227.850	↑	246,450	=	246,450	=	246,450
Annual opportunities[4]	-	↑	$115,585	↑	$227,720	↑	$313,977
Other opportunities							
Reduction in boar inventory	-	↓	33%	↓	66%	↓	85%
Savings in boar stud costs	-	↑	$90,750	↑	$181,500	↑	$233,750

[1]PC-AI, post-cervical AI: SFT-AI, Single fixed-time AI using the PC-AI technique.
[2]Average index based on a relative index of 100: opportunity value set at $US 7 cents per index point.
[3]PSY, pigs produced per sow per year, based on 2.4 litters per year.
[4]Based on increased production plus increased average boar index points x 7 cents.

In a major collaborative project that used the above approach in the 40,000 sow Holden Farms system with two internally controlled boar studs of 100 boars, data from 67 boars initially evaluated, representing a total of 3,675 single-sire matings and approximately 50 matings per boar, showed some interesting trends. Comparing the highest and lowest performing boars, there was over a 30% difference in pregnancy rate at day 30 and in farrowing rate, a difference of more than 5.5 pigs total born per litter. This resulted in a difference of more than 600 total pigs produced per 100 sows bred. Because the initial reduction from 3 to 2 billion of sperm per AI dose allows the culling of less productive boars from further commercial production, overall herd productivity increased. Of the first 30% of boars that were no longer required, about half of these boars were culled because of low fertility/productivity and the other half because of a relatively low genetic index. The 13.0% (9/67) lower-producing boars produced a day 30 pregnancy rate of 80.4% and averaged 12.2 pigs total born, compared to 93.9% and 13.3 pigs, respectively, for the more productive boars. Indeed, the most fertile boars tested in several ongoing AI-based collaborations achieve close to a 100% farrowing rate and high numbers of pigs born, even at reduced sperm numbers per AI dose. The latest summary of relative boar productivity from the Holden Farms project is shown in Table 4. These results reinforce the conclusion that one of the limitations in existing AI programs is an underestimation of the high fertility of contemporary commercial sows. In terms of lost production, the initial removal of the lowest 15 to 20% of boars brings sow productivity to a new level and is major contributor in Phase 1 of the project to the revenue opportunity available. In contrast, Phases 2 and 3 of the project are largely affecting the genetic merit of the progeny produced, although continued savings in boar stud costs continue to contribute to the cost benefit opportunity available.

At the present time, all incoming boars in the Holden Farms system are now routinely evaluated at 2 billion sperm per AI dose using the post-cervical AI technique in mature sows. After successful implementation of Phase 2 at the breeding farms used for initial boar evaluation, all commercial farms in the system are adopting the use of post-cervical AI for sows, but using conventional catheters for breeding gilts. Phase 3 of the project has just been evaluated and data on the use of single-fixed time AI in mature weaned sows is being finalised. At the time of writing, one of the two original 100-boar AI studs has been

closed, representing another important cost saving component of this type of project. Other collaborations with commercial customers suggests that implementation of at least Phases 1 and 2 of the proposed AI strategy presents few difficulties in farms with acceptable breeding herd performance at the outset of the project.

Table 4. *Results on production performance of sows bred to the first cohorts of boars (n=190) subjected to single-sire evaluation at entry to the commercial stud. Data are based on a minimum of 50 breedings per boar using standard AI techniques in mature sows with a WEI between 2 and 6 days, 2 billion sperm per AI dose, and semen that was used between 2 and 5 days after collection. Boars categorised as Low, Medium or High fell in the top, middle or highest third of the population based on pregnancy rate at day 30 (University of Alberta/PIC/Holden Farms,* unpublished data, *2012).*

Category	Low	Medium	High	Pooled SEM
Day 30 pregnancy rate (%)	85.9[a]	94.1[b]	98.1[c]	0.6
Farrowing Rate (%)	82.3[a]	91.1[b]	95.0[c]	0.7
Total born	13.0[a]	13.5[b]	13.5[b]	0.1
Total born alive	11.6[a]	12.3[b]	12.2[b]	0.1
Pigs produced[1]	1,071[a]	1,233[b]	1,280[c]	15.3

[1]Estimated per 100 sows bred; [a,b,c]Means in a row not having the same superscript are significantly different (P<0.05); SEM, standard error of the mean.

The evaluation of relative fertility amongst commercial AI boars and a move to single-sire AI programs, in combination with advanced AI techniques, holds significant potential economic benefits to the swine industry. Data collected from initial boar evaluations would allow for elimination of the less fertile boars at an early stage. The characterisation of AI boars that maintain high productivity at even lower numbers of sperm per AI dose then allows the industry to capitalise on established and emerging AI technologies like post-cervical, and single, fixed-time, insemination. These changes would be made without any loss in productivity, as measured in terms of pigs born per sow per year. The boars retained for commercial use would then have the highest genetic merit among boars available at any point in time, and would be used across a greater number of gilts and sows. Results to-date suggest that the relative value of commercial progeny could be increased by between US $0.80 and $1.30 per pig born, and would largely reflect the genetic merit of elite boars in terms of growth performance and feed utilisation efficiency of their offspring.

If single-sire inseminations were routinely adopted for commercial production, there would be additional opportunities to identify boar traits that would bring value to pork production. These might include: (1) Use of AI strategies (low litter size boars) to limit intra-uterine crowding in early gestation, and (2) use of AI strategies (high litter survivability boars) to mitigate effects of a low litter birth weight phenotype. Finally, from a genomic perspective, the accumulation of production data from single-sire matings, and information on ejaculate quality and other semen characteristics for the boars under evaluation in large populations of commercial line boars, has enabled the first steps in completing a genomic analysis based on boar DNA, and proteomic analyses of the seminal plasma. Both can be linked to fertility results with low sperm numbers per AI dose and routine data on ejaculate quality and sperm characteristics measured routinely in the stud. Association analyses will be completed with the goal of identifying genetic markers of boar fertility for use in sire-line selection programs and proteomic markers of semen quality that would provide a better prediction of ejaculate quality than is possible at the present time. A comparable study using sperm DNA fragmentation as a phenotypic trait to identify high and low fertility populations of AI boars associated with differences in gene transcription in testicular tissue is also being directed at the eventual identification of SNPs that would be predictive of relative boar fertility (Van Som *et al.*, 2013).

Conclusions

The main focus of this presentation, and the strategic goal of the research discussed, is to find short-term ways of closing the gap between the excellent genetic potential of existing sire-line and dam-line breeding stock and the realised value of the progeny currently being produced. In the case of the boar, immediate adoption of a number of achievable steps to dramatically reduce the number of sperm used to produce terminal-line progeny will have two effects: The use of boars with proven fertility will increase the overall productivity of the sow herd, and the use of substantially fewer boars with the highest genetic index will substantially improve the value of their progeny. However, in the longer-term, these strategies offer opportunities to find valuable markers of boar fertility. In the case of the sow, a better understanding of the interactions at sow level that determine the variation in litter birth phenotype suggests short-term strategies

that might address or reduce this variation in litter quality. Again, in the longer-term, the collection of relevant phenotypic data on the component reproductive traits that determine litter birth weight will enable the identification of useful genetic markers that will allow a more appropriate balance between selection for litter size and selection for litter quality.

References

ALM, K., PELTONIEMI, O.A., KOSKINEN, E. and ANDERSSON, M. (2006). *Reproduction in Domestic Animals.* **41:**210-213.

AMANN, R.P. (1989). *Journal of Andrology*. **10:**89-98.

AMANN, R.P., KATZ, D.F. and WANG, C. (1995). In: "The Handbook of Andrology". American Society of Andrology, Schaumburg, IL.

ARDON, F., DOHRING, A., LE THI, W., WEITZE, K.F. and WABERSKI, D. (2003). *Reproduction in Domestic Animals.* **38:**161-165.

BIDANEL, J-P. (2011). In "The Genetics of the Pig", 2nd edition, pp.218-241, eds. M.F. Rothschild and A. Ruvinsky (CABI Cambridge, MA, USA).

BRAHMKSHTRI, B.P., EDWIN, M.J., JOHN, M.C., NAINAR, A.M. and KRISHNAN, A.R. (1999). *Animal Reproduction Science.* **54:**159-168.

BRAUNDMEIER, A.G. and MILLER, D.J. (2001). *Journal of Dairy Science.* **84:**1915–1925.

COLENBRANDER, B., GADELLA, B.M. and STOUT, T.A. (2003). *Reproduction in Domestic Animals.* **38:**305-311.

CORREA, J.R., PACE, M.M. and ZAVOS, P.M. (1997). *Theriogenology*. **48:**721-731.

DHAKAL, S. (2011) MSc Thesis. University of Saskatchewan, Saskatoon, Canada .

DEKKERS, J.C.M., MATHUR, P.K. and KNOL, E.F. (2011). In "The Genetics of the Pig", 2nd edition, pp.390-425, eds. M.F. Rothschild and A. Ruvinsky (CABI Cambridge, MA, USA).

FLOWERS, W.L. (1997). *Journal of Reproduction and Fertility. Supplement* **52:**67-78.

FOXCROFT, G.R. (2012). *Reproduction in Domestic Animals.* **47**(Suppl. 4**):**313–319.

FOXCROFT, G.R., BELTRANENA, E., PATTERSON, J. and WILLIAMS, N. (2005). In: Proceedings of the 2005 Allen D. Leman Swine Conference, University of Minnesota, pp.130–138.

FOXCROFT, G.R., BEE, G., DIXON, W., HAHN, M., HARDING, J., PATTERSON, J., PUTMAN, T., SARMENTO, S., SMIT, M., TSE, W-Y. and TOWN, S. (2007a). In "Paradigms of Pig Science", pp.207-229, eds. J. Wiseman, M.A. Varley, S. McOrist and B. Kemp. (Nottingham University Press, Nottingham, UK).

FOXCROFT, G.R., VINSKY, M.D., PARADIS, F., TSE, W-Y., TOWN, S.C., PUTMAN, C.T., DYCK, M.K. and DIXON, W.T. (2007b). *Theriogenology*. **68**(Suppl. 1)**:**S30–S39.

FOXCROFT, G.R., DIXON, W.T., DYCK, M.K., NOVAK, S., HARDING, J.C.S. and ALMEIDA, F.C.R.L. (2009). *Society of Reproduction and Fertility, Supplement.* **66:**213–231.

FOXCROFT, G., PATTERSON, J. and DYCK, M. (2010). Improving Production Efficiency in a Competitive Industry. *Proceedings of the Manitoba Swine Seminar,* 81-98.

GERRITSEN, R., SOEDE, N.M., LANGENDIJK, P., DIELEMAN, S.J., HAZELEGER, W. and KEMP, B. (2008a). *Reproduction in Domestic Animals.* **43:**1–8.

GERRITSEN, R., SOEDE, N.M., LANGENDIJK, P., TAVERNE, M.A.M. and KEMP, B. (2008b). *Reproduction in Domestic Animals.* **43:**59–65.

GERRITSEN, R., SOEDE, N.M., LAURENSSEN, B.F.A., LANGENDIJK, P., DIELEMAN, S.J., HAZELEGER, W. and KEMP, B. (2008c). *Animal Reproduction Science.* **103:**379–384.

GIBSON, C.D. (1989). *Veterinary medicine*. **84:**200.

KEMP, B. and SOEDE, N.M. (2012). *Reproduction in Domestic Animals.* **47**(Suppl. 4**):**320-326.

KNAP, P.W., van der STEEN, H.A.M. and PLASTOW, G.S. (2001). *Livestock Production Science.* **72:**43–48.

KNOL, E.F. (2003). In "Paradigms of Pig Science", pp.11-24, eds. J. Wiseman, M.A. Varley, S. McOrist and B. Kemp (Nottingham University Press, Nottingham, UK).

KNOL, E.F., MATHUR, P. and FOXCROFT, G.R. (2010). *Advances in Pig Production*. **21:**277–286.

LANGENDIJK, P., BERKEVELD, M., GERRITSEN, R., SOEDE, N.M. and KEMP, B. (2007). In "Paradigms of Pig Science", pp.359-383, eds. J. Wiseman, M.A. Varley, S. McOrist and B. Kemp (Nottingham University Press, Nottingham, UK).

PATTERSON, J., WELLEN, A., HAHN, M., PASTERNAK, A., LOWE, J., DEHAAS, S., KRAUS, D., WILLIAMS, N. and FOXCROFT, G.R. (2008). *Journal of Animal Science*. **86:**1996–2004.

PATTERSON, J.L., SMIT, M.N., NOVAK, S., WELLEN, A.P. and FOXCROFT, G.R. (2011). *Reproduction Fertility Development.* **23:**889–898.

POPWELL, J.M. and FLOWERS, W.L. (2004). *Animal Reproduction Science*. **81:**97-113.

RODRIGUEZ-MARTINEZ, H. (2003). *Reproduction in Domestic Animals.* **38:**312-318.

RODRÍGUEZ-MARTINEZ, H., KVIST, U., SARAVIA, F., WALLGREN, M., JOHANNISSON, A., SANZ, L., PEÑA, F.J., MARTÍNEZ, E.A., ROCA, J., VÁZQUEZ, J.M. and CALVETE, J.J. (2009). *Society of Reproduction and Fertility, Supplement* **66:**1-21.

RUIZ-SANCHEZ, A.L. (2006). PhD Thesis. University of Alberta, Edmonton, Canada.

SAACKE, R.G., DALTON, J.C., NADIR, S., NEBEL, R.L. and BAME, J.H. (2000). *Animal Reproduction Science.* **60:**663-677.

SILVA, P.V. (2012). PhD Thesis. Universidade Federal de Viçosa, Brazil.

SMIT, M.N. (2007). MSc Minor Thesis. Wageningen Agricultural University, The Netherlands.

SMIT, M.N. (2013). PhD Thesis. University of Alberta, Edmonton, Canada.

SMIT, M.N., PATTERSON, J.L., WEBEL, S.K., SPENCER, J.D., CAMERON, A.C., DYCK, M.K., DIXON, W.T. and FOXCROFT, G.R. (2013a). *Animal*. **7:**784-792.

SMIT, M.N., SPENCER, J.D., ALMEIDA, F.R.C.L., PATTERSON, J.L., CHIARINI-GARCIA, H., DYCK, M.K. and FOXCROFT, G.R. (2013b). *Animal*, **7:**1681-1689.

SOEDE, N.M., HAZELEGER, W., GERRITSEN, R., LANGENDIJK, P. and KEMP, B. (2009). *Society of Reproduction and Fertility, Supplement* **66:**117–186.

TARDIF, S., LAFOREST, J P., CORMIER, N. and BAILEY, J.L. (1999). *Theriogenology*. **52:**447-459.

TOWN, S.C., PUTMAN, C.T., TURCHINSKY, J., DIXON, W.T and FOXCROFT, G.R. (2004). *Reproduction.* **128:**443-454.

VAN SOM, M, GAUSTAD, A.H., ANDERSEN-RANBERG, I., MYROMSLIEN, F. and GRINDFLEK, E. (2013). EAAP Book of Abstracts No. 19, Session 29, Poster 16, p395. (Wageningen Academic Publishers, The Netherlands).

VINSKY, M.D., NOVAK, S., DIXON, W.T., DYCK, M.K. and FOXCROFT, G.R. (2006). *Reproduction Fertility and Development.* **18:**347–355.

WATSON, P.F. and BEHAN, J.R. (2002). *Theriogenology*. **57:**1683-1693.

WILLENBURG, K., DYCK, M., FOXCROFT, G. and PATTERSON, J. (2012). In "Proceedings of the 11th Annual London Swine Conference", London, Ontario, pp9-14.

XU, X., POMMIER, S., ARBOV, T., HUTCHINGS, W., SOTTO, W. and FOXCROFT, G.R. (1998). *Journal of Animal Science.* **76:**3079-3089.

ZAK, L.J., COSGROVE, J.R., AHERNE, F.X. and FOXCROFT, G.R. (1997). *Journal of Animal Science*. **75:**208–216.

We acknowledge the financial contributions of NSERC, ALMA and numerous industry partners to the Swine Reproduction-Development Program at the University of Alberta and to the development of the research reported in this review.

SYMPOSIUM: Feeding pregnant sows for optimum productivity: past, present and future perspectives

R.O. Ball and S. Moehn

University of Alberta, Edmonton, Alberta, T6G 2P5, Canada.

Abstract

A review of experiments that have manipulated amino acid and/or energy allowances for sows, either during specific phases in pregnancy or the entire gestation, shows that optimal results for lifetime performance is likely achieved by feeding pregnant sows close to requirements. Both undersupply and oversupply of nutrients and energy in pregnancy have the potential to negatively impact subsequent sow and piglet performance, with gilts more sensitive to improper nutrition than older sows. Gilts also benefit more than sows from increased protein and energy intake in late pregnancy. We have conducted a number of experiments in which we directly measured the requirements of sows during gestation for energy, using indirect calorimetry, and for amino acids using indicator amino acid oxidation. When we began this research there were no good models to predict pregnant sow amino acid and energy requirements. There are now several models that are based on the growth of body components, i.e., maternal body and products of conception during gestation. These models add up the amino acid and energy deposition in these body components, together with estimates of maintenance, and then apply estimates of the efficiency of amino acid and energy utilisation to calculate requirements. These models can calculate requirements for a wide range of physical and performance characteristics. A key output of these models is that the requirements for amino acids and energy are much greater in late than early pregnancy, which is in agreement with our empirical studies specifically designed to determine the effects of stage of pregnancy and age of sows on amino acid and energy requirements. However, there is disagreement regarding the magnitude of requirement changes during late gestation and between gilts and sows, indicating that some of the assumptions used in the models may need reconsideration. A general weakness of both models and most empirical data is their strict focus on pregnancy without considering possible effects of nutrition during gestation on sow and piglet performance following parturition. For practical application, parity-segregated phase feeding is ideal to feed all sows to their individual needs, and thus achieve optimal performance and economic return from both sows and offspring.

Introduction

Sows are of central importance to pork production not only because they are the reproductive unit of the swine herd, but also because their productivity and genetic potential defines the maximum potential productivity and economic return of the entire system. Thus an investment in sow feeding, just like sow genetics, is an investment in the productivity and profitability of the entire herd. Although sows represent a numerically small fraction of the total pig herd, sows consume 20% of the feed in farrow-to-finish pork production and thus have a very large impact on the overall feed cost per kg of pork produced. Improper diets have many negative effects on sow performance, including poor rebreeding success, fewer pigs per litter born and weaned, lower piglet birth and weaning weights, greater variation in piglet weight and growth potential, and in reduced sow longevity (Dourmad *et al*., 1994).

Traditionally, assessments of nutrient requirements are based on empirical studies. However, studies in pregnant animals are much more complex than experiments in growing animals, with the result that there are comparatively few empirical results available for nutrient requirements during gestation in pigs. The use of mathematical modelling can combine the results of a body of empirical research and derive relationships between requirements and several factors impacting them. The NRC (1998) first used this approach in a quite simple and rudimentary model to estimate the nutrient requirements of pregnant sows. Since then, availability of new data and refinement of the modelling approach has resulted in second-generation models for nutrient requirements of pregnant sows. Recent models from France (Dourmad *et al.*, 2008), Germany (GfE, 2008), and United States (Kim *et al*., 2009; NRC, 2012) are based on estimates of growth of body components, i.e., maternal body, placenta, foetuses, and mammary growth. These models calculate amino acid requirements based upon estimates of the amino acid composition of protein deposited in these body components, and for maintenance, modified with an estimate of the efficiency of amino acid retention. However, a model is of necessity an abstraction and simplification of complex and integrated physiological processes, with the results that requirement values created by models may differ from *in vivo* observations, and may not take into account consequences beyond the initial physiological state the model was devised for. In pregnant sows, these additional consequences comprise carry-over

effects into lactation, rebreeding and subsequent pregnancies, as well as the performance of the offspring of the sows.

In this review, we will briefly mention the major nutritional concepts applicable to sow feeding during gestation and the implications of a sub-optimal feeding program. The assumptions and calculations used by recent models will be discussed along with recent results from direct measurements of amino acid and energy requirements of sows of various ages during early and late gestation. The predicted requirements from models will be compared to our empirical results and combined with an assessment of additional consequences of nutrient intakes in pregnant sows. Finally, a revised feeding strategy for pregnant sows will be proposed with the goal of optimising the lifetime performance of sows and their offspring.

Concepts in gestation feeding

Until recently, most authorities recommended constant nutrient and energy intake during gestation (e.g., NRC, 1998). Feeding a constant level of amino acid and energy during gestation has always been metabolically incorrect because it assumed an equal distribution of nutrient demand throughout gestation. However, this simplistic approach was universally applied because it was easy to manage and the innate adaptability of the sow often enabled her to overcome this deficient feeding paradigm. However, sow productivity began to increase dramatically about 12 years ago (CCSI, 2007) and with this increased productivity the sow became less able to overcome the deficiencies of a constant feeding program during gestation. As a result, practical experience with feeding modern, more prolific sows showed that it was often beneficial to increase nutrient intake during late gestation (Boulot *et al*., 2008).

A constant nutrient intake during gestation is incorrect because the metabolic focus of the sow changes dramatically during gestation, beginning with the recovery of sow body tissue following weaning, to primarily maintenance during the mid gestation period, to rapid synthesis of foetal tissue in late gestation. For example, foetal weight, foetal protein content and mammary protein content increased 5-, 18- and 27-fold, respectively, in the last 45 d of gestation (McPherson *et al.*, 2004; Ji *et al.*, 2006). These dramatic increases in foetal weight and protein gain indicate that the requirement for amino acids and energy must be greater in late gestation compared to early gestation.

In addition, the sows' maternal growth rate during gestation decreases with age, becoming almost zero in adult animals, with the consequence that mature sows have lower amino acid and energy requirements during gestation than gilts that are still growing vigorously and sows producing the second and third litter. Ignoring these various dynamics by applying a single-phase feeding program for sows of all ages will lead to overfeeding during early gestation and for older sows, and underfeeding during late gestation and for younger sows. Intake of excess nutrients during early gestation creates excess deposition of lipid and protein which, with a constant feeding paradigm, are mobilised in the subsequent period of nutrient deficit, i.e., late pregnancy (Close *et al*., 1985). These processes have energetic efficiencies of 0.5 to 0.8 (Dourmad *et al*., 2008), meaning that failure to meet nutrient requirements is energetically and nutritionally wasteful, and thus also economically wasteful. In addition, underfeeding in late gestation leads to sows entering lactation in a severe catabolic state that may impair piglet viability at birth and reduce milk production of sows (Clowes *et al.*, 2003), and lead to poor re-breeding (Whittemore and Kyriazakis, 2006). Therefore, a constant feed allowance for pregnant sows is metabolically, nutritionally and economically incorrect and should be avoided.

Feeding sows the correct protein and energy intake at the correct time also results in lower nitrogen and CO_2 excretion (Möhn *et al*., 2003), and thus also has environmental implications. Finally, feeding sows in a manner that requires them to become catabolic in late gestation has animal welfare implications that must be considered.

Until recently, the nutrition of sows had been neglected, relative to their importance in pork production, with the result that although these concepts were well recognised, insufficient data existed to create new and more accurate feeding programs. New data and new models are now available and sow feeding programs can now be revised to improve productivity and economic return of the sow herd.

Models for pregnant sow requirements

The NRC (2012) model

The NRC (2012) model is based on the INRA-Porc model (Dourmad *et al.*, 2008), and uses a similar conceptual approach and structure. The NRC (2012) model is: *dynamic*, calculating pool sizes and their growth on a daily basis; *mechanistic*, being based on the utilisation of key nutrient pools; and *deterministic*, in that it calculates requirements for a given sow.

To estimate amino acid requirements, the NRC (2012) model identified six discreet sow body pools, for which weight and protein content are calculated on a day-by day basis. Thus, daily growth of each pool can be calculated for any user-defined period in pregnancy. For each of the pools, as well as for maintenance, its own specific amino acid pattern is applied so that retention of amino acids can be estimated as the sum of all pools. Standardised ileal digestible (SID) lysine requirements are calculated by adjustment for the efficiency of amino acid utilisation. This efficiency of 0.75 represents the minimum and inevitable amino acid losses of lysine based on serial slaughter experiments in growing pigs (Bikker *et al*., 1994; Möhn *et al*., 2000) and applied to sows, based on the observation that the serial slaughter experiments did not show an effect of body weight on the efficiency of lysine utilisation. In addition, an 'adjustment to account for inter-animal variability' (NRC, 2012) is applied in the calculation of SID lysine requirements. The efficiency of utilisation of other amino acids was estimated by dividing their retention (NRC, 2012) by the requirement predicted using the NRC (1998) model. Because the efficiency of utilisation was deemed too high for tryptophan and valine, i.e., greater than 0.75, and too low for isoleucine, additional adjustments were made for these amino acids. The contents of these amino acids in conceptus, mammary tissue and uterus pools differ substantially from maternal protein pools. Because this information was not available when the NRC (1998) model was developed, the NRC (1998) model may have incorrectly estimated the requirements for these amino acids. Nevertheless, the use of 0.75 as a yardstick for the efficiency of amino acid utilisation must be questioned because the efficiency of utilisation differs among amino acids (Heger *et al*. 2002). However, the severe lack of data for efficiency of utilisation of amino acids other than lysine made this approach necessary.

The protein pools are (see NRC, 2012, Figure 8.5) conceptus (i.e., uterus, placenta and fluids), mammary, foetal, and maternal protein pools. The foetal and mammary protein pools increase exponentially with a sharp increase in the third trimester of pregnancy, and contribute most to the increase in amino acid and energy requirements in late gestation. In comparison, uterus protein content contributes little to the total requirements. The pool of placenta and fluids shows an increase in protein content between day 40 and 70 of pregnancy but remains fairly constant before and after that period. Combined, the conceptus protein content changes little until mid pregnancy, but accelerates rapidly after day 70.

Maternal protein growth was separated by the NRC (2012) into energy-dependent plus time-dependent protein deposition. Maternal protein growth is, firstly, dependent on energy intake, and the assumption was made that the relationship between protein deposition and energy intake above maintenance was linear and constant across stages of gestation. The NRC (2012) model treats energy (feed) intake as a user input, and suggests as the default value an increase of feed allowance of 400 g/d after day 84 of pregnancy. As a consequence, energy-dependent maternal protein deposition increases after that time-point for the default input values. The NRC (2012) model for pregnant sows does not contain an upper limit for protein deposition (PD_{max}), as proposed by Williams *et al.* (1985). Although this omission may be regarded as a conceptual shortcoming, it is unlikely that PD_{max} can be achieved due to the restrictive feeding of pregnant sows. A range of experiments (Willis and Maxwell, 1984; Etienne, 1991; King and Brown, 1993; Dourmad *et al.*, 1996) failed to reach a plateau in protein deposition in both gilts and multiparous sows so that PD_{max} has little practical relevance for feeding pregnant sows.

The second component of maternal protein deposition is the time-dependent protein gain, which was defined as 'whole-body nitrogen retention that could not be associated with energy intake or reproductive tissues' (NRC, 2012). This means that the sum of energy-dependent protein deposition and conceptus protein deposition was less than the whole-body protein deposition observed in a range of experiments, and thus a correction factor had to be introduced. This approach has several problems. Firstly, the need to include a correction factor to account for the unexplained changes in protein deposition indicates that there are important changes in protein metabolism of the sow that we still do not understand; these must be investigated if we are to improve the accuracy and application of the NRC (2012) model. Secondly, N-balance, which was used in the key experiments of Dourmad *et al.* (2008) upon which much of the NRC (2012) model was based, overestimates maternal protein deposition (Clowes *et al*., 2003). In addition, because conceptus protein deposition was modeled based on data obtained in serial slaughter experiments, this leaves an unexplained amount of maternal protein deposition that may be an artifact of the scaling of protein deposition based on N-balance experiments. Finally, although the time-dependent protein deposition was derived mainly from experiments in gilts, it was applied to sows of all ages. It is possible that the time-dependent protein deposition in older sows may represent the re-gaining of body protein lost in the preceding lactation. However, body protein loss during lactation differs widely among sows due to genetics, number of piglets, feeding program, environment, etc., therefore a blanket correction is not appropriate. It would be much more preferable if the NRC (2012) model contained a separate term for protein deposition to regain lactation body tissue loss that could be scaled by the user to the actual loss observed.

The age of sows was accounted for in NRC (2012) by assuming that energy dependent maternal protein deposition decreases from parity 1 to 4, becoming zero in parity 5, when sows reach their adult age and effectively stop growing. This was achieved by including a scaling factor for the energy dependent protein deposition. The scaling factor decreases with increasing parity number but cannot become less than zero. The time-dependent protein deposition, however, remains at the same magnitude for all parities, which contradicts our data on protein deposition (Figure 1), where sows are clearly depositing protein at different rates in 2nd, 3rd and 4th parities.

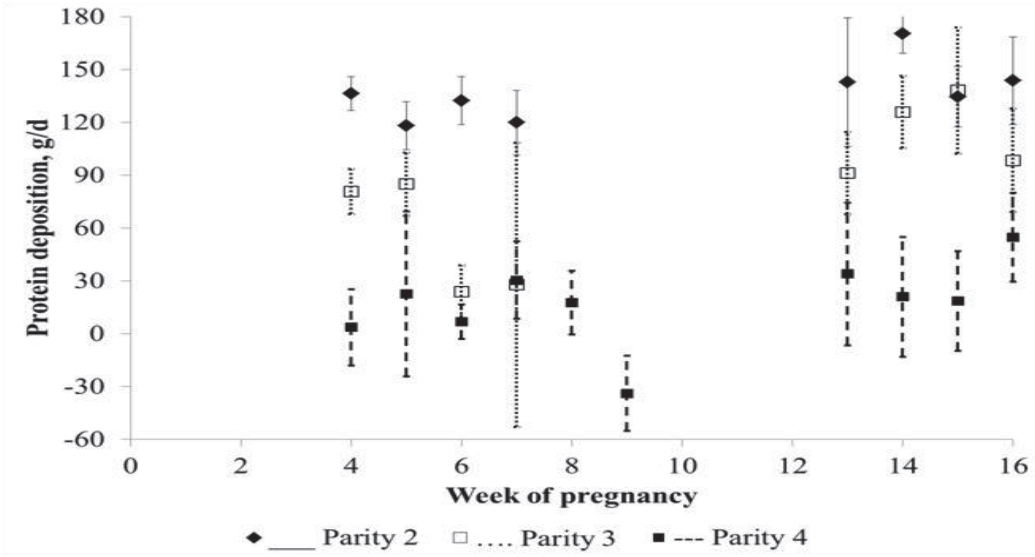


Figure 1. *Weekly protein deposition of pregnant sows at amino acid intake above the requirement.*

For energy utilisation, the NRC (2012) gave priority to maintenance functions and growth of conceptus over maternal growth. In maternal growth, protein deposition has priority over lipid deposition, so that lipid deposition becomes a residual that occurs only when all other demands for energy have been met. The standard energy maintenance requirement (418 kJ/kg$^{0.75}$ body weight) can be adjusted if sows spend more than 4 h/d standing, or are exposed to ambient temperature below the lower critical temperature. This adjustment is useful if sows are housed in groups and thus expend more energy in exercise; however, more data on energy expenditure in group-housed sows is necessary to accurately calculate energy requirement. Opposed to protein deposition, products of conceptus are regarded as a single pool for energy requirement. The energy need for conceptus growth is expressed as a function of gestational age and increases exponentially as sows approach parturition. However, the NRC (2012) program did not include the option of calculating the energy needed to achieve a certain performance level. Instead, energy intake was treated as a user-defined input.

The NRC (2012) does not state explicitly how mobilisation of body lipid is handled. Lipid mobilisation can occur in gilts and sows in late gestation when they are fed a constant amount of feed in pregnancy (Close *et al.*, 1985). If a limiting energy (feed) intake is entered for late pregnancy, the model calculates a slight reduction in maternal protein deposition, while foetal protein and lipid deposition are not affected by variation of energy intake. This means that conceptus growth is only impaired if sows are severely malnourished, which is agreement with Dourmad *et al* (1994). However, because litter size has increased dramatically in the last decade, the impact of energy intake on conceptus growth should be re-evaluated in sows with large litter size.

The Excel-based NRC (2012) model is versatile in its application. User-defined performance inputs are sow body weight at breeding, parity, expected litter size, piglet birth weight and gestation length. In addition, the user can define four feeding phases in pregnancy. Both the day in pregnancy when each phase starts as well as the feed amounts and feed energy and fibre content within each phase can be varied. The entered feed energy and fibre content, however, remain the same for all phases, unless a feeding program is entered for evaluation. Thus, the model can be used to predict nutrient requirements for most situations encountered in commercial pig production, or can be used to compare actual to predicted sow body weight and back fat gain to predicted values, if this option is chosen.

The GfE model

The recommendations of the German Society for Nutrition Physiology (GfE, 2008) for amino acid intake in pregnant sows are based on the same principles as the NRC (2012) model. Based on lysine, the daily amino acid retention is the sum of maintenance requirement, and lysine retention in products of

conception (foetuses, fluids, placenta), uterus, mammary gland and maternal protein gain. Similar to NRC (2012), the lysine retention is subjected to an efficiency factor to yield daily lysine requirements. The requirements for other amino acids are estimated by applying specific amino acid ratios, relative to lysine, to each of the components of requirement. Because the method of requirement calculation by GfE (2008) and NRC (2012) are analogous, recommendations are often similar. Both sets of recommendations agree in principle: amino acid requirements are greater in late pregnancy and decrease as sows approach maturity.

However, the recommendations sometimes differ in their absolute values. This occurs because the assumed amino acid composition of the pools and the efficiencies of deposition sometimes differ between GfE (2008) and NRC (2012). For example, GfE (2008) assumed that SID lysine occurred with an efficiency of 63% whereas NRC (2012) assumed an efficiency of 75%, but added 'an adjustment to account for between-animal variation' of 0.63, resulting in a final value for efficiency of 49% for NRC (2012). While GfE (2008) based its estimate of efficiency on the work by Dourmad and Etienne (2002), NRC (2012) based the efficiency of lysine utilisation on the efficiency for maintenance (75%), and reduced this estimate by 34.7%. The resulting efficiency of 49% 'agrees reasonably well with that of Everts and Dekker (1995), who estimated a lysine efficiency of 0.46' (NRC, 2012). In part, the reduced efficiency of amino acid utilisation in pregnant sows appears logical considering that NRC (2012) calculated a decrease of efficiency when the body weight in growing pigs increased. However, reports that pregnant sows can retain a greater portion of the ingested nitrogen (Rombauts, 1962; Close *et al*., 1985) appear to contradict the conclusion of NRC (2012). In addition, placental and foetal amino acid metabolism results in amino acid losses; foetal oxidation of non-dispensable amino acids may be between 4 and 20% of the infused tracer (Cetin, 2001). Finally, pregnancy anabolism (Rombauts, 1962), which would increase efficiency of utilisation, seems to have been ignored. It is possible that pregnancy anabolism is offset by the metabolism in conceptus tissues, however a good mechanistic model will account for all possible metabolism even if they offset each other. Because the efficiency values chosen contribute greatly to the amino acid requirements of pregnant sows, targeted research is needed to resolve the issue of efficiency of amino acid retention in pregnancy.

GfE (2008) does not treat energy requirements as a user input, contrary to NRC (2012), but calculates energy requirements using a factorial approach. The factors used in calculation of energy requirements are the same as used for amino acid requirements. This results in similar trends, i.e., both amino acid and energy requirements increase rapidly in the last quarter of pregnancy. The GfE (2008) recommendations also show that energy requirements increase from the first to third parity due to the combined effect of increasing sow weight and decreasing growth rate as sows age. The decrease in energy requirement from the third to fourth parity is caused by the assumed cessation of maternal growth so that energy requirements of mature sows are only driven by the requirements for maintenance plus pregnancy.

Experimentally determined requirements of pregnant sows

Very few studies of amino acid and energy requirements of pregnant sows have been performed in the last 10 years. Srichana (2006) used the nitrogen (N) balance technique to determine the lysine requirement of gilts in early, mid and late pregnancy. Zhong *et al.* (2009) reported the true ileal digestible lysine requirement in gilts up to day 84 of pregnancy as 0.69% in a diet containing 12.5 MJ metabolisable energy (ME). In contrast, Dourmad and Etienne (2002) did not report differences in lysine and threonine requirements in four consecutive nitrogen balance periods in pregnant sows. The experimental data from our research group include the requirements in early and late gestation for lysine (Samuel *et al.*, 2012), threonine (Levesque *et al.*, 2011a), isoleucine (Franco *et al.*, 2013) and tryptophan (Moehn *et al.*, 2012a). We also simultaneously measured energy requirement, protein synthesis and breakdown, and metabolomic profiles during all of these experiments.

Conduct of indicator amino acid oxidation studies

The conventional approach to measuring nutrient requirements in sows was to feed a group of sows a control and one or two dietary treatments, during one or two reproductive cycles, and measure numerous outputs such as the number of piglets born and weaned, rebreeding rates, culling rates, and weights of sows (e.g., Cooper *et al*., 2003). This approach allows only one treatment per animal, requires a very large number of sows, a long period of time and a great deal of money, and reveals almost nothing about the metabolism of the sows and seldom results in a definitive answer. In addition, these methods do not to allow an estimate of the effects of important factors that significantly influence the sow's requirement, such as litter size and piglet weights, lactation output, age, body weight, body composition, and carry-over effects between cycles. Obviously, if we desired to make any rapid and significant progress in sow nutrition, new methods would need to be developed and applied. We therefore chose to apply the indicator amino acid oxidation technique (Ball and Bayley, 1984; Pencharz and Ball, 2003) combined with indirect calorimetry (Samuel, 2010) to examine requirements.

Contrary to conventional methods that provide only an average estimate of requirement for the entire group of animals, the indicator amino acid oxidation technique (IAAO) enables us to determine the requirements of individual pigs and thus the mean and variability in requirements among individuals (Bertolo *et al.*, 2005; Moehn *et al.*, 2008). Using the IAAO also offered the opportunity to determine phenylalanine kinetics, for the calculation of rates of protein synthesis and breakdown, during pregnancy. Because sows need to be confined in respiration chambers to determine the rate of labeled CO_2 production, this method also allows the simultaneous determination of energy expenditure, using indirect calorimetry, to calculate energy requirements.

The amino acid requirements of pregnant sows were determined in early gestation (day 23 to 60) and late gestation (day 85 to 113). The same sows were used to determine the requirements in early and late pregnancy within test amino acids, and some sows were used in multiple parities. Sows were fitted with venous catheters and subcutaneous injection ports (Swindle *et al.*, 2005; Levesque *et al.*, 2011a) for frequent blood sampling to determine plasma AA concentrations and phenylalanine (Phe) kinetics after oral application of the indicator amino acid. In each experiment, six to eight sows received each of six test diets in random order in both early and late gestation. Feed allowance was kept constant throughout gestation for comparability to past research. Amino acid requirements were determined using the indicator amino acid oxidation technique (Pencharz and Ball, 2003) simultaneously with indirect calorimetry (Samuel, 2010) to measure energy expenditure.

The diets were based on corn, cornstarch, and sugar. Corn was included to meet the lowest intake of test amino acid in each basal diet. Thus, the inclusion of corn differed among experiments. The amino acid availability in corn was determined in a similar group of sows (Levesque *et al.*, 2011b). The test amino acid was provided at 20% to 120% of the NRC (1998) requirement in the early gestation period, and 60% to 180% in late pregnancy. This range of test amino acids was chosen after the experiments for lysine (Samuel *et al.*, 2012) and threonine (Levesque *et al.*, 2011a) showed greater differences between early and late pregnancy than expected. Other indispensable amino acids were provided in an ideal amino acid pattern (NRC, 1998) relative to the maximum inclusion (i.e., 120 or 180%) of the test amino acid.

The tracer amino acid, L-[1-^{13}C]phenylalanine, was selected because of its tightly controlled pool-size (Flaim *et al.*, 1982) and quick turnover rate (Neale and Waterlow, 1974). Thus, phenylalanine as a tracer responds rapidly to changing test amino acid intakes and short adaptation periods and infusion protocols (Zello *et al.*, 1993, Elango *et al.*, 2009). In fact, the full response of IAAO was observed only one day after changing the protein intake of sows (Moehn *et al.*, 2004). The sows received oral doses of 1 or 2 mg/(kg of BW·h) of L-[1-^{13}C] phenylalanine (99% enrichment; Sigma Aldrich, Mississauga, ON, Canada) for 4 h divided into eight, 0.5-h feedings. A priming dose equal to 1.75 times the hourly dose was given along with the first 0.5-h dose. The sows consumed all the feed provided before administration of the next 0.5-h feed allowance.

Two independent airtight respiration chambers were constructed to allow rapid air exchange in the chambers and placed in a temperature-controlled room and fitted with a drop tube to feed sows in the chambers. Air was drawn through the chambers at a rate of 240 L/min to maintain CO_2 concentration below 1.0% and to create a slight negative pressure to prevent the loss of labelled CO_2. Before entering the respiration chambers, the subcutaneous vascular port that had been surgically installed in the sow was accessed and a 1.5-m extension set was then externalised from the chamber to enable blood sampling during collection of expired air. Sows were placed in the respiration chambers at least 30 min before the beginning of the collection of expired air to allow the air in the chamber to equilibrate with the ventilating air stream. Expired air was collected in 30-min intervals into 11-mL 1 *N* NaOH solution. Background $^{13}CO_2$ enrichment was measured for three 30-min periods before administration of the isotopic tracer, followed by 8 30-min samples after administration of the isotopic tracer. Expired air and plasma were analysed for $^{13}CO_2$ and ^{13}C-Phe enrichment, respectively. Plateaus in $^{13}CO_2$ production were determined to define isotopic steady state. Requirements were determined as the breakpoint of $^{13}CO_2$ production during isotopic steady state using 2-phase linear models.

Empirical results for amino acid requirements of pregnant sows

Srichana (2006) found no difference in the lysine requirement of 15.0 g/d between early and mid gestation, but reported an increased requirement of 20.0 g/d in late gestation (Table 2). Samuel *et al.* (2010) showed that the total lysine requirement of second parity sows was 13.1 g/d and 18.7 g/d in early and late gestation, respectively. For third parity sows, the dietary total lysine requirement was 8.2 g/d and 13.0 g/d for early and late gestation, respectively (Samuel *et al.*, 2010). Levesque *et al.* (2011a) found that second parity sows required 7.2 g/d total threonine in early gestation (day 35 to 53) and 13.6 g/d threonine in late gestation (day 92 to 110), based on indicator amino acid oxidation. In multiparous sows (Levesque *et al.*, 2011a), the total threonine requirement more than doubled from 5.0 g/d in early gestation to 12.3 g/d in the

last third of gestation. The tryptophan requirement of second parity sows increased from 1.7 g/d to 2.6 g/d from early to late gestation. The isoleucine requirement of fourth parity sows increased from 3.6 g/d to 9.6 g/d from early to late gestation.

These requirement values need to be considered with respect to sow performance as affected by stage of gestation and age of sows. The body weight of sows increased from early to late gestation (Table 1), regardless of parity, and increased from parity 2 to 4. Litter size and weight increased marginally over three parities. Protein deposition was greater in late than early gestation, across all parities, which is in accord with the foetal growth that occurs predominantly in late gestation. Thus, foetal growth drives amino acid requirements in late gestation, whereas maintenance and maternal growth are the principal factors affecting amino acid requirements in early gestation. Because maternal growth markedly decreases in the fourth compared to second and third parity, older (adult) sows will require amino acids in early gestation predominantly for maintenance. In fact, the isoleucine requirement for maintenance in early gestation (2.2 g/d based on 35 mg/kg$^{0.75}$ body weight; Moehn *et al.*, 2012b) was only 1.4 g/d less than the measured early gestation requirement of 3.6 g/d (Table 2). The largely similar foetal growth over three parities coupled with reduced maternal growth can explain the greater difference between early and late gestation requirements in younger versus older sows.

Table 1. *Sow performance during amino acid requirement studies in early (EG) and late gestation (LG) over three parities (adapted from Moehn* et al.*, 2011).*

Gestation and parity	AA studied	BW (kg)	Maternal gain (kg)	ME intake (MJ/d)	Protein retention (g/d)	Energy retention (MJ/d)	Litter size	Litter weight (kg)
EG 2	Lys	177	44	34.2	32	3.0	13.8	19.5
LG 2	Thr Trp	215		34.5	126	-0.7		
EG 3	Lys	205	40	36.1	38	1.2	13.6	20.1
LG 3	Thr	244		36.0	119	-0.9		
EG 4	Thr	240	25	35.6	4	1.5	15.8	22.1
LG 4	Ile	266		35.5	64	-1.3		

Table 2. *Total lysine[1], threonine[2], tryptophan[3] and isoleucine[4] requirements of gestating sows in their first, second and third and fourth parities.*

		1st parity	2nd parity	3rd parity	4th parity
Lysine	Early gestation	15.0	13.1	8.1	n/a
	Late gestation	20.0	18.4	13.0	n/a
Threonine	Early gestation	n/a	7.0	5.0	n/a
	Late gestation	n/a	13.6	12.3	n/a
Tryptophan	Early gestation	n/a	1.7	n/a	n/a
	Late gestation	n/a	2.6	n/a	n/a
Isoleucine	Early gestation	n/a	n/a	n/a	3.6
	Late gestation	n/a	n/a	n/a	9.7

[1]Srichana (2006) for 1st parity; Samuel *et al.* (2010) for 2nd and 3rd parity; [2]Levesque *et al.* (2011a); [3]Moehn *et al.* (2012a); [4]Moehn *et al.* (2012b); n/a - not available.

A second aspect to consider is the ileal digestibility of amino acids in sows. Stein *et al*. (2001) showed that the SID values were greater for sows than for growing-finishing pigs for some feedstuffs (e.g., corn, soybean meal) but not others (e.g., wheat and most amino acids in barley). Conversely, Levesque *et al*. (2011b) found greater metabolic availability of threonine in corn and barley in sows compared to growing pigs. This confirms the conclusion by Stein *et al*. (2001) that it is not possible to extrapolate data from growing pigs to sows, from one feed ingredient to another, or even between batches of the same ingredient. Accurate diet formulation for pregnant sows is therefore dependent on more data for amino acid digestibility, and more knowledge of the diet and animal factors that affect amino acid digestibility in sows.

Energy expenditure and protein turnover

Heat production was calculated using indirect calorimetry simultaneous with IAAO. The 5.5 h measurement of heat production was extrapolated to 24 h, and adjusted for the greater energy expenditure during frequent feeding of 17% compared to full 24 h measurements (Samuel, 2010). Heat production (Table 3) increased from early to late pregnancy and from parity two to four due to the increase in sow body weight, so that energy retention decreased because of the constant feed allowance for individual sows.

As Table 3 shows, body protein breakdown was more affected by stage of pregnancy and parity of sows than protein synthesis, and should, thus, be regarded as the driving force for protein deposition. Special emphasis should be put on the interactive effect of stage of pregnancy and parity on body protein breakdown: while the younger, growing sows reduced body protein breakdown in late pregnancy, older sows increased it. As a consequence, maternal protein retention actually can become negative in older sows. This is in agreement with Close *et al.* (1985) who showed that increasing portions of protein deposition occur in conceptus when gilts approached parturition. This is in addition to the observation that protein retention decreased as sows aged while it increased from early to late pregnancy. Lipid retention was lower and negative in late pregnancy in all three parities. Therefore, sows were clearly in a catabolic state energetically and may also become catabolic with regards to protein stores when entering lactation, which would exacerbate possible body tissue loss in lactation.

Additional energy in late pregnancy is needed to cover the additional maintenance requirement of heavier sows, and to support energy retention similar to early pregnancy. In our experiments, the sows gained 38 kg, 39 kg and 26 kg from early to late pregnancy in the second, third and fourth parity, respectively (Table 1). This leads to an increase of the energetic maintenance requirement of 3.5, 3.5 and 2.2 MJ/d in the second, third and fourth parity, respectively. In addition, the energy retention of sows decreased from early to late pregnancy by 1.0 to 1.5 MJ/d. This is equivalent to an additional ME need of 1.6 to 2.2 MJ/d, assuming efficiency of energy utilisation to be 0.7 (ARC, 1981). To meet this additional energy need, sows would need an additional energy intake of 5.6, 5.7 and 3.8 MJ/d in the second, third and fourth parity, respectively. This additional energy need can be covered by 400, 400 and 275 g/d of a corn-soybean meal diet in the second, third and fourth parity, respectively. Nutritionists should consider slightly exceeding this minimum increase in feed allowance if they wish to assure positive energy balance throughout pregnancy.

Table 3. *Parameters of energy metabolism of sows in early and late pregnancy over three parities.*

	Parity 2: Stage of pregnancy[1]		Parity 3: Stage of pregnancy[1]		Parity 4: Stage of pregnancy[1]		SEM	Significance		
	Early n=93	Late n=89	Early n=37	Late n=39	Early n=62	Late n=61		Stage	Parity	Parity x stage
ME intake (MJ/d)	34.2	34.5	36.1	36.0	35.6	35.5	0.11	0.55	0.001	
Heat production (MJ/d)	30.7	32.6	33.2	34.6	35.9	37.0	0.22	0.001	0.001	
Energy retention (MJ/d)	3.44	1.97	2.88	1.35	-0.36	-1.48	0.216	0.001	0.001	
Respiratory quotient	1.00	0.98	1.00	0.98	1.05	1.00	0.004	0.001	0.002	
Protein synthesis (g/d)	50.0	50.6	51.6	44.5	48.3	50.4	0.53	0.21	0.29	0.012
Body protein breakdown (g/d)	45.5	43.4	48.9	41.8	47.8	51.3	0.59	0.14	0.003	0.006
Protein retention[2] (g/d)	104.1	146.9	76.2	84.4	-8.7	19.5	4.09	0.001	0.001	0.088
Lipid retention[3] (g/d)	25.0	-38.5	26.9	-16.8	-3.9	-49.1	5.33	0.001	0.073	

[1]Early pregnancy, day 23-61; late pregnancy, day 83-113; [2]Based on phenylalanine kinetics: phenylalanine intake – phenylalanine oxidation; Phenylalanine digestibility assumed as 90%; Phenylalanine content of body protein assumed as 4.2 g/16 g N (NRC, 2012); [3]Calculated as energy retention–protein energy retention; energy content of body protein and lipid assumed as 23.8 kJ/g and 39.6 kJ/g (Schiemann *et al.*, 1971).

Comparison of determined requirement values to model estimates

These comparisons were based on our determined requirements for each amino acid, stage of pregnancy and parity studied. The requirements based on NRC (2012) were calculated by entering the actual feed intake and sow data from our experiments into the model, while the GfE (2008)

recommendations were chosen using the appropriate parity and stage of gestation. The requirement values determined by our research group were correlated to the values calculated using the NRC (2012) program with r=0.85 (17 observations, P=0.001; Figure 2) and to the GfE (2008) with r=0.82, while NRC (2012) and GfE (2008) values were correlated with r=0.94. Thus, there is a large degree of agreement between empirically determined and modelled amino acid requirement values. The general agreement of the SID amino acid requirements according to NRC (2012) and GfE (2008) is shown in Table 4. However, this table also shows that requirements show considerable differences in some cases.

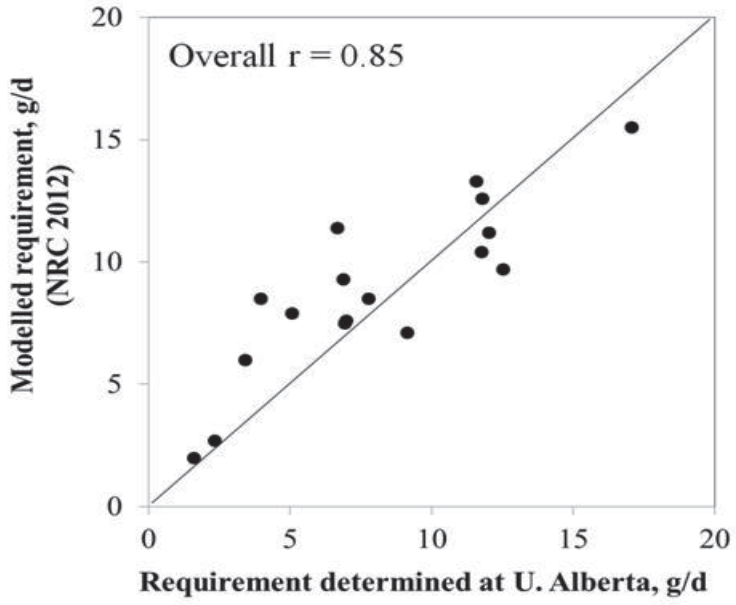


Figure 2. *Comparison of determined requirements to modelled estimates (NRC 2012).*

Another common feature among the determined and modelled amino acid requirements was that the requirements for late gestation were always significantly greater (P<0.001) than for early gestation. However, the degree of requirement increase differed among sets of recommendations. The greatest differences in amino acid requirements were found in the empirical data by Moehn *et al.* (2011; 77% increase in late pregnancy over early pregnancy values), followed by the data modelled by GfE (2008, 61% increase), while the NRC (2012) recommendations showed the least difference at 22% increase. A possible reason for the differences in requirement increase is the time-dependent protein deposition that was included in the early gestation requirement in the NRC (2012) model as a factor to adjust the output to more closely match predicted protein deposition to N-retention measured using the N-balance technique. Inclusion of the time-dependent protein deposition in turn increased the lysine requirements (NRC, 2012) in early pregnancy. This component of protein deposition is not a feature of our empirical data or the GfE (2008) model, which may explain our larger differences between early and late pregnancy. Our data (n=192) did not show any impact of day of gestation on phenylalanine retention (protein synthesis) between day 23 and 60 of pregnancy. Figure 1 shows that the mean weekly protein deposition determined in our experiments did not change significantly (P>0.4) between weeks four and nine of pregnancy. The absence of the 'time-dependent protein deposition' in our data has the consequence that the amino acid requirements determined in our experiments during early gestation were lower than predicted by NRC (2012). However, our experiments were not designed to study protein retention in the first 30 d after breeding so that the existence and extent of the time-dependent protein deposition during this period cannot be fully verified. Because of its large impact on amino acid requirements, the time course of protein deposition needs to be studied directly after weaning in gilts as well as in sows.

In early pregnancy, our measured requirement values were lower (P=0.04) than those proposed by NRC (2012), while those modelled by GfE (2008) were intermediate (Table 4). In late pregnancy, our empirical values were similar to those modelled by GfE (2008), and both these sets of recommendations were greater (P<0.1) than the proposed values by NRC (2012). This is despite NRC (2012) and GfE (2008) basing their amino acid requirements on increased energy intake in late pregnancy, while our experiments were conducted with a constant feed allowance. It is possible that increased energy intake by sows will increase daily amino acid requirements (Pettigrew and Yang, 1997), as it does in growing pigs (NRC, 2012).

However, the lack of data on the relationship between energy intake and amino acid requirements means that this needs to be determined in sows.

To elicit reasons for the differences among the recommendations, we need to look at amino acids individually. Table 4 shows that our measured requirements and the requirements modelled using the NRC (2012) program agree quite well for lysine and tryptophan, but less so for threonine and isoleucine. In addition, the modelled (NRC, 2012) and determined requirement values for lysine and tryptophan were similar to the recommendations by GfE (2008) in both early and late gestation (Table 4). For threonine, the late gestation values were similar for all three recommendations, while in early gestation our empirical values and the recommendations of GfE (2008) were lower ($P<0.1$) than the recommendations by NRC (2012). Large differences were found in the isoleucine requirement of adult sows, ranging from 1.8 to 5.0 g/d in early and from 4.4 to 9.7 g/d in late gestation. This indicates that the agreement among requirement values is close for more frequently studied amino acids or when sow growth is a large component of requirements. For the little-studied amino acids in adult sows, however, large discrepancies between requirement values are evident, indicating that more research is necessary on these amino acids.

Table 4. *Comparison of modelled and determined SID amino acid requirements of gestating sows in early and late pregnancy in several parities.*

			Source of requirements		
Amino Acid	Parity	Stage of pregnancy	NRC (2012)[1]	GfE (2008)[2]	Ball[3]
Lysine	2	Early	11.3	9.4	11.8
		Late	14.0	14.6	17.1
	3	Early	9.9	8.2	6.7
		Late	11.7	13.4	11.6
Threonine	2	Early	8.0	6.6	6.6
		Late	9.5	9.6	9.7
	3	Early	7.6	6.2	4.0
		Late	9.6	9.1	12.0
	4	Early	6.7	3.9	7.0
		Late	8.6	6.8	11.8
Tryptophan	2	Early	2.0	1.8	1.6
		Late	2.7	2.8	2.3
Isoleucine	4	Early	5.0	1.8	3.4
		Late	6.0	4.4	9.1

[1]Calculated using sow and litter performance, feed intake and days in pregnancy as in each experiment; [2]Mean values given by GfE for parity and stage of pregnancy; [3]Standardized ileal digestibility calculated assuming NRC (2012) digestibility for amino acids in corn and 100% digestibility for free amino acids.

There is good agreement between the NRC (2012) and GfE (2008) recommendations and our empirical data for the necessary increase in feed (energy) allowance in late gestation. The default input for the NRC (2012) model suggests that the feed intake be increased by 400 g/d after day 90, while our data indicate increases of 600 g/d for gilts and 400 g/d for older sows. The suggested increases in energy intake by GfE (2008) are equivalent of 570 g/d and 430 g/d of a corn-soybean meal diet for gilts and fourth parity sows, respectively.

In conclusion, there is general agreement between modelled and empirically determined energy and amino acid requirements of sows, however there are important questions that need further investigation. Despite this, the current recommendations represent requirement values that reflect the changing physiology of pregnant sows. To meet these requirements, parity-segregated phase feeding is the ideal nutritional regimen for pregnant sows.

Beyond pregnancy: carry over effects of pregnancy feeding regimen

Although the recommendations discussed above are significant improvements over past feeding programs for pregnant sows, gestation feeding regimens have many consequences for lactation and offspring performance that must also be considered. Feed allowance in pregnancy may affect lactation feed intake of sows and may thus have impacts on milk production and piglet growth and rebreeding (Dourmad *et al.*, 1994). In addition, litter size, piglet birth weight (Coffey *et al.*, 1994) and its variability (Campos *et*

al., 2012) and growth performance of the sows' offspring may also be affected by pregnant sow nutrition (Metges *et al*., 2012).

Although current recommendations (NRC, 2012; GfE, 2008) indicate that offering a constant feed energy allowance is not appropriate for pregnant sows, this constant feeding regimen has been used in a large body of research into effects of energy intake on sows, with the consequence that much of this research is no longer relevant. In addition, research that simply manipulated feed intake during gestation must be interpreted very carefully since we know that the ideal amino acid ratios and optimum protein: energy ratios are different between early and late gestation.

Concentrating on extremes of energy intake, Dourmad *et al.* (1994) concluded that constant feed intake providing less than 22 MJ digestible energy (DE)/d will impair sow longevity through reduced reproductive performance. Exceeding a daily energy intake of 36 MJ DE reduced the number of sows that completed four parities (Castaing *et al*., 1983), mainly due to culling for locomotion problems. However, Hoppe *et al*. (1990) did not report a substantial reduction in number of sows culled when receiving an average of 37.6 MJ DE/d. Therefore, it appears that there is a fairly narrow range of energy intake during pregnancy that supports optimal sow and offspring performance and that there may be considerable benefits to lifetime sow performance to better defining this range.

Increasing the feed allowance in pregnancy leads to a decrease in daily lactation feed intake (Coffey *et al*., 1994; Eissen *et al*., 2000). Eissen *et al*. (2000) suggested that the reduction in lactation feed intake was a result of body fat and protein tissue, hormonal status of the sow and metabolic demands due to the level of milk production. The reduction in lactation feed intake observed in several studies (Eissen *et al*., 2000) was approximately equivalent to the increase in daily gestation feed allowance, and was similar for gilts and sows. It appears that there is a certain tolerance to feed intake levels because Mahan (1998) observed no adverse effects over five parities when the feed allowance was increased from 1.82 kg/d to 1.95 kg/d. The impact of gestation feeding on lactation feed intake also depends on the body protein to lipid ratio. Sinclair *et al*. (2001) observed that increased body fatness did not impact lactation feed intake if body protein mass was also increased. It therefore appears that the appropriate ratio of dietary energy to protein is as important as the level of feed intake on sow lactation performance. This also implies that the commonly accepted idea, i.e., that additional energy intake in late gestation has negative consequences on lactation feed intake, may be due to incorrect intake of amino acids in these experiments during late gestation. This also demonstrates that feeding a single diet in pregnancy supplies incorrect ratios among the amino acids combined with incorrect protein:energy ratio in late pregnancy.

In some studies, extra feed was given to sows at specific periods during gestation to determine if piglet quality, e.g. piglet birth weight and its variability, could be improved. The timing of increased feed allowance was chosen to either facilitate re-growth of body tissue lost in a preceding lactation or targeted specific stages of foetal development. In early and mid pregnancy, most studies aimed to assess nutritional influences on the differentiation of muscle fibres into oxidative, oxidative-glycolytic, or glycolytic fibres, which occurs up to day 20 of pregnancy (Bee, 2004), or the development of secondary muscle fibres, which occurs from day 54–90 (Lawlor *et al*., 2007). Nutritional manipulation in late pregnancy was aimed at improving the final stages of foetal growth to increase piglet and litter weight at birth.

For the very early stage of pregnancy, reduced feed allowance was suggested because some studies (Jindal *et al*., 1996; De *et al*., 2009) showed reduced embryonic survival in gilts when feed allowances were increased. In multiparous sows, this effect was not observed (Toplis and Ginesi, 1983; Varley and Prime, 1993). Also, in recent studies with hyperprolific gilts (Quesnel *et al*., 2010; Hoving *et al*., 2012), increasing the daily feed from 2.0 kg/d to 4.0 kg/d during early pregnancy had no effect on embryo survival. It therefore appears that a reduction in feed allowance during the first few days after insemination is not warranted for sows or gilts of modern genotypes.

Manipulating feed allowance in mid pregnancy often aims to improve muscle fibre development to enable piglets to achieve better growth in the growing-finishing period. Bee (2004) studied the effect of feeding 2.8 or 4.0 kg of a control diet (10.7 MJ DE/kg) or 2.8 kg of a reduced energy and protein diet (6.6 MJ DE/kg, 7.8% crude protein) during the first 50 d of pregnancy in multiparous sows. Bee (2004) found reduced growth rate and gain:feed and greater body fatness in offspring of sows fed 4.0 kg/d compared to offspring of sows fed the low energy intake. The number of muscle fibres was not affected by dietary treatments. Lawlor *et al*. (2007) compared offering approximately 2.3 kg/d (30 MJ DE/d) throughout the pregnancy of multiparous sows to offering 4.6 kg/d of the same diet (60 MJ DE/d) on days 25-50, 50-85 and 25-85. Increasing feed allowance had no effect on daily gain and feed efficiency of offspring but decreased carcass back fat. Offering the higher feed allowance (4.6 kg/d) from day 25-85 decreased lactation feed intake. Nissen *et al*. (2003) found that *ad libitum* feeding the same diet from day 25-50 or from day 25-70 had no beneficial effect compared to a control-feeding regimen (2.0 kg/d), and may

actually impair post-natal muscle growth. Heyer *et al*. (2004) fed 2.3 kg of a standard gestation diet or 35%, 70% or 100% more of the same diet from day 25-85. The increased feed allowance increased litter size in sows but not gilts, and did not affect the variability in piglet birth weight. Similar to Nissen *et al*. (2003) and Bee (2004), Heyer *et al*. (2004) found that the growth performance of offspring decreased with increasing feed intake. Cerisuelu *et al*. (2009) offered two levels of the same gestation diet to gilts and multiparous sows between day 45 and 85 of pregnancy, and found no effect of feed intake on litter size or piglet weight at farrowing and on day 18 of lactation. Weight gain was greater during the nursery but not growing finishing phase for offspring of sows given the higher feed allowance. The number of muscle fibres was reduced for the high feeding level but growth performance was not significantly affected. Common to all these experiments was the simple manipulation of feed intake, so that it is not possible to determine whether any observed effects were caused by protein or energy intake. The consensus of these experiments is that increasing the feed allowance between day 25 and 85 over a control amount has little beneficial effect on sow and offspring performance or muscle characteristics in offspring, and can therefore not be recommended for commercial pig production. This conclusion is in agreement with our results and both the NRC (2012) and GfE (2008) models, which indicate that during this mid-pregnancy period nutrient intake greater than that required for maintenance is unnecessary.

Increasing feed allowances in late pregnancy, as opposed to early and mid gestation, is warranted given the increased nutrient requirements of conceptus in the last trimester. However, experimental evidence for beneficial effects of increased feed intake is conflicting and interpretation needs to consider that we have only recently recognised that the amino acid ratios and the protein:energy ratios need to be different during late gestation and therefore much of the research used incorrect amino acid intakes. Cromwell *et al.* (1989) reported the results of a collaborative study from several research stations where the regular ration of 1.82 kg/d (summer) or 2.27 kg/d (winter) was supplemented with 1.36 kg/d of the same feed from day 90 onwards. Cromwell *et al*. (1989) found the litter size and piglet weight at birth and weaning in multiparous sows were greater in the supplemented group. Lactation feed intake and wean-to-oestrus interval were not affected by the supplemental feed. Feeding 0.8 kg/d more during the last 2 weeks of pregnancy, Quiniou (2007) found no effect on litter size or piglet weight at birth but reported that supplemented sows were heavier at farrowing and consumed less feed in lactation than non-supplemented sows. Increasing the feed intake from 2.3 kg/d to 3.9 kg/d of a diet containing 12.6 MJ DE/kg and 5.6 g/kg lysine after day 100 of pregnancy, Miller *et al*. (2000) found no effect on piglet birth weight, litter gain on the sow, sow lactation feed intake or wean-to oestrus interval in first to third parity sows. While sows on the lower feed allowance lost backfat in late pregnancy, sows on the higher feeding level maintained their backfat thickness (Miller *et al*., 2000). Shelton *et al.* (2009) increased the feed allowance of corn-soybean meal diets containing 13.6 MJ ME/kg and 6.6 g/kg total lysine by 0.9 kg/d after day 90 of pregnancy, and reported interactions of feeding level with the age of sows. In gilts, the increase in feed allowance increased the birth weight of piglets, their weight gain during lactation, piglet weaning weight and conception rate after weaning, whereas in sows in their second and greater parity it did not. Conversely, increased gestation feed intake reduced lactation feed intake in gilts but not in sows. When the same feeding regimen was applied in the subsequent parity, Shelton *et al*. (2009) found greater piglet birth weights in second parity sows but not older sows, while piglet weight gain and piglet weaning weight was increased in all sows when given additional feed in late gestation during the first reproductive cycle on test. This indicates that an increased feed allowance in late pregnancy may have longer lasting positive effects. Soto *et al*. (2011) also reported differential responses to additional feed in late pregnancy between gilts and sows: feeding 0.91 kg/d or 1.82 kg/d more of a diet containing 5.5 g/kg total lysine in the last 2 weeks of pregnancy had no effect on litter size or weight in sows. In gilts, however, feeding 1.82 kg more increased litter weight and piglet weight at birth over the control feed intake of 1.82 kg/d throughout pregnancy, while increasing the feed allowance by 0.91 kg/d only had no effect.

In the first instance, it appears that gilts are more responsive to increased feed amounts in late pregnancy than older sows. However, the impact of amino acid (lysine) and energy intake need consideration to elicit the true impact of increasing feed allowance in late pregnancy. The lysine intake provided by the control groups for sows (Cromwell *et al*., 1989; Miller *et al*., 2000) and for gilts (Shelton *et al*., 2009; Soto *et al*., 2011) was below the late gestation requirements shown in Table 2. Increasing the feed allowance met or exceeded the amino acid requirements but also resulted in a less than optimal amino acid: energy ratio (Cromwell *et al*., 1989; Miller *et al*., 2000; Soto *et al*., 2011) - at the highest feed intake. This did not cause improved piglet characteristics in the experiment by Miller *et al*. (2000), possibly because the total additional feed was less than 20 kg total compared to over 25 kg (Cromwell *et al*., 1989; Soto *et al*., 2011). A similar relationship applies when comparing the results in gilts by Shelton *et al*. (2009) and Soto *et al*. (2011): increasing the feed allowance by 0.9 kg/d still resulted in a limiting lysine intake of 16.4 g/d and 15.0 g/d, respectively, in late gestation. However, the total additional feed given by Shelton *et al*. (2009) was over 25 kg and resulted in improved litter characteristics while Soto *et al*. (2011) offered less than 20 kg extra feed and did not see a response. Conversely, the feed allowance given by

Shelton *et al*. (2009) to sows of parity 2 and greater provided 17.0 g/d lysine, which was already close to or in excess of the requirements shown in Table 2, so that further increase in lysine intake (to 20.0 g/d) failed to elicit a positive response in the litters. This indicates that a minimum increase of total feed eaten in late pregnancy may be needed to yield positive effects on litters, be it as moderate feed increase over a longer period of time or a larger increase over a shorter period. However, this positive effect only becomes evident if the amino acid limitation in late pregnancy is either eliminated or drastically curbed and energy intake is not excessive. This reiterates the importance of using the correct amino acid balance in late gestation.

Manipulation of the protein to energy ratio in pregnant sow diets during late gestation is probably necessary to yield beneficial results. Hoving *et al*. (2011) fed, from day 3 to 32, either 2.5 kg/d or 3.25 kg/d of a control diet or 2.5 kg/d of a diet that supplied 30% more ileal digestible amino acids than the control diet. The 30% increase in feed allowance during the first quarter of pregnancy improved sow body weight recovery after the preceding lactation and increased litter size. Conversely, increasing only the protein intake had no effect on sow recovery or the subsequent litter size (12.7 to 14.0 piglets born; Hoving *et al*., 2011) and weight. The lack of response to increased amino acid intake indicates that the amino acid content in the control diet was at least adequate, and increased energy intake was necessary to utilise the additional amino acid intake. Offering 2.5 kg/d of diets of similar energy content containing 12.5, 10.0 or 7.0% crude protein from day 0 to 75 (lysine intakes of 13.8, 11.0 and 8.3 g per day, respectively) showed similar new-born and weanling litter size, body weight of piglets, and the interval of postpartum oestrus from first to sixth parity (Abe *et al*., 2002), but reducing the feed allowance of the 7% crude protein diet to 2.0 kg/d reduced the birth weight of piglets (Abe *et al*., 2002). This indicates that the protein to energy ratio in the 7.0% crude protein diet was adequate but reducing the feed allowance of this diet below 2.5 kg/d impaired foetal growth of piglets. This indicates that a minimum protein intake is necessary to maintain foetal development in early to mid pregnancy, as observed by several other studies (Campos *et al*., 2012). Sinclair *et al.* (2001) studied gilts in a later phase of pregnancy, i.e., from day 42 onwards, supplementing the basal diet with either 1.64 kg/d corn starch-soybean oil mix or 1.76 kg additional feed. The treatments had no effect on litter size or performance, probably because the amino acid balance was incorrect for late gestation. Additional energy intake supported less body weight gain in pregnancy but decreased lactation feed intake compared to additional feed (protein plus energy) intake. Both experiments indicate that the correct balance of energy and amino acids is needed to support optimal sow and litter performance.

Kusina *et al.* (1999) showed that increasing dietary protein from 5.5% to 9.8% and 15.6% at constant energy intake during gestation increased sow lactation feed intake and milk yield and piglet weight gain. This is further illustrated by the study of Metges *et al*. (2012) who offered the same energy allowance with diets that contained 6.5%, 12.0% or 32% crude protein. The control diet (12%) supported greater sow body weight gain in pregnancy and greater piglet and litter birth weights than both the excess and deficient protein diets. The authors concluded that protein excess caused drastically reduced body lipid while the low-protein diet reduced sow and piglet performance by imposing amino acid deficits. In fourth and fifth parity sows, Sabioni *et al*. (2007) found no effect of feeding diets containing 10%, 13.5% or 17% crude protein at constant rates throughout pregnancy on sow and litter performance but observed the best energetic efficiency of sows at the intermediate protein level; an improper amino acid balance for late gestation may have affected their results. Similarly, Mahan (1998) found no effect of increasing dietary protein contents from 13% to 16% for five parities on sow and litter performance. This indicates that optimal sow and litter performance can be achieved by feeding sows to requirement in early and mid pregnancy, while inadequate protein supply in late pregnancy may cause reduced birth weight and greater variability of piglet birth weights (Campos *et al*., 2012). Conversely, achieving the correct balance of energy and amino acid intake will lead to lower variability in birth weights and better lactation performance. Therefore, the correct balance of energy and amino acid intake appears necessary to optimize sow and litter performance.

The recent recommendations (GfE, 2008; NRC, 2012) that sows in late pregnancy require more amino acids means that researchers need to assess the effect of this newer feeding program (i.e., increased protein intake with optimal amino acid balance) on sow and litter performance following gestation. Clowes *et al*. (2003) offered sows over three parities either a 15% crude protein diet throughout pregnancy, or diets containing 12% (day 0–38), 13% (day 39–75) or 16% crude protein (day 75 onwards) at constant feed allowance, and found no effect on sow and litter performance in the following lactation, however, these diets did not have the optimal ratio of amino acids. Kleisiary (2007) showed increased piglet birth weight when the dietary protein content was increased from 11 to 13% for the last 30 d of pregnancy. Everts and Dekker (1994) imposed a phase-feeding regimen over three parities where the feed allowance was increased from 2.5 kg/d to 3.0 kg/d after day 86 at dietary protein contents of 12.1% or 17.8% for the entire pregnancy. Increasing the dietary protein in early and mid pregnancy had no effect on N retention but reduced heat production and energy and lipid retention in gilts. In late pregnancy, increasing the protein

content increased N retention in all three parities but had no effect on energy metabolism. Litter size and litter birth weight was not affected by dietary treatments, although variability in individual litter weights was not reported. This indicates that increasing the feed intake with greater protein content was the optimal dietary regimen, which is in accord with current recommendations (GfE, 2008; NRC, 2012). However, increasing dietary protein content too much in late pregnancy may be detrimental. Tydlitat *et al.* (2007) showed that offering diets with 18% or 21% crude protein from day 100 onwards increased piglet losses and the incidence of mastitis, metritis and agalactia (MMA) and decreased the conception rate for the following pregnancy compared to diets containing 13% or 15% crude protein. It is possible that the excess protein induced an energy deficit due to its low energetic efficiency, or that the amino acid balance was not correct for these sows in late pregnancy. Whether this would be true or not if the amino acid balance was optimal needs to be re-examined. In conclusion, it appears that phase-feeding crude protein in pregnancy has no negative effects on the reproductive performance if extremes of dietary protein and energy are avoided. Phase-feeding crude protein offers the advantage of reducing crude protein intake for the entire pregnancy without affecting sow performance, while reducing N excretion and feed cost.

Consideration of sow performance in lactation and subsequent pregnancies and performance of offspring indicates that the current nutritional recommendations are suitable to support optimum offspring performance. In fact, exceeding or falling short of the recommended amino acid and energy allowances has the potential to impair sow and offspring performance. This is despite the fact that NRC (2012), GfE (2008), and our own results were based strictly on sow performance in pregnancy. Therefore, feeding sows to their requirements - both amino acids and energy - during gestation appears to be the best option to achieve optimal lactation and offspring performance, as well as sow longevity.

Feeding recommendations for pregnant sows

To meet the requirements of pregnant sows throughout their lifetime, parity-segregated phase feeding is the best option. Such a regimen addresses both the increase of nutritional requirements from early to late pregnancy as well as the change of requirements during the productive life span of sows. Because amino acid requirements increase to a much greater degree in late gestation than energy requirements, it is impossible to satisfy the requirements by simply feeding more of the same diet in late gestation. Therefore, diets with different amino acid contents should be formulated and blended to meet the entire range of amino acid requirements, from late gestating gilt to early gestating adult sow.

The preferred strategy for parity-segregated phase feeding is to formulate just two diets – one corresponding to the highest, the other to the lowest, amino acid needs – that can be mixed in appropriate ratios. With this approach, individual sows can be fed to their specific and changing nutrient needs. Thus, nutrient deficiency or excesses are minimised, which allows sows to perform to their potential while eliminating oversupply of nutrients. Although using 2 diets ('high' and 'low') and blending them is optimal from a nutrition and feed cost point of view, its implementation incurs some costs to upgrade existing feeding equipment. However, such 'blend-feeding' can be cost-effective (Clowes *et al.*, 2003) if suitable technology, e.g., electronic sow feeders, is available at acceptable cost.

If a two-phase feeding system is used in gestation, the question is when to change diets. McPherson *et al.* (2004) showed no change in foetal growth up to day 70 of gestation. Our results (Levesque *et al.*, 2011b) indicate that the threonine requirement between day 63 and 73 of gestation was similar to that in early gestation, so that a change in gestation diets before day 73 is not warranted. When offering constant feed allowance in pregnancy, whole body protein retention in pregnancy is unsuitable to determine the ideal time to change feed. Close *et al.* (1985) showed that protein retention under these conditions changed little between day 70 and day 100 of pregnancy but a greater fraction of protein deposition occurred in conceptus late in pregnancy. Alternatively, energy expenditure may be a suitable parameter to indicate when an increase in feed intake is warranted. Our data showed that heat production of sows changed little during early and mid gestation but increased in late gestation, with an intersection of both phases between days 85-90 of pregnancy. Because heat production in early and mid gestation will decrease if feed allowances are reduced by approximately 10% in a phase feeding regimen (see below), intersection of heat production in early and the late gestation will occur earlier in pregnancy. Therefore, the suggestion of GfE (2008) appears appropriate, to use an early gestation diet from breeding to day 84 of pregnancy, and a late gestation diet from day 85 to farrowing.

Because sow growth and fatness is controlled by the amount of feed offered, feed allowances need to be considered first when devising a phase feeding regimen. When given a constant feed allowance in gestation, sows deposit body fat in early and mid gestation that is mobilised to supplement the inadequate energy intake in late gestation. This is energetically wasteful because both tissue deposition and mobilisation have efficiencies of 0.5 to 0.8 only (Dourmad *et al.*, 2008). Because feeding a single feed allowance in pregnancy results in good condition sows, the inherent energetic efficiency in such a regimen

mean that in phase feeding the allowance in early and mid gestation can be reduced. This reduction is partly offset by the increase in feed allowance in late gestation while slightly reducing the total feed eaten by a phase-fed pregnant sow.

In the absence of electronic sow feeders, an alternative would be to top dress a base diet during late pregnancy when amino acid requirements increase. The amino acid contents in this base diet may be formulated to be lower than if a single diet was used throughout pregnancy, but should be sufficient to cover the requirement of most animals during early and mid gestation. A protein-rich custom mix may be top dressed to supplement the base diet during late gestation, with the top dressing amount derived from the additional energy needs in late pregnancy and the amino acid contents of the top dressing mix adjusted to supply amino acids as required. Because top dressing of necessity represents a compromise between the requirements of sows of differing age and may incur inaccuracies associated with hand feeding, this option is less than ideal. The advantage of top dressing, however is that it does not require the purchase of any additional feeding equipment.

If neither of these options is possible or wanted, sows can be grouped according to similar requirement. In this case, a decision needs to be made how to segregate diets. One option would be to use one diet for gilts in early and late gestation and for second parity sows in late gestation and a second diet could be used for second parity sows in early gestation and for older sows throughout pregnancy. A second option would be parity segregation, with one diet for gilts and second parity sows and second diet for older sows. In either case, most of the benefits of parity segregated phase feeding will be lost.

The obvious benefit of parity-segregated phase feeding is decreased feed cost, which we estimate to be about $5/sow per gestation cycle, which is mainly caused by the slight reduction in feed allowance. Reducing dietary amino acid contents in the first ¾ of pregnancy may reduce diet cost by an additional $5.00 to 10.00 per tonne. Although the diet for late gestation must be formulated to greater amino acid contents than current single diets for sows and will be more expensive, the diet for early and mid gestation, of which more is used, can be made less expensive.

However, these feed cost savings are smaller than the anticipated production benefits. The benefit of more robust piglets and more even litters, which may result in better post-weaning pig performance, cannot easily be quantified. Parity segregated phase feeding probably will result in lower culling rates for sows, because of improved rebreeding after the 1st litter (Shelton *et al.*, 2009) and because sows will not be entering lactation in a severely catabolic state due to underfeeding in late gestation, especially after the first to third litters. An increase of the average litters per sow from 3.5 to 4.0 litters would increase the lifetime performance per sow by half a litter and reduce the replacement rate by 10%. Sasaki *et al.* (2012) calculated that each additional litter after the third parity increased the net income per sow by approximately US$300, so that an increase of the lifetime performance of 0.5 litters carries a value of $150 per sow place. In addition, if sows had 4.0 instead of 3.5 litters per sow, savings of approximately $30.00 in replacement costs per sow can be realized. The financial potential is too great to be ignored. Although there will be an investment cost in equipment to implementing parity-segregated phase feeding the payback should be significant.

The question remains: how much more energy should be provided in late pregnancy? The necessary amounts apparently change with the age of sows. Close *et al.* (1985) showed that first-litter gilts lost 140 g/d of body lipid in late gestation when given a constant feed allowance. McMillan (2003) and Samuel *et al.* (2007) found that second parity sows lost backfat in late gestation. Samuel *et al.* (2007) showed that second parity sows increased heat production by 4.0 MJ/d from early gestation to late gestation and were in negative energy balance at day 105 of gestation. Ramonet *et al.* (2000) and McMillan (2003) showed that multiparous sows maintained positive energy balance throughout gestation when given constant amounts of feed. It therefore appears that first and second parity sows clearly need additional feed allowance in late gestation while third parity and older sows may need less adjustment to the feed allowance. To prevent the lipid loss of 140 g/d reported by Close *et al.* (1985), an additional energy intake of approximately 7.5 MJ ME/d would be needed. The additional heat produced by second parity sows in late gestation (Samuel *et al.*, 2007) consisted of 1.5 MJ of energy to account for the increased maintenance requirement of heavier sows in late gestation and of 2.5 MJ of heat associated with maternal and foetal tissue gain, so that almost 10 MJ/d would be needed to support the same energy retention as in early gestation. However, the increase in energy intake in late gestation must fall within a fairly narrow window because excess energy will inhibit the necessary transition to a catabolic state when lactation starts. The GfE (2008) suggested increasing energy intake by 8 MJ ME/d in late gestation for first to third parity sows and by 6 MJ ME/d for fourth parity sows. The default increase of 0.4 kg/d of a corn-soybean meal diet after day 90 for all parities (NRC, 2012) amounts to approximately 5.5 MJ ME/d. Our recommendations, based upon indirect calorimetry, are between GfE (2008) and NRC (2012) with an increased feed allowance for first, second and third parity and older sows of 0.6 kg/d, 0.5 kg/d and 0.4 kg/d, respectively, during the last 4 weeks of

gestation. It should be noted that these increases in feed allowance are less than that shown to achieve an increase in piglet and litter weight at birth (see discussion above on feed intake in late pregnancy). However, the suggested increases in late gestation feed allowance would be low enough to avoid excessive sow fatness at parturition and impaired lactation feed intake (Miller *et al.*, 2000). These considerations lead to the suggested feed amounts shown in Table 5 for corn-soybean meal diets. These feed amounts apply to sows of average body weight and condition; they should be modified for sows that are too lean or too fat, heavier or lighter than average, and more or less efficient in their nutrient utilization, and with larger or small litter size.

Table 5. *Daily feed allowance in kg/d of a corn-soybean meal based diet for average sows in good condition in early and late gestation. The metabolizable energy intake (MJ/d) is included in parentheses.*

	Parity (and approximate body weight at breeding; kg)		
	1st (140)	2nd (180	3rd and older (220)
Early gestation (day 1 to 84)	1.8 (21.0)	2.2 (30.8)	2.4 (33.6)
Late gestation (day 85 to 112)	2.4 (33.6)	2.7 (37.8)	2.8 (39.2)
Average daily feed:			
Phase feeding	1.95 (27.3)	2.32 (32.5)	2.50 (35.0)
Constant allowance	2.00 (28.0)	2.40 (33.6)	2.50 (35.0)

The feed allowances in Table 5 were chosen to meet the energy requirement of sows; Table 6 shows the necessary amino acid contents at these respective energy intakes. In addition, an example is given for the amino acid contents that can be found in a single diet for pregnant sows, as used in the Swine Research and Technology Centre at the University of Alberta. It becomes clear that a single diet is not adequate to provide sufficient amino acids in late pregnancy for young sows or for gilts throughout pregnancy. Conversely, a single diet provides excess amino acids throughout pregnancy for older sows. The consequence is that the use of single diet for all breeding female may impair performance of young sows and will waste money because it is over-formulated for older sows.

Table 6. *Total dietary amino acid contents (g/kg) in parity-segregated phase feeding compared to feeding a single diet for all sows.*

		Phase feeding		Single diet
		2nd parity	3rd parity	
Early gestation	Lysine	0.60	0.34	0.65
	Threonine	0.32	0.21	0.55
Late gestation	Lysine	0.68	0.46	0.65
	Threonine	0.50	0.44	0.55

In addition to amino acid contents, the ratios among amino acids need consideration. The changes in amino acid requirement with age of sow and stage of pregnancy (Table 2) have important consequences. Firstly, the magnitude of change in requirements makes it impossible to satisfy the requirements using a single diet during gestation. Secondly, these data show that the amino acid ratios change as pregnancy progresses and as sows' age. Threonine and isoleucine requirements increased, relative to lysine, from early to late gestation while the tryptophan to lysine ratio showed little change. The threonine to lysine ratio was greater for both early and late gestation in the third versus second parity. These changes in ideal amino acid ratios for sows are probably caused by the changing contributions of requirements for maintenance and maternal and foetal growth to total amino acid requirements. Because the ideal amino acid patterns differ for maintenance, maternal growth and conceptus (NRC, 2012), the ideal amino acid ratios in complete diets can be expected to change as well. That means that lysine may not be the first-limiting amino acid for older sows. This last point deserves further consideration in that the familiar order of limitation in growing finishing pigs may not apply to pregnant sows. In fact, Levesque *et al*. (2010), based on IAAO and rates of protein turnover, showed that threonine was likely the first limiting amino acid in multiparous sows in late gestation, tryptophan second limiting, and lysine and branched-chain amino acids third limiting.

Clearly, a single diet for all sows in all stages of gestation is inadequate for optimal sow nutrition. Diet formulation must account for: a) markedly different ideal amino acid ratios compared to growing pigs, b)

that different amino acids are first-limiting in the diets; c) differences in amino acid digestibility/availability of ingredients for sows compared to growing pigs; d) increased amino acid and energy requirements in late versus early gestation; e) different protein(amino acid):energy ratios during early compared to late gestation; and, f) decreasing amino acid and energy requirements as sows age.

Conclusions

Sow productivity can be expected to continue to increase with genetic selection with the consequence that better sow nutrition will become more and more important. Economic realities will always demand that we seek to reduce costs while optimising performance. New approaches to sow feeding during gestation are therefore required. Completely different methods – mathematical modelling and experimental animal research - agree that amino acid and energy requirements of sows increase significantly in late pregnancy. Despite the recent major steps forward in research and modelling of sow nutrition, a number of questions needing further study remain such as definition of the amino acid requirements immediately after breeding, amino acid requirements for first litter gilts, the relationship between energy intake and amino acid requirements in late pregnancy, and amino acid availability of ingredients for sows. To supply nutrients and energy at the right amounts and at the right time for sows of all ages, parity-segregated phase feeding is the most appropriate approach. Such a feeding regimen will minimise feed costs for pregnant sows and can be expected to improve sow and piglet performance.

References

ABE, N., SUGIURA, C. and NAKAMURA, K. (2002). *Japanese Journal of Swine Science*. **39:**71-78.

ARC (1981). "The Nutrient Requirements of Pigs". Agricultural Research Council, Commonwealth Agricultural Bureaux, Slough, U.K.

BALL, R.O. and BAYLEY, H.S. (1984). *Journal of Nutrition*. **114:**1741-1746.

BEE, G. (2004). *Journal of Animal Science.* **82:**826–836.

BERTOLO, R.F., MOEHN, S., PENCHARZ, P.B. and BALL, R.O. (2005). *Journal of Animal Science.* **83:**2535-2542.

BIKKER, P., VERSTEGEN, M.W.A., CAMPBELL, R.G. and KEMP, B. (1994). *Journal of Animal Science.* **72:**1744-1753.

BOULOT, S., QUESNEL, H. and QUINIOU, N. (2008). *Advances in Pork Production.* **19:**213-220.

CAMPOS, P.H.R.F., SILVA, B.A.N., DONZELEJ, L., OLIVEIRA, R.F.M. and KNOL, E.F. (2012). *Animal.* **6:**797-806.

CASTAING, J., COUDURE, R., FEKETE, J. and LEUILLET, M. (1983). *Journées Recherche Porcine en France.* **15:**267-284.

CCSI. (2007). Canadian Centre for Swine Improvement Annual Report 2007. www.ccsi.ca.

CERISUELO, A., BAUCELLS, M.D., GASA, J., COMA, J., CARRION, D., CHAPINAL, N. and SALA, R. (2009). *Journal of Animal Science.* **87:**729–739.

CETIN, I. (2001). *Pediatric Research.* **49:**148–154.

CLOSE, W.H., NOBLET, J. and HEAVENS, R.P. (1985). *British Journal of Nutrition*. **53:**267–279.

CLOWES, E.J., KIRKWOOD, R., CEGIELSKI, A. and AHERNE, F.X. (2003). *Livestock Production Science.* **81:**235–246.

COFFEY, M.T., DIGGS, B.G., HANDLIN, D.L., KNABE, D.A., MAXWELL, C.V. Jr., NOLAND, P.R., PRINCE, T.J. and CROMWELL, G.L. (1994). *Journal of Animal Science*. **72:**4-9.

COOPER, D.R., PATIENCE, J.F., ZIJLSTRA, R.T. and RADEMACHER, M. (2003). *Journal of Animal Science.* **79:**2367–2377.

CROMWELL, G.L., HALL, D.D., CLAWSON, A.J., COMBS, G.E., KNABE, D.A., MAXWELL, C.V., NOLAND, P.R., ORR Jr., D.E. and PRINCE, T.J. (1989). *Journal of Animal Science*. **67:**3-14.

DE, W., AI-RONG, Z., YAN, L., SHENG-YU, X., HAI-YAN, G. and YONG, Z. (2009). *Journal of Animal Physiology and Animal Nutrition.* **93:**577-585.

DOURMAD, J.Y. and ETIENNE, M. (2002). *Journal of Animal Science.* **80:**2144-2150.

DOURMAD, J.Y., ETIENNE, M., ALAIN VALANCOGNE, A., DUBOIS, S., VAN MILGEN, J. and NOBLET, J. (2008). *Animal Feed Science and Technology*. **143:**372–386

DOURMAD, J.Y., ETIENNE, M. and NOBLET, J. (1996). *Journal of Animal Science*. **74:**2211–2219.

DOURMAD, J.Y., ETIENNE, M., PRUNIER, A. and NOBLET, J. (1994). *Livestock Production Science.* **40:**87–97.

ELANGO, R., HUMAYUN, M.A., BALL, R.O. and PENCHARZ, P.B. (2009). *Journal of Nutrition.* **139:**1082-1087.

ETIENNE, M. (1991). *Journées Recherche Porcine en France.* **23:**69–74.

EISSEN, J.J., KANIS, E. and KEMP, B. (2000). *Livestock Production Science*. **64:**147–165.

EVERTS, H. and DEKKER, R.A. (1994). *Animal Production.* **59:**293-311.

EVERTS, H. and DEKKER, R.A. (1995). *Livestock Production Science*. **43:**27–36.

FLAIM, K.E., PEAVY, D.F., EVERSON, W.V. and JEFFERSON, L.S. (1982). *Journal of Biological Chemistry*. **257:**2932–2938.

FRANCO, D.J., JOSEPHSON, J.K., MOEHN, S., PENCHARZ, P.B. and BALL, R.O. (2013). *Journal of Animal Science*. **91**:3859-3866.

GfE. (2008). DLG Verlags GmbH, Frankfurt, Germany.

HEGER, J., VAN PHUNG, T. and KŘÍŽOVÁ, L. (2002). *Journal of Animal Physiology and Animal Nutrition.* **86:**153-165.

HEYER, A., ANDERSSON, H.K., LINDBERG, J.E. and LUNDSTROM, K. (2004). *Acta Agriculturae Scandinavica*. **A54:**44–55.

HOPPE, M.K., LIBAl, G.W., WAHLS~M., R.C. (1990). *Journal of Animal Science*. **68:**2235-2242.

HOVING, L.L., SOEDE, N.M., FEITSMA, M. and KEMP, B. (2012). *Theriogenology*. **77:**1557-1569.

HOVING, L.L., SOEDE, N.M., VAN DER PEET-SCHWERING, C., GRAAT, E.A., FEITSMA, H. and KEMP, B. (2011). *Journal of Animal Science.* **89:**3542–3550.

JI, F., HURLEY, W.L. and KIM, S.W. (2006). *Journal of Animal Science.* **84:**579-587.

JINDAL, R., COSGROVE, J.R., AHERNE, F.X. and FOXCROFT, G.R. (1996). *Journal of Animal Science.* **74:**620–244.

KIM, S.W., HURLEY, W.L., WU, G. and JI, F. (2009). *Journal of Animal Science.* **87:**E123-E132.

KING, R.H. and BROWN, W.G. (1993). *Journal of Animal Science.* **71:**2450–2456.

KLEISIARI, M. (2007). In "XIII International Congress In Animal Hygiene", June 17–21, 2007, Tartu, Estonia. pp.778-784.

KUSINA, J., PETTIGREW, J.E., SOWER, A.F., WHITE, M.E., CROOKER, B.A. and HATHAWAY, M.R. (1999). *Journal of Animal Science.* **77:**931-941.

LAWLOR, P.G., LYNCH, P.B., O'CONNELL, M.K., MCNAMARA, L., REID, P. and STICKLAND, N.C. (2007). *Archives of Animal Breeding.* **50:**82–91.

LEVESQUE, C.L., MOEHN, S., PENCHARZ, P.B. and BALL, R.O. (2010). *Applied Physiology, Nutrition and Metabolism.* **35**:402

LEVESQUE, C.L., MOEHN, S., PENCHARZ, P.B. and BALL, R.O. (2011a). *Journal of Animal Science*. **89:**93-102.

LEVESQUE, C.L., MOEHN, S., PENCHARZ, P.B. and BALL, R.O. (2011b). *Journal of Nutrition.* **141:**406-410**.**

MAHAN, D.C. (1998). *Journal of Animal Science.* **76:**533–541.

MCMILLAN, D.J. (2003) MSc Thesis. University of Alberta, AB, Canada.

MCPHERSON, R.L., JI, F., WU, G., BLANTON, J.R. Jr. and KIM, S.W. (2004). *Journal of Animal Science*. **82:**2534-2540.

METGES, C.C., LANG, I.S., HENNIG, U., BRŰSSOW, K.-P., KANITZ, E., TUCHSCHERER, M., SCHNEIDER, F., WEITZEL, J.M., STEINHOFF-OOSTER, A., SAUERWEIN, H., BELLMANN, O., NÜRNBERG, G., REHFELDT, C. and WINFRIED OTTEN, W. (2012) *PLoS One.* **7**(2)**:**e31390

MILLER, H.M., G.R. FOXCROFT and F.X. AHERNE. (2000). *Animal Science.* **71:**141-148.

MOEHN, S., BERTOLO, R.F., PENCHARZ, P.B. and BALL, R.O. (2004). *Journal of Nutrition.* **134**:836-841.

MOEHN, S., FRANCO, D., JOSEPHSON, J.K., PENCHARZ, P.B. and BALL, R.O. (2012a). *Journal of Animal Science*. **90 (**Suppl. 2)**:**61.

MOEHN, S., FRANCO, D., JOSEPHSON, J.K., PENCHARZ, P.B. and BALL, R.O. (2012b). *Journal of Animal Science*. **90** (Suppl. 2)**:**61.

MOEHN, S., FRANCO, D., LEVESQUE, C.L., SAMUEL, R. and BALL, R.O. (2011). In "Proceedings of the 72nd Minnesota Nutrition Conference", Sept 20-21, 2011. Owatonna, MN, pp. 216-227.

MOEHN, S., SHOVELLER, A.K., RADEMACHER, M. and BALL, R.O. (2008). *Journal of Animal Science.* **86:**364-369.

MÖHN, S. ATAKORA, J.K.A. McMILLAN, D.J. and BALL, R.O. (2003). In "Progress in research on energy and protein metabolism". pp 425-427. (Wageningen Academic Publishers, Wageningen, The Netherlands).

MÖHN, S., GILLIS, A.M., MOUGHAN, P.J. and DE LANGE, C.F.M. (2000). *Journal of Animal Science.* **78:**1510-1519.

NEALE, R. J., and WATERLOW, J.C. (1974). *British Journal of Nutrition*. **32**:11–25.

NISSEN, P.M., DANIELSEN, V.O., JORGENSEN, P.F. and OKSBJERG, N. (2003). *Journal of Animal Science*. **81:**3018–3027.

NRC. (1998). "Nutrient Requirements of Swine" (10th Ed.). National Academy Press. Washington, DC.

NRC. (2012). "Nutrient Requirements of Swine" (11th Ed.). National Academy Press. Washington, DC.

PENCHARZ, P.B. and BALL, R.O. (2003). *Annual Review of Nutrition.* **23:**101-116.

PETTIGREW, J.E. and YANG, H. (1997). *Journal of Animal Science*. **75:**2723-2730.

QUESNEL, H., BOULOT, S., SERRIERE, S., VENTURI, E. and MARTINAT-BOTTÉ, F. (2010). *Animal Reproduction Science*. **120:**120–124.

QUINIOU, N. (2007). *TechniPorc*. **30:**11-17.

RAMONET, Y., VAN MILGEN, J., DOURMAD, J.Y., DUBOIS, S., MEUNIER-SALAÜN, M.C. and NOBLET, J. (2000). *British Journal of Nutrition*. **84**:85–94.

ROMBAUTS, P. (1962). *Annales de Zootechnie.* **11**:39-52.

SABIONI, K.S., BRUSTOLINI, P.C., DE OLIVEIRA SILVA, F.C., FERREIRA, A.S., DONZELE, J.L., KILL, J.L. and NUNES SILVA, B.A. (2007). *Revista Brasileira de Zootecnia*. **36**:403-410.

SAMUEL, R.S. (2010). PhD Thesis, University of Alberta, Alberta, Canada.

SAMUEL, R.S., MOEHN, S., PENCHARZ, P.B. and BALL, R.O. (2007). In "Energy and protein metabolism and nutrition". pp.519-520, eds. I. Ortigues-Marty, N. Mireaux and W. Brand-Williams. (Wageningen Acadamic Publishers, Wageningen, The Netherlands).

SAMUEL, R.S., MOEHN, S., PENCHARZ, P.B. and BALL, R.O. (2010). In "Energy and protein metabolism and nutrition", pp.111-112, ed. G.M. Crovetto. (Wageningen Acadamic Publishers, Wageningen, The Netherlands).

SAMUEL, R.S., MOEHN, S., PENCHARZ, P.B. and BALL, R.O. (2012). *Journal of Animal Science.* **90:**4896-4904.

SASAKI, Y., McTAGGART, I. and KOKETSU, Y. (2012). *Journal of Veterinary Epidemiology*. **16**:37-45.

SCHIEMANN, R., NEHRING, K., HOFFMANN, L., JENTSCH, W. and CHUDY, A. (1971). *Energetische Futterbewertung und Energienormen.* VEB Deutscher Landwirtschaftsverlag Berlin.

SHELTON, N.W., DEROUCHEY, J.M., NEILL, C.R., TOKACH, M.D., DRITZ, S.S., GOODBAND, R.D. and NELSSEN, J.L. (2009). Swine Day, Manhattan, KS, November. Retrieved 2 Feb 2013 from: http://krex.k-state.edu/dspace/handle/2097/2155

SINCLAIR, A.G., BLAND, V.C. and EDWARDS, S.A. (2001). *Journal of Animal Science.* **79:**2397–2405.

SOTO, J., GREINER, L., CONNOR, J. and ALLEE, G. (2011). *Journal of Animal Science.* **89**(E-Suppl.)**2:**124.

SRICHANA, P. (2006). PhD Thesis 479. University of Missouri, Columbia, USA.

STEIN, H.H., KIM, S.W., NIELSON, T.T, and EASTER, R.A. (2001). *Journal of Animal Science*. **79:**2113–22.

SWINDLE, M.M., NOLAN, T., JACOBSON, A., WOLF, P., DALTON, M. and SMITH, A.C. (2005). *Contemporary Topics in Laboratory Animal Science*. **44:**7–17.

TOPLIS, P. and GINESI, M.F.J. (1983). *Animal Production*. **37:**45-48.

TYDLITAT, D., VINKLER, A. and CZANDERLOVA, L. (2007). *Acta Veterinaria Brno.* **76:**585-593.

VARLEY, M.A. and PRIME, G.R. (1993). *Livestock Production Science*. **34:**267–279.

WHITTEMORE, C.T. and KYRIAZAKIS, I. (2006). Whittemore's Science and Practice of Pig Production, 3rd Edition, Wiley-Blackwell, UK.

WILLIAMS, I.H., CLOSE, W.H. and COLE, D.J.A. (1985). In: "Recent Advances in Animal Nutrition", pp.133–147, ed. D.J.A. Cole. (Nottingham).

WILLIS, G.M. and MAXWELL, C.V. (1984). *Journal of Animal Science*. **58:**647–656.

ZELLO, G. A., PENCHARZ, P.B. and BALL, R.O. (1993). *American Journal of Physiology Endocrinology and Metabolism.* **264:**E677–E685.

ZHONG, Z.Z., JIANG, S., XIAO, R., WANG, J.Y., OU, X.Q., ZHOU, X.R., WANG, C., SHI, Y. and SONG, D.J. (2009). *Chinese Journal of Animal Science.* **21:**625-633.

Funding for this research has been received from: Alberta Pork, Ontario Pork, ALIDF, ACAAF and the Ajinomoto Amino Acid Research Program. Amino acids have been generously provided by Heartland Lysine (Ajinomoto).

SYMPOSIUM: Maximising productivity in the modern sow: Constraints to realising the genetic potential of the breeding herd and targeting nutrition for optimal productivity: Conclusions

C.L. Collins

Rivalea (Australia), Corowa, NSW, 2646

Genetic selection has driven significant improvements in sow productivity over the years with further gains possible through the use of targeted selection pressures and nutritional programs. This symposium has provided valuable insights into the reproductive and nutritional constraints currently limiting sow productivity and proposed several key areas for future improvement.

Foxcroft *et al.* (2013) highlighted the limitations of both the sow and boar components of current breeding programs in realising the full genetic potential of the breeding herd. The continuing maternal focus on the number of pigs born per sow per year has had a substantial impact on increasing litter size, however the impacts of such selection pressures on litter quality indicators such as birth weight variability and survivability require future focus. On the boar side, the use of the most fertile boars of known genetic potential is critical in maximising sow productivity with the authors proposing several innovative changes to current breeding programs to improve efficiencies.

Advances in reproductive performance have also coincided with a focus on the nutritional requirements of the modern sow. Ball and Moehn (2013) outlined the most recent research on sow energy and amino acid requirements throughout the various stages of gestation and across multiple parities. The authors outlined the changes in nutritional requirements as sows' age and highlighted the benefits of a targeted nutritional regime. Implementation of such a feeding program will depend on sow housing and feeding systems, with the move away from gestation stalls to group housing for many producers providing some challenges. Such challenges must however be overcome in order to meet the nutritional demands of the modern sow in the face of continued genetic selection for improved sow productivity.

Lactational oestrus can be induced using piglet separation and/or boar exposure in multiparous commercial breeding sows

E.J. McDonald[1], J.I. Wilkinson[1], C.L. Collins[2], J.J. Lievaart[3] and J.A. Downing[1]

[1]University of Sydney, Camden, NSW 2570. [2]Rivalea Australia Pty Ltd, Corowa, NSW 2646. [3]Charles Sturt University, Wagga Wagga, NSW 2650.

A commercially acceptable lactational oestrus induction protocol could be used to modify current weaning and mating management systems within the pork industry. Piglet separation and boar exposure during lactation have previously resulted in an 85% pregnancy rate (Downing *et al*., 2009). A suitable induction protocol can potentially delay weaning, increase weight gain of piglets after weaning (Thompson *et al*., 1981), and help in the management of 'weaner setback'. Our hypothesis was that from d 21 of lactation, either full boar contact or piglet separation for 8h/d for 3 d will be sufficient to induce oestrus.

One hundred and ninety two multiparous sows (Large White x Landrace, PrimeGro™ Genetics, Rivalea Australia Pty Ltd) with a mean parity of 3.5±1.4 (mean±SEM), were selected at entry into the farrowing house. On an average d 21±1.7 of lactation, sows were allocated to one of four treatment groups based on parity and suckling litter size; A: controls, conventionally weaned litters d 21, sows moved from the farrowing crate to an adjoining shed. Sows were given full boar exposure in a designated mating area for 10 min daily, for 8 consecutive days post-weaning; B: three consecutive nights of 16 h piglet separation (Sep) from the sow; C: three consecutive d of 8 h piglet Sep; and D: 30 min of full boar exposure daily for 8 consecutive d, with no piglet separation. All treatment B, C and D sows were exposed to 10 min of fence-line boar contact within the farrowing crates, twice daily from the start of treatment for 8 consecutive d. During treatment, all sows that displayed standing oestrus were mated (up to three times) by artificial insemination. Sows that were not mated during lactation were mated at their first post-weaning oestrus. Weaning of piglets in treatments B, C and D was delayed until an average age of 28 d. All piglets were offered creep feed daily from the start of treatment. Data analyses were undertaken using SPSS, by post-hoc Bonferroni methods of analysis, with the sow considered the experimental unit.

Table 1. *Influence of oestrous induction on reproductive performance and piglet growth (mean ± SD).*

	Control (A) (n=47)	16 h Sep (B) (n=50)	8 h Sep (C) (n=47)	Boar (D) (n=48)
% sows mated within 28 d of farrowing	93.6	82.0	53.2[a]	75.0
% sows farrowed (mated within 28 d of farrowing)	79.5[*]	92.3	71.4	73.5
Piglet growth rate d 21-28 (g/d)	71±10[b]	193±10[b]	218±8[c]	244±9[c]
Litter size at d 21	9.6±1.63	9.4±1.70	9.4±1.57	9.5±1.56
Piglet weight at 28 d (kg)	7.0±1.08	8.2±1.14	8.6±1.22	9.0±1.15
Total piglets born in subsequent parity	8.89[a] (n=31)	9.00[a] (n=36)	3.45[b] (n=15)	6.42[b] (n=25)

[a]Mean value is significantly different to other values within the same row (P<0.05). [b,c]Means within a row not having the same superscript are significantly different (P<0.05); [*] Percentage of control sows mated within 8 d after weaning (d 21 of lactation).

The percentage of sows mated within 28 d of parturition was less (P=0.002) in sows that were subjected to 8 h piglet separation in comparison to all other treatment groups. Of those sows mated within 28 d of parturition, 92.3% of 16 h Sep treatment sows subsequently farrowed. Despite this, subsequent litter size was markedly reduced in all treatment groups. It is thought that suckling intensity during lactation could have had a negative effect on ovarian follicular growth and hence the potential subsequent litter size. The results of this experiment support our hypothesis. However, low subsequent litter sizes warrants further investigation into ovarian follicular development and embryo survival associated with lactational oestrus.

DOWNING, J.A., BROEK, D., SMITS, R.J. and GILES, L.R. (2009). In "Manipulating Pig Production XII", p. 144, ed R.J. van Barnevald. (Australasian Pig Science Association: Werribee).

THOMPSON, L. H., HANFORD, K. J. and JENSEN, A. H. (1981). *Journal of Animal Science.* **53**:1419-1423.

Supported in part by Pork CRC Limited Australia.

Porcine spermatozoa interact with the uterine epithelium and modulate endometrial gene expression

A. Bergmann, U. Taylor and D. Rath

Institute of Farm Animal Genetics (FLI), 31535 Neustadt am Rübenberge, Germany.

Successful artificial insemination (AI) in pigs requires 1.5 to $3x10^9$ sperm per dose. This very high concentration, compared to other farm animal species, leads to an inefficient crop of boar ejaculates and impedes the development of new reproductive technologies such as sperm sexing where the sperm number is the main limiting factor. Based on previous research (Taylor *et al*., 2008), we hypothesised that one of the reasons for this phenomenon could be that following insemination a large number of vital sperm undergo a transient binding with the endometrium, thus establishing a uterine sperm population and therefore ensuring a continuous supply of viable sperm for the functional reservoir in the oviduct. Evidence also indicated a sperm-mediated influence on the expression of genes relevant for the maternal immune response and reproductive success (Taylor *et al*., 2009).

To increase understanding of the initial processes in the porcine uterus after breeding, a cell culture model from primary porcine uterine epithelial cells (UEC) was established (Bergmann *et al*., 2012). To investigate uterine-specific sperm binding patterns, the cultured UEC were co-incubated with spermatozoa. In parallel a control monolayer of porcine foetal fibroblasts was treated identically. The sperm rich fraction of four German Landrace (GL) boars was collected and extended in cell culture medium with 20% heat inactivated foetal bovine serum to $100x10^6$ sperm/ml. Sperm suspension (500 µl) was released onto a UEC and fibroblast monolayer, respectively and incubated for 10 min at 37 °C. Loose sperm were removed by washing with PBS and the monolayers observed under a phase contrast microscope. Images (two repeats/boar) were divided into fields of 61.6 μm^2 and the area with and without sperm was counted. For statistical analysis a Mann-Whitney Rank Sum Test was performed. Sperm binding density (μm^2) was higher ($P=0.002$) in UEC (15924±2657.9, median±SEM) than in fibroblasts (3018±638.1). For UEC, sperm binding could be observed within 5 min. It was noted that while in some cases clusters of sperm attached to a single UEC along the complete perimeter of the cell, other cells were not populated by the sperm at all. Binding occurred at the sperm head and bound sperm remained motile.

After demonstrating sperm binding *in vitro*, we wanted to specify the influence of bound sperm on gene expression *in vivo*. A custom-made microarray (384 immune-relevant genes) studied gene expression in the porcine endometrium after contact with various inseminate components. Synchronised GL gilts (n=5 per treatment) were inseminated either with spermatozoa diluted in seminal plasma, spermatozoa in PBS, epididymal sperm in PBS, seminal plasma only, PBS only, or kept unbred. The doses contained fresh semen from one fertile GL boar washed in PBS and extended to $3x10^9$ sperm/100 ml of the respective extender. The gilts were slaughtered 2 h post AI and the abundance of specific mRNAs in the endometrial samples was measured with a Geniom Biochip (*febit group*, Heidelberg, Germany). Statistical analysis was carried out using the Empirical Bayes approach (software: *R* Version 2.15.2). Treatments without sperm did not shift gene expression ($P>0.05$). The presence of spermatozoa led to a significant differential expression in 37 of the 384 analysed genes ($P<0.05$), and mostly led to a lower expression of the respective gene compared to the negative control (31 of out of 37 differentially regulated genes).

In conclusion, spermatozoa play a major part in the containment of immunological reactions in the uterus after breeding. Pathway analyses of the obtained data are underway for a deeper understanding of the mechanisms behind this finding. *In vitro* trials exposed an endometrium-specific binding of spermatozoa, which strengthens the hypotheses that (i) sperm binding is the cause of the found shift in gene expression *in vivo*, and (ii) that a uterine sperm reservoir is formed, secondary to the one in the oviduct. On-going research aims to characterise the participating surface molecules on the sperm as well as the uterine epithelium. Since interactions between sperm and oviduct epithelium as well as the zona pellucida are lectin-mediated (Töpfer-Petersen, 1999), we currently test whether sperm-UEC binding is also due to lectin-carbohydrate interactions.

BERGMANN, A., JUNGE, S., TAYLOR, U. and RATH, D. (2012). *Reproduction in Domestic Animals*. **47:**(Suppl 2)**:** 11.
TAYLOR, U., RATH, D., ZERBE, H. and SCHUBERTH, H.J. (2008). *Reproduction in Domestic Animals*. **43:**166-175.
TAYLOR U., ZERBE H., SEYFERT H.M., RATH D., BAULAIN U., LANGNER K.F. and SCHUBERTH H.J. (2009). *Animal Reproduction Science*. **115**:279-89.
TÖPFER-PETERSEN, E. (1999). *Human Reproduction Update*. **5**:314-29.

Financial support through IMV Technologies, L'Aigle, France and Besamungsverein Neustadt an der Aisch, Germany.

Effects of parity and supra-nutritional dietary antioxidants on the lactation performance of sows during summer

F. Liu[1], C.L. Collins[2], D.J. Henman[2] and F.R. Dunshea[1]

[1]The University of Melbourne, Melbourne, VIC 3010. [2]Rivalea Australia, Corowa, NSW 2646.

Lactating sows are susceptible to heat stress and the severity may depend on parity (Black *et al*., 1993). Heat stress causes oxidative stress that can be ameliorated in sheep by feeding supra-nutritional dietary antioxidants, in the form of organic selenium and vitamin E (Chauhan *et al*., 2012). This study tested the hypothesis that supra-nutritional dietary antioxidants ease heat stress in lactating sows.

The study involved 170 sows (Large White x Landrace, PrimeGro™ Genetics, Corowa, NSW), which consisted of 103 second parity and 67 third parity sows. Sows in each parity were equally and randomly allocated to two dietary regimes that were a control (CON) and supra-nutritional antioxidant diet (AO). The control wheat-based pre-farrowing and lactation diets contained 0.3 ppm Selplex® (selenium yeast), 0.4% betaine and 130 and 90 IU/kg respectively of vitamin E, as is normal practice for the farm during summer. The AO diet consisted of the CON diet supplemented with 0.3% EconomasE, an algae-based antioxidant containing selenium yeast (dietary selenium of 0.8 ppm), 1% Nupro® (a yeast nucleotide product) and 0.1% Actigen™ (a yeast mannan product). Selplex®, EconomasE®, Nupro® and Actigen™ are from Alltech Inc., Nicholasville, KY. The sows in each dietary group were fed on the pre-farrowing diet for an average of 9 d followed by the lactation diets from farrowing to weaning (average of 27 d). The experiment was conducted at Corowa, NSW, Australia from 14 January to 4 March, 2013. The average daily temperatures (minimum 16.1 ± 4.3 °C and maximum 32.9 ± 3.8 °C; mean±SEM) were beyond the thermal comfort zone (12-22 °C) for sows (Black *et al*., 1993). Daily feed intake, body weight and back fat were measured from 9 d before farrowing and weaning. The litter size and weight were recorded and cross-fostering performed at 2 d to equalise litter size across parities and diets. Data were analysed using a linear mixed model (GenStat, 15th Edition; UK).

Table 1. *Lactation performance of sows and piglets according to different parities and diets fed.*

Parity (P)	Parity two		Parity three		SED[a]	Significance		
Diet (D)	CON	AO	CON	AO		P	D	DxP
ADFI[b] (kg/day)	5.52	5.49	5.79	5.56	0.21	0.27	0.46	0.51
Weight change (kg)	-33.6	-34.8	-25.4	-37.6	3.74	0.33	0.04	0.04
Back fat change (mm)	-4.35	-4.65	-2.27	-3.68	0.79	0.01	0.18	0.32
Piglets born alive	10.8	11.0	12.5	11.8	0.62	0.01	0.70	0.29
Litter size, post fostered	11.2	11.2	11.2	11.1	0.18	0.98	0.86	0.77
Litter size, weaned	9.96	9.92	9.38	9.52	0.33	0.04	0.90	0.70
Total litter weight, post- fostered (kg)	18.5	17.8	16.6	17.0	0.61	0.01	0.50	0.22
Total litter weight weaned (kg)	79.0	76.0	77.1	74.3	3.27	0.43	0.21	0.97
Total litter weight gained (kg)	60.5	58.2	60.5	57.4	3.01	0.82	0.21	0.85
Average piglet weight, post-fostered (kg)	1.66	1.60	1.49	1.53	0.05	0.01	0.58	0.16
Average piglet weight, weaned (kg)	7.96	7.69	8.11	7.87	0.24	0.34	0.12	0.94
Average individual piglet weight gain (kg)	6.15	6.09	6.62	6.34	0.26	0.06	0.42	0.53

[a]SED (standard error of difference) for parity x diet; [b]ADFI, average daily feed intake; P, parity; D, diet.

Second and third parity sows had identical ADFI (P=0.27), but second parity sows lost more back fat (P=0.01) than third parity sows. While the AO sows lost more weight (P=0.04) than the CON sows, this was only evident in the parity three sows as evidenced by a diet x parity interaction (P=0.04). Parity three sows had more born alive pigs than parity two (P=0.01). Cross-fostering achieved standardised litter size for the parities (P=0.98). However, the post-fostering litter weight and average piglet weight of parity two sows were higher than those of parity three (P<0.01), presumably because of their higher born alive. At weaning, parity two sows had larger (P=0.04) litter size but a similar (P=0.43) total litter weight compared with parity three sows. Average piglet weight gain tended to be higher in parity three (P=0.06), although total litter weight gain was similar (P=0.82). Overall, parity two sows mobilised more fat and weaned more piglets than parity three sows during lactation in summer. In conclusion, an AO diet did not change ADFI intake but altered sows' weight loss in a parity-dependant manner. Parity three AO sows lost more body weight than those on the CON diet, but the extra mobilised body reserves did not contribute to increase piglet growth. Further research needs to be done to identify the destination of energy flow.

BLACK, J. L., MULLAN, B. P., LORSCHY, M. L. and GILES, L. R. (1993). *Livestock Production Science*. **35**:153-170.
CHAUHAN, S., CELI, P., LEURY, B. and DUNSHEA F. (2012). *Journal of Animal Science*. **90** (Suppl. 3):668.

Supported by Rivalea Australia, Alltech Australia and Australian Pork Limited.

Improving piglet birth weight viability through better maternal hygiene and nutrition in gestation

E.C. Greenwood[1], W.H.E.J. van Wettere[1], R. Kirkwood[1] and P.E. Hughes[2]

[1]The University of Adelaide, Roseworthy, SA 5371. [2]South Australian Research and Development Institute, Roseworthy, SA 5371.

Evidence suggests that high level feeding in late gestation is beneficial, increasing both piglet birth weight and survival and maintaining sow condition (Shelton *et al*., 2009). However, recent work (Hughes and van Wettere, *unpublished data*) suggests that increased feed intake in mid to late gestation is of no benefit. An activated immune system increases the nutritional requirements of the sow, and this change may explain the variation in effectiveness of studied feeding strategies (Saker, 2006). We hypothesised that an increase in feed allowance, from day 86 to 112 of gestation, would not alter piglet birth weight or survival when gestating sows were housed in high hygiene conditions, but that differences would be observed in sows housed in standard hygiene conditions, due to the increased nutrient requirements of their immune system.

The study used 123 Large White x Landrace gestating sows (parity 3.1±1.3; mean±SEM) weighing 275±6.4 kg at commencement. The experiment was a 2 x 2 factorial design, with two hygiene levels (High and Standard) and two feed levels (High and Control). The animals were randomly allocated into pens of four. The High Hygiene pens were pressure cleaned, disinfected (Virkon S, diluted; as per manufacturer's instructions) and the floors covered with hydrated lime prior to the sows entering, and then pressure cleaned every 2-3 d throughout the trial period. Standard Hygiene pens were not cleaned prior to sow entry or during the trial. Within housing treatment, sows received either 2.3 kg/day (29.9 MJ digestible energy (DE)/d, 15.9 g available lysine/d; Control) or 3.3 kg/day (42.9 MJ DE/d, 22.8 g available lysine/d; High) of the same gestation diet, which they received as one drop in the morning. A gestating sow needs between 25.1 MJ and 41.8 MJ DE per day (Noblet *et al*., 1990). Sows were weighed and P2 back fat depth measured at 83±1 days of gestation, and prior to entering the farrowing house at 110±2 days gestation. Blood samples were taken at these time points to calculate neutrophil:lymphocyte ratios. Farrowing duration and the numbers of stillborn and mummified fetuses were recorded at farrowing. Piglets (including stillbirths) were weighed individually at birth. At 72 h after completion of farrowing, piglet deaths were recorded. Data were analysed using mixed and general linear models (IBM SPSS, Version 20.0; USA). Parity, sow body weight and litter size were covariates and pen, block and treatment were fixed effects.

Table 1. *Piglet weight, its variation, and the percentage of piglets under 1 kg at birth, for sows with High and Standard hygiene and High (3.3 kg/d) and Control (2.3 kg/d) feed intake in late gestation (P>0.05) (least-squares means ± SEM).*

	High Hygiene		Standard Hygiene	
	High Feed (n=31)	Control Feed (n=28)	High Feed (n=28)	Control Feed (n=26)
Piglet birth weight (kg)	1.46 ± 0.041	1.47 ± 0.042	1.52 ± 0.045	1.47 ± 0.047
Within-litter variation (kg)	0.30 ± 0.015	0.30 ± 0.017	0.27 ± 0.016	0.28 ± 0.017
Piglets < 1 kg at birth (%/litter)	9.2 ± 1.9	7.0 ± 2.1	7.1 ± 2.0	8.1 ± 2.1

The pattern of feeding during late gestation and hygiene conditions had no effect (P>0.05) on piglet weights (Table 1). There was no increase (P>0.05) in immune response observed by change in neutrophil:lymphocyte ratios from d 83 to d 110 (P>0.05), however the ratios were highly variable (0.41±0.45). The proportion of the litter to survive 72 h after farrowing was not significantly altered by treatment (0.94±0.3), neither the farrowing duration (18.1 min/piglet ±3.6), numbers of stillborn (0.49±0.9) and mummified foetuses (0.15±0.65) per litter. Slight variation in the experiment might have been introduced by not controlling feed measurements based on individual sow weight. In conclusion, feeding at an increased level during mid to late gestation to maximise piglet survival is unnecessary. Hygiene levels did not alter sow immune activation or their nutritional requirements. An increase in commercial pen hygiene practices, during late gestation, is not proven to alter sow immune status or improve piglet survival.

NOBLET, J., DOURMAD, J.Y. and ETIENNE M. (1990). *Journal of Animal Science.* **68**:562-572.
SAKER, K.E. (2006). *Veterinary Clinics Small Animal Practice.* **36**:1199-1224.
SHELTON, N.W., DEROUCHEY, J.M., NEILL, C.R., TOKACH, M.D., DRITZ, S.S., GOODBAND, R.D. and NELSSEN J.L. (2009). *Kansas State University Swine Day 2009*, Report of Progress 1020:38-43.

Supported by the University of Adelaide, Pork CRC Limited Australia and the Lienert Family.

Manipulating Pig Production XIV

Post-mating but not pre-mating dietary restriction decreases embryo survival of group-housed gilts

P.C. Condous, R.N. Kirkwood and W.H.E.J. van Wettere

The University of Adelaide, Roseworthy, SA 5371.

Unintentional variation in feed intake in group housing systems may be enough to exert a negative effect on pregnancy rate and possibly litter size (Kongsted, 2005). High variation in feed intake between sows, due to competition for feed, is evident in gestation group housing systems, with low ranking sows thought to receive less feed than their higher ranking counterparts (Andersen *et al*., 1999). The low ranking sows are more likely to be parity one sows (Hoy *et al*., 2009), with these sows also commonly suffering nutritional challenges prior to mating due to the energy demands from lactation. Using an established gilt model for parity one sows (Almeida *et al*., 2000), this study tested the hypothesis that post-mating, but not pre-mating, feed restriction will negatively affect embryo survival.

A total of 49 gilts (Large White x Landrace) were selected at 171 d of age. Puberty was stimulated with PG600 (400 IU equine chorionic gonadotrophin and 200 IU of human chorionic gonadotrophin; Intervet, Australia) and daily boar contact. From d 1 to d 14 of the second oestrous cycle gilts were fed 0.8 x maintenance [PreL; mean intake of 1.05±0.07 (mean±SEM) kg feed] or 1.0 x maintenance (PreH; mean intake of 1.32±0.1 kg feed) of a gilt developer diet (13.2 MJ digestible energy (DE); 15.1% crude protein (CP); 0.69% lysine). Gilts were housed individually in stalls during this treatment period. From d 15 of the second oestrous cycle until exhibition of their third oestrus, gilts were fed the gilt developer diet *ad libitum* to represent feeding management during the weaning to oestrus interval. Gilts were artificially inseminated at detection of their third oestrus. From d 1 post-insemination until slaughter, gilts were fed 1 x maintenance (PostL; mean intake of 1.37±0.09 kg feed) or 1.5 x maintenance (PostH; mean intake of 2.06±0.14 kg feed) of a gestation diet (12.99 MJ DE; 13.8% CP; 0.55% lysine). From d 15 of the second oestrous cycle until slaughter, gilts were housed in fixed groups of six in a grower shed, with a space allowance of 1.25 m^2 per pig. From insemination at their third oestrus until slaughter, gilts were moved daily to individual stalls where they received the post-insemination feed intake and, once consumed, returned to their group pens. Gilts were slaughtered on day 25.5±0.22 post-insemination and reproductive tracts were collected. Data were analysed using a general linear model in SPSS, version 19 (IBM).

Table 1. *Reproductive characteristics on d 25 of pregnancy in gilts fed 1 x maintenance (PreH) or 0.8 x maintenance (PreL) from d 1 to 14 of the oestrous cycle prior to insemination(PreM), and 1.5 x maintenance (PostH) or 1 x maintenance (PostL) after mating (PostM).*

	PreH		PreL			Significance		
	PostH (n = 11)	PostL (n = 12)	PostH (n = 12)	PostL (n = 11)	SEM	PreM	PostM	PreM PostM*
Embryo weight (g)	0.90	0.94	0.76	0.81	0.05	NS	NS	NS
No. of embryos	13.2	11.7	14.8	11.8	0.50	NS	0.006	NS
Embryo Survival (%)	86.6	78.1	90.0	77.6	2.50	NS	0.026	NS

SEM, standard error of the mean; NS, not significant at (P>0.05); PreM, pre-mating diet; PostM, post-mating diet; *Interaction between the PreM and PostM dietary treatments.

There was no interaction (P>0.05) between the pre-mating and post-mating feed intake on any reproductive measure (Table 1). Prior to mating, PreL gilts lost more weight (P<0.05) than PreH gilts (3.7±0.71 versus 6.7±0.84 kg). After mating, gilts in the PostL treatment lost 0.5±1.02 kg live weight (LW) while those in the PostH group gained 5.7±0.90 kg LW (P>0.05). The pre-mating treatment had no effect (P>0.05) on any reproductive measure. Embryo survival was higher (P<0.05) in the PostH compared to PostL groups (88.4±2.52 versus 77.8±3.98 %), resulting in more (P<0.05) embryos (14.0±0.63 versus 11.7±0.68). These data show that reducing post-mating feed intakes to maintenance levels impaired embryo survival, and demonstrate the importance of ensuring sows receive adequate post-mating nutrition, which may require optimisation of feeding regimens in group housing systems during this stage of gestation.

ALMEIDA, F., KIRKWOOD, R.N., AHERNE, F.X. and FOXCROFT, G.R. (2000). *Journal of Animal Science*. **78**:1556-1563.
ANDERSEN, I.L., BØE, K.E. and KRISTIANSEN, A.L. (1999). *Applied Animal Behaviour Science*. **65**:91-104.
HOY, S., BAUER, J., BORBERG, C., CHONSCH, L. and WEIRICH, C. (2009). *Applied Animal Behaviour Science*. **121**:103-107.
KONGSTED, A.G. (2005). *Livestock Production Science*. **97**:13-26.

Supported in part by Pork CRC Limited. Australia.

The effects of season and moderate feed restriction on oocyte developmental competence in cycling gilts

A.M. Swinbourne [1], J.M. Kelly[2], K.L Kind[1], D.J. Kennaway[3] and W.H.E.J van Wettere[1]

[1]The University of Adelaide, Roseworthy, SA 5371. [2]South Australian Research and Development Institute, Rosedale, SA 5350. [3]The University of Adelaide, Adelaide, SA 5005.

It is common for sow fertility to be impaired during summer/early autumn due to the effects of photoperiod on gonadotrophin release, resulting in reduced production rates (Auvigne *et al.*, 2010). Sow fertility is also impaired when fed severe restrictive diets (Ferguson *et al.*, 2003;); however, the effects of moderate nutritional restriction are still unclear (van Wettere *et al*., 2011). Equally, the effect of season on cycling gilt fertility has not been investigated. Our hypothesis is that a moderate feed restriction during summer/early autumn will increase the effects of seasonal infertility on the characteristics of the preovulatory follicle pool and the ability of the preovulatory oocyte to complete maturation *in vitro*.

Sixty-one individually housed F2 terminal sire line gilts [live weight (LW) 126 ± 1.3 kg and 214 ± 0.55 d of age; mean ± SEM] were used in summer (S; January to March; n = 29) and winter (W; June to August; n = 32). Within season, gilts received one of two feeding levels during their second oestrous cycle; high (H; 2.5 times maintenance) versus moderate (M; 1.5 times maintenance). All gilts received the same diet; 13.0 MJ of digestible energy (DE)/kg and 16% crude protein, with LW recorded on d 1 (first day of standing heat) and 19 of the second oestrous cycle prior to being slaughtered and reproductive tracts collected. The diameter of all follicles ≥1 mm was recorded and follicular contents were aspirated from all follicles ≥4 mm. All aspirated cumulus-oocyte complexes were matured *in vitro* for ~ 44 hours and the proportion of oocytes at the metaphase II (MII) stage of meiosis assessed. Follicular fluid (FF) was frozen for later analysis of luteinising hormone (LH), progesterone (P4) and oestradiol (E2) concentrations. A general linear model was used to determine the effect of treatment on all variables (SPSS, version 19).

Table 1. *Effect of season, summer versus winter, and feeding level, high (H) versus moderate (M), on the number of ovarian follicles, oocyte maturation to metaphase II and follicular fluid hormone concentrations.*

	Summer		Winter		Pooled	Significance		
	H	M	H	M	SEM	S	D	S*D
Number of gilts	14	15	16	16				
Total Follicles/gilt (>1 mm)	59.7	58.7	53.5	49.1	6.3	0.240	0.607	0.809
Small follicles (<4 mm) (%)	55	54	53	46	1.4	0.280	0.479	0.562
Medium (≥ 4 -5.99 mm) (%)	24	20	11	13	0.04	<0.01	0.574	0.404
Large (≥6 mm) (%)	21	26	36	41	0.06	<0.01	0.989	0.077
Oocytes at MII (%)	71	72	71	68	0.06	0.904	0.997	0.895
FF Progesterone (ng/ml)	274.4	169.7	78.8	233.5	49.31	0.265	0.912	<0.05
FF Oestradiol (pg/ml)	7.4	1.2	24.3	31.1	4.42	<0.011	0.948	0.197
FF Luteinising hormone (ng/ml)	40.0	40.6	6.9	16.0	5.48	<0.011	0.385	<0.01
FF Oestradiol : Progesterone	0.15	0.02	0.48	0.26	0.01	<0.011	0.405	0.392

High (H), Moderate (M); Metaphase II (MII); Follicular Fluid (FF); Season (S); Diet (D); Season*Diet (S*D); SEM, standard error of the mean.

Between d 1 and 19, LW gain was higher in summer compared to winter (S: 0.65±0.04 versus W: 0.39±0.04 kg/d, P<0.05) and for high compared to moderate fed gilts (H: 0.83±0.05 versus M: 0.24±0.04 kg/d, P<0.05). During summer, there was a higher proportion of medium follicles (P <0.01) and a lower proportion of large follicles (Table 1). In summer, FF concentration of E2 and the ratio of E2:P4 were reduced, while concentrations of LH were increased (P<0.01). There was no effect of season or nutrition (P>0.05) on the proportion of oocytes at the MII stage of meiosis following *in vitro* maturation.

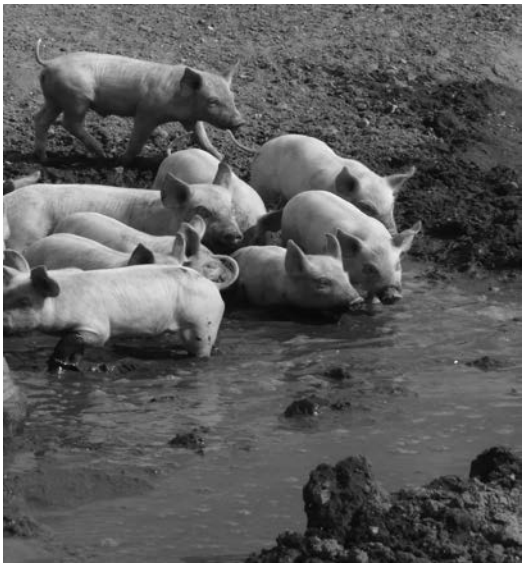
Our data demonstrates that season alters or delays LW gain, peri-ovulatory follicle development and intra-follicular conversion of P4 to E2. Whilst moderate nutritional restriction does not affect follicular development or MII attainment in cycling gilts, it is possible that the effects on oocyte competence may not manifest until the embryonic stage development, and further investigation on moderate feed restriction on fertilisation and embryo development needs to be conducted.

AUVIGNE, V., LENEVEN, P., JEHANNIN, C., PELTONIEMI, O. and SALLE, E. (2010). *Theriogeneology*. **74:**60-66.
FERGUSON, E.M., ASHWORTH, C.J., EDWARD, S.A., HAWKINS, N., HEPBURN, N. and HUNTER, M.G. (2003). *Reproduction.* **126:**61-71.
VAN WETTERE, W.H.E.J., MITCHELL, M., REVELL, D.K. and HUGHES, P.E. (2011). *Theriogenology*. **75:**1301-1310.

This project was funded by Pork CRC Limited Australia.

CHAPTER 8

Herd and Pig Health and Production



REVIEW: Use of oral fluids to monitor health and immunity in pig herds

J. Zimmerman

Iowa State University, Ames, IA 50011, USA.

Abstract

In both human and porcine medicine, infectious agents and antibodies to these agents have been detected in oral fluid samples. Oral fluid samples offer the advantage of sampling groups of animals within a population instead of sampling individuals within a population. The natural behaviour of pigs to chew new objects means that a large proportion of pigs within a pen chew on cotton ropes within a short period and sufficient volumes of oral fluids are collected for laboratory testing. Oral fluid samples are cost-effective and welfare friendly to collect, and analyte or antibody detection can be more sensitive in oral fluids than in serum because the probability of detecting a positive pen is higher with an oral fluid sample than with an individual serum sample. Oral fluid samples are currently used in the USA to perform disease surveillance and (or) monitor herd immunity.

Introduction

Over the course of the 20th century, the majority of pig production metamorphosed from small, extensive, labour-dependent enterprises into intensive, capital-dependent, multi-site production systems. These changes produced safe, wholesome, inexpensive food for consumers. Epidemiologically, however, the same infrastructure and production characteristics that led to improved efficiencies, i.e., large herds with high throughput and extensive pig movement between sites, left the industry vulnerable to the rapid dissemination of infectious diseases. The premise of this paper is that control of infectious diseases - endemic, transboundary, or exotic - cannot occur without effective surveillance. However, effective on-farm surveillance needs to account for the unique features of populations on swine farms:

1. **Subpopulations:** Physical segregation is a necessary part of managing the large populations in modern pork systems. That is, animals on farms are spatially separated by age, production stage and (or) function, with little interaction between these subpopulations. A consequence of physical separation is the creation of subpopulations that differ markedly in disease status. The non-uniform distribution of disease among subpopulations should be expected and accounted for in surveillance;

2. **Extensive, dynamic population change:** In sociology, "population change" is defined as "natural change" (total births minus total deaths) plus migration into (+) or out of (–) the population. In human populations, this change is typically small, i.e., populations are relatively stable. For example, the state of Nebraska recorded a population change of +8.4% in the *decade* between 1990 and 2000 (Deichert, 2001). This consisted of a natural change (births minus deaths) of +5.4% and net migration of +3.1%. In contrast, a finishing barn on a typical farm will experience ~250% *annual* population change as groups of animals enter into the facility, grow, and are sent to market. Although more stable than growing pig populations, ~40% of females in sow herd populations are replaced annually (PigChamp® Benchmark, 2011). The rate of population change in swine populations is important because of its destabilising effect on herd immunity and the need this creates for *continuous* surveillance in highly vulnerable populations;

3. **Metapopulations:** In many parts of the world, large numbers of pigs are transported from distant farms to fill spaces (buildings) vacated as animals are marketed. Economically, it is more efficient to move young pigs to feed than the reverse. Epidemiologically, this practice connects "metapopulations" and facilitates the movement of infectious agents across space.

Large, physically segregated pig populations with high population turnover rates favour pathogens because herd immunity becomes tenuous and unstable under such circumstances (Evans *et al*., 2010). Transport of large numbers of animals from sow farms to finishing sites provides the means for pathogens to rapidly reach geographically distant populations. Under such conditions, surveillance needs to be effective and continuous, if accuracy and timeliness is to be achieved. However, surveillance needs to be easy if it is to be carried out on a routine basis.

Basis of oral fluid diagnostics

In humans: Diagnostics based on oral fluid specimens, as opposed to serum or other specimens, are used extensively in humans (Prickett and Zimmerman, 2010). Human oral fluid diagnostics began in earnest with the isolation of human T-cell leukaemia virus type III (later renamed human immunodeficiency virus - HIV) from buccal specimens from patients with AIDS (Groopman *et al*., 1984), and then the detection of antibodies against HIV in oral fluids (Archibald *et al*., 1986). In 1996, the Food and Drug Administration approved the first oral fluid HIV antibody test (Anonymous, 1996). Thereafter, commercial oral fluid-based assays were developed and commercialised for a wide variety of infectious and non-infectious diseases, drugs, hormones, and disease markers (Mandel, 1993; Tabak, 2007). These tests have been used in large surveillance studies on a variety of infectious diseases of humans, e.g., HIV in Africa (Fylkesnes and Kasumba, 1998; Connolly *et al*., 2004) and Thailand (Frerichs *et al*., 1994), varicella zoster virus in Europe (Quinlivan *et al*., 2013), and measles in Europe (Ramsay *et al*., 1997), Ethiopia (Nigatu *et al*., 1999), Brazil (Oliveira *et al*., 1998) and Africa (Ohuma *et al*., 2009).

In swine: Discoveries in swine mirrored those reported in humans. Corthier (1976) first described the detection of antibodies in swine oral fluids. Either intranasal or intramuscular inoculation with classical swine fever virus (CSFV) produced antibody responses in serum and oral fluid (Corthier and Aynaud, 1977). DeBuysscher and Dubois (1978) found that either oral or Thiry-Vella loop inoculation produced plasma cells in submandibular and sublingual salivary glands of pigs inoculated with *E. coli*. DeBuysscher and Berman (1980) repeated this experiment with transmissible gastroenteritis virus (TGEV) with similar results. In pigs infected with *Actinobacillus pleuropneumoniae* (APP), Loftager *et al*. (1993) found that IgA was detectable in oral fluid before it appeared in serum and concluded that an oral fluid IgA-based assay could be used to screen for APP infection. Ultimately, most swine pathogens and (or) antibodies against them have been reported in porcine oral fluids. In addition to those mentioned above, a partial list would include: African swine fever virus (ASFV) (Greig and Plowright, 1970; Mur *et al*., 2013), *Erysipelothrix rhusiopathiae* (Bender *et al*., 2010), foot and mouth disease virus (FMDV) (Eblé *et al*., 2004), influenza A virus (IAV) (Goodell *et al*., 2013a,b), porcine circovirus (PCV2) (Prickett *et al*., 2008a), porcine reproductive and respiratory syndrome virus (PRRSV) (Prickett *et al*., 2008a,b; Kittawornrat *et al*., 2010), torque teno virus (Pogranichniy *et al*., 2010), and vesicular stomatitis virus (Stallnecht *et al*., 1999). Cumulatively, the data suggest that oral fluid-based diagnostics could be developed to detect most, and perhaps all, infectious agents of swine.

The rationale for oral fluid-based surveillance in swine

The primary roadblock to effective surveillance in swine has been the inconvenience and cost of collecting and testing statistically appropriate numbers of blood, faeces, or nasal swab specimens from individual pigs. In contrast, oral fluid specimens: (1) are collected by a single person, (2) can be collected as frequently as desired without stress to pigs or people, and (3) provide a higher probability of analyte detection with fewer samples than serum (Figure 1; Olsen *et al*., 2013).

Oral fluid is collected from pigs by suspending a length of rope in an accessible location (video at http://vetmed.iastate.edu/vdpam/disease-topics/oral-fluids; see Figure 2). Ropes are hung in a clean area of the pen for 20-60 minutes. Oral fluids are deposited as the pigs chew on the rope. The sample is extracted by inserting the bottom (wet) end of the rope into a plastic bag, compressing the rope to release the fluid, and decanting the fluid into a tube. The volume depends on the age and number of pigs contributing to the sample, but Kittawornrat *et al*. (2010) reported an average sample volume of ~16 ml per animal in a study of PRRSV in individually housed boars. In pens of approximately 20 pigs, Seddon *et al*. (2012) reported that 70% or more of the pigs would interact with rope within in 20 minutes.

Oral fluid sample collection is possible because it coincides with pig behavior. Pigs instinctively test new objects by chewing (Kittawornrat and Zimmerman, 2011). Pigs prefer flexible, destructible, chewable objects, adjectives that exactly describe rope (Feddes and Fraser, 1994; Zonderland *et al*., 2001). For these reasons, oral fluid sampling can be done across a range of pig populations such as piglets on the sow, nursery pigs, grower pigs, pen-housed sows, and individually-housed boars (Kittawornrat *et al*., 2010). These behavioural imperatives operate even during acute infection. Hence, sample collection success rates were unchanged during acute IAV (Millman *et al*., 2009) and PRRSV (Kittawornrat *et al*., 2010) infections.

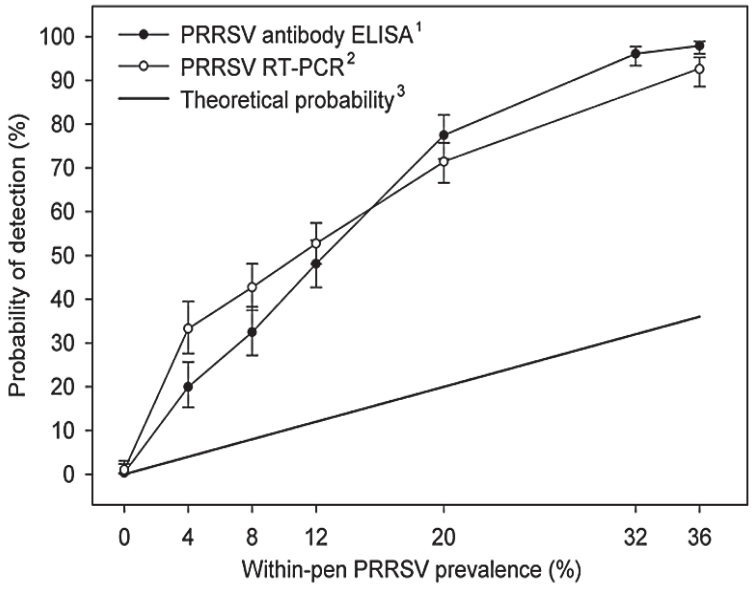


Figure 1. *Probability of a PRRSV-positive oral fluid result as a function of within-pen prevalence modeled using logistic regression. The analysis was based on testing at eight laboratories, with six laboratories performing antibody assays and six laboratories performing nucleic acid assays (from Olsen* et al.*, 2013). NOTE: The simple probability of detecting a positive pen by randomly selecting and testing one pig in a pen is shown by the straight line at the bottom of the graph. The area between the simple probability line and the logistic regression lines is the improved probability of detection using oral fluid.*

Oral fluid assay performance characteristics

Oral fluid-based assays are capable of excellent diagnostic performance. In humans, a recent meta-analysis showed that the OraQuick® Advance rapid (20 minute) HIV-1/2 antibody assay displayed 99.1% sensitivity and 99.6% specificity (Delany *et al*., 2006). In swine, the diagnostic sensitivity and specificity of a commercial serum antibody ELISA modified to detect PRRSV IgG antibodies in pen-based oral fluid specimens was estimated at 94.7% (95% CI: 92.4, 96.5) and 100% (95% CI: 99.0, 100.0), respectively (Kittawornrat *et al*., 2012).

Olsen *et al*. (2013) estimated the probability of detecting PRRSV infection in pen-based oral fluid samples using pens of known PRRSV prevalence. Twenty-five pens were assigned to one of five levels of PRRSV prevalence (0%, 4%, 12%, 20%, or 36%) by placing a fixed number (0, 1, 3, 5, or 9) of PRRSV-positive pigs (14 days post PRRSV MLV vaccination) in each pen. Among the 100 samples from pens containing ≥1 positive pig (≥4% prevalence) and tested at the six laboratories, 62% were positive for PRRSV RNA and 61% for PRRSV antibody (Figure 2).

Routine use in the field

The Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) introduced routine oral fluid testing in 2010. The numbers of samples tested indicates that swine producers and veterinarians have rapidly adopted this approach, i.e., the ISU-VDL performed 10,329 tests on swine oral fluids in 2010, 32,517 in 2011, 60,192 in 2012, and anticipate >80,000 samples in 2013. Likewise, other veterinary diagnostic laboratories in North America specialising in swine diagnostics also offer swine oral fluid testing. Commercial assays for the detection of antibodies or nucleic acids in swine oral fluids are reaching the market, e.g., the IDEXX® PRRS Oral Fluid Ab ELISA (IDEXX Laboratories, Inc.) was released in 2012. Meanwhile, oral fluid assay research and development continues, as does commercialisation of new diagnostic products for swine oral fluids.

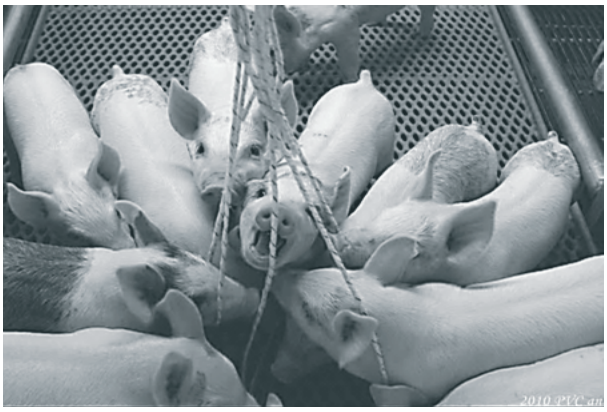


Figure 2. *Oral fluid specimens are collected in a welfare-friendly process that both caretakers and pigs enjoy (photo courtesy of Gordon Spronk, DVM).*

Conclusions

The purpose of disease surveillance is to control infectious diseases, assure animal health and welfare, improve producer profitability, and protect valuable national assets. Although serum is the traditional ante-mortem specimen, oral fluid samples have become a commonly used alternative in surveillance. As reviewed, a series of research and field studies have shown that pen-based oral fluids can meet or exceed the performance of serum for the detection of a variety of pathogens using either PCR- or antibody-based assays. On the farm, integration of surveillance data with herd records will provide the means to: (1) identify the circulation of specific pathogens; (2) quantify their effects on pig health and productivity; (3) target interventions to the correct pathogen and population; and (4) time the intervention for maximum effect. At the regional level, oral fluid-based surveillance would make producer-driven area control programs more practical and affordable. At the national level, a surveillance infrastructure based on assays optimized for the oral fluid matrix will facilitate rapid collection of data for national control-and elimination-programs or containment of transboundary and (or) foreign animal disease pathogens.

References

ANONYMOUS. (1996). *AIDS Alert.* **11:**94.

ARCHIBALD, D.W., ZON, L., GROOPMAN, J.E., McLANE, M.F. and ESSEX, M. (1986). *Blood.* **67:**831-834.

BENDER, J.S., SHEN, H.G., IRWIN, C.K., SCHWARTZ, K.J. and OPRIESSNIG, T. (2010). *Clinical and Vaccine Immunology*. **17:**1605-1611.

CONNOLLY, C., SHISANA, O., COLVIN, M. and STOKER, D. (2004). *South African Medical Journal*. **94:**776-781.

CORTHIER, G. and AYNAUD, J. (1977). *Annals of Veterinary Research*. **8:**159-165.

CORTHIER, G. (1976). *Annals of Veterinary Research.* **7:**361-372.

DEBUYSSCHER, E. and BERMAN, D. (1980). *American Journal of Veterinary Research*. **41:**1214-1220.

DEBUYSSCHER, E. and DUBOIS, R. (1978). *Advances in Experimental Medicine and Biology*. **107:**593-600.

DEICHERT, J. (2001). *Focus: Nebraska.* **1:**1-10. (Center for Public Affairs Research, University of Nebraska at Omaha, NE, USA).

DELANEY, K.P., BRANSON, B.M., UNIYAL, A., KERNDT, P.R., KEENAN, P.A., JAFA, K., GARDNER, A.D., JAMIESON, D.J. and BULTERYS, M. (2006). *AIDS.* **20:**1655-1660.

EBLÉ, P., BOUMA, A., DE BRUIN, M., VAN HEMERT-KLUITENBERG, F., VAN OIRSCHOT, J. and DEKKER, A. (2004). *Vaccine.* **22:**1372-1378.

EVANS, C.M., MEDLEY, G.F., CREASEY, S.J. and GREEN, L.E. (2010). *Preventive Veterinary Medicine*. **93:**248-257.

FEDDES, R. and FRASER, D. (1994). *Transactions of the American Society of Agricultural Engineers.* **37:**947–950.

FRERICHS, R.R., SILARUG, N., ESKES, N., PAGCHAROENPOL, P., RODKLAI, A., THANGSUPACHAI, S. and WONGBA, C. (1994). *AIDS*. **8:**885-894.

FYLKESNES, K. and KASUMBA, K. (1998). *AIDS*. **12:**540-541.

GOODELL, C.K., PRICKETT, J., KITTAWORNRAT, A., ZHOU, F., RAUH, R., NELSON, W., O'CONNELL, C., BURRELL, A., WANG, C., YOON, K.-J. and ZIMMERMAN, J.J. (2013a). *Veterinary Microbiology*. **166:**450-460.

GOODELL, G.K., PRICKETT, J., KITTAWORNRAT, A., JOHNSON, J., ZHANG, J., WANG, C. and ZIMMERMAN, J. (2013b). *Transboundary and Emerging Diseases*. (submitted).

GREIG, A. and PLOWRIGHT, W. (1970). *Journal of Hygiene*. **68:**673-682.

GROOPMAN, J., SALAHUDDIN, S., SARNGADHARAN, M., MARKHAM, P., GONDA, M., SLISKI, A. and GALLO, R. (1984). *Science.* **226:**447-449.

KITTAWORNRAT, A., PRICKETT, J., CHITTICK, W., WANG, C., ENGLE, M., JOHNSON, J., PATNAYAK, D., SCHWARTZ, T., WHITNEY, D., OLSEN, C., SCHWARTZ, K. and ZIMMERMAN, J. (2010). *Virus Research*. **154:**170-176.

KITTAWORNRAT, A., PRICKETT, J., WANG, C., OLSEN, C., IRWIN, C., PANYASING, Y., BALLAGI, A., RICE, A., MAIN, R., JOHNSON, J., RADEMACHER, C., HOOGLAND, M., ROWLAND, R. and ZIMMERMAN, J. (2012). *Journal of Veterinary Diagnostic Investigation*. **24:**262-269.

KITTAWORNRAT, A. and ZIMMERMAN, J. (2011). *Animal Health Research Reviews*. **12:**25-32.

LOFTAGER, M., ERIKSEN, L. and NIELSEN, R. (1993). *Research in Veterinary Science*. **54:**57-62.

MANDEL, I.D. (1993). *Annals of the New York Academy of Sciences*. **694:**1-10.

MILLMAN, T., BROOKS, R. JR., ZIMMERMAN, J. and IRWIN, C. (2009). *Journal of Animal Science*. **87:** E-Suppl:iii.

MUR, L., GALLARDO, C., SOLER, A., ZIMMERMAN, J., PELAYO, V., NIETO, R., SÁNCHEZ-VIZCAÍNO, J.M. and ARIAS, M. (2013). *Veterinary Microbiology*. **165:**135-139.

NIGATU, W., NOKES, D.J., ENQUSELASSIE, F., BROWN, D.W.G. and COHEN, B.J. (1999). *Journal of Virological Methods*. **83:**135-144.

OHUMA, E.O., OKIRO, E.A., BETT, A., ABWAO, J., WERE, S., SAMUEL, D., VYSE, A., GY, N., BROWN, D.W. and NOKES, D.J. (2009). *Epidemiology and Infection*. **137:**227-233.

OLIVEIRA, S.A., SIQUEIRA, M.M., BROWN, D.W., CAMACHO, L.A., FAILLACE, T. and COHEN, B.J. (1998). *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **92:**636-638.

OLSEN, C., WANG, C., CHRISTOPHER-HENNINGS, J., DOOLITTLE, K., HARMON, K., ABATE, S., KITTAWORNRAT, A., LIZANO, S., MAIN, R., NELSON, E., OTTERSON, T., PANYASING, Y., RADEMACHER, C., RAUH, R., SHAH, R. and ZIMMERMAN, J. (2013). *Journal of Veterinary Diagnostic Investigation*. **25:**328-335.

PigCHAMP Benchmarking. (2012). USA 2012 - Second quarter summary. (Ames, IA, USA).

POGRANICHNIY, R.M., PRICKETT, J., MAIN, R., CLARK, A. and ZIMMERMAN, J. (2010). In "International Scientific and Practical Conference on Modern systems of Biosecurity and Biosafety as the Basis for Infectious Diseases Control Strategy in Veterinary and Human Medicine", pp. 52-53 (Feodosia, Ukraine).

PRICKETT, J., SIMER, R., CHRISTOPHER-HENNINGS, J., YOON, K.-J., EVANS, R.B. and ZIMMERMAN, J. (2008b). *Journal of Veterinary Diagnostic Investigation*. **20:**156-163.

PRICKETT, J., SIMER, R., YOON, K.-J., KIM, W.-I. and ZIMMERMAN, J. (2008a). *Journal of Swine Health and Production*. **16:**86-91.

PRICKETT, J.R. and ZIMMERMAN, J.J. (2010). *Animal Health Research Reviews*. **11:**207-216.

QUINLIVAN, M., SENGUPTA, N., PAPAEVANGELOU, V., SAUERBREI, A., GRILLNER, L., ROUSSEVA, R., HAGUE, R., LUTSAR, I., JOGI, P., LECA, A., GRYTCHOL, R., ALAIN, S. and BREUER, J. (2013). *Journal of Infectious Diseases*. **207:**588-593.

RAMSAY, M., BRUGHA, R. and BROWN, D. (1997). *Bulletin of the World Health Organization*. **75:**515-521.

SEDDON, Y.M., GUY, J.H. and EDWARDS, S.A. (2012). *Veterinary Journal*. **193:**180-184.

STALLKNECHT, D., HOWERTH, E., REEVES, C. and SEAL, B. (1999). *American Journal of Veterinary Research*. **60:**43-48.

TABAK, L.A. (2007). *Annals of the New York Academy of Sciences*. **1098:**7-14.

ZONDERLAND, J., VERMEER, M., VEREIJKEN, G., and SPOOLDER, M. (2001). In "Proceedings of the International Symposium of the C.I.G.R. Animal Welfare Considerations in Livestock Housing Systems", pp. 147-156, 2nd Technical Section (Technical University of Zielona Góra, Poland).

REVIEW: Managing poultry health in Australia: Lessons for the pig industry

P.C. Scott

Scolexia Pty Ltd, Moonee Ponds, VIC 3039.

Abstract

The Australian poultry industry consists of the chicken meat and egg sectors, and the smaller duck and turkey sectors. The chicken meat industry has been particularly prominent by its rapid and successful growth into an industry with high quality capital investment and productivity outputs equivalent to world best standards. Poultry health programs have also meant that endemic disease is generally well controlled and the need for therapeutic intervention is minimal. This contrasts to some parts of the Australian pig industry that finds itself with old infrastructure, productivity not to the world's best standards, and a heavy reliance on the use of therapeutics. This paper aims to provide the reasons why the growth and overall standards of the poultry industry exceed those generally seen in the pig industry, and offers some insights for the pig industry in how improvements to pig health and welfare may be attained.

Introduction

Historical perspectives of the poultry industry in Australia

The prominent two entities in the Australian poultry industry are the chicken meat (broiler) and commercial table egg sectors. The turkey and duck meat sectors are relatively small by comparison, only accounting for about 3% of the total poultry meat consumed or equivalent to less than 2 weeks of broiler production in terms of numbers. Chicken meat per capita consumption for 2012/2013 is estimated to be around 45 kg, now surpassing beef (36 kg) and higher than pork (25 kg). Table egg consumption, despite undergoing a substantial decline in the 1980s because of perceived health concerns, has recovered back to a per capita consumption of around 220 eggs (ACMFI 2013).

The chicken meat industry was originally based on spent layers and layer cockerels in an era in which the consumption of chicken meat was low (around 4 kg/head) and the now banned (since the mid 1950s) use of chemical caponisation was practiced. The consumption of chicken meat was an uncommon meal and one considered for special occasions. In the 1960s and 1970s the development of meat strains lead to the beginning of the chicken meat industry as known today. Then in the 1980s, with the development of the Steggles-type bird of "more meat less fat", chicken meat consumption was becoming a commodity meal, but with annual per capita consumption figures of around 20 kg, consumption was still modest by today's standards.

Initially the majority of broiler houses were naturally ventilated sheds until the introduction of controlled environment cross-flow sheds that enhanced air quality. The sealed sheds had extraction fans positioned down one length of the shed, and had an adjustable inlet flap lengthwise down the other side of the shed. By varying the number of operating fans, either initiated through minimal ventilation, temperature and (or) time pulsing, and concurrently controlling the size of the inlet flap in proportion to the number of fans operating, the shed was able to create air speed across the width of the shed. This airflow enhanced air quality by creating an admixture of air in the shed, assisted in drying litter and also created a chill factor in the hotter weather. Cooling pads (water walls) were also incorporated in the inlet flaps for evaporative cooling in the hot weather. These sheds inherently required backup generators in the case of power failure and were expensive to run with inefficient electric fan motors. Combination tunnel controlled environment sheds, as discussed later in this paper, have replaced cross-flow sheds more recently.

The industry was also commencing to experience some consolidation of the major producers, with the buyout particularly of the smaller hatcheries. The table egg industry was still relatively unchanged, with the majority of layers being housed in conventional cages in naturally ventilated sheds, with large numbers of small producers. Importantly for both sectors, profitability was good with chicken meat selling for more than 40% of today's prices and egg prices similar to today's farm gate prices. The issues of regulatory compliance, environment and welfare were not high priorities. This started to change significantly in the early 1990s with community concerns about the environment, residential encroachment and odour in the broiler industry, and "battery cages" in the layer industry. It would be correct to say that both industries underestimated what impact these community and consumer reactions would have on the Australian poultry industry.

The growth of the chicken meat and table egg industries started to diverge due to a decline in egg consumption and a rapid growth in chicken meat consumption. This directly reflected on the capital investment in the industry, with egg producers declining in numbers and some consolidation of the industry through closure and buyouts. Conversely the chicken meat industry commenced an unprecedented expansion of broiler shedding, hatcheries and processing plants. Contracted growers and not the producer entities to which the growers were contracted primarily funded the growth in the broiler shedding.

However, all poultry sectors realised that there was a need for improved efficiencies in the industry, not only through better facilitation and health programs, but also by the introduction of international genetics through the importation of hatching eggs. The Australian poultry sector realised that with such a small domestic gene pool, it was not feasible to achieve the performance gains required, and regular imports began once the Biosecurity Australia protocols were in place. The chicken meat industry currently imports Great Grandparent (GGP) and Grand Parent (GP) hatching eggs as regularly as every 6 months, while the egg layer and the non-chicken poultry sectors import every 3 to 5 years. Concern that the importation of hatching eggs would open the Australian industry to chicken meat imports was considered, but the industry's negative status for virulent infectious bursal disease (IBDV) or Gumboro disease, which is now present in all countries except New Zealand and Australia, provided an effective biological trade barrier (DAFF 2013). Pasteurised egg powder is permitted for importation into Australia, something that was initiated by the egg producers to control short falls in local product production.

The growth of the turkey meat industry has not experienced that of chicken meat, with less than 5 million birds produced annually. The market is still highly geared for the Christmas period but even here the tradition of having a whole bird on the table is declining. For ducks there has been a steady increase, with consumption at around 10 million birds per year.

Drivers of the industry approach to poultry health

The closed border policy to poultry imports in the period from the 1960s to 1980s did, by default, have a favourable outcome on the Australian poultry industries' approach to poultry health. The industry decided to undertake its own research and development in the development of vaccines specifically suited to the Australian poultry industry. As a consequence of this, the contribution to the understanding of poultry diseases and vaccine development by Australian veterinarians and scientists on the international stage was significant. This legacy was particularly as a consequence of the many regional veterinary laboratories in each state, and industry and government financial support through the establishment of the Chicken Meat Program, Rural Industry Research and Development Corporation (RIRDC) program and the Egg Industry Research and Development Corporation (EIRDC). University contributions were significant also with the development of many quality vaccines against diseases such as Newcastle disease (NDV) (Bell *et al*., 1991), Infectious laryngotracheitis (ILT), Infectious bronchitis (IBV) (Hewson *et al*., 2012), *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS) and a live attenuated *Pasteurella multocida* vaccine (Scott *et al*., 1999). A number of these vaccines are now used worldwide (Morrow and Whithear, 2011). These vaccines can be traced through the Australian Pesticides and Veterinary Medicines (APVMA) web site (www.apvma.gov.au/). This legacy still continues with Australia having one home-grown international poultry vaccine company (Bioproperties, 2013). The poultry industry is thought fortunate compared to the pig industry in that most poultry diseases are viral in origin, making control through vaccination easier. In comparison, many pig diseases are associated with chronic bacterial colonisation, which is more difficult to control by vaccination.

Optimising poultry health has also been driven by significant internal domestic competition, which reduces margins and makes it uneconomical to grow and medicate poor performing and disease affected flocks. The use of strategic in-feed and water medications in broilers, like that which occurred in the 1980s, is uncommon, if non-existent today, with extensive vaccination programs, sophisticated and quality facilitation and high level husbandry replacing the need. The sophistication of modern poultry shed housing is complemented by the sophistication of the facilitation of the sheds, including the drinking and feeding systems, efficient gas heaters, energy efficient high capacity fans, static pressure controlled inlet vents and controllers that coordinate all this supply, and environmental management equipment to optimise shed conditions for a particular phase of the bird's growth and (or) stage of production. These controllers monitor all aspects of the shed operations and environment. Growers are notified of failure of the shed systems, back up operation and optimal bird safety and environment. The latter particularly drives the contracted growers who operate in a pool-based grow-fee and efficiency system, where grower payments are reduced for poor performance and continued "inefficient" performance results in loss of contract. In contrast, growers who perform at the top of the pool receive higher grow fees (the difference coming from their poor performing fellow growers), and are allowed by processors to increase the number of contracted sheds. This expansion in shedding is currently at the rate of 3 to 5% per annum in Australia.

Another driver of improved health has been the government pressure through the Emergency Animal Health Cost Sharing Agreement, which was in part initiated by the federal and state governments. Governments were dissatisfied with continued fundamental failures in biosecurity, resulting in Emergency Animal Disease (EAD) outbreaks, and the inability of a number of the livestock industries to operate under best practice. During this period the government was responsible for all costs associated with the eradication and cleanout program, which included compensation to the affected producer. For the poultry industry this involved ongoing outbreaks of NDV and avian influenza (AI). Under the new cost sharing agreement, the chicken meat and egg industries were required to assure the government that biosecurity manuals and best management farming practices were developed and implemented through industry training courses. Additional drivers to improved performance were local authority and community pressures to close down older and poorer performing facilities that were now experiencing reverse buffer encroachment from lifestyle residential living in the peri urban areas, such as the Mornington Peninsular in Victoria. These older style sheds were considered, even if only by perception in some cases, to have a reduced ability to control litter moisture and thus odour generation, both in cold and hot weather when fogging. The loss of these farms was not considered negative by processors who did not want to be responsible for the grief associated with these contracted farms, in conjunction with wanting newer style shedding on large farms with improved access to B-double transport vehicles. This termination of older-style contracted shedding has continued by processors and is coordinated with the ongoing line of private investors who want to build modern broiler complexes.

The egg industry, in contrast, has been essentially forced by consumer welfare concerns, and thus retailer requirements, to move production systems to less efficient systems with the rapidly increasing demand for free-range eggs, but at profit margins that are becoming less attractive. In the early 2000s, it was legislatively agreed that cage facilities would stay for many years in Australia, provided facilities containing non-compliant cages were phased out and all new facilities had cages at 550 cm^2 per bird. Producers spent large amounts of capital building large and sophisticated cage facilities. This decision was based on the new cage code, but erroneously did not consider the consumer sentiment towards cage eggs compared to free-range eggs. Thus with an almost exponential increase in demand for free-range eggs at the expense of cage eggs, the price paid for cage eggs is now unprofitable. Cage egg producers are caught in a downward profit spiral, with high capital quality cage facilities that no one wants to purchase and existing debt. The paradoxical aspect of this is that from a performance efficiency and bird health perspective, free-range production is inferior to cage production.

Achieving poultry health through multifactorial inputs

Optimisation of poultry health in a sustainable manner can only be achieved from a multifactorial approach. The various input factors must be cohesively coordinated, and importantly by a cooperative and functionally competent management team that includes the senior management, veterinarians, nutritionists, farm serviceman and managers, sales and marketing staff and even the accountants. Without this holistic approach to poultry management, no satisfactory and long-term outcome can be achieved for the poultry business, which is fundamentally driven by optimising the costs of producing the final poultry product, whether this is meat or eggs. Importantly, each division of the company needs to realise its impact, and thus responsibility, in achieving a good poultry health outcome. The factors that directly impact on poultry health and are influenced by each of the sectors include the following:

- Genetics selected for the business model;
- Quality of the housing and the facilitation;
- Primary health status of the livestock;
- Poultry health program;
- Quality of nutrition;
- Husbandry skills and their implementation by service staff and farm managers;
- Market profile and how it impacts on stocking densities and depopulation policies;
- Correct application of expenditure within the company.

The failure not to consider all of the above points, and to not recognise each for their importance in any livestock health strategy, will mean that there will never be an optimal and sustainable outcome for the livestock business. The Australian poultry industry has generally been successful in recognising the need to achieve best practice standards in all sectors. The poultry industry has continued to regularly import the best current world genetics that is both of a high health status and selected for liveability. Liveability is a

term used by the poultry industry to reflect the robustness of the bird to live under commercial conditions and to demonstrate low mortality from metabolic, growth-related and even infectious disease (Fanatico *et al*., 2008). These birds are then placed in modern world-class facilities and supplied with optimally specified feed, with quality ingredients. In addition, rigorous preventative health programs are applied to disease free breeding stock in the rearing phase (the period from day of age to the onset of sexual maturity and production). Associated with this is the technical and husbandry input of proficient staff that have been both mentored within the company and trained externally through such avenues as Certificate III training courses, workshops, seminars and internships. There is also a coordinated understanding with marketers that high stocking densities, placement and depopulation strategies that do not prioritise single age farms and product balance can affect flock liveability. Within the Australian poultry industry it is also recognised that money is spent wisely on quality cleanouts and sanitation programs, strategic maintenance, specialist contractors such as vaccination crews and regular facility upgrades. All of these factors ensure a more favourable outcome in regard to poultry health and importantly food safety (Gradel, 2003).

Genetics

Unlike the pig industry, all sectors of the Australian poultry industry regularly import new genetics in the form of hatching eggs through Post Arrival Quarantine (PAQ) facilities, which are either privately or government operated. Not only does this ensure that the performance of the Australian poultry livestock is to world standards, but the inclusion of other selection criteria such as liveability and welfare soundness ensure suitability of the birds for the specific production systems and consumer requirements (Fanatico *et al.*, 2008). The importation of new "clean" genetic stock also allows producers to more rapidly address an existing endemic change in health status that may be affecting performance. Avian leukosis virus subgroup J (ALV-J) for example was introduced into Australia through imported hatching eggs prior to it being recognised as a significant disease entity, and it caused significant production losses. The removal of this virus from the Australian breeder industry was only made possible by importing new genetics free of J leucosis. (Bagust *et al*., 2004). The free movement of this imported livestock throughout the entire Australian poultry industry also means that all companies experience the benefits, which encourages and allows a more uniform and consistent approach to husbandry.

Shedding and facilitation

Unlike some parts of the pig industry, the chicken meat sector has continued to spend major capital on shedding, facilities and equipment. This has been driven not only by the increase in consumption of chicken meat by more than 3% per year, but also because of the drive by processors and growers to have world standard shedding and equipment. It is not uncommon for poultry company owners to reduce the take on profits, and use this money instead to upgrade facilities using the "latest and the best" technologies. The need to do this is also driven by the small margins in the business that essentially mandates the optimising of performance efficiencies. Typically for broiler growers this means having larger farms with larger sheds and more automation. A farmer in the 1970s would typically have sheds with 20,000 birds and farm sizes of 40,000 to 60,000. Now, the model farm operated by a single manager is 320,000 birds with sheds containing 50,000-60,000 birds.

The majority of these new sheds have a controlled environment, as combination tunnel (transitional ventilation) sheds. These sheds have both side wall or ridge fans and end wall extraction fans that operate separately or in a transitional manner to allow for optimal ventilation during the birds growth cycle under variable ambient conditions. Combi-Tunnel broiler sheds are best practice chicken meat sheds that utilise the concept of Minimal Transitional Tunnel (MTT) ventilation. The sheds are operated under negative pressure. The sheds are facilitated with a two-component ventilation system that allow for optimal maintenance of shed conditions under different ambient and livestock conditions. The first component is termed minimal ventilation (MV) where a series of fans are installed either along the side walls or ridge line of the shed. These fans operate under conditions where only minimal air exchange is required to ensure optimal growth and performance of the broiler bird, while concurrently maintaining optimal environmental conditions within the shed. When the shed is operating under MV, air is brought into the shed via a series of small adjustable openings called mini vents that are positioned in an elevated position along the side walls. The size of the aperture of these mini vents is controlled usually via a static pressure sensor or in some cases the number of fans operating.

The second component of the ventilation system is a bank of fans at one end of the shed that have the capacity to provide the maximum ventilation required by the birds. Air enters into the shed under negative pressure at the opposite end of the shed to the side wall apertures. These are adjustable and usually incorporate evaporative cooling pads for hot weather operation. This type of ventilation is called tunnel ventilation (TV) and air speeds of approximately 2 to 3 metres per second are achieved, which results in a chill factor of up to 6 °C that further assists in the cooling of the bird by removal of metabolic heat.

Computerised controllers in these sheds are programmed to selectively operate the components of the ventilation system, which are determined by bird age, weight and ambient conditions. The controller also allows the shed ventilation system to transition from MV to TV, hence the term MTT. Generally the shed operates in MV when the birds are small, transitioning into TV as the birds increase in weight. The shed may move between MV and TV during this transitional period depending on the ambient conditions. In the later part of the batch cycle, after the shed has been partly depopulated and the total bird mass has been reduced, the shed may also operate in MTT. Over the life of the batch, the great majority of ventilation is via the end wall extraction or tunnel ventilation fans.

Concurrent with this is the need for optimal feed conversion and mortality but with efficient shed running costs, which are achieved with sophisticated control systems incorporating multiple sensors, monitoring capabilities and critical back up and alarm systems. The required brooding temperatures can be achieved under all ambient conditions and temperature step-down curves accurate to within 0.5 ºC. Ventilation systems allow optimal air quality and litter moisture control when correctly applied in conjunction with a good husbandry understanding. All facilities such as feeder space, drinker numbers and flow rate, air speed, fan capacity per kilogram live body weight and heating and cooling capacity are all strictly prescribed by processors' minimum standards, which must be met before contracts are supplied. These minimum standards have been developed over decades by the poultry industry and the summation of these findings is referenced in the current poultry industry manuals produced by the breeder companies, for example Aviagen and Cobb Vantress (www.aviagen.com; www.cobb-vantress.com). Failure to meet these standards or system failures in sheds result in either, or both, the loss of contract and financial penalties. This need for quality facilitation and shed design is not only prescribed by processors but also by the responsible planning authorities, who legislatively enforce the minimum standards in broiler codes which are usually incorporated into state planning legislation.

Companies have similarly applied the same world best practice policies to rearing and breeding sheds and hatcheries. The technologies in such facilities allow the specific allocation of nominated feed quantities to breeding stock, the automatic measurement of water intake (down to minutes each day), and also automatic weighing. This technology has advanced to allow modem reporting back to the administration office, where for broilers, the reported weights assist to coordinate pickups.

The modern Australian hatcheries also now include the technology to undertake *in ovo* vaccination, which allows the young chick to develop active immunity even before hatching. This technology is now advancing to provide nutrient supplements to the young developing chicken in the egg (Islam *et al*., 2000; van den Wijngaard, 2002; Uni *et al*., 2005).

Primary health status

Pivotal in the production of all parent breeding stock is to have pathogen-free-status for all important poultry pathogens. Thus in Australia, all parent breeder and laying stock are negative for Mycoplasma, historically the cause of chronic respiratory disease. At the GG and GP level, all stock is free of Salmonella and where there is a change of status, these flocks are depopulated. The strict Australian border controls and protocols in the PAQ import facilities assist Australia to be one of the best poultry health status countries in the world, after New Zealand. In the layer industry it is common practice for clients buying reared pullets to be provided with the results of flock testing for Salmonella, Mycoplasma and serological evidence that the vaccination program has been efficacious prior to delivery. Generally about 2 to 3 weeks prior to the transfer of the reared flock to the production farm, blood samples are taken for serology to confirm the absence of a number of avian pathogens (of both domestic and wild birds) and also to see if the required immunological response has been achieved after vaccine delivery. Microbiological swabs of the environment will also be taken to reflect the status of the flock. Polymerase chain reaction (PCR) may be used where a differentiation between wild type and vaccine strain is required. Following such testing a flock of known desirable status can then be transferred with confidence. The rearer of the birds usually covers the cost of testing the birds whether this is a supplier, a contractor, or an in house farming section.

Poultry health program

Under the premise and the accepted standard that a poultry health program commences with a disease-free, day-old chicken, the aim of the poultry veterinarian is to ensure that the bird remains free not only of clinical disease, but remains free of being colonised by avian pathogens. To achieve this, fundamental principles are applied in the Australian poultry industry:

- Breeder flocks at all levels are monitored regularly throughout production for any change in health status, and this is usually undertaken by serology or microbiological culture of the bird or environment, and in some cases PCR. The majority of the veterinary diagnostic testing is done at private laboratories, with declining levels of testing done at government and university

laboratories. Large poultry companies also do some basic serological and microbiologically testing in house.

- Further monitoring is undertaken at the hatchery particularly for Salmonella.
- All shedding (rearing, production and broiler) prior to placement has all litter removed and a dry clean, wet detergent-based wash down, disinfection and insecticide treatment (Gehan *et al*., 2009). The disinfectants used are of all the common categories and the choice of product, while ideally based on efficacy against all poultry pathogens, will in reality be chosen as a compromise between cost, occupational health and safety reasons, and efficacy under low or high organic loads. Water lines are flushed and sanitised and the rat-baiting program replenished. Sheds may be microbiologically tested for the effectiveness of the cleanout and wash-down prior to placement. In the large integrated chicken meat companies, the growers are mandated to follow prescriptively the cleanout and disinfection program supplied by the processor. In the layer industry and the other poultry sectors, cleaning programs are usually devised in-house and implemented with varying levels of efficacy and quality. In the chicken meat (broiler) sector, where farms are single age and all-in all-out, a total removal of used litter and a full wet wash down and disinfection is undertaken at the end of each batch, that is every 7 to 10 weeks. In the layer sector, where some sheds are multiple age and most farms are multiple age, the cleanout and disinfection programs are significantly varied. Generally though, all rearing of young birds is in single age sheds with a full cleanout, wash down and disinfection between batches.
- All shed placements of birds to be reared are single age and preferably, but not invariably, on a single age site. Birds are vaccinated in the rearing sheds for essentially all endemic viral poultry diseases in Australia. Bacterial diseases such as fowl cholera are included in the vaccination program if there is a history of this disease on the production site. Birds are ideally not transferred to the production farm until adequate time for protective immunity to develop after vaccination, around 4 weeks, and until the vaccination efficacy serology has been completed.
- Generally all breeder and rearing farms have strict biosecurity visitation conditions and have shower on facilities.
- Prior to the transfer of birds to productions farms, they are serologically tested to ensure the vaccination has been efficacious, as indicated and implied by an appropriate antibody response. Where the vaccination response is considered inadequate the birds are revaccinated. The shed deep litter or slatted floor environment is also tested for Salmonella by utilising drag swabs. While the number of bloods taken for testing is practically considered to be not statistically significant, the numbers are limited because of the economics of testing. However, the outcomes of the testing are usually indicative because it is expected that all birds in the flock (close to 100%) should be immune as a consequence of vaccination, and this should be reflected even in the small number of bloods. Environmental drags swabs are extremely sensitive to identifying very low levels of environmental contamination and thus will identify very low numbers of infected birds. Veterinarians may not always be involved in a serological testing program, often this is undertaken and interpreted by the experienced farm operator
- For long-lived poultry, birds are re vaccinated in lay to boost their immunity.

It is routine in the Australian poultry industry for long-lived birds, such as broiler breeders and commercial layers, to be vaccinated for the majority of endemic poultry diseases as a matter of course, regardless of whether disease is absent on the rearing or production site. The birds are still vaccinated to ensure that any wild avian pathogen challenge is displaced and fails of colonise the population. Thus vaccination is an insurance policy, and this policy has the benefit of displacing wild type organisms from the operation. Vaccination is considered one aspect of biosecurity and a method by which avian pathogens are restricted from colonising a site. While control of viral diseases through vaccination is relatively efficacious, control of bacterial diseases such as Mycoplasma and *Pasteurella multocida* by vaccination will be continued well beyond presence or failure to detect the agent. Long-lived birds will usually be vaccinated to protect them against about 12 to 14 avian pathogens, which are either applied at the hatchery by *in ovo* or coarse aerosol spray, and in the field by drinking water, eye drop or injection.

Considering the number of birds involved and the number of vaccines applied, external vaccination crews are usually contracted to do the work for the vaccines where the birds need to be picked up and handled. They will concurrently undertake activities like beak trimming and grading in some operations. The poultry industry has produced training booklets and training workshops for vaccination crews and

Certificate III training courses are also available. Each company usually maintains a batch summary sheet for each flock that records the historical treatments, vaccinations and testing that was done in each flock. This includes a sign off and recording of vaccine batch numbers. Post vaccination, and several weeks before transfer (or production onset on day old to death sites), a total of 15-25 birds is bled for each 6,000- to 30,000-bird flock. These bloods are forwarded to one of several veterinary diagnostic laboratories where the bloods are serologically tested using either haemagglutination inhibition (HI), ELISA and occasionally agar gel diffusion for antibody levels, where cut off points determine the efficacy of the vaccination program and application. Testing can also concurrently determine if there has been a wild type challenge for some of the avian pathogens. The differentiation of a wild type or vaccination response is dependent on the avian pathogen. Some vaccines, such as the live Mycoplasma vaccines, give a minimal serological response and thus any high titres suggest a wild type response. For other agents, it can be the size of the antibody response in conjunction with the timing of the vaccination that will assist in differentiating a wild type response. Bacterial culture, PCR detection or sequencing of pathogens is used as the gold standard to differentiate wild-type infections from vaccination.

Birds' health is assessed prior to coming into lay. For diseases such as classic infectious bursa disease (IBD) and avian encephalomyelitis (AEV), vaccination of breeders also protects their progeny. In the case of IBD, it is the antibody titre levels that are important, because maternal antibodies are required to protect the young chick up to about 21 days of age. For diseases like NDV, it is mandatory in most states of Australia for producers to vaccinate. Governments introduced this mandate as a condition if the poultry industry wanted to have NDV remain in theEAD cost sharing agreement.

Broilers are generally only vaccinated *in ovo* at the hatchery for Marek's Disease and by coarse aerosol spray for infectious bronchitis (IBV) and, in most states, for NDV using live vaccines. While not all broilers are vaccinated for MDV and NDV all are for IBV, which equates to 10 million doses per week.

The success and effectiveness of avian viral vaccinations is generally good provided they are applied correctly and serological testing undertaken to monitor the efficacy of vaccination. An example where this outcome has not occurred is in the occurrence of ILT over the last 5 years in the chicken industry. The control of the disease has been limited by a combination of factors including the limited and inconsistent supply of vaccine, poor vaccine applications and inappropriate vaccination programs. Historically the industry has applied this "respiratory" vaccine via the drinking water as an easier means of application instead of the recommended and preferred method of eye drop. The historical vaccination for ILT appeared to be effective but in reality it was often of limited effectiveness, and the reason that clinical disease was not seen was because there was no challenge. With the occurrence of ILT as an emerging disease, vaccination failure became evident but not because of the failure of the vaccine to protect, as the industry thought, but because of poor vaccination technique. Since the industry has moved back to eye dropping long-lived birds several times commencing in early rearing, the incidence of the disease has declined. However, ILT still remains a problem in broilers that are still vaccinated by water, as it is not feasible and (or) cost effective to pick up broilers in the 100,000s per week in the various operations.

It has recently been demonstrated that innovative water administration methods can overcome to some degree the problems of water vaccination for this respiratory-based disease. The ILT vaccine was originally developed and registered for eye drop administration to expose the conjunctiva and upper respiratory tract, which are the normal colonisation sites of the ILT virus. More recently ILT vaccination was done via the drinking water due to the large number of vaccinations needed in meat chickens (10,000s of broilers). The prescribed traditional method of withdrawing water from the birds for several hours to encourage them to consume the vaccine in drinking water in less than 2 hours results in an uneven distribution of the vaccine dosage in the birds, which is counterproductive for a vaccine where every bird has to receive an immunising dose. The consumed vaccine directly enters the gastrointestinal tract in the majority of birds, and thus makes no contact with the normal ILT receptor sites. The problem was resolved by avoiding withholding water from the birds, other than to allow time to flush the vaccine in water to the end of the drinker line. Birds could then calmly consume the drinking water containing half the prescribed vaccine dose over 2 to 4 hours and the remainder of the vaccine was delivered in water at another time period, that day or the following day. This drinking water vaccine administration procedure allows a better opportunity for all birds to get a bioequivalent dose of vaccine, and increases the chance of the vaccine contacting the pharyngeal and upper respiratory tract area while it is being calmly and repeatedly consumed. Serological testing and field protection against challenge has validated the improved drinking water vaccination technique (Scott, *personal communication*).

Vaccination against MG and MS using the Australian developed temperature sensitive mutants ts-11 and MS-H (Bioproperties) has, since their introduction in the late 1980s, dramatically changed the status of mycoplasma in the Australian poultry industry. What were once endemic diseases associated with significant chronic respiratory disease are today a very uncommon finding (Morrow and Whithear, 2011).

Like the pig industry, bacterial diseases are more difficult to control using vaccination. While Salmonella infections are normally asymptomatic in poultry, there are significant food safety issues associated with Salmonella in eggs and chicken meat. The Australian poultry industry, unlike in Europe, has not yet had the pressures to control Salmonella at the bird level through the use of vaccination and in feed additives. There is only one registered vaccine in Australia for use in drinking water or coarse aerosol spray, and autogenous vaccines can be used. The uptake by the industry though has generally been low. Attempts to limit egg-associated food safety cases include phytosanitary procedures, in-feed synbiotics, egg handling including washing, and the preference for people to not use raw eggs in ready-to-eat food (Matlho *et al*., 1997; DEFRA 2009; Jones 2011).

With the consumer pressure to move birds back into extensive or free-range systems there has been an emergence of "old" diseases such as fowl cholera (FC), caused by *Pasteurella multocida*, external parasites such as lice and mites, and internal parasites including both nematodes and tapeworms. Also emerging are Erysipelas and Black Head, the latter caused by *Histomonas meleagridis.* A disease called Spotty Liver is a significant problem in young free-range layer flocks and, while responding to antibiotic therapy, the aetiological cause has not been identified despite extensive studies including metagenomics (Grimes and Reece, 2011; AHVALA, 2012; Scott, 2012). Control of FC in chickens is usually achieved by vaccination, provided the vaccine used is the correct protector type and the associated husbandry stressors are identified and minimised. In turkeys the situation is very different with control dependent on repeated vaccination. Erysipelas is controlled by improving hygiene and particularly rodent control and "off labelling" a ruminant vaccine. Black Head control is now difficult with no therapeutic product for the treatment registered in Australia. Dimetridazole was deregistered because of concern it was potentially genotoxic, APVMA (2007) and control is now attempted through controlling the caecal worm that acts as an intermediate host, and the use of phytogenics such as oregano (Scott, personal communication).

Unlike the chicken meat industry, where there is still a wide range of antibiotics that can be used provided the withholding periods are followed, the egg industry has very few therapeutics it can use either in rearing or in production. Currently the registered label for amoxycillin states as a contraindication, *"EGGS: DO NOT USE in birds which are producing or may in the future produce eggs or egg products for human consumption"*. Thus according to the label one cannot medicate day-old chickens with amoxycillin if the eggs from production birds are for human consumption, even up to the age of depopulation at more than 70 weeks. Thus in Australia currently, amoxil cannot be used in layers even with a withholding time, but in New Zealand it can be used in-lay with a nil withholding period. Similar contraindication labels apply to the sulfur-based antibiotics in Australia. The only antibiotics that are registered for use in Australian layers with a nil holding time are chlortetracycline (CTC) and lincospectin soluble. The latter is cost prohibitive being around 10 fold the cost of CTC to medicate a flock. With the tetracycline resistance patterns emerging for FC and *E. coli* and the lack of response of Spotty Liver to CTC treatment, the industry is facing a dilemma. Compounding this for birds in extensive systems is the absence of any all-purpose registered wormer in Australia with a nil withholding period. Tapeworm (*Raillietina spp.)* is an emerging significant problem and the poultry industry has no registered treatment available. While "off labelling" with members of the benzimadazole family occurs, a zero MRL should apply for all meat and eggs during and after treatment. Attempts to register flubendazole in Australia for use in poultry with a nil withholding time has been progressing since 1986, but the residues section of the APVMA is still seeking more data, which the primary international registrant does not have. Australia is the only country in the world where flubenzadole is not registered.

The same situation applies to insecticide use in poultry houses or on birds, with most products now not registered for this purpose, and "off labelling" is not allowed with insecticides. Thus the control of external parasites is limited. Paradoxically, consumers and legislators want poultry free-ranging for welfare considerations, but as a consequence more disease is occurring with subsequent suffering and the inability to treat. Despite efforts through the APVMA, RSPCA, state authorities and the Bureau of Animal Welfare there has been no resolution to this problem. This creates commercial and animal welfare responsibility pressures on poultry owners to attempt to resolve issues themselves.

Quality nutrition

The marginal nature of the chicken meat industry means 1 or 2 points in feed conversion efficiency (FCR) can make the difference between profit and loss. There is a need to feed young birds where bioavailability is less and to feed older long-lived birds where consistency in both formulation and specification is critical. The specification of the feed and quality of raw materials is particularly important in limiting health problems associated with metabolic disease. The primary liveability issue in meat chickens can be metabolic disease and not infectious disease. The way the feed is provided during the growing or production cycle is critical in achieving improved performance in broilers by limiting early intake with the use of lighting programs, and then using compensatory growth strategies. In layer birds the

diet specifications are changed with changing formulations throughout the laying period to ensure optimum production costs, quality eggs and also controlling metabolic disease.

Raw materials are regularly tested for protein, metabolisable energy (particularly cereal grains at the start of the season), biogenic amines, mycotoxins and soybean meal digestibility. The enzyme additives are commonly used and the synbiotics are more commonly used in layers and broiler breeders but not broilers.

Husbandry skills and practices

While the poultry industry is experiencing a resource shortage of skilled workers and is using more professional immigrants to do this work, it has still maintained a relatively high skill base where managers are aware of the husbandry requirements of the birds. In contrast to some aspects of the pig industry, the need for the intervention of therapeutics is seen in the poultry industry as a failure in the husbandry and health programs. It is rare in the poultry industry for routine or long term antibiotic programs to be used. The industry is essentially cost-averse to using medication programs other than those for control of coccidiosis. While the use of in-feed growth promotants continues in broilers, it is rare or absent in the layers and the broiler breeders. With the advent of free-range chicken meat the use of growth promotants is decreasing in broilers (Scott, personal communication).

Companies and technical managers supply to producers, broiler growers and farm mangers operational guidelines that must be strictly adhered to. All companies have technical service staff to ensure compliance with company recommendations. Included in this are biosecurity and sanitation programs. With the exception of some cage layer facilities, all poultry sheds are dry-cleaned, wet-detergent washed down, disinfected, treated with an insecticide and then undergo a final inspection to ensure there is satisfaction with the cleanout and preparation for the next delivery. Some sheds may undergo post-disinfection swabbing to assess the quality of the cleanout and check for pathogens such as Salmonella. Some companies will cancel placement if the findings are unsatisfactory. Contractors usually do cleanout, wash-down and disinfection. In the broiler grow-fee model, cleanout costs are included and thus compliance expected.

The access to ongoing training for mangers is available to the poultry industry. While the role of the agricultural colleges has declined, bodies like the Poultry CRC have workshops, training exercises and internships. The Australian Egg Corporation provides limited funds for extensive Certificate III training courses and poultry breeding companies such as Aviagen® and hatchery equipment firms regularly provide both domestic and international training workshops. There will be a need to have training reinforced, as the trend in sentiment towards farming is being lost to marketing philosophies and corporate management. The issue of fatigue with compliance requirement is also adding a new burden to farm managers.

Marketing and the interaction with poultry husbandry

It is all too familiar with producers that what the market invariably wants is not necessary compatible with best farming practices and poultry health outcomes. In the chicken meat industry there is a requirement for large birds and more weight coming out of sheds, creating pressure on kilograms produced per m^2. Producers have already recognised though that by reducing placement stocking densities they achieve overall better outcomes in regard to liveability, FCR and quality (Sekeroglu *et al.*, 2011; Zuowei *et al.*, 2011). Similar findings have been identified for growing pigs (Gonyou, 2005). So despite paying contracted growers higher fees for lower productivity, and requiring more shedding due to decreased occupancy capacity, the gains in overall productivity outweigh this. With layers, the industry itself changed the market by creating markets for egg sizes that were more compatible with egg production profiles.

The only area where there has been market-enforced change, which has led to reduced productivity, is the move to free range. While the payments for this market are at a premium, producers are happy to adapt to the requirement but when this becomes the norm and margins again reduce, then the attitude of the producers will certainly change. Retailers though, one might say surprisingly, are aware of this potential margin squeeze and do not want to see it impact on the sustainability of producers.

Fortunately in Australia there have not been the overt pressures from the consumers on inputs such as antibiotics and GMO soybean meal. Generally the regulators and even the scientists are balanced in that they see a need for limited therapeutics but under tight regulatory control. The question is whether or not this balance extends to the current use of antibiotic use in the pig industry.

Expenditure on capital and maintenance

The Australian poultry industry contrasts to some parts of the pig industry when comparing the capital that has been spent on expanding, replacing, modernising and upgrading facilities over the last three decades. This has been done by the poultry producers themselves and not driven by outside forces, with the exception of free range. Grower shedding, whether owned by contracted growers or companies, is always

progressing and being maintained to and above best world standards. All new shedding has to be built to a minimum standard including all ancillary items like generators, silo space, water supply quality, roads and amenities. As mentioned previously there is a culture amongst growers and producers of wanting the best available and a "must have attitude". Currently within the chicken meat industry there are more people willing to invest in shedding than shedding required, although the dynamics of this can change rapidly.

Quality maintenance within both the chicken meat and egg industry is a necessity because equipment failure can quickly result in losses of productivity and product flow. Maintenance divisions within companies are often ranked as equal in importance to all other divisions like accounting and marketing. However, a skill shortage is developing in this area.

The upgrade of processing plants is another very high capital input area and is driven by change in product type and profile, demand for higher quality product and the need for more automation to reduce labour costs. This expenditure can be risky for poultry companies if it is undertaken just prior to a market down turn and lower prices, as the returns on funds employed are more based on remaining competitive in the market, unlike new shedding which creates greater and more efficient turnover.

Conclusions

The poultry industry in Australia has not only grown dramatically over the last decade but has done so in a manner that incorporates world best practice standards and facilities. It cannot be argued that the reason for this is because it is a highly profitable business, protected from imports, unlike the pig industry. Competition within the poultry industry has resulted in very marginal returns and in some cases with non-profitable sectors. Despite its substantial improvements in productivity gains, the poultry industry has given returns back to the consumer, with corrected farm gate egg prices the same as they were in the 1970s and real chicken meat prices now 40% less than they were in the 1980s. Compared to the pig industry, there is what could be termed a different "culture" in the way the poultry industry has progressed. The poultry industry has been more focused on achieving productivity gains through improved facilities, genetics and husbandry, including quality vaccines, with less reliance on therapeutics such as antibiotics. In contrast the pig industry has remained with existing genetics, invested less capital in lower cost facilities, and relied more on the use of therapeutics. Despite the distinct disadvantages in FCR and reproductive performance of the Australian pig genetics, the industry has not entertained importing new genetics. There are biological technical reasons for this, commercial reasons and import risk assessment protocols to consider, but these all need to be considered collectively in regard to the competiveness of the Australian pork industry. The Australian poultry industry brings in new genetic stock of a variety of commercial poultry species multiple times a year and yet Australia still maintains free of fresh and par-cooked poultry meat and fresh egg imports from all countries, except New Zealand, because of the rigid import requirements, particularly related to vIBD. Paradoxically Australia now allows processed pig meat imports from four countries and yet we do not have the genetics in our domestic pigs that allow us to at least compete on performance and quality aspects.

The Australian poultry industry has always had a culture of achievement in regard to all aspects of poultry health and husbandry. It has historically for a small country been over represented with leading poultry scientists, and has world recognised poultry Emergency Animal Disease programs such as the AUSVETPLANS, has world best standard facilities, and now has the leading avian genetics company Aviagen® using Australia as not only one of its major supply hubs for genetic stock into south east Asia and the subcontinent, but also as a backup for the rest of the world. This does not mean that the industry has always been profitable as margins are often small, and these pressures have resulted in changes of industry ownership with consolidation in both the meat and egg sectors.

Poultry health programs are not just aimed at being free of clinical disease but are aimed at maintaining a negative status for avian pathogens. Thus vaccination and other preventive programs including biosecurity and in-feed synbiotics are used to avoid the initial colonisation of avian pathogens and thus displace wild type organisms from operations. Even on isolated, new greenfield rearing sites, day-old chicks still are vaccinated for the majority of recognised avian pathogens. Producers want to provide all the insurance possible to maintain their flock negative status, understanding that all the various horizontal contacts within a poultry operation can introduce status changing poultry pathogens. Vaccination by default is a biosecurity tool because if there are no susceptible hosts, the invading organism cannot find a site/host to colonise. This approach has been particularly successful with the widespread use of the live MG vaccination (Vaxsafe MG, Bioproperties). The incidence of MG in chicken operations in the mid 1980s was almost 100%, causing ongoing expenditure on Tylosin and other antibiotics extending into millions of dollars to control CRD. Today no chicken or layer breeding stock is positive for MG and the identification of MG in chickens is a rarity. Consequently the use of Tylosin is very uncommon in the poultry industry in Australia. The use of vaccination for displacement of wild type organisms has also been

seen in the National NDV program, where NDV vaccination was mandated across the industry in 2003. This program was implemented after repeated outbreaks of virulent NDV (vNDV) of Australian origin from 1998 to 2002. Since the program's implementation there have been no vNDV outbreaks in Australia.

While control of bacterial diseases is more difficult, there needs to be an appreciation that combining all aspects of a preventive health program will invariably lead to disease control and an overall improvement of the herd status. With regard to the Australian pig industry, the implementation of only parts of the program, inconsistent implementation, short-term implementation or cessation of a program after the cessation of clinical signs without a change in status will cause a failure to achieve the desired aims and no return on the funds employed. The simple concept is to ensure clean breeding stock, wean into clean single-age nursery groups and use preventative programs to protect young pigs from future challenges during the grow out period. During this stage, immunologically sound pigs should be grown out in single-age groups under minimal pathogen load, sound husbandry and quality facilities. Quality facilities require the basic acceptable conditions being achieved with regard to ventilation, air quality and temperature control.

The Australian pig industry does know how to implement such programs like those used in the poultry industry. The implementation of such complete programs though is limited for reasons that are related to operational activities, historical and philosophical perspectives, workplace endeavour (staff effort) and (or) working capital. To not attempt change will, and is resulting in, the ongoing poor performance and reduced profitability in the pig industry. As an example of change; in the poultry industry back in the 1980s many layer operations ran their rearing sites, and even sheds, as multiple age for reasons of convenience, reduced work load, saving on cleanout costs and continuity of egg size flow when the birds were in production. Many producers were unwilling to change these practices. The occurrence of virulent MDV soon changed this as producers could not survive with 30% mortalities and compromised production. The move to single-age rearing and improved vaccination led not only to the cessation of clinical MDV but overall improved performance as a consequence of improvement in general health, the uniformity and quality of rearers, and the improved ability to manage birds in lay because of more appropriate lighting and feeding programs.

Where sectors of the Australian pig industry better understand the pathogen status of their herd through autopsies, abattoir surveys and laboratory testing (know what microbes are killing their pigs and affecting performance), they have a much better opportunity to strategically medicate and treat their herd at the correct time and with the correct therapeutic to optimise the treatment impact and cost. The existing security blanket of medications in feed that can be demonstrated to be ineffective because of no response to treatment and antibiotic resistance patterns is a husbandry ethos that needs attention in the pig industry. Once the epidemiology of the diseases' process is understood, then a strategic treatment program can be augmented with effective vaccination programs in sows and progeny or both, which can and will replace the need for medication. This is provided that husbandry programs such as single-age rearing, all-in all-out production and suitable facilitation and nutrition are provided, which are a pivotal component of any disease control program and especially for those pig diseases such as *Actinobacillus pleuropneumoniae* (APP) and Pasteurella. It is appreciated that these changes will be harder for some sectors of the pig industry than others because of the nature of their existing infrastructure, the quality of resources, operational restraints and the need for some capital. This, however, should not impede the commencement of the process but just the understanding that it will take longer and the gains realised slower; it is the only way forward.

Experience with producers that have taken this holistic approach to husbandry and health programs indicate they are seeing improvement in pig productivity through reduced mortalities, better growth rates and improved feed conversion efficiency. These early improvements positively augment the program by reducing the overall pathogen load and improving uniformity, and in some cases providing more growing space, which assists the ability to maintain single-age grow out groups. Savings through reduced medication costs and lower processing rejects are significant.

References

ACMFI (2013). Australian Chicken Meat Federation Inc, www.chicken.org.au/page.php?id=4#Consumption

AHVALA (2012). *Information for Farmers and Vets in Great Britain,* Animal Health and Veterinary Laboratories Agency, www.defra.gov.uk/ahvala p1.

APVMA (2007). Australian Pesticides and Veterinary Medicines Authority, (http://www.apvma.gov.au/products/review/docs/dimetridazole_review_findings.pdf)

BAGUST, T., FENTON, S. and REDDY, M. (2004). *Australian Veterinary Journal*. **82**:701-706.

BELL, I., NICHOLLS, P., NORMAN, C., COOPER, K and CROSS, G. (1991). *Australian Veterinary Journal*. **68:**85-89.

Bioproperties (2013). Bioproperties publications, www.bioproperties.com.au

DAFF (2013). Australian Government, Department of Agriculture, http://www.daff.gov.au/ba/ira/final-animal/chicken-meat

DEFRA. (2009). *A Guide to the National Control Programme for* Salmonella *in laying flocks*.

FANATICO, A., PILLAI, P., HESTER, P., FALCONE, C., MENCH, J., OWENS, C. and EMMERT, J. (2008). *Poultry Science*. **87:**1012-1021.

GEHAN, M., ANWER, W., AMER, H., EL-SABAGH, I., REZK, A. and BADAWY, E. (2009). *International Journal of Poultry Science*. **8**:237-241.

GONYOU, H. (2005). In "Proceedings of the 5th London Swine Conference Proceedings, pp. 89-93.(London, Ontario, Canada).

GRADEL, K. (2003). *Disinfection in Animal Production. Symposium, Aarhus, Denmark.* p.1.

GRIMES, T. and REECE, R. (2011). In "Proceedings of the 60th Western Poultry Disease Conference, pp. 53-56 (Sacramento, California, USA).

HEWSON, K., SCOTT, P., DEVLIN, J., IGNAJATOVIC, J. and NOORMOHAMMADI, A. (2012). *Vaccine*. **30:**4190-4199.

ISLAM, A., WALKDEN-BROWN, S., WONG, C., BURGES, S. and GROVES, P. (2000). *Proceedings of the Australian Poultry Science Symposium.* **12**:190-193.

JONES, F. (2011). *Journal of Applied Poultry Research*. **20**:102-113.

MATLHO, G., HIMATHONGKHAM, S., RIEMAN, H. and KASS, P. (1997). *Avian Diseases.* **41**:58-61.

MORROW, C. and WHITHEAR K. (2011). *International Hatchery Practice*. **25:**13-15.

SCOTT, P. (2012). In "Proceedings of the PIX Conference", pp.167-171. (Poultry Information Exchange Association Inc.; Wamuran, Queensland, Australia).

SCOTT, P., MARKHAM, J. and WHITHEAR, K. (1999). *Avian Diseases.* **43**:83-88.

SEKEROGLU, A., SARICA, M., GULAY, M. and DUMAN, M. (2011). *Journal of Animal and Veterinary Advances*. **10**:246-250.

UNI, Z., FEKET, P., TAKO, E. and KEDAR, O. (2005). *Poultry Science*. **84**:764-770.

VAN DEN WIJNNGAARD, J. (2002). *Lohmann Information.* **26**:1-4.

ZUOWEI, S., YAN, L., YUAN, L., JIAO, H., SONG, Z., GUO, Y. and LIN, H. (2011). *Poultry Science*. **90**:1406-1415.

Pork producers can use their industry knowledge to prioritise exotic diseases

V.J. Brookes[1], M. Hernández-Jover[2], R. Neslo[3], B. Cowled[4], P. Holyoake[5], M.P. Ward[1]

[1]The University of Sydney, Camden, NSW 2570. [2]Charles Sturt University, Wagga Wagga, NSW 2650. [3]University Medical Center Utrecht, The Netherlands. [4]AusVet Animal Health Services, Conjola Park, NSW 2539. [5]Department of Environment and Primary Industries, Bendigo, VIC 3551.

Protection from transboundary diseases cannot be guaranteed despite Australia's geographic isolation, trade restrictions and biosecurity. Prioritisation of exotic diseases is required to direct resources for contingency planning. Previously, prioritisation of livestock diseases has used disease experts to value impacts. In this study it was hypothesised that although pork producers are not experts in exotic diseases, they are experts in disease impacts. The objective of this study was to prioritise exotic diseases for the pig industry in Australia according to both scale and importance of potential disease impacts to pork producers.

Four hundred and thirty producers registered with Australian Pork Limited were asked to complete an online survey between December 2011 and March 2012, in which they ranked four groups of test disease scenarios and one group of real disease scenarios, according to the level of concern to themselves. The scenarios comprised disease criteria (Table 1) from which producers could infer potential disease impacts. Weights of importance for the criteria were extracted from the test scenario ranks using probabilistic inversion and validated against the real disease ranks (Neslo and Cooke, 2011). These weights were combined with actual criterion measurements for exotic diseases, using a multi-criteria decision analysis framework to give an overall score for each disease. Diseases were ordered by mean score.

Table 1. *Disease criteria used to prioritise exotic diseases for the domestic pig industry in Australia.*

	Mean criterion weights	
Criteria	Producers prioritising livestock diseases	Producers prioritising zoonotic diseases
Cost contribution to industry by government (0–100%)	0.32	0.07
Pork markets loss (0–100%)	0.39	0.15
Attack rate[a] in pigs (0–100%)	0.55	-0.10
Length of clinical disease in pigs (0-42 d)	0.40	0.03
Case fatality rate[b] in pigs (0–100%)	0.25	0.65
Ruminant market loss (0–100%)	0.23	0.13
Incidence in humans (0–100%)	0.38	0.02
Disability weight for humans[c] (0-1)	0.02	0.52
Case fatality rate[b] in humans (0–100%)	0.04	0.30

[a]Attack rate: the percentage of pigs affected by the disease during the outbreak. [b]Case fatality rate: the percentage of the population with the disease who died due to the disease. [c]Disability weight: World Health Organisation value to indicate the severity of disease in humans.

The response rate for the survey was 11.6% and, based on differences in survey responses, the producers were divided into two groups (Table 1). The largest group (38 producers) considered attack rate and length of clinical disease in pigs, and pork market loss, to be the most important criteria. Their highest priority diseases were the vesicular diseases, followed by African swine fever, classical swine fever and highly pathogenic porcine reproductive and respiratory syndrome. Their weights for the criteria could be validated against their ranks for real diseases. The other group of 12 producers valued case fatality rate in pigs, and disability weight and case fatality rate in humans. This group were less consistent about their choices, ranking diseases with zoonotic impacts with increasing priority as they completed the survey. Validation of their criterion weights against their ranks for real diseases was poor. Their highest priority disease was rabies, with Nipah, Eastern equine encephalitis and Japanese encephalitis also high priority.

These results demonstrate that producers can use their industry knowledge to prioritise livestock diseases based on scale importance of potential impacts to themselves. However, producer opinion needs to be characterised further for zoonotic disease prioritisation. Validating criterion weights against participant responses was designed to reduce hypothetical bias. Other sources of bias in this study include response and selection bias which may limit inference to the target population: pig producers in Australia.

NESLO, R.E.J. and COOKE, R.M. (2011). *Applied Stochastic Models in Business and Industry*. **27:**115-130.

Supported in part by Australian Pork Limited.

The potential value of MLVA to porcine *Salmonella* surveillance in Australia

T.R.D. Weaver[1,2], G.G. Hogg[1], K. Dimovski[1], M. Valcanis[1], A. Kiermeier[2], H. Billman-Jacobe[1] and D.H. Hamilton[2]

[1]The University of Melbourne, Parkville, VIC 3010. [2]South Australian Research and Development Institute, Urrbrae, SA 5064.

Australian surveillance of porcine *Salmonella* has primarily used methods of serotyping, bacteriophage typing and pulsed-field gel electrophoresis to further differentiate taxonomic groups. Multilocus variable number of tandem repeats analysis (MLVA) presents the possibility of more refined differentiation of bacteria by determining the number of repeat units in a base sequence at a specific locus on the bacterial genome or plasmid, and may have potential in salmonellosis source attribution (as proposed by Best *et al*., 2007). We hypothesised that use of MLVA could increase differentiation of *Salmonella* subspecies *enterica* serovar Group B strains found in Australian pig farms and could assist Australian epidemiological investigations of porcine and human salmonellosis. The study employed MLVA on a Group B *Salmonella* serovar and phage type (PT) isolated from three Australian pig farms and compared the resulting patterns.

Faecal samples were collected on three farms with previously confirmed presence of *Salmonella* and clinical salmonellosis; the farms were located in two Australian states and were geographically isolated from one another. Sampled pens housed batched terminal-generation pigs selected to be representative of the respective herd. Samples were collected from individual, undisturbed stool pats on pen floors. Single batches of samples were collected from farms A (n=14) and B (n=13) from pigs aged 5 to 20 weeks. Five batches of samples were collected from a third farm, C (n=71), over 14 months from pigs aged between 4 and 22 weeks. All samples were cultured; where *Salmonella* were isolated, six to 10 colony picks were randomly selected for serotyping and phage typing. Isolates of a common serovar and phage type found on all three farms then underwent DNA extraction, multiplex PCR and MLVA of the standard loci identified by Lindstedt *et al*. (2004). MLVA patterns are presented in the standard Australian MLVA nomenclature.

At farm A, a Group B *Salmonella* serovar and PT was isolated from 8 of 14 samples and two MLVA patterns, pattern (II) 04-15-12-00-490 and pattern (III) 04-15-13-00-490, were present (Table 1). For farm B the same *Salmonella* serovar and PT was found in nine of 13 samples; MLVA identified pattern (I) 04-15-11-00-490. The same serovar and PT was isolated in 42 of 71 samples collected over 14 months from farm C and 13 MLVA patterns were identified, including patterns (I), (II), and (III). On farm C, variation in the frequency and persistence of different MLVA patterns was observed; two MLVA patterns were found in every sample batch, pattern (I) and a pattern not found on farms A and B. The MLVA patterns (I), (II), and (III) exhibit variation at locus STTR6; this locus is regarded as highly polymorphic and, therefore, potentially highly discriminatory, however, high variability may also hamper source attribution. Further research into the reasons for, implications of, and utility of, this variability are part of a related 3-year study.

Table 1. *Common MLVA patterns from* Salmonella *isolates from three Australian pig farms; 'X' indicates the respective MLVA pattern's presence on the identified farm.*

MLVA Pattern	(I)	(II)	(III)
MLVA Code	04-15-11-00-490	04-15-12-00-490	04-15-13-00-490
Farm A		X	X
Farm B	X		
Farm C	X	X	X

We found a number of MLVA patterns within a *Salmonella* serovar and phage type present on three Australian pig farms. Three closely related MLVA patterns on separate farms suggest either a common source or common genetic shifts among these *Salmonella* strains. The results also suggest MLVA patterns may be associated with bacterial phenotype; two MLVA patterns were found in all sample batches collected from farm C, suggesting a more persistent strain. Comparisons of porcine *Salmonella* MLVA patterns with MLVA patterns obtained from human and other production animal isolates may assist investigations of ultimate source in human salmonellosis cases. MLVA may aid disease risk management and reveal further insights into the origin, ecology and evolution of porcine *Salmonella* in Australia.

BEST, E., LINDSTEDT, B. A., COOK, A., CLIFTON HADLEY, F.A., THRELFALL, E.J. and LIEBANA, E. (2007). *Journal of Applied Microbiology*. **103**:565-572.

LINDSTEDT, B.A., VARDUND, T., AAS, L. and KAPPERUD, G. (2004). *Journal of Microbiological Methods*. **59**:163-172.

Supported in part by Australian Pork Limited.

Prevalence and molecular characterisation of *Clostridium difficile* in neonatal piglets in Australia

D.R. Knight[1], M. Squire[1] and T.V. Riley[1,2]

[1]The University of Western Australia, Crawley, WA 6009. [2]PathWest Laboratory Medicine, Crawley, WA 6009.

Clostridium difficile is a Gram-positive, spore-forming bacterium, well-known as an enteric pathogen of humans and recognised outside Australia as the causative agent of high morbidity enteritis in piglets aged 1-7 d (Songer and Anderson, 2006). Recently the incidence of human *C. difficile* infection has increased in the community and indistinguishable strains have been isolated from humans and pigs in The Netherlands (Bakker *et al*., 2010) suggesting food-borne transmission. *C. difficile* has been isolated from meats and other produce in North America and Europe (Rupnik and Songer, 2010). Environmental contamination may also be a spillover source as *C. difficile* spores persist in the environment and survive in treated piggery effluent (Squire *et al*., 2011). In Australia *C. difficile* has been isolated from scouring neonatal piglets but there are no systemic prevalence data. Our objective was to determine the prevalence and molecular types of *C. difficile* in Australian neonatal pigs.

A total of 229 rectal swabs was obtained from piglets aged 1-7 d from 21 farms designated by veterinarians as scouring or non-scouring in NSW (n=3), Queensland (n=5), Victoria (n=6), SA (n=3) and WA (n=4) from June 2012 to March 2013. Farms were either breeder only or farrow-to-finish units and ranged from 300 to 9,000 sows per farm (mean=1,657). Selective culture for *C. difficile* was performed directly onto *C. difficile* chromogenic agar and also by selective enrichment in broth for 48 h. *C. difficile* isolates were then characterised by PCR-ribotyping and PCR detection of toxin genes *tcdA* (toxin A), *tcdB* (toxin B) and *cdt* (binary toxin) as previously described by Knight *et al*. (2013).

Overall, *C. difficile* was isolated from 52% of samples by direct culture and 67% by enrichment culture. The majority (87%, 130/154) of strains were toxigenic. There was no significant difference (P=0.14, χ^2) in recovery of *C. difficile* between scouring and non-scouring farms. After comparison to international databases, 23 different ribotypes (RTs) were detected, including RT237 (detected in a previous Australian study by Squire *et al*., 2013). RT014 (23%, 36/154) was most commonly isolated. This RT, together with RT237 and seven other RTs isolated from pigs in this study, causes disease in humans (Bauer *et al*. 2009). RT078, the predominant strain in pigs worldwide (Rupnik *et al*., 2008) was not found. However, the second most commonly identified strain RT033 (13%, 20/154) is, according to Stabler *et al*. (2012), genetically related to RT078.

In summary, toxigenic *C. difficile* strains are prevalent in Australian neonatal pigs from farms with and without scour. Ribotying revealed overlap between pig and human strains of *C. difficile* including uniquely Australian RTs (e.g. RT237) and international RTs (e.g. RT014). This association needs to be confirmed by whole genome sequencing of representative human and pig isolates. Strains associated with recent human outbreaks in Australia (RT244) (Eyre *et al*. submitted) were not isolated from pigs. These results indicate three issues that warrant further attention. First, planned studies to determine the association between *C. difficile* and scouring in neonatal piglets should be completed. Second, the contribution of environmental contamination with *C. difficile* spores shed by scouring and clinically normal piglets to local human disease needs investigation. Finally, imported pork meat should be examined as a possible source of human outbreak strains.

BAKKER, D., CORVER, J., HARMANUS, C., GOORHUIS, A., KEESSEN, E.C., FAWLEY, W.N., WILCOX, M.H. and KUIJPER, E.J. (2010). *Journal of Clinical Microbiology*. **48**:3744-3749.

BAUER, M., VEENENDAAL, D., VERHOEF, L., BLOEMBERGEN, P., DISSEL, J.V. and KUIJPER, E.J. (2009). *Clinical Microbiology and Infection*. **15**:1087-1082.

KNIGHT, D., THEAN, S., PUTSATHIT, P., FENWICK, S. and RILEY, T.V. (2013). *Applied and Environmental Microbiology*. **9**:2630-5.

RUPNIK, M. and SONGER, J.G. (2010). *Advances in Food Nutrition and Research*. **60C**:53-66.

RUPNIK, M., WIDMER, A., ZIMMERMANN, O., ECKERT, C. and BARBUT, F. (2008). *Journal of Clinical Microbiology.* **46**:1963–1964.

SONGER, J. and ANDERSON, M. (2006). *Anaerobe.* **12**:1-4.

SQUIRE, M., LIM, S., FOSTER, N. and RILEY, T.V. (2011). In "Manipulating Pig Production XIII", p. 215, eds D.P. Hennessy and P.D. Cranwell. (Australasian Pig Science Association: Werribee).

SQUIRE, M., CARTER, G., MACKIN, K., CHAKRAVORTY, A., NORÉN, T., ELLIOTT, B., LYRAS, D. and RILEY, T.V. (2013). *Emerging Infectious Disease*. **19**:790-792.

STABLER, R., DAWSON, L., VALIENTE, E., CAIRNS, M., MARTIN, M., DONAHUE, E., RILEY, T.V., SONGER, J., KUIJPER, E., DINGLE, K. and WREN, B.W. (2012). *PLoS ONE*. **7**:1-12.

Funded in part by Australian Pork Limited. Sample collection coordinated by Dr Hugo Dunlop, Chris Richards and Associates.

Manipulating Pig Production XIV

Variation in the number of *Lawsonia intracellularis* shed in commercial pig herds over time

A.M. Collins and I.M. Barchia

Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568.

Lawsonia intracellularis, the bacterium that causes ileitis, is found in the majority of Australian herds, but most pigs are sub-clinically affected. Both clinical and sub-clinical ileitis cause reduced growth rate, poorer feed efficiency and increased days to slaughter, reducing net revenue by $8 to $13 per pig (Holyoake *et al*., 2010). A quantitative polymerase chain reaction (qPCR) was developed to quantify the numbers of *L. intracellularis* excreted in faeces by individual pigs, which correlated well with scouring and histopathology and negatively with pig growth (Collins *et al*., 2012). However, for qPCR to be a useful herd health monitoring test, the variation in *L. intracellularis* shedding over time required investigation in commercial herds.

Three batches of pooled faecal samples were collected from two commercial herds over a 6-month period to determine the variability in *L. intracellularis* numbers. In each batch, between 10 and 20 pooled faecal samples (five pigs per pooled sample) were collected from grower and finisher pigs at six different ages between 7 and 18 weeks of age. Samples were collected from the same age groups in subsequent batches 7 and 14 weeks later. The DNA was extracted from equal weights of pooled faeces and the number of *L. intracellularis* per gram of faeces was determined by qPCR. The number of *L. intracellularis* detected was transformed (natural log) to normalise data. Due to small sample sizes these sample distributions (means and standard deviations) were used to generate results for much larger populations (50,000) using the Monte Carlo method (Metropolis and Ulam, 1949). Differences in the population means over time were analysed by comparing quantiles (divisions of the total population frequency into a given number of equal proportions). We chose quantiles for two standard deviations (quantiles Q2.5 and Q97.5) and for one standard deviation (quantiles Q25 and Q75) from the mean for each population.

The mean number of *L. intracellularis* detected in both herds over time is outlined in Table 1, without qPCR negative data, where the mean number of bacteria was below the qPCR detection limit ($< 1 \times 10^3$). No significant variation in *L. intracellularis* numbers over time occurred in either herd at any age group when population means were tested at two standard deviations from the mean (range between Q2.5 and Q97.5). However, if the variation in *L. intracellularis* numbers over time was evaluated using one standard deviation from the mean (range between Q25 and Q75), then Herd 1 shed significantly fewer ($P<0.05$) *L. intracellularis* between the second and third batch of 16-week samples. Reduced stocking density and a change in antibiotic medication in Herd 1 between the second and third batches may partly explain the variation in *L. intracellularis* numbers. No management changes were recorded for Herd 2 over the same period.

Table 1. *Population mean, standard deviation and quantiles (Q2.5, Q97.5; Q25, Q75) of* L. intracellularis *numbers (natural log transformed) detected in pooled faeces by qPCR in two commercial herds.*

Herd	Batch	Age (weeks)	Mean	SD	Q 2.5	Q 97.5	Q 25	Q 75
1	1	16	12.53	2.416	7.79	17.26	10.90	14.16
1	2	16	14.19	2.366	9.55	18.83	12.59	15.78
1	3	16	6.94	2.704	1.64	12.24	5.12	8.77
2	2	11	7.81	1.713	4.46	11.17	6.66	8.97
2	3	11	11.17	2.115	7.02	15.32	9.74	12.60
2	1	13	8.64	2.276	4.18	13.10	7.10	10.17
2	2	13	7.48	2.128	3.31	11.65	6.05	8.92
2	3	13	7.76	1.905	4.03	11.49	6.48	9.05

SD, standard deviation.

This sampling protocol and qPCR assay can be used to quantify the effect of management changes, as variability in *L. intracellularis* numbers was minimal in the absence of change. Further studies are underway to test the strength of association between the qPCR and weight gain in commercial herds.

COLLINS, A., FELL, S. and DONAHOO, M. (2012). Proceedings of 22nd International Pig Veterinary Congress, Jeju, Korea, p.116.

HOLYOAKE, P., COLLINS, A. and MULLAN, B.P. (2010). Proceedings of 21st International Pig Veterinary Congress, Vancouver, Canada, p.233.

METROPOLIS, N. and ULAM, S. (1949). *Journal of the American Statistical Association*. **44**:335–341.

Supported in part by Pork CRC Limited Australia.

Optimal pooling of faeces to quantify *Lawsonia intracellularis* in clinically and sub-clinically affected pigs

A.M. Collins and I.M. Barchia

Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568.

Although the majority of finisher pigs in Australian herds are infected with *Lawsonia intracellularis*, not all infected pigs are compromised with respect to intestinal damage and growth rates. Disease severity in individual pigs correlates well with *L. intracellularis* numbers in faeces (Collins *et al*., 2012), as determined by a quantitative polymerase chain reaction (qPCR). In order to convert this research test into a useful herd health monitoring tool, faecal samples would need to be pooled. The aim of this study was to determine the number of faecal samples that could be pooled to provide an accurate quantification of *L. intracellularis* numbers in herds both clinically and sub-clinically affected with ileitis.

Pools of faeces were prepared to mimic populations with 10%, 20%, 40%, 60% or 100% of pigs with either sub-clinical ileitis (not scouring, but excreting 2 x 10^5 *L. intracellularis*/g faeces) or clinical ileitis (scouring and excreting 7 x 10^6 *L. intracellularis*/g faeces). In addition, individual faecal samples from pigs experimentally challenged with *L. intracellularis* or negative control pigs were mixed into pools of 1, 5 or 10 samples per pool. The DNA was extracted from 0.1 g of each of the above samples and the number of *L. intracellularis* per 0.1 gram of faeces was determined by qPCR.

The number of *L. intracellularis* (LI) increased in pooled faecal samples as the proportion of clinically affected pigs (c) increased, according to the linear relationship LI = 1.41c + 5.6 (R^2=0.88). The qPCR was sensitive enough to detect a single clinically affected animal in a pool of nine negative animals. However, at least six of 10 pigs (60%) had to be sub-clinically affected for detection by the qPCR. In individual faecal samples, *L. intracellularis* numbers varied widely from zero to 7.6 x 10^8, with a mean of 2.1 x 10^7 *L. intracellularis* per gram of faeces. The number of *L. intracellularis* detected in all 121 individual or randomly pooled samples was log transformed to achieve normal distribution. The variation in the number of *L. intracellularis* detected in individual samples versus pooled samples was analysed using 95% confidence intervals from means and standard deviations for each pool size.

For faecal pools of five pigs, the range in the mean number of *L. intracellularis* (log transformed with 95% confidence intervals) was between 4.87 and 7.06 (Table 1). Pooling five samples provided a representative estimate of the number of *L. intracellularis* since the mean *L. intracellularis* numbers shed by individuals (4.94) was within the range of the pools of five samples. However, pooling 10 samples provided a poor representation of *L. intracellularis* numbers, as the range for 10 pooled faeces (between 5.18 and 6.77) did not include the population mean for individual samples.

Table 1. *The mean and standard deviation (SD) of L.intracellularis numbers detected in individual or pooled faecal samples (log transformed) and the 95% confidence intervals.*

Pool size	1	5	10
Number of samples	69	27	25
Sample mean (in log)	4.94	5.97	6.0
Sample SD (in log)	1.24	0.56	0.41
Exp(Mean – 2*SD) 95% CI	2.50	4.87	5.18
Exp(Mean + 2*SD) 95% CI	7.38	7.06	6.77

In conclusion, a pool of five faecal samples was optimal for accurate quantification of *L. intracellularis* by qPCR in pigs with both clinical and sub-clinical ileitis. Pooling 10 faecal samples would only be suitable if at least one of the 10 pigs was clinically affected.

COLLINS, A., FELL, S. and DONAHOO, M. (2012). Proceedings of the 13th International Pig Veterinary Congress, Jeju, Korea, p.116.

Supported by Pork CRC Limited Australia.

The effects of intraperitoneal vaccination with *Lawsonia intracellularis* on immune responses in weaner pigs

M.G. Nogueira[1], D. Emery[1], A.M. Collins[2] and H. Dunlop[3]

[1]The University of Sydney, Sydney, NSW 2006. [2]Department of Primary Industries, Menangle NSW, 2567. [3]Chris Richards and Associates Pty Ltd, Bendigo VIC, 3550.

Oral vaccines reliably induce immunity against enteric pathogens by directly accessing mucosal surfaces of the intestine and draining mesenteric lymph nodes (Muir *et al.*, 1995). In pigs, proliferative enteropathy (PE) is an important enteric disease caused by the Gram-negative bacterium *Lawsonia intracellularis*. Oral and intramuscular vaccination with *L.intracellularis* induced specific responses and protection against challenge (Nogueira *et al*., 2013). Previously, intraperitoneal (IP) vaccination protected pigs from lung lesions due to *Mycoplasma hyopneumoniae* (Djordjevic *et al.*, 1997). An immune correlate which correlates with the successful induction of mucosal immunity and predicts protection against *L.intracellularis* has been difficult to measure. Since the route of vaccination is one factor that determines the magnitude, duration and quality of immune response at mucosal sites, we hypothesised that IP vaccination should produce immune responses similar to those induced by oral administration of *L. intracellularis*.

Thirty piglets, Landrace x Large White, were selected 5 d before weaning (weaned at 26 d of age) and grouped into three treatments: oral, IP and control. The distribution of pigs was performed by randomly selecting three offspring from one sow and allocating to each of the three groups, and this was repeated for 10 sows until each group had 10 piglets. The trial was performed on a commercial piggery with all-in/all-out production flows. The farm was a 650 farrowing to finish farm with an additional two grow-outs sites. After weaning (d 0), pigs were inoculated orally via drench gun and IP using syringe with a 2 mL dose [$10^{5.9}$ $TCID_{50}$ (50% tissue culture infective dose) organisms] of Enterisol® Ileitis vaccine (Boehringer Ingelheim, USA). The control group remained unvaccinated. Antibiotic was removed from feed and water 3 d prior to vaccination. Blood was collected from each pig at d 0, 8 and 17 post-vaccination and *L. intracellularis*-specific antibodies were determined as percent inhibition (PI; Bioscreen Ileitis ELISA; GmbH, Germany). Ileal mucosa were collected at necropsy (17 d post-vaccination) to determine IgG/IgA titres (Nogueira *et al.*, 2013) and the following cytokine levels: IFN-γ, TNF-α, IL-6, IL-10, TGF-β1 (Quantikine®ELISA kit; R&D Systems, US). Data were analysed using a restricted maximum likelihood test procedure (GenStat, 13th Edition; UK).

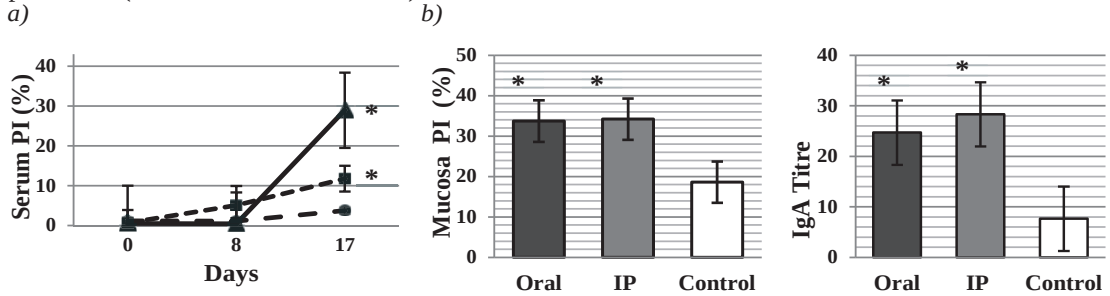


Figure 1. *Mean for* Lawsonia intracellularis*-specific IgG (PI value, %) and/or IgA (Titre) in serum (a) and ileal mucosa (b) within oral (▲), IP (intraperitoneal) (■) and control (●).[1] Asterisks indicate statistically significant differences (P<0.05) between each vaccinated group and unvaccinated controls in each figure.*

Protective immune responses against bacteria (e.g., IgG and IgA) at mucosal surfaces can block microbial attachment or neutralise virulence factors (Muir *et al*., 1995). In this study, IP vaccination mimicked the oral route and generated increased (P<0.05) serum and mucosal *L. intracellularis*-specific IgG and IgA (Figure 1). Local mucosal cytokine responses were increased (P<0.05) in oral and IP vaccinated groups respectively for TNF-α (83 and 31 pg/mL), and TGF-β1 (285 and 174 pg/mL), when compared with the control group (0.2, 0.5 pg/mL, respectively). The comparable spectrum of immune responses generated after vaccination and the subsequent protection after challenge in previous experimental trials (Nogueira *et al.*, 2013) suggests that significant protection against *L. intracellularis* would be anticipated. However, further study to verify protection is yet to be performed.

DJORDJEVIC, S.P., EAMENS, G.J., ROMALIS, L.F. and NICHOLLS, P.J. (1997). *Australian Veterinary Journal.* **75**:504-511.
MUIR, W.I., BRYDEN, W.L. and HUSBAND, A.J. (1995). *Avian Pathology.* **24**:679-692.
NOGUEIRA, M., COLLINS, A., DONAHOO, M. and EMERY, D. (2013). *Veterinary Microbiology.* **164**:131-138.

A comparison between meloxicam and ketoprofen in assisting the recovery of weaner pigs from illness or injury

R.L. Wilson[1], P.K. Holyoake[2], G.M. Cronin[3] and R.E. Doyle[1]

[1]Charles Sturt University and E.H. Graham Centre, Wagga Wagga, NSW 2678. [2]Department of Environment and Primary Industries, Bendigo, VIC 3551. [3]The University of Sydney, Camden, NSW 2570.

The Model Code of Practice for the Welfare of Animals – Pigs (Anon., 2008) states that sick, weak or injured pigs must be treated. Administration of analgaesic/anti-inflammatory drugs is appropriate for pigs suffering pain. Recent studies have suggested that ketoprofen may have a greater efficacy than meloxicam in young pigs (Fosse *et al*., 2011), but there have not been any studies to test this in a commercial setting. This study aimed to determine if the administration of ketoprofen as an adjunct to antibiotic treatment was more effective than the administration of meloxicam and an antibiotic on young pigs being admitted into a hospital pen. It was hypothesised that the administration of ketoprofen would decrease recovery time compared to animals treated with meloxicam.

The experiment was conducted at an intensive commercial piggery using 136 3- to 5-week old weaner pigs (Large White x Landrace). Pigs were housed in three hospital pens and commingled with animals not included in the study. Sick and injured pigs were matched for gender and disease type upon admittance to a hospital pen and allocated to one of two treatment groups (meloxicam or ketoprofen); the assessor was blind to the treatment groups. Pigs were administered an antibiotic suitable for their condition (equally distributed between treatments) and either meloxicam (0.02 mL/kg; Metacam, Boehringer Ingelheim Vetmedica, North Ryde, NSW, Australia) or ketoprofen (0.03 mL/kg; Merial, North Ryde, NSW, Australia) was given daily for 3 d. Recovery of individual pigs was assessed daily by rectal temperature (RT), infrared eye temperature (IET) (image taken 45 cm from eye, temperature measured using dot point analysis), time spent lying (measured every 15 min from 1200 to 0700 h on d 1 and 4), and body weight (BW) change (between d 1 and 4). Behavioural data were analysed using binomial generalised linear mixed models and the other measures with linear mixed models (GenStat, 13th Edition; UK), all addressing the parameters of normality. To investigate the usefulness of IET as a non-invasive indicator of core temperature, a correlation between RT and IET was determined using Pearson's correlation analysis.

There was a difference (P=0.006) between treatments for time spent lying, with the ketoprofen treatment group spending less time lying than pigs treated with meloxicam. There were no differences (P>0.05) in any of the other measures (Table 1). A weak but significant correlation was identified between RT and IET (r=0.30, P<0.001).

Table 1. *Results (mean±SEM) for time spent lying, rectal temperature, infrared eye temperatures and BW change.*

Treatment	Ketoprofen	Meloxicam	Significance
Time spent lying	87%±0.12	90%±0.12	P=0.006
Rectal temperature	39.55°C±0.06	39.60°C ±0.06	P=0.39
Infrared eye temperature	34.12°C±0.13	34.22°C±0.13	P=0.44
Body weight	0.29kg±0.11	0.26 kg±0.11	P=0.97

BW, bodyweight.

These results indicate that pigs administered ketoprofen were more active than pigs administered meloxicam, suggesting a greater analgaesic effect of ketoprofen in young pigs. Indeed, illness often results in an increase in the amount of time spent lying (Dantzer and Kelley, 2007). Further investigation of this finding and how it relates to other measures of illness and recovery is needed. The correlation between RT and IET also suggests that further investigation is needed to determine if this non-invasive health assessment measure could be useful in the detection of illness in a commercial setting.

ANON. (2008) *Model code of practice for the welfare of animals: Pigs*. CSIRO Publishing: Collingwood, VIC.
DANTZER, R. and KELLEY, K.W. (2007). *Brain, Behavior, and Immunity*. **21:**153-160.
FOSSE, T., HORSBERG, T., HAGA, H., HORMAZABAL, V. and RANHEIM, B. (2011). *Journal of Veterinary Pharmacology and Therapeutics*. **34:**153-159.

This project was funded by Australian Pork Limited.

Porcine colostrum supplementation increases serum immunoglobulin concentration of light piglets

C.F. Hansen[1], R. Müller[1], E. Kanitz[2], M. Tuchscherer[2] and F.Thorup[3]

[1]University of Copenhagen, Denmark. [2]Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany. [3]Pig Research Centre, Copenhagen, Denmark.

Sows are improved genetically to farrow large litters, but increased litter size leads to longer duration of farrowing and a decrease in birth weight of the piglets (Baxter *et al*., 2013; Rutherford *et al*., 2013). Simultaneously the concentration of immunoglobulins (Igs) in colostrum declines rapidly after birth of the first piglet (Klobasa *et al.*, 1987). As newborn piglets depend on colostrum as a source of energy and Igs for passive immunity and survival there is a risk that small and (or) piglets born later might not be able to obtain sufficient colostrum. The aim of the current experiment was to investigate if supplementing newborn piglets with porcine colostrum after birth could increase serum IgG levels of light birth weight and late born piglets. The hypothesis tested was that additional colostrum would increase serum IgG levels in the supplemented piglets.

The completely randomised experiment was conducted in one Danish piggery and included 431 live-born piglets delivered by 27 Landrace x Yorkshire sows. Litter size varied between 6 and 24 total born piglets per litter with an average of 16.0 live-born piglets per litter. At birth piglets were weighed, ear-tagged and the sex, time and birth order was recorded. Piglets with an odd ear-tag number served as untreated controls whereas even piglets were tube-fed with 25 mL porcine colostrum at 6 and 9 h after birth. The porcine colostrum originated from the same piggery and had been obtained by hand milking farrowing sows prior to the start of the experiment. Collected colostrum was bulked, stored at -20ºC and then reheated to 39ºC prior to use. When piglets were 24 h old a blood sample was collected via jugular vein puncture for subsequent analysis of circulating IgG using a porcine specific ELISA. Data were analysed univariately in SAS (version 9.2) using the mixed procedure with piglet as the experimental unit.

Table 1. *Effect of birth weight (BW) and colostrum supplementation (SUP) on serum IgG concentration in 24 h-old suckling piglets. Values are least-squares means.*

	Light <1.1 kg		1.1≤Normal ≤1.5 kg		Heavy > 1.5kg			Significance		
	Con	Colos	Con	Colos	Con	Colos	SE	SUP	BW	SUP×BW
n	61	60	82	80	60	80				
Serum IgG (g/L)	28.4[a]	34.6[b]	34.0[b]	34.8[b]	32.9[b]	33.6[b]	1.5	0.007	0.059	0.043

[a,b]Means in a row not having the same superscript are significantly different (P<0.05); Con, untreated controls; Colos, piglets supplemented with colostrum; SE, standard error.

Table 2. *Effect of birth order (BO) and colostrum supplementation (SUP) on serum IgG concentration in 24 h-old suckling piglets. Values are least-squares means.*

	BO 1-9		BO 10 and above			Significance		
	Con	Colos	Con	Colos	SE	SUP	BO	SUP×BO
n	117	114	103	96				
Serum IgG (g/L)	33.1	36.0	31.0	32.6	1.3	0.015	0.004	0.506

Con, untreated controls; Colos, piglets supplemented with colostrum; SE, standard error.

Circulating serum IgG concentration was lower in light birth weight piglets; however, supplementation with colostrum increased the serum IgG concentration to the same levels as the normal and heavy birth weight piglets (Table 1). Serum IgG concentration was higher (P=0.004) in piglets of birth order 1 to 9 compared with piglets that were born later (Table 2). Supplementing piglets with extra colostrum increased (P=0.015) circulating IgG concentration independent of birth order (Table 2). In conclusion, supplementing newborn piglets with porcine colostrum increased serum IgG levels especially for the light piglets.

BAXTER, E., RUTHERFORD, K.M.D., D'EATH, R.B., ARNOTT, G., TURNER, SP., SANDØE, P., MOUSTSEN, V.A., THORUP, F., EDWARDS, S.A. and LAWRENCE, A.B. (2013). *Animal Welfare.* **22**:219-238.

KLOBASA, F. and BUTLER, J.E. (1987). *American Journal of Veterinary Research.* **48**:176-182.

RUTHERFORD, K.M.D., BAXTER, E., D'EATH, R.B., TURNER, SP., ARNOTT, G., ROEHE, R., ASK, B., SANDØE, P., MOUSTSEN, V.A., THORUP, F., EDWARDS, S.A., BERG, P. and LAWRENCE, A.B. (2013). *Animal Welfare.* **22**: 199-218.

This project was supported by the EU and Ministry of Food, Agriculture and Fisheries of Denmark Grant 3663-U-11-00183.

Method analysis for a novel trait: comparing locations for haemoglobin sampling in piglets

K.M. Tickle[1], C.L. Collins[1] and S. Hermesch[2]

[1]Rivalea Australia Pty Ltd, Corowa, NSW 2646. [2]Animal Genetics and Breeding Unit, a joint venture of NSW Department of Primary Industries and University of New England, UNE, Armidale, NSW 2351.

Haemoglobin (Hb) testing on farm is a new technology within the pig industry that can be used for improving survival in piglets (Rootwelt *et al.*, 2012) and as a selection criterion for iron content in pork (Hermesch and Jones, 2012). The testing requires a 10-µL sample of blood to be collected from the piglet. The most logical time to collect this sample is at litter processing when piglets are already being handled, thereby minimising stress on the piglet. The aims of the study were to determine first whether the location from which the blood sample was collected from the piglet (ear versus tail) influenced Hb measurement, and second, whether the order of collection (ear or tail first) had any impact on Hb measurement. The sow of the litter was also included as an effect. It was hypothesised that the ear would be the optimal location for blood collection and that it would not have an effect on the Hb levels.

Haemoglobin levels were measured on 186 12-hour-old piglets (Large White and Landrace, PrimeGro™ Genetics, Corowa, NSW) prior to iron injection. All litters were represented by two male and two female piglets (one light, two medium, and one large). Each piglet had four blood samples taken, two ear samples and two tail samples. Piglets were alternated as to which measurement was to be taken first. A HemoCue® Hb201$^+$ was used to measure Hb concentration in the four samples immediately after collection. This machine has been previously validated to show accurate results on farm in neonatal pigs when compared to laboratory analysis (Kutter *et al.*, 2012). Piglet ears veins were pricked with small needles and tails were excised, to create small droplets of blood; these were then collected using cuvettes and were inserted into the HemoCue® for measurement. Both tests were done twice. This process took four minutes per piglet. Data were analysed by ANOVA for the main effects of location, order, sow and their interactions using GenStat (VSN International, Oxford UK). Pearson's correlation analysis was undertaken to obtain repeatability of the two samples taken at each location.

Table 1. *Influence of blood sample collection location and order of sampling on Hb concentrations.*

	Ear		Tail		Significance					
Sample number	1st (n=94)	2nd (n=92)	1st (n=92)	2nd (n=94)	L	O	S	L x O	S x O	S x L
Hb (g/L)	88.6	92.7	83.6	82.6	<0.001	0.405	<0.001	0.121	NS	NS
SEM	1.58	1.60	1.84	1.82						

SEM, standard error of the mean; L, Location (Tail or Ear); O, Order (1st measurement or 2nd measurement); S, Sow of the litter; NS, not significant (P>0.05).

Haemoglobin concentration was significantly higher (P<0.001) in blood collected from the ear (90.7 g/L) compared to the tail (83.1 g/L). The SEM were higher in the tail (1.84, 1.82) than the ear (1.58, 1.60) indicating larger measurement errors (Table 1). The order of sample collection did not influence (P>0.05) Hb concentration although the interaction of location and order showed a trend (P=0.12) towards significance due to the increase in Hb levels at the ear from the first to second sample. The second Hb sample collected at the ear was taken after the tail was cut. Although the Sow had a highly significant effect on Hb concentration, it had no effect on Hb when interacting with Location or Order (Table 1). Pearson's correlations showed that the duplicate samples collected from the ear were highly correlated, regardless of order of sampling (ear sampled first, r = 0.92; ear sample second, r = 0.86). In comparison, lower correlations between duplicates were found for tail samples (tail sampled first, r = 0.83; tail sampled second, r = 0.84).

Blood sampling from the ear of the piglet resulted in higher mean Hb concentrations and more consistent results when compared to samples obtained from the tail. Using the ear of the piglet to obtain a blood sample for Hb testing could be easily implemented by industry. This would allow Hb levels to be used as a trait for selection and management to increase survivability in piglets and iron content in pork.

HERMESCH, S. and JONES, R.M. (2012). *Animal*. **6:**1904-1912.

KUTTER. A.P.N, MAUCH, J.Y., RIOND, B, MARTIN-JURADO, O, SPIELMANN, N, WEISS, M, and BETTSHART-WOLFENSBERGER, R .(2012). *Laboratory Animals*. **46:**65-70.

ROOTWELT, V., REKSEN, O., FARSTAD, W. and FRAMSTAD, T. (2012). *Journal of Animal Science*. **90:**1134-1141.

Supported in part by Australian Pork Limited.

Feeding live *Saccharomyces cerevisiae* CNCM I-1079 to the sow improves the vitality of piglets at birth: A multi-analysis of five trials

D. Guillou, E. Chevaux, Y. Le Treut and A. Sacy

[1]Lallemand Animal Nutrition, Blagnac, France.

Despite the efforts undertaken to improve maternal traits of sows (e.g., colostrum production and quality, functional teats, milk production) and farrowing area design, pre-weaning mortality of piglets remains high, especially in highly prolific herds. Increased farrowing duration and decreased body energy reserves have been considered to reduce innate capacity of the last-born and low birth-weight piglets to thrive. Fortifying sow feed with *Saccharomyces cerevisiae* CNCM I-1079 (SB) has been recommended to increase piglets' weaning weight and reduce sow body losses (Bertin *et al*., 1997). Producers have reported improved piglet vitality and hence, data from five trials were compiled to study the effect of SB on vitality at birth.

The five studies were conducted in commercial farms in France (n=3) or Canada (n=2) in 2009 or 2010. Genotype, housing, management, feeding schedule and other factors differed between trials. All trials compared local control diets (C) to SB added to gestation and lactation diets (1.10^9 colony forming units/kg) and started 3 weeks before expected parturition, when sows were allocated to treatment based on parity. Measurements in the various farms were performed by different operators but following the same procedure, namely recording individual birth time and order, scoring vitality (V), and weighing piglets individually within 5 min of life. Vitality score was described by Baxter *et al.* (2008) and was assessed in the first 15 s after birth: piglet not breathing was scored 0, piglet breathing but not moving was scored 1, moving the head 2, trying to get up 3. The piglet's gender and sow parity were recorded. Altogether, full data from 304 litters (C: 154, SB: 150) were retrieved, corresponding to 4,341 total born piglets. Birth order (BO) and birth weight (BW) were further transformed into categorical data (BO-cat: 1-4, 5-10, 11+; BW-cat: <1 kg, then by step of +0.25 kg until 1.75+). Data were first studied per trial, then the full dataset was analysed with a mixed model of analysis of variance (SPSS 19.0), including a random effect of trial to account for uncontrolled factors. The effects of treatment, piglet's gender, sow parity, and their interactions on V were studied first with BO and BW, then BO-cat and BW-cat in the model.

The proportion of piglets born alive, stillborn and mummified piglets did not differ across treatments. Distribution of born alive piglets across V marks was: 2% mark 0, 29% mark 1, 55% mark 2 and 14% mark 3. Within trial, several variables affected V significantly namely the birth interval (BI), BO and BW, while sow parity or piglet's gender did not. The BO was better related to V than cumulative farrowing duration, as V increased with BW and decreased linearly when BO increased. The significance of BI was trial dependent: in one trial, lowest V score was related to BI over 20 min, while in other trials, no difference (P>0.05) was found. The BO and BW were not statistically related, and were included as covariates in the general model, where they did not reach significance. Replacing them by the categorical variables (BO-cat and BW-cat) allowed reproduction of the effects observed in the individual trials.

Table 1. *Influence of feeding* S. cerevisiae *CNCM-I 1079 (SB) to the sow on piglet vitality at birth.*

Treatment	C	SB	SE	Significance[a]			
				TRT	BO-cat	BW-cat	BO-cat*BW-cat
V	1.66	1.75	0.023	<0.0001	<0.0001	<0.0001	0.018

[a]Model including random effect of Trial; C, control; SB, *Saccharomyces cerevisiae* CNCM-I 1079; V, vitality score; TRT, treatment; BO-cat, BW-cat, see text; non-significant interactions between TRT and BO-cat, TRT and BW-cat (not shown); SE, standard error.

Vitality score at birth was improved with SB by 0.09 units (5%) irrespective of birth order and birth weight categories (Table 1). A significant interaction was found between BO-cat and BW-cat: V of heavier piglets did not depend on BO-cat. Altogether, the results of these trials confirm a positive effect of feeding live *Saccharomyces cerevisiae* CNCM-I 1079 to the sow on the vitality of neonatal piglets, and support the potential of a reduction in pre-weaning mortality of piglets through sow nutritional management.

BAXTER, E.M., JARVIS, S., D'EATH, R.B., ROSS, D.W., ROBSON, S.K., FARISH, M., NEVISON, I.M., LAWRENCE, A.B. and EDWARDS S.A. (2008). *Theriogenology*. **69:**773-783.

BERTIN, G., BRAULT, M., MERCIER, M., BAUD, M. and TOURNUT, J. (1997). In "Proceedings of the VII[th] International Symposium on Digestive Physiology in Pigs", pp.450-453, ed. J.-P. Laplace. (EAAP Publication No. 88: Saint-Malo).

CHAPTER 9

Production, Disease, Environment and Product Quality

Relationships between *in vitro* fertilisation and *in vivo* mating outcomes with boar semen

F.J. McPherson[1], B.M.Gadella[2] and P.J. Chenoweth[3]

[1]Charles Sturt University, Wagga Wagga, NSW 2650. [2]Utrecht University, The Netherlands. [3]Chenovet Pty Ltd, Wagga Wagga, NSW 2650.

Although assessments such as sperm motility, morphology, membrane integrity and DNA status are used in attempting to predict boar semen fertility, these do not assess the functional capability of sperm in terms of fertilising oocytes and producing viable embryos (Aitken, 2006). The aim of this study was to determine if *in vitro* fertilisation (IVF) outcomes for boar semen could be used to predict *in vivo* breeding outcomes.

Single-sire semen samples (n=56) obtained in autumn/winter from 44 Large White and Duroc boars were used to fertilise abattoir-derived sow oocytes obtained by needle aspiration of follicles measuring 3-6 mm. Oocytes were then matured *in vitro* in maturation media in a 5% CO_2 incubator at 38.5ºC and 96% humidity for 2 d before fertilisation. Fertilisation was achieved using 1 million sperm/ml at a rate of 1000 sperm/oocyte after denuded oocytes had been equilibrated in IVF media for 1 hour. Presumptive zygotes were assessed 3 d post-IVF for fertilised or non-fertilised status and again as viable or non-viable blastocysts at 7 d post-IVF. Temperature of the sow ovaries (cystic, diseased and non-active ovaries were discarded) was taken at aspiration (range 30-36ºC). Accessory sperm number, fertilisation and blastocyst formation rates were assessed microscopically. The semen batches used for IVF were used to inseminate oestrous sows (n=240) within 4 d of collection. Semen was also concurrently evaluated for sperm motility (computer-assisted sperm analyser), morphology (wet mount, differential interference contrast microscopy), membrane integrity (eosin-nigrosin staining) and DNA integrity (DiffQuick staining). Data were statistically analysed (GraphPad® Prism Version 4).

Two sperm traits influenced IVF results; sperm tail defects (R^2=0.177) and normal sperm DNA (R^2=0.125) (both P<0.05). In addition, accessory sperm number helped predict both the percentage of fertilised oocytes (R^2=0.975) and the percentage of normal blastocysts (R^2=0.702) (both P<0.01). As expected, ovarian temperature influenced both the percentage of fertilised and unfertilised oocytes (both P<0.05, R^2=0.187) since oocytes degenerate rapidly when ovarian temperature becomes lower than optimum (37°C) (Yuge *et al*., 2003).

Litter size and pigs born live were predicted by percent normal blastocysts (both P<0.01) but not percent oocytes fertilised (Table 1). There was no correlation (P>0.05) between stillbirths and either the percentage of fertilised oocytes or the percentage of normal blastocysts formed. Farrowing rate was not influenced by any IVF trait. Sire line influenced litter size and stillbirths (P<0.05) but not pigs born alive or any of the IVF traits (data not shown).

Table 1. *Correlation relationships between* in vivo *breeding outcomes and IVF results.*

In vivo outcomes	% Oocytes fertilised	% Blastocysts formed	% Blastocysts hatched
Litter size	NS	P<0.01; R^2=0.24	NS
Pigs born alive	NS	P<0.01; R^2=0.24	NS
Stillbirths	NS	NS	NS

NS, not significant.

As the percent normal blastocysts helped predict the breeding outcomes of litter size and live births, it is feasible that sperm morphology and DNA/chromatin assessment could prove useful in improving breeding outcomes in commercial pig breeding enterprises if made cost and time effective. Sperm morphology and sperm DNA assessment using DiffQuick stain are possible screening tools that can be pursued by boar studs to monitor boar fertility. However, IVF is too time-consuming and expensive to be useful on a regular commercial basis at boar studs, so the use of porcine IVF is currently limited to research purposes.

AITKEN, R.J. (2006). *International Journal of Andrology*. **29**:69-75.

YUGE, M., OTOI, T., NII, M., MURAKAMI, M., KARJA, N.W., RAJAEI, F., AGUNG, B., WONGSRIKEAO, P., MURAKAMI, M. and SUZUKI, T. (2003). *Cryobiology*. **47**:102-108.

Supported in part by Pork CRC Limited Australia.

Boar exposure and split weaning used in a commercial herd to induce oestrus in lactation

R. Terry[1], K. Kind[1], D.S. Lines[2], T.E. Kennett[2], P.E. Hughes[3] and W.H.E.J. van Wettere[1]

[1]The University of Adelaide, Roseworthy, SA 5371. [2]Australian Pork Farms Group, Stirling, SA 5152. [3]South Australian Research and Development Institute, Roseworthy, SA 5371.

Stimulating sows to ovulate and conceive during lactation enables piglet weaning age to be increased and the piglets to achieve greater growth potential (Cabrera *et al*., 2010), whilst maintaining sow reproductive efficiency. This trial tested the hypothesis that daily boar exposure and a reduction in the suckled litter size on d 18 of lactation would increase the lactation oestrous expression for both multiparous (MP) and primiparous (PP) sows.

The study was conducted in winter/spring 2012 using a total of 299 MP (parity 2.5±0.03, mean±SE; range 2-3) and 303 PP sows. Prior to shed entry, sows were allocated randomly within parity to receive either boar exposure (BE) or no BE (No BE), and housed by treatment in separate farrowing sheds. On d 1 of lactation litter size was standardised to 11 piglets per sow. Within parity group, sows previously assigned to receive BE were stratified according to suckled litter size and sow weight loss on d 17 of lactation and allocated to one of two litter size treatments: litter size unchanged (BE) or litter size reduced to the seven lightest piglets (BESPW7) on d 18 of lactation. From d 18 of lactation until weaning, sows in both BE treatments were taken daily to a detection mating area where they received 15 min of full physical BE. At the first observed oestrus during lactation or after weaning, sows were artificially inseminated (AI). On d 2 after weaning, a blood sample was collected from No BE sows and assayed for progesterone (P4) by radioimmunoassay to determine if a spontaneous lactation ovulation had occurred. Sows were weaned on d 30.7±0.05 of lactation. The cumulative proportion of sows expressing oestrus during and after lactation and associated farrowing rates were analysed as χ^2. Subsequent litter size data were analysed using an ANOVA with experimental replicate accounted for (GenStat, 10th Edition; UK).

Table 1. *Proportion of sows showing oestrus in response to treatment, and subsequent farrowing rate (FR) and total pigs born, in sows mated during lactation (Lact) or post-weaning (Pw).*

Treatment	No BE			BE			BESPW7			Pooled SEM
Parity	MP	PP	Both	MP	PP	Both	MP	PP	Both	
Number	74	75	149	151	151	302	75	77	152	
Lact. Oestrus[1] (%)	24[a]	08[b]	16[c]	76[a]	47[b]	62[d]	89[a]	61[b]	75[e]	
Pw oestrus[2]	72/74	73/75	145/149	35/36	78/80	113/116	8/8	27/30	35/38	
FR, AI Lact (%)	na[3]	na[3]	na[3]	73[*]	82	77	73[*]	77[*]	75[*]	
FR, AI Pw (%)	83	83	83	83[*]	81	82	88[*]	96[*]	94[*]	
Pooled FR rate (%)	83	83	83	76	82	79	75	84	79	
TB, AI lact[4]	na	na	na	10.9	11.2	11.0	11.9	11.8	11.9	0.21
TB, AI pw[4]	12.5	11.5	12.0	11.6	11.5	11.6	12.8	11.1	11.8	0.20

[a,b]Means in a row with differing superscripts indicate parity differences within treatment (P<0.05); [c,d,e]Means in a row with differing superscripts indicate main treatment effects (P<0.05); *Within columns, indicate differences between lactation and post-weaning mating (P<0.05); [1]No BE lactational oestrus percentage based on P4 ≥4ng/ml on d 2 post-weaning; MP, Multiparous sows; PP, Primiparous sows; [2]Anoestrus sows at weaning expressing oestrus post-weaning; [3]Sows in the No BE treatment were not AI whilst lactating; [4]TB, total born piglets in subsequent litter.

Providing sows with BE increased the incidence of lactational oestrus, with a further increase observed when litter size was reduced to seven piglets (Table 1). In all groups, the incidence of lactation oestrus was higher in MP compared to PP sows. Spontaneous ovulation in the No BE treatment is supported by the longer weaning to oestrus intervals compared to sows which did not ovulate during lactation, with similar litter sizes (16.1±1.4 d and 9.5±0.3; 4.8±0.6 d and 9.3±0.1; P<0.05). Compared to their counterparts mated in lactation, farrowing rates were higher for BE MP sows and all BESPW7 sows mated post-weaning (P<0.05). Subsequent TB was not affected by treatment or timing of mating (Table 1). These data suggest that MP sows provided with BE is effective at stimulating a lactational oestrus whilst PP sows require, in addition to BE, a reduction in suckled litter size to seven piglets. The incidence of spontaneous oestrus in only 16% of sows in the "No BE" group suggests luteinising hormone release during lactation is increasing.

CABRERA, R.A., BOYD, R.D., JUNGST, S.B., WILSON, E.R., JOHNSTON, M.E., VIGNES, J.L. and ODLE, J. (2010). *Journal of Animal Science*. **88**:2265-2276.

Supported in part by Pork CRC Limited Australia.

Oestrus induced in primiparous sows by intermittent suckling shows a bimodal distribution

T.Y. Chen[1], A.L. Knight[2], E.G. Bouwman[1], D. Turpin[3] and P. Langendijk[1]

[1]South Australian Research and Development Institute, Roseworthy, SA 5371. [2]The University of Adelaide, Roseworthy, SA 5371. [3]Murdoch University, Murdoch, WA 6150.

Lactating sows usually only conceive after weaning with conventional lactation management. However, intermittent suckling (IS), with sows separated from piglets for a number of hours daily, allows multiparous sows to ovulate during lactation, by reducing the suckling-induced inhibition of gonadotrophin secretion, and allows sows to be mated during lactation. Previous work in multiparous sows has shown that if IS starts around 20 d after farrowing, reproductive performance is not compromised (Soede *et al*., 2011). Primiparous sows, however, may have problems with post-weaning reproductive performance and there is a lack of information regarding IS regimens in this parity. Therefore, the current study evaluated the effects of IS compared to conventional weaning on follicle development and reproductive performance in primiparous sows.

Primiparous sows (Large White and Large white x Landrace; n=34) were randomly allocated to two treatments within each replicate and were weighed after farrowing (206±2.7 kg, mean±SEM) and at 3 weeks of lactation. Litters were standardised to 11 piglets within 3 d after farrowing. In the IS treatment (n=15), sows were separated from piglets for 8h/d and had daily boar exposure from d 7 before weaning on d 28, whereas piglets in the conventional weaning treatment (CW; n=19) had continuous access to sows until weaning, and then had daily boar exposure. Sows were artificially inseminated at their first standing oestrus and this was repeated every 24 h until ovulation. Follicle diameter and ovulation were monitored every 12 h from the first standing oestrus using trans-rectal ultrasonography. All statistical analyses were performed using GLM procedures (SAS®; USA).

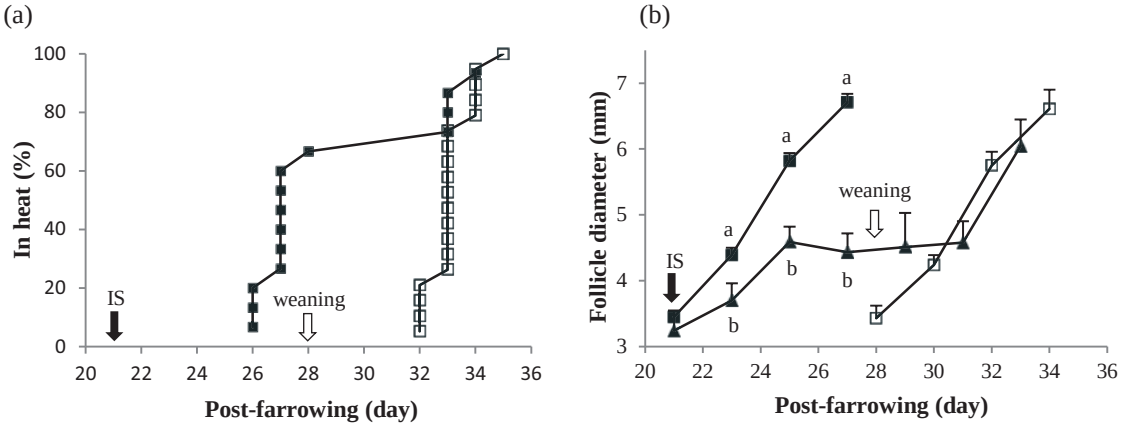


Figure 1. (a) *Timing of oestrus (expressed as percentage of sows in heat) (CW=□, n=19; IS=■, n=15), and (b) follicle diameter (CW=□, n=19; IS with oestrus during lactation=■, n=10; IS with oestrus after weaning=▲, n=4), relative to farrowing (days).*

All (19/19) CW sows showed oestrus within 7 d after weaning. Of the IS sows showing oestrus, 71% (10/14) showed oestrus during lactation and 4/14 sows showed oestrus after weaning. One IS sow remained anoestrous. The timing of oestrus thus showed a bimodal pattern in IS (either 5-7 d after start of IS or after 5-7 d after weaning). Body weight loss during lactation did not differ (P>0.05) among IS response categories, but from d 23 follicle diameter was larger (P<0.01) in IS sows that came into heat during lactation than in those coming into heat after weaning. Pregnancy rate for sows that were mated during lactation or after weaning was 90% versus 100%. In conclusion, the response to a limited nursing regime is an all-or-none event, with two distinct responses and "non-responders" completing follicle growth only after weaning.

SOEDE, N.M., LAURENSSEN, B., ABRAHAMSE-BERKEVELD, M., GERRITSEN, R., DIRX-KUIJKEN, N., LANGENDIJK, P. and KEMP, B. (2011). *Animal Reproduction Science*. **130**:74-81.

Supported in part by Pork CRC Limited Australia.

High growth rates during early pregnancy positively affect farrowing rate in parity one and two sows

R.Z. Athorn, K. S. Sawyer, C.L. Collins and B.G. Luxford

Rivalea Australia Pty Ltd, Corowa, NSW 2646.

Younger first and second parity sows entering the gestation unit in a negative energy balance often show a reduced farrowing rate and/or a reduced litter size (Hoving *et al*., 2011). An increase in feed intake during the first month of gestation may aid in the recovery of previous bodyweight loss in lactation. This period is especially important for young sows because they are still growing towards their mature body size. Hoving *et al*. (2011) reported that first and second parity sows which received 30% more feed during the first month of gestation compared to 'control' sows (3.25 versus 2.5 kg/d) had improved sow body weight recovery and an increased litter size. Similarly, it has also been reported that first parity sows with growth rates > 740 g/d had a 100% farrowing rate, compared to 94% for sows with an intermediate growth rate and 92% for sows with a low growth rate (P=0.09) during the first 25 d of gestation (Athorn *et al*., 2011). This suggests that sows entering the gestation unit in a negative energy balance and not receiving enough feed to achieve a positive energy balance and support further growth, may be at a higher risk of pregnancy failure or decreased litter sizes. Therefore, this study tested the hypothesis that high growth rates during early pregnancy would positively affect farrowing rate and litter size in parity one and two sows in commercial practice in Australia.

At d 2 of gestation, 796 first and second parity sows (Large White x Landrace F1, PrimeGro™ Genetics, Corowa, NSW) were randomly assigned to either a 2.4, 2.7 or 3.0 kg/d feed allowance until d 28 of gestation, in order to manipulate growth rates. The study observed sows gestating between July and October 2012. Sows were housed in mixed parity groups of either 90 or 45 sows and were fed individually via an electronic sow feeder (ESF) system. All three feeding levels were represented within each pen. Sows were individually weighed just prior to entry to the gestation housing and approximately 28 d later to measure growth over the trial period. High, intermediate or low growth rates were determined according to the mean growth rate of each sow parity and the 25% of sows with the highest growth rates were classified as having a high growth rate, the middle 50% an intermediate growth rate, and the lowest 25% the lowest growth rate. Data were analysed using ANOVA or a Chi squared test (farrowing rate) (IBM SPSS, Version 21.0; USA).

Table 1. *Effect of average daily gain (g) from d 2 to 28 of gestation on reproductive performance in parity one and two sows.*

	Low[1] (n=185)	Intermediate[2] (n=383)	High[3] (n=196)
Farrowing rate[#] (%)	92 (185/201)[a]	97 (383/394)[b]	98 (196/200)[b]
Total born per litter (mean±SEM)	12.1 ± 0.2[x,y]	12.1 ± 0.1[x]	12.6 ± 0.2[y]
Born alive per litter (mean±SEM)	11.1 ± 0.2[x]	11.3 ± 0.1[x,y]	11.6 ± 0.2[y]

[1]Parity 1, ≤ 464 g/d; Parity 2, ≤ 293 g/d; [2]Parity 1, 465-930 g/d; [2]Parity 2, 294-793g/d; [3]Parity 1, ≥ 931 g/d; Parity 2, ≥ 794 g/d; [#]At farrowing, excluding sows that did not farrow for non-reproductive reasons; [a,b]Means in a row not having the same superscript are significantly different (P < 0.01); [x,y] Means in a row not having the same superscript tend to differ (P < 0.10): SEM: standard error of the mean.

A clear difference in farrowing rate was seen between the high and low growth rate groups (Table 1). Total number of piglets born tended to differ between the high and intermediate growth rates, but no difference was seen between the high and low categories. However, the number of piglets born alive tended to differ between the high and low categories. In conclusion, an increase in growth rate during early pregnancy, through an increase in feed intake, leads to an improvement in farrowing rate and may also improve litter size in early parity sows.

ATHORN, R.Z., STOTT, P., SMITS, R.J. and LANGENDIJK, P. (2011). In "Manipulating Pig Production XIII", p.81, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

HOVING, L.L., SOEDE, N.M., VAN DER PEET-SCHWERING, C.M.C., GRAAT, E.A.M., FEITSMA, H. and KEMP, B. (2011). *Journal of Animal Science*. **89:**3542-3550.

Effect of split weaning on subsequent blastocyst development rates *in vitro* and embryonic gene expression

A.C. Weaver[1], K.L. Kind[1], G.R. Foxcroft[2], M.K. Dyck[2], S. Tsoi[2] and W.H.E.J. van Wettere[1]

[1]The University of Adelaide, Roseworthy, SA 5371. [2]The University of Alberta, AB, T6G 2P5, Canada.

Suckling normally prevents sow ovarian follicle growth, ovulation and luteinizing hormone (LH) release during lactation (Langendijk *et al.*, 2007). Previous work has demonstrated that boar contact during lactation results in high incidences of lactational oestrus (Terry *et al.*, 2011). However, sows suckling 10 piglets had a reduced subsequent litter size (8.9±1.1; mean±SEM) compared to sows suckling seven and five piglets (13.1±1.1 and 12.5±1.0, respectively) which may be due to reduced oocyte quality resulting from decreased LH secretion. Therefore, we hypothesized that a reduction in suckled litter size from d 18 of lactation would increase subsequent blastocyst development rates *in vitro* and alter gene expression from *in vitro* derived blastocysts.

Thirty-nine multiparous (parity 3.1±0.3) Large White x Landrace sows were studied over four replicates. On d 3 of lactation, litter size was standardised to 11 piglets per sow and maintained at this level until d 18. Sows were randomly allocated to one of two treatments: control (litter size maintained at 11) and split-wean 7 (SW7; litter size reduced to seven on d 18). From d 18 to 21, sows received 15 min of full physical boar contact daily. Sows were slaughtered on d 21 of lactation and for each sow, ovaries were collected. Follicles larger than 4 mm were aspirated and recovered cumulus oocyte complexes were matured and fertilised *in vitro* (Kelly *et al.*, 2010). Cleavage rate was recorded 48 h post-fertilisation and stage of embryonic development assessed on d 6 post-fertilisation. From four sows per treatment, total RNA was extracted from pools of 5-10 embryos using the Arcturus® PicoPure® RNA Isolation Kit and amplified using the RiboAmp HS^{Plus} kit (Applied Biosystems, CA, USA), and compared to a reference sample in a custom designed porcine embryo-specific microarray (EMPV1: EmbryoGENE Porcine Array Version 1) (Tsoi *et al*., 2012). This array comprises 43,795 probes which targets more than 20,000 unique genes. Microarray data were analysed with the Flexarray 1.6.1 software. All remaining embryos were stained using Hoechst 33342 to determine total cell number. Data were analysed using a univariate general linear model with sow as the experimental unit (SPSS Science Inc., Chicago, IL, USA).

Table 1. *Effect of split weaning on the number and size of follicles ≥ 4 mm present on d 21 post-farrowing, and embryo cleavage, blastocyst development rates and total cell numbers* in vitro.

	Control (n=20)	SW7 (n=19)	SEM	Significance
Mean number of follicles ≥ 4 mm in diameter	29.7	30.7	2.28	0.425
Mean size of follicles ≥ 4 mm in diameter	6.0	6.1	0.49	0.632
% cleaved	69.0	69.6	7.26	0.664
% blastocysts[1] from cleaved embryos	48.9	50.4	5.17	0.512
Expanded blastocyst cell number	44.2	49.0	3.17	0.168

SEM, standard error of the mean; [1]Mean number of blastocysts generated per sow/treatment, control (6.1), SW7 (7.2), SEM 0.81.

There was no difference (P>0.05) between control and SW7 sows in follicle characteristics, embryo cleavage rates, blastocyst development rates or blastocyst total cell numbers (Table 1). Analysis of microarray data indicated no differentially expressed genes between the two treatments. These results indicate that the reduced subsequent litter size observed in previous work may not be due to a reduction in oocyte quality. However, sows were slaughtered 3 d after boar contact commenced, which may have been too early for split-weaning effects on oocyte quality to be detected. Alternatively, split-weaning may lead to changes in the maternal environment important for oocyte maturation, fertilisation and early embryo development. Further work could investigate *in vivo*- instead of *in vitro*-derived blastocysts. Oocyte quality and litter sizes need to be maintained if lactation oestrus is to be a commercially viable option.

KELLY, J.M., WEAVER, A.C., KLEEMANN, D.O., FRAZER, L.M., KIND, K.L., VAN WETTERE, W.H.E.J. and WALKER, S. K. (2010). *Reproduction, Fertility and Development.* **23**:206-207.

LANGENDIJK, P., DIELEMAN, S.J., VAN DEN HAM, C.M., HAZELEGER, W., SOEDE, N.M. and KEMP, B. (2007). *Theriogenology.* **67**:1076-1086.

TERRY, R., KIND, K.L., HUGHES, P.E. and VAN WETTERE, W.H.E.J. (2011). In "Manipulating Pig Production" p.210, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

TSOI, S., ZHOU, C., GRANT, J., PASTERNAK, J., RIGAULT, P., NIEMINEN, J., SIRARD, M-A., ROBERT, C. and FOXCROFT, G. (2012). *BMC Genomics.* **13**:370.

Supported in part by Pork CRC Limited Australia.

Variability in growth and backfat of finisher pigs grown in superior and poor environments

S. Hermesch and L. Li

Animal Genetics and Breeding Unit, a joint venture of NSW Department of Primary Industries and University of New England, University of New England, Armidale, NSW 2351.

Environmental stressors (ES) such as heat or social stress lead to inferior growth performance (Hyun *et al*., 1998) and increased fatness in some studies (Black *et al*., 2001). However, whether ES also affect variability of performance is less well understood. Individual effects of stressors are additive and mean performance of a group of pigs can be used to define superior and poor environments. This study aimed to establish whether variation in growth and backfat of pigs differed between superior and poor environments.

Data included 265,165 Large White, Landrace and Duroc pigs recorded from 2000 to 2010 in nine herds of the National Pig Improvement Program in Australia (http://npip.une.edu.au). Average daily gain (ADG) and backfat at the P2 site (BF) were measured at an average live weight of 92.8 kg. Analyses conducted with ASReml (Gilmour *et al*., 2009) involved two steps to obtain firstly an estimate of the environmental variable (EV) and secondly to estimate residuals of pigs in each environmental class. The first model fitted sex, birth parity, breed, live weight (BF only) and contemporary group (CG) defined as month of birth within herds as fixed effects. Animal was fitted as a random effect. The solution for each CG was the EV used in the second model, which fitted the same fixed effects as model one, a fixed linear regression on EV, and intercept and linear regression on EV for the random sire effect. Environmental classes were based on steps of 20 g/day for ADG and 0.5 mm for BF with good representation across herds.

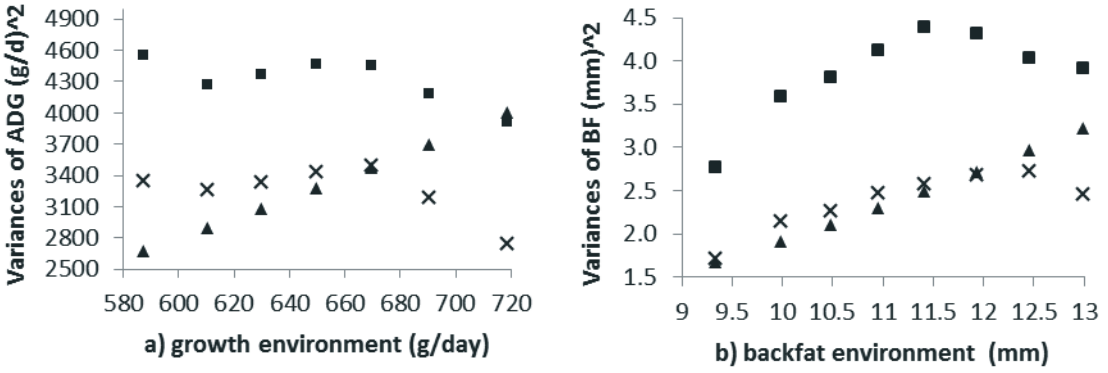


Figure 1. *Observed (■), residual (×) and expected- residual (▲) variances of lifetime average daily gain (ADG) and backfat (BF) for each growth (a) and backfat (b) environment.*

Observed and residual variances for ADG showed a general downward trend for superior environments (Figure 1a), which was contrary to the expected increase in variances due to scaling effects, i.e., a higher mean is related with a larger variance. For BF, variances showed an upward trend with higher EVs that corresponded well with expectations (Figure 1b). The expected-residual variances across the environmental trajectory were based on the average coefficient of variation for residual variances across environmental classes of 0.088 for ADG and 0.138 for BF. The difference between observed and residual variances represents the proportion of variation that was explained by the model.

Environmental stressors mostly affect growth leading to inferior performance. This study found higher variability than expected for ADG in poor environments. This higher variability further adds to the costs of inferior environments for production. In addition, it could be used as an additional environmental parameter to describe the environment better for future analyses of genotype by environment interactions.

BLACK, J.L., GILES, L.R., WYNN, P.C., KNOWLES, A.G., KERR, C.A., JONES, M.R., STROM, A.D., GALLAGHER, N.L. and EAMENS, G.J. (2001). In "Manipulating Pig Production VIII", pp. 9-36, ed P.D. Cranwell. (Australasian Pig Science Association: Werribee).

GILMOUR, A.R., GOGEL, B.J., CULLIS, B.R. and THOMPSON, R. (2009) "ASReml user guide. Release 3.0." (VSN International Ltd., Hemel Hempstead, UK).

HYUN, Y., ELLIS, M., RISKOWSKI, G. and JOHNSON, R.W. (1998). *Journal of Animal Science*. **76**:721-727.

Supported in part by the Pork CRC Limited Australia.

Actinobacillus pleuropneumoniae – a diagnostic update

C. Turni, R. Singh, L. Omaleki and P. Blackall

The University of Queensland, Brisbane, St Lucia, QLD 4072.

Actinobacillus pleuropneumoniae (*App*) is the causative agent of porcine pleuropneumonia and contributes to substantial economic losses in the swine industry worldwide. There are currently a total of 15 serovars of *App* (Chiers *et al*., 2010). In recent years there have been observations of submissions from farms previously free of *App*. There has also been an increase in submissions of *Actinobacillus*-like isolates. In this paper the prevalence of *App* serovars over 11 years and the occurrence of the related species are reported. It was hypothesised that serovar 5 isolates had limited genetic diversity.

The 336 isolates of *App* submitted from February 2002 until April 2013 were serotyped via gel diffusion, indirect haemagglutination and multiplex PCR. Genotyping of the serovar 5 isolates was performed with the enterobacterial repetitive intergenic consensus (ERIC) PCR. Partial sequencing of the 16S rDNA gene and blast analysis was done to determine the species of the *Actinobacillus*-like isolates, with the gene being first amplified with primers 27F, 519R, 530F and 1525R. The serovars of *App* were identified over the 10 years, 2002-2011 and the year 2012/13 (Table 1). The data suggest a shift in serovars in 2012 with serovar 12 submissions from outbreaks being more prominent than in previous years. Serovar 12 was first seen in 2007, then in 2008 and two samples in 2011. There was also an apparent increase in serovar 7 compared to the previous years. When determining the genotype of serovar 5 isolates in the period from 2003 to 2013, all 27 isolates, originating from 11 farms from three Australian states, displayed the same genetic fingerprint. This suggests that the isolates are a single clone and of one origin. Twenty-eight *Actinobacillus*-like isolates were submitted in the period of October 2011 until November 2012. Thirteen of these isolates had the same ERIC profile, yet four of them were identified (minimum of 96% identity) as *A. indolicus*, six as *A. porcitonsillarum* and three as *A. porcitonsillarum/minor* complex. The remaining 15 isolates with 13 different ERIC profiles were identified as *A. indolicus* (8), *A. minor* (2), *A. porcinus* (2), *A. porcitonsillarum* (1) and *A. porcitonsillarum/minor* complex (2). One of the 13 ERIC matched isolates and one of the ERIC differing isolates were further analysed by full sequencing of the 16S rDNA gene. This revealed that the first isolate (HS 3674) was closely related to *A. porcitonsillarum/minor* and the other (BR1169) to *A. indolicus* (Figure 1).

Table 1. *Isolated strains submitted to the laboratory from outbreaks (2002 -13).*

Serovar	Feb 2002 to Dec 2011 (%)	Jan 2012 to April 2013 (%)
1	27 (9)	2 (4)
5	55 (19)	5 (11)
7	76 (26)	19 (42)
12	4 (1)	7 (16)
15	102 (35)	10 (22)
3	1	
4	1	
8	2	
12/5	2	
3/9/15	1	
4/7/10/15	1	
6/8	7 (2)	
6/9	1	
NT	11 (4)	2 (4)
Total	291	45

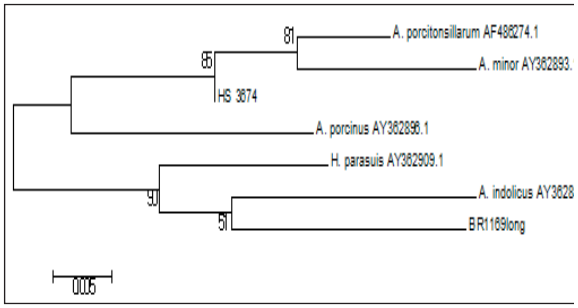


Figure 1. *Maximum likelihood tree for 16S rDNA gene*

The serotyping results are based on diagnostic submissions and hence are not a reflection of the full field population. Nevertheless, the results suggest a shift in *App* serovars in the last year with an increase in serovar 7 and 12. Several explanations could be the cause: 1) the serovars are being spread (most likely for serovar 7); 2) serovar 12, which is regarded as having a low virulence (Marsteller and Fenwick 1999), has changed in terms of virulence; 3) sow herd immunity status; or 4) management processes have changed. A single clone of serovar 5 was detected, suggesting that one strain has entered the country and has been spread among Australian pig herds. Further investigations into the *Actinobacillus*-like isolates that have emerged in the last year are necessary to define the species of these isolates.

CHIERS, K., DE WAELE, T., PASMANS, F., DUCATELLE, R. and HAESEBROUCK, F. (2010). *Veterinary Research*. **41**:65.
MARSTELLER, T. and FENWICK, B. (1999). *Swine Health Production*. **7**:161-165.

Detection of twelve *Escherichia coli* virulence genes by real time polymerase chain reaction

T.M. Limm, L.R. Fitt, R.L. O'Meara and A.M. Vanderfeen

ACE Laboratory Services, Bendigo, VIC 3550.

It is known that *Escherichia coli (E. coli)* can cause disease, morbidity and mortality in pigs (Ngeleka *et al.*, 2003). Current methods for *E. coli* virulence gene detection are time consuming and do not include all the relevant virulence genes of interest for pigs in eastern Australia. The aims of this study were to develop a real time PCR multiplex method for the detection of 12 *E. coli* virulence genes, and to analyse and compare the prevalence of the virulence gene profiles found. Previously, this laboratory has observed pure growth of non-haemolytic *E. coli* with virulence genes in clinical cases. Hence, both β haemolytic *E. coli* (HEC) and non-haemolytic *E. coli* (NHEC) have been included in this study

Primers and Taqman probes were chosen after analysis of traditional PCR products (Ngeleka *et al.*, 2003; Casey and Bosworth, 2009), using Genbank and Applied Biosystem's Primer Express program (Table 1). Each amplification (single and multiplex) was confirmed using typed isolates provided by OIE *E. coli* Reference Laboratory, University of Montreal, Canada (EcL) or the University of South Australia. Cycling conditions consisted of a 95°C 10 min hold and 40 cycles of 95°C 15 s, 60°C 30 s. Clinical diagnostic samples from pigs of all ages, with illnesses including ill thrift, diarrhoea, vomiting, coughing, septicaemia and sudden death, were received from all eastern states of Australia. Specimens included faeces, tissue, swabs and blood. Where *E. coli* was observed as the predominant culture by standard tests (Ngeleka *et al*., 2003), the *E. coli* colonies were lysed in 100µL water by boiling for 10 min at 98°C, and then used directly in the PCR.

Table 1. *Amplicon details.*

Multi-plex	Gene	Genbank accession	Amplicon position
1	ST1	M58746	325-500
	ST2	M35586	459-612
	LT1	V00275	148-221
2	STx2e	AM939641	999-1105
	EAST	AB042004	1-111
	EAE	AF099076	20-172
3	F4	M35954	502-571
	F5	M35282	286-418
	AIDA	JQ044410	1815-1941
4	F6	M35257	328-482
	F18	M61713	524-645
	F41	M21788	112-183

Table 2. *Prevalence of* E. coli *virulence gene profiles.*

E. coli	Major Australian virulence gene profiles (>1%)	Prevalence%[(1)]	
		ACE	EcL
HEC	LT1/ST1/ST2/EAST/F4	12.5	
	ST1/ST2/F4	4.0	
	AIDA/F18	1.5	1.4
	LT1/ST1/ST2/EAST/AIDA/	1.5	
	EAST/F18	1.1	
	LT1/ST2/EAST/F4	1.1	3.8
	ST1/ST2/Stx2e/AIDA/F18	1.1	
NHEC	ST2/EAST/AIDA	2.6	2.4
	ST1/F5	1.1	1.7
HEC and NHEC	EAST	19.5	12.4
	LT1/ST2/EAST	1.8	
	ST1/ST2	1.1	

[(1)]ACE – this study, EcL – Ngeleka *et al*., (2003).

Of the 272 *E. coli* isolates analysed, 86% (78/91) of the HEC and 36% (66/181) of the NHEC isolates tested positive for virulence genes. Four virulence gene profiles were observed in the NHEC alone (two were <1%), while three profiles were observed in both HEC and NHEC isolates.

Profile frequencies were compared with those published for Canada (Ngeleka *et al.*, 2003) (Table 2). Some profiles were observed in one country and not the other; the most outstanding of these was for the profile LT1/ST1/ST2/ EAST/F4, which was isolated from faecal, tissue and blood specimens. This was the most frequent HEC profile observed in Australia (12.5%), but was not observed in the Canadian study. Isolates with this profile were tested by EcL and returned completely concordant results.

This real time PCR is a fast, specific and reliable tool for the detection of *E. coli* virulence genes. Considering that 36% of the NHEC had *E. coli* virulence genes, and were the predominant microbial agent observed, NHEC must be considered for future diagnostic specimens.

NGELEKA, M., PRITCHARD, J., APPLEYARD, G., MIDDLETON, D. and FAIRBROTHER, J. (2003*). Journal of Veterinary Diagnostic Investigation*. **15**:242–252.

CASEY T. and BOSWORTH B. (2009). *Journal of Veterinary Diagnostic Investigation.* **21**:25-30.

Porcine circovirus 1 detection in a New Zealand investigation into increased mortalities and abortions

N. Brangenberg[1], S. Dobbinson[2], E. Neumann[3], A. Suddith[4], R. Hesse[4], W. Stanislawek[1] and K. Tham[1]

[1]Ministry for Primary Industries, Wallaceville, New Zealand. [2]FreshPork, Christchurch, New Zealand. [3]Massey University, Palmerston North, New Zealand. [4]Kansas State University, Manhattan, KS, 66045 USA.

From 2008 to 2012, there were sporadic epizootics of abortions during late gestation in gilts and sows on two commercial outdoor piggeries on the South Island of New Zealand (NZ). During this time the same two farms experienced an increase in mortality characterised by pulmonary oedema in gilts occurring around the time of first mating. An investigation was undertaken to rule out disease agents known to be exotic to NZ (e.g., porcine reproductive respiratory syndrome and classical swine fever viruses). A comparative study of the affected (n=2) and an unaffected nearby farm (n=1) was then conducted from January to July 2012 to assess the association of several endemic infectious organisms with the observed clinical syndromes. All three farms vaccinated unmated gilts with a commercially available porcine circovirus vaccine 4-6 weeks prior to their anticipated date of first mating.

Tissue and serum samples were tested (i.e., virology, histopathology, serology, and bacteriology) from 14 pigs on the two affected farms [average age 1.7 years (not including foetal samples)], which met the case definitions during the study time, and 25 abattoir samples from the unaffected farm. The unaffected farm had no mortalities or abortions that met the case definitions during this time. Information was recorded on a total of 19 abortions and 29 acute deaths.

In five out of six abortion cases and three out eight acute death cases that were tested, porcine circovirus type 1 (PCV1) was detected in tissue using PCR. Furthermore, PCV2 was detected in one of the six abortion cases and in four out of eight acute deaths (Table 1). A real-time PCV PCR assay (Fenaux *et al.*, 2000) was used with amplicons purified and sequenced to determine homology with published sequences of PCV1 and PCV 2. Other viruses detected by PCR on tissue included bocavirus, torque teno virus, and porcine parvovirus. The PCR analysis for generic pestivirus, pan-corona and influenza, and virus isolation, were all negative. Serum and tissue abattoir samples collected from 85 kg pigs and cull sows from the unaffected farm were also positive for PCV1, bocavirus, and torque teno virus.

Internationally, approximately 2-3% of PCV-associated disease (PCVAD) cases are concurrently estimated to be infected with PCV1 while 75-82.6% is estimated to be infected with PCV2 (Puvanendiran *et al*., 2011, Shen *et al*., 2012). The current study suggests that PCV1 may be a component cause or associated with certain clinical presentations of PCVAD more than previously recognized. We suggest that monitoring of PCV1 strains may be of benefit to determine if they remain non-pathogenic.

Table 1. *Prevalence of PCV1 and PCV2 in tissue samples from cases on affected farms tested between January and July 2012.*

Farm	Clinical expression	PCV1 (PCR, %)	PCV2 (PCR, %)	Annualised prevalence of clinical disease	
				Pre-outbreak: Jan-Jul 2011 (%)	Outbreak: Jan-Jul 2012 (%)
1	*Abortion*	5/6 (83.3)	1/6 (16.7)	0.9	2.7
	Acute death	1/8 (12.5)	3/8 (37.5)	2.5	1.4
2	*Abortion*	0/6 (0)	0/6 (0)	0.3	0
	Acute death	2/8 (25)	1/8 (12.5)	2.4	1.5

It is not possible due to the small sample sizes to determine causality. However, the high prevalence of PCV1 detection in the tissue of affected pigs in this small outbreak is unique relative to previous reports in the literature. The cause of the pulmonary oedema nor the sudden death could not be explained nor could it be specifically related to the increased abortions or presence of PCV1. Further sequencing work of the PCV1 detected is underway in light of the current interest in the phylogenetics of circulating circovirus strains.

FENAUX, M., HALBUR, P.G., GILL, M., TOTH, T.E. and MENG, X.J. (2000). *Journal of Clinical Microbiology.* **38**:2494-2503.
PUVANENDIRAN, S., STONE, S., YU, W., JOHNSON, C.R, ABRAHANTE, J., JIMENEZ, L.G., GRIGGS, T., HALEY, C., WAGNER, B. and MURTAUGH, M.P. (2011). *Virus Research.* **157**:92-98.
SHEN, H.G., HALBUR, P.G. and OPRIESSNIG, T. (2012). *Journal of General Virology*. **93**:1345-1355.

Polymerase chain reaction detection of *Actinobacillus pleuropneumoniae* in pigs using pooled oral fluids

N.M. Dron[1], R. E. Doyle[1], M. Hernandez-Jover[1] and P.K. Holyoake[2]

[1]Charles Sturt University, Wagga Wagga, NSW 2678. [2]Department of Environmental and Primary Industries, Epsom, VIC 3551.

Actinobacillus pleuropneumoniae (APP) is endemic on many pig farms in Australia and is the most common cause of pleuropneumoniae in pigs in the country (Lee, 2012). Current methods of APP diagnosis in live pigs rely on the collection of either tonsillar swabs for bacterial isolation or polymerase chain reaction (PCR) testing, or blood samples for detection of APP-specific antibodies. Testing of pooled oral fluids (OF) by PCR allows large numbers of animals to be sampled in an easy, cost-effective, and welfare-friendly manner (Costa *et al*., 2012). The aim of the following experiment was to determine the sensitivity of APP PCR when used to test grouped OF.

Two separate experiments were conducted to determine the sensitivity of a PCR for detecting the *OmlA* gene of APP in OF samples from pigs. Firstly, OF was collected from a group of APP-free pigs using the new TEGO^{TM} swine oral fluid collection kit. The OF sample along with a diluent control were serially spiked with a known concentration of APP bacteria (APPAlive^{TM} Vaccine, Pork CRC Ltd, Australia). Conventional PCR was used to amplify the *OmlA* sequence. Additionally the DNA spiked OF was added to the PCR well at three different concentrations (1, 3 and 6 µl) to determine if there was an inhibitory affect from the saliva or the APPAlive^{TM} vaccine. In the second experiment, tonsillar swabs, OF, and blood samples were collected from 25 (16-week-old) grower pigs from a farm with a known positive APP status. Tonsil and OF samples were tested using *OmlA*, and blood serum tested using ELISA to determine the APP status of the individuals and compare the sensitivity of testing for APP by the different methods.

The PCR test was successful in detecting APP bacteria in spiked OF to a concentration of $1.26x10^4$ colony forming units (CFU)/ml, and was tenfold more sensitive ($1.26x10^3$ CFU/ml) when OF was purified and homogenised with diluent prior to PCR testing. The difference in sensitivity suggests that there was a slight inhibitory effect from the saliva. This was further supported when the test sensitivity increased after the quantity of DNA-spiked OF added to PCR wells was reduced from 6 µl to 3 µl and to 1 µl consecutively. The results of this experiment indicate that PCR testing of OF for APP using the *OmlA* gene sequence is a sensitive method of detection when compared to bacterial culture isolation (10^3 CFU/ml), supporting the future use of grouped OF collection for the diagnosis of APP within a herd (Gagné *et al*., 1998). However, the constituents of saliva could reduce the PCR sensitivity. Methods of saliva and DNA purification including columns, sieves, or centrifugation to remove the supernatant could overcome the potential inhibitory effect of saliva impurities on PCR methods.

In the second experiment, APP was detected by PCR in the OF of 1 of the 25 pigs, another pig was seropositive, whilst all the tonsillar swabs were negative. These results suggest that APP bacteria can be detected using PCR testing of OF, and may even be more sensitive than tonsillar swabbing. OF has a potential place in APP diagnostics; however, better understanding of the pathology of APP is necessary. The efficacy of PCR and ELISA testing of OF is determined by the presence of the bacteria within the oral-nasal cavities and respiratory tract. Previous investigations have recorded variances in the number of APP forming bacteria found in the OF depending on serotype, and infection stage (Costa *et al.*, 2011). This investigation supports the potential use of OF for APP diagnostics, with the limitation of APP bacteria being continuously found in OF during all stages of infection and across all serotypes.

COSTA, G., OLIVEIRA, S., TORRISON, J. and DEE, S. (2011). *Veterinary Microbiology*. **148**:246-251.

COSTA, G., OLIVEIRA, S., and TORRISON, J. (2012). *Journal of Swine Health and Production.* **20**:78-81.

GAGNÉ, A., LACOUTURE, S., BROES, A., D'ALLAIRE, S. and GOTTSCHALK, M. (1998). *Journal of Clinical Microbiology.* **36**:251-254.

LEE, A. (2012). APP pleuropneumonia in pigs. NSW Department of Primary Industries Primefact 1222(1).

Supported in part by Pork CRC Limited Australia, Charles Sturt University, and Bendigo PHRU DPI.

Correlation between mounting activity and leg injuries in sows housed in groups after weaning

L.U. Hansen[1], P.M. Weber[1,2], S-L. Schild[1] and C.F. Hansen[2]

[1]Pig Research Centre, Copenhagen, Denmark. [2] University of Copenhagen, Denmark.

Since 1997, Danish pig producers have been able to obtain a premium for their pork for the UK market if engaging in branded production. One of the requirements for this production is that sows must be loose-housed from weaning until farrowing. However several studies conducted by the Pig Research Centre have shown that young sows in particular that are housed in groups after weaning have reduced reproductive performance. For instance, Hansen and Jensen (2005b) showed that young sows gave birth to 0.6 more total born piglets per litter if they were housed in homogenous groups compared with young sows housed in heterogeneous groups with sows of mixed parity. In addition, sows weaned into loose groups had a higher frequency of leg problems compared with sows that were confined during oestrus (Hansen and Jensen, 2005a).

The aim of the current study was first to investigate the correlation between mounting activity and leg problems in sows housed in groups after weaning, and second, to examine if older sows mounted more than younger animals. Our hypotheses were first, that a positive correlation would exist between mounting activity and leg injuries and second, that older sows would mount more compared with younger sows.

A total of 921 sows (Landrace × Yorkshire) in three different piggeries with group housing from weaning until farrowing were included in the study. The group size varied between the different piggeries. In herd A the sows were housed in groups of 20, in herd B the group size was 25 and in herd C there were 50 animals in a group. In all herds the mating units had free access eating and inseminating stalls, a non-slippery floor in the activity area (approximately 25 cm straw bedding) and an area per sow of 4 m^2 including the stalls. All sows were loose from weaning and assessed for leg injuries on d 3 using visual gait scoring. Only sows with no leg injuries on d 3 were included in the study, meaning that leg injuries that occurred due to mixing and establishment of the hierarchy were not included. These animals were removed from the group and housed elsewhere in the herd. On d 4 and 6 after grouping the mounting activity was recorded for all sows from 0900 to 1200 and again from 1300 to 1500. Mounting activity included mounting and mounting attempts and the frequency was recorded continuously during the two observations periods. A mount or mounting attempt was recorded when an animal placed the head or at least one leg on the back of a group member. On d 7 after grouping, the sows were again evaluated for leg injuries based on gait.

Collected data were analysed using logistic regression in SAS (version 9.2), and sows in the same group were considered as replicates. Using logistic regression it was examined if herd, sow parity and day of recording (d 4 or d 6) were related to the number of mountings. Leg problems on d 7 were evaluated using the same logistic regression approach but with the addition that the number of delivered and received mountings were included as explanatory variables.

Contrary to our hypothesis there was no correlation (P=0.786) between sows involved in mounting activities (both delivering and receiving) on d 4 and d 6 on leg injuries at d 7 after weaning. Furthermore there was no link (P=0.606) between parity of the sow and leg injuries on d 7. A total of 10% of the sows had leg problems recorded on d 7. Nonetheless it has to be acknowledged that sows with leg injuries that occurred before d 3 were not included in this study as the focus was on mounting behaviour and not aggression *per se*.

However, as expected sows of parity 3 or higher mounted more than younger sows (P=0.033). In relation to sows that were mounted, there was no correlation with parity (P=0.214). In addition, more than twice as many sows (n=314) were mounting on d 6 compared with d 4 after weaning (n=136) (P<0001). Of the sows that were studied on d 4 and d 6 after weaning, 26% did not participate in mounting behaviour, 43% were involved in mounting on one of the days, and 31% took part in mounting on both days.

In conclusion, this study failed to show a correlation between mounting behaviour and leg injuries in sows during/over the first week after weaning under loose-housing conditions.

HANSEN, L.U. and JENSEN, H.K. (2005a). *www.vsp.lf.dk*. Report no. 697.
HANSEN, L.U. and JENSEN, H.K. (2005b). *www.vsp.lf.dk*. Report no. 698.

This project was supported by the Pig Research Centre.

Adoption of three technologies by Victorian pig producers

P. Holyoake and K. Moore

Department of Environment and Primary Industries Victoria, Bendigo, VIC 3551.

Innovation contributes to improving farm productivity and sustainability. This paper focuses on three technologies chosen based on their age. ProHand® is considered a relatively old technology, APPAlive® is medium-term and pond covers a new technology. ProHand® was considered a "universal" technology with application to all farms, whilst APPAlive® and pond covers would only benefit farms with uncontrolled pleuropneumonia and effluent ponds, respectively. The aim of this study was to identify the drivers and barriers to the adoption of these three technologies (ProHand®, APPAlive® and effluent pond covers) on pig farms.

An anonymous self-administered questionnaire was developed consisting of 20 (mostly closed) questions relating to the three technologies (ProHand®, APPAlive® and effluent pond covers), a question asking producers how they received information about new technologies, and another asking their preferred method for receiving this information. The questionnaire was posted to 458 individuals in Victoria who had an active registration on the APL PigPass database. The questionnaire was completed by the owner or manager of the farm. A second questionnaire was posted 4 weeks later to capture non-responders from the first mail-out.

One hundred of the 458 surveys sent out (22%) were returned in a completed format that could be analysed. Summary statistics were calculated to describe farm characteristics and producer responses in relation to the three technologies. Logistic regression was used to examine the association between farm size and awareness of ProHand®. The odds ratio (OR) and 95% confidence interval (CI) are reported. Data from the questionnaire was analysed using Stata/IC 11.1 (StataCorp LP). This is a low response rate for a paper-based survey (Cook *et al*., 2000). The 100 farms represented a total of approximately 27,540 sows or 50% of Victoria's 55,583 sow population (ABS, 2012). Thirty-eight respondents (38%) indicated that they had outdoor herds, 25 respondents had indoor only and the remainder consisted of a mix of housing types.

Table 1 outlines producer awareness and participation in the three technologies. Producers were aware of ProHand® from APL communications (41%), industry seminars/workshops (44%) and industry journals (35%). Consultants and pig farmers were other sources of information (both 28%). Compared to producers with small herds, producers with larger herds were more likely to have heard of ProHand® (OR 3.7; 95% CI 2.2-6.2, P<0.001) and to have used ProHand® (OR 2.7; 95%CI 1.7-4.4, P<0.001). Reasons cited for not adopting ProHand® included lack of time (53%), no benefit (40%), high cost (26%) and lack of access to training (20%). Reasons for non-adoption of APPAlive® included absence of pleuropneumonia (64%), against veterinary advice (21%), or because current disease control methods were effective (21%). Producers were made aware of pond covers mostly from industry journals (63%), APL (46%) and seminars (25%). Most commonly cited reasons for using pond covers included to reduce energy costs (90%) and to generate income through carbon trading (80%).

Table 1. *Percentage of producers with awareness of, and participation in, three technologies.*

	ProHand®	APPAlive®	Pond covers
Awareness	32	25	62
Participation*	53	4	15

*Expressed as a percentage of those aware of the technology.

"Written material" was the most frequent source of information by 38% of producers, followed by "other pig farmers" (30%) and "non-written" material from APL (23%). Almost one fifth of producers ranked the Internet and their veterinarian as sources of information. Most producers (60%) preferred receiving information on new technologies in writing, with electronic sources the preferred method by 36% of producers and from private veterinarians by 25% of producers (both small and large-scale).

This study highlights the importance of using a variety of communication methods to enhance producers' awareness of new technologies. Despite the variety and availability of electronic media, written and face-to-face communication remains a favourite tool for promoting awareness and adoption of technologies.

ABS (2012). Agricultural Commodities, Australia, 2010–11. (Cat. No.7121.0).
COOK, C., HEATH, F., and THOMPSON R.L. (2000). *Educational and Psychological Measurement.* **60**:821–836.

Supported in part by Australian Pork Limited.

The use of sloped walls in the dunging area of PigSAFE farrowing pens over summer to improve piglet survival

R.S. Morrison[1], B.E. McLeod[1], M. Farish[2], M.C. Whyte[1] and E.M. Baxter[2]

[1]Rivalea Australia, Corowa, 2646, NSW. [2]Scottish Agricultural College, Edinburgh, EH9 3JG, UK.

The PigSAFE (Piglet and Sow Alternative Farrowing Environment) loose farrowing pen has been developed in the United Kingdom. This system is designed to allow the sows to perform maternal behaviours such as isolation, nest building and bonding with piglets. The pen design incorporates a nest area with solid flooring, the provision of nesting material and sloped walls against which the sow can slide slowly to ground level for suckling (which lowers the chance of piglets being trapped and killed). A heated creep area is easily accessed by piglets from the nest, and a separate slatted dunging area is bounded by walls with barred panels to adjacent pens to discourage farrowing outside the nest and allow physical contact between sows. A feeding crate is included where the sow can be locked away to allow safe inspection or treatment of the piglets (Baxter *et al*., 2011). An Australian pen design includes a spray mister and cooling fan over the dunging area, which is activated during summer. There is anecdotal evidence that the sow will spend more time in the dunging area over summer, which may put piglets at risk as there are no protected zones within this area. The aim of this experiment was to compare piglet survival in the PigSAFE farrowing pen with and without a small sloped wall in the dunging area, with the hypothesis tested that less piglet deaths would occur in the dunging area compared to other areas of the pen in the treatments with a sloped wall in the dunging area.

Ninety sows (Large White x Landrace-PrimeGro™ Genetics) were randomly selected prior to entry to their farrowing accommodation. There were 45 sows allocated to each treatment over three time replicates. The experiment began in December 2012 and finished in February 2013. Sow reproductive performance (i.e., number born alive) and the location and cause of death (i.e., overlying by sow, unthrifty piglet) of individual piglets was recorded on a daily basis. The piglets were weaned when they were approximately 25 d of age. Chi-square analysis was used to determine if there were differences in number and cause of deaths and location of piglet deaths. There was no difference ($P>0.05$) in total number of deaths between treatments. There were less ($P<0.05$) piglet deaths in the dunging area in the sloped wall treatment.

Table 1. *Litter characteristics and location of death in the PigSAFE pen with and without a sloped wall in the dunging area.*

	No sloped wall	Sloped wall	Significance and (Chi-squares statistic)
Number litters farrowed	44	44	-
Total number piglets born alive	513	488	-
Total piglet deaths	115	96	0.287 (1.13)
Number of deaths (overlying)	85	58	0.037 (4.36)
Number of deaths (unthrifty etc.)	30	38	0.037 (4.36)
Location: (number of dead piglets in area)			
Dunging area	30	13	0.024 (5.07)
Entrance door	6	4	0.721 (0.13)
Open nest	14	18	0.185 (1.76)
Under sloped wall in nest	6	9	0.242 (1.37)
Creep area	19	19	0.538 (0.38)
Near kickboard in nest	5	2	0.360 (0.84)
Pop hole between nest and dunging area	8	3	0.212 (1.55)
Unknown/Piglet euthanised	27	28	0.349 (0.88)

These data suggest that sloped walls in the dunging area afford greater piglet protection in this area, therefore the hypothesis was accepted. However, with overall piglet mortality not different between treatments, adding this extra feature may not be cost effective. Further investigation of additional modifications that will decrease overall piglet mortality in summer will increase the commercial viability of PigSAFE systems.

BAXTER, E.M, ADELEYE, O.O., JACK, M., ISON, S. and EDWARDS, S.A. (2011) In. "Manipulating Pig Production XIII", p.239, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

The financial support of Rivalea Australia and the technical support of the Rivalea Research and Innovation and Production management and staff is gratefully acknowledged.

Cereal base of the diet does not influence meat colour

R.J.E. Hewitt[1] and R.J. van Barneveld[2]

[1]CHM Alliance Pty Ltd, Millmerran, QLD 4350. [2]Barneveld Nutrition Pty Ltd, Loganholme, QLD 4129.

Sorghum is an important grain for northern pig producing regions, where it is often abundant and very price competitive relative to other cereal grains. Sorghum is likely to become more important for other pig producing regions as demand for cereals more suited to human consumption increases. However, sorghum differs in its rate of starch digestion relative to other grains (Al-Rabadi *et al.*, 2009). These differences are unlikely to have a significant effect on the growing pig where feed is available on an *ad libitum* basis. However, when pigs are taken off feed prior to slaughter the differing pattern of digestion may influence the replenishment of muscle glycogen reserves, affecting the rate of pH fall and thus colour development.

There is limited information in the scientific literature of differences in meat colour, or eating quality aspects, resulting from different ingredients in pig diets. D'Souza *et al*. (2011) found a difference in ultimate pH between barley-lupin and maize-soybean diets, although this did not translate into differences in muscle colour. This research was in contrast to the results of Zraly *et al.* (2007) who reported no differences between lupin and barley-wheat diets. The objective of this experiment was to measure the differences in meat colour as a result of feeding sorghum or wheat as the primary dietary ingredient, with the hypothesis that diet ingredients do not affect meat colour.

Female pigs (Hybrid slaughter generation, PIC Australia Pty Ltd, Grong Grong, NSW) were grown on a commercial farm in south-east Queensland. Twenty pens (40 pigs/pen) were allocated to one of two treatments arranged in a randomised block design with entry weight as a blocking factor (~55 d, 20 kg). One treatment utilised sorghum as the cereal base and the other wheat. Pigs received four diets over their growth period: a weaner diet (14.5 MJ digestible energy (DE)/kg, 0.80 g available lysine (AvL)/MJ DE), grower 1 (14.0 MJ DE/kg, 0.71 g AvL/MJ DE), grower 2 (13.8 MJ DE/kg, 0.65 g AvL/MJ DE) and a finisher diet (13.5 MJ DE/kg, 0.64 g AvL/MJ DE). In the wheat diets, wheat comprised 65, 74, 70 and 70% of the diets respectively, whilst in the sorghum diets, sorghum comprised 20, 40, 66 and 60% of the diets. Pigs within a pen were identified with a common tattoo. On the day of slaughter an equal number of treatment pens, matched for weight, were transported to an abattoir (~400 km) and held overnight, with total time off feed being 23 h. Slaughter took place over three consecutive days. Meat colour assessment was conducted on the flank muscle (*Rectus abdominis*) 20-22 h post-slaughter, with a single measure using a Minolta Chromameter CR-400 (C lighting, 2° standard observer, 8 mm measuring aperture), calibrated to a standard white tile. The flank muscle was assessed due to its common use as an indicator muscle and care was taken to avoid measuring areas of adipose or connective tissue. Colour and slaughter data were pooled on a pen basis and data were analysed via GLM ANOVA (GenStat 15th ed., VSNI Ltd, Hemel Hempstead, UK), and differences were determined by least significant difference (LSD) ($P<0.05$).

Table 1. *Mean (SEM) hot standard carcase weight (HSCW; kg), backfat depth at the P2 site (mm) and the CIE 1976 (L*, a*, b*) colour space coordinates for pigs fed wheat or sorghum based diets.*

				Significance		
	Wheat	Sorghum	SED	Diet (D)	Slaughter day (S)	D x S
HSCW	83.3 (1.59)	83.3 (1.82)	3.61	0.466	0.000	0.930
P2	11.5 (0.16)	11.6 (0.31)	0.51	0.730	0.000	0.225
CIE 1976 L*	42.0 (0.28)	41.6 (0.46)	0.82	0.574	0.001	0.188
CIE 1976 a*	15.4 (0.43)	14.5 (0.27)	0.42	0.119	0.355	0.819
CIE 1976 b*	10.1[a] (0.27)	9.4[b] (0.18)	0.35	0.042	0.181	0.999

[ab]Means in a row not having the same superscript are significantly different ($P<0.05$); SED, standard error of difference.

There was no difference in lightness (L*) or redness (a*), as measured on the flank muscle, from pigs fed sorghum or wheat-based diets (Table 1), however, wheat pigs were more yellow (b*). Whilst slaughter day effects were significant ($P<0.001$) for HSCW, P2 and L*, there was no interaction between slaughter day and diet. The results from this experiment indicate that the inclusion of sorghum at the expense of wheat in growing pig diets has minimal influence on meat colour and it is therefore not necessary to exclude sorghum from the ingredients matrix when formulating diets for markets that show sensitivity to meat colour.

AL-RABADI, G.J., WILLIAMS, B.A., TORLEY, P., BRYDEN, W.L., NIELSEN, S. and GIDLEY, M.J. (2009) In "Manipulating Pig Production XII", p. 58, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

D'SOUZA, D.N., DUNSHEA, F.R. and MULLAN, B.P. (2011) In "Proceedings of the 57th International Congress of Meat Science and Technology", p.596 (Ghent, Belgium).

ZRALY, Z., PISARIKOVA, B., TRCKOVA, M., HERZIG, I., JUZL, M. and SIMEONOVOVA, J. (2007). *Veterinarni Medicina.* **52:**29-41.

Divergent selection for residual feed intake alters whole body tissue accretion rate in growing pigs

A.J. Harris, J.K. Grubbs, S.M. Lonergan, J.F. Patience, J.C.M. Dekkers and N.K. Gabler

[1]Iowa State University, Ames IA, 50011 USA.

Residual feed intake (RFI) index is a measure of feed efficiency (FE) we define as the difference between the actual feed intake of a pig and its expected feed intake based on a given amount of growth and backfat. After six generations of selecting Yorkshire pigs based on RFI at Iowa State University, a unique resource population of pigs has been created to study the genetic and physiological basis of FE (Young and Dekkers, 2012). Therefore, selecting pigs with a low RFI has resulted in a more feed efficient animal for a given rate of growth. Our objective was to determine the extent to which whole body tissue accretion rates contributes to FE differences in pigs divergently selected for RFI in the seventh generation.

From 96 high RFI (HRFI) and 96 low RFI (LRFI) gilts equally housed across 12 pens each containing an electronic FIRE-feeder (16 pigs/pen), 24 HRFI and 24 LRFI gilts were matched in pairs by littermate (within line only), age, and weight [60±7 kg bodyweight (BW), mean ± SE]. Twelve of these gilts per line were selected as an initial slaughter group (ISG) and their respective littermates were then used in the final slaughter group (FSG) after 6 weeks of feeding. Pigs were fed a corn-soybean meal-DDGS diet (13.8 MJ/kg digestible energy, 0.83% standardised ileal digestible lysine) and had free access to water and feed. Whole body chemical composition (fat, protein, and ash) was determined on both the ISG and FSG. Tissue accretion was calculated using backfat ultrasound scans and BW in a regression analysis to estimate the initial body composition of the FSG. The PROC MIXED procedure of SAS (version 9.2) was used to analyse all data with pen as a random effect and pig, line and slaughter date as fixed effects.

Table 1. *Performance and body composition of pigs divergently selected for residual feed intake.*

Treatment	End weight (kg)	ADG[3] (kg/d)	ADFI[4] (kg/d)	G:F[5]	Backfat (mm)	Whole-body tissue accretion (g/d)		
						Protein	Fat	Bone
HRFI[1]	94.6	0.75	2.06	0.37	16.5	125	258	19
LRFI[2]	94.0	0.73	1.83	0.40	13.6	142	213	27
SEM	4.98	0.032	0.133	0.011	0.74	9.8	110.1	6.7
Significance	0.898	0.671	0.097	0.032	0.001	0.089	0.462	0.040

[1]HRFI, High residual feed intake; [2]LRFI, Low residue feed intake; [3]ADG, average daily gain; [4]ADFI, average daily feed intake; [5]G:F, gain to feed ratio; SEM, standard error of the mean.

No differences (P>0.05) were found for start BW, end BW and ADG over the 6-week performance period between gilts divergently selected for RFI (Table 1). As expected, selection for the LRFI line tended to have lower ADFI (P=0.10) and improved FE by 8% (P=0.032) compared to the HRFI line. Body composition of both the ISG and FSG LRFI gilts showed a decrease in back fat (P=0.001), whole body fat (P<0.02, data not shown) and gross energy (P<0.001, data not shown) compared to their HRFI counterparts. The LRFI gilts also tended to have decreased total empty viscera weights (6.22 versus 6.49 kg, P=0.09). Tissue accretion rates (Table 1) indicated that LRFI gilts tended (P=0.089) to have increased whole body protein accretion, greater (P=0.040) bone accretion and no difference (P>0.05) in fat accretion compared to the HRFI gilts. These data indicate that whole body composition and tissue accretion rates may explain some of the differences seen in finishing pigs divergently selected for RFI. Mechanistically, this may be a result of LRFI pigs having reduced tissue reactive oxygen species production (Grubbs *et al*., 2013), calpain and ubiquitin proteasome system activities (Cruzen *et al*., 2013) and using feed nutrients and energy more efficiently (Harris *et al*., 2012) than HRFI pigs.

CRUZEN, S., HARRIS, A., HOLLINGER, K., SELSBY, J., GABLER, N.K., LONERGAN, S. and HUFF-LONERGAN, E. (2012). In "Proceedings of the 58th International Congress of Meat Science and Technology", p. 59. Montreal, Canada.

GRUBBS, J.K., FRITCHEN, A.N., HUFF-LONERGAN, E., DEKKERS, J.C.M., GABLER, N.K. and LONERGAN, S.M. (2013). *Journal of Animal Science*. **91:**2133-2140.

HARRIS, A.J., PATIENCE, J.P., LONERGAN, S.M., DEKKERS, J.C.M. and GABLER, N.K. (2012). *Journal of Animal Science*. **90(Suppl 4):**164-166.

YOUNG, J.M. and DEKKERS, J.C.M. (2012). In "Feed efficiency in swine", pp. 153-166, ed. J.F. Patience. (Wageningen Academic Publishers: Wageningen).

Supported by USDA NIFA grant #2010-65206-20670.

Higher glycogen stores in outdoor bred pigs pre-slaughter correlate with a faster rate of pH decline in pork

A. Suckling[1,2], C.G. Jose[3] and J.R. Pluske[1]

[1]Murdoch University, Murdoch, WA 6150. [2]Linley Valley Pork, Wooroloo, WA 6558. [3]Department of Agriculture and Food WA, South Perth, WA 6983.

Variation in pork quality has been proposed to be mostly due to different rates of post-mortem muscle pH decline (Ryu and Kim, 2006) and its influence on tenderness (Pomponio *et al.* 2010), which is an important eating quality discriminator. The variable nature and inconsistency of meat quality attributes, such as meat colour and pH, in outdoor bred pigs in Western Australia, and the related quantity of stored muscle glycogen at slaughter, led to this investigation. High levels of muscle glycogen pre-slaughter increases potential lactic acid production, the extent of pH decline, and the lightness of pork. The effect of high glycogen levels on the rate of pH decline is controversial (Scheffler and Gerrard, 2007) and has never been examined in outdoor bred pigs in Australia. The aim of this experiment was to investigate the influence of loin glycogen stores pre-slaughter on post-slaughter pH decline in outdoor bred pigs in Western Australia.

Seven groups of outdoor bred pigs (Narrogin, Western Australia; mixed barrows and gilts; Large White-Landrace x Duroc; n=350) were used in this experiment. The pigs were fed the same diets and housed in either intensive (indoor) sheds or in eco-shelters from weaning to finishing. From April to August 2012 pigs were trucked to a commercial abattoir for slaughter. Following slaughter, split carcasses were chilled and loin pH measured at 24 h (pH_{24}). Samples of muscle from the *longissimus dorsi* (LD) were taken and frozen at -20 ^{o}C for analysis of residual glycogen and lactate content. Residual glycogen and lactate values reflected the amount of glycogen and lactate in the LD at 24 h post-slaughter, and total glycogen level pre-slaughter was determined by combining these. Data were analysed using a linear mixed effects model (SAS®; USA) incorporating the fixed effects of sex and housing type within kill-group and the continuous variables of lactate, residual glycogen, total glycogen and pH_{24}.

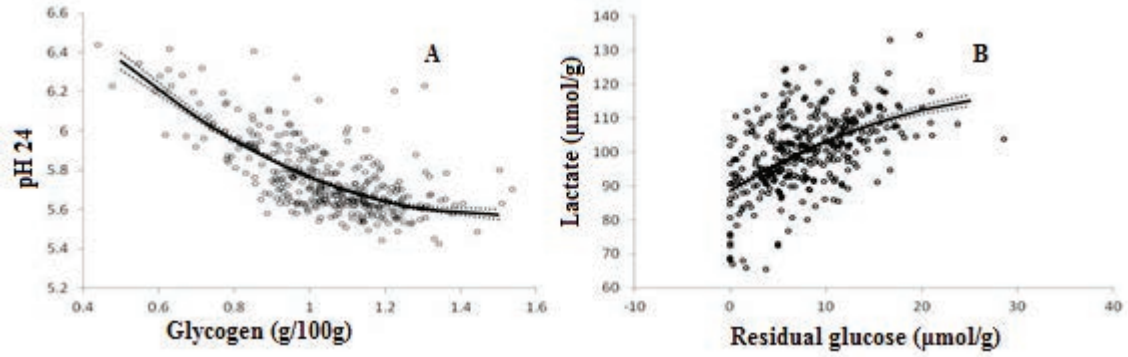


Figure 1. *Relationships (a) between pH_{24} and pre-slaughter glycogen; (b) between lactate at 24 h and residual glycogen, in the LD of outdoor bred pigs. Points are residuals of least squared means.*

As pigs from the different housing systems (eco-shelter and indoor) were slaughtered on different days, it was not possible to determine a housing effect and data were subsequently pooled. A negative relationship between pH_{24} and total glycogen at slaughter was observed (r=-0.56; Figure 1a), together with lactate at 24 h post-slaughter and pH_{24} (r=-0.6). This suggested that higher glycogen levels at slaughter caused a lower pH_{24} and thus a faster decline of pH from slaughter to 24 h. This was further evidenced by the relationship between residual glycogen and lactate at 24 h post-mortem (r=0.37; Figure 1b), indicating that muscles with larger residual glycogen pools had higher levels of lactate at 24 h. Higher residual glycogen pools correlated strongly with higher total glycogen (r=0.86). It is therefore likely that higher glycogen stores in muscle pre-slaughter caused lower muscle pH_{24}. Further investigation is needed to determine if this adversely affects sensory attributes of pork as hastened pH declines have been implicated in poorer meat quality attributes (Huff-Lonergan *et al.*, 2002). Further research of the muscle glycogen reservoir may allow for the alteration of management strategies to optimise pork quality.

HUFF-LONERGAN, E., BAAS, T.J., MALEK, M., DEKKERS, J.C.M., PRUSA, K. and ROTHSCHILD, M.F. (2002). *Journal of Animal Science.* **80**:617-627.

POMPONIO, L., ERTBJERG, P., KARLSSON, A.H., NANNI COSTA, L. and LAMETSCH, R. (2010). *Meat Science.* **85**:110-114.

RYU, Y.C. and KIM, B.C. (2006). *Journal of Animal Science*. **84**:894–901.

SCHEFFLER, T.L. and GERRARD, D.E. (2007). *Meat Science*. **77**:7–16.

Supported in part by Pork CRC Limited Australia.

Depletion-repletion of dietary iron increases total muscle and liver iron contents, but not aerobic capacity, in pigs

C.G. Jose[1,2], J. Mansfield[1], M. Trezona[2], J.C. Kim[2], R.G. Campbell[3], D.W. Pethick[1], G. Smith[2] and J.R. Pluske[1]

[1]Murdoch University, Murdoch, WA 6150. [2]Department of Agriculture and Food WA, South Perth, WA 6151. [3]CRC for High Integrity Australian Pork, Roseworthy, SA 5118.

Current iron (Fe) levels in fresh pork are below requirements to claim for a source of Fe (Anonymous, 2013). Dietary studies to increase muscle Fe in pork have generally failed, although an increase in muscle redness, associated with increased haem Fe, was observed (Apple *et.al* 2007). Under normal feeding conditions about 10% of dietary Fe is absorbed, however this value increases markedly under Fe deficiency (West and Oates 2008). This study tested the hypothesis that feeding lower levels of dietary Fe (depletion) followed by feeding higher levels of dietary Fe (repletion) to pigs will increase muscle Fe levels.

A total of 48 female pigs was allocated to one of two grower-stage diets differing in Fe content (High; 239 ppm or Low; 50 ppm) at 10 kg and fed *ad libitum* for 8 weeks; eight pigs per group were then slaughtered. The remaining 32 pigs were allocated to a cross-over design during the finisher period. Half of the High pigs were fed a high Fe diet (248 ppm; High-High) while the other half were fed a low Fe diet (71 ppm; High-Low). The same design was applied to the Low pigs (Low-High and Low-Low treatments). Pigs were slaughtered after another 7 weeks of supplementation. Liver, heart and muscle samples (*m.longissimus dorsi*-LD; *m.rectus femorus*-RF) were collected. Myoglobin (Mb) and haem Fe were measured in both muscles, and redness in the LD only. Data were analysed using the GLM procedure (SAS®; USA).

Table 1. *Least-square means for iron (Fe) and myoglobin (Mb) concentrations in the* m. longissimus dorsi, m. rectus femoris *and liver (Fe only).*

Dietary Treatments	High	Low	High-High	High-Low	Low-Low	Low-High	SE
m.longissimus dorsi							
Fe (mg/kg)	4.7^{ab}	4.3^{ab}	5.1^{a}	4.3^{ab}	4.0^{b}	4.2^{ab}	0.39
Mb (mg/g)	0.9^{ab}	0.8^{a}	1.7^{d}	1.4^{cd}	1.2^{bc}	1.3^{cd}	0.12
m.rectus femorus							
Fe (mg/kg)	7.0^{ab}	5.5^{a}	7.2^{ab}	8.5^{b}	7.1^{ab}	9.3^{b}	0.94
Mb (mg/g)	1.9^{ab}	1.4^{b}	2.8^{a}	2.9^{a}	2.1^{ab}	2.4^{ab}	0.35
Liver							
Fe (mg/kg)	169.4^{b}	54.1^{a}	294.4^{d}	198.5^{b}	181.5^{b}	247.3^{c}	15.38

[a,b] Significant differences between treatments within rows (P<0.05); SE, standard error.

Muscle Fe levels in the Low-High treatment were of a level high enough to be considered a source of Fe (>8.8 mg/kg), but only in the RF, thus partially supporting our hypothesis. Muscle Fe levels in the LD did not change (P>0.05) during the finisher period compared to levels in the grower pigs. Liver Fe levels increased (P<0.05) during the finisher period; pigs in the Low-High group deposited almost 5-fold more Fe during the finisher period. Heart Fe levels were maintained (P>0.05) across all treatments (data not presented). The Mb levels in finisher pigs across both muscles were similar (P>0.05), however finisher pigs from the High grower treatment generally trended towards higher Mb levels. Redness was increased in High-High samples (data not presented; P<0.05).

Muscle Mb increases with age and with high dietary Fe supplementation, from grower to finishing. The liver acts as a sink for Fe, and it appeared that absorption of Fe was increased by the depletion of body Fe, as observed in the Low-High pigs. Because heart Fe did not differ between treatments, this suggests that the liver will continually supply Fe to tissues with an absolute requirement. These data also suggest that more oxidative muscles (RF) responded in a greater manner to the dietary manipulations tested. Assuming Mb is a measure for oxidative capacity and is correlated with total muscle redness (Lindahl *et al.*, 2001), this dietary depletion/repletion model did not increase total muscle oxidative capacity nor did it detrimentally affect meat redness. Thus, dietary depletion/repletion offers a method for increasing free Fe and keeping pork the 'other white meat'. However, further investigations to optimise these responses are required.

ANONYMOUS (2013). *Australia New Zealand Food Standards Code – Standard 1.2.1* p20.

APPLE, J.K., WALLIS-PHELPS, W.A., MAXWELL, C.V., RAKES, L.K., SAWYER, J.T., HUTCHINSON, S. and FAKLER, T.M. (2007). *Journal of Animal Science.* **85:**737-475.

LINDAHL, G., LUNDSTROM, K. and TOMBERG, E. (2001). *Meat Science.* **59**:141-151.

WEST, A.R. and OATES, P.S. (2008). *World Journal of Gastroenterology.* **14:**4101-4110.

Supported in part by Australian Pork Limited and Pork CRC Limited Australia.

Soy lecithin decreases plasma total and LDL cholesterol but neither lecithin nor lupins has an effect on lean tissue or fat cholesterol levels in finisher pigs

J.P.A. Sweeny, G.M. Smith, M.D. Langridge, J.C. Kim, K.L. Moore, B.P. Mullan and M. Trezona

Department of Agriculture and Food WA, South Perth, WA 6151.

Lupins have been reported to reduce both plasma total and low density lipoprotein (LDL) cholesterol in pigs (Martins *et al.*, 2005), but few studies have measured their effect on tissue cholesterol levels. The aim of this experiment was to measure the cholesterol reducing properties of lupins against the known serum cholesterol reducing properties of lecithin in finisher pigs (Kim *et al.*, 2008). The hypothesis tested was that pigs fed a lupin-based or lecithin-fortified diet during the finishing period would have lower cholesterol levels in both the plasma and tissue when compared to pigs fed a control diet.

The experiment was a 2 x 3 factorial design, with respective factors being cholesterol content (low and high) and basal diet (control, soy lecithin and lupin). The low and high cholesterol content diets (containing 0.5% or 4.5% tallow and 0% or 18.5% full cream milk powder, respectively) had calculated cholesterol contents of 0 and 0.2 g/kg, respectively. The diets were balanced for available lysine (0.7%), but contained different digestible energy concentrations (Table 1). Sixty individually-housed pigs (Large White x Landrace; immunocastrates) weighing 39.6±0.49 kg [live weight (LW) ± SEM] were randomly allocated to six dietary treatments. Blood samples collected from pigs at the start of the experiment and 24 h pre-slaughter were analysed for total, LDL and high density lipoprotein (HDL) cholesterol using an automated clinical chemistry analyser (Olympus AU400). Pigs were slaughtered at a commercial abattoir at 95.9±0.8 kg LW. Lean tissue [*m.longissimus dorsi* (loin) and *m.biceps femoris* (ham)] and belly fat samples were collected at 24 h post-slaughter. Cholesterol analyses of muscle tissue were performed using a high-performance liquid chromatography (HPLC) method (Katsanidis and Addis, 1999). Belly fat cholesterol content was determined by a modified HPLC method (Dinh *et al.*, 2008) as the high levels of triglyceride present in fat tissue inhibited the recovery of cholesterol. Data were analysed by two-way ANOVA (Genstat v15). Both slaughter date and duration on treatment diet (42–63 d) were assessed for their impact on cholesterol measurements, although neither had an impact (P>0.05) on treatment effects.

Table 1. *The effects of dietary cholesterol (C) content and diet type on the total and LDL cholesterol contents in plasma and total cholesterol content in the loin, ham and belly fat from finisher pigs.*

Diet (D)	Control		7.5% Soy lecithin		30% Lupins			Significance	
Cholesterol (C)	Low	High	Low	High	Low	High	SEM	C	D
Calculated DE (MJ/kg)	14.5	15.0	15.5	17.0	14.5	15.2	-	-	-
Plasma C (Initial)	2.67	2.45	2.65	2.53	2.79	2.70	0.137	0.112	0.172
Plasma C (Final)	2.67	2.70	2.53	2.35	2.77	2.70	0.126	0.265	0.002
Plasma C, % change	-0.1	11.3	-4.4	-6.2	-1.5	-1.9	5.94	0.339	0.040
Plasma LDL (Initial)	1.70	1.54	1.65	1.61	1.66	1.747	0.105	0.110	0.467
Plasma LDL (Final)	1.61	1.56[b]	1.33	1.28	1.55	1.64	0.082	0.379	0.001
Plasma LDL, % change	-2.2	2.5	-18.8	-19.5	-4.8	-5.6	6.89	0.242	0.001
Loin (mg/100g)	43.6	45.8[b]	41.7	43.5	42.1	43.7	0.102	0.049	0.153
Ham (mg/100g)	48.9	50.6[b]	43.3	47.4	43.9	48.1	0.057	0.042	0.062
Belly fat (mg/100g)	70.4	75.2	66.1	66.3	68.7	70.1	0.045	0.474	0.153

SEM, standard error of the mean for Cholesterol x Diet (C*D, interaction means presented); No significant interactions between C*D.

Neither lecithin nor lupins had an effect on plasma HDL cholesterol levels (P=0.590). As expected, lecithin lowered both plasma total (P=0.040) and LDL (P=0.001) cholesterol in low and high cholesterol diets when compared to the control groups. Lupins had no effect on the plasma cholesterol content. Pigs fed high cholesterol diets had higher (P>0.05) tissue cholesterol content in the loin and ham (P<0.05). Although lecithin decreased plasma cholesterol levels, neither lecithin nor lupins reduced loin, or belly fat tissue cholesterol content (P>0.05). These results indicate that the amount of cholesterol in pork is likely to be regulated by dietary cholesterol content.

DINH, T.T.N., BLANTON, J.R., BROOKS, J.C., MILLER, M.F. and THOMPSON, L.D. (2008). *Journal of Food Composition and Analysis*. **21**:306-314.

KATSANIDIS, E. and ADDIS, P.B. (1999). *Free Radical Biology and Medicine*. **27**:1137-1140.

KIM, W.-T., SHINDE, P., CHAE, B. (2008). *Canadian Journal of Animal Science*. **88:**283-292.

MARTINS, J.M., RIOTTOT, M., DE ABREU, M.C., VIEGAS-CRESPO, A.M., LANCA, M.J., ALMEIDA, J.A., FREIRE, J.B. and BENTO, O.P. (2005). *Journal of Lipid Research*. **46**:1539-1547.

Supported in part by the Pork CRC Limited Australia.

Eating quality of pork shoulder roast and stir fry outperform cuts from the loin and silverside in male pigs

H.A. Channon[1,2], D.N. D'Souza[1], A.J. Hamilton[2] and F.R. Dunshea[2]

[1]Australian Pork Limited, Barton, ACT 2600. [2]The University of Melbourne, Parkville, VIC 3010.

It is well known that the administration of the boar taint vaccine can effectively reduce androstenone and skatole concentrations to minimise risks associated with boar taint and increase intramuscular fat levels (D'Souza *et al.,* 2000), as well as reduce male socio-sexual behaviours on-farm. Channon *et al*. (2013), in a study comparing the effect of ageing period, cut type, cooking method and final internal cooking temperature on eating quality attributes of pork, identified that overall liking was lower from entire male than surgically castrated males, with females intermediate. Previous studies involving immunocastrated males have not investigated such effects on eating quality compared to entire males and this information is needed as part of the cuts-based eating quality system for the Australian pork industry. Therefore, this study aimed to determine the effect of ageing period, cut type, cooking method and endpoint cooking temperature on eating quality attributes of pork from entire male (EM) and immunocastrated (IC) males.

Forty Large White x Landrace (PrimeGro™) pigs (EM and IC; n=20) targeting carcass specifications of 60-75 kg (Trim 1) and 8-13 mm P2 were slaughtered at 22 weeks of age. At 24 h post-slaughter, loin, silverside and shoulder primals from both sides of the carcase were collected, prepared into roast and stir fry cuts (all primals) and steaks (loin only) and vacuum packed. Cuts were aged for either 2 or 7 d and cooked to endpoint temperatures of 70 or 75 °C. Consumers (n=360) assessed 2,240 samples for overall liking (0=dislike extremely to 100=like extremely) and quality grade (1=unsatisfactory; 2=below average; 3=average; 4=above average; 5=excellent). Fail rate (%) for quality grade was determined (scores of <3). Pork cuts were allocated to ageing and temperature treatments and data analysed by ANOVA (R: Free Software Foundation's GNU General Public License) using models previously described by Channon *et al.* (2013). Chi-squared analysis was used to determine cut x cooking method effects on fail rate.

Table 1. *Means and standard error of difference (SED) of overall liking scores† due to ageing period (A), endpoint temperature (T), cut (C), cooking method (roast or stir fry; CM), between loin steaks and all other cuts (S) and fail rates (%) for quality grade for cut x cooking method.*

Ageing	Temp	Shoulder		Loin			Silverside		SED	Significance
(d)	(°C)	Roast	Stirfry	Roast	Stirfry	Steak	Roast	Stirfry		
2	70	69.6	72.3	58.1	62.0	58.9	55.0	54.7	1.66	A*
2	75	69.8	75.0	57.2	57.1	51.2	55.2	52.0		CM, C, S***
7	70	67.8	75.8	62.5	68.4	56.9	52.7	59.0		CMxC*
7	75	71.7	82.4	60.3	59.2	52.8	54.4	56.6		TxC**
										AxCM***
Fail rate (%)		5.6	5.3	19.1	13.4	25.0	26.9	21.9		P<0.05

EM – entire male; IC – immunocastrate; *P<0.05, **P<0.01, ***P<0.001; †0= dislike extremely to 100=like extremely.

No effect of EM or IC males on overall liking was found and fail rates of pork from EM were only 2.1% higher than IC (17.8 versus 15.7%, respectively; P>0.05). Average overall liking scores for EM were higher than those reported by Channon *et al*. (2013) and may reflect lower androstenone and skatole levels (data not shown) as well as consumer differences. Shoulder stir fry obtained the highest overall liking scores compared to all other cuts and, together with shoulder roasts, achieved low fail rates. Overall liking of loin steaks were 7.7 units lower (P<0.001) than the average of the other cut x cooking methods and scores were also higher for 7 d aged roasts than those aged for 2 d. The interaction between endpoint temperature and cut type (P<0.001) was identified; cooking loins to 75 °C reduced overall liking compared to 70 °C, with the converse found for shoulder. In conclusion, cuts from the pork shoulder, irrespective of EM or IC males, were more acceptable to consumers than leg and loin cuts. Further work to optimise eating quality pathways to achieve low fail rates for loins and silversides, as found for shoulder cuts, is still required.

CHANNON, H.A., D'SOUZA, D.N., HAMILTON A.J. and. DUNSHEA. F.R. (2013). In "Manipulating Pig Production XIV", p.235, eds. J.R. Pluske and J.M. Pluske. (Australasian Pig Science Association, Werribee).

D'SOUZA, D.N., HENNESSY, D.P., DANBY, M., McCAULEY, I. and MULLAN, B.P. (2000). *Journal of Animal Science.* **78(Suppl. 1):**158.

Supported in part by Pork CRC Limited Australia.

Diet and slaughter age have minimal impact on pork eating quality

H.A. Channon[1,4], D.N. D'Souza[1], R.J. van Barneveld[2], R.J.E. Hewitt[3], A.J. Hamilton[4] and F.R. Dunshea[4]

[1]Australian Pork Limited, Barton ACT 2600. [2]Barneveld Nutrition Pty Ltd, Loganholme QLD 4129. [3]CHM Alliance Pty Ltd, Millmerran QLD 4357. [4]The University of Melbourne, Parkville VIC 3010.

Improving the positioning of Australian pork through differentiation on an eating quality basis is required to both maintain and develop our markets. It is not known whether dietary composition and slaughter age typical of pigs in North America (NA) and in Europe (E) could provide opportunities for them to differentiate on an eating quality basis against Australian pork (AU), produced to meet carcass specifications of 60-75 kg and P2 8-13 mm (Channon *et al*., 2013). We hypothesised that increased age at slaughter may increase intramuscular fat (IMF) content of pork and, together with a longer ageing period (as would occur with chilled shipping for export), overall liking of loin steaks (LD) and silverside roasts (BF) could be improved.

Sixty Large White x [Landrace x (Duroc x Large White)] gilts were randomly allocated at 16 weeks of age to one of three treatments (n=20 per diet) matched for digestible energy (DE) (13.6 MJ DE/kg) and total lysine (0.93%). These were Diet A (NA): 77% corn and 16% soybean meal (SBM) [14.9% crude protein (CP)], slaughtered at 24 weeks; Diet B (E): 71% wheat and 10% canola meal (16.3% CP; restricted animal material-free) slaughtered at 21 weeks; and Diet C (AU): 46% wheat and 30% sorghum (16.0% CP) slaughtered at 20 weeks. The Diet A group received a second diet from 21 weeks: 81% corn and 12% SBM (13.4 MJ DE/kg, 0.76% total lysine, 13.0% CP). At 24 h post-slaughter, LD and BF were removed and samples obtained for IMF determination. Cuts were vacuum packaged and aged for either 7 or 28 d. LD steaks were cooked to an endpoint temperature of 70 °C and BF roasts to 75 °C. Consumers (n=240) assessed 1,200 samples for overall liking (0=dislike extremely to 100=like extremely). Quality grade was rated (1=unsatisfactory; 2=below average; 3=average; 4=above average and 5=excellent), with fail rate (%) determined as scores < 3. Data were analysed by ANOVA using the statistical package, R (http://www.R-project.org). Due to heterogeneity in variances, IMF data were log-transformed for analyses.

Table 1. *Effect of dietary treatment (A: Corn/soy; B: RAM-free wheat; C: Wheat/sorghum), slaughter age and ageing period on overall liking and quality grade scores and IMF content of loin steaks and silverside roasts. Values for IMF in parentheses are back-transformed means.*

Diet, Age	Cut	A, 24 weeks		B, 21 weeks		C, 20 weeks		SED	Significance
Final LW		110.7[a]		89.1[b]		86.2[b]		1.59	<0.001
HCW (kg)		88.2[a]		70.0[b]		67.8[b]		1.25	<0.001
P2 (mm)		13.4[a]		9.4[b]		9.6[b]		0.67	<0.001
Log IMF (%)	LD	-0.304 (0.50)[a]		-0.447 (0.36)[ab]		-0.659 (0.21)[b]		0.1210	0.017
	BF	0.314 (2.06)[a]		0.237 (1.73)[ab]		0.040 (1.09)[b]		0.1103	0.045
Ageing period		7 d	28 d	7 d	28 d	7 d	28 d		
Overall liking	LD	61.9	65.4	68.9	66.6	68.2	66.7	2.64	Cut <0.0001
	BF	52.9	56.2	53.3	54.5	57.0	53.2		
Quality grade	LD	3.46	3.55	3.79	3.56	3.66	3.69	0.11	Cut <0.0001
	BF	3.08	3.21	3.08	3.23	3.19	3.08		

[a,b]Means in a row not having the same superscript are significantly different (P<0.05); LW, live weight; HCW, hot carcass weight; LD, loin steaks; BF, silverside roasts; SED, standard error of difference.

Carcasses from pigs from treatment A were heavier (P<0.001) and fatter (P<0.001) than those from treatments B and C (Table 1). Diet/age treatments influenced IMF in both muscles (log transformed data). Neither diet/age nor ageing period influenced overall liking or quality grade scores. Overall liking scores for 7-d aged LD from Treatment A were lower (P<0.001) than the average scores from Treatments B and C, although reasons for this are unclear. Overall liking and quality grade scores for LD were 10.8 and 0.38 units higher (P<0.001), respectively, than SS. The LD had a lower fail rate than BF (11.5% versus 22.7%; P<0.05). Scores for overall liking were comparable for cuts aged for 7 and 28 d, indicating that improvements in eating quality from post-slaughter ageing had plateaued by 7 d. The response to different treatments imposed in this study may have been influenced by the genotype of pigs used. Finishing female pigs at 20, 21 or 24 weeks did not impact on overall liking of pork loin steaks or silverside roasts.

CHANNON, H.A, D'SOUZA, D.N., HAMILTON A.J. and. DUNSHEA. F.R. (2013). In "Manipulating Pig Production XIV", p.235, eds. J.R. Pluske and J.M. Pluske. (Australasian Pig Science Association: Werribee).

Supported in part by the Pork CRC Limited Australia.

Pig mortalities during transportation in Australia

S.Willis[1], J.Riley[2], G.Pope[3] and K.Bell[1]

[1]Department of Agriculture, Fisheries and Forestry (Qld), Toowoomba, QLD 4350. [2]JCR Associates International, Pittsworth, QLD 4356. [3]Graeme Pope Consulting, Nuriootpa, SA 5355.

The rationalisation of the abattoir sector in Australia necessitates that pigs are transported over longer distances for slaughter. As the distance between farm and abattoir increases, the pork producer, consumer and community at large could consider transport as a developing welfare issue. Currently, the industry has limited information to counteract adverse comment by the consumer and community regarding pig welfare in transit. The aim of this study was to examine the impact of transportation time, time of year, stocking density, genetic source, time off feed and transport arrangements on pig deaths between loading on farm and arrival at the abattoir destination.

The study represented 197,451 market-weight pigs sold in 2011/12 by 19 Australian producers representing 14,260 sows. The pigs were transported by six independent transport companies and nine owner-operators to three abattoirs located in QLD, NSW and SA. Herd size of participating production units ranged from 220 sows to 4,000 sows. Across the survey there were 71 pig deaths in transport out of 197,451 pigs sent to the abattoirs (0.036%). This level of mortality was low compared with previous investigations in Australia and other international studies. Shorthose and Dickinson (1982) reported an annual mortality loss of approximately 0.24% in Australia. Higher death rates have been reported in The Netherlands, Western Germany, Denmark and Belgium, occasionally exceeding 1.0%, and approximately 0.22% of all pigs transported in the USA die during transport from farm to abattoir (Ellis *et al*., 2004).

The overall percentage of loads with deaths was 5.4% (57 out of 1,050 loads). The percentage of loads with deaths in transit increased from 2.04% for short trips (less than 2 h) to 5.93% for medium trips and 6.74% for long trips (more than 4 h); all means in this study that are different are significantly different at the 5% level unless otherwise mentioned. The percentage of loads with deaths was lower for short trips than for medium or long trips. The average time in transit was 4.2 h with only 23% of loads in transit for less than 2 h. The percentage of loads with deaths was higher in summer (7.3%) compared with winter (3.5%). Forty-two percent of loads were transported at a low stocking density, (>0.50 m^2/pig) while 56% at a medium stocking density (0.35-0.50 m^2/pig). At the low stocking density, the number of loads with deaths in transit was lower (2.3%) compared to 8.0% of loads where a medium stocking density was recorded. The average time off feed was 10.2 h. Overall there were 8.0% of the loads with deaths when pigs were off feed for short periods (<6.5 h) and 7.0% of the loads with deaths for a medium time off feed (6.5-13 h), which were higher than 1.8% deaths for the loads with long times off feed (>13.5 h). In this survey of seven genetic sources (GS), GS1 had a higher number of loads with deaths (20.4%) compared with other genetic sources. GS2 had the next highest percentage of loads with deaths (7.5%), then GS3 (1.8%), GS4 (1.6%), GS5 (1.4%) and other genetic sources not specified (0.8%).

Deaths in transit recorded in this survey were lower than in previous Australian and international surveys. All the production businesses participating in the study were certified by the Australian Pork Industry Quality Assurance Program (APIQ√®), which requires staff to be trained in animal welfare including the movement and handling of pigs. The transport companies were all species-specific and stated they trained their employees in the movement of pigs. In addition, all three destinations were export-accredited abattoirs and it is possible that the producers and transporters were more rigorously interpreting the APL's 'Is It Fit to Load?' guide, in selecting pigs for loading. The survey undertaken only recorded deaths in transit. Further work should investigate the level and reasons for condemnations in both export accredited and domestic abattoirs.

ELLIS, M., MCKEITH, F.K., and RITTER, M.J. (2004). *American Meat Science Association Pork Quality Symposium*, Columbia, MO. **4**:1-3.

SHORTHOSE, W.R. and DICKINSON, F.R. (1982). *Australian Society of Animal Production*. **14:**674.

Supported in part by Australian Pork Limited.

Quantitative assessment of odour, dust and noise emission from free-range piggeries

T.M. Banhazi

University of Southern Queensland, Toowoomba QLD 4350.

Odour, dust and noise emission/pollution from outdoor piggeries has been acknowledged as a potential issue for the free-range pig industry (Banhazi, 2013). However, data that can help the industry to assess the likely odour, dust and noise emissions from free-range piggeries are not readily available in Australia. Thus, the main objective of this study was to quantify the generally encountered odour emission rates, dust concentrations and noise levels at a number of free-range pig farms and thus allow the industry to develop appropriate responses, if required.

A well-targeted survey was executed on three representative free-range piggeries in New South Wales, Queensland and Victoria. Data-loggers with built-in sensors were used to measure temperature and relative humidity, and a SoundPro sound pressure meter (Quest™ Technologies, Oconomowoc, W.I., USA) was operated to collect representative sound pressure samples on farms during data collection (Banhazi, 2013). Odour sampling was undertaken using an isolation flux hood, and odour samples were collected by applying the "lung method" (Banhazi, 2013). Forced-choice dynamic olfactometry was used to test the odour samples (Banhazi, 2013). A DustTrack™ light-scattering instrument was deployed to monitor intermitted dust concentrations on all study farms (Banhazi, 2013). The statistical analyses were undertaken using GLM and regression methods (Statistica, StatSoft®, 2011) and aimed at establishing if the recorded variables were influenced by differences between farms and climatic conditions (Table 1).

Table 1. *Means and likely sources of environmental variables surveyed during the study period, their variation between farms, and their relationship with air temperature and relative humidity.*

Aspects/Variables	Odour emissions ($OU/m^2/s$) (n=30)	Dust concentrations (mg/m^3) (n=9)	Noise levels (dB) (n=9)
Study Mean (±SD)	*0.036 (±0.029)	0.014 (±0.009)	37 (±5.06)
Farms' effects	P=0.29	P<0.05	P=0.14
Temperature effects	P=0.06	P=0.08	P=0.41
Humidity effects	P=0.002	P=0.15	P=0.92
Likely sources	Soils, grass	Farm machinery, work activity	Farm machinery, birds, insects
Comments	Low levels detected	Low levels detected	Low levels detected
Abatement action	No immediate action required	No immediate action required	No immediate action required

*Outliers removed (data points three or more standard deviations from the mean).

Results indicated that odour emission rates and noise levels measured on free-range pig farms were generally low (Banhazi, 2013) and not affected by farm differences (Table 1). The results of this study also demonstrated that free-range piggeries on average are quieter places than traditional piggery buildings (Talling *et al*., 1998). While there was a difference (P<0.05) demonstrated in dust concentrations between farms, essentially on all farms very low dust concentrations were measured (mean 0.014 mg/m^3). The difference in dust concentration levels is attributed to the differences observed in farm management (Banhazi, 2013). Air temperature did not influence (P>0.05) any of the measured variables and relative humidity only affected odour emissions rates (Table 1). Based on the study means, it was concluded that free-range piggeries are not likely to be major sources of noise, odour or dust pollution, especially when compared to conventional piggeries and other livestock facilities (Banhazi, 2013).

BANHAZI T.M. (2013) Data collection to underpin the quantitative assessment of odour, dust and noise emission from free-range piggeries APL Final Report, Canberra, Australia. pp. 39.

STATSOFT I. (2001) Statistica, StatSoft, Inc., Tulsa, OK, USA

TALLING J.C., LINES J.A., WATHES C.M. and WARAN, N.K. (1998). *Journal of Agricultural Engineering Research.* **71:**1-12.

The author would like to acknowledge the important contribution of the managers and staff of collaborating farms, the technical/scientific assistance of odour consultants, Dr Valeri Lenchine (Science and Assessment, EPA of SA), Dr. Neale Hudson (National Institute of Water and Atmospheric Research Ltd), Mr. Mick O'Keefe (FSA Consulting) and Mr. Mick Atzeni (Agri-Science Queensland, DEEDI). Financial assistance provided by Australian Pork Limited.

Temperature effects on outdoor sow skin temperature: A measure of heat stress

A. LeMoine and H.M. Miller

University of Leeds, School of Biology, LS2 9JT, UK.

The majority of sow research is conducted on indoor animals. However, in the UK over 40% of the breeding herd resides outdoors. These sows are different from their indoor counterparts both genetically and in terms of their environment, since for the duration of their lifetime they are exposed to cooler conditions year-round and extreme temperatures, not only throughout the year but also in a single day. It was therefore postulated that outdoor lactating sows might succumb to heat stress at lower temperatures than those published for indoor sows (22 °C; Black *et al.*, 1993). As such, the aim of this study was to use skin temperature (ST; °C) to establish an upper critical temperature (UCT; °C) for outdoor lactating sows.

Lactating Landrace x Duroc sows and gilts [n = 380; 3±2.2 (mean parity±SD)] were studied between July 2011 and July 2012 on an outdoor farm in North Yorkshire, UK. Sows were kept on fields under commercial conditions and were scored for body condition at the beginning and end of lactation. Skin temperature was taken four times a day, twice a week throughout lactation. Wean to service intervals (WSI) and conception rates (CR) were recorded following weaning. A weather station was set up to record temperature (°C), rainfall (mm), wind speed (mph), wind direction, relative humidity (RH; %) and solar radiation (W/m^2). Temperature and RH were combined to form a temperature humidity index (THI (°C); Zumbach *et al.*, 2008). The effects of THI on ST were analysed using piecewise linear regression from the *segmented* package in R (Mueggo, 2003), using other meteorological variables as covariates and either with or without season.

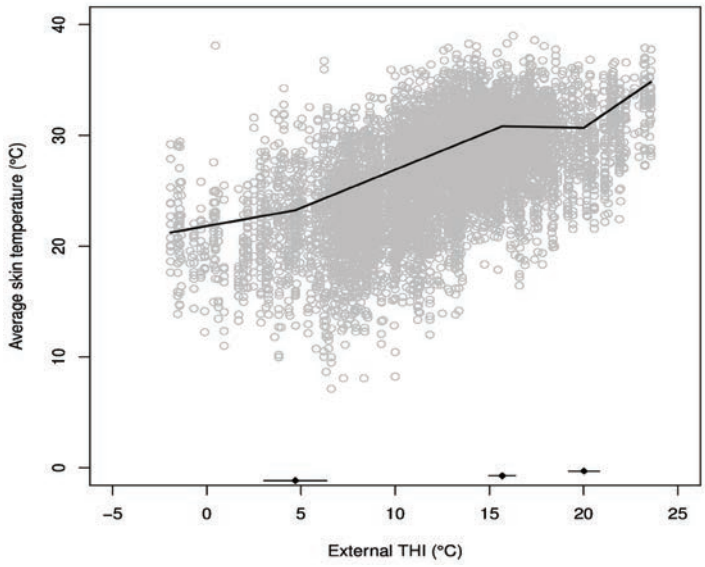


Figure 1. *Piecewise regression line of best fit for sow skin temperature in response to external temperature humidity index, with 95% confidence intervals around estimated breakpoints.*

Overall, sow ST was found to increase in a non-linear manner with THI (Figure 1), since three breakpoints best fitted the data. The final rise in ST was found to occur at a lower temperature than those cited for indoor animals (Black *et al.*, 1993), making 20 °C the UCT for these sows. If separated into seasons, THI affected sows at a lower threshold in spring than in other seasons, suggesting that they have acclimatised to the cold over winter and are therefore more susceptible to rises in THI when the weather improves. Other meteorological conditions were found to moderate the perceived temperatures. No links between THI and reproductive ability (WSI and CR) could be established, although previous work suggests they do exist at lower temperatures (Lemoine, 2013) than previously cited (Black *et al.*, 1993).

BLACK, L.J., MULLAN, B.P., LORSCHY., M.L. and GILES L.R. (1993). *Livestock Production Science*. **35**:153-170.
LEMOINE, A. (2013). University of Leeds, PhD Thesis.
MUEGGO, V.M.R. (2003). *Statistics in Medicine*. **22**:3055-3071.
ZUMBACH, B., MISZTAL, I., TSURUTA, S., SANCHEZ, J.P., AZAIN, M., HERRING, W., HOLL, J., LONG, T. and CULBERTSON, M. (2008). *Journal of Animal Science*. **86**:2082-2088.

Supported by the British Pig Executive and Yorkshire Agricultural Society. Thanks to Dents Ltd for the use of their farm.

CHAPTER 10

Meat Science and Technology, Sow Housing and Welfare

Gender, cut type, cooking method and endpoint temperature influence eating quality of different pork cuts

H.A. Channon[1,2], D.N. D'Souza[1], A.J. Hamilton[2] and F.R. Dunshea[2]

[1]Australian Pork Limited, Barton, ACT 2600. [2]The University of Melbourne, Parkville, VIC 3010.

Previous research has investigated the effects of different production, processing and cooking factors on eating quality of Australian pork (Channon and Warner, 2011). However, few studies have reported these effects on different muscles, prepared and cooked as different cuts, as part of a multi-factorial study involving pigs of different genders. This information is crucial to our understanding of the effects of, and interactions between, different cut types, cooking methods, endpoint cooking temperature and ageing period of pork. This is needed to successfully implement a cuts-based eating quality pathway system to deliver high quality Australian pork. This study aimed to determine the effect of gender, ageing period, cut type, cooking method and endpoint cooking temperature on pork eating quality attributes.

Sixty Large White x Landrace (PrimeGro™) pigs (entire male, female and surgical castrates; n=20 per sex) targeting carcass specifications of 60-75 kg (Trim 1) and 8-13 mm P2 were slaughtered at 22 weeks of age. At 24 h post-slaughter, the loin, silverside and shoulder were obtained from both sides of each carcase and prepared into roast and stir fry cuts (all primals) and steaks (loin only). Individually vacuum-packed cuts were then aged for 2 or 7 d and cooked to endpoint temperature of 70 or 75 °C. Ageing and endpoint temperature treatments were allocated across two carcases within gender and all cuts from two sides within gender were included in each session. Four evaluations per cut x cooking method treatment per side were made (n=40 per treatment combination). Consumers (n=480) assessed 3,360 samples for overall liking (0=dislike extremely to 100=like extremely). Quality grade was rated for each sample (1=unsatisfactory; 2=below average; 3=average; 4=above average and 5= excellent) and fail rate (%) was determined (scores < 3). ANOVA (R: Free Software Foundation's GNU General Public License) was used to determine treatment effects of this 2x2x2x7 factorial design on overall liking of pork with sources of variation within and between cut x cooking method, sides, pigs, sessions and consumers taken into account in the model. The cut x cooking interaction was further analysed as a 2x3 (cooking method (roast or stir fry) x cut type (shoulder, loin or silverside)) + 1 (loin steak). Chi-squared analysis was used to determine treatment effects on fail rate.

Table 1. *Means and standard error of difference (SED) for overall liking (OL) scores† for effect of endpoint cooking temperature (T), cut (C), cooking method (roast or stir fry; CM), between loin steaks and all other cuts (S), and quality grade fail rate (%) for cut x cooking method*

	Temp	Shoulder		Loin			Silverside		SED	Significance
	(°C)	Roast	Stirfry	Roast	Stirfry	Steak	Roast	Stirfry		
OL	70	62.6	70.6	56.2	58.5	51.4	46.1	54.3	1.53	C, CM, S***
	75	63.5	71.5	57.1	59.4	45.8	47.1	55.2		C x CM**
										T x S**
Fail rate (%)		12.1	5.4	19.2	15.2	30.2	36.0	21.5		P<0.05

*P<0.05, **P<0.01, ***P<0.001; †0= dislike extremely to 100=like extremely

Lower overall liking scores were obtained for pork from entire males than surgical castrates, with females intermediate (55.2, 59.5 and 56.6, respectively; SED 1.45, P=0.018). Cut (P<0.001) and cooking method (P<0.001) influenced overall liking, but not ageing period or endpoint temperature. Shoulder stir fry obtained higher overall liking scores than all other cuts, which may reflect anatomical differences (Table 1). Overall liking scores for roasts were 6.2 units lower (P<0.001) than for stir fry (53.9 versus 60.2, respectively; SED 1.53). The interaction between loin steaks and all other cut x cooking treatments and endpoint temperature was significant (P<0.01) for overall liking - scores for loin steaks were 6.6 and 13.2 units lower than the average of all other cut x cooking methods when cooked to 70 and 75 °C, respectively. Although the fail rate of pork from entire males was higher than pork from females and surgical castrates, this was not significant (23.0, 19.1 and 17.7%, respectively; P>0.05). However, lower (P<0.05) fail rates for shoulder stir fry were found compared with all other cuts. Based on the lower overall liking scores, entire males should not be included in eating quality pathway systems being developed for Australian pork. Further work to optimise pathway parameters to deliver consistently high quality pork is still required, particularly for the loin and silverside and to extend these outcomes to include immunocastrated males.

CHANNON, H.A. and WARNER, R.D. (2011). In "Manipulating Pig Production XIII", p. 262-293, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

Lupins reduce carcass yield and increase the PUFA:SFA ratio in loin, ham and belly fat tissue of finisher pigs

J.P.A. Sweeny, A.J. van Burgel, M.D. Langridge, J.C. Kim, K.L. Moore, B.P. Mullan and M. Trezona

Department of Agriculture and Food Western Australia, South Perth, WA 6151.

There are misconceptions with consumers that meat consumption is linked with health risks (Latvala *et al.*, 2012). Previous research has shown that lecithin in the diet improved the fatty acid profile of pork by reducing the saturated fatty acid (SFA) content, thereby improving nutritional value (D'Souza *et al.*, 2005). The hypothesis of this study was that feeding finisher pig diets with either a high lupin content [30%; lupins contain high levels of polyunsaturated fatty acids (PUFA)] or soy lecithin (7.5%) would increase the PUFA:SFA ratio in pork, enhancing its nutritional value without influencing carcass or pork quality.

Sixty individually-housed pigs (Large White x Landrace; immunocastrates) weighing 39.6±0.49 kg (mean ± SEM) were allocated by liveweight (LW) to a 2 x 3 factorial experiment (n=10) with respective factors being two dietary PUFA:SFA ratios (low versus high) and three basal diets (control, 7.5% soy lecithin and 30% lupins). The low PUFA:SFA diets were equivalent to commercial finisher diets with low tallow content (~0.5%), and the high PUFA:SFA ratio diets contained 4.5% tallow and 18.5% full cream milk powder to increase the proportion of SFA. Diets were balanced for available lysine (0.7%), but contained different digestible energy concentrations due to the addition of both tallow and full cream milk in the high PUFA:SFA ratio diets (Table 1). Pigs were fed the control diet one week before *ad libitum* feeding of experimental diets until slaughter. Pigs were slaughtered at a commercial abattoir at 95.9 kg LW (range 91.0–101.4 kg) and carcass weight, dressing percentage and P2 depth determined. Twenty-four h post-slaughter, *m.biceps femoris* (ham), belly fat and the *m.longissimus dorsi* (loin) were collected. Fatty acid analyses of the loin, ham and belly fat were analysed by capillary gas chromatography. Data were analysed by two-way ANOVA (Genstat v15, VSN International Ltd).

Table 1. *The effects of dietary PUFA:SFA ratio (PS) and basal diet type (D) on dressing percentage, the fatty acid content of the loin, ham and belly fat, and the PUFA:SFA in immunocastrated pigs.*

Diet (D)	Control		7.5% Soy lecithin		30% Lupins			Significance		
PUFA:SFA ratio (PS)	High	Low	High	Low	High	Low	SEM	PS	D	PS*D
PUFA:SFA	2.60	1.38	2.51	1.90	2.61	1.63	-	-	-	-
Calc. DE (MJ/kg)	14.5	15.0	15.5	17.0	14.5	15.2	-	-	-	-
Dressing percentage	68.1	68.1	68.3	68.1	67.0	66.5	65.6	0.533	0.008	0.070
Loin Total fat (g/kg)	27.3	31.4	23.9	25 .2	23.2	25.7	3.08	0.285	0.192	0.906
SFA (g/kg)	9.5	12.3	8.1	8.8	7.5	9.3	1.17	0.075	0.064	0.684
PUFA (g/kg)	5.2	5.7	6.5	7.1	5.8	6.3	0.43	0.657	0.013	0.362
PUFA:SFA	0.66	0.49	0.84	0.83	0.9	0.70	0.072	0.031	0.001	0.351
Ham Total fat (g/kg)	32.4	40.1	33.4	35.3	31.7	31.5	0.52	0.467	0.680	0.738
SFA, g/kg	10.6	15.0	10.5	11.5	9.8	10.6	0.17	0.149	0.316	0.497
PUFA, g/kg	6.9	7.2	9.6	10.5	7.2	6.9	0.11	0.972	0.016	0.775
PUFA:SFA	0.70	0.50	0.97	0.93	0.86	0.72	0.040	0.001	0.001	0.146
Belly Total fat (g/kg)	802	805	825	822	809	819	11.0	0.714	0.190	0.836
SFA, g/kg	275^{b}	33^{e}	278^{bc}	295^{cd}	250^{a}	298^{d}	6.1	0.001	0.001	0.004
PUFA, g/kg	155	123	223	216	182	163	6.2	0.001	0.001	0.132
PUFA:SFA	0.57	0.37	0.81	0.74	0.73	0.55	0.032	0.001	0.001	0.129

SEM, standard error of the mean for PUFA:SFA ratio x Diet (PS*D. interaction means presented); ^{abcde} Means in a row not having the same superscript are significantly different (P<0.05).

Dietary PUFA:SFA ratio and diet type had no effect on carcass weight, P2 depth, objective pork quality (data not presented) or total fat content of the loin, ham and belly fat (P>0.05). The dressing percentage of pigs fed lupin diets was lower (P<0.05) than pigs fed control and lecithin diets. Unlike control or lupin diets, the lecithin diet maintained SFA content in the belly fat when fed a diet containing a low PUFA:SFA ratio (P=0.004). Dietary inclusion of either lecithin or lupins increased (P<0.05) the PUFA:SFA ratio in the loin, ham and belly fat, along with the total amount of PUFA in belly fat, when compared to pigs fed the control diet (P<0.05). Soy lecithin increased the PUFA:SFA ratio the most in all tissues. Lupins can possess further advantages other than being a nutrient source for pigs, as their inclusion in finisher diets can increase the PUFA content and thereby improve the nutritional value of pork.

D'SOUZA, D.N., MULLAN, B.P., MCLEISH, J., PETHICK, D.W. and DUNSHEA, F.R. (2005). In "Manipulating Pig Production X", p. 272, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

LATVALA, T., NIVA, M., MÄKELÄ, J., POUTA, E., HEIKKILÄ, J., KOTRO, J. and FORSMAN-HUGG, S. (2012). *Meat Science*. **92**:71-77.

Supported in part by the Pork CRC Limited Australia.

Development of immunoassay technology for the detection of ractopamine in pork products

L.Y.J. Tung[1], N. Ferguson[2], G.S.H Lee[1] and G.K. Tay[1]

[1]The University of Western Australia, Crawley, WA 6009. [2]Westpork Pty Ltd, Maylands, WA 6051.

β-adrenoceptor agonists (BAGs) are used in livestock production to enhance muscle growth by stimulating lipolysis, increasing protein synthesis and reducing protein breakdown in muscle (Baker *et al*., 1984; Emery *et al*., 1984). BAGs have been shown to improve feed efficiency and increase carcase leanness in pigs (Watkins *et al*., 1990). Ractopamine (RAC) is approved for use in pigs in Australia. Detection of RAC in pork products is necessary to ensure that customer requirements, both in our domestic and export markets, are being met. In Australia, testing for veterinary chemical residues, including BAGs, in meat is expensive and confined to a few expert laboratories with concomitant high cost. Thus there is a need to develop a rapid and sensitive detection method for residues. The overall aim of this project was to develop a portable lateral flow device based using a custom made chip technology to simultaneously screen different veterinary chemicals and BAGs.

The first step in this process is to assess the sensitivities of the currently available screening technologies based on immunoassays for BAGs against highly sensitive mass spectrometric techniques such as Liquid Chromatography Mass Spectrometry (LC-MS/MS). To validate the LC-MS/MS technology, 8 groups of Improvac treated males (n=32) and eight groups of females (n=32) with average weight of 78.6kg were fed with 0, 5, 7.5, 10, 15, 20, 5/10, 10/15ppm RAC, respectively. Ractopamine treatment commenced 28 d prior to slaughter. Samples of faeces, urine, saliva and blood were collected weekly, while meat, liver and kidney were collected when the pigs were sacrificed.

A LC-MS/MS method was developed for pig meat analysis. In summary, a 2.5 g sub-sample of the meat was treated with protease/Tris buffer and incubated with β-glucuronidase. The pH was adjusted and the analytes extracted into ethyl acetate/isobutanol. After evaporation of the solvent, the extract was dissolved in methanol/phosphate buffer and cleaned up with a mixed mode cation-exchange solid phase extraction column. After eluting with ethyl acetate/ammonia, the sample was dissolved in 1 % acetic acid and analysed by LC-MS/MS set to multiple reaction monitoring mode. Results were confirmed by LC-MS/MS and detection of daughter ions formed in the mass spectrometer. The presence of a suspected residue is confirmed if two characteristic daughter ions of the relevant parent are observed to occur simultaneously (+2 seconds in the chromatogram) at the retention time (within 2.5% of the RT of the nearest standard) and in the correct relative abundance. To meet the EU criteria for confirmation, the ratio of the abundance of the smaller daughter ion to that of the larger daughter ion must be the same in the sample as that observed in either a calibration standard or sample spiked at a similar concentration and measured under the same conditions. To test the LC-MS/MS methodology, 15 samples of ham, bacon and roast pork were obtained from retail outlets around Perth and analysed. The results are given in Table 1.

Table 1. *Detectable RAC levels can be found in commercial small goods.*

Sample type	Bacon	Ham	Roast Pork
Number of sample tested	9	5	1
RAC level above 0.0001 mg/kg	3 (33.3%)	2 (40%)	0 (-)

Results in Table 1 indicate that trace amounts of RAC presented in five out of 15 samples. Ractopamine levels in the bacon samples were 0.0003, 0.00047 and 0.00088 mg/kg, then 0.00031 and 0.00059 mg/kg in the ham samples. Ractopamine levels in the rest of the samples were below the limit of detection of the method employed.

BAKER, P.K., DALRYMPLE, R.H., INGLE, D.L. and RICKS, C.A. (1984). *Journal of Animal Science*. **59**:1256-1261.
EMERY, P.W., ROTHWELL, N.J., STOCK, M.J. and WINTER, P.D. (1984). *Bioscience Reports*. **4**:83-91.
WATKINS, L.E., JONES, D.J., MOWREY, D.H., ANDERSON, D.B. and VEENHUIZEN, E.L. (1990). *Journal of Animal Science*. **68**:3588-3595.

Supported in part by Australian Pork Limited.

A quantitative microbial risk assessment of *Salmonella* spp. infection from consumption of Australian pork burgers

P.M. Gurman[1,2], T. Ross[2] and A. Kiermeier[1]

[1]South Australian Research and Development Institute, Urrbrae, SA 5064. [2]University of Tasmania, Hobart, TAS 7001.

In Australia, annual human foodborne infections from *Salmonella* spp. are second highest after *Campylobacter* spp., and pork products may be a route of infection.A quantitative microbial risk assessment was undertaken to better understand the risk to consumers from *Salmonella* spp. associated with pork products and how it can be managed. A risk assessment is a formalised process of finding and synthesising data and knowledge from diverse sources in order to evaluate risk. The Codex risk assessment framework, which consists of Hazard Identification, Exposure Assessment, Hazard Characterisation and Risk Characterisation steps, has been used to develop a preliminary Monte Carlo simulation model that describes the risk of salmonellosis from consumption of Australian pork burgers. This model has been developed using a range of data sources to simulate the preparation and consumption of Australian pork burgers from retail mince. This paper presents preliminary results, and as such would benefit from increased input from industry to improve the accuracy and reliability of the model.

Prevalence of *Salmonella* spp. in retail mince was found in Hamilton *et al*. (2011), who reported that 1.4% of 148 mince samples were positive for *Salmonella* (in 25 g) This prevalence was modelled as a Beta distribution with parameters $\alpha = 1.97297$ and $\beta = 144.027$, and this was used to 'contaminate' individual burgers using a Bernoulli distribution. As Hamilton *et al*., (2011) did not test the concentration of *Salmonella* on the positive mince samples, *E. coli* concentration data was used instead as it was considered to be similar (Baker and Dougan, 2007). The log concentration per gram for each contaminated serving was described by a normal distribution with a mean of 1.65 log CFU /g and standard deviation 0.97 log colony forming units (CFU)/g. These values are conservative (describe the "worst case scenario") because the concentration of *Salmonella* spp. is expected to be lower than *E. coli* as shown for Irish pork by Prendergast *et al*. (2009). The cooking model for *E. coli* O157:H7 in beef burgers (Juneja *et al*., 1997) was used to simulate the effect of cooking pork burgers because of the lack of a similar inactivation model for *Salmonella* spp., and assuming that *E. coli* O157:H7 and *Salmonella* spp. will respond similarly to cooking. The internal temperature after cooking was obtained from US consumer data for cooking of beef burgers (EcoSure, 2008) due to the lack of Australian-specific data. The Beta-Poisson dose response model (FAO, 2002) was used with parameters $\alpha = 0.2767$ and β randomly drawn from a normal distribution with mean of 21.159 and standard deviation of 20, truncated at zero and 60. The subsequent probability of illness was determined using a Bernoulli distribution to simulate whether each burger resulted in illness. This model was implemented using the statistical programming language R.

The model was run for a total of 1,000,000 simulated servings. The number of cases of salmonellosis from the consumption of Australian pork burgers was estimated to be 1.7 illnesses per 100,000 servings, giving a mean probability of illness for per serving as 1.7×10^{-5}. Despite the conservative assumptions made in this model due to lack of suitable data, the results were similar to the upper range of estimates of EFSA (2010) which estimated the mean probability of illness to range between 2.24×10^{-5} and 8.84×10^{-7} per serve of pork burgers in four unnamed member states of the European Union.

This model is being extended to a farm-to-fork model that can be used to inform industry risk managers about the risk of salmonellosis from a range of production methods, cooking methods and product types. As part of this process, critical data gaps will be filled. The models' structure, data and validity will be subject to ongoing peer review by relevant stakeholders so that it can be used with confidence to assess the efficacy of interventions, help inform marketing strategies and assess the likelihood of market access issues.

BAKER, S. and DOUGAN, G., (2007). *Clinical Infectious Diseases.* **45:**S29–33.

ECOSURE, (2008). EcoSure 2007 Cold Temperature Database http://foodrisk.org/exclusives/EcoSure/ (accessed 5.9.13).

EFSA, (2010). Quantitative microbiological risk assessment on *Salmonella* in slaughter and breeder pigs: final report. Report Prepared by VLA in Consortium with DTU and RIVM. 437pp.

HAMILTON, D., HOLDS, G., MAY, D., FLINT, R., SLADE, J., PALLANT, L., THOMPSON, A. and KIERMEIER, A. (2011). Food Safety Priorities - Baseline Survey of Carcases and Mince and *Toxoplasma gondii* Genotyping (APL Project 2009/2306).

JUNEJA, V.K., SNYDER, O.P., WILLIAMS, A.C. and MARMER, B.S (1997). *Journal of Food Protection*. **60:**1163–1166.

PRENDERGAST, D.M., DUGGAN, S.J., GONZALES-BARRON, U., FANNING, S., BUTLER, F., CORMICAN, M. and DUFFY, G. (2009). *International Journal of Food Microbiology.* **13:**233–239.

FAO (2002). Risk assessments of *Salmonella* in eggs and broiler chickens. Food and Agriculture Organisation of the United Nations. 302pp.

Supported in part by Australian Pork Limited.

The behaviour of sows towards piglets in farrowing crates and farrowing pens in New Zealand

K.L. Chidgey, P.C.H. Morel, I.W. Barugh and K.J. Stafford

Massey University, Palmerston North 4442, New Zealand.

Previous studies have highlighted the effect that the farrowing accommodation can have on sow and piglet performance (Marchant *et al.*, 2000) and the expression of behaviour (Baxter *et al.*, 2011).This has led to the development of many farrowing pen designs, some of which have the potential to replace farrowing crates. The aim of this study was to compare the behaviour of sows that were housed in farrowing pens with those housed in farrowing crates.

This study was based at a 1,200-sow production unit in New Zealand. Data was collected from 16 sows (parity 2–10) and their litters (n=164 piglets weaned). Sows farrowed in either a farrowing crate (n = 8 sows) or a farrowing pen (n=8 sows). The pens (Combi Flex, Vissing Agro) had a space allowance of 5.84 m^2 (including creep area). Sows housed in pens were confined within a temporary crate from 3 d pre-farrowing until d 4 post-farrowing. The farrowing crates (Big Dutchman®) measured approximately 3.2 m^2 (crate + piglet areas). Sow and piglet behaviour in both systems was observed for 6 d post-farrowing. Behaviour was recorded using fixed interval scan sampling. Each day, 100 x 30-sec observations were performed on each sow (50 observations in the AM and PM) between 0700 and 1600. The following piglet-directed sow behaviours were recorded as binomial variables: nursing vocalisation, vocalising at piglets, investigating piglets (turning towards, approaching and watching piglets) and touching piglets. The frequency of observed behaviours was analysed using a logit model (PROC Genmod) (SAS®; USA) with fixed effects of farrowing system, day, and their interactions, with parity and litter size as covariates. Significant differences between systems (P<0.02) were observed for all parameters; however, these differences were not systematic over the 6 d (Figure 1).

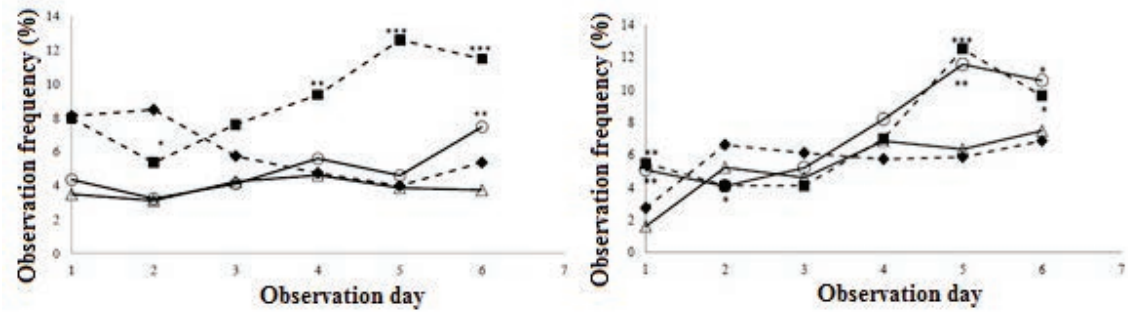


Figure 1. *A) Sow vocalisations towards piglets (dashed lines; ◆ Crate; ■ Pen) and nursing vocalisations (solid lines; △ Crate; ○ Pen). B: Sow investigations of piglets (solid lines; △ Crate; ○ Pen) and touching of piglets (dashed lines; ◆ Crate; ■ Pen). *P<0.05, **P<0.01, ***P<0.0001.*

Sows in farrowing pens investigated their piglets more frequently (P<0.0001) than crated sows. This difference was significant on d 1, 5 and 6 post-farrowing. Penned sows also had more piglet contact (P= 0.0009) than those in crates. After these sows were given access to the entire pen at d 4 post-farrowing, there was an increase (P<0.05) in observations of sows touching piglets that was statistically different (P<0.05) to sows in crates during the same period. Observations of nursing vocalisations increased in penned sows over time, and were more frequent at d 6 post-farrowing versus crated sows on the same day (P=0.0006) Vocalisations directed towards piglets were performed more often by penned sows than crated sows on d 2 (P<0.05), and d 4, 5 and 6 post-farrowing (P<0.01). Our results show that the farrowing accommodation can influence the expression of sow maternal behaviour. More frequent vocalisations, and increased physical contact and investigations of piglets by the sow, indicate that sows with greater freedom during lactation are more interactive with their litter than sows in crates. This may translate to a different experience for the piglets reared in either a pen or a crate system.

BAXTER, E.M., LAWRENCE, A.B. and EDWARDS, S.A. (2011). *Animal*. **5:**580–600.

MARCHANT, J.N., RUDD, A.R., MENDL, M.T., BROOM, D.M., MEREDITH, M.J., CORNING, S. and SIMMINS, P.H. (2000). *Veterinary Record*. **147:**209–214.

Supported by the New Zealand Pork Industry Board.

Effects of gestation housing system on sow performance and longevity over three reproductive cycles

X. Li[1], G.C. Shurson[1], S.K. Baidoo[2], Y.Z. Li[3] and L.J. Johnston[3]

[1]University of Minnesota, St. Paul, MN, USA 55108. [2]Southern Research and Outreach Centre, Waseca, MN, USA 56093. [3]West Central Research and Outreach Centre, Morris, MN, USA 56267.

Selection of housing systems for pregnant sows has become an important issue for pork producers throughout the world. Many people perceive that individual stalls compromise sow welfare due to restricted space allowance, lack of exercise, and reduced socialisation. In contrast, group pens provide more space for sows to move and exercise, and more social interactions (Spoolder *et al*., 2009). However, aggression, injury, and stress usually result when sows are housed in group pens. Well-being of sows may affect sow performance and longevity. Anil *et al*. (2006) reported similar sow performance and longevity when sows were housed in stalls or group pens during gestation for one reproductive cycle. However, few long-term studies which evaluated sow performance and longevity between housing systems have been reported. Therefore, we hypothesised that housing sows in individual stalls or group pens would not affect productivity and longevity over three reproductive cycles.

To evaluate this hypothesis, 401 (n=311 for parity 0; n=90 for parity 1) sows (English Belle, GAP Genetics, Winnipeg, Manitoba, Canada) with an initial body weight of 163±22 kg (mean±SE) were assigned randomly to either individual stalls (2.1 m long × 0.6 m wide) or dynamic group pens on totally slatted floors equipped with electronic sow feeders (15.2 m long × 7.6 m wide; 50 sows/pen; 2.2 m^2/sow). Sows were introduced to pens within 7 d after mating and each sow experienced two mixing events during gestation (d 7 post-mating and 8 weeks later). Sows were maintained in their assigned housing treatment for up to three reproductive cycles. All sows farrowed in individual stalls (2.13 m long × 0.97 m high × 0.66 m wide). Sows were fed 2.25 kg of standardised lactation diets starting on d 109 of gestation until farrowing and allowed *ad libitum* access during lactation, which averaged 19 d. Within each reproductive cycle, sows were culled only if they failed to conceive after the second post-weaning service, were anoestrous longer than 21 d post-weaning, or were lame. Longevity data were analysed using PROC PHREG of SAS. Sow and litter performance data were analysed using general linear models with repeated measures in time. Total number of piglets born alive and weaned were analysed using a Poisson regression in PROC GLIMMIX of SAS.

Table 1. *Performance and longevity of gestating sows housed in individual stalls or dynamic pens for three reproductive cycles.*

Trait	Stalls	Pens	SE[1]	Significance
Pigs born live/litter	11.5	11.0	0.27	0.07
Pigs weaned/litter	10.2	9.9	0.09	<0.05
Total pigs over three cycles:				
Born live	28.4	25.2	0.52	<0.05
Weaned	25.2	23.1	0.49	<0.05
Sows completing:				
First cycle (%)	88.6	89.6	--	0.32
Second cycle (%)	80.0	68.2	--	0.06
Third cycle (%)	68.9	55.8	--	<0.05

[1]Standard error.

Group-housed sows tended to farrow and wean smaller litters compared with stall-housed sows (Table 1). Stall housing tended to increase the completion rate of sows through the second reproductive cycle and increased the completion rate of sows through the third reproductive cycle compared with group housing. Stall-housed sows farrowed and weaned more pigs compared with group-housed sows over three reproductive cycles (Table 1). In conclusion, long-term housing of sows in group pens decreased litter size, sow longevity, and sow productivity over the three reproductive cycles studied, in contrast to our hypothesis.

ANIL, L., ANIL, S., DEEN, J., BAIDOO, S. and WALKER, R. (2006). *Canadian Journal of Veterinary Research.* **70**:128-136.
SPOOLDER, H.A.M., GEUDEKE, M.J., VAN DER PEET-SCHWERING, C.M.C. and SOEDE, N.M. (2009). *Livestock Science.* **125**:1-14.

The authors express appreciation to the National Pork Board for financial support of this study.

The duration of parturition is similar for confined and loose-housed sows

J. Hales[1], V.A. Moustsen[2], A.M. Devreese[1] and C.F. Hansen[1]

[1]University of Copenhagen, Denmark. [2]Housing and Environment, Pig Research Centre, Denmark.

Confinement of sows in farrowing crates limits the ability of sows to perform nest-building behaviour and affects physiology as well as maternal behaviour (Jarvis *et al.*, 2004). Increased physiological stress can adversely affect the process of parturition, and restricting sows in crates during the nest-building phase may thus have a negative impact on the progress of parturition, resulting in prolonged farrowing and longer birth intervals. The hypothesis tested in this study was that sows loose-housed in farrowing pens would have a shorter duration of parturition and shorter birth intervals compared to sows in farrowing crates.

This experiment was conducted using 123 multiparous sows (Landrace x Yorkshire). The animals were randomly allocated to two treatment groups, either confined in farrowing crates or loose-housed in farrowing pens before and during parturition. All sows were housed in identical pens and were fed and managed equally, so the only difference was whether the sows were confined or loose before and during parturition. Farrowings were video recorded and piglet birth details were collated from observation of the video records. Duration of parturition was defined as the time between the expulsion of the first and the last born piglet, and as the time between expulsion of the first and last live born piglet. Birth interval was calculated as the time between expulsion of two succeeding piglets and birth duration as the time from birth of the first to the n'th piglet. Duration of parturition, birth intervals and birth durations were square-root transformed to ensure homogeneity of variance and normal distribution of the data and subsequently analysed by use of generalised linear mixed models using SAS (ver. 9.3).

As expected, the total number of piglets born and parity of sows did not differ between treatments. The mean (±SE) number of total born piglets was 18.4±0.4 piglets per litter and mean parity of sows was 3.4±0.2. There were no differences in duration of parturition, birth duration or birth interval (Table 1). Birth interval was, however, shorter for piglets born to younger (parity one and two) compared to older (parity three or more) sows ($P<0.05$). Moreover, birth interval was longer for piglets born in litters with 7-16 total born piglets compared to litters with 21-28 total born piglets ($P<0.05$).

Table 1. *Duration of parturition, birth interval and birth duration for confined and loose-housed sows [values are presented as medians and numbers in parentheses are quartiles, (P25; P75)].*

	Crates	Pens	Significance
Number	63	60	
Duration of parturition (min)			
First piglet to last born piglet	390 (264; 646)	417 (234; 583)	0.52
First piglet to last live born piglet	353 (249; 528)	390 (225; 506)	0.30
Birth interval (min)	11 (5; 25)	11 (5; 26)	0.59
Birth duration (min)	188 (94; 318)	168 (86; 307)	0.46

In the current experiment, in which both confined and loose-housed sows had access to straw, the progress of parturition was similar for the two treatments. These results differ from Oliviero *et al.* (2008) who found longer farrowing duration in crates compared to pens. However, only sows in pens were provided straw in that study. Jarvis *et al.* (2004) found that housing sows in pens or crates did not influence the progress of parturition and they suggested that provision of rooting material might be more important than provision of space for nest building. Our results were in accordance with Jarvis *et al.* (2004) as space alone did not influence progress of parturition. In conclusion and contrary to our hypothesis, confining the sows in crates before farrowing did not affect the progress of parturition.

JARVIS, S., REED, B.T., LAWRENCE, A.B., CALVERT, S.K. and STEVENSON, J. (2004). *Animal Welfare*. **13**:171-181.
OLIVIERO, C., HEINONEN, M., VALROS, A., HÄLLI, O. and PELTONIEMI, O.A.T. (2008). *Animal Reproduction Science*. **105**:365-377.

Comparison of the behaviour of piglets housed in loose pens and farrowing crates

C. Singh and P.H. Hemsworth

The University of Melbourne, Parkville, VIC 3010.

Housing of sows in crates during farrowing and lactation is commonly used and alternatives that improve welfare without compromising productivity are under development. Parturient and lactating sows are highly motivated to express maternal behaviours, which facilitate successful birth, nursing and rearing of piglets (Gonyou, 2001). Confinement limits the expression of maternal behaviour but the implications of this on behavioural development in piglets are poorly understood. Rearing piglets in loose housing reduced belly nosing, chewing, nibbling and sucking but increased play behaviour in piglets (Oostindjer *et al.*, 2011). This experiment tested the hypothesis that housing sows and piglets in loose pens in comparison to farrowing crates during lactation increases sow-piglet interaction and play and reduces 'harmful' piglet behaviour.

Twenty-four crossbred Large White x Landrace sows of mixed parity and their litters (standardised to 11 piglets) were removed from farrowing crates on the third day of lactation (d 1 of treatment) and randomly allocated to either a farrowing crate or a loose pen until weaning at 28 d (d 26). The loose pen was 2.5 x 1.8 m (l x w), 4.8 m^2 in area and allowed sows to turn round. Crates were 1.9 x 0.6 m (crate) and 1.9 x 1.5 m (total area 2.85 m^2). Both pens and crates had heated creep areas, slatted flooring and no bedding material. The behaviour of 96 focal piglets (four per litter) was observed weekly on d 2, 9 and 16 with the use of video records. The frequency of sow-piglet interaction, defined as close nasal contact (within 10 cm), was observed for 2 min before, during and 2 min after each suckling bout from 0700 to 1700 h, and the frequency of play and 'harmful' piglet behaviour was observed in a total of 60, 30-second scans per day per pen/crate between 0800 and 1130 h. Play was defined as shaking head, pivoting, jumping or running with bouncy movements, whereas 'harmful' behaviour was defined as nibbling, sucking or chewing another piglet. Using SPSS, an ANOVA for repeated measures was used to examine treatment effects as well as time effects on piglet behaviour. All data were tested for normality prior to analysis and a square root transformation applied to data sets that were non-normally distributed.

Table 1. *Effects of housing treatment on piglet behaviour.*

	Sow-piglet interaction (Interactions/suckling bout)			Play behaviour (Proportion of observations)			'Harmful' piglet behaviour (Proportion of observations)		
Week	1 (d 2)	2 (d 9)	3 (d 16)	1 (d 2)	2 (d 9)	3 (d 16)	1 (d 2)	2 (d 9)	3 (d 16)
Mean (crates*)	2.59	2.11	1.92	0.02	0.05	0.04	0.02	0.08	0.06
Mean (pens*)	2.68	2.69	2.64	0.02	0.07	0.07	0.01	0.05	0.05
SEM	0.18	0.14	0.14	0.01	0.02	0.02	0.01	0.02	0.02
Treatment effects		P<0.01			P=0.03			P=0.02	
Time effects		P<0.01			P<0.01			P<0.01	

*Transformed means presented.

The increased interaction observed between sows and piglets in the loose pens provides evidence of behavioural restriction in crates. Due to less physical restriction and increased space, loose pens provided more opportunity for interaction, possibly also creating more environmental and social stimulation for piglets, encouraging social contact including play. The more frequent 'harmful' piglet behaviour observed in crates may indicate overcrowding. While these results suggest an improvement in piglet welfare in the loose pens, determining the long-term effects on piglet welfare will clearly require further and more extensive observations, both pre- and post-weaning.

GONYOU, H.W. (2001). In "Social Behaviour in Farm Animals" pp. 147-176, eds L.J Keeling and H.W. Gonyou (CABI Publishing: Wallingford).

OOSTINDJER, M., VAN DEN BRAND, H., KEMP, B. and BOLHUIS, J. (2011). *Applied Animal Behaviour Science*. **134**:31-41.

Supported in part by Pork CRC Limited Australia and Australian Pork Limited.

Behaviour of sows is dynamic at mixing into groups with free access shoulder stalls

T. Clarke, J.R. Pluske, D.W. Miller, T. Collins, A.L. Barnes and P.A. Fleming

Murdoch University, Murdoch, WA, 6051.

Traditionally, the main focus in behavioural studies of group-housed sows has been how aggression changes over time, with little attention paid to other (not necessarily negative) aspects of behaviour. For example, it has been established that, compared with open group pens, the presence of partial feeding stalls results in reduced levels of aggression over the first 90 min of mixing (Barnett *et al*, 1992), but there are few data regarding other behaviour. Behaviour is dynamic and negative interactions are not the only welfare-relevant actions, and therefore other behaviour such as investigation (sniffing/interacting with pen or another animal), time spent walking, standing (in the group or stalls) or lying should also be quantified to provide a more holistic picture of sow activity at mixing into groups. There are also limited studies on sows mixed at 5 d post-mating. We predicted that behaviour of sows changes over the 90 min post-mixing and that the expression of aggression is transient as the animals establish their social structure.

Ten groups of 10 mixed-parity sows (Large White x Landrace) were weaned at 21 d of lactation and spent 5 d in mating stalls before being randomly assigned into groups. Pens featured 10 free-access feeding stalls (0.58 m^2 available floor space per sow) and a common area with a slatted floor (1.24 m^2 per sow). Water was provided *ad libitum* in troughs, but no food was provided at mixing. Due to the nature of the recording system at the piggery, we were unable to track which animals had been housed together previously. Cameras were mounted high in a corner to capture activity in the common area for the first 2 h post-mixing. Continuous video footage was scanned at 1-min intervals for the proportion of animals visible showing each of six behavioural categories, and these values were then averaged for each 10-min interval and arcsine squareroot-transformed. Each behavioural category was analysed by mixed-model ANOVA (Statistica, StatSoft®, 2011) with group as a random factor and time (10-min interval) as the fixed factor.

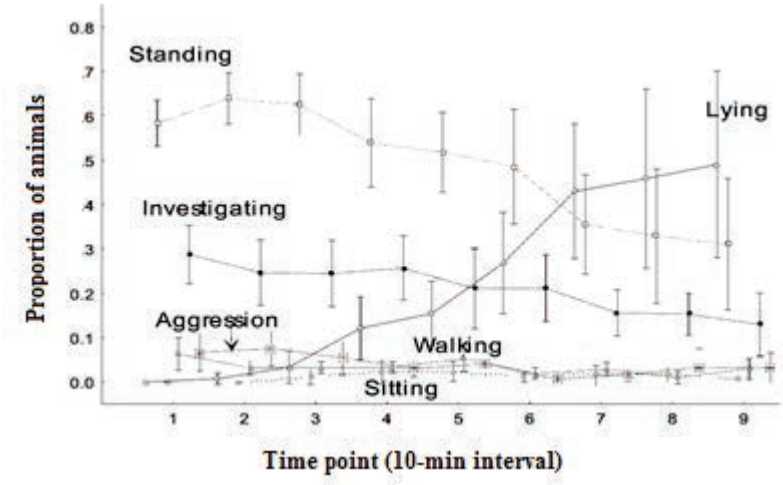


Figure 1. *The proportion of sows performing each of six behaviours over 90 min post-mixing.*

There were significant differences over time for the proportion of sows investigating, standing, lying and interacting aggressively. Standing and investigating decreased over time, while the proportion of sows lying increased dramatically after 50 min. An average of 4% of the observations were categorised as aggressive; the number of aggressive interactions decreased over time from 8% over the first 20 min post-mixing to a minimum of 1% at 60 min post-mixing. The proportion of sows observed walking or sitting did not change significantly over time.

It is important to recognise positive welfare and not only negative welfare states (Mellor, 2012). This study demonstrated only a small proportion of interactions at mixing into free-access shoulder stalls were aggressive behaviour and these reduced over time. This study also highlights that the time point for preforming a behavioural assessment is important for future investigations.

BARNETT, J.L., HEMSWORTH, P.H., CRONIN, G.M., NEWMAN, E.A., MCCALLUM, T.H. and CHILTON, D. (1992). *Applied Animal Welfare Science*. **34:**207-220.

MELLOR, D. (2012). *New Zealand Veterinary Journal*. **60:**1-8.

Supported in part by Australian Pork Limited.

The impact of stocking density on gilt and piglet performance and welfare in group lactation housing

A. Martyniuk[1], P.E. Hughes[2], K. Plush[1] and W.H.E.J. van Wettere[1]

[1]The University of Adelaide, SA 5371. [2]South Australian Research and Development Institute, Roseworthy, SA 5371.

The farrowing crate was introduced to restrict sow movement so as to minimise piglet mortality from crushing. Confinement of sows resulted in impaired sow welfare (Moustsen *et al*., 2013) and therefore confinement-free options are being investigated. Group housing later in lactation may improve sow welfare, but this improvement may be to the detriment to piglet welfare from increased mortality rates. This experiment investigated the effect of stocking density of sows housed in groups during lactation on piglet mortality and performance and sow behaviour. We hypothesised that piglet performance will be compromised before weaning however will be increased after weaning.

Thirty-five first lactation Large White x Landrace sows were used over four replicates. All animals farrowed in a conventional farrowing crate. Based on litter size (mean 9.9, range 8–12) and sow weight (mean 209 kg, range 173-257), sows were allocated to either remain in the conventional farrowing crate throughout lactation (control), or be moved to group housing containing either three (G3) or four (G4) sows and litters on d 14 of lactation. Sow weight was not different between treatments. The group housing treatments consisted of a pen measuring 5.4 by 2.6 m (14 m^2), allowing 4.8 m^2 (G3) or 3.6 m^2 (G4) per sow and litter. Each group pen also contained an adjacent heated creep area (1.1 x 2.6 m) accessible only by the piglets. Sow P2 was measured at the P2 site and live weight (LW) was recorded along with litter LW on d 14 and 28 post-farrowing, with piglet LW also measured on d 33 and 41. Weaning occurred on d 28±0.1 (mean±SEM) post-farrowing. Scan behavioural sampling, a single observation taken on each animal at 15 min intervals for 6 h, was conducted on d 14, 17, 21 and 28 post-farrowing. Aggressive behaviours (including fighting, biting, head thrusting) and normal behaviours (including eating, lying, sow-sow interaction) were recorded in scan sampling. Piglet mortality, age, location and cause of death were recorded. Piglet mortality was analysed using a Chi-squared test and all other variables were tested using an unbalanced ANOVA with fixed effects of block, day and treatment and group litter size fitted as a covariate (GenStat, 15th Edition; UK).

Number of suckling bouts tended to be lower (P=0.1) in group compared to individually (crate) housed sows. Sow LW and the P2 loss were similar (P>0.05) for all treatments (-9.5±1.8kg; -2.1±0.5mm). There was no effect of housing on the incidence of sow-to-sow aggression. Although pre-weaning LW gain of group-housed piglets was lower (P<0.05) than those in crates, growth rate after weaning was higher (P<0.05; Table 1). Piglet mortality was higher (P<0.05) in the G4 compared to the control treatment.

Table 1. *The effect of stocking density [4.8 m^2 (G3) or 3.6 m^2 (G4) first lactation sows] in group lactation pens on sow behaviour and piglet and pig performance.*

	Treatment		
	Control (mean±SEM)	G3 (mean±SEM)	G4 (mean±SEM)
Suckling bouts per observation	0.18 ±0.02	0.14 ±0.01	0.15 ±0.01
Sow aggression per observation	0.00 ± 0.005	0.01±0.004	0.01 ±0.003
Piglet LW (d 14)	4.4 ±0.1[a]	4.2 ±0.1[b]	4.0 ±0.1[c]
Piglet LW (d 28)	8.5 ±0.3[a]	7.3 ±0.2[b]	7.1 ±0.1[b]
Piglet LW (d 41)	9.9 ±0.3	9.3 ±0.2	9.3 ±0.2
LW gain pre-weaning (g/day)	268 ±11.9[a]	242 ±8.3[b]	215 ±7.3[c]
LW gain post-weaning (g/day)	108 ±8.1[a]	152 ±5.6[b]	165 ±5.0[c]
Piglet mortality (%)	0[a]	2.6[a]	6.9[b]

[a,b,c]Means in a row not having the same superscript are significantly different (P<0.05 P<0.10.

The current data demonstrated that group housing in lactation tended to reduce suckling frequency, possibly resulting in minimal sow-to-sow aggression, and improved pig growth after weaning. However, the higher stocking density resulted in more piglet deaths compared to litters housed in a conventional farrowing pen. These data suggest that group housing sows and litters during late lactation might be a viable alternative to conventional housing, but further work required to determine the optimal space allowance and group sizes.

MOUSTSEN, V.A., HALES, J., LAHRMANN, H.P., WEBER, P.M., and HANSEN, C.F. (2013). *Animal*. **7**:648-654.

Supported in part by the Pork CRC Limited Australia.

Aggressive strategies in grouped sows: The relationship between individual aggressive behaviour and welfare

M. Verdon[1], R. S. Morrison[2], M. Rice[1] and P.H. Hemsworth[1]

[1]The University of Melbourne, Parkville VIC 3010. [2]Rivalea Australia Pty Ltd, Corowa, NSW 2646.

The welfare of some group-housed sows could be more compromised than others during gestation. There is limited evidence in gilts that individual aggressive behaviour is related to welfare and reproductive performance (Mendl *et al*., 1992). Consequently, the aim of the current experiment was to provide a better understanding of the relationship between individual sow aggression and welfare. The hypothesis was that, in comparison to subdominant and dominant sows, submissive sows would have the lowest welfare, defined in terms of aggression received, skin lesions, liveweight (LW) gain, cortisol and litter size.

Four replicates of 50 gilts (n=200) were randomly mixed into pens of 10 (1.8 m²/gilt) within 7 d of insemination and floor-fed four times per day (07:30, 09:00, 11:00, 15:00 h). Records were taken on aggression (delivered, received) at feeding, fresh skin lesions and plasma cortisol concentrations on d 2, d 9 and d 51 post-mixing, LW gain (from d 2 to d 100) and litter size (total). Pigs were classified at d 2 as 'Submissive' (SM) if they delivered no aggression, 'Subdominant' (SD) if they received more aggression than delivered, and 'Dominant' (D) if they delivered more aggression than received. After weaning, first-litter sows were inseminated and randomly mixed into pens as for gestation 1, and the same welfare outcomes recorded. Cortisol data were $\log_{10}$ transformed, and aggression and lesions data were square root transformed. Within gestation relationships were examined using a one-way ANOVA with classification as the between-subjects factor, controlling for pen as a random factor. Multiple comparisons between means were performed using the Least Significant Difference (LSD) test (SPSS Inc., Version 17.0: USA).

Table 1. *Relationships within gestations between submissive (SM), subdominant (SD) and dominant (D) aggressive classifications on d 2 and a selection of welfare outcomes* (*means and overall SEM).*

	Gestation 1				Gestation 2			
	SM	SD	D	SEM	SM	SD	D	SEM
d 2 aggression delivered*	1.0[a]	2.6[b]	6.3[c]	0.18	1.0[a]	2.6[b]	5.6[c]	0.15
d 2 aggression received*	4.0[ab]	4.3[a]	3.7[b]	0.09	4.0[a]	4.1[a]	2.8[b]	0.09
d 2 lesions*	4.9	5.0	4.5	1.39	4.8	5.0	5.4	0.13
d 2 cortisol (ng/ml)*	1.2	1.1	1.2	0.02	1.1[a]	1.2[b]	1.1[a]	0.02
LW gain (kg)	69.5[ab]	66.9[a]	73.9[b]	0.97	67.4[ab]	63.6[a]	71.1[b]	1.4
Total litter size	9.8	10.5	10.8	0.20	11.0	11.0	11.9	0.21

[a,b]Means in a row within a gestation not having the same superscript are significantly different (P<0.05);*Transformed means; LW, live weight; SEM, standard error of the mean.

Relationships between classifications and aggression delivered and received on d 2 are shown in Table 1. Similar relationships were observed on d 9 and d 51. In both gestations, D pigs delivered more (Gestation 1 P<0.001; Gestation 2 P<0.001) and received less (Gestation 1 P<0.01; Gestation 2 P<0.001) while SD pigs gained the least LW (Gestation 1 P<0.001; Gestation 2 P<0.01). There were no differences (P>0.05) in lesions on d 2 (Table 1), however on d 9 the D pigs had fewer lesions (Gestation 1 P<0.01; Gestation 2 P<0.001), and on d 51 the SM pigs had the most (Gestation 1 P<0.001; Gestation 2 P<0.001). The SM pigs tended to have the highest cortisol on d 9 in gestation 1 (P=0.077) whereas SD pigs had the highest on d 2 of gestation 2 (P=0.013). There were no significant relationships (P>0.05) with litter size (Table 1).

At mixing, aggressive sows engage in fights for dominance, while others do not deliver aggression. Once a hierarchy is established, D sows have reduced risk of receiving aggression and gain priority access to resources (Edwards, 1992), and consequently have lower injuries and increased LW gain. Reduced LW gain and high cortisol suggest that the SD sows may be more stressed due to continual engagement in aggression without gaining dominance. However, the SM sows had more injuries in the long term. In conclusion, sows that engage in aggression at mixing and gain dominance have increased weight gain and reduced injuries and stress. However, while the SM sows sustain more lesions, the SD sows could be more stressed.

EDWARDS, S.A. (1992). *Pig Veterinary Journal.* **28**:40-51.
MENDL, M., ZANELLA., A.J. and BROOM, D. M. (1992). *Animal Behaviour.* **44**:1107-1121.

Supported in part by Australian Pork Limited, Rivalea Australia and Pork CRC Limited Australia.

Attitudes of citizens and conventional pig farmers about pig husbandry in The Netherlands

T.J. Bergstra[1], H. Hogeveen[1] and E.N. Stassen[2]

[1]Wageningen University, Chair Group Business Economics, 6707 KN No.1, Wageningen. [2]Wageningen University, Chair Group Animals in Society, 6708 WD No.1, Wageningen.

There is evidence that people place importance on environment and nature, animal welfare and the need for environmentally-friendly food production (Krystallis, *et al*., 2009). However, this level of importance can differ between stakeholder groups, e.g., pig farmers and citizens (Tuyttens *et al*., 2010). As a result, differences in attitudes about pig production can result in misunderstanding between stakeholder groups with regard to production. Consequently, citizens may express little support for some production practices implemented by pig farmers. For pig farms to maintain citizens' support, it is important to understand differing attitudes between stakeholder groups when decisions are made with regard to improving pig production. With respect to citizens and conventional pig farmers, the objective of this study was to determine and compare some specific concerns about pig production.

An internet questionnaire was randomly distributed throughout The Netherlands with closed questions about different entities of pig production, i.e., animal, human (animal keeper and consumer) and environment. For each entity, respondents could indicate how much additional care (AC), i.e., extra attention compared to the current situation, they found necessary for aspects related to pig production e.g. animal welfare, animal housing, farmer income, costs consumer and environmental waste. Respondents could indicate their AC level on a Likert-scale of 1 (no AC necessary) to 10 (utmost AC necessary). For the analyses, AC levels were reduced to a five-point Likert scale to have acceptable numbers per category. Descriptive statistics (frequencies) and ordered multinomial logistic regression (OMLR) were respectively used to determine and compare AC levels of the different stakeholder groups. Descriptive statistical analysis, (IBM SPSS, Version 19.0; USA) was used to calculate the percentage of respondents that chose a particular AC level for each of the relevant aspects related to pig husbandry. The impact of stakeholder group membership on the score given to the aspects was estimated using OMLR, (EViews 6, IHS Global Inc., 2013).

Table 1. *Percentage of respondents per additional care (AC) level per stakeholder group for different aspects per entity related to pig production. AC-levels: N: no AC, L: little AC, M: moderate AC, S: strong AC, U: utmost AC. (Significant difference P<0.01 in AC levels between citizens and conventional pig farmers for all aspects. The greyvertical line represents the median, and the grey highlights represent the highest percentage of respondents per group for an aspect.*

	Citizens					Conventional pig farmers				
Entity and Aspect	N	L	M	S	U	N	L	M	S	U
Animal: Able to go outside	3.3	4.4	22.7	30.2	39.4	73.0	11.0	11.0	2.2	2.2
Animal: Use of antibiotics	3.0	2.6	21.1	22.8	50.5	13.7	13.3	27.1	22.7	22.1
Animal keeper: Income	3.9	6.0	33.6	41.4	15.2	3.9	0.6	5.5	21.0	69.1
Consumer: Price product	7.9	10.8	35.8	31.6	13.9	8.8	3.9	11.0	23.2	53.0
Consumer: Food safety risks	3.0	4.6	23.3	30.8	38.3	30.9	12.7	16.6	16.6	21.5
Environment: Waste	2.4	3.6	20.3	37.3	36.4	33.7	18.2	21.5	16.1	9.9

For almost all aspects of pig production, citizens and conventional pig farmers indicated different AC levels (Table 1). The AC levels given by citizens for the aspects related to the animal, for environmental waste and for food safety risks were higher (P<0.01) than the AC levels given by conventional pig farmers. In comparison to citizens, pig farmers gave higher (P<0.01) AC levels to income for the farmer and the price of the product for consumers. The indicated levels of AC can be representative for levels of concern, as it is likely that respondents with higher concerns give higher AC levels.

Results from the current study show that concerns about aspects of pig production differ between citizens and conventional pig farmers in The Netherlands. Conventional pig farmers had the highest concerns about aspects related to business. Citizens had the highest concerns about aspects related to the animals and to human health. Understanding these differences is important if pig farmers wish to obtain support from citizens.

KRYSTALIS, A., DE BARCELLOS, M.D., KUGLER, J.O., VERBEKE, W. and GRUNERT, K.G. (2009). *Livestock Science.* **126:**46-56.

TUYTTENS, F.A.M., VANHONACKER, F., VAN POUCKE, E. and VERBEKE, W. (2010). *Livestock Science*. **131:**108-114.

Funded by the Dutch Ministry of Economic Affairs (EZ) and the Product Board for Livestock, Meat and Eggs (PVE).

CHAPTER 11

Environmental Microbiology, Environment and General Production

REVIEW: Genomic approaches for characterising and quantifying microbial communities to the benefit of the pig industry: An environmental perspective

S.N. Jenkins

The University of Western Australia, Crawley, WA 6009.

Abstract

The advent of genomic approaches for the identification and quantification of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in microbiology has significantly improved our understanding of microbial diversity and function in both natural and engineered environments. Polymerase chain reaction (PCR)-based analysis of genes and shotgun metagenomic studies have been used to identify a) the environmental factors and management practices that influence microbial community dynamics, and b) the role microbes play in environmental processes (e.g., methanogenesis, nitrification). Recently, the introduction of next generation sequencing (NGS) means it is now possible to fully sequence (at high taxonomic resolution and low costs) the complex microbial communities found within different environmental samples. These technologies have the potential to lead to more sustainable waste and environmental management practices within the pork industry through improved understanding, diagnostic tools, treatment and mitigation. In this review, a cost effective sequencing approach is demonstrated for underpinning the microbial dynamics and function within covered anaerobic pond (CAP) digesters used to treat piggery wastes. The information gained has benefitted the industry by identifying management practices that enhance waste treatment and biogas recovery, and in highlighting conditions that induce system failure. Additionally, the effectiveness of using molecular monitoring tools as indicators of soil quality for assessing the risks and benefits of recycling of manure and waste by-products as soil improvers and fertilizers is evaluated.

Introduction

The Australian pork industry has been developing sustainable management practices that minimise environmental impacts [e.g., mitigating greenhouse gas emissions (GHG), reduced leaching and pathogen survival] whilst maximising the benefits (e.g., decreased fertilizer use, pathogen suppression, bioenergy recovery, nutrient recapture) associated with manure storage and its application to land. This includes improved manure storage systems, such as pelletising or composting deep-litter spent bedding and covering effluent ponds to create low-cost covered anaerobic pond (CAP) digesters that both treats the waste and recaptures the biogas and nutrients. However, there is limited knowledge about the microorganisms that govern the waste degradation process and how management practices can be altered to make the conditions more favourable for biogas recovery and nutrient recapture. Also, there are currently no widely available tools to evaluate the best management practices for maintaining pond health, avoiding pond failure and applying waste by-products as soil improvers. Genomic approaches offer the possibility of: a) identifying the key microbial processes and mechanisms driving soil and pond processes; b) determining the impact of management (e.g., antimicrobial use) on waste treatment, biogas and nutrient recovery and crop yields; and c) developing molecular tools as indicators of pond stability or soil quality. Guidelines and microbial monitoring tools can then be developed to enhance bioenergy recovery, predict pond failure and ensure sustainable manure applications to land.

Genomic approaches and the advent of next generation sequencing

Global microbial diversity and distribution patterns are being examined at levels that were unthinkable only a decade ago, principally due to the next generation sequencing (NGS) revolution (Sogin *et al.*, 2006; Lozupone and Knight, 2007). Polymerase chain reaction (PCR)-based analysis of taxonomical (16S and 18S ribosomal DNA) and functional (*amo*, *nir*) genes (Lane *et al.*, 1985; Olsen *et al.*, 1986) and shotgun metagenomic studies have characterised diverse microbial communities in soils (Jenkins *et al.*, 2010; Bartram *et al.*, 2011), oceans (Caporaso *et al.*, 2012), the atmosphere (Bowers *et al.*, 2011), the human gastrointestinal tract (Kuczynski *et al.*, 2012) and engineered environments (Supaphol *et al.*, 2011; Whiteley *et al.*, 2012). Following the introduction of PCR, traditional culturing methods were superseded by genomic approaches that provided a more rapid and better representation of microbial community structure and diversity (Amann, 1995). Prior to the NGS revolution, 'fingerprinting' technologies such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) were used to gain insights into how microbial communities responded to environmental gradients

and perturbations (Fromin *et al.*, 2002; Jenkins *et al.*, 2009; Jenkins *et al.*, 2010). Whilst analysing clone libraries with traditional Sanger sequencing methods assessed microbial diversity, the effort and costs involved meant that this technique is not applicable to large multi-factorial studies. Quantitative PCR (qPCR) was also developed to monitor gene amplification in real-time so that gene copy number could be used to estimate the population size (gene abundance) of a specific target microbial group (Jenkins *et al.*, 2009; Jenkins *et al.*, 2010; Supaphol *et al.*, 2011).

In addition to PCR-based approaches, metagenomic sequencing (the analysis of whole community genomes extracted from natural environments) is a more powerful approach for characterising the microbial community structure and its metabolic potential based on genes, pathways, and systems without PCR bias (Meyer *et al.*, 2008). Shotgun metagenomic sequencing also provides a better genomic coverage of rarer community members and metabolic traits (Martin *et al.*, 2006). Functional genomic analysis can be extended to include whole genome RNA transcripts (metatranscriptomics), protein (proteomics) and metabolites (metabolomics), which highlights the functionally relevant individuals involved in metabolic pathways (Wilmes and Bond, 2006). To date, such studies have recovered unique microorganisms and novel functional genes coding for metabolic pathways from a variety of environments (Martin *et al.*, 2006; Xu, 2006; Lipson *et al.*, 2013). The integration of these methods will provide a greater insight into the microbial diversity and functional activities (Beja *et al.*, 2001; Wellington *et al.*, 2003; Fierer *et al.*, 2012) that control waste degradation pathways during manure storage and application to land. Initially, metagenomic studies were laborious requiring the construction of bacterial artificial chromosome (BAC) or fosmid clone libraries that were analysed using Sanger sequencing methods (Xu, 2006). With the introduction of NGS techniques, it is now possible to fully sequence most known habitats on Earth to unprecedented sequence levels.

New technological advancements in NGS that enable high throughput solutions for studying microbial ecology means it is more accessible to microbiologists. Developments include pyrosequencing (Margulies *et al.*, 2005), barcoding and multiplex analyses (Hamady *et al.*, 2008), ultra-high-throughput sequencing (Bartram *et al.*, 2011; Caporaso *et al.*, 2012) and improved storage, computational processing and sequence analysis (Lozupone and Knight, 2005; Meyer *et al.*, 2008; Caporaso *et al.*, 2010). Recently, a light-independent, ion-sequencing method has been developed and commercialised via the introduction of the Ion Torrent Personal Genome Machine (PGM) (Life Technologies). During ion sequencing, DNA sequence composition is determined by measuring slight changes in pH as hydrogen ions are released when nucleotides are incorporated during DNA strand synthesis (Rothberg *et al.*, 2011). Compared to pyrosequencing, ion sequencing is a more affordable sequencing option due to the substantially reduced costs since a light detection system and associated reagents are not required (Glenn, 2011). Consequently, the PGM has been receiving increased attention due to the inherent scalability within the system, relatively low costs and appreciable levels of sequence outputs. Recently, it was demonstrated that the Ion Torrent platform was a suitable NGS platform for studying microbial community dynamics and function associated with a piggery waste treatment system (Whiteley *et al.*, 2012). These authors developed Ion Torrent protocols for both PCR amplified 16S rRNA or metagenomic community sequencing analysis and then used these protocols to assess community structure, temporal stability and key taxa during the waste treatment process. This included the development of Golay barcoded ion tags for multiplex analyses of microbial communities. The Ion Torrent sequence output was equivalent to pyrosequencing technologies such as 454 Titanium, but benefits from a relatively low machine and reagent cost, has scalable analyses levels, and also has a fast sequence turnaround.

Understanding the microbial communities in covered anaerobic ponds (CAPs) to enhance bioenergy and nutrients recapture

The Australian pork industry has been proactive for a number of years in developing low-cost sustainable waste management practices and methane mitigation technologies. This approach concurs with new government policies and legislation on climate change (e.g., Carbon Farming Initiative; CFI). Effluent waste treatment ponds have been identified as a major source of methane emission in piggeries, accounting for 66% of all Greenhouse gas (GHG) emissions from the pork supply chain (Wiedemann *et al.*, 2010). One simple and affordable option gaining attention is the possibility of covering these effluent ponds with geosynthetic materials to create a CAP digester that both treats the waste and captures the biogas via the process of anaerobic digestion (AD) (Craggs *et al.*, 2008; Heubeck and Craggs, 2010). A recent Australian Pork Limited (APL) study showed that emissions from the effluent treatment might be reduced by 62-80% by doing this (Skerman and Collman, 2012). The biogas can then be used either directly as a fuel or converted to electricity via a motor generator. CAP systems offer a multitude of benefits including, GHG mitigation, renewable energy, soil improvers and improved community amenity via odour control. Currently, 7% of production is capturing methane (CH_4) with another 16% in the planning/design stage, and this number is likely to increase with rising energy costs and new government incentives such as

carbon credits and Renewable Energy Certificates (RECs). Nevertheless, the amount of biogas generated from manure can vary considerably, especially under suboptimal operating conditions where the AD process within CAPs becomes unstable (Heubeck and Craggs, 2010). Maintaining process stability is therefore essential for maximising CH_4 yields and avoiding pond failure. However, knowledge about suitable operating management, environmental conditions and the underlying microbiology in CAPs that governs the bioconversion of waste into biogas is limited.

Anaerobic digestion (AD) is a complex multistep process that occurs under the absence of oxygen (O_2) in which microorganisms degrade piggery waste into CH_4 and carbon dioxide (CO_2). It involves several successive stages of metabolic reactions mediated by different microbial groups that work closely together: (i) hydrolysis; (ii) acidogenesis; (iii) acetogenesis; and (iv) methanogenesis (Demirel and Scherer, 2008; Rittmann *et al*., 2008). Yet, the identities of the microorganisms involved in these AD pathways are often overlooked with the AD process being treated as a 'black box' (Supaphol *et al*., 2011). This limits engineering and management of the AD process for optimal bioenergy production. There is a pressing need to understand these complex microbial interactions during the waste degradation process and how management practices could be altered to make the conditions more favourable for biogas recovery. Also, the prevailing environmental conditions that induce system failure must be identified. However, there are currently no universal tools for evaluating the best management practices for maintaining pond health. Therefore, microbial diagnostic tools need to be developed for monitoring microbial communities in CAPs, to enable early warning detection of impending pond failure.

Recent advancements in molecular biology including the development of NGS approaches (e.g., metagenomic sequencing, pyrosequencing or ion tag sequencing) can start to elucidate the link between microbial identify and metabolic potential based on common genes, pathways, and systems in AD environments (Lueders *et al*., 2004; Karakashev *et al*., 2006; Goh *et al*., 2009; Jenkins *et al*., 2010; Supaphol *et al*., 2011). In other words, it is possible to ascertain which microbes are doing what, where and when, and what conditions increase their growth or inhibit their activity. This information could be used to develop microbial indicators and (or) biosensors for CAP pond stability. By changing the prevailing environmental conditions inside the CAP through management practices, it may be possible to enhance the microbial activity and therefore the bioconversion efficiency (Amani *et al*., 2010). With the introduction of low-cost NGS platforms that are rapid, cost-effective and high-throughput there is the possibility of providing a pond monitoring service at a centralised sequencing facility for end users. Best management practices and monitoring services that lead to improved pond stability and biogas yields will increase the reliability and profitability of CAPs. Improving the economic feasibility of CAPs will encourage more producers to adopt the technology leading to reduced GHG emissions and increased on-farm profits through bioenergy, RECs and carbon credits. Ultimately, this will enhance the competitiveness of the Australian pork industry and highlight its environmental credentials.

Recently, Ion Torrent sequencing protocols for community sequencing were developed and used to assess the basic community structures, temporal stability and key taxa within the CAP system. It was demonstrated that the Ion Torrent sequencing platform could be used to fully sequence (at high taxonomic resolution and low cost) the complex microbial communities found within different piggery environmental samples (Whiteley *et al*., 2012) making it a good candidate for providing a pond monitoring service. PCR-based analysis of genes (e.g., 16S rRNA) and shotgun metagenomic studies were used in this study to explore the microbial dynamics and function. The results show that after an initial period of acclimatisation (following pond covering), microbial communities become both temporally and spatially stable. The microbial community was also found to be highly resilient being able to tolerate a range of temperatures, loading rates, feed additions and substrate quality (Figure 1).

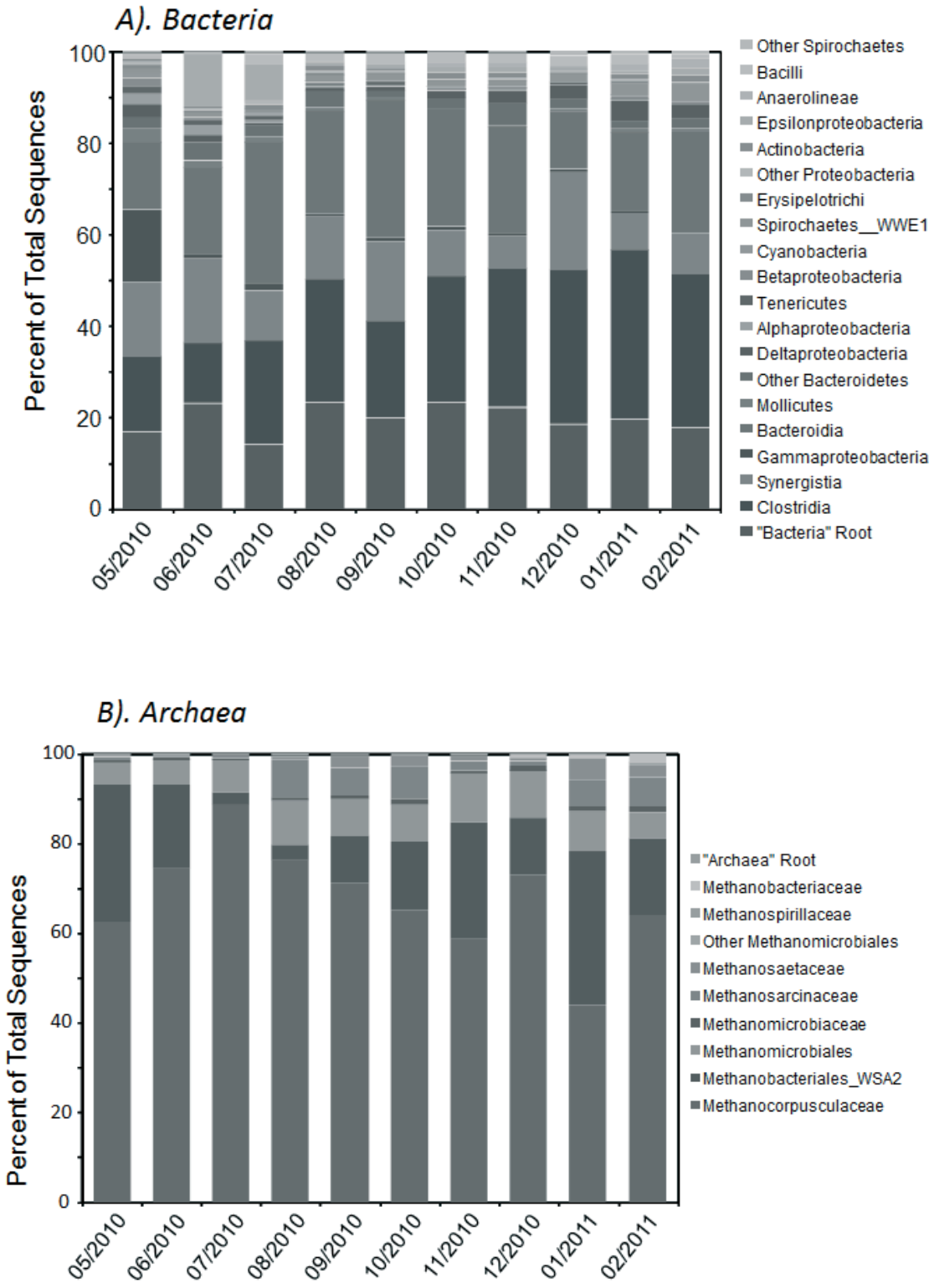


Figure 1. *Muliplex PCR analyses of microbial community structure showing temporal changes in Bacteria (A) and Archaea (B) over a 10-month period within the covered anaerobic pond. Bacterial and archeal communities underwent PCR amplification using 200 b.p. V3 region and sequences were analysed on a 316 chip (adapted from Whiteley* et al.*, 2012).*

The microbial communities within the CAP were dominated by the bacterial taxa *Clostridia*, *Synergistia* and *Bacteroidia*, which in turn supported a consortium of syntrophic bacteria and methanogens (Whiteley *et al*., 2012). These taxa are metabolically versatile (Müller *et al*., 2011) and resilient to

perturbation, periods of starvation and environmental fluctuations (Tang *et al*., 2011; Kampmann *et al*., 2012), which probably accounts for their temporal stability in CAPs. These taxa are often isolated from piggery waste treatment systems (Cook *et al*., 2010; Patil *et al*., 2010; Talbot *et al*., 2010) and other anaerobic digesters (Riviere *et al*., 2009; Supaphol *et al*., 2011; Tang *et al*., 2011; Kampmann *et al*., 2012), making them a good candidate for "indicator" taxa. Hydrogenotrophic methanogenesis was the dominant methanogenic pathway in CAPs consistent with other AD systems treating piggery waste (Kim *et al*., 2010; Patil *et al*., 2010; Talbot *et al*., 2010). *Methanocorpusculaceae*, *Methanobacteriales* and *Methanomicrobiales*, which produce CH_4 from H_2/CO_2 and formate, prevailed throughout the sampling period. A large number of syntrophic bacteria were recovered from the CAP, in particularly the butyrate oxidising bacteria *Syntrophus* spp. and *Syntrophomonas* (Müller *et al*., 2011). These fatty acid degraders can only grow syntrophically in partnership with hydrogenotrophic methanogens (Müller *et al*., 2011), and their presence suggests an accumulation of butyrate in the CAP during the summer. The information gained so far will benefit the industry by identifying management practices that enhance biogas recovery and conditions that induce system failure.

Evaluating the effectiveness of different manures and their by-products as fertilizers or soil improvers and quantifying the risks and benefits of applying them to land

Plant growth is often constrained by phosphorus (P) and nitrogen (N) availability and with the global population size expected to reach between 8 and 10.5 billion by 2050, there will be increasing demand on agricultural production. Currently, large quantities of synthetic fertilizers are applied to soils in order to maintain crop productivity and profitability (Guppy and McLaughlin, 2009). However, synthetic N fertilizers are very expensive to produce via the Bosch-Haber process (Garnaut, 2008) and P fertilizers are produced from a finite resource (phosphate rocks), which is expected to be exhausted within 60 to 90 years (Hammond *et al*., 2004). There is therefore an urgent need for more sustainable fertilizer use without compromising crop performance, and pig manure, which is characteristically high in P and N content, is an attractive alternative.

The application of animal manures to soil enhances crop performance, soil quality and microbial activity (O'Donnell *et al*., 2001; Edmeades, 2003). Despite these benefits, there is still some concern surrounding the re-use of manures such as odour and GHG emissions, leaching, pathogen survival (e.g., *Clostridium difficile*) and toxicity from contamination with heavy metals, salts and antimicrobials (Conacher and Conacher 1998; Peigne and Girardin, 2004; Wang *et al*., 2004; Abell *et al*., 2009; Semenov *et al*., 2010; VanderZaag *et al*., 2011). However, the true extent of their benefits and risks associated with manure amendments has not been fully investigated or quantified. To minimise these risks the pork industry is adopting management practices and technologies (e.g., anaerobic digestion including CAPs and composting) that enable odour and GHG mitigation, pathogen removal and stabilisation of organic solids. Another benefit of these improved manure systems is the creation of a versatile range of by-products including renewable energy (biogas), anaerobically treated effluents and sludges, pellets and composts. Nevertheless, further research is needed to develop these by-products as P fertilizers or soil improvers and to ensure they are deployed in ways that are economical, sustainable and practical from an operations perspective.

Recycling manures and by-products has the potential to increase on-farms profits through nutrient recapture, bioenergy, reduced fertilizer use and creation of new saleable products. In addition, and under the new CFI, farmers and landholders have the opportunity to earn carbon credits for sale in domestic and international market through activities that sequester carbon and reduce GHG emissions in soils. This will encourage more producers to adopt sustainable manure management practices including the more expensive storage options such as CAPs and composting. However, before these manure management practices can be widely adopted, a greater understanding of soil biology is needed. Soil microorganisms play a central role in in providing soil processes such as nutrient (N, P, S, C) cycling, disease suppression, pollutant mitigation. and improved soil structure and water holding capacity (Johnston, 1986; Lazarovits *et al.*, 2000; O'Donnell *et al.*, 2001; Bailey and Lazarovits, 2003; Young and Crawford, 2004; O'Donnell *et al.*, 2007; Ojeda *et al.*, 2010; Cytryn *et al.*, 2011). It is speculated that manure additions to soil stimulate the growth of these beneficial microorganisms either directly by providing nutrients or indirectly by stimulating plant growth and enhancing root carbon flow (Buyanovsky and Wagner, 1986). However, soils are complex with multi-component interactions and understanding why they respond and how to bioengineer these responses in a systematic and predictive way remain a significant challenge for agricultural research and development.

With the advent of NGS methods for characterising and quantifying community structure and function, it may be possible to determine the microorganisms, their mode(s) of action and the mechanisms involved. Elucidating the link between soil function and manure applications offers the possibility of manipulating

the size, activity and diversity of the beneficial soil biota through changes in manure management (O'Donnell *et al.*, 2001; Kemmitt *et al.*, 2008; Jenkins *et al.*, 2009; Jenkins *et al.*, 2010). Ultimately, this could increase productivity and profitability of agricultural soils through improved crop yields and soil quality, and better fertilizer, pesticide and herbicide use efficiency. For example, management-induced changes in the population sizes of bacteria were investigated using qPCR (Figure 2). The bacterial population (as judged by 16S rRNA gene abundance) was greater in the soils receiving manure applications compared to the synthetic fertilizer treatment suggesting that organic inputs support a greater microbial biomass and possibly select for different functional components of the microbial community. However, this effect was lost when manure was applied in combination with synthetic fertilizer. Moreover, irrespective of the fertilizer used, management inputs decreased the bacterial population size in the soil relative to the unamended treatment (control) suggesting that pristine soils harbour a greater microbial density.

NGS also offers the possibility of identifying and developing microbial indicators of soil quality. Potential soil quality indicators would target key microbial pathways responsible for soil processes (e.g., biogeochemical cycling and disease suppression) enabling them be used as monitoring tools that quantify the risks and benefits of applying different piggery manures or by-products to soil. The knowledge gained will then be used to develop guidelines for end users on best management practices that enhance soil quality whilst maintain crop productivity and profitability.

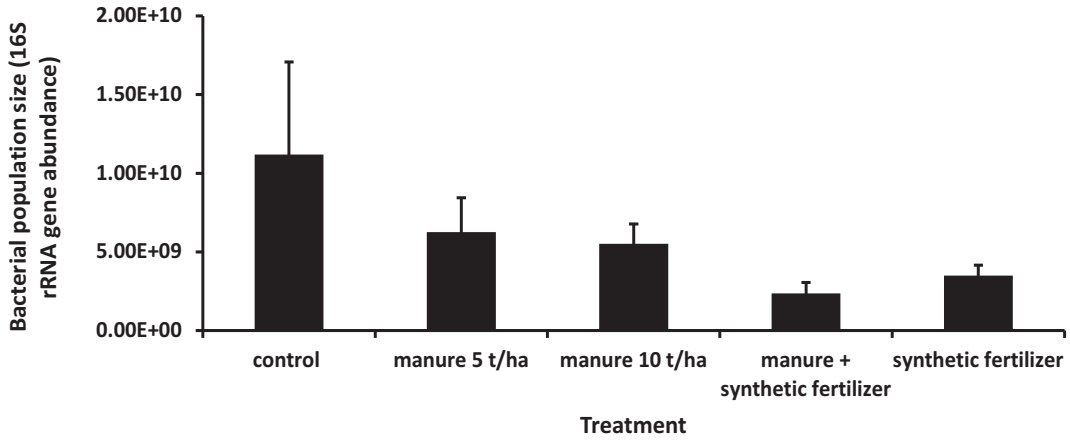


Figure 2. *Quantification of bacterial abundance based on 16S rRNA gene copies (gene abundance) in clay soil amended with five treatments: untreated control; 5 t/ha of piggery eco-shelter manure bedding; 10 t/ha of eco-shelter manure bedding; 10 t/ha of eco-shelter manure bedding plus conventional fertilizer; and conventional fertilizer. All qPCR reactions were done in triplicate and error bars indicate the standard error where n=3. (Jenkins,* unpublished data*).*

Role of P-mediating microbes in P responsive and P non-responsive Australian soils

Mycorrhizal fungi, P solubilising microbes (PSM) and P mineralising microbes (PMM) play crucial roles in mobilising insoluble phosphates into plant available forms via direct and indirect mechanisms (Richardson and Simpson, 2011). Manure applications to soil enhance P cycling, with increases in phosphatase production (P mineralisation), biological activity and microbial P biomass being reported (Colvan *et al.*, 2001). However, little is known about the diversity and abundance of these P-mediating microbes in soils and the factors regulating their activities due to methodological constraint (Richardson, 2007). Overcoming this issue may be fundamental to understanding the mechanisms involved in P responsive and P non-responsive Australian soils. Consequently, there is interest in developing molecular tools for monitoring these P-mediating microbes (Cardon and Gage, 2006). The P-mediating microbes produce a range of enzymes (e.g., dehydrogenases, phytases and phosphoesterases) that release plant-friendly soluble inorganic forms of P from organic P and mineral P sources within the soil (Lim *et al.*, 2007; Rodríguez *et al.*, 2007; Jorquera *et al.*, 2008). Genes controlling the expression of these enzymes are good candidates for molecular biomarkers and the development of monitoring tools (Cardon and Gage, 2006). Historically, molecular tools targeting P mediating microbes have been limited, non-specific or poorly designed due a lack of sequence information on the databases (Wasaki and Maruyama, 2011). However, new technological advancements in NGS mean that a large number of sequences encoding P cycling genes are now available and can be used to design molecular monitoring tools (Lim *et al.*, 2007; Jorquera *et al.*, 2008) for elucidating the role of P-mediating microbes in P responsive and P non-responsive soils.

Mitigating GHG emissions in soils amended with piggery manure

Manure application to soil could result in GHG emissions, namely nitrous oxide (N_2O), CO_2 and CH_4 VanderZaag *et al*., 2011). The magnitude of these emissions depends on the characteristics of the excreta (e.g., N availability and pH), the housing and production system(s) used to rear the pigs, and how the manure is stored and applied to land (Petersen and Sommer, 2010; Chadwick *et al*., 2011). The CFI is encouraging the development and adoption of GHG mitigation technology, abatement methods and regulations that decrease GHG from Australia's agricultural sector. Consequently, Australian piggeries have been looking at ways to modify their production systems in order to reduce GHG emission during the storage of manure.

Currently, solid manure and spent bedding material from animals housed in deep-litter systems is often stockpiled during storage. One mitigation option is to compost or pelletise both solid manure and spent bedding, which has been shown to reduce odour and GHG emission (VanderZaag *et al*., 2011). The development of low-cost AD technologies (e.g., CAPs) for treating piggery effluent wastes has already been discussed. Manures generated under these improved storage systems are expected to contain more stable forms of C and N thereby reducing their GHG potential. Nevertheless, those manures high in N and ammoniacal N (Güngör and Karthikeyan, 2008) could accelerate N_2O production in soils through microbial nitrification and (or) denitrification processes. Also, some land application practices may induce anaerobic conditions that are favourable for methanogenesis within soils leading to increased CH_4 emissions. These risks can be managed by using an appropriate GHG abatement method during manure application, such as: surface spreading followed by immediate incorporation (Petersen and Sommer, 2010, VanderZaag *et al*., 2011), shifting the timing of manure application to avoid summer rainfall events (Barton *et al*., 2010; Gleeson *et al*., 2010; VanderZaag *et al*., 2011), and liming acidic soils to reduced N_2O emission from nitrification (Barton *et al*., 2013a). However, there is limited published data that evaluates the effectiveness of different GHG abatement methods at reducing GHG emission in Australian soils receiving manure amendments.

Developing GHG mitigation strategies for soils amended with manure or by-products requires an improved understanding of how the underlying microbial processes responsible for GHG emissions are linked to environmental variables and management (Giles *et al*., 2012). However, identifying the microorganisms and mechanisms involved and determining the factors regulating their activities remains a significant challenge (O'Donnell *et al*., 2007). To date, the abundance and distribution of microbial populations involved in N_2O production in Australian soils has largely been investigated using qPCR approaches targeting functional genes involved in N transformations (Gleeson *et al*., 2010; Hayden *et al*., 2010; O'Sullivan *et al*., 2011; Barton *et al*., 2013a; O'Sullivan *et al*., 2013). Nitrogen transformation pathways that lead to N_2O emissions include dissimilatory nitrate reduction to ammonia (DNRA), nitrification, and denitrification. These pathways are catalysed by different enzymes encoded by genes that can be used as proxies for determining their contribution to N_2O emissions in soils.

Ammonia oxidising bacteria (AOB) and Archaea (AOA) mediate the first stage of nitrification using ammonia monooxygenase, which is encoded by the *amo* gene (Brown *et al*., 2012). Many factors affect the distribution of AOB/AOA, nitrification rates and N_2O production in Australian soils including pH, total N content, organic carbon content, soil type, climate and seasonal variations (Hayden *et al*., 2010; O'Sullivan *et al*., 2011; Barton *et al*., 2013a; O'Sullivan *et al*., 2013). Denitrification is catalysed by a series of enzymes including nitrate reductase, nitrite reductase, nitric oxide reductase, and N_2O reductase (nosZ), which are encoded by the genes *nar*, *nir*, *nor*, and *nos*, respectively. Environmental conditions often limit the gene expression of *nosZ* resulting in the production of N_2O. Nitrous oxide is also released as by-product during DNRA, catalysed by nitrite reductase and encoded by the *Nrf* gene (Giles *et al*., 2012). Denitrification and DNRA are influenced by a number of factors including availability of oxidants (NO_2^- or NO_3^-), carbon availability, soil moisture content, pH, O_2 concentrations and the size and community structure of nitrate-reducing organisms responsible for the processes (Colloff *et al*., 2008; Giles *et al*., 2012; Barton *et al*., 2013a). The N_2O emissions from agricultural soils have been attributed to both denitrification and nitrification, with a predominance of AOA throughout Australia with the exception of Western Australian soils where AOB prevails (Barton *et al*., 2013a; O'Sullivan *et al*., 2013).

Despite the increased understanding of GHG emission from Australian soils, the impact of manure amendments on N transformation pathways and GHG emissions has been largely overlooked. Different biochemical scenarios may occur in soils amended with manure depending upon soil conditions and GHG abatement practice used. For example, manure applications to soil could increase the rate of nitrification due to increased availability of NH_4^+ in the manure, leading to elevated N_2O emissions. Alternatively, increased availability of carbon in the manure may lead to a) increased microbial N mineralisation and immobilisation, thereby decreasing N_2O emission, or b) increased denitrification by stimulating the growth of denitrifiers, thereby increasing N_2O emissions. Another major limitation of current studies is that the

qPCR assays were performed on only a small subset of N cycle genes and therefore key N transformation pathways responsible for N_2O emissions could be missed. This can be overcome with low-cost NGS that offers the potential to fully explore these complex microbial communities and interactions. A metagenomic approach can be used to investigate the metabolic capacity of the entire soil community by examining multiple genes, enzymes and taxa involved in the C and N pathways responsible for GHG emissions.

Recently, metagenomic analysis undertaken as part of an APL/Department of Agriculture "Filling the Research Gap" project indicated that the potential for GHG emissions in Australian soils depends on the soil type and manure management, in terms of the form, combination (applied singly or mixed with synthetic fertilizer) and loading amount. Nitrogen transformation pathways in soil that lead to N_2O emissions included dissimilatory nitrate reduction to ammonia (DNRA), nitrification and denitrification (Figure 3). In the manured soils there was an increase in the ammonia-oxidising *amo* gene relative to the control suggesting a high potential for nitrification. This was surprising since it was expected that the increased availability of carbon in the soil following manure application would stimulate the growth of nitrate-reducing microroganisms thereby increasing N_2O emissions via denitrification or DNRA pathways. A possible explanation is that manure applications to soil increased nitrification by stimulating the ammonia-oxidising microorganisms due to increased availability of NH_4^+ leading to the elevated N_2O emissions. Previously, N_2O emissions in Western Australia were shown to be related to summer rainfall events and NH_4^+ from soil organic mineralisation, rather than applied fertilizer-N, was the likely source (Barton *et al.*, 2008). Raising the pH by liming or applying a Ca^{2+}-or Mg^{2+}-rich manure to the soils is a good abatement option for N_2O derived from nitrification (Barton *et al.*, 2013a).

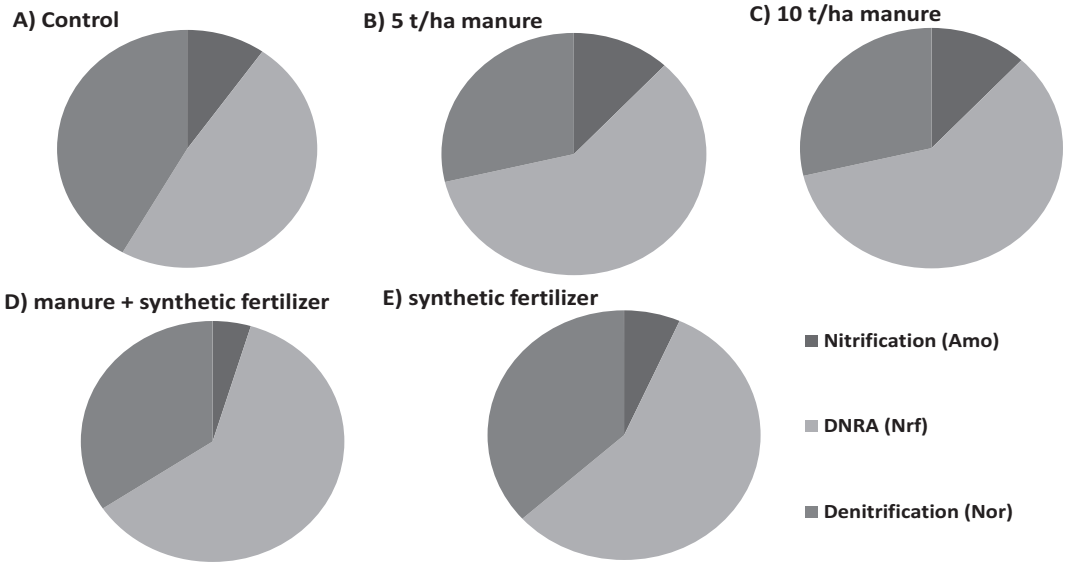


Figure 3. *Functional gene composition showing the relative contribution of N cycle genes involved in N_2O emissions including nitrification, denitrification and dissimilatory nitrate reduction to ammonia (DNRA) in clay soil amended with five treatments: A, untreated control; B, 5 t/ha of piggery eco-shelter manure bedding; C, 10 t/ha of eco-shelter manure bedding; D, 10 t/ha of eco-shelter manure bedding plus conventional fertilizer (D); E, conventional fertilizer. Total DNA for each soil sample was sequencing using a 316 chip by shotgun metagenomics (PCR independent method) (from Barton* et al.*, 2013a).*

Aside from N_2O, CH_4 is another potent GHG produced by Archaea during the process of methanogenesis. Although CH_4 emissions from Australian agricultural soils have been reported (Dalal *et al.*, 2008; Barton *et al.*, 2010; Biswas *et al.*, 2010; Denmead *et al.*, 2010), the identities of the microorganisms involved and factors regulating their activities remain unresolved. Interestingly, a reduction in CH_4 production was observed in semi-arid soils after liming which warrants further investigation (Barton *et al.*, 2010). Methane oxidation by soil methanotrophic bacteria is the only biological sink for CH_4 but the microorganisms and mechanisms involved are not fully understood (Stiehl-Braun *et al.*, 2011). The interaction between methanogenesis and CH_4 oxidation was explored in soils receiving different manure and fertilizer regimes as part of a current APL/Department of Agriculture grant.

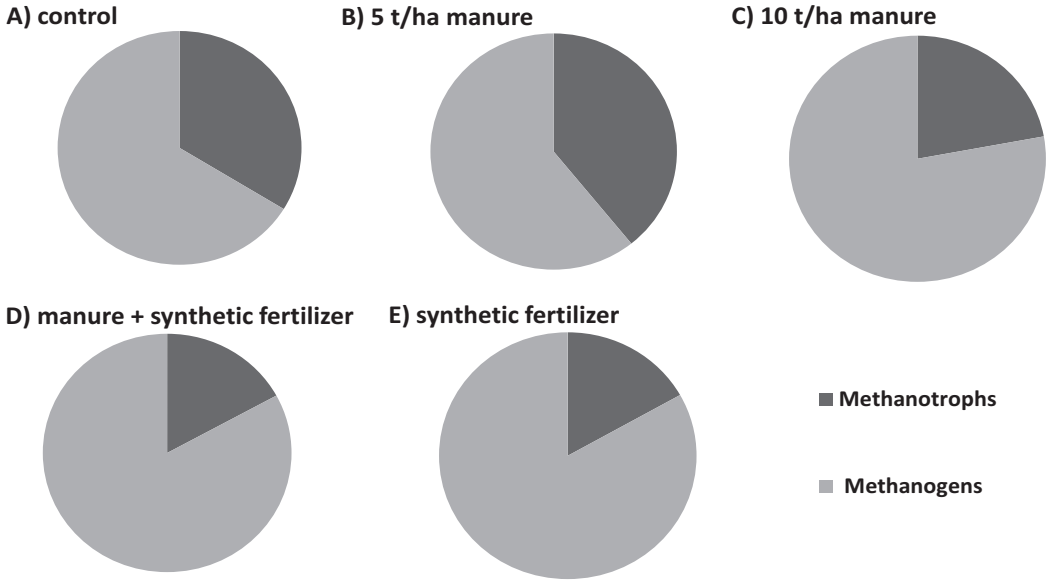


Figure 4. *Functional gene composition showing the relative contribution of CH_4-producing taxa (methanogens) and CH_4-oxidising taxa (methanotrophs) in clay soil amended with five treatments: A, untreated control; B, 5 t/ha of piggery eco-shelter manure bedding; C, 10 t/ha of eco-shelter manure bedding; D, 10 t/ha of eco-shelter manure bedding plus conventional fertilizer (D); E, conventional fertilizer. Total DNA for each soil sample was sequencing using a 316 chip by shotgun metagenomics (PCR independent method). (Jenkins,* unpublished data*).*

Figure 4 shows that adding 5t/ha of manure to soil appears to increase the relative abundance of CH_4-oxidising bacteria, however this effect is lost at higher manure loading rates (10t/ha). The methanotroph population is further reduced in the soils receiving synthetic fertilizer inputs. It appears, therefore, that management-induced changes in methanogens and methanotrophs are related to both the amounts and type of manure inputs. Agricultural manures and wastes, such as the piggery manure used here, have been shown to have a liming effect since they usually contain basic cations such as Ca^{2+} or Mg^{2+} that serve to neutralise soil acidity (Walker *et al*., 2004). More recently, liming was shown to be an effective GHG abatement strategy by lowering on-farm GHG emissions from arable soils in semi-arid environments fertilised with synthetic N via decreased N_2O fluxes and increased CH_4 uptake (Barton *et al*., 2013b). Thus, the benefits of liming as a strategy to abate GHG arising from CH_4 are two-fold: first, suppression of acidophilic methanogens and thereby CH_4 emissions, and second, stimulation of CH_4-oxidising bacteria and increased CH_4 removal. Knowledge gained from the molecular, chemical and gas analysis about how microbial communities are linked to GHG flux, fertilizer use, manure type and storage and soil type will benefit the industry by identifying the most promising mitigation methods for inclusion in the CFI approved methodologies. Ultimately, this will lead to the development of cost-effective mitigation strategies that decrease national livestock emissions with minimal impact on productivity and profitability.

Conclusions

Genomic approaches are currently being used to identify more economically and environmentally sustainable management practices for treating, storing and recycling manure to land. This includes identifying the microbial processes and mechanisms involved in soil and pond processes (e.g., GHG emission and disease suppression) and developing molecular tools as indicators of pond stability or soil quality. Long-term, these tools will be used as part of a pond and soil monitoring services for end-users to a) enhance biogas yields and avoid pond failure in covered anaerobic ponds, b) quantify the risks and benefits of applying manure to soil, and c) select best manure management practices for their operation and soils. Overall, genomic research will contribute to improving the sustainability of the Australian pork industry and increase its resilience to climate variation.

References

ABELL, G.C.J., REVILL, A.T., SMITH, C., BISSETT, A.P., VOLKMAN, J.K. and ROBERT, S.S. (2009). *ISME Journal*. **4:**286-300.

AMANI, T., NOSRATI, M. and SREEKRISHNAN, T.R. (2010). *Environmental Reviews.* **18:**255-278.

AMANN, R.I. (1995). *Molecular Ecology*. **4:**543-554.

BAILEY, K.L. and LAZAROVITS, G. (2003). *Soil Tillage Research.* **72:**127-132

BARTON, L., GLEESON, D.B., MACCARONE, L.D., ZÚÑIGA, L.P. and MURPHY, D.V. (2013a). *Soil Biology and Biochemistry.* **62:**28-35.

BARTON, L., KIESE, R., GATTER, D., BUTTERBACH-BAHL, K., BUCK, R., HINZ, C. and MURPHY, D.V. (2008). *Global Change Biology.* **14:**177-192.

BARTON, L., MURPHY, D.V. and BUTTERBACH-BAHL, K. (2013b). *Agriculture Ecosystems and Environment.* **167:**23-32.

BARTON, L., MURPHY, D.V., KIESE, R. and BUTTERBACH-BAHL, K. (2010). *Global Change Biology Bioenergy.* **2:**1-15.

BARTRAM, A.K., LYNCH, M.D.J., STEARNS, J.C., MORENO-HAGELSIEB, G. and NEUFELD, J.D. (2011). *Applied and Environmental Microbiology.* **77:**3846-3852.

BEJA, O., SPUDICH, E.N., SPUDICH, J.L., LECLERC, M. and DELONG, E.F. (2001). *Nature.* **411:**786-789.

BISWAS, W. K., GRAHAM, J., KELLY, K. & JOHN, M. B. (2010). *Journal of Cleaner Production.* 18**:** 1386-1392.

BOWERS, R.M., SULLIVAN, A.P., COSTELLO, E.K., COLLETT, J.L., KNIGHT, R. and FIERER, N. (2011). *Applied and Environmental Microbiology.* **77:**6350-6356.

BROWN, J., BLANKINSHIP, J., NIBOYET, A., GROENIGEN, K., DIJKSTRA, P., ROUX, X., LEADLEY, P. and HUNGATE, B. (2012). *Biogeochemistry.* **109:**85-100.

BUYANOVSKY, G.A. and WAGNER, G.H. (1986). *Plant Soil.* **93:**57-65.

CAPORASO, J.G., KUCZYNSKI, J., STOMBAUGH, J., *et al*. (2010). *Nature Methods.* **7:**335-336.

CAPORASO, J.G., LAUBER, C.L., WALTERS, W. A., *et al*. (2012). *ISME Journal.* **6:**1621-1624.

CARDON, Z.G. and GAGE, D.J. (2006). *Annual Review of Ecology Evolution and Systematics.* **37:**459-488.

CHADWICK, D., SOMMER, S., THORMAN, R., FANGUEIRO, D., CARDENAS, L., AMON, B. and MISSELBROOK, T. (2011). *Animal Feed Science and Technology.* **166-167:**514-531.

COLLOFF, M.J., WAKELIN, S.A., GOMEZ, D. and ROGERS, S.L. (2008). *Soil Biology and Biochemistry.* **40:**1637-1645.

COLVAN, S.R., SYERS, J.K. and O'DONNELL, A.G. (2001). *Biology and Fertility of Soils.* **34:**258-263.

CONACHER, J. and CONACHER, A. (1998). *Biological Agriculture and Horticulture.* **16:**145-171.

COOK, K.L., ROTHROCK, M.J., LOVANH, N., SORRELL, J.K. and LOUGHRIN, J.H. (2010). *Anaerobe.* **16:**74-82.

CRAGGS, R., PARK, J. and HEUBECK, S. (2008). *Australian Journal of Experimental Agriculture.* **48:**142-146.

CYTRYN, E., KAUTSKY, L., OFEK, M., MANDELBAUM, R.T. and MINZ, D. (2011). *Applied Soil Ecology.* **48:**160-167.

DALAL, R.C., ALLEN, D.E., LIVESLEY, S.J. and RICHARDS, G. (2008). *Plant and Soil.* **309:**43-76.

DEMIREL, B. and SCHERER, P. (2008). *Reviews in Environmental Science and Biotechnology.* **7:**173-190.

DENMEAD, O.T., MACDONALD, B.C.T., BRYANT, G., NAYLOR, T., WILSON, S., GRIFFITH, D.W.T., WANG, W.J., SALTER, B., WHITE, I. and MOODY, P.W. (2010). *Agricultural and Forest Meteorology.* **150:**748-756.

EDMEADES, D.C. (2003). *Nutrient Cycling in Agroecosystems.* **66:**165-180.

FIERER, N., LEFF, J.W., ADAMS, B.J., NIELSEN, U.N., BATES, S.T., LAUBER, C.L., OWENS, S., GILBERT, J.A., WALL, D.H. and CAPORASO, J.G. (2012). *Proceedings of the National Academy of Sciences of the United States of America.* **109:**21390-21395.

FROMIN, N., HAMELIN, J., TARNAWSKI, S., ROESTI, D., JOURDAIN-MISEREZ, K., FORESTIER, N., TEYSSIER-CUVELLE, S., GILLET, F., ARAGNO, M. and ROSSI, P. (2002). *Environmental Microbiology*, **4:**634-643.

GARNAUT, R. (2008). The Garnaut Climate Change Review. Final Report. (Melbourne).

GILES, M.E., MORLEY, N.J., BAGGS, E.M. and DANIELL, T.J. (2012). *Frontiers in Microbiology*, **3:**407.

GLEESON, D.B., MULLER, C., BANERJEE, S., MA, W., SICILIANO, S.D. and MURPHY, D.V. (2010). *Soil Biology and Biochemistry.* **42:**1888-1891.

GLENN, T.C. (2011). *Molecular Ecology Resources.* **11:**759-769.

GOH, S.H.M., MABBETT, A.N., WELCH, J.P., HALL, S.J. and McEWAN, A.G. (2009). *Letters in Applied Microbiology.* **48:**486-492.

GÜNGÖR, K. and KARTHIKEYAN, K.G. (2008). *Bioresource Technology.* **99:**425-436.

GUPPY, C. N. and MCLAUGHLIN, M.J. (2009). *Crop and Pasture Science.* **60:**116-123.

HAMADY, M., WALKER, J.J., HARRIS, J.K., GOLD, N.J. and KNIGHT, R. (2008). *Nature Methods.* **5:**235-237.

HAMMOND, J.P., BROADLEY, M.R. and WHITE, P.J. (2004). *Annals of Botany.* **94:**323-332.

HAYDEN, H.L., DRAKE, J., IMHOF, M., OXLEY, A.P.A., NORNG, S. and MELE, P.M. (2010). *Soil Biology and Biochemistry.* **42:**1774-1783.

HEUBECK, S. and CRAGGS, R.J. (2010). *Science and Technology.* **61:**1019-1026.

JENKINS, S.N., RUSHTON, S.P., LANYON, C.V., WHITELEY, A.S., WAITE, I.S., BROOKES, P.C., KEMMITT, S., EVERSHED, R.P. and O'DONNELL, A.G. (2010). *Soil Biology and Biochemistry.* **42:**1624-1631.

JENKINS, S.N., WAITE, I.S., BLACKBURN, A., HUSBAND, R., RUSHTON, S.P., MANNING, D. C. and O'DONNELL, A.G. (2009). *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology.* **95:**319-334.

JOHNSTON, A.E. (1986). *Soil Use and Management.* **2:**97-105.

JORQUERA, M.A., HERNANDEZ, M.T., RENGEL, Z., MARSCHNER, P. and DE LA LUZ MORA, M. (2008). *Biology and Fertility of Soils.* **44:**1025-1034.

KAMPMANN, K., RATERING, S., KRAMER, I., SCHMIDT, M., ZERR, W. and SCHNELL, S. (2012). *Applied and Environmental Microbiology.* **78:**2106-2119.

KARAKASHEV, D., BATSTONE, D.J., TRABLY, E. and ANGELIDAKI, I. (2006). *Applied and Environmental Microbiology.* **72:**5138-5141.

KEMMITT, S.J., LANYON, C.V., WAITE, I.S., WEN, Q., ADDISCOTT, T.M., BIRD, N.R.A., O'DONNELL, A.G. and BROOKES, P.C. (2008). *Soil Biology and Biochemistry.* **40:**61–73.

KIM, W., LEE, S., SHIN, S.G., LEE, C., HWANG, K. and HWANG, S. (2010). *Water Research.* **44:**4900-4907.

KUCZYNSKI, J., LAUBER, C.L., WALTERS, W.A., PARFREY, L.W., CLEMENTE, J.C., GEVERS, D. and KNIGHT, R. (2012). *Nature Reviews Genetics.* **13:**47-58.

LANE, D.J., PACE, B., OLSEN, G J., STAHL, D.A. and SOGIN, M.L. (1985). *Proceedings of National Academy of Sciences of the United States of America.* **82:**6955-6959.

LAZAROVITS, G., TENUTA, M. and CONN, K. L. (2000). In "Proceedings of the International Symposium on Chemical and Non-Chemical Soil and Substrate Disinfestation", pp. 59-64, eds. M.L. Gullino, J. Katan and A. Matta. (ISHS Working Group on Soil-Borne Pathogens: Leuven, Belgium).

LIM, B.L., YEUNG, P., CHENG, C. and HILL, J.E. (2007). *ISME journal.* **1:**321-330.

LIPSON, D.A., HAGGERTY, J.M., SRINIVAS, A., RAAB, T.K., SATHE, S. and DINSDALE, E.A. (2013). *PLoS ONE.* **8:**e64659.

LOZUPONE, C. and KNIGHT, R. (2005). *Applied and Environmental Microbiology.* **71:**8228-8235.

LOZUPONE, C.A. and KNIGHT, R. (2007). *Proceedings of the National Academy of Sciences of the United States of America.* **104:**11436-11440.

LUEDERS, T., POMMERENKE, B. and FRIEDRICH, M. W. (2004). *Applied and Environmental Microbiology.* **70:**5778-5786.

MARGULIES, M., EGHOLM, M., ALTMAN, W. E., et al. (2005). *Nature.* **437:**376-380.

MARTIN, H.G., IVANOVA, N., KUNIN, V., et al. (2006). *Nature Biotechnology.* **24:**1263-1269.

MEYER, F., PAARMANN, D., D'SOUZA, M., OLSON, R., GLASS, E.M., KUBAL, M., PACZIAN, T., RODRIGUEZ, A., STEVENS, R., WILKE, A., WILKENING, J. and EDWARDS, R.A. (2008). *BMC Bioinformatics.* **9:**386.

MÜLLER, N., WORM, P., SCHINK, B., STAMS, A.J.M. and PLUGGE, C.M. (2011). *Environmental Microbiology Reports.* **2:**489-499.

O'DONNELL, A.G., YOUNG, I.M., RUSHTON, S.P., SHIRLEY, M.D. and CRAWFORD, J.W. (2007). *Nature Reviews Microbiology.* **5:**689-699.

O'DONNELL, A.G., SEASMAN, M., MACRAE, A., WAITE, I. and DAVIES, J.T. (2001). *Plant Soil.* **232:**135-145.

OJEDA, G., MATTANA, S., ALCANIZ, J.M., MARANDO, G., BONMATI, M., WOCHE, S.K. and BACHMANN, J. (2010). *Geoderma.* **156:**399-409.

OLSEN, G.J., LANE, D.J., GIOVANNONI, S.J., PACE, N.R. and STAHL, D.A. (1986). *Annual Review of Microbiology.* **40:**337-365.

O'SULLIVAN, C.A., WAKELIN, S. ., FILLERY, I.R.P., GREGG, A.L. and ROPER, M.M. (2011). *Soil Research.* **49:**715-724.

O'SULLIVAN, C.A., WAKELIN, S.A., FILLERY, I.R.P. and ROPER, M.M. (2013). *Soil Research.* **51:**240-252.

PATIL, S.S., KUMAR, M.S. and BALL, A.S. (2010). *Applied Microbiology and Biotechnology.* **87:**353-363.

PEIGNE, J. and GIRARDIN, P. (2004). *Water Air and Soil Pollution.* **153:**45-68.

PETERSEN, S.O. and SOMMER, S.G. (2010). *Animal Feed Science and Technology.* **166:**503-513.

RICHARDSON, A.E. (2007). In "First International Meeting on Microbial Phophsate Solubilization", pp. 85-90, eds. E. Velazquez and C. Rodrigues-Barrueco. (Springer: The Netherlands).

RICHARDSON, A.E. and SIMPSON, R.J. (2011). *Plant Physiology.* **156:**989-996.

RITTMANN, B.E., KRAJMALNIK-BROWN, R. and HALDEN, R.U. (2008). *Nature Reviews Microbiology.* **6:**604-612.

RIVIERE, D., DESVIGNES, V., PELLETIER, E., CHAUSSONNERIE, S., GUERMAZI, S., WEISSENBACH, J., LI, T., CAMACHO, P. and SGHIR, A. (2009). *ISME Journal.* **3:**700-714.

RODRÍGUEZ, H., FRAGA, R., GONZALEZ, T. and BASHAN, Y. (2007). In "First International Meeting on Microbial Phophsate Solubilization", pp. 15-921, eds. E. Velazquez and C. Rodrigues-Barrueco. (Springer: The Netherlands).

ROTHBERG, J.M., HINZ, W., REARICK, T.M., et al. (2011). *Nature.* **475:**348-352.

SEMENOV, A.M., KUPRIANOV, A.A. and VAN BRUGGEN, A.H.C. (2010). *Microbial Ecology.* **60:**239-249.

SKERMAN, A. and COLLMAN, G. (2012). Methane Recovery and Use at Grantham Piggery". RIRDC Publication No 12/064, RIRDC Project No PRJ-005672.

SOGIN, M.L., MORRISON, H.G., HUBER, J.A., WELCH, M.D., HUSE, S.M., NEAL, P.R., ARRIETA, J.M. and HERNDL, G.J. (2006). *Proceedings of the National Academy of Sciences of the United States of America.* **103:**12115-12120.

STIEHL-BRAUN, P.A., POWLSON, D.S., POULTON, P.R. and NIKLAUS, P.A. (2011). *Soil Biology and Biochemistry.* **43:**1034-1041.

SUPAPHOL, S., JENKINS, S.N., INTOMO, P., WAITE, I.S. and O'DONNELL, A.G. (2011). *Bioresource Technology.* **102:**4021-4027.

TALBOT, G., ROY, C.S., TOPP, E., KALMOKOFF, M.L., BROOKS, S.P.J., BEAULIEU, C., PALIN, M.F. and MASSE, D.I. (2010). *Water Science and Technology.* **61:**1147-1155.

TANG, Y.-Q., JI, P., HAYASHI, J., KOIKE, Y., WU, X.-L. and KIDA, K. (2011). *Applied Microbiology and Biotechnology.* **91:**1447-1461.

VANDERZAAG, A.C., JAYASUNDARA, S. and WAGNER-RIDDLE, C. (2011). *Animal Feed Science and Technology.* **166:**464-479.

WALKER, D.J., CLEMENTE, R. and BERNAL, M.P. (2004). *Chemosphere.* **57:**215-224.

WANG, H.L., MAGESAN, G.N. and BOLAN, N.S. (2004). *New Zealand Journal of Agricultural Research.* **47:**389-403.

WASAKI, J. and MARUYAMA, H. (2011). *Phosphorus in Action.* **93**:93-111.

WIEDEMANN, S. G., MCGAHAN, E.J., GRIST, S.L., and GRANT, T. (2010). "Environmental Assessment of Two Pork Supply Chains Using Life Cycle Assessment". RIRDC Publication No 09/176, RIRDC Project No PRJ-3176 and PRJ-4519.

WELLINGTON, E.M.H., BERRY, A. and KRSEK, M. (2003). *Current Opinion in Microbiology.* **6:**295-301.

WHITELEY, A.S., JENKINS, S.N., WAITE, I., KRESOJE, N., PAYNE, H.G., MULLAN, B.P., ALLCOCK, R. and O'DONNELL, A. (2012). *Journal of Microbiological Methods.* **91:**80-88.

WILMES, P. & BOND, P. L. (2006). *Trends in Microbiology.* **14:** 92-97.

XU, J. (2006). *Molecular Ecology.* **15:**1713-1731.

YOUNG, I.M. and CRAWFORD, J.W. (2004). *Science.* **304:**1634-1637.

This work was funded by grants from Australian Pork Limited and Department of Agriculture, Fisheries and Forestry (DAFF) under the FarmReady Industry scheme, Science and Innovation Awards for Young People in Agriculture, Fisheries and Forestry program and Carbon Farming Futures "Filling the Research Gap" Program, and funds from The University of Western Australia.

REVIEW: Options for anaerobic digestion of piggery waste

D.J. Batstone, P. Gopalan, P.D. Jensen and S. Tait

The University of Queensland, Australia, QLD 4072

Abstract

Anaerobic digestion is one of the best ways to generate renewable energy, treat wastewater, reduce greenhouse gas emissions, and value-add to a variety of modes of pig rearing. In the future, it may enable recovery of nutrients as saleable concentrated fertilisers as well as energy. This has been enhanced by publication of a range of carbon farming initiative (CFI) methodologies specific to piggeries, as well as better tools for project feasibility analysis. Engineered digesters and covered anaerobic lagoons are popular for intensive pig rearing, and new technologies are emerging for conversion of spent litter into methane energy. This review covers the broader application of anaerobic digestion to the Australian pig industry. It particularly focusses on new science being applied to existing technologies such as lagoon systems, including maximising methane yield, and assesses the applicability of emerging technologies with engineered digesters as an example for intensive piggeries, and solid-phase batch digesters for deep litter piggeries.

Introduction

Anaerobic digestion, which is biological conversion of organic matter in piggery waste (manure, spent litter, by-products) into methane and carbon-dioxide, has become a particularly attractive option for piggeries to value-add to their manures at a variety of scales. A key driver is increases in energy costs, with substantial historical increases, an additional 20-30% expected in the next 5 years, and increased costs associated with uncertainty and international demand for specific energy resources such as natural gas. Implementation of carbon farming initiative (CFI) methodologies for converting manures in covered lagoons (CFI Methodology F2012L01501) and engineered digesters (F2013L00124) are also a driver. The revenue from carbon capture has enhanced existing income available from renewable energy production, and in the future, the possibility for nutrient capture and sale will likely add considerable additional income to producers. While both of the CFI methodologies apply specifically to liquid pig manures, there is a substantial amount of research being done on anaerobic digestion options for deep litter piggeries.

There has been a substantial amount of research in the last 5 years contextual to the Australian pork industry that has considerably reduced risk around anaerobic digestion projects. This review is aimed at providing a basic understanding of the processes of anaerobic digestion, and providing contextual information for the Australian pork industry.

Anaerobic digestion processes

Anaerobic digestion relies on microbes to convert organic matter to the most chemically-reduced and most chemically-oxidised forms of carbon, methane and carbon-dioxide. While aerobic digestion relies on oxygen, as the major externally supplied electron acceptor (oxidant), anaerobic digestion has no such electron acceptor. This is important, as it means that the energy inherent in the organic matter in waste being converted [measured as chemical oxygen demand (COD) or electron equivalents] will flow to additional products, such as methane, instead of being consumed during the growth of additional microbes (as is the case with aerobic digestion). That is, the COD entering the reactor/lagoon will either be in the treated effluent or in the gas product stream as methane. While the gas product may contain hydrogen, the levels will be insignificant and therefore methane is the only component in the gas with significant levels of COD; this then makes it relatively easy to estimate the amount of gas produced from a given amount of feed. For example, if 1 tonne of dry straw is converted to biogas it will produce approximately 500 m^3 of biogas per day at 50% methane, which is approximately 7 GJ of energy. Yield on this in a cogeneration engine is normally approximately 35% or more. Electricity input is mostly for mixing in engineered systems, sludge pumping and reactor heating, and is 0.15 kwh $m^{-3}day^{-1}$ (m^3 reactor volume) (Batstone and Jensen, 2011). Lagoons that are unmixed usually need much longer solids retention times due to lower prevailing temperatures, which slows the breakdown of the organic solid matter in the waste.

Anaerobic digestion is a multi-step process, and many aspects in performance are determined by the nature of the steps (Pavlosathis and Girlado-Gomez, 1991; Figure 1). The most common rate limiting steps in high-solids, or solid-phase, digesters are either hydrolysis (enzymatic breakdown of solids or complex

chemical structures) or aceticlastic methanogenesis, which is the final step that turns acetic acid (vinegar) into methane. For piggeries, either hydrolysis or methanogenesis can be rate limiting. Because the rate at which these processes (hydrolysis and aceticlastic methanogenesis) occur influences the rate at which piggery waste can be turned into methane, these processes (hydrolysis and aceticlastic methanogenesis) are recognised as critical, and are covered in more detail below and are the subject of on-going Pork Cooperative Research Centre (CRC)-funded research.

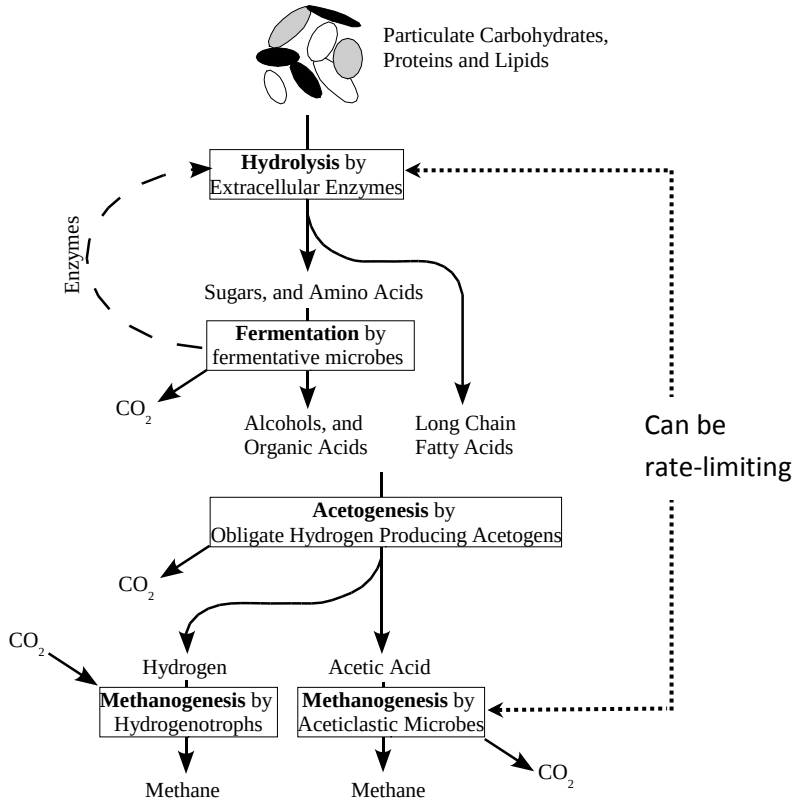


Figure 1. *Anaerobic digestion is a multi-step process (modified from Batstone and Jensen, 2011). In high nitrogen/particulate systems, either hydrolysis or aceticlastic methanogenesis can be rate-limiting (Copyright preserved).*

Hydrolysis

Degradation of carbohydrate-type wastes, and particularly agricultural residues, is a heavily researched area. Specific projects are discussed in a later section, but an overview of the hydrolysis process is given here. Straw is composed of predominantly cellulose, hemicellulose, lignin, mineral solids and other organic compounds (Hashimoto, 1986). Enzymatic hydrolysis of cellulose and other carbohydrates have a number of steps (Figure 2):

1. Production of enzyme (1); production rate can decrease when there is excessive soluble substrate available (Ramsay, 1997);
2. Transport processes (2, 3 and 6) can be limited with large particles, or in solid phase systems with inadequate carrier liquid;
3. Adsorption (4) processes are limited by surface area;
4. Reaction rates (5) are limited by surface area and enzyme concentrations;
5. Deactivation (7) can be excessive away from optimal temperature and pH.

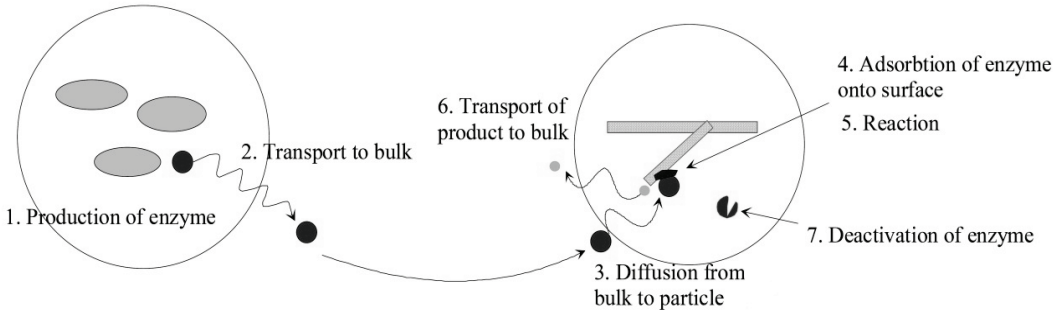


Figure 2. *Steps in enzymatic hydrolysis (modified from Batstone and Jensen, 2011). (Copyright preserved).*

While there have been complex physico-biological-chemical models that attempt to present hydrolysis as this complex process (e.g., Humphrey, 1979), in practice it is very difficult to properly validate these models, and the most commonly used model is thus first order (linear dependence of rate of breakdown on the available organic matter). The use of first order models has been justified as "an empirical expression that reflects the cumulative effect of all the microscopic processes occurring…' (Eastman and Ferguson, 1981). First order (or slightly more complex) has also been found to be just as effective as more complex models (Vavilin, 1996). Where hydrolysis is rate-limiting (the majority of agricultural wastes), its net methane generation potential is commonly expressed in the amount of methane it ultimately generates per unit amount of organic solids/volatile solids (VS) fed into the system (mL CH_4 gVS^{-1} at standard temperature and pressure; 25 °C, 1 bar).

Hydrolysis commonly becomes rate-limiting when either:

- In a continuous mixed digester system: hydraulic loading rate becomes too high (there is not enough time to hydrolyse the solids). Mass loading rate is generally not an issue, and higher concentrations allow higher loading rates.
- In a batch system: insufficient batch time. Batch digesters have a higher volumetric efficiency, due to kinetic considerations (breakdown is initially fast when a large amount of organic matter is available to breakdown).
- In a plug-flow system: insufficient reactor volume. Plug-flow digesters are also highly efficient on a volumetric basis. Time of contact with the active biomass can also be an issue.

'Well-mixed' refers to a reactor where the contents are consistent throughout whereas a plug-flow digester has little mixing along its length, such that the contents are less degraded at the feed side of the reactor and progressively more degraded towards the outflow end. The first-order kinetic parameter for hydrolysis of carbohydrates ranges from 0.05 d^{-1} to 1 d^{-1} (Eastman and Ferguson, 1981; Gujer and Zehnder, 1983; Pavlosathis and Girlado-Gomez, 1991; Angelidaki *et al*., 1999; Gavala *et al*., 1999; Batstone *et al*., 2002), which means that solids have to be retained for between 30-50 d (0.05 d^{-1}) and 2-4 d (1 d^{-1}) to achieve about 90% breakdown.

For piggery manures, hydrolysis rates vary substantially. Manure itself has a moderate speed of breakdown into methane, averaging at a hydrolysis rate of around 0.1 d^{-1} (Gopalan *et al*., 20913), which would normally require approximately 30 d to degrade fully and require a minimum retention time of 20 d for reasonable operation. Degradation extent and potential is good, with a national greenhouse emissions reporting scheme (NGERS) potential of 0.45 $Nm^3CH_4.kgVS^{-1}$, and observed potentials (Figure 3) of 0.170.6 $Nm^3CH_4.kgVS^{-1}$ (Gopalan *et al*., 20913)

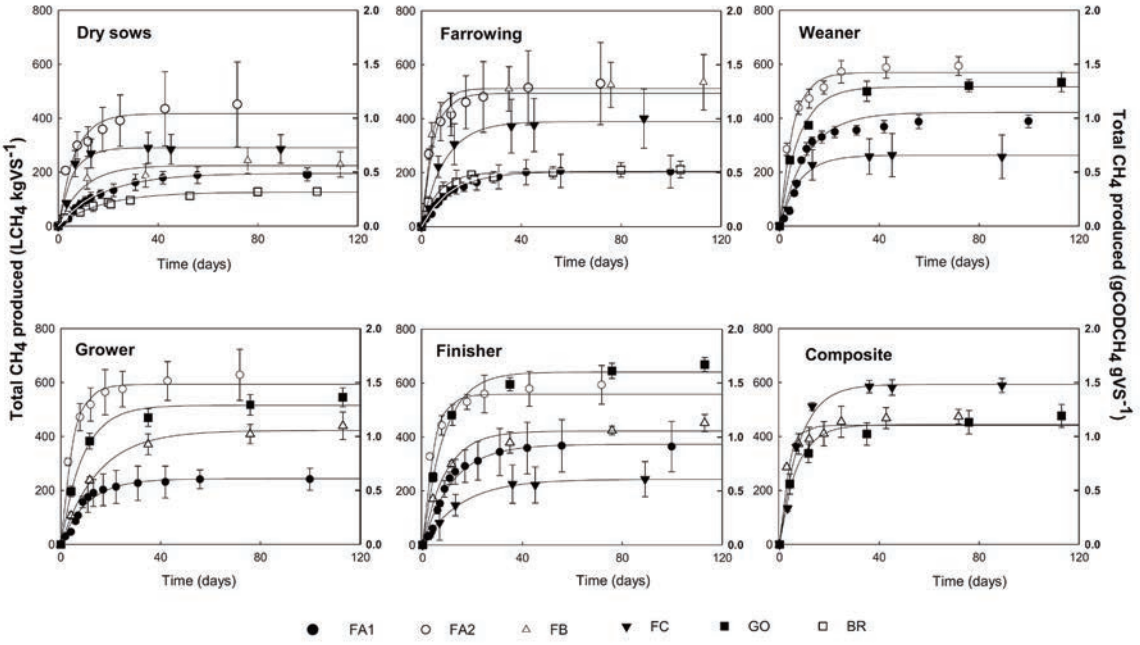


Figure 3. *Methane yields in batch tests from Australian piggeries at different stages (from Gopalan* et al.*, 2013). (Used with permission and copyright preserved).*

For litters, straw breaks down much slower with hydrolysis rates of between 0.03 and 0.1 d^{-1} (Moller *et al*., 2004), with minimum reactor retention times of 25-50 d. Degradation extent is relatively good with reasonable methane yields of 0.2-0.3 $m^3CH_4.kgVS^{-1}$ fed (Hashimoto, 1983, 1986; Gavala *et al*., 1999; Moller *et al*., 2004). Data found on spent bedding indicates that moisture and nitrogen content is far higher than in raw straw (Nicholas *et al*., 2006), with moisture content increasing to approximately 50% from a background of 15% in unused straw. Pigs generally have a substantial and strong impact on straw bedding, such that spent litter has an increased degradability resulting in improved conversion to methane. However the base substrate (straw as opposed to rice husks) needs to have a reasonable degradability to make digestion of litter an economic proposition (Tait *et al*., 2009). That is, pig-soiled straw can be readily digested while pig-soiled rice hulls do not produce sufficient methane.

Pre-treatment (such as mechanical, thermal hydrolysis or chemical treatment) of organic wastes target a partial breakdown of the waste to simplify and improve subsequent biodigestion and methane recovery, and works well for domestic wastewater sludges (Carrère *et al*., 2010). The microstructure of the waste material broken down by thermal, mechanical or chemical pre-treatment becomes more exposed to allow access by enzymes for hydrolysis and thus making enzymatic hydrolysis faster. However, these methods are generally not applicable to piggery applications due to high capital cost. Milder physical methods (cutting or milling) may have relatively low capital costs, but does not impact the microstructure of the waste, and consequently does not significantly improve digestion rate or extent (Hashimoto, 1983; Moller *et al*., 2004). Milling or cutting can improve overall mechanical handling of the waste material.

Methanogenesis

Aceticlastic methanogenesis (the final step in the breakdown of organic matter in the waste into methane) can become rate-limiting under inhibitory conditions such as with poor mixing or with substrate overload (feeding too much waste into the digester). Manure systems are commonly inhibited by ammonia (Karakashev *et al*., 2005) in an engineered configuration, where ammonia is the nitrogen that originates from the chemical structure of the waste and transfers into the liquid portion as the waste is broken down. Ammonia inhibition is caused by the free-form of ammonia (i.e., NH_3, not NH_4^+). It is therefore heavily pH dependent, with the acidity constant of ammonium being 9.25. Therefore, at pH of 7.25, 1% of the total ammonia/ammonium is in the free form (not very inhibitory), while at a pH of 8.25, 10% of the total ammonia/ammonium is in the free form (very inhibitory). The ammonia/ammonium system is also heavily temperature dependent, with increasing free ammonia with increasing temperature (Batstone *et al*., 2002). The impact of this is shown in Figure 4 (including short-term inhibition impacts). At lower temperatures, the pKa value for the ammonia weak-acid-base reaction increases, so for a given pH at a lower temperature there will be less free ammonia and thus less toxicity. For this reason, thermophilic digesters (digesters that operated at higher temperatures) are generally more prone to inhibition by ammonia. Ammonia inhibition has two impacts:

- In the short term, it causes reversible inhibition of aceticlastic methanogenesis. This causes a rise in acetic acid concentrations (the 'food' of the aceticlastic methanogens). Inhibition is approximately 50% at 0.0018M NH_3 (Siegrist *et al*., 2002; 1400 mg N/L total $NH_3/NH4^+$ at pH 7.25), but excess capacity means that volatile fatty acids levels (such as acetic acid) often do not rise until total ammonia is above 3000 mgN/L. Microbes may be more tolerant to free ammonia at higher temperatures, which compensates for the physico-chemical changes. Digested liquid pig manure has ammonia nitrogen concentrations at around 1000 mgN/L (Gopalan *et al*., 2013), but spent litters can have concentrations of up to 7000 mgN/kg waste (Tait *et al.*, 2009), so the impact of ammonia is important when the waste is not diluted before anaerobic treatment.
- In the long term (e.g., manure digesters), ammonia toxicity has been found to cause a fundamental change in the methanogen population converting acetate to methane. The most common pathway is cleavage of the acetate to methane and carbon dioxide by *Methanosaeta* – a highly specialised genus of microbes. In the long term, ammonia prevents growth of *Methanosaeta*, and instead, acetate is first oxidised to hydrogen and carbon dioxide, potentially by several different microbes, and the hydrogen-carbon dioxide mix is subsequently converted to methane by a different group of microbes. The two-step oxidation process is slower and less efficient than the cleavage step (Karakashev *et al*., 2006), hence slowing down the overall conversion to methane. The two-step oxidation process is made more efficient by operation at higher temperatures.

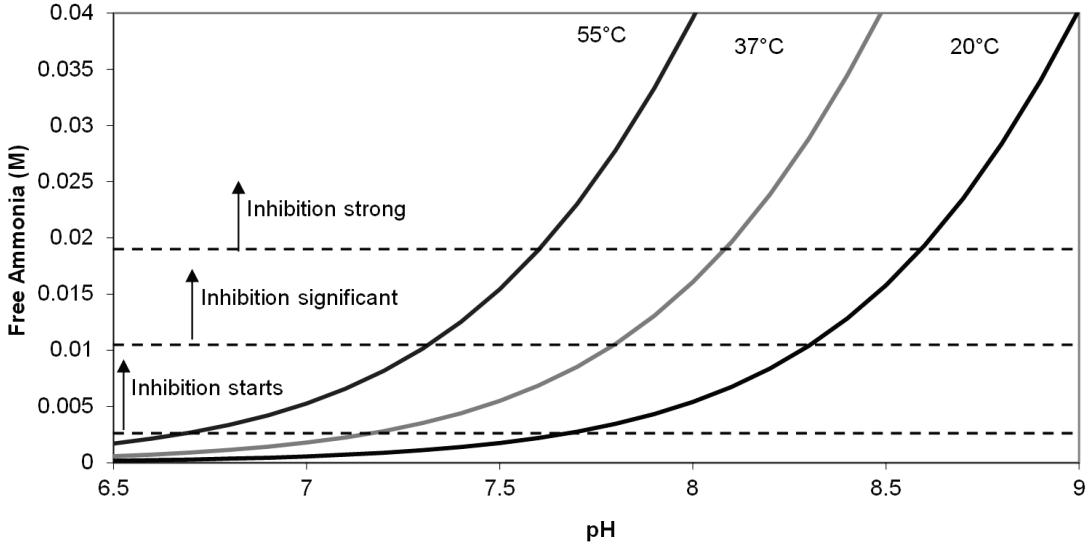


Figure 4. *Free ammonia levels, and ammonia inhibition at different temperatures and pH levels based on a total ammonia of 2000 mgN L^{-1} (calculations based on thermodynamic coefficients provided in Batstone* et al.*, 2002).*

Despite its negative impacts, ammonia is also important for pH control. Maintaining a pH greater than 6.5 is vitally important for anaerobic digesters. This is because the fermentation step produces acids, which decreases the pH. At a pH of below 6.5 methanogenesis slows down dramatically (Batstone *et al*., 2002), but fermentation continues at a similar rate, and the system enters an acid overload from which it is very difficult to recover (expensive alkali dosing is required). Ammonia acts as an alkaline substance that neutralizes organic acids being produced and keeps the pH at a high level. Ammonia is particularly helpful with co-digestion of a poorly buffered feed such as straw (Hashimoto, 1983), which makes digestion of deep litters (ammonia-rich manure component and carbon-rich base bedding component) through anaerobic processes more favourable.

Effect of temperature

Apart from influencing ammonia inhibition, temperature is very important to anaerobic digestion and is often a key economic factor in determining the feasibility of digestion projects. Reaction rates are dependent on temperature and can approximately double for every 10 °C increase in temperature (van Lier *et al*., 1997). In effect, this means that a 1 ML reactor at 40 °C can have the same digestion capacity as a 2-4 ML reactor at 20 °C, which is relevant for mixed digesters versus lagoons. However, heating requires energy (which is often available in excess with pigs), such that the increased capital cost for a larger low-

temperature digester/lagoon needs to be balanced against the energy requirements for heating and mixing, and also considering the heat exchanger capital costs of a high temperature system. If cogeneration is available, approximately 50% of the total energy produced as methane is available as low quality heat for heating piglets or weaners or is normally adequate for reactor heating. This assumes that that feed solids concentration is 2% or higher and, under these conditions, feed can be heated by 15 °C or more. However, in pure breeder piggeries, this may be too valuable internally, which may shift the balance in process selection towards a no-heat system such as a covered lagoon. Farrow-to-finish piggeries usually have excess energy potential available in the manure and spent litter.

Gas utilisation

Due to ever-increasing rises in the cost of energy, payback periods can be good at 5 years or less, provided the energy produced can be fully utilised. Renewable energy certificates usually cover internal maintenance and operational costs and CFI credits can pay up to 20% of capital for 1 year of operation, although the impact of carbon credits is subject to market conditions. There is still further work needed on routes to electricity generation at small and medium scale. A farm with 20,000 pigs on deep litter will produce approximately 15 dry tonnes per day of spent bedding (Kruger *et al*., 2006; Nicholas *et al*., 2006). This matter can be digested to produce 3500 $m^3.d^{-1}$ of CH_4. A similar sized intensive piggery will produce approximately 1300 $m^3.d^{-1}$ of CH_4 through a covered anaerobic lagoon (Casey *et al*., 2000) or 1900 $m^3.d^{-1}$ of CH_4 for an engineered digester, because the straw or other bedding material provided more methane in the litter scenario. An appropriately sized cogeneration engine would be 400-500 kW. Co-generation engines (internal combustion type) are relatively intolerant of fluctuations in flow, becoming less efficient below 70% capacity, and are relatively intolerant of sulphide which is a component of biogas. The classical method to address sulphide in internal combustion engines has been to clean biogas using commercial scrubbing media, which can also introduce an expensive maintenance requirement because the scrubbing media becomes spent after a certain duty. Lower cost options such as use of industrial wastes rich in iron are currently being considered through Australian research projects funded by the Pork CRC and Australian Pork Limited (APL). Access to the iron in the scrubbing media is key with porosity controlling effectiveness of a scrubbing medium. One developing option that avoids this issue is gas cogeneration microturbines, which are approximately 2-4 times the capital cost of smaller cogeneration engines, but are much more tolerant to variable flow and sulfide. Microturbines do not tolerate siloxanes, but these are not known to be contaminants in piggery biogas. Alternatively, if all heat can be used, direct-fired heating is suitable as illustrated by Skerman and Collman (2013). Further work is required to demonstrate the routes to biogas usage at Australian piggeries.

Anaerobic digestion technology

Key technologies relevant to the piggery industry are summarised in

Table **1**. These can be summarised as those suitable for liquid manures (lagoon, mixed, or engineered digester) and those suitable for solid manures and litter (engineered digester, liquid plug-flow, and solid phase). At this point, only lagoon and engineered digesters are well established for liquid manures. Australian liquid manures are generally too dilute for plug-flow digesters. There are no established options for solid phase manures (litters), though there are currently joint Department of Agriculture, Fisheries and Forestry/Pork CRC initiatives to develop litter based batch digesters for the Australian pork industry. Key issues around each of the options of lagoon, engineered digester, and batch solid phase are also summarised in the following sections

Lagoons

Piggery lagoons (Figure 5) have been widely applied in Australia without covers, and are being increasingly covered to capture methane and reduce emissions. To date, methane from manure of about 8% of the national herd is being captured and burnt (Based on the Pork CRC Bioenergy Support Program data). By far the most popular technology at present is covered lagoons, although heated engineered digesters will likely become more prominent with time because of the size benefits mentioned above. Gopalan *et al.* (2013) conducted extensive analysis of Australian piggery lagoons and found that:

- Compared with engineered digesters, longer retention times are required due to poorer mixing and lower temperatures;
- Solids pre-removal can be required with husky feeds to avoid sludge crusting under the lagoon cover;
- In-pond sludge management is critical, but can be effectively managed by good lagoon hydraulics or intermittent sludge extraction through pipework;

- The two lagoons (assessed in detail by Gopalan *et al*., 2013) achieved about 70% removal of organic solids (a portion of which was converted to methane). Approximately 10% additional methane could be expected from an engineered digester.

Table 1. *Summary of reactor configurations for piggery litter/piggery liquid manure digestion.*

Technology	Principle	Advantages	Disadvantages
Lagoon	Direct conversion at 1-2% post screening. Retention time of 40-100 days. Widely used in piggeries	Established technology. Low capital.	Difficult to control. Sludge accumulation requiring intermittent sludge extraction Not suitable for spent litter
Engineered mixed digester	Feed at 3-6%, and continuous feed in mixed tank. Retention of 20 days or more. Used across many industries	Established technology. Easy to control. Continuous gas production.	Expensive tanks. Need dilution liquid for litter piggeries. Bedding needs milling. Liquid (not solid) residue. Operator input
Liquid plug-flow (RCM)	Dilution to 15%, and feed through a liquid plug-flow reactor	Very high loading rates. Continuous gas production.	Only suitable for concentrated feeds. Need dilution liquid for litter piggeries Poor contact with active biomass. Liquid residue. Bedding probably needs milling Stratification (float and sink layers)
Batch solid phase	Fill and react in a solid phase reactor. Can be an engineered landfill (but must be properly sealed). System is loaded, enclosed, and leachate/inoculum circulated intermittently.	Can be very cost-effective. Very high loading rates. Good gas conversion due to retention of active biomass. Easy to control via leachate. No milling required.	Only suitable for litter with a bulky base bedding material that prevents bed collapse Non-continuous system (gas flow changes in quality and flow over time). Can be difficult to seal (gas seals). Needs mechanical loading and unloading
Continuous dry solid phase (plug-flow)	Continuous feed of solid through a system. Recirculation of leachate around solid phase.	Continuous gas and nutrient-rich residue production. Do not need dilution liquid Very good loading rates.	Only suitable for spent litter (not liquid manures). Extremely high capital costs, and only really practical at very large scale. Very complicated mechanical mixing system. Potential solids handling issues.

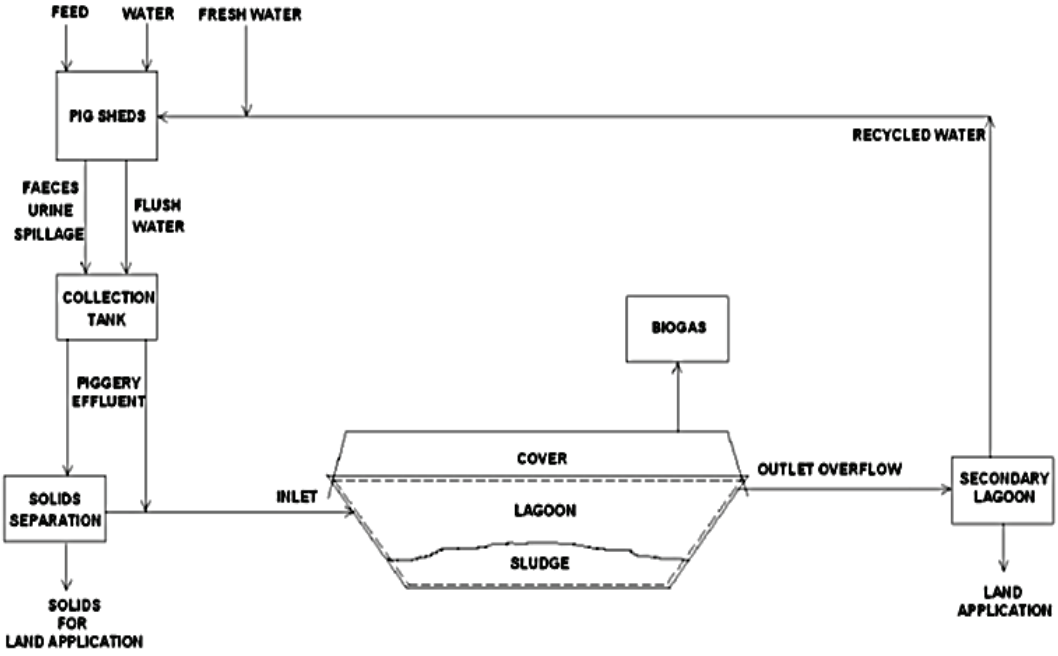


Figure 5. *Application of lagoons in the Australian piggery industry. Solids removal can be critical for lagoons fed with husky manures (from Gopalan, 2013). (Copyright preserved).*

In particular, the issue of solids was found to be critical, and assessed in detail by computational fluid dynamic modeling. Figure 6 shows a long-term model of a large lagoon (150 m × 40 m × 6m), which contained a stable sludge layer at 3% induced by floor based desludging and effective hydraulic design. Most lagoon failures can be traced to either low retention time resulting in overloading, poor hydraulic design resulting in solids accumulation or poor mixing, or otherwise poor sludge management. Emerging options include sonar to detect sludge layers and hydraulic assessment through embedded salt tracers to determine effective volume.

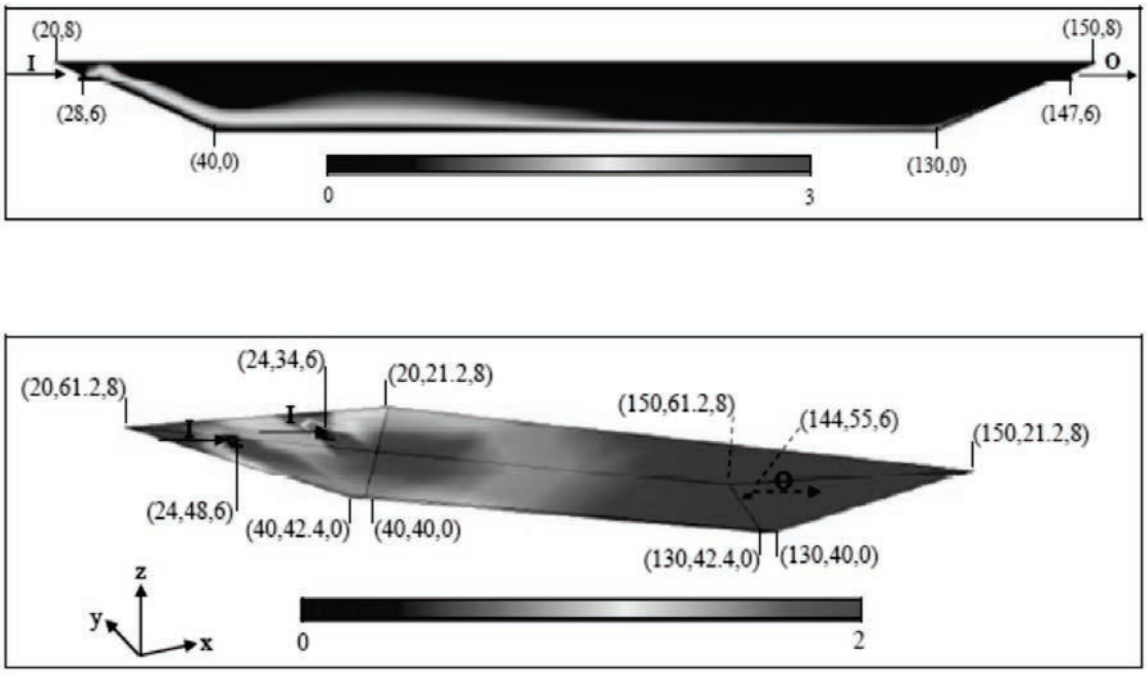


Figure 6. *CFD based analysis of sludge accumulation in a large lagoon (from Gopalan, 2013). (Copyright preserved).*

Engineered Digesters

Mixed tank reactors are the most widely used engineered anaerobic digesters in the world. They rely on continuous feed, and contact with biomass retained in the digester. The solids retention time is the same as hydraulic retention time because the solids are thoroughly mixed through the liquid bulk and thus solids retention is completely dependent on liquid bulk flow. In large-scale systems, mixing is normally by gas recirculation. In smaller systems, mixing is by impeller. Feed can be continuous, but is almost always semi-batch (periodic feed, with simultaneous gravity discharge). In this regard:

- The technology and process understanding is very well developed;
- To maintain fluid viscosity, process solids need to be maintained below 4%. Given 50% organic solids destruction, this means feed must be below 6% (Batstone and Jensen, 2011), which can require large quantities of dilution water for a predominantly solid waste such as spent litter;
- Because the process liquid is homogeneous, process control and monitoring is relatively straight forward, but does require substantially more operator input than lagoons;
- Power input for mixing is approximately 0.15 $kWh.m^{-3}d^{-1}$ (Batstone and Jensen, 2011);
- Straw would need hammer milling to <approximately 30 mm in a mixed process if feed with litter;
- Manure can be slow to degrade and a design retention time of >20 days is generally required. This makes capital costs high in comparison to lagoons (Lim, 2005).

Overall, engineered digesters are likely to be a viable future option (in comparison with lagoons), particularly if land price is at a premium or with substantial energy demand (such as with a feed-mill or abattoir) or if a sufficient market is available for energy exports (making the recovered methane more valuable). In addition, engineered digesters are likely to be more responsive to operator input and readily controlled in comparison with a lagoon system.

Batch Solid Phase

This process is only applicable to litters. The solid feed is loaded into a digester and inoculation liquid is recirculated over the biomass, with liquid also digested in a sidestream reactor. Gas is collected directly from the digester and from the side-stream reactor and the end of digestion is indicated by a decline in the gas flow (it is a batch system with a certain amount of available substrate). An example of a pilot digester used in Europe is shown in Figure 7.

Figure 7. *Example of 10 m^3 batch solids phase digester. Note the heating coils at the base of the digester, and the cage for holding biomass. This system was loaded and unloaded using small trucks on rails. (Copyright preserved).*

While this is a process currently in-development, it offers advantages and a stabilisation process for litters, which is currently not available. The high methane recovery potential may also be highly profitable where there is a need for off-grid power or heat energy, because the leach bed system has relatively low energy costs, and a continuous gas stream from the batch process. Additional points/advantages of the leach bed system are:

- A batch process gains kinetic advantages over a mixed tank process due to first order kinetics (with high concentration of degradable substrate at the start of the batch giving higher rates of first order decay);
- Gas flow and gas quality varies over the batch life of the project, which is a consideration for engine loading;
- There is no need to mill straw or bulky bedding materials, and it is actually beneficial to not do so, maintaining the structure of the straw bed (Svensson *et al*., 2006);
- Volumetric loading rates are similar to liquid plug-flow (which is high), since the optimal straw bed density is 100 kg m^{-3}. Above this, the bed blocks, and below this, the bed collapses (Svensson *et al*., 2002);
- Typical batch times are 30 days at 30°C, and 90 days at 15-20°C (Svensson *et al*., 2002);
- Maintaining a good gas seal can be problematic;
- It is easier to operate at elevated temperatures, since the system has a much lower thermal volume than mixed or plug-flow;
- The system is highly flexible. More material can be added mid-batch, a dedicated leachate reactor can be implemented (biofilm methanogenic reactor to remove organic acids), and pH or buffer capacity can be directly altered in the recirculation liquid;
- The process allows for recovery of nitrogen and phosphorous from the recirculation stream, and indeed, this is required to avoid ammonia overload;
- Logistics include loading and unloading.

Conclusions

Anaerobic digestion is a mature technology that offers the ability to reduce piggery environmental impact while generating renewable energy. There is now a range of technology options being developed to address both conventional intensive and deep litter piggeries, with future potential to recover nutrients as well as energy to fully close the nutrient cycle and take advantage of the nature of piggeries as point sinks (and sources) of agricultural resources.

References

ANGELIDAKI, I., ELLEGAARD L. and AHRING BK. (1999). *Biotechnology and Bioengineering* **63:**363-372.

BATSTONE, D.J. and JENSEN, P.D. (2011). In: "Treatise on Water Science", pp. 615-640, eds. W. Peter, R. Peter, U. Stefan, F. Fritz and H. Keisuke. (Academic Press. Oxford, U.K.).

BATSTONE, D.J., KELLER, J., ANGELIDAKI, I., KALYUZHNYI, S.V., PAVLOSTATHIS, S.G., ROZZI, A., SANDERS, W.T.M., SIEGRIST, H. and VAVILIN, V.A. (2002). "Anaerobic Digestion Model No. 1 (ADM1), IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes.". London: IWA Publishing.

CARRÈRE, H., DUMAS, C., BATTIMELLI, A., BATSTONE, D.J., DELGENÈS, J.P., STEYER, J.P. and FERRER, I. (2010). *Journal of Hazardous Materials.* **183:**1-15.

CASEY, K.D., MCGAHAN, E., ATZENI, M.A., GARDNER, E.A. and FRIZZO, R.E. (2000). PigBal: A nutrient balance model for intensive piggeries. DEEDI (Toowoomba: Queensland).

EASTMAN, J.A. and FERGUSON, J.F. (1981). *Journal of Water Pollution Control Federation.* **53:**352-366.

GAVALA, H.N., SKIADAS, I.V. and LYBERATOS, G. (1999). *Water Science and Technology.* **40:**339-346.

GOPALAN, P. (2013). PhD Thesis. The University of Queensland, St Lucia, QLD.

GOPALAN, P., JENSEN, P.D. and BATSTONE, D.J. (2013). *Biomass and Bioenergy.* **48:**121-129.

GUJER, W. and ZEHNDER, A.J.B. (1983). *Water Science and Technology.* **15:**127-167.

HASHIMOTO, A.G. (1983). *Biotechnology and Bioengineering.* **25:**185-200.

HASHIMOTO, A.G. (1986). *Biotechnology and Bioengineering.* **28:**1857-1866.

HUMPHREY, A.E. (1979). In "Hydrolysis of Cellulose: Mechanisms of Enzymatic and Acid Catalysis", pp. 25-53, eds. R.D. Brown and L. Jurasek (American Chemical Society. Washington: DC, USA).

KARAKASHEV, D., BATSTONE, D.J. and ANGELIDAKI, I. (2005). *Applied and Environmental Microbiology.* **71:**331-338.

KARAKASHEV, D., BATSTONE, D.J., TRABLY, E. and ANGELIDAKI, I. (2006). *Applied and Environmental Microbiology.* **72:**5138-5141.

KRUGER, I., TAYLOR, G., ROESE, G. and PAYNE, H.G. (2006). "Primefact 68: Deep-litter housing for pigs." New South Wales Department of Primary Industry (Sydney, Australia).

LIM. B. (2005). Final Report for Project No. 1915, "Renewal Energy Industry Development" (Australian Pork Limited: Canberra, ACT)

MOLLER, H.B., SOMMER, S.G. and AHRING, B.K. (2004). *Biomass and Bioenergy.* **26:**485-495.

NICHOLAS, P., REDDING, M., DEVEREUX, J., KELSEY, G., MCGAHAN, E., TUCKER, R. and HEINRICH, N. (2006). Final Report for Project No. 1969, "Developing Guidelines for Use of Spent Deep Litter Bedding" (Australian Pork Limited: Canberra, ACT).

PAVLOSTATHIS, S.G. and GIRALDO-GOMEZ, E. (1991). *Critical Reviews in Environmental Control.* **21:**411-490.

RAMSAY IR. (1997). "Modelling and Control of High-Rate Anaerobic Wastewater Treatment Systems, PhD Thesis" [PHD]. University of Queensland. Brisbane 270 p.

SIEGRIST, H., VOGT, D., GARCIA-HERAS, J. and GUJER, W. (2002). *Environmental Science and Technology.* **36:**1113-1123.

SKERMAN, A., and COLEMAN, G. (2013) " Methane Recovery and use at Grantham Piggery" RIRDC Publication No. 12/064., RIRDC, Canberra.

SVENSSON, L.M., BJÖRNSSON, L., BATSTONE, D.J. and MATTIASSON, B. (2002). *The effect of packing density on the startup and operation of an anaerobic single stage fixed wheat straw bed digester.* In: P. A. Wilderer PA, ADSW2002, Anaerobic Digestion of Solid Waste, Munich.

SVENSSON, L.M., BJÖRNSSON, L. and MATTIASSON, B. (2006). *Journal of Chemical Technology and Biotechnology.* **81**:1729-1735.

TAIT, S., TAMIS, J., EDGERTON, B. and BATSTONE, D.J. (2009). *Bioresource Technology.* **100:**2210-2218.

VAN LIER, J.B., REBAC, S. and LETTINGA, G. (1997). *Water Science and Technology.* **35:**199-206.

VAVILIN, V.A., RYTOV, S.V. and LOKSHINA, L.Y.A. (1996). *Bioresource Technology.* **56:**229-237.

The Pork CRC Limited Australia and Australian Pork Limited have funded work leading to this review through both individual projects and general fellowship support through the Pork CRC Limited Australia bioenergy support programme. Australian Pork Limited also partly funded Preethi Gopalan's PhD through the Methane to Markets initiative. Eugene McGahan and Alan Skerman have contributed through discussion and advice.

Impact of DNA extraction method for faecal microbiome sequencing

Y. Lu, P. Hugenholtz and D.J. Batstone

The University of Queensland, Brisbane, QLD 4072.

Faecal microbiome sequencing is being applied to assess complex interactions with the host organism, including nutrition, welfare, and infectious disease control. While it has been extensively assessed in humans, there is high potential for the faecal microbiome to have an impact on domestic animal health, nutrition, and welfare. One of the key issues, particularly with mixed microbial populations in a new host, is selection of an optimal methodology for DNA extraction. Methods that require extensive mechanical or enzymatic digestion can destroy DNA of less robust organisms, while methods with insufficient digestion can fail to extract DNA from bacteria with capsules or spores. There is also a question of the repeatability in sampling of DNA from faecal and soil samples, which can have a high degree of heterogeneity. For the purposes of whole sample sequencing, the key objective is to minimise variation in comparative sequence identification for the same sample, rather than maximising quantity of DNA. This study aims to compare three extraction techniques with different levels of mechanical and enzymatic processing for microbiome identification on faecal samples from three different pigs.

Faecal samples were collected from the pigs 2 weeks into their growing stage (10 weeks total age). Two of the pigs were raised on a wheat diet and one on a barley diet. The three pigs were chosen from a larger pool of 16 pigs based on variation in DNA band size in initial extractions with FastDNA® Spin Kit For Soil (see below). The DNA extractions were done in triplicate with a FastDNA® Spin Kit For Soil (F), which had moderate mechanical and enzymatic digestion, a PowerSoil® DNA Isolation Kit (P) (MO BIO, US), which reduced mechanical disruption, and a traditional extraction method (C) (Tang *et al*., 2008), which increased enzymatic disruption. A total of 300 ng extracted DNA from each sample was sequenced by Ti454 Pyrosequencing at the Australian Centre for Ecogenomics, University of Queensland. Results were clustered based on Bray-Curtis dissimilarity by multiple dimensional scaling.

Samples were defined as high (H), medium (M) and low (L) based on intensity of the DNA band from initial extraction on 2D gel electrophoresis. MDS analysis indicated that samples clustered mainly according to the host pig, with only L samples showing substantial variation between different extraction methods (Fig 1. A). The pyrosequencing results of triplicate samples extracted by the Power soil kit clustered well with the samples extracted by the conventional method (detailed result not shown). Variation across extraction methods is indicated by the large error bars (Fig 1. B). Triplicates produced by the Fast Spin kit had a higher degree of variation (for example *E. coli* in H and *Clostridium* spp. in M in Fig 1. B), indicating lower technical repeatability. Fast spin extraction caused Gram-negative bacteria such as *Prevotella* spp. to be under-represented (sample L in Fig 1. B), which in turn led to an over-representation of Gram-positive pig pathogens such as *Streptococcus* and *Clostridium* spp.

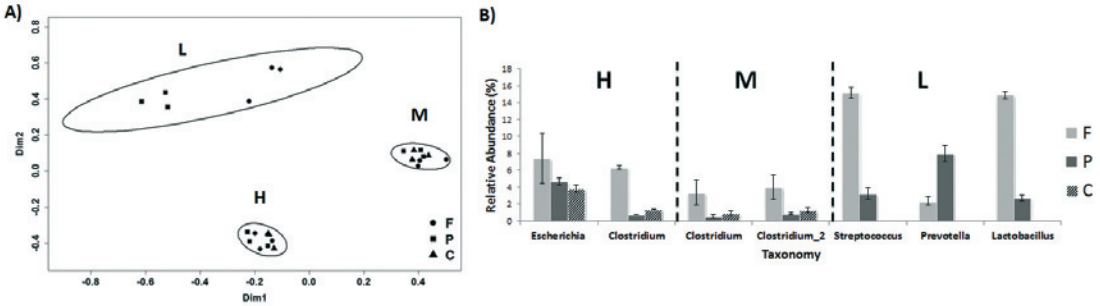


Figure 1. ***A)*** *Multidimensional scaling analysis of pyrosequencing results from all samples extracted with different methods. Replicates and different extraction methods are clustered by sample.* ***B)*** *Major taxonomy with difference between extraction methods. High (H), Medium (M) and Low (L) quality samples were extracted by F: Fast Spin kit, P: Power Soil kit and C: Conventional method.*

The conventional method is time consuming and fails to generate quality DNA for amplification of low quality samples. Based on repeatability and quality, power soil extraction is a faster and lower cost method producing a complete community profile with lower variability between replicates.

TANG, J., ZENG, Z., WANG, H., YANG, T., ZHANG, P., LI, Y., ZHANG, A., FANG, W., ZHANG, Y., YANG, X., ZHAO, S., TIAN, G. and ZOU, L. (2008). *Journal of Microbiological Methods.* **75:**432-436.

Supported in part by Pork CRC Limited Australia.

Sizing pumps for desludging of covered piggery ponds

S. Tait[1], S. Birchall[2] and M. O'Keefe[3]

[1]The University of Queensland, St Lucia, QLD 4072. [2]AgSystems Design, Shepparton, VIC 3631. [3]FSA Consulting, Horsham, VIC 3401.

This paper presents a common method to size pumps to pump sludge from covered anaerobic piggery ponds (CAP). The analysis uniquely considers the influence of solids concentration on pipe pressure losses.

Sludge was extracted from a piggery CAP at a depth of 4-4.5 m through sludge extraction pipes through the pond bank. While extracting, sludge flow velocity (V, with units of m/s) and pipe pressure loss were measured for a 430 m length of PN10 110 mm HDPE pipe (0.096 m internal diameter ID). Samples of the extracted sludge were collected for offline laboratory analysis. In the laboratory, the solids content of the samples were measured by wet and dry weights and flow characteristics were determined with a concentric cylinder rheometer operated in steady-shear mode at 15 and 25 °C (relevant temperatures for CAPs; Birchall, 2010). Prior to testing of flow characteristics, coarse solids in the samples were removed with a 500 μm standard sieve. For one sample, the solids content was artificially up-concentrated with a centrifuge. The laboratory measurements were fitted with a Herschel-Bulkley rheology model and the corresponding approaches described by Skelland (1967) were used to express all the data on a common/general basis.

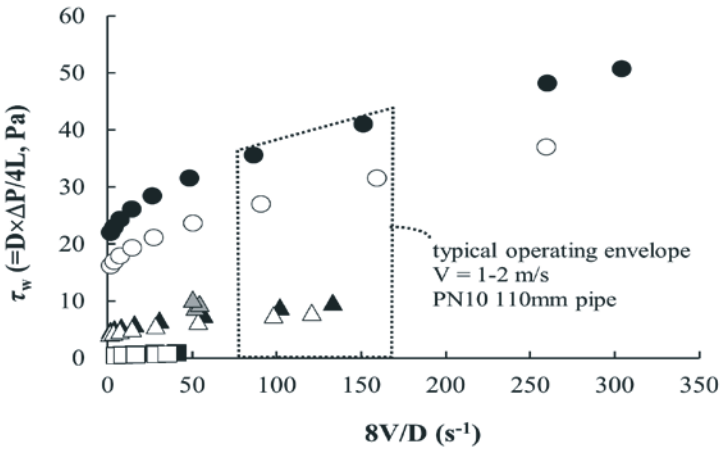


Figure 1. *Sludge pipe flow characteristics. Field (grey symbols) and laboratory (black-white symbols) data are presented for sludge with 3% (squares), 7% (triangles) and 10% (circles) solids concentrations (by wet mass) and test temperatures of 15 °C (closed symbols) and 25 °C (open symbols).*

Figure 1 shows the data on a general plot of pipe flow wall shear stress τ_w (= DΔP/4L) versus 8V/D, where ΔP is pipe pressure drop with units of Pascals, L is pipe length in metres, and D is the pipe ID also in metres. Note that the datasets are valid for ID 96 mm or smaller, and direct extrapolation of the results to other CAPs should be done with great caution. Good agreement between field and laboratory measurements at 7% solids gave confidence that the laboratory measurements appropriately represented in-field conditions for this CAP. To size a pump, a pipe flow velocity V is arbitrarily selected (say at 1-2 m/s) and the value 8V/D is calculated. The value of τ_w is then read off Figure 1 for a specific solids concentration and the pipe pressure loss ΔP estimated as τ_w multiplied by 4L/D. For example, V was 0.6 m/s for the field measurements, so 8V/D was 8×0.6/0.096 = 50 s^{-1}, so τ_w from Figure 1 was about 10 Pa and ΔP was 10×4×430/0.096 = 179,200 Pa or 1.8 bar (measured value was 1.7 bar). An appropriately sized pump would provide the required flow and overcome the pipe pressure loss ΔP. The calculation of ΔP is highly dependent on the value of τ_w which in turn is strongly influenced by solids concentration. A higher solids concentration causes a higher pipe pressure loss. For instance, in the example above, increasing solids concentration from 7% to 10% would increase τ_w from 10 Pa to 20 Pa (at 25 °C) or 30 Pa (at 15 °C), which would double or triple the pipe pressure loss. Using similar measurements/data, the practicality of pumping sludge at a particular solids concentration can be assessed and, because solids concentration usually increases with less frequent desludging, the required desludging frequency can also be assessed.

BIRCHALL, S. (2010). "Biogas Production by Covered Lagoons", (RIRDC Publication No. 10/023: Canberra).
SKELLAND, A.H.P. (1967). "Non-Newtonian Flow and Heat Transfer", (Wiley: New York).

Supported in part by Australian Pork Limited and Pork CRC Limited Australia. Sludge samples were from Bears lagoon Don KRC.

Waste production recorded in PigBal model validation trial

A.G. Skerman[1], S. Willis[1] and E.J. McGahan[2]

[1]Department of Agriculture, Fisheries and Forestry, Toowoomba, QLD 4350. [2]FSA Consulting, Toowoomba, QLD 4350.

A replicated trial was undertaken to determine the influence of diet on the waste production of grower-finisher pigs fed four diets formulated using ingredients commonly available to the Australian pig industry. This trial was primarily undertaken for the purpose of generating data to be used in validating the PigBal model (Casey *et al*., 1996), which is widely used for estimating piggery manure and greenhouse gas (GHG) production in Australia.

Sixteen male pigs (four diets x four replicates), selected from weaner pigs bred at the University of Queensland Gatton piggery, were housed in individual metabolic raised pens at the Centre for Advanced Animal Science. The manure output of the individual pigs was collected, weighed, sub-sampled and analysed during six alternate sampling weeks, over an 11-week trial period. The pigs were weighed at weekly intervals and daily feed intakes were recorded. The mean pig age and liveweight (LW) at the start of the first sampling week were 9.7 weeks and 21.5 kg, respectively.

The diets, which were formulated with a digestible energy (DE) content of 14.0 MJ /kg and an available lysine content of 0.65 g/MJ DE, were prepared by a commercial feed company in bagged, pelleted form. The main ingredients in the four diets (% mass as-fed) were: diet A: 72% wheat, 10% barley; diet B: 55% sorghum, 20% wheat; diet C: 65% wheat, 10% mung beans, 10% sorghum; diet D: 48% barley, 20% wheat and 20% mung beans. The mean average daily gain was 619 g/day (from birth), with a mean live weight of 90.0 kg at the end of the trial. The mean feed conversion ratio over the trial period was 2.09.

Table 1. *Mean pig manure characteristics over the 11-week trial period for the four diet treatments.*

Diet	TS (kg/d)	VS (kg/d)	VS/TS	FS (kg/d)	COD (kg/d)	CH_4 yield (L/kg VS)	N (g/d)	P (g/d)	K (g/d)
A	0.225 ^{b}	0.162 ^{b}	0.72 ^{c}	0.063 ^{b}	0.254 ^{b}	362 ^{b}	33.29 ^{a}	6.67 ^{a}	8.39 ^{a}
B	0.239 ^{b}	0.163 ^{b}	0.68 ^{d}	0.076 ^{a}	0.246 ^{b}	355 ^{b}	27.38 ^{b}	6.33 ^{a}	7.36 ^{b}
C	0.223 ^{b}	0.170 ^{b}	0.76 ^{b}	0.054 ^{c}	0.258 ^{b}	365 ^{b}	31.17 ^{ab}	3.73 ^{c}	8.52 ^{a}
D	0.293 ^{a}	0.232 ^{a}	0.79 ^{a}	0.061 ^{b}	0.322 ^{a}	407 ^{a}	16.40 ^{c}	4.87 ^{b}	6.61 ^{c}
SEM	0.0089	0.0080	0.008	0.0016	0.0129	5.9	1.212	0.272	0.191
Sign.	<0.001	<0.001	<0.001	<0.001	<0.010	<0.050	<0.001	<0.001	<0.001

[a,b,c]Means in a column not having the same superscript are significantly different (P< 0.05); SEM, standard error of the mean; Sign., significance; TS, total solids; VS, volatile solids; FS, fixed solids or ash; COD, chemical oxygen demand; CH_4 yield, methane yield or degradability (B_o) determined from biochemical methane potential (BMP) analyses; N, nitrogen; P, phosphorus; and K, potassium.

Table 1 shows significant differences between the manure characteristics recorded for the four trial diets. Pigs fed diet D (predominantly barley) produced higher (P<0.01) masses of manure TS, VS and COD. This outcome is consistent with barley having a lower dry matter digestibility (gross energy/digestible energy - derived from values published in the Premier Atlas, 2008) compared to the other major grains used in the trial diets. The BMP analyses also showed that diet D manure produced a higher (P<0.05) methane yield. There were also significant differences between the manure N, P and K masses for the four diets (<0.001).

The results of this trial have demonstrated the need for a scientifically validated model to accurately predict piggery manure production, based on different dietary inputs. These predictions are essential for designing piggery waste treatment systems, estimating GHG emissions, evaluating the energy potential and economic viability of anaerobic digestion systems, and planning sustainable effluent reuse systems. Accordingly, the data generated by this trial has been used to validate the PigBal model, as described in Skerman *et al*. (2013).

CASEY, K.D., McGAHAN, E.J., ATZENI, M.A., GARDNER, E.A. and FRIZZO, R.E. (1996). PigBal version 1.0 - A nutrient mass balance model for intensive piggeries. Department of Primary Industries (Queensland).

PREMIER NUTRITION PRODUCTS LTD (2008). Premier Atlas: Ingredients Matrix, Redwood Business Ltd, UK.

SKERMAN, A.G., COLLMAN, G.D., KNIGHT, R., WILLIS, S., McGAHAN, E.J. and BATSTONE, D.J. (2013). Final Report prepared for Australian Pork Limited, APL Project No. 2010/1011.334.

Supported in part by Australian Pork Limited.

Application of veterinary thermography for the detection of sow lameness

T.L. Muller[1], S.K.J. Peucker[1], R.J.E. Hewitt[1] and R.J. van Barneveld[2]

[1]CHM Alliance Pty Ltd., Loganholme QLD 4129. [2]Barneveld Nutrition Pty Ltd, Loganholme QLD 4129.

Thermography has been used as a veterinary diagnostic tool to detect differences in topographical temperature resultant of localised trauma in bone, soft-tissue or nerve damage. Its use for analysing foot health has been widely demonstrated in horses and proved efficient on detecting inflammation in the hooves of dairy cows (Nikkah *et al.*, 2005). The objective of this experiment was to investigate the usefulness of thermal imaging technology to facilitate early detection of sow lameness. The ability to successfully detect lameness in its early stages would then allow for remedial treatments that may prevent the early removal of that sow from the herd.

Mixed parity sows (n=146; Camborough^{TM} 29, PIC Australia Pty Ltd, Grong Grong, NSW) were held in fully-slatted group housing, approximately 62 sows per pen, within a conventional dry sow shed. Pens were fitted with an electronic sow feeder (ESF) and four nipple drinkers. A survey of sows was conducted by randomly selecting individuals upon entry into a gestation pen (from d 1 post-mating). Selected sows were walked a distance of 10 m to enable a gait assessment using a standardised gait score system (0-4), hoof lesion score (0-4) and claw length assessment followed by thermal imaging of all four limbs (FLIR E60, FLIR Systems, Inc., Wilsonville, OR, USA). Images were analysed using the camera's spotmeter marker tool to locate hotspots associated with significant changes in temperature.

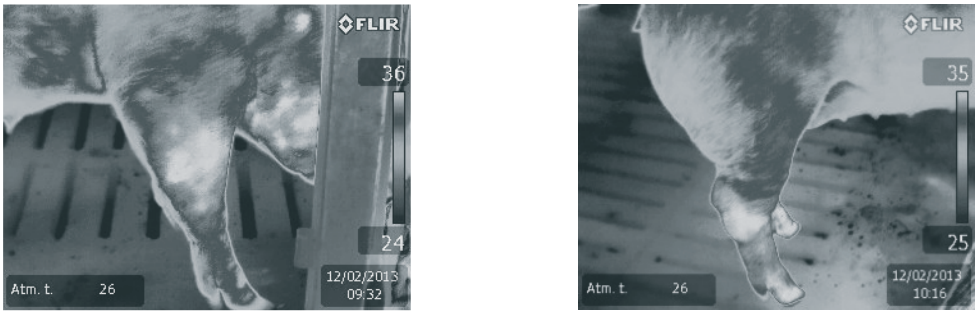


Figure 1. *Thermal image of a sow's front right limb (left) with a gait score of 1 (mild lameness) and a sow's right hind leg (right) with a gait score of 0 (no lameness). Both sows have no visual injury but show a temperature hotspot.*

Data showed that 45% of sows displayed thermal images with one or more areas of increased temperature within one or more of the limbs, illustrating an area hotspot. From these, the majority (70% of sows) were identified as having a gait score of 0 and therefore not showing signs of lameness. A series of Chi Square analyses (GenStat 15th ed., VSNI Ltd, Hemel Hempstead, UK) revealed that a compromised gait (χ^2=28.09, df=2, P<0.001), lesion presence (χ^2=15.63, df=1, P<0.001) and an increase in claw length (χ^2=7.58, df=2, P=0.023) were significantly associated with the presence of a thermal hotspot in one or more limbs. Although sample size was small, sows revealing a compromised gait on assessment commonly displayed the presence of one or more hotspots within that limb. Whilst it was evident that thermal imaging detected changes in limb temperature within the joints and muscle of sows with a compromised gait, hotspots could also be found in many sows with a normal gait. These hotspots may be related to early signs of joint inflammation or potentially, osteochondritis from mechanical overloading (Keenslide *et al.*, 2006).

Whilst this study showed a significant, yet confounding, association between the presence of a hotspot and lameness, the low number of sows in this study with abnormal gait scores (13.5%) suggests that either gait is not a sensitive measure of lameness, or more likely, the presence of inflammation resulting in a hotspot does not necessarily manifest as lameness. Further research is required to assess whether early identification of hotspots in the absence of changes in gait correlates with a higher incidence of lameness as the sow ages.

KEENSLIDE, J., GAMROTH, A., BYSTROM, J. and PERRY, A. (2006). *Advances in Pork Production.* **17**:187-192.
NIKKAH, A., PLAIZIER, J., EINARSON, M., BERRY, R., SCOTT, S. and KENNEDY, A. (2005). *Journal of Dairy Science.* **88**:2749-2753.

Supported in part by Pork CRC Limited Australia.

Lameness in culled sows related to distal limb morphology and pathology

J.F. Bonnicci and P.L. Cakebread

The University of Melbourne, Parkville Vic 3010.

Sow longevity is limited by a high rate of post-weaning culling. A major contributor to culling is lameness (Anil *et al.,* 2009). The prevalence of sow lameness can be up to 13.8% (Karlen *et al.,* 2006). Lameness in sows is due to a wide variety of causes but lower limb abnormalities may be a major contributor (Kilbride *et al., 2009*). Chronic lameness produces morphological changes to lower limb structures. Lower limb abnormalities are commonly seen, but relationships of lower limb abnormalities to detectable lameness have not been previously investigated. This study aimed to assess the relationship between distal limb characteristics and lameness of culled sows, and hypothesised that moderately lame culled sows would exhibit more lower limb pathology and bone dysplasia than non-lame/mildly-lame culled sows. The study also identified parameters that may be used to identify lameness-associated abnormalities in the lower limbs.

Fifty-seven Large White and Landrace sows, from an Australian piggery, had been selected for culling post-weaning. The sows had been kept in pens on concrete flooring. They'd had at least two parities and were an average age of 2.8 years. Gait assessment of culled sows, based on a system devised by Karlen *et al.* (2006), was performed before slaughter. Moderate lameness was defined as having an obvious limp. The distal limbs were collected at slaughter and were examined for hoof pathology, scars and bruises, and then scored using a foot lesion scoring system devised by Anil *et al.* (2008). The thickness of the bones was measured in the transverse plane using callipers and was then sectioned through the central axes of the digits and photographed. The photographs of the sectioned surfaces were used to determine the lengths and thicknesses of the bones and joints. The discrete data was compared applying two-table contingency Chi-Squared analysis and the continuous variables were compared using two-sample t-tests (Minitab®, Version 15.0; USA). Both defined significance at $P<0.05$. No correction for age was made.

Sixteen per cent (19 of the 57) of the sows were moderately lame. The rest were mildly or not lame and none were severely lame. Only the statistically significant results are presented (Table1).

Table 1. *Age and morphological characteristics of moderately lame (n=19), and mildly lame or not lame (n=38) commercial sows.*

Variables	Moderately lame (mean±SD)	Mildly lame or not lame (mean±SD)	Significance
Age (years)	3.0±0.80	2.6±0.78	0.028[z]
Hind Limb			
Number of foot ulcers	0.10±0.311	0.01±0.115	0.024[z]
Number of 2nd and 3rd phalangeal lesions	0.7±0.46	0.5±0.503	0.032[z]
Lateral metatarsal proximal end thickness	9.6±3.83	3.4±8.02	0.043[y]
Lateral metatarsal diaphysis thickness (cm)	9.5±2.95	3.4±8.19	0.032[y]
Lateral metatarsal distal end thickness (cm)	10.4±3.30	4.1±8.53	0.012[y]
Lateral 1st phalanx diaphysis thickness (cm)	12.3±3.41	3.2±10.70	0.022[y]
Medial 2nd phalanx diaphysis thickness (cm)	10.0±2.62	4.0±11.80	0.005[y]
Front Limb			
Lateral metacarpal distal end thickness (cm)	10.2±4.22	8.4±4.2	0.037[y]
Medial metacarpal proximal end thickness (cm)	12.7±4.16	10.7±4.06	0.024[y]
Medial metacarpal diaphysis thickness (cm)	12.8±4.12	11.2±3.57	0.04[y]
Lateral 2nd phalanx diaphysis thickness (cm)	11.5±2.98	12.9±3.58	0.03[y]

[z] Chi-Squared Test; [y] t-test.

These results indicate there are anatomical and pathological changes in the lower limbs that relate to moderate lameness. They are generally consistent with changing weight bearing from the medial to the lateral aspects of the lower legs due to chronic lameness.

ANIL, S. S., ANIL, L. and DEEN, J. (2009). *Journal of the American Veterinary Medical Association.* **235:**734-738.
ANIL, S.S., ANIL, L., DEEN, J., BAIDOO, S.K. and WALKER, R.D. (2008). *Journal of Swine Health and Production.* **15:**78-83.
KARLEN, G., HEMSWORTH, P.H. GONYOU, H.W., FABREGA, E., STROM, A.D. and SMITS, R.J. (2006) *Applied Animal Behaviour Science*. **105:**87-101.
KILBRIDE, A. L., GILLMAN, C. E. and GREEN, L.E. (2009). *Animal Welfare.* **18:**215-224.

Incidence of foot lesions in sows subjected to foot trimming interventions as gilts

R.J.E. Hewitt[1], S.K.J. Peucker[1], M.E. Wilson[2] and R.J. van Barneveld[3]

[1]CHM Alliance Pty Ltd, Millmerran, QLD 4350. [2]Zinpro Corporation, Eden Prairie, MN, USA 55344. [3]Barneveld Nutrition Pty Ltd, Loganholme, QLD 4129.

Lameness is the single biggest contributor to premature culling of sows (Hughes and Smits, 2002), with early culling adding significant costs to pig producers. Lameness and sow foot health can be influenced by various factors including gilt rearing practices, the environment of the pen and flooring, sow nutrition and how she is managed in both gestation and lactation (Kroneman *et al.*, 1993). Thus it is difficult to identify individual factors that contribute to poor sow foot health without any interaction with other causal agents.

Sow foot health can be characterised by the incidence of foot lesions and associated lameness. Foot lesions observed in sows vary in severity and can include heel over-growth and erosions (HOE), separation along the white line (WL), cracks in the sole (HSC) or walls (CWH, CWV) of the feet or irregularities in toes (T) or dew claws (DC; Gjein and Larssen, 1995). Whilst physical damage to the feet can result in lameness almost immediately, many of the foot lesions seen develop over the life of the sow (Gjein and Larssen, 1995). The ability to remediate such lesions at a relatively young age in the sow's productive life may arrest their development. It is hypothesised that the physical inspection and trimming of gilts early in their first gestation will affect the development of foot lesions in subsequent parities.

Gilts (n=500, parent gilt, PIC Australia Pty Ltd, Grong Grong, NSW), group-housed (n=40) in fully-slatted gestation pens, were walked, during their sixth week of gestation, to an inspection pen fitted with a purpose built lifting chute. Gilts were loaded into the chute and raised to allow the inspection of the feet. Lesions on all four feet were assessed; the Zinpro® Feet First™ Lesion Identification Guide (Zinpro Corp., Eden Prairie, MN USA) was used to classify the lesion to type. Gilts were then allocated to a trimming or untrimmed (control) group using a completely randomised design. In the trimmed group, all four feet were trimmed to restore normal claw conformation and weight distribution. This consisted of trimming the toes, straightening the claw wall, balancing the sole and heel and trimming of dew claws, if required. During the sixth week of their third gestation, after udder involution but before expansion of the gravid uterus, a similar inspection and trimming occurred. Differences in the pattern of lesion prevalence, at each parity, between treatments were analysed by Chi-Squared analysis (GenStat 15th ed., Hemel Hempstead, UK).

Table 1. *The prevalence (%) of foot lesions in parity 1 (P1) and parity 3 (P3) sows which were trimmed (Trim) or left untrimmed (Control) after initial assessment.*

			HOE	HSC	WL	CWH	CWV	T	DC	χ^2	Significance
Front feet	P1	Control	31.9	22.6	32.5	0.6	9.3	2.9	0.0	3.77	0.708
		Trim	42.2	21.4	24.6	0.6	9.1	1.3	0.6		
	P3	Control	76.9	4.9	16.4	0.0	2.0	0.0	0.0	13.26	0.039
		Trim	56.6	7.7	22.2	4.4	8.8	0.0	0.0		
Rear feet	P1	Control	70.4	11.6	10.5	1.8	5.3	0.6	0.0	1.89	0.929
		Trim	75.9	10.3	8.4	0.6	3.2	1.3	0.0		
	P3	Control	72.1	2.9	16.3	2.0	4.8	1.0	1.0	4.70	0.583
		Trim	65.6	6.6	20.0	1.1	6.7	0.0	0.0		

HOE, heel over-growth and erosion; HSC, heel-sole crack; WL, white line; CWH, cracked wall horizontal; CWV, cracked wall vertical; T, toes; DC, dew claws.

Trimming in P1 modified ($P<0.05$) the prevalence of foot lesions in the front feet of P3 sows (Table 1), and no effect was seen in the rear feet. At initial inspection lesions did not differ between treatments, with the predominant lesion being HOE in the rear feet and HOE, HSC and WL on front feet. The observed reduction in the prevalence of HOE in front feet is important as HOE has been idenified as a major factor leading to lameness, and to the development of HSC, CWH, CWV and WL (Ossent, 2010). There was no significant difference between treatments in survival to third parity ($\chi^2=2.55$, df=1, $P=0.110$). This intervention has shown that trimming can change the incidence of foot lesions, however, at least in this herd, there was no impact on survival of sows to the third parity.

GJEIN, H. and LARSSEN, R.B. (1995). *Acta Veterinaria Scandinavica.* **36:** 433-442.
HUGHES, P.E. and SMITS, R. (2002). Project No 1611. Report prepared for the Pig Research and Development Corporation.
KRONEMAN, A., VELLENGA, L., VAN DER WILT, F.J. and VERMEER, H.M. (1993). *Veterinary Quarterly.* **15:**26-29.
OSSENT, P. (2010). "An Introduction to Sow Lameness, Claw Lesions and Pathogenesis Theories." (Zinpro Corp.,Eden Prairie, MN).

Supported in part by Pork CRC Limited Australia.

Feet trimming interventions in gilts and reproductive performance over the subsequent three parities

R.J.E. Hewitt[1], S.K.J. Peucker[1], M.E. Wilson[2] and R.J. van Barneveld[3]

[1]CHM Alliance Pty Ltd, Millmerran, QLD 4350; [2]Zinpro Corporation, Eden Prairie, MN, USA 55344; [3]Barneveld Nutrition Pty Ltd, Loganholme, QLD 4129.

Lameness is normally associated with early culling and the loss of productivity associated with the removal of the animal, however several studies have shown reduced rates of reproduction and production when comparing lame and healthy sows. Grandjot (2007) reported both fewer litters (<3.0 versus 4.5 litters per sow) for lame sows, and higher pre-weaning mortality (PWM) in lame sows (27 % PWM versus 12.4 %), whilst another cohort study (Anil *et al.*, 2009) showed that lame sows had a lower number of piglets born alive, which were of lower weight than progeny from their healthy counterparts. It is thought that the basis for this poorer performance is multifactorial, but often sows with lameness have decreased feed intake and that the inflammation associated with lameness shifts nutrient availability, with the nutrient flow to reproduction being reduced (Wilson *et al.*, 2009).

Whilst lameness can be for multiple reasons, claw lesions have been shown to be evident in 97% of lame sows (Ossent, 2010). The majority of lesions arise from trauma, inflammation or other mechanical factors. Treatments for trauma and inflammation are readily available but remedies for mechanical factors are more involved. Lesions such as heel overgrowth and erosion (HOE) are associated with overloading issues. This leads to increased keratinisation causing increases in the overloading issue and a vicious circle ensues. We have shown (Hewitt *et al.*, 2013) that trimming can reduce the incidence of significant lesions like HOE that often lead to lameness. It is hypothesised that the reduction in prevalence of severe foot lesions through remedial trimming will lead to a reduction in reproductive losses.

Allocation of gilts (n=500) to treatment, feet assessment and lesion scoring and feet trimming were previously described (Hewitt *et al.*, 2013). Lifetime reproductive data were extracted from the herd recording system. Data were analysed using a GLM ANOVA (continuous data) or Chi-Squared analysis (categorical data, GenStat 15th ed., Hemel Hempstead, UK).

Table 1. *Relationship between foot trimming interventions, trimmed (Trim) or left untreated (Control), applied in gilts during early gestation and subsequent reproductive performance to three parities.*

	Parity 1			Parity 2				Parity 3			
	TB	Wean	WOI	TB	Wean	WOI	FR	TB	Wean	WOI	FR
Control	12.5	10.6	8.8	11.5	9.8	6.1	84.9	12.9	9.7	6.7	89.0
Trim	12.3	10.5	6.7	11.3	9.9	5.4	84.4	13.1	9.7	5.9	84.6
SED	0.40	0.17	1.24	0.50	0.19	0.65		0.51	0.21	0.94	
Sign.	0.651	0.713	0.091	0.739	0.839	0.308	0.934	0.611	0.949	0.343	0.441

TB, total born per litter; Wean, number of pigs weaned per litter; WOI, wean-to-oestrus interval; FR, farrowing rate; SED, standard error of difference; Sign., significance.

Trimmed gilts tended (P<0.10) to have a shorter wean-to-oestrus interval (WOI) than the control group after first weaning (Table 1). If we accept a 90% confidence interval for a commercial herd, these results are commercially significant. This difference in WOI between trimmed and control sows did not continue into subsequent parities (P>0.05). There was no difference (P>0.05) in total pigs born per litter, number weaned per litter or farrowing rates between control and trimmed sows. The reduction in WOI observed in trimmed first-parity sows may be due to possible increased lactation intake, however direct measurement of intakes would be required to test this hypothesis. While WOI may be influenced by trimming, the additional cost associated with remedial trimming is unlikely to pay dividends from a reproductive perspective alone, although any benefits to the sow from a welfare perspective need to be quantified.

ANIL, S.S., DEEN, J., ANIL, L., BAIDOO, S.K., WILSON, M.E. and WARD, T.L. (2009). In "Manipulating Pig Production XII", p. 108, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

GRANDJOT, G. (2007). *SUS-Schweinezucht und Schweinesmast.* **5:**28-31.

HEWITT, R.J.E., PEUCKER, S.K.J., WILSON, M.E. and VAN BARNEVELD, R.J. (2013), In "Manipulating Pig Production XIV", p. 277, eds. J.R. Pluske and J.M. Pluske. (Australasian Pig Science Association: Werribee).

OSSENT, P. (2010). "An Introduction to Sow Lameness, Claw Lesions and Pathogenesis Theories." (Zinpro Corp., Eden Prairie, MN).

WILSON, M.E., WARD, T.L. and RAPP, C. (2009). *Proceedings of the Allen D. Leman Swine Conference*, Saint Paul, MN, USA, pp.132-125.

Supported in part by Pork CRC Limited Australia.

Late gestation feeding: Effects on gilt performance

W.H.E.J. van Wettere[1], T. E. Kennett[2], D.S. Lines[2], P. Herde[3] and P.E. Hughes[3]

[1]The University of Adelaide, SA 5005. [2]Australian Pork Farms Group, SA 5152. [3]South Australian Research and Development Institute, Roseworthy, SA 5371.

Altering sow nutrient intake during late gestation affects maternal body composition and metabolic status at farrowing, and can alter lactation voluntary feed intake and milk production (Campos *et al*., 2012). Changes in all these factors can influence mobilisation of body reserves during lactation, and thus subsequent reproductive function. The current study tested two hypotheses: one, that low feed intakes in late gestation will increase body tissue loss during lactation and impair subsequent reproduction of gilts; and, two, that the negative effect of low level feeding in late gestation will be more severe for gilts with a large gestated litter size, and therefore higher fetal nutrient demand.

A total of 337 first gestation sows (gilts) were used in this study. The experimental design was a 2 x 2 factorial, incorporating two litter size groups (LS; Small, < 12 versus Large, ≥ 12) and two feeding levels from d 85 of gestation to farrowing (FL; High, 2.8 kg/d versus Low, 2.2 kg/d). Litter size groups were determined, retrospectively, based on the median value (12) for the herd's total litter size. The diet contained 14.0 MJ digestible energy (DE)/kg and 0.70% available lysine and was delivered via electronic sow feeders. Gilts were housed in straw-filled ecoshelters in groups of approximately 100. Gilt live weight (LW) and P2 backfat (P2) were measured on a subset of animals at d 85 of gestation, d 1 of lactation and weaning [d 25.4±0.28, (mean±SEM); n = 215 sows]. Subsequent reproductive performance was recorded for all gilts, namely weaning-to-oestrus interval (WOI), farrowing rate and the total number of piglets born (TB) at the second farrowing. Proportions were analysed using a χ^2 test, with all other data analysed using an ANOVA, unbalanced design (Genstat, 10th Edition, Harpenden, UK).

Gilt LW and P2 on d 85 of gestation were 182±0.90 kg and 13.5±0.11 mm. On d 1 of lactation, High fed sows had more P2 than Low fed sows (13.8±0.21 versus 13.2±0.21 mm; P=0.07), but similar LW (190.4±1.23 kg). The High feeding level increased (P=0.04) P2 gain during late gestation and P2 loss during lactation, but did not affect LW change (Table 1). Gilts gestating a large litter lost P2 and gained less LW during gestation, but also weaned more piglets. The WOI was longer (P<0.01) and TB at the second parity tended to be lower (Table 1; P=0.09) for gilts previously gestating a Large compared to a Small litter (7.6±0.68 versus 4.9±0.71 d and 10.2±0.40 versus 11.2±0.45, respectively). Farrowing rates were 0.70 and 0.68 for High and Low fed gilts (P>0.10) and 0.73 versus 0.64 for gilts gestating Large versus Small litters (P>0.05).

Table 1. *Effect of litter size and late gestation feeding level on the subsequent performance of gilts.*

Litter size (LS)	Small (< 12)		Large (≥12)		Pooled SEM[A]	Significance[B]		
Feeding level (FL)	Low	High	Low	High		LS	FL	LSF
Number of gilts	82	87	85	83				
Total piglets born	8.7	9.2	13.2	13.4	0.15	<0.01	0.18	0.37
Number of piglets weaned	7.4	7.2	9.1	9.0	0.19	<0.01	0.85	0.98
P2 change d85 to farrow (kg)	-0.1	0.6	-0.7	-0.1	0.16	0.04	0.04	0.78
LW change d85 to farrow (kg)	11.2	11.0	4.6	8.1	0.99	0.02	0.35	0.34
P2 change, d1 to wean (mm)	-1.0	-1.8	-0.9	-1.5	0.18	0.57	0.04	0.78
LW change, 1 to wean (kg)	-11.5	-13.1	-11.1	-15.2	1.03	0.68	0.17	0.54
WOI (d)	4.5	5.4	8.5	6.8	0.50	<0.01	0.61	0.18
Total born, 2nd litter	11.3	11.1	10.3	10.0	0.25	0.09	0.72	0.81

[A]SEM for litter size x gestation feeding level; [B]Litter size (LS) and Gestation feeding level (FL).

Overall, the current data indicated that feeding gilts either 2.2 or 2.8 kg of feed during the last 4 weeks of gestation had little effect on sow performance. However, gilts gestating Large litters gained less LW and lost more P2 backfat during the last four weeks of gestation, and also took longer to return to reproductive function post-weaning and tended to farrow fewer piglets at their second litter. Although gilts farrowing Large as opposed to Small litters weaned more piglets, lactation P2 and LW loss were not different. It is, therefore, suggested that mobilisation of P2 during late gestation could have contributed to the impaired reproductive function of gilts gestating a large litter.

CAMPOS, P.H.R.F., SILVA, B.A.N., DONZELE, J.L., OLIVIERA, R.F.M. and KNOL, E.F. (2012). *Animal.* **6:**797-806

This project was funded by Pork CRC Limited Australia.

Late gestation feeding: Effects on sow performance

W.H.E.J. van Wettere[1], T. E. Kennett[2], D.S. Lines[2], P. Herde[3] and P.E. Hughes[3]

[1]The University of Adelaide, SA 5005. [2]Australian Pork Farms Group, SA 5152. [3]South Australian Research and Development Institute, Roseworthy, SA 5371.

Sow body composition and metabolic status at farrowing can affect lactation voluntary feed intake and milk production during lactation (Campos *et al*., 2012), which determine mobilisation of body reserves during lactation and subsequent reproductive function. The current study tested two hypotheses: one, that low feed intakes in late gestation will increase body tissue loss during lactation and impair subsequent reproduction of multiparous (MP) sows; two, that the negative effect of low level feeding in late gestation will be more severe for sows with a large gestated litter size, and therefore higher fetal nutrient demand.

A total of 296 parity 2.8±0.06 sows (mean±SEM; range 1-4) sows were used in this study. The experimental design was a 2 x 2 factorial incorporating two litter size groups (LS; small, < 12 versus Large, ≥ 12) and two feeding levels from d 85 of gestation to farrowing (FL; High; 2.9 kg/d versus Low; 2.3 kg/d). Litter size groups were determined, retrospectively, based on the median value for total litter size (12) for the herd. The diet consisted of 13.1 MJ digestible energy (DE)/kg and 0.5% available lysine and was delivered via electronic sow feeders. Sows were housed in straw-filled ecoshelters in groups of approximately 100. Sow liveweight (LW) and P2 backfat (P2) were measured on d 85 of gestation, d 1 of lactation and weaning (d 22.1±0.10). Subsequent reproductive performance was recorded, namely weaning-to-oestrus interval (WOI), farrowing rate and the total number of piglets born (TB). Proportions were analysed using a χ^2 test, with all other data analysed using an ANOVA, unbalanced design (Genstat, 10th Edition, Harpenden, UK).

Sows gestating Large versus Small litters were heavier (255±2.0 versus 243±2.2 kg; P<0.01) but had similar P2 (14.3±0.26 and 14.2±0.29 mm; P=0.81) on d 85. High feeding increased LW gain during late gestation, but did not affect any other measure (Table 1). Treatment did not affect weaning LW (245±2.2 kg) or P2 (13.7±0.26 mm). Litter size (Large vs Small) decreased LW gain during late gestation and increased the number of piglets weaned. Sows gestating large litters also gave birth to more piglets at their subsequent farrowing (13.0±0.30 versus 11.1±0.32; P<0.05). There were significant interactions between LS and GF such that Low fed sows gestating small litters had a longer WOI than their High fed counterparts. Subsequent farrowing rates were lower for Low versus High fed sows which gestated Large litters (Table 1).

Table 1. *Effect of litter size and late gestation feeding level on the subsequent performance of sows.*

Litter size (LS)	Small (< 12)		Large (≥12)		Pooled SEM[A]	Significance[B]		
Feeding level (GF)	Low	High	Low	High		LS	FL	LSxFLF
Number of sows	69	86	65	76				
Total number of piglets born	8.9	8.6	14.6	14.2	0.20	<0.01	0.23	0.66
Number of piglets weaned	7.9	8.2	9.6	9.5	0.15	<0.01	0.90	0.55
P2 change d85 to farrow (kg)	-0.9	-0.2	-0.6	-0.7	0.23	0.77	0.62	0.36
LW change d85 to farrow (kg)	14.3	20.5	8.3	8.9	0.92	<0.01	<0.05	0.11
P2 change, d1 to wean (mm)	0.3	-0.4	0.2	0.5	0.23	0.51	0.78	0.31
LW change, d1 to wean (kg)	-14.9	-18.1	-18.4	-18.2	1.00	0.57	0.36	0.41
WOI (d)	5.8	4.1	4.5	4.8	0.24	0.53	0.22	<0.05
Adj. Farrowing rate ($\%_1$)	86%	83%	72%	82%		>0.05	>0.05	<0.05
Total born, 2nd litter	11.0	11.2	12.9	13.1	0.23	<0.01	0.49	0.93

[A] SEM for litter size x gestation feeding level; [B]Litter size (LS) and Gestation feeding (GF)

Sows which farrow large litters continue to do so, regardless of feeding level in late gestation, suggesting inherent fertility is a major determinant of reproductive output. However, the extended WOI of Low fed sows gestating Small litters indicates that reproductive function of inherently less fertile animals may be more sensitive to reduced nutrient intake during gestation. Equally, the decreased farrowing rates of Low fed sows gestating Large litters suggests that imbalances between metabolic demand and nutrient intake during late gestation may affect farrowing rate. Our data suggested that some reproductive parameters are impaired by lower feeding levels in late gestation, depending on gestated litter size.

CAMPOS, P.H.R.F., SILVA, B.A.N., DONZELE, J.L., OLIVIERA, R.F.M. and KNOL, E.F. (2012). *Animal.* **6:**797-806.

This project was funded by Pork CRC Limited Australia.

Feeding level during early pregnancy in sows: Effects on litter size and farrowing rate

G. Sørensen

Pig Research Centre, 1609 Copenhagen, Denmark.

High feeding levels during the first four weeks after mating affects the reproductive performance of sows (Quesnel *et al.*, 2010; Athorn 2011; Hoving 2012), although differences in study outcomes have been observed leading to the question: what is the right recommendation? In Denmark, future legislation from 2015 states that sows should be kept in new built, loose housing systems in the period from weaning to farrowing. This means that aggressive behaviour between sows in the critical four weeks after mating is possible and may not be beneficial for reproductive results. One way to reduce aggressive behaviour is to increase the daily feed intake. In this study, the hypothesis tested was that feeding sows with a high energy intake during the first four weeks of gestation would reduce farrowing rates but not affect litter size.

Only multiparous DanAvl sows in normal heat 3-7 d after weaning were included, and they were mated twice with AI. The study took place in two production herds where sows were housed individually in crates and received a set ratio of feed twice daily (07:30 and 15:30 h) for the first four weeks after mating. After four weeks all sows were moved to loose housing and fed individually by electronic sow feeding to achieve the same body condition score at farrowing. Just after mating the sows were allocated to one of three energy levels blocked by parity: Low [28 MJ digestible energy (DE)/d], Medium (42 MJ DE/d) and High (56 MJ DE/d). The same diet was used for all sows (13 MJ DE/kg and 12.2% protein). Sows were weighed and scanned for backfat depth at P2 site at mating and four weeks after mating. At farrowing the number of total born piglets per litter was recorded and the farrowing rate was calculated. Litter size was analysed in a linear model by ANOVA under the GLM procedure, while farrowing rate was analysed by logistic regression in the MIXED procedure (SAS®; USA). The covariates were parity, bodyweight (BW) and P2 backfat depth at mating.

Table 1. *Effect of extra energy during the first four weeks of gestation on litter size and farrowing rate.*

Items	Dietary treatment (DE/d)			Significance
	Low (mean±SD)	Medium (mean±SD)	High (mean±SD)	
Number	917	893	950	
Average parity	3.5	3.4	3.6	
BW at mating (kg)	233 ± 23	235± 25	230± 22	
BW gain during the first 4 weeks (kg)	-12± 8	1± 6	7± 5	0.030
Backfat P2 at mating (mm)	13.1± 2	13.2± 2	13.1± 2	
Backfat P2 gain during the first 4 weeks (mm)	0.2 ± 0.4	0.7± 0.4	1.5± 0.3	0.030
Total born piglets per litter	17.5± 0.3	17.3± 0.3	17.5± 0.3	0.800
Farrowing rate (%)	85	87	88	0.100

As expected, the extra energy during the first four weeks resulted in an increased weight and back fat gain in the sows. Despite this, there were no significant differences (P>0.05) in litter size or farrowing rate between the groups. This is in agreement with results by Athorn *et al.* (2011) who also found no differences, but are in contrast to findings by Hoving (2012) where 3.25 kg of feed per day for the first 4 weeks after service gave significantly more total born piglets in the subsequent litter compared to sows receiving 2.5 kg of feed per day. Hoving (2012) only included second parity sows and it can be speculated that they are perhaps more sensitive to high energy levels than older sows. In conclusion, in this study, a high feeding level was not detrimental to litter size or farrowing rate.

ATHORN, R Z., STOTT, P., SMITS, R.J. and LANGENDIJK, P. (2011). In "Manipulating Pig Production XIII", p.81, ed. R.J. van Barneveld. (Australasian Pig Science Association: Werribee).

HOVING, L. (2012). The second parity sow, PhD Thesis. Wageningen University, The Netherlands.

QUESNEL, H., BOULET, S., SERRIERE, S., VENTURI, E. and MARTINAT-BOTTE, F. (2010). *Animal Reproduction Science.* **120:**120-124.

This project was supported by EU and Ministry of Food, Agriculture and Fisheries of Denmark Grant 32101-U-12-00197.

CHAPTER 12

Index of Authors and Notes

Index of Authors

Index of Authors

Index of Authors

Index of Authors

Notes

14th
APSA
2013
conference
melbourne australia

www.apsa.asn.au

ISBN: 978-0-9806880-3-0