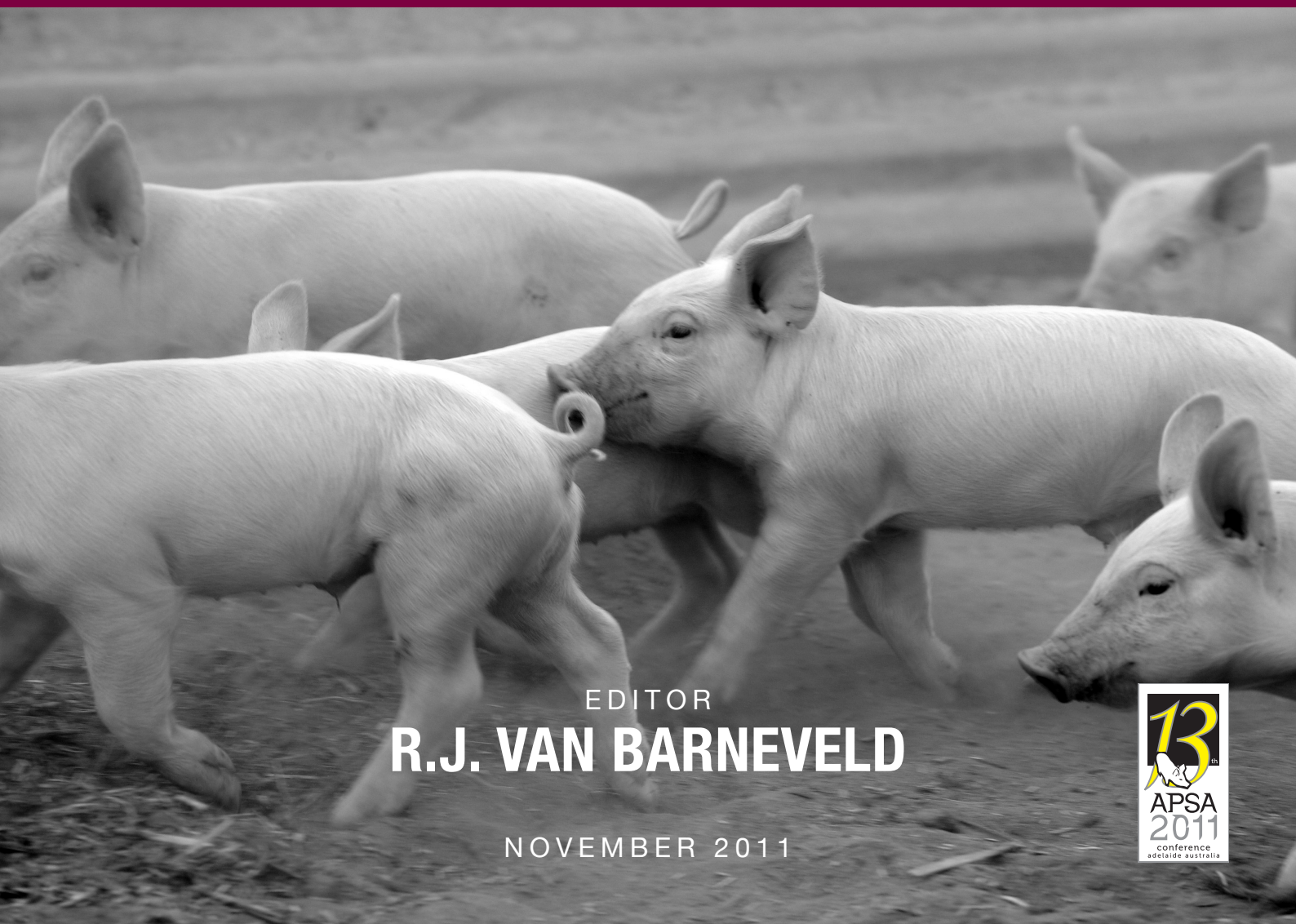
AUSTRALASIAN PIG SCIENCE ASSOCIATION

MANIPULATING PIG PRODUCTION XIII



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R.J. VAN BARNEVELD

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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 was a true pioneer in the field of pig nutrition. His initial research was with poultry, and he actually resisted his superiors when directed to work with pigs, but as with most things in his life he was soon applying his logical thought processes towards his new target species. His initial research at the NSW Agriculture Wollongbar Research Station with Dr John Holder investigated variation in meat meals, a valuable product for use in pig diets that suffered from a lack of quality control at the point of manufacture. While Ted identified variation in bone content as a major driver of variability in meat meals, he also realized that heat application to proteins could be affecting their quality and value in pig diets and that the total amino acid content did not necessarily reflect the quantity available to the pig. In the mid-1970's Ted commenced a PhD at the University of Melbourne under the supervision of Tony Dunkin with the aim of developing an in vivo assay using rats and pigs capable of measuring available lysine in a meat meal and other protein sources and cereals. On completion of his PhD, Ted returned to Wollongbar and undertook world-renowned research into the availability of amino acids in feedstuffs for pigs, and extended this to some of the first with the use of supplementary amino acids in pig diets – a practice that is commonplace in modern pig nutrition.

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. The award is conferred with the aim 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 include:

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)

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 include:

Dr Ray King	(2007)
Dr David Hennessy	(2007)
Dr Michael Taverner	(2009)

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.

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Acknowledgements

The biennial conference of the Australasian Pig Science Association (APSA) continues to go from strength to strength and is now widely regarded as one of the main international conferences for pig science. From its humble beginnings in Albury, New South Wales in 1987, APSA now represents one of the must attend pig science conferences within the Asia-Pacific region, attracting delegates from all the major pig producing countries globally. This has come about as a consequence of the hard work and dedication of many people over more than twenty years.

As with previous conferences, considerable time and effort by a willing group of scientists and support people has ensured that the 2011 conference retains the same high standards now expected of APSA. The continued support of members and others associated with pig science and production through submission of papers to these proceedings is acknowledged. Of course no conference is a success without a good number of delegates and the APSA Committee thank all those who have attended the 2011 conference, especially those from overseas.

Given the number of international delegates now attending APSA and the global nature of pork production, the organising committee aims to invite a number of international speakers to each conference, and the contributions from Cornelius De Lange and Mick Hazzledine are greatly appreciated. In addition, I acknowledge the following people from Australia who also contributed to the success of the symposia and reviews: David Emery, Eugeni Roura, John Black, Trish Holyoake, Darren Trott, Tony Edwards, Rebecca Morrison, Heather Channon and Robyn Warner. The A.C. Dunkin Memorial Lecture is an important part of any APSA conference, and the Committee thanks John Keniry for accepting the honour of presenting the 2011 Lecture. APSA also thanks this year's chairpersons and judges for their important contribution to the success of the Conference.

There are very few conferences now held where the proceedings are produced prior to the conference and to such a high editorial and scientific standard. The contribution and dedication of the Editor, Robert van Barneveld, in ensuring this happens is acknowledged. The team from Barneveld Nutrition Pty Ltd including Kylie Franzmann are also thanked for their on-going support and contribution to this process. I would also like to point out that it is becoming increasingly difficult to find people who are prepared to act as referees, and the contributions of these people (named elsewhere in the proceedings) are gratefully acknowledged.

The XIIIth Biennial Conference would not have been possible without the generous support of our many sponsors. APSA has always had a strong relationship with Australian Pork Limited, and this has now also extended to the Pork CRC Ltd. It is most pleasing to see Australian Pork Limited and the Pork CRC Ltd combine to be the joint Principal sponsors for the event. All of the Sponsors are listed on the Sponsors page, and their contribution to the success of the 2011 APSA conference is gratefully acknowledged.

Finally, the organising committee has worked hard for the last two years to organise this conference. Accordingly my thanks go to Dave Cadogan (Vice President), Neil Gannon (Immediate Past President), Karen Moore (Secretary), Megan Trezona (Treasurer), Heather Channon, Cherie Collins, Patricia Holyoake and John Pluske. The 2011 conference was organised in conjunction with YRD who have acted as the secretariat for the last three conferences, and it has been a pleasure to work with Kate Murphy, Mary Sparksman, Jill Simmons and Angeline Deo. In recognition of the contribution that the past APSA Committee's have made to the current success of APSA, the membership of past Committees is now documented in the Proceedings.

Dr Darryl D'Souza, President

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Acknowledgements to Referees

The proceedings of the thirteenth biennial conference of the Australasian Pig Science Association, 'Manipulating Pig Production XIII', contain 107 one-page papers, 4 reviews, 2 symposia and a summary of the Dunkin Memorial Lecture. As is the policy of the Association, all one-page papers, reviews and symposia were reviewed by external referees. The APSA committee and Editor gratefully acknowledge the assistance generously given during 2011 by the following referees:

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Preface

Since it's commencement in 2005, the Cooperative Research Centre (CRC) for an Internationally Competitive Pork Industry and now the newly established CRC for High Integrity Australian Pork have rejuvenated the pig research and development landscape, and this success is being celebrated through both the scientific program and venue for the thirteenth biennial conference of the Australasian Pig Science Association (APSA). From its base at the University of Adelaide Roseworthy Campus, the Pork CRC has better positioned the Australian pork industry to compete on a global basis and will now work to ensure Australian pork is one of the most ethically produced, healthy, low-environmental impact meats available. The A.C. Dunkin Memorial lecture at this year's conference provides participants with an overview of a very successful R&D investment model that has certainly had a beneficial impact on the Australian pork industry.

The global pork industry has and continues to be subjected to many factors that contribute to the volatility surrounding pork supply and demand. These factors include; the global financial crisis, animal welfare, feed costs, pig health, environmental management, product quality and consumer preference. Animal welfare and climate change are rapidly becoming global issues and the resultant government policies are sure to have significant impacts on pork production, including Australia, which has recently voluntarily banned the use of sow gestation stalls by 2017. The use of minimal pig confinement production systems is reviewed in these proceedings. The many papers presented at the APSA Conference and the resultant discussions around these important issues are certain to provide the industry and its stakeholders with a better understanding of the factors influencing the key drivers of pork production including productivity, sustainability and responsibility. It can be guaranteed that the APSA Conference program and related papers have something for everyone associated with the pork industry. I urge you to read these papers and fully appreciate the multi-disciplinary benefits that APSA provides.

Looking back to the inaugural APSA conference, it aimed to provide a forum dedicated to the various pig research disciplines, enabling them to foster in-depth examination of research findings and industry problems. The thirteenth APSA Conference certainly upholds these aims and provides a real opportunity for researchers to present their findings to their global peers. This is particularly important for junior scientists, and it is extremely encouraging to see the number of new gradates attending and presenting at APSA. The support of these new graduates through private and publicly funded research programs is greatly appreciated.

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

Dr Darryl D'Souza, President

A.C.Dunkin Memorial Lecture

Was the Cooperative Research Centre (CRC) for an Internationally Competitive Australian Pork Industry Worth the Investment?

J. S. Keniry AM
Cooperative Research Centre for High Integrity Australian Pork, Roseworthy, SA 5371.

Cooperative Research Centres arise from a competitive research funding program initiated in the late 1980s by the Federal Labor Government. Since then, the Cooperative Research Centre (CRC) program has been instrumental in funding a range of industries to better foster collaborative research between industry and research providers. The Australian pork industry successfully applied to the CRC program in 2004 to establish the CRC for an Internationally Competitive Pork Industry (Pork CRC). This paper provides key insights into the CRC model for research and development and the substantial impacts the Pork CRC has had on the Australian pork industry. This paper also outlines the outcomes from the Pork CRC and its impact on the research capability and capacity of the pork industry. The benefits outlined in this paper are defined in terms of tangible economic benefits to the Australian pork industry, as well as less-readily quantifiable benefits that have enabled the pork industry to work collaboratively to address the new challenges facing the Australian industry.

Background

The Cooperative Research Centre (CRC) program had its origins in the late 1980's. The Australian Government of the time, led by Prime Minister Bob Hawke, brought down a tough budget, which included substantial cuts to spending on scientific research. The science community, and the Minister for Science at the time, Barry Jones, expressed significant concern. The Prime Minister responded with a well-funded new program, the CRC Program that was developed by the then Chief Scientist, Professor Ralph Slatyer. Essentially, the program involved the Commonwealth offering substantial sums of money to induce industry and research organisation's to undertake collaborative research, and then to implement or commercialise the research outputs. Cash grants were awarded on a competitive basis. Typically, the Commonwealth provided a total of $20 to $30 million to each Centre over seven years, and those funds could leverage up to $100 million in cash and in-kind from universities, industry, state agencies and other research organisations. The seven year funding model was very attractive, and encouraged participants to tackle large opportunities with good basic science. So far, the program has funded 190 CRC's, and the total Commonwealth investment in CRC's has averaged about $150 million per annum, or about $3 billion over the 20 years of the program to date. Whilst these are very large amounts of money, it is important to recognise that the Commonwealth's investment in CRC's currently represents only about 2% of its total investment in science and innovation.

Like any Government program, the CRC Program has, over its 20 year life, been reviewed several times. Changes have been made from time-to-time, but the essential features have remained unchanged and the CRC remains a highly successful program. CRC's work extremely well in agriculture and natural resource areas where successful adoption of research outcomes relies on rapid public dissemination of research results, and intellectual property issues are less of an impediment to rapid uptake of research than they are in many other industries.

Australian Pork Industry

Like many intensive agricultural industries in Australia, and most Australian manufacturing industries for that matter, the Australian pig production and processing sector developed with a significant degree of protection from import competition that was provided by tariffs and/or quarantine restrictions on imports. Tariff barriers were gradually reduced commencing in the 1970's, and Australia's accession to the World Trade Organisation (WTO) free trade agreement placed other barriers to imports under intense scrutiny from the late 1980's onwards. Throughout the 1990's the pig production sector in Australia experienced very tough times indeed. Successive changes to quarantine rules allowed the importation of processed pork and uncooked, frozen, de-boned pork for cooking and processing in Australia. Furthermore, the local industry suffered some significant cost disadvantages compared with imports from some other countries. For example, carcase weights were generally lower and processing plants were smaller than overseas, meaning unit slaughter costs were higher; feed costs, based mainly on wheat, were higher than those of Australia's competitors that were based mainly on corn and soybean meal; and producers had to market the products from entire pigs and compete with imports from countries which could export at discounted prices those portions of the animal that were surplus to their domestic market requirements. By the end of the 1990's, the Australian pig production sector had shrunk by 12% compared with 1990, even though total domestic pork production had grown over the same period by some 13%. The industry was clearly not cost competitive on an international scale, and

was surviving because remaining quarantine restrictions prevented imports from taking all of its domestic market. Importantly also, the industry had little in the pipeline by way of research and development that might address its cost position. Its focus and investment on research and development had diminished amidst the commercial and re-structuring turmoil of the 1990's.

CRC for an Internationally Competitive Australian Pork Industry

Fortunately, some leaders in the industry were determined to not throw in the towel, and decided to address head on some of the factors that were adversely affecting the industry's international competitiveness. This was addressed via the bid for the CRC for an Internationally Competitive Pork Industry. The bid was put together by a team led by Dr Robert van Barneveld. Consistent with its title, the Pork CRC's research focus was on driving international competitiveness under three broad headings: to drive down feed costs, to improve herd performance, and to improve pork quality and consumer appeal. The three research programs were often described as better feed, better pigs and better pork. The bid was submitted in 2004 and was awarded to commence on 1 July 2005. The successful bid provided that, over the seven year life of the Pork CRC, the Commonwealth would contribute $25.7 million, further cash contributions of $ 10.7 million would be made by Participants, and in-kind contributions from Participants would amount to $43.2 million; a total investment of cash and in-kind of $79.6 million. By pork industry standards, this represented a huge incremental investment in research, development and extension, and potentially, in training and development of the research leaders of the future.

Was the Pork CRC Investment Worth it?

Was the Pork CRC investment worth it? - the short answer is an emphatic yes. To quote Dr Roger Campbell in a report to the Commonwealth Department of Industry, Innovation, Science and Research: "During almost six years of operation the Pork CRC has revitalised pig and pork research in Australia, and enhanced the global competiveness of the Australian pork industry, through the development of an unprecedented range of new technologies and information. The outcomes have exceeded participant and industry expectations and demonstrate the value of the CRC model for aligning industry needs with Australia's research capabilities to develop collaborative research and development programs to enhance industry productivity and at the same time achieve excellence in science". Those few words sum up the achievements of the Pork CRC very appropriately. This paper attempts to demonstrate the same conclusion in an analytical way. The remainder of this paper deals with the basis for the conclusion that the $79 million investment has been very worthwhile. Before attempting to put an economic value on the Pork CRC's outputs, it is necessary to determine just what has been achieved by the Pork CRC in a physical sense, and of those achievements, how much has been adopted by industry participants, and what might be adopted by industry participants in future.

Program 1 - Securing More Reliable and Consistent Supplies of Protein and Energy for Pig Diets

Program 1 addressed priorities around better feed, and dealt with securing more reliable and consistent supplies of protein and energy for pig diets. The program had two quantified targets. Firstly, to reduce diet costs by 10% by 2012; and secondly, to improve the digestible energy (DE) content of grains by 1.0 MJ/kg by 2012. The outputs from Program 1 include a range of new plant varieties in the form of triticale, barley and pulses which, when compared with their conventional counterparts, demonstrate superior agronomic (yield) performance, and superior DE content in pigs. For the first time, the Australian pig industry has access to feed raw materials that are specifically tailored to pig performance, rather than crop materials (eg. wheats that were grown for a human consumption purposes but ended up being downgraded to feed). The results from National Variety Trials conducted in New South Wales, Victoria and South Australia between 2004 and 2010 have shown that the Pork CRC triticale (Berkshire) consistently out yields the bench mark variety (Tahara) by 8%-17%. Berkshire also contains 0.5 MJ more DE per kg than other triticale varieties and in animal experiments supports similar performance levels to wheat. The Pork CRC pea varieties (Maki and CRC Walana) are the highest yielding varieties currently available to growers in Australia. The latest release, CRC Walana, has also been shown to have a more consistent yield across environments than any other pea variety currently available. Uptake for commercial production of these new varieties has so far been limited by the availability of seed for commercial supply. However, the licensee for the most advanced variety, Berkshire triticale, and those involved in closed loop or contract growing arrangements harvested 12,000 tonnes of the grain in 2010-2011 and ample grain should be available for planting in 2012. Reaction from large producers who have grown limited quantities of Berkshire triticale for their own consumption has been enthusiastic.

A further output from Program 1 has been the development of Near Infrared Reflectance Spectroscopy (NIRS) techniques for rapid estimation of the DE content of feed grains, to supplement the usual NIR estimations for moisture and protein content. The Pork CRC has licensed 17 companies, including stockfeed and commercial pig producing companies, to use this technology (marketed under AusScan), and expects wide-scale application of it as a routine practice in grain buying and feed formulation in coming years. This technology also has application in poultry feeds.

A third output of Program 1 has been the discovery that conventional hammer-milling of grains results in significant proportions of particles having a size greater than 1 mm and that further comminution of this fraction prior to diet formulation can significantly increase the DE of the feed for pigs (Table 1). This technology has not yet been implemented commercially, but is under trial with a commercial feed company throughout 2011/12. In the meantime, producers and feed millers are now more actively assessing the efficacy of their grain processing and in particular ensuring larger particles are minimised.

Table 1. *Effects of grain type, particle size and diet form on average daily intake (ADI), rate of gain (ROG) and feed conversion ratio (FCR) of pigs from 0-28 days starting at 23 kg (from Black et al., 2010)*

Treatment	ADI (±SEM) (kg, as fed)	ROG(±SEM) (kg/d)	FCR(±SEM) (kg:kg)
B-G-M	1.62^{a} ± 0.069	0.801^{a} ± 0.030	2.04^{a} ± 0.05
B-G-P	1.66^{a} ± 0.071	0.841^{a} ± 0.031	1.96^{ab} ± 0.051
B-R-M	1.60^{a} ± 0.071	0.855^{a} ± 0.032	1.88^{b} ± 0.052
B-R-P	1.62^{a} ± 0.077	0.852^{a} ± 0.035	1.90^{b} ± 0.055
S-G-M	1.84^{a} ± 0.074	0.850^{ab} ± 0.033	2.20^{a} ± 0.054
S-G-P	1.60^{b} ± 0.069	0.795^{b} ± 0.033	2.02^{b} ± 0.050
S-R-M	1.72^{ab} ±0.069	0.866^{a} ±0.030	1.98^{bc} ± 0.050
S-R-P	1.59^{b} ± 0.072	0.810^{ab} ± 0.031	1.92^{c} ± 0.052

B, barley; S, sorghum; G, ground once; R, re-ground large fraction; M, mash; P, pellet; SEM, standard error of the mean. ^{abc} Means in a column with different superscripts differ significantly ($P<0.05$).

Whilst adoption of Program 1 outputs is still in relatively early stages, there seems little doubt that the milestones for this program will be met, and indeed exceeded.

Program 2 - Improving Herd Feed Conversion Efficiency

Program 2 addressed priorities around improving whole herd feed conversion ratio (ie. efficiency; HFCR). The target was to reduce HFCR from 4.3 to 3.6 over the life of the Pork CRC. The Pork CRC has developed new technologies and new information that, if fully implemented, would reduce HFCR well below that target. In terms of implementation, the Pork CRC benchmarking project, which involves collection of data from pig producers which together represent 30% of Australia's sows, shows that by 2010 the average HFCR had been reduced to 3.7, with individual enterprises achieving HFCR values of 3.4. The latter is world class, and at 2004 feed costs, the improvement achieved by these individual producers would reduce average feed cost by 20 cents/kg carcase weight (equivalent to a reduction in feed costs of 9.1% or $15/pig, or some $51 million annually across the industry).

The gains in Program 2 have come both from incremental improvements in pig production practices as well as from some step changes in nutritional and pig husbandry practices. Those changes include:

a) Demonstration under controlled conditions that Australian pig genetics have similar growth and feed conversion performance capabilities to those available internationally, a finding which removed industry concerns that Australian genetics were a constraint on international competiveness.

b) Derivation of revised nutritional requirement guidelines for modern pig genetics, which are higher than previous commercial recommendations.

c) Demonstration of practices for the use of natural metabolism modifiers (Ractopamine and Porcine Somatotropin (pST)) that improve feed efficiency and carcase gain in the last 4-5 weeks of growth by 10% to 24%.

d) Development of new weaner nutritional and management programs that reduce overall costs and enhanced survival and performance.

e) Discovery of nutriceuticals that enhance the feed intake of pigs immediately after weaning by 20%-80%, as well as enhancing gut development and immune competence, and reducing pre- and post- weaning mortality, particularly in gilt progeny. These technologies have been adopted widely by industry and are the only effective interventions developed for enhancing the health and survival of light birth weight piglets and gilt progeny.

f) The development of a novel and effective vaccine against *Actinobacillus pleuropneumonia* (APP). Whilst there have been some teething problems with the vaccine, it continues to be used by two of Australia's largest commercial producers. The vaccine will be further refined by the recently established CRC for High Integrity Australian Pork (Pork CRC II).

g) The development of a range of diagnostic tests for common diseases of pigs in Australia and globally. Specifically the Pork CRC developed a quantitative polymerase chain reaction (PCR) test for ileitis and is in the final stages of refining a pen side strip test for the same disease organism. An ELISA kit has been developed (and patented) for swine dysentery, and PCR and other tests have been developed for Glasser's disease. Alternatives to antibiotics to control *E.coli* infections particularly in young pigs have also been developed. These include Bacterophages and probiotic strategies based in the genotype of the pathogenic *E.coli* strains. Both the latter technologies will form part of Program 2 of Pork CRC II.

h) The discovery and development of a range of new science and technologies for enhancing the reproductive performance of sows, which challenge existing science and form the basis of Program 1 of Pork CRC II.

i) The establishment of procedures and economics for the use of Improvac® (Pfizer Animal Health, New Jersey, USA) vaccine technology for the immunisation of intact male pigs against gonaotrophin releasing hormone (GnRH). This technology is being adopted globally.

It is believed that all piggeries have been impacted by Pork CRC programs to some extent, with the level of adoption and the impact of various outcomes dependant on a combination of factors including the piggeries structure, labour and feed resources, plus its marketing approach. The value of the technologies and new information developed by the Pork CRC within Program 2, when combined, could conservatively be worth $0.35/kg carcase weight or $116 million per annum across the Australian industry. The implementation of the new technologies by the three commercial participants in the Pork CRC improved net margins over the three organisations by $14 million in 2010 (Campbell, 2011).

Program 3 - Enhancing Capacity to Deliver Nutrients that Promote Health and Well-Being Through Pork

Program 3 addressed priorities around enhancing capacity to deliver nutrients in pork that promote health and well-being in consumers. The targets were, firstly, to increase export and domestic sales volumes by 10% by 2012 and secondly, to achieve a $1.00/kg increase in returns for 10% of the product sold into the higher value markets by 2012.

The major outcomes from Program 3 have been the discovery that selenium enhanced pork reduces the incidence of colon cancer in a rat model, and the identification of a range of human health attributes of Australian pork. The latter, all of which require further follow-up research, but which have potentially major ramifications, include:

a) A possible role for pork in the control of Type -2 diabetes. Pork CRC research showed that, when combined with exercise, inclusion of fresh pork in the diet resulted in greater weight loss than subjects on a starch-based diet and that pork prevented the marked decline in thiamine status exhibited by subjects on the starch-based diet.

b) A possible role for pork in the diets of overweight and obese people. Pork CRC research showed that increasing pork consumption some 10-fold over a six month period had no adverse effect on cardiometabolic health, but significantly reduced body weight and body fat loss compared to subjects who remained on their normal diet. These findings will be followed up in Pork CRC II.

c) Establishing that including pork in the diets of young women improved their haemoglobin status and enhanced plasma Vitamin B and folate level.

d) Investigating, more recently, the genetics of muscle iron levels in pork and nutritional and other means of increasing muscle iron levels. The research outcomes have identified a potential (and very positive) unexpected effect of iron supplementation on the development of internal organs and the gastrointestinal tract. The research is ongoing and the findings will be fully evaluated, and if warranted, followed up in the near future.

e) Studying the effects of lecithin on the cholesterol and fatty acid composition of pork. The results to date are intriguing in that they are showing marked changes in the fatty acid composition of pork, a reduction in cholesterol and an unexpected effect on carcase weight gain. These studies will be completed in 2011.

Overall, the outcomes from Program 3 have markedly enhanced the knowledge of consumer and human health experts on the health attributes of pork. These outcomes exceeded the program expectations and indicate that the general negative perceptions of pork are unfounded, and highlight the lack of good science and research on the subject in the past. The findings have been widely communicated to human nutritionists, wholesale and retail participants and organisations and now form part of the pork promotion campaigns for the Australian and New Zealand pork industries. It should be noted that the industry is seeking to quantify these outcomes against the initial targets.

Return on Investment

The Pork CRC has produced many worthwhile achievements, and, many of these achievements have been rapidly adopted by a significant proportion of industry, and further adoption will occur for several years at least. There are many individual investors in the Pork CRC: Universities, Commonwealth and State Governments, commercial producers and other industry participants. Each of those individual participants would have to judge for itself whether its individual investment has been worthwhile for its organisation. In the next section of the paper the economic returns on the investment are considered from two perspectives. Firstly, has it been worthwhile in the national interest and secondly, has it been worthwhile from the perspective of the pork industry?

Pork Industry Return on Investment

In this section of the paper, the investment returns from a pork industry perspective are considered. These are summarised in Tables 2 and 3 (Campbell, 2011). Research by the Pork CRC has provided Australian producers with an extensive range of new technologies and information that have and will continue to contribute to improving the cost effectiveness and competitiveness of the industry.

As regards the national interest, the CRC program has spent considerable effort to establish a framework and methodology, known as the "Economic Impact Tool", to determine the benefits flowing from investments in CRC's. The Economic Impact Tool is used to evaluate new CRC applications, as well as to assist in Third Year Reviews of CRC's and to evaluate the return on the investment in the Pork CRC from a national perspective. The Economic Impact Tool evaluates the impacts over a 15 year investment period, commencing at the end of Year 1 (2005/06 for the Pork CRC), returning a Benefit:Cost Ratio (BCR) for each Program, and for the overall Pork CRC research portfolio. The tool provides an estimated value of the impact multiplied by the probability of impacts being achieved, less cost of delivery and usage.

For the Pork CRC impacts, adoption rates from Program 1 were assumed to increase from 20 to 30% commencing between 2008 and 2009 to 70 to 80% by 2015 for technologies such as NIRS and grain processing, and from 2 to 5% in 2009-2010 to a maximum of 30% in 2015/16 for new grains. For Program 2 impacts that were nutrition based, such as the nutritional requirements of modern genetics, Ractopamine and pST, the initial adoption rates were based on changes in the sales of products where applicable, and on feedback from industry nutritionists. The adoption rates for both metabolism modifier technologies were assumed to decline over time to zero in some cases by 2012. Health and reproduction impacts were assumed to have low adoption rates initially, and to increase over time to 60-70% by 2015, and to remain constant thereafter. For Program 3 impacts, information was provided by the marketing department of Australian Pork Limited (APL) on the value of the human health outcomes on domestic consumption and price over time, and this information was used in the Economic Impact Tool. The outputs from the Impact Tool are shown in Table 2 for the period to 2010, and in Table 3 for the fifteen year period to 2020. The Impact tool showed over the 15 year period that the BCR of the Pork CRC program averaged 4.33 with an average of 3.33, 3.61 and 15.07 for Programs 1, 2 and 3, respectively. The extremely high value for Program 3 reflects the value of increasing the demand for Australian pork. So, from the national perspective, the investment has been a resounding success.

Table 2. *Benefits, costs and benefit:cost ratio (BCR) of the three Pork CRC programs through to 2010 (from Campbell, 2011).*

Program	Benefits (B)	Cost (C)	BCR
1	$78,249,719	$38,909,817	2.01
2	$131,639,596	$44,193,598	2.98
3	$82,119,783	$7,219,279	11.38

Table 3. *Benefits, costs and benefit:cost ratio (BCR) of the three Pork CRC programs over 15 years (from Campbell, 2011).*

Program	Benefits (B)	Cost (C)	BCR
1	$129,604,111	$38,909,817	3.33
2	$159,497,968	$44,193,598	3.61
3	$108,759,426	$7,219,279	15.07

So, there is clear evidence that, from an economic viewpoint the investment in the Pork CRC has been very rewarding from the perspectives of both the Commonwealth and the pork industry. However, to leave the discussion at that point would be to short-change the issue, because there are many real, but less-readily quantifiable benefits that have flowed, and will continue to flow, from the Pork CRC that are not taken into account in the standardised modelling. These are considered in the next section of this paper.

Other Substantial Pork Industry Benefits

The pork industry had gotten itself on the back foot through the 1990's as imports increased and pig prices came under increasing downwards pressure. Contrast that with today, when, in spite of continued pressure from imports, the domestic industry is well and truly on the front foot, and willing to invest in new marketing initiatives, new technologies, and new production systems. This turnaround cannot obviously be attributed entirely to the Pork CRC, however, the Pork CRC has been a significant influence in bringing to the industry a much needed "can do" mentality.

Secondly, and a benefit for which the Pork CRC can take the majority of the credit, has been the renewed willingness on the part of major industry participants to invest in collaborative research, and to collaborate with each other and with governments to enhance the technological and economic viability of the industry. Clear evidence for this lies in the willingness of major producers to support and invest in the second Pork CRC. It is evidenced also by the establishment by the Pork CRC and APL of long term arrangements to identify, and then underwrite, the provision of research facilities in both public and private organisations that are then available to researchers from other organisations to work on pork industry projects. Whilst some other agricultural industries have attempted similar arrangements in the past, the pork industry appears to be the only industry that has achieved the level of cooperation and organisational efficiency.

Thirdly, the Pork CRC's contribution to enhancing the skills base of the industry needs to be highlighted. This has been done in several ways. Like all CRC's, the Pork CRC operated programs for honours students, PhD students and Post-Doctoral researchers. Over the life of the Pork CRC, 50 honours students, 23 PhD students and 5 Post-Doctoral researchers were supported. Many of those students (23), have remained working in the pork industry since graduation, and will form the nucleus of young researchers and production management personnel that will carry the industry forward in future years. In addition to those education programs, the Pork CRC pioneered a process by which organisations were invited to bid to undertake projects in areas identified to be of interest to the Pork CRC. This process resulted in many new researchers coming into the industry, and bringing with them knowledge gained from research in other industries that has proven to be very worthwhile for the pork industry. And finally on the human resources front, the Pork CRC was very proactive in engaging with industry practitioners, veterinarians and consultants to facilitate adoption of new technology. Many of those adoption practices that were pioneered by the Pork CRC have proven to be of lasting value.

Fourthly, whilst some of the industry's research personnel have always had global recognition from their overseas peers, the proliferation of significant research outcomes reported in the Pork CRC newsletters and journal papers has raised the international recognition of Australian pig research to new levels, and has led to important new collaborations with leading overseas researchers in Pork CRC II. These collaborations will stand us in good stead for many years to come.

Fifthly, whilst Pork CRC II has very few similarities with the Pork CRC, it is very important to recognise that it was research carried out in the Pork CRC that provided the inspiration, and the underlying science and justification, for a major part of the work to be undertaken in Programs 1, 2 and 3 of Pork CRC II. Whilst there is still much science and application to be done in Pork CRC II, the preliminary work done in the first Pork CRC on sow conception, bacteriophages and pork nutrient qualities went a long way to generating the confidence to put together the new bid, and then to justify its scientific merit.

And finally, in terms of industry benefits, the new Pork CRC will provide to industry and research organisations a further $20 million of Commonwealth funds over the next eight years. Without the enviable track record of the Pork CRC for achieving, and frequently exceeding, its research and adoption milestones, and for communicating its results effectively, there is no doubt that the Pork CRC II bid would have failed. So, intangible benefits of the Pork CRC are aplenty to add to the very substantial economic benefits.

Conclusion

The benefits of the Pork CRC outlined in this paper are significant and are defined in terms of tangible economic benefits to the Australian pork industry, as well as less-readily quantifiable benefits that have enabled the pork industry to work collaboratively to address the new challenges facing the Australian pork industry. Pork CRC II has an inspirational program with many 'research mountains' to climb to achieve the new targets set, not just in one program, but in all four programs. Going forward, if Pork CRC II achieves these targets, the Australian pork industry will in fact be transformed to create an internationally competitive Australian pork industry and an industry that produces high integrity Australian pork.

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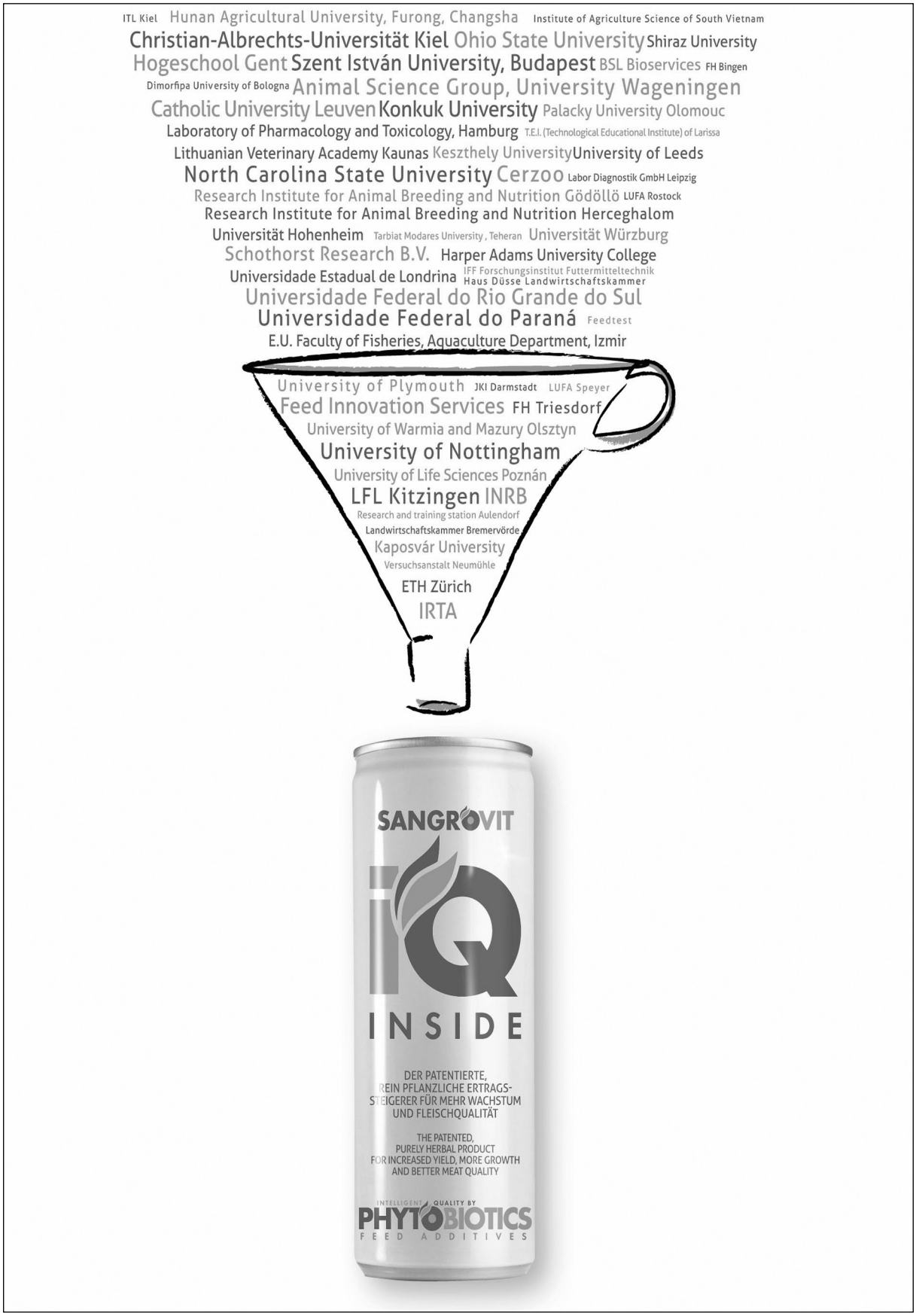
CHAPTER 1

Immunology and the Gut Microbiota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STEIGERER FÜR MEHR WACHSTUM
UND FLEISCHQUALITÄT
THE PATENTED,
PURELY HERBAL PRODUCT
FOR INCREASED YIELD, MORE GROWTH
AND BETTER MEAT QUALITY
INTELLIGENT QUALITY BY
PHYTOBIOTICS
FEED ADDITIVES

Development of the Gastrointestinal Ecosystem and Mucosal Immunity in the Neonatal Pig

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Abstract

Major changes to gastrointestinal immunophysiology occur in the young piglet in the first eight weeks of life. This review documents the interactions between the developing intestinal microflora, nutrients and prebiotics in colostrum and milk and the developing gut and gut associated lymphoid system (GALT) in the perinatal period. Antibodies, cells and trophic components in colostrum provide passive immunity and assist development of full immune competence in the innate and acquired immune systems, all while discrimination between commensal microbes and pathogens and oral tolerance of dietary antigens is established. With the incentive to reduce antibiotic usage by vaccines or feed additives, the best opportunities for probiotics are examined within the current knowledge on the developing gut ecosystem in several species. Finally, since the majority of antibiotic cover post-weaning aims to prevent proliferative enteropathy (PE) and other bacterial enteric infections, progress towards induction of mucosal immunity against an intracellular pathogen is presented through vaccination against the causal agent of PE, *Lawsonia intracellularis*.

Introduction

The gastrointestinal tract and immune apparatus of the newborn pig is immature with rapid development occurring postnatally. Neonatal piglets are classed as immunologically naïve because the epitheliochorial nature (uterine epithelium and chorion in contact) of the placental tissue in sows prevents the transfer of maternal immune cells to the foetal circulation (Blecha, 1998). Therefore, the soluble and cellular components of innate immune defences, together with the absorption of specific antibodies and bioactive peptides (and perhaps cells) in maternal colostrum and milk in the immediate postnatal period are critically important for the protection of the newborn piglet from endemic infectious agents. The structure, composition and activity of the porcine immune system and the interactions occurring for acquired immune responses by pathogens and vaccines have been described in several excellent and recent reviews (see Roth and Thacker, 2006; Bailey, 2009; Burkey *et al*., 2009) and will be discussed only briefly here. Since most of the principal pathogens inflicting death and production losses assault the growing piglet across mucosal surfaces, this review will concentrate in the mechanistic interplay between innate immunity, passive immunity and the acquired mucosal immune systems, and its links to nutrition and the intestinal microbial flora in the piglet during the first 2-3 months of life. During this important period, "foetal" gut epithelial cells are progressively replaced, the gastrointestinal microflora rapidly colonises the lumen to establish a unique ecology by 3-4 weeks postnatally (Thompson *et al*., 2008), many immune cells develop full functional competency and differential reactivity against food, commensal bacterial versus pathogens are established (Bailey 2009). At the cellular level, intestinal epithelial cells, myofibroblasts, stromal cells, T cells, B cells, myeloid cells in the lamina propria together with the lumenal microbiota all participate in a multi-faceted and redundant network of interactions regulating the inflammatory tone and digestive capability of the intestine. The complexity of this interaction is slowly being appreciated (Garrett *et al*., 2010; Kinross *et al*., 2011), and is relevant to optimise health and production for intensively-farmed livestock.

Innate and Acquired Immune Competence at Birth

The late gestational development of the gastrointestinal tract of pigs show remarkable similarities with other livestock including cattle, horse, sheep and goat, all of which exhibit relatively high levels of perinatal mortality around 8–25%. Many of the rapid anatomical and physiological changes in the neonatal gut have already commenced late *in utero*, so that these may be critical in determining neonatal survival and especially following premature births (Sangild, 2001). The "permeability" of the intestine post-natally enabling the absorption of maternal immunoglobulins is a specific maturational event commencing late in gestation in livestock; colostrum also appears central to gut closure (Sangild, 2001). While limited absorption of macromolecules can occur before birth, the "foetal" gut epithelial cells with higher endocytic activity together with increased porosity of the intestinal tract enable uptake of large molecules, including IgG and IgA for 24-36 h after birth before closure. This is critical for passive immunity and protection against endemic environmental pathogens in the naïve piglet. Within 24 hours of life, the serum IgG levels of suckling neonates are often similar to those of their dam (Butler and Kerhli, 2005).

Differences in the health, survival and growth performance of gilt and sow progeny in the first few weeks of life indicates that dam parity may influence pathogen exposure and immune response in the newborn and neonatal pig. Pigs born to or reared on gilts have increased mortality, increased acute phase proteins and reduced growth relative to sow born or reared progeny (Piniero *et al*., 2006). Higher total IgG in sow's serum and their progeny also suggests that sows are better able to protect their progeny than gilts pre-weaning (Klobasa *et al*., 1986). Burkey *et al*., (2008) proposed that sow progeny had more IgG receptors on intestinal cells than gilt progeny as a way to explain the higher serum IgG concentrations in sow-reared versus gilt-reared piglets given equivalent IgG concentrations in colostrum. In contrast, Miller *et al*. (2011) observed no difference in specific IgG in the colostrum or milk of gilts and sows when they were vaccinated pre-farrowing with a novel antigen, or in the circulating antibodies of their progeny. In studies aimed at separating the effects of birth and rearing dam, piglets were removed from their birth dams and gilt and sow litters created with half gilt-born and half sow-born progeny. Under these conditions, birth dam parity did not influence measures of innate immunity (number or activity of phagocytes or lymphocytes; Miller *et al*., 2011), but gilt reared progeny had higher concentrations of major acute phase proteins, suggesting that gilt progeny are exposed to a greater antigenic challenge (Morales *et al*., 2006). Although Miller *et al*. (2011) observed no difference in specific passively acquired antibodies pre-weaning, gilt born progeny had reduced IgG concentrations in response to vaccination post weaning, suggesting that the birth dam may influence the adaptive immune response of their progeny. Immunizing sows late in gestation will increase the transfer and passive absorption of maternal immunoglobulin, but will have little effect on the piglet's own immunity once maternally acquired antibodies have waned.

Development and Changes to the Neonatal Gut

The Intestinal Epithelium

The adult intestinal epithelium is composed of highly specialized cells that digest and absorb nutrients, provide a barrier to bacterial and viral pathogens and prevent commensal microorganisms that ferment undigested nutrients from becoming opportunistic pathogens. The epithelium comprises absorptive enterocytes (epithelial cells), mucus-producing goblet cells, hormone-producing (enteroendocrine) cells and Paneth cells, all of which arise from a common progenitor with proportions determined by local concentrations of "wingless-integration" (WNT), Notch and Hedgehog differentiation factors (Yen and Wright, 2006).

The growth of the intestinal tract and the gut-associated lymphoid tissue (GALT) and immune apparatus occurs rapidly post-natally. Physiologically, the rapid development of the neonatal gut is critically dependent on oxygen supply through increased blood flow and the supply of energy, endogenous hormones (particularly cortisol), nutrients and growth factors from colostrum and milk for its full maturation of mass and functional activity by 12 weeks of age (reviewed by Sangild, 2001). In the gastrointestinal tract (GIT) epithelium, foetal-type enterocytes with high endocytotic activity are gradually replaced by new adult-type cells in the intestine by 3–4 weeks of age (Baintner, 1986). Independent of immune input but associated with the onset of nutrition, goblet cells increase in number and with acidomucin secretion, bolster luminal barrier function during epithelial renewal (Conour *et al*., 2002). While acid levels decline for the first five days after birth, stomach development is not affected by food intake in the first week after birth. However, the intestinal mass of parenterally fed piglets is around 40% lighter than cohorts fed orally (Sangild *et al*., 2000), indicating the importance of ingesta to gut development.

In colostrum, growth factors including insulin-like growth factors (IGFs) are important bioactive moieties for postnatal intestinal growth, but trophic factors are also produced endogenously. For example, glucagon-like peptide-2 (GLP-2) is produced in the ileal L-cells and is released in response to meals high in fat and carbohydrate. In the testing of prebiotics, exogenous GLP-2 (96 μg/kg/day) for six days enhanced mucosal growth by 50% (Petersen *et al*., 2001), but had little effect to promote increased intestinal enzyme levels. For this effect, colostrum initiated changes in mucosal villus structure which led to the positioning and detection of functional protease and other enzymes into the brush-border membrane (Dudley *et al*., 1996) and was associated with the rapid postnatal increases in the absorption of glucose and amino acids.

Postnatal gut changes could be effected by other means. Feeding piglets crude red kidney bean lectin (phytohaemagglutinin- PHA) preparation (400 mg/kg body weight, containing 25% pure lectin) from 12-14 days reduced the size of lysosomal vacuoles and the number of vacuolated enterocytes/villus, suggesting that PHA may induce maturation of the enterocytes in suckling piglets (Biernat *et al*., 2001). This is likely to be caused by production of inflammation, stress and apoptotic responses as has been shown from a proteomic analysis of 28 d piglet intestines after feeding soybean allergen (β-conglutinin) for 18 d (Chen *et al*., 2011). In addition, PHA is a leucocyte mitogen (Stokes *et al*., 1987). Many other plants and cereals contain similar lectins and may instigate changes in digestive enzymes (eg. declines in lactase) associated with alterations in dietary carbohydrates and other substrates. In fact, in early weaning models, feeding soyabean meal before withdrawal of milk prevented malabsorption, diarrhoea and *E.coli* infection (Kiers *et al*., 2001), probably by promoting an anti-microbial, inflammatory milieu in the mucosa as evidenced by transiently reduced crypt area and villus size (Stokes *et al*., 1987).

The Innate Immune System

Composition and Functional Development of the Innate Immune System

Within the innate immune system, levels of complement are low at birth and rapidly increase in serum with faster increases in normal piglets compared with colostrum-deprived cohorts (Roth and Thacker, 2006). The cellular components of the innate immune system which consist of blood and tissue leucocytes, dendritic cells and phagocytes (monocytes and macrophages) express a variety of receptors directly related to their function. Porcine polymorphonuclear leucocytes (PMN), monocytes and macrophages express receptors for complement (C3), IgG-1 and 2 and a separate receptor for IgG-2, and will ingest IgG-coated microbes at birth (Roth and Thacker, 2006). While cells of the innate immune system such as mononuclear phagocytes and leucocytes are present at birth at reduced numbers, their populations and migratory and functional activity gradually matures to adult levels over the next four weeks (see Roth and Thacker, 2006). Natural-killer (NK) cell activity was not detected in blood from foetal piglets and had developed by two weeks post-partum (Yang and Schultz, 1986). However, interferon-alpha (INF-α) was detected in foetal lymphoid tissues which had been challenged *in utero* with coronavirus (Splichal *et al.*, 1997), indicating that the low levels of NK activity at birth simply reflected lack of infection or antigenic challenge.

To protect the growing piglet from the insult of pathogens in the immediate period after birth while the whole interactive system is maturing and vulnerable, colostral bioactive molecules and specific antibodies which should be most relevant to cover endemic pathogen challenge will integrate with the innate immune system to reduce pathogen load (by direct killing or uptake, or interference with attachment or establishment) or ameliorate pathogenic effects (by neutralization of toxins) and hold the host-pathogen balance in favour of the developing piglet. Concomitantly, the GIT microbiome is also enabled to the exclusion of introduced pathogens. In addition to antibodies, colostrum and milk contain a variety of bioactive/trophic peptides which have been shown to enhance gut development and exhibit anti-microbial activity. These peptides include IGF and epidermal growth factor (EGF) and bear resemblance to many moieties found in serum and inducible secretions that constitute the innate immune system. Most of the antimicrobial compounds documented in the innate immune system such as lectins, C-reactive proteins, defensins, macroglobulins and complement are present in sow colostrum (see Salmon *et al.*, 2009), or have been produced by the foetal liver and are present at reduced levels in serum and secretions at birth. These opsonise (neutralize) invasive pathogens prior to ingestion by phagocytic cells. More than 10 anti-microbial peptides have been isolated from porcine tissues (Boman, 1995) and cercropin P1 and PR-39 are found in the small intestine and two β-defensins (1 and 2) have been extracted from intestinal epithelial cells (Veldhuizen *et al.*, 2007). Indicative of their potential protective effects for the piglet, several antibacterial peptides such as β-defensin-1 have been shown to neutralize infectious bursal disease virus (IBDV; Sun *et al.*, 2010), reduce pathogen burdens and enhance growth rates when fed to poultry in drinking water (Bao *et al.*, 2009). Maternal cells (up to 10^5 per ml colostrum) are also absorbed with colostrum and have been detected using fluorescent and radioactive markers in intestinal mucosa, mesenteric lymph node (MLN), circulating blood, lungs, liver and spleen (Tuboly *et al.* 1988; Le Jan, 1996; Salmon *et al.*, 2009). Their functional activity and relevance for protection and immune responses are not clear.

Mechanistically, tolerance to dietary antigens must also be established postnatally (Bailey, 2009). The B-cell repertoire is substantially generated in ileal-peyers patches (IPP; Butler *et al.*, 2009) while the jejunal PP (JPP) are primarily involved in the generation of acquired immune response resulting from antigen presentation through specialized M-cells and colostral or piglet IgG- or IgA-bound antigens. The exact interplay between GI tract epithelium the GI microflora and the developing immune system under the influences of bioactive peptides (in milk) is complex and dynamic, and much is required of the operation to protect the health and growth of the piglet through optimal digestion, nutrient partitioning and a functional gut ecosystem.

The "Toll-like" Receptor (TLR) System and Functional Development in the Piglet

The TLR system is rapidly assuming prominence in the regulatio n of the gut milieu (Abreu, 2010). Leucocytes and intestinal epithelial cells express a variety of TLRs that are activated by binding to antigens commonly expressed on microbial pathogens. TLRs are vital components of the "innate sensing mechanism" to detect pathogen-associated molecular patterns ("pamps") on potential pathogens. By this means, the interplay between soluble and cellular components of the innate immune system will reduce the severity of any microbial challenge breaching the skin or mucosal surfaces of gut and lung by capturing (or opsonising) invading organisms for phagocytosis or removal. To regulate exuberant activity, local inflammation engendered by activated TLRs can be dampened by negative regulators of TLR expression (such as "tollip"and "TIR8") produced by intestinal epithelial cells that act in association with regulatory T-cells to limit collateral damage particularly at mucosal surfaces (Abreu, 2010). The secondary effect following ingestion and intracellular lysis of pathogens will be to stimulate the acquired immune system. In a linkage

analysis, TLR4 was associated with resistance to infection with *Salmonella enterica serovar typhimurium* where 16 days after a challenge infection, 64% of chickens that were homozygous for "SS" alleles survived infection compared with 82% of the "RS" genotype (Leveque *et al*., 2003).

Toll-like receptors (TLRs) 1-10 have been reported and described on a range of porcine cells (reviewed by Uenishi and Shinkai, 2009). TLRs 1,2,4,5,6 and 10 are membrane bound and recognise extracellular bacterial components and TLR5 recognises bacterial flagellin, while TLRs 3,7, 8 and 9 are intracellular and recognise nucleic acids (TLRs 3,7 and 8) or unmethylated CG dinucleotides/CpG motifs (TLR 9; Uenishi and Shinkai, 2009). Binding of bacteria and bacterial components to TLRs activates NF-κB with downstream transcription of proinflammatory cytokines (see Burkey *et al*., 2009) and expression of the antimicrobial peptide, β-defensin-2 (Vora *et al*., 2004). The detection of "pamps" can also occur intracellularly by nucleotide-binding oligomerization domain (NOD)-like receptors (Meylan *et al*., 2006) and porcine NOD1 and NOD2 receptors have been identified (Tohno *et al*., 2008).

As in other species, the importance of TLRs in the development of porcine immunity has been reported. In young pigs, expression of TLR-2 and TLR-9 mRNA was localised to M-cells and was highest in mesenteric lymph node and IPP (Tohno *et al*., 2006). In response to infection, TLR-4 expression on primary cultures of neonatal porcine intestinal cells in vitro was increased after exposure to LPS or enterotoxigenic *E.coli* (ETEC; Moue *et al*., 2008), indicating an early competence to respond. A heterodimer of TLR 2/6 on porcine alveolar macrophages binds *Mycoplasma hyopneumoniae* (Muneta *et al*., 2003), while PCV2 interacts with nucleic acid binding TLR 9 to induce (and suppress) production of IFN-α and inflammatory cytokines (IFN-γ, IL-6, TNF-α and IL-12; Vincent *et al*., 2007). Most intriguingly, transcriptional profiling of the intestinal mucosa of 6-7 week-old piglets discovered only limited increases in mRNA for IL-8 (innate- inflammation) at 4h and STAT-3 (anti-inflammatory) at 4 and 8h after infection with *Salmonella typhimurium ex vivo* (Niewold *et al*., 2007), indicating that Salmonella could possibly suppress local inflammation. In stark contrast, the addition of 1μg *S.typhimurium* LPS to cultured neonatal jejunal cells (IPEC-J2) and adult boar ileal cells (IPI-2I) induced 3- and 7-fold increases, respectively, in TLR-2 mRNA after 3h incubation and a 2-fold increase for TLR-4 mRNA in both cultured cell types (Arce *et al*., 2010). The adult cells also elaborated higher levels of mRNA for TLRs 1,3,6 and 9 than IPEC-J2, and the reverse for TLRs 8 and 10 (Arce *et al*., 2010). IPEC-J2 cells also produced high levels of IL-8 and TNF-α after 2h incubation whereas adult cells showed increased TNF-α (Arce *et al*., 2010). These data could indicate that neonatal (foetal-type) intestinal epithelial cells are quite capable of gene transcription (and produce a different response to adult cells), although the continuous culture *in vitro* may affect their maturational status and evidence of protein secretion was not provided. Similar studies have recorded TLR-4 activity on cultured intestinal cells (Gribar *et al*., 2008) as part of the interactive and constant sensing of the luminal environment by the gut epithelium and microbiome. *In vivo*, the model using tissue explants to define cellular responses is complicated by the variety of fixed and migratory cells types that are present in the biopsy. Interestingly, *E.coli* has been reported to undergo clathrin-mediated endocytosis in the neonatal IPEC-J2 cells (Rasschaert *et al*., 2010), as might be expected from an opportunist pathogen.

Regulation of Inflammation

Increased Toll receptor expression or engagement is usually associated with inflammation which must be strictly regulated in the gut, since ongoing induction of pro-inflammatory cytokines reduce growth rates through villus flattening and reduced appetite (Johnson, 1997). The fascinating intricacies of the mutual regulation of inflammation, injury and repair (as well as epithelial cell regeneration) by intestinal epithelial cells, stromal cells and commensal microbes have been reviewed (Abreu, 2010). This highlights the close interaction and balance between "hyper-reactivity" in disease states such as colitis and the regulatory milieu required for intestinal integrity, a functional microbiome and digestive capability. TLR agonists have been proposed as immune-enhancing agents for vaccines. However, since significant polymorphisms exist in porcine TLR genes, these are unlikely to contribute any large genetic effect to disease resistance, but imbalances may have a profound effect on intestinal function. In addition, while many components from microorganisms bind to TLRs (*ipso facto*) as part of the process to activate acquired immune responses, it is speculative whether isolated TLR-binding antigens or agonists will act as potent adjuvants for adaptive immunity. For example, LPS is a poor antigen except in oil adjuvants and reactivity to cell-bound LPS in the gut is dampened through reduced TLR-4 / MD2 expression on epithelial cells (Abreu, 2010).

Porcine Natural Killer (NK) and γδ+Tcells.

Porcine NK cells are CD2+ CD8+ CD3-, large granular leucocytes with receptors that can activate or inhibit cytotoxicity against malignant or viral- infected cells (Toka *et al*., 2009). Receptors such as NKR-P1 (Sharma *et al*., 2008) and NCR1 (NKp46; Jozaki *et al*., 2010) have been cloned from pig leucocyte libraries, but function has not been determined. NK-mediated cytotoxicity has been reported against porcine transmissible gastroenteritis virus (TGEV)

by intra-epithelial lymphocytes (IEL) and peripheral blood mononuclear cells (PBMC) isolated from 2-week old but not 1-week old piglets (Cepica and Derbyshire 1984). Incubation of porcine NK cells from 3-4 month old pigs with TLR-7 or -8 agonists (quinoline derivatives) in vitro induced IFN-γ and minimal cytotoxicity against FMDV-infected cells: cytotoxicity and intracellular perforin storage were augmented if activated macrophages were also present (Toka *et al*., 2009). So while NK cell activity was not detected in peripheral blood at birth, cytotoxicity could be induced by incubation of PBMC *in vitro* with IFN-γ or *in vivo* after inoculation of IFN-γ inducers (poly I:C; Lesnick and Derbyshire, 1988). NK-activity against K562 and PI3 infected Vero target cells was apparent by two weeks of age in blood (but not in splenic, MLN or thymic cells) and full functional capacity had developed by three months of age (Yang and Schultz, 1986).

Compared to T-cells involved in the acquired immune response that also express CD3, porcine γδ+ T cells are considered components of the innate immune system (Burkey *et al*., 2009). This subpopulation of T cells comprises three subsets: CD2+CD4-CD8-, CD2+CD4-CD8^{low} and CD2-CD4-CD8- and are found predominantly in blood and intestinal tissues where they account for up to 35% of PBMC in young pigs (Yang and Parkhouse, 1997). The CD2+CD4-CD8^{low} γδ T cells are also CD3+ and capable of cytotoxic activity and differ from CD3- NK cells (Yang and Parkhouse, 1997). While low numbers of γδ T-cells occur in the foetus, rapid expansion occurs just before birth (Trebichavsky *et al*., 1995). After birth, CD2+4-8- γδ T-cells cells enter the intestine; CD2+4+ ("helper T-cells"; Th) cells appear in pigs aged three weeks while CD2+8+ (Th1) cells are found around seven weeks (Vega-Lopez *et al*.,1995; Rothkotter, 2009). Intraepithelial γδ+ T cells appear to be involved in tissue repair, lysis of damaged epithelial cells, and inflammatory cell recruitment (see Burkey *et al*., 2009).

Structure and Immunophysiology of the Gut-Associated Lymphoid Tissue (GALT)

The porcine mucosal immune system represents an exquisite interaction between epithelial cells lining the luminal surface of the gastrointestinal tract, IEL and interdigitating dendritic cells (DC) that sample the luminal contents and may present antigens to lymphocytes present in the lamina propria and lymphoid follicles. The organised GALT is comprised of jejunal and ileal Peyer's patches (JPP and IPP) in the intestinal lamina propria and the adjacent mesenteric lymph nodes (MLN). In the small intestine, specialized epithelial cells called M cells reside in the follicle-associated epithelium overlying PPs. These cells transport antigens through pinocytosis to the PPs and particular pathogens including *Yersinia enterocolitica* and *Salmonella typhimurium*, use receptors on M cells to transit across the mucosal barrier (Mims *et al*., 2002). Immediately beneath the layer of intestinal epithelial cells, smooth muscle cells provide the structural support for epithelial cells. All of these cell types may contribute to the innate immune response to pathogens and parasites. The lamina propria is also populated by macrophages, blood leucocytes, natural-killer (NK) cells and the specialised dendritic cells that participate in the adaptive immune response by antigen presentation to B-cells and both CD4+ and CD8+ T-cells in the GALT (Husband, 2000).

Peyer's Patches (PP)

Porcine PP structure and function is similar to other domestic species, with multiple, isolated, JPP and a single, continuous IPP (Liebler-Tenorio and Pabst, 2006). The numbers and morphology of PPs present in pigs at birth is equivalent to those of adults. The mesenteric lymph nodes (MLN) are well formed and lymphocyte infiltration has been observed in the lamina propria during this time (Chapman *et al*., 1974). Despite the anatomical development, local immunity in the GALT of neonatal pigs appears functionally immature. The synthesis of secretory IgA does not occur until the second week postpartum (Allen and Porter, 1973). Within the pig jejunum, 11–26 discrete PP have been reported at birth, each containing multiple B-cell follicles separated by interfollicular areas dominated by T-cells (Brown and Bourne, 1976). The IPP contains predominantly follicles with very little migration of cells through it (Binns and Licence, 1985) and several studies have suggested that the IPP may be a site of early differentiation and selection of immature B-cells (Butler *et al*., 2009), similar to the sheep (Reynolds, 1997). In contrast, the more numerous and proximal JPPs may be the inductive sites for acquired immune responses as pathogens such as *E.coli* (Snoek *et al*., 2006) move downstream with the intestinal flow. Nerve fibres are in close proximity to porcine enterocytes and PP (Vulchanova *et al*., 2007), so that neural inputs to the gut and immune function can occur as has been demonstrated in sheep (Stewart *et al*., 1996). The lack of circulation and its predominantly B-cell nature may predispose the IPP and local area to invasion by ingenious intracellular pathogens such as Mycobacteria and *Lawsonia intracellularis*.

Activated cells from immune responses generated in PPs or adjacent MLNs enter the systemic circulation via lymphatic vessels and then recirculate through blood, circulate systemically and migrate back to the mucosa via specialized blood vessels (Mowat and Vigny, 1997; Rothkotter *et al*., 1999). Lymphocytes generated in the periphery are less likely to enter the mucosa, hence parenteral immunization is less common against enteric pathogens.

T-cell Function, Location and Postnatal Development

For two decades, a paradigm of polarised Th1/Th2 reactivity has dominated the generation and activity of T-cell responses orchestrated by Th1 (inflammation/graft rejection) and Th2 (allergy) subpopulations of CD4+ (helper) T-cells (Brown *et al*., 1994). Operationally, CD4+ Th1-cells produce cytokines including IFN-γ, TNF-α that activate CMI by stimulating CD8+ cytotoxic T cells to promote direct or indirect killing of intracellular bacterial and protozoal parasites and virus-infected cells, while Th2 cells produce cytokines that include IL-4 and IL-5 to promote differentiation of B cells and accessory leucocytes such as eosinophils to aid in clearance of extracellular pathogens such as parasites or to neutralize microbial attachment and exotoxins.

With respect to porcine Th1-2 immune responses, Crawley *et al*. (2003) cultured pig B-cells in the presence of Th1- and Th2-type cytokines, finding that IL-10 promotes IgG1 over IgG2, while IFN-γ biased IgG2 synthesis, consistent with the rodent paradigm if this extrapolates authentically for porcine Ig isotypes. As has been demonstrated in cattle (Brown *et al*., 1994), the alternative approach profiles gene transcription during chronic diseases of pigs, one with an intracellular pathogenesis (toxoplasmosis) for Th1-type immunity and *Trichuris suis*, a nematode that should engender a Th2 response with IgE production. As anticipated, preferential increases in a range of Th1-associated genes from donors with *T. gondii* infection and increased expression of Th2-type genes in *T. suis* infections were found using real time PCR in mucosal-associated tissues (Dawson *et al*., 2005; Kringel *et al*., 2006). *In vitro*, porcine T-cells exposed to monocyte-derived dendritic cells (DC) that were pulsed with avian lyzozyme, expressed increased levels of mRNA for IL-13, while pulsing DC with Mycobacteria (intracellular pathogen) induced mRNA for IFN-γ (Raymond and Wilkie, 2004). Both reports support the operation of the Th1-2 paradigm in pigs, as the mRNA expression profiles could be modified by exogenous cytokines, at least *in vitro* (Raymond and Wilkie, 2004).

For most acquired responses, a combination of Th1 and Th2 pathways are detected, with more complexity and regional immune regulation from regulatory T-cells (T-reg) and Th-17 T-cells (see Bailey 2009). T-reg are CD4+ T-cells that secrete predominantly TGF-β or IL-10 (Fontenot *et al*., 2003) and at the mucosa, contribute to maintaining oral tolerance to food or experimental antigens or participate in the control of experimental colitis in mice (Groux *et al*., 1997). T-reg also express the transcription factor FoxP3 and the surface receptor CD25 (the IL-2 receptor α-chain). Systemically, T-reg contribute to maintaining "peripheral tolerance" to self-antigens (Fontenot *et al*., 2003) as well the capability to use a range of mechanisms to suppress activity of a range of immune cells including NK cells, T and B cells, dendritic cells, granulocytes, macrophages and monocytes (Garrett *et al*., 2010). Porcine CD4+ CD25+ peripheral T-cells also express FoxP3 *in vitro*, produce IL-10 and suppress mitosis of CD4+ CD25- Th-cells (Kaser *et al*., 2008). During development of T-regs in the thymus and peripherally, TGF-β appears crucial, while CD103+ dendritic cells can incite development of "inducible T-reg" through secretion of TGF-β and with additional retinoic acid (Coombes *et al*., 2007).

More recently, another murine CD4+ effector T-cell (Th17 cell), characterized by secretion of IL-17 and the expression of CCR6 and IL-23R, has been found, particularly at mucosal interfaces where they contribute to control of bacterial and fungal infections (Weaver *et al*., 2007). Th17 cells are related to γδ-T-cells and some NK cells, and produce IL-17A (IL-17), IL-17F, and IL-22, which influence neutrophilia, tissue remodelling and repair, and production of antimicrobial proteins (Littman and Rudensky, 2010). Like pigs and ruminants, murine γδ T-cells are prominent in mucosal tissues, where they also express IL-23R constitutively and have been reported to differentiate into IL-17-producing cells early after exposure to IL-23 (Roark *et al*., 2008). This may allow some flexibility of T-cell activity during differing types of inflammatory responses (Littman and Rudnesky, 2010). Malfunctional Th17 responses have also been implicated in the pathogenesis of colitis in mice and humans (Neiss *et al*., 2008). The cytokines IL-17 and IL-22, presumably secreted from Th17 cells, are required to protect mice from pathology induced by orally administered *Citrobacter rodentium* (Ouyang *et al*., 2008), a murine pathogen with similar pathology to that induced by infection with *Lawsonia intracellularis* in pigs and rodents. Once again, commensal bacteria, particularly segmented filamentous bacteria (SFB), stimulate the development and accumulation of Th17 cells in the gut mucosa (Neiss *et al*., 2008) and mRNA for porcine IL-17 is strongly expressed in mucosal tissues (Katoh *et al*., 2004). Colonisation of germ-free mice with SFB enhanced resistance to challenge with *Citrobacter rodentium* (Ivanov *et al*., 2009), while colonization of normal mice with SFB from a different source induced accumulation of CD4+ T cells, including Th1, Th17, and T-reg cells in the intestinal lamina propria (Gaboriau-Routhiau *et al*., 2009). The various roles and relationships between these T-cell types in a range of disease states in different species are slowly becoming clearer, but much less is known about the activity of T-reg and Th-17 T-cells in pigs, except that mucosal location is important. It is testament to the complexity and demands of mucosal reactivity that several options for cellular function and regulation are in place. The comparative results also emphasise the importance of basic studies in pig-pathogen relationships, as extrapolations from rodent models are not always reliable.

Dendritic cell (DC) characterisation and functional activity in pigs and porcine GALT is complicated at present. Four main subsets have been determined by MHC II expression and co-expression of CD11R1 and CD172 from immunohistological studies in tissues from 15 month old gilts (Bailey, 2009). Around 35% of M-cells in jejunal PP were adjacent to DCs (Bimczok *et al*., 2006). Similar kinetic studies during postnatal development would be very instructive to examine the impact of the microbial flora, milk, dietary bioactives, antibiotics and infectious agents during early gut and GALT development.

In the GALT, T-cell locations reflect the fixed and mobile elements of the adaptive immune system and are found in the gut tissue between villus epithelial cells and mucosa, lamina propria and conventional lymphoid organs (PP and MLN). In the intestinal tissue, IEL are mostly negative for markers CD2, 4 and 8 at birth. During the first few weeks postnatally, typical γδ-T-cells or NK cells (CD2+CD4- CD8-) appear but CD8+ IEL have not been found until around seven weeks onwards (Whary *et al*., 1995). In pigs less than three weeks old, Stokes *et al*. (2001) reported that less than 50% of CD2+ cells were positive for CD3 (expressing the αβ T-cell receptor), while the majority co-express both in adult pigs. Amongst IEL in more mature pigs, the majority of IEL express the αβ T-cell receptor (type a), while type b IEL express T-cell receptors that are γδ+, γδ+CD8αα+, or αβ+CD8αα+ (Hayday *et al*., 2001). Both types of IEL are cytolytic effectors that secrete cytokine and chemokine mediators. Secretion of high levels of IL-2, IL-4, IFN-γ and TNF-α by CD2+ lamina propria cells indicates that the acquired immune system is capable of activation by three weeks of age (Stokes *et al*., 2001). In support of this timing, IEL from piglets aged two to three weeks were not stimulated by mitogens, but IEL from cohorts aged nine to 11 weeks were reactive (Wilson *et al*., 1989). Associated with the maturation of CD4+ T-cell help, synthesis of IgA can occur by two weeks of age, mature B-cells (sIgM+ and later IgA+ plasma cells) are found in PP and lamina propria in the first four weeks of life, and the numbers of B lymphocytes reach adult levels in pigs over the same period (Roth and Thacker, 2006). By this time, around 80% of IEL express the CD8+ surface marker, and act as cytotoxic/ suppressor T cells (Mowat and Viney 1997; Rothkotter, 2009). It has been suggested that IELs are able to enter the lumen, sample antigen and re-enter the LP, stimulating effector T-cells in the process (Husband, 1990). Brundage *et al*. (1980) detected only weak and localized cell mediated immune (CMI) responses such as MHC- restricted cytotoxicity in the intestine of five week old pigs infected with porcine enterovirus compared to rapid CMI responses observed to similar challenge and oral antigens in adult pigs. Together these results indicate a staged and advancing maturation of immune competence throughout the first month of life. While this indicates that postnatal piglet is capable of active immune responses to live virus and to dietary components at three weeks of age (Bailey *et al*., 1994), tolerance to dietary proteins takes up to eight weeks to be completed and then the reactivity to novel dietary components is reduced with age (Wilson *et al*., 1989; Miller *et al*., 1994). As mentioned above, disturbances to this staged and developing interaction as the appropriate responses to dietary proteins or commensal bacteria are regulated and consolidated over the first month of life coupled with the "trauma" of weaning may contribute to postweaning diarrhoea in early-weaned piglets.

Lymphocyte Homing

Reagents developed specifically for pig lymphocytes and adhesion molecules have been used to demonstrate that homing of effector T-cells and Ig+ cells to mucosal sites in the pig is comparable to rodents (see Bailey, 2009). To this extent, γδ T-cells generated in the gut mucosa rapidly recirculate as in sheep and sites of priming appear to influence recirculation patterns (see Bimczok and Rothkotter, 2006). To demonstrate the ontogeny of recirculation, expression of mRNA for the migration ligand CCL25 was higher in 30 day old compared to newborn piglet intestine, while levels of CCL28 were low in newborns but higher in intestines than in lungs in older piglets (Meurens *et al*., 2006). The receptors for ligands CCL25 and CCL28, that bind to homing receptors CCR9 and CCR10, respectively, were expressed in multiple piglet mucosal sites especially MLN, and were highest by 30 days of age (Meurens *et al*., 2006). The findings are consistent with the developing migratory capacity potential of leucocytes in the piglet mucosal immune system during the first month after birth, particularly those receptors on migratory cells in the adaptive immune system to disseminate gut immune responses.

Development of the Microbiome - Importance and Relation to Mucosal Development

Establishment of the Microbiome

By young adulthood, the microbiological content of the mammalian gut comprises around 10^{12} viable bacteria per gram of colonic content, consisting of 500–1000 microbial species (Mazmanian *et al* ., 2005). Repeated pyrosequencing (bTEFAP) of faeces from piglets starting from 21 days of age has predicted over 800 bacterial species in the ileum (Dowd *et al*., 2008). In humans, the phenology of the microbiome has been proposed in the etiology of asthma and atopy, through modulation of the innate immune response (Bjorksten, 2004; Vael and Desager, 2009).

Recent metabolic profiling approaches in several disease processes in humans including hypertension, ischemic heart disease, diabetes and obesity (lifestyle diseases?) have implicated the intestinal microbiome in determining the metabolic response of the host to environmental stimuli and consequent disease (see Kinross *et al*., 2011). In addition, the microbiome affects drug and nutrient metabolism, and may be predictive of adverse reactions (Sousa *et al*., 2008). The precision and specificity of similar analyses for resistance or susceptibility to infectious disease may be more challenging given the dynamic and transient nature of infections in the life span of the individual and its control by a mobile and specific acquired immune response. Early environmental exposures are key determinants of the adult gut microbiome and there does not appear to be a "core" gut microbiota defined by abundant organismal lineages in humans (Garrett *et al*., 2010). Turnbaugh *et al*. (2009) also concluded that a core microbiome based on species or strain data may not be present in humans, as no bacterial phylotype was detectable at a sufficiently high frequency in 154 human gut samples. By comparison, phylogenetic and metabolic similarities between the metagenomes of dogs, cats and mice (all monogastrics) have been reported from hierarchical clustering analyses (Kinross *et al*., 2011), thus permitting for pigs, extrapolation or "piggy-backing" of the results from research in other species. For pigs, it needs to be appreciated that the microbiome may be site/ litter/ housing dependent within the limits of the current technical approaches. This may have interesting consequences for endemic pathogens/diseases on different farms.

The microbial flora of the immediate environment is critical during the early colonisation of the gut, with bacteria being transferred from feed and the mother's vagina, GIT, mammary glands and skin (Pedersen *et al*., 1992; Mandar and Mikelsaar, 1996; Lindberg *et al*., 2004). Using DGGE with 16S rRNA gene eubacterial primers, Thompson *et al*. (2008) found that from the first two weeks of life, the faecal communities of cohabiting piglets became significantly more similar over the ensuing two weeks, and differed from those found in separated siblings. This establishment of a relatively stable microbiome occurred in concert with the development of the gut and competency of the local acquired immune response, the latter evidenced by the initial detection of faecal IgA at four weeks of age (Thompson *et al*., 2008). In another study, Inman *et al*. (2010) reported that siblings raised on the dam possessed a more diverse microbial flora at 21-28 days than piglets reared in (hygienic) isolators, while dendritic cells accumulated more slowly and T-cells made more IL-4 under the higher bacterial challenge of the maternal environment. However, once established, the local gastrointestinal "ecosystem" appears to resist immigrants, as the therapeutic "transplantation" of heterologous microflora appears to be predominantly rejected over a period of three to six months in human recipients (Kinross *et al*., 2011).

Microbial colonization of the small and large intestine is influenced by cell types and distribution, substrate availability, luminal mucus and the mucins in the glycocalyx of epithelial cells (Kelly and King, 2001). Luminal mucus contains mucin glycoproteins, antimicrobial molecules and secreted immunoglobulins, which are produced respectively by goblet and Paneth cells in the epithelium and B lymphocytes in the lamina propria. There is an inner and outer layer of mucus in the lumen, with concentrated antimicrobials in the inner layer to protect the epithelium, and a thicker outer layer with lower antimicrobial concentrations to allow commensals to survive in the lumen. Pathogens are able to breach the mucus barrier through motility or enzymatic degradation of the mucus, but APC, T lymphocytes and granulocytes secrete cytokines and other factors that modulate the production, constituents, biophysical properties and release of mucins to protect the host cells (McGuckin *et al*., 2011). Cytokines produced by T helper 1 and 2 cells (including IL-1β, IL-4, IL-6, IL-9, IL-13, IFN-γ and TNF-α) can increase mucus production, upregulate mucin expression and induce goblet cell hyperplasia in response to parasitic and bacterial infection (Artis *et al*., 1999; Dabbagh *et al*., 1999; Enss *et al*., 2000; Andrianifahanana *et al*., 2007; Sugimoto *et al*., 2008). Reduced viscosity or thickness of the mucus layer, caused by infection, can lead to reduced concentrations of antimicrobial molecules and secretory IgA in mucus, reducing the protective capacity of the intestine.

The intestinal microbiome is dominated by four operational bacterial phyla comprising Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria (Kinross *et al*., 2011). In humans, the composition of these phyla has been found relatively specific and stable in an individual over a two year period (Kinross *et al*., 2011). With the results from pigs (Thompson *et al*., 2008), this implies that each host has a unique biological relationship with its gut microbiota. The gut microbial flora contributes substantially to the total metabolic products (the metabolome) of the intestine, especially in the production of short-chain fatty acids essential for ileal function and epithelial integrity and repair (Scheppach, 1994).

The Microbiome, Probiotics and Mucosal Immunity

In addition to the provision of nutrients and metabolic activity, increasing evidence indicates that the microbiome influences the activity and maturation of the innate immune system through interactions with TLRs (Fukata and Abreu, 2007). Germ-free neonates in many species suffer retarded gastrointestinal development and function and are more susceptible to infection (Shanahan, 2002). Using TLRs and NOD-like receptors, epithelial cells, M cells,

and DCs can directly sense the intestinal contents and microbial products (pamps, LPS, lipopeptides and nucleic acids) and communicate information about the microbiota to other subsets of immune cells (Garrett *et al*., 2010). In mice, intestinal colonisation of germ-free recipients with Bacteroides fragilis restored normal levels of splenic CD4+ T-cells through the effects of bacterial (capsular) polysaccharide (Mazmanian *et al*., 2005). To emphasise the higher order interaction, mice deficient in TLR5 develop an altered microbiome and consequent clinical signs consistent with metabolic syndrome (altered fat metabolism) in humans (Vijay-Kumar *et al*., 2010). However, because of the close proximity and high density of pathogen-associated molecular patterns ("pamps") to intestinal epithelium, several mechanisms including reduced TLR expression, tolerance and antibacterial peptide secretion have also evolved to regulate any deleterious gut inflammation in the absence of pathogens (Abreu *et al*., 2005). In this respect, development of inflammatory responses including delayed-type hypersensitivity (DTH) and cytotoxic T-cell activity in the intestinal epithelium are reduced and more potent in lamina propria and GALT, especially when the mucosal integrity is breached (Davies and Parrott, 1981; Stokes *et al*., 2001; D.Emery, unpublished).

During the early development of porcine immune responses, reactivity to intestinal commensal bacteria has been documented. Jejunal explants from mature pigs cultured in vitro for 6 h produced IgM and IgA in relatively equivalent quantities *de novo*, much of which demonstrated anti-glycosyl activity and was ostensibly activated by gut microbes (Hansen *et al*., 2006). Blood and saliva samples collected from 21 day-old piglets already contained IgA reactive with *Lactobacillus rhamnosis* (an approved probiotic), arising from responses to commensal species by this time, while feeding *L. sobrius* stimulated specific IgA within two weeks (Casini *et al*., 2007) and prevented *E.coli* K88+ attachment (Roselli *et al*., 2007). Trevisi *et al*., (2007) detected *Bifidobacterium spp* DNA in liver extracts from five week old pigs that had been fed $10^{7\text{-}11}$ bacteria daily for two weeks after weaning, but it was not determined if intact bacteria had traversed the intestinal epithelium. While interactions between commensal organisms and the developing immune system appear well documented, the concomitant diminution of responses to dietary antigens (oral tolerance) also occurs (Bailey, 2009). In the practice of feeding probiotics to promote immune development and coverage, as well as to replace antibiotics in feed, the evidence would suggest that their effect could be optimised in late gestational sow diets and in the first month of pig's lives as the gut microflora and its interactions with the immune system and gut development becomes established. Probiotic strains of *Lactobacillus acidophilus* and *Bifidobacterium lactis* fed to pregnant sows seven days pre-farrowing were transferred vertically, even though the pigs were removed from the sow immediately after delivery (Buddington *et al*., 2010). The probiotic strains were present within 24 hrs and colonized the GIT of the majority of neonates for at least two weeks. However, colonization and post-weaning persistence of probiotic strains in the GIT was variable between neonates.

Immunomodulation by Commensal Bacteria and Dietary Components

Commensal bacteria are also vital for modifying the immune response. Intestinal isolate *Lactobacillus johnsoni* induced mainly TGF-β in human CaCo-2 cells while pathogenic ETEC initiated transcription of TNF-α (Haller *et al*., 2000). Although a "cultured cell", this confirms that intestinal epithelial cells can synthesise those regulatory cytokines ascribed to the innate immune system and are therefore part of it (Abreu, 2010). Repopulation of IL-10 gene-deficient mice with *Lactobacillus sp* have been shown to correct the cytokine deficiency (Haller *et al*., 2000), arguing for direct immunoregulation by these intestinal commensals. The induction of TGF-β has important implications for the local development of T-reg cells (Fontenot *et al*., 2003) which reduce expression of inflammatory reactions in the gut. Commensal bacterial signalling through TLRs on epithelial cells appears to induce cytokines such as TGF-β which dampen gut Th1 and Th-17 responses in mice (Zaph *et al*., 2008). In mice, binding of bacterial pamps with enterocyte TLRs promotes extension of processes from dendritic cells in the lamina propria between enterocytes in the villus (see Bailey, 2009) and engagement of Paneth cell TLR promotes increased synthesis of anti-microbial peptides including α-defensin and lectins (Abreu, 2010).

The regulation and interplay of all gut components (epithelium, GALT, innate and adaptive immunity) are relevant to the use of probiotics to combat pathogens and to mitigate "weaning stress", especially in gilt progeny. In this regard, the probiotic *Bifidobacterium infantis* was examined in the rat maternal separation model where offspring treated with *Bifidobacteria* or citalopram exhibited a "normalised" immune response, reversed behavioral deficits after maternal separation, "motivation" and restored concentrations of noradrenaline in the brainstem (Desbonnet *et al*., 2010). Other clinical trials with *Bifidobacterium spp*. and *Lactobacillus spp*. have reportedly improved mood and reduced stress symptoms in human patients with chronic fatigue syndrome and inflammatory bowel syndrome (Rao *et al*., 2009). Modification of immune reactivity using probiotics has also been described in pigs. Neonatal piglets dosed orally with 109 CFU *Lactobacillus lactis* probiotic daily from one to seven days of age, then weekly to 35 days showed reduced skin responses to allergic sensitization with ovomucin when challenged at 45 days: this was accompanied by increased levels of antigen-specific IgG1, IgG2 and IgE (Rupa *et al*., 2011).

Dietary components may act directly or indirectly to modulate immunity. Oral administration of the TLR2 ligand, Tri-palmitoyl3CSK4 protects mice against dextran-induced colitis by induction of trefoil factor 3 from goblet cells (Abreu, 2010). In pigs, feeding of pregnant gilts and their progeny with n-6 (safflower oil) polyunsaturated fatty acids (FAs) resulted in significant reductions in birthweight, liveweight gain, feed intake and health to finishing compared to pigs fed n-3 FAs (fish oil extracts) or saturated fats (Wilkinson *et al*., 2011). This was attributed to the establishment of a "pro-inflammatory" milieu in the piglet gut emanating from n-6 precursors for the production of ecosanoids and leucotrienes (Innis and Jacobson, 2007). Postnatally and possibly during gestation, these could have affected the intestinal integrity, the quality of the microbiome and the metabolome of the intestine (R. Newman, *pers. comm*.). It was noted that the cull/mortality rate was 13-fold higher in the piglets on the n-6 diet when housed conventionally, but that substitution of n-6FAs at weaning (4 weeks) with saturated FA produced equivalent weights in all treatment groups by 19 weeks of age (Wilkinson *et al*., 2011). Although changes to the microbiome were not assessed in the study, the outcome is consistent with reports that gut inflammation is ameliorated by n-3 FAs (Innes and Jacobsen, 2007).

Dietary fibre, including resistant starches (RS) and non-starch polysaccharides (NSP) are fermented by bacteria in the large intestine producing short-chain fatty acids (SCFAs). Resistant starches fed to rats increase concentrations of butyrate, acetate and propionate in the caecum and colon, contributing energy to the host and playing a role in maintaining intestinal health. Dietary resistant starches in combination with the probiotic *Bifidobacterium lactis* were able to protect rats against coloreactal cancer (Le Leu *et al*., 2010). SCFAs bind to the G-coupled-protein-receptor 43 and in a mouse model of dextran-induced colitis where oral acetate reduced the local inflammation (Maslowski *et al*., 2009). In addition, SCFAs can directly inhibit the growth of many pathogens such as *E.coli* and *Clostridium perfringens* that prefer neutral or slightly alkaline environments (Wang and Gibson, 1993) and SCFAs have been added to weaner diets as alternatives to antibiotics. Resistant and butyrylated starches fed to rats modified the dominant faecal microbiota to a potential probiotic *Lactobacillus sp* (Abell *et al*., 2011).

Significant increases in liveweight gain, jejunal villus height and crypt depth and caecal concentrations of SCFAs were noted in early weaned piglets fed sodium gluconate (microbial fermentation of glucose), mannan (NSP) or potassium diformate (organic acid) for 16 weeks (Poeikhampha and Bunchasak, 2011) and maintenance of the microbiome was a possible mechanism. As the technology becomes more available, the effects of dietary and medical manipulations of the porcine microbiome will allow a more holistic approach to amelioration or prevention of disease in the weaner piglet.

Dietary modulation of the intestinal microflora will also affect host protection through changes to the mucus layer and the intestinal epithelium. Diets high in phytate (soybean meal with high NSP) fed to poultry are reported to cause hypersecretion of gastric and intestinal mucin (Cowieson *et al*., 1994). Cowieson *et al*., (2009) suggested that phytate bound to and reduced solubility of proteins at low gastric pH, requiring increased secretion of HCl and pepsin for digestion. The increased endogenous amino acids from mucin would be lost in the faeces unless re-absorbed before entering the ileum. The increase in unabsorbed amino acids in the small intestine could lead to increased microbial fermentation in the large intestine and changes in the intestinal microbiome and immune status. In this respect, supplementation of high-phytate (0.44%) poultry diets with 500 phytase units (FTU)/kg of feed significantly ($P<0.05$) increased the percentages of $CD4^+$and $CD8^+$ T lymphocyte subsets and specific antibodies against Newcastle disease virus three to four weeks after intranasal or intraocular vaccination. In comparison, 500 FTU phytase significantly ($P<0.05$) increased the total levels of intestinal secretory IgA for the initial three weeks after poultry were fed diets either high or low in phytate (Liu *et al*., 2008).

The incidence and severity of enteric diseases in pigs can be influenced by fibre in the pig's diets. Diets low in soluble non-starch polysaccharides (sNSP) and resistant starches (RS), such as cooked rice, can prevent or reduce the severity of swine dysentery and intestinal spirochaetosis (Siba *et al*., 1996; Pluske *et al*., 1996; Pluske *et al*., 1998; Hampson *et al*., 2000). Conversely, diets high in sNSP and RS increase the viscosity of the digesta, reduce pig growth and encourage microbial fermentation in the large intestine. The addition of carboxymethylcellulose (CMC) to a low sNSP diet increased the viscosity of the digesta and increased the duration of *Brachyspira pilosicoli* faecal shedding. Pigs fed the diet containing CMC were also more susceptible to a natural haemolytic *E.coli* challenge with significant increases in proliferation and faecal shedding of haemolytic *E.coli* (McDonald *et al*., 2000). The severity of swine dysentery was exacerbated (mucohaemorrhagic) by both diets high in NSP (ethanol extracted and heat treated soybean meal and hulls) and stress associated with group housing (Jacobsen *et al*., 2004).

The effect of dietary fibre on proliferative enteropathy (PE) is less clear. Diets low in soluble NSP and relatively high in insoluble NSP (including 10% distillers dried grain with solubles (DDGS)) reduced the severity and incidence of PE in pigs challenged with moderate doses of *L.intracellularis*, but increased PE severity in pigs challenged with higher doses of *L.intracellularis* (Whitney *et al*., 2006a,b).

Weaning

Feral piglets are normally weaned at three to four months of age whereas farmed piglets are usually weaned between two to four weeks old. Weaning is a time of increased pig morbidity and mortality and disturbances to the microbiome often occur in this period (Kelly and King, 2001). Weaning procedures impact differentially on piglets in timing, duration and severity. These include infection with endemic pathogens (enteric bacteria such as *E.coli*, *Salmonella sp*. and *L.intracellularis*, and rotaviruses); changes to housing and temperature, diet causing inappettance, the stress of removal of the sow and loss of milk, nurture and mixing with other pigs, altered microenvironment of the gut anatomy and function (Pluske, 2001). Dietary changes inducing villus atrophy and crypt hypoplasia lead to reduced nutrient absorption with concomitant changes in enzymic profiles (Pluske, 2001). As one example, piglets weaned at one, two and three weeks of age and measured at 28 days for serum IgA and intestinal cytokine mRNA revealed significant early reductions in serum IgA in the early weaning groups that was rectified by d 28; cytokine mRNA showed no consistent trends and FoxP3 (T-reg cells) was highest in piglets weaned at 28 days (Levast *et al*., 2010).

To maintain growth and productivity in commercial piglets, a range of nutritional supplements have been trialled. Most of these provide energy supplementation to facilitate all facets of growth (including immunity) or reduce/change microbial profile, particularly pathogens. For example, addition of 1.8% Formi™-LHS (potassium diformate; Addcon Group, Bonn, Germany) to diets reduced the counts of total anaerobic bacteria, lactic acid bacteria and yeasts throughout the GIT without affecting pH (see review by Stein, 2007). The effect was speculated to arise from the activity of anionic and proton (oxidant) products from formic acid metabolism within the bacteria which then disrupt bacterial protein synthesis (Canibe *et al*., 2001).

The effect of dietary organic acids on the control of proliferative enteropathy (PE) is less clear. Lactic acid (2.4%) and formic acid (1.8%) reduced microscopic lesions of PE, but did not reduce faecal shedding of *L.intracellularis* nor significantly improve growth performance (Boesen *et al*., 2004). With the advent of non-culture methods to analyse the gut microbiome and metabolome, greater accuracy to document the effects of various supplements with adverse or beneficial outcomes on growth, immunity and the microbiome will be possible (see Kinross *et al*., 2011). This will enable the costs of infectious diseases to be determined or modelled with more precision as well.

The Pathogenesis of Infectious Disease

Once established around three weeks of age, the microbiome (total microbial flora) of the intestine or lung and the mucosal epithelium have been described as acting similarly to an ecosystem that is refractory or antagonistic to the establishment of new invaders unless compromised. In addition, it is recognized that small numbers of commensal bacteria probably traverse the mucosal epithelium under normal conditions (Abreu, 2010). The continuous cellular division (in crypt region of epithelium), migration, maturation and desquamation of cells into the lumen together with mucus production helps to limit pathogens from colonizing the epithelium (Gaskins, 1998). However, microbial pathogens may gain access indirectly to target cells or organs by changes to the gut environment as occurs with changes in nutrition, host physiological status (eg. stress and weaning, gut stasis), or gut microflora with antibiotic treatment and interactions between these. Microbial or protozoal pathogens must "gain a foothold" to multiply, invade and produce disease. Most actively achieve this by:

- Binding to specific receptors to gain entry to cells or create local damage (*E.coli* F18 and F4 [K88] fimbriae, intimin and Cholera toxin CTB, endotoxin-LPS; Pizzaro-Cerda and Cossart, 2006);
- Producing exotoxins which devitalize regional cells or defences to enable establishment of a local niche (*E.coli* enterotoxin; Finlay and McFadden, 2006);
- Penetration of the mucus layer in the large intestine and microerosion of the epithelium post attachment (*Brachyspira hyodysenteriae*);
- Inhibiting normal cellular processes such as lysosome fusion (*Mycobacteria*) or apoptosis (*Mycobacteria, Cryptosporidium sp*); or,
- Subverting (Type-3 secretory systems: Cornelius, 2006) or disrupting host responses (*Yersinia sp* "invasin" protein) or taking advantage of host inflammation and disruption to enable cell entry (eg. *Salmonella:* Brown and Price, 2008), and *Balantidium coli*.

Decline in Passive Immunity and Susceptibility to Disease

During the course of lactation in pigs, IgA constitutes over 50% of total maternal antibodies by the end of the first lactation week and 80-95% by mid lactation (Butler and Kehrli, 2005). Large epithelial cells filled with fat globules, secretory components and sIgA make up over 60% of the total cell count of sow milk (Le Jan, 1996), contributing to both the increased fat content of milk and the high concentration of sIgA. After weaning, pigs around three to eight weeks of age are susceptible to a number of opportunistic mucosal pathogens because of various combinations of environmental and nutritional stress (Stokes *et al*. 2004), the decline in passively-acquired maternal immunity and the ongoing maturation of immune competence described above. The half life of maternal antibodies in neonatal piglets varies, (IgM and IgA, 3-4 days; IgG, 6.5-22 days) depending on the isotype and antigen-specificity (Blecha, 1998). A reduction in mean antigen-specific titres to *Lawsonia intracellularis* in piglet serum from three to six weeks of age was attributed to declining levels of passively acquired immunoglobulins (Holyoake *et al*., 1994). The loss of passively acquired immunoglobulins is more likely to be due to weaning than to the sow's inability to continue production and transfer of specific antibodies in milk. Piglets weaned from sows recently infected with *L.intracellularis* were susceptible to an experimental challenge, while litter mates that remained suckling on the sow were protected from challenge (Pozo *et al*., 2000). The most common pathogens causing post-weaning intestinal disease in pigs include *S.typhimurium*, *E.coli*, *Brachyspira hyodysenteriae* (swine dysentery), *B.pilosicoli* (intestinal spirochaetosis) and *L.intracellularis* (Moxley and Duhamel, 1999).

Acquired Immunity to Enteric Pathogens

The duration of antibody-mediated memory is often limited, requiring re-exposure to antigens (natural exposure or booster immunization) for the recruitment of new clones to increase the longevity of the memory response. Generally, immune memory persists longer if the initial clonal expansion is larger following a more "severe" first encounter with the pathogen, or higher doses of antigen are given (Beverley 2002). While many natural infections do not stimulate a durable immunity, both humoral and cell-mediated responses appear to be important for protection against most intracellular pathogens (Casadevall, 1998). Antigen-specific antibodies neutralize virulence factors such as LPS and toxins from pathogens such as *Listeria monocytogenes* and *Rickettsia spp*, rotavirus and TGEV (Casadevall 1998; Mims *et al*., 2002; Snoeck *et al*. 2006) and block motility or attachment of *Salmonella* and *Chlamydia* to the intestinal epithelium (Casadevall, 1998; Snoeck *et al*., 2006). Antibodies may also contribute to the intracellular elimination by mediating cytolysis of bacteria-infected host cells or macrophages by NK cells or monocytes as has been reported for *Shigella spp* and *Ehrlichia chaffeensis* (Winslow *et al*. 2000). Additional mechanisms of cell mediated immunity are required for intracellular pathogens such as viruses, coccidial protozoans, mycobacteria and *L.intracellularis* and for larger nematode parasites. While antibodies may block pathogen attachment or establishment in a particular niche, activated NK cells, macrophages or specific cytotoxic T-lymphocytes (CTL) may be needed alone or in concert to destroy infected host cells through mechanisms such as perforin-mediated lysis or production of IFN-γ (Beverley, 2002).

Induction of Mucosal Immunity by Vaccination (The *Lawsonia intracellularis* Model)

Strategies for the induction of mucosal immunity in pigs have been reviewed (Roth and Thacker, 2006). The "holy grail of vaccinology" is knowledge of the protective mechanism to be induced by the vaccine- the "immune correlate" (Emery, 1996) to facilitate rational vaccine design, formulation and delivery. Understanding disease pathogenesis is critical to this process. For successful vaccination protocols and formulations, it is well documented that specific maternal antibodies may interfere with the induction of immunity. Compared to control pigs, Hodgins *et al* (1999) showed significantly lower levels of IgA and IgG antibody secretory cells (ASC) in intestinal and lymphoid tissue of gnotobiotic piglets given high titres of maternal antibodies to rotavirus via serum, colostrum and milk and a reduced immunity to a subsequent challenge infection. Strategies to avoid such interference include vaccination at 4-6 weeks after maternal antibodies decline, DNA-based vaccines (see Roth and Thacker, 2006) and utilizing dual vaccines with two recombinant antigens, one given prepartum to the sow and the other to the neonate as has been used against *Taenia ovis* in sheep (Rickard *et al*., 1995).

Lawsonia intracellaris

Progress in the formulation and delivery of a vaccine to induce protective immunity in the porcine mucosa is exemplified by infection with *L.intracellularis* (LI). The bacterium is a Gram-negative, obligate intracellular organism most commonly described in pigs but reported from a range of host species including hamsters, rabbits, deer, horses, birds, rodents and primates (Frisk and Wager, 1977; Drolet *et al*., 1996; Hotchkiss *et al*., 1996; Williams *et al*., 1996; Cooper *et al*., 1997; LeMarchand *et al*., 1997; Collins *et al*., 1999; Klein *et al*., 1999). In pigs, ingestion of LI causes proliferative enteropathy (PE) or ileitis: the proliferation of immature crypt cells containing LI in the apical

cytoplasm. Adenomatosis occurs primarily in the terminal ileum, but also in the large intestine, and is manifest as a non-haemorrhagic or haemorrhagic clinical forms (McOrist and Gebhart, 2006). The former affects weaner and grower pigs six to 20 weeks of age with variable clinical signs including anorexia, diarrhoea, poor growth and variable appetite (McOrist and Gebhart, 2006) and recovery within four to 10 weeks after infection. The second form is an acute proliferative haemorrhagic enteropathy (PHE) which predominantly affects young breeding stock and finisher pigs (4-12 months of age; Love *et al*., 1977). Commensal gut flora are speculated to influence both the colonisation of enterocytes by *L.intracellularis* (McOrist *et al*., 1994) and infections with *Brachyspira hyodysenteriae* (Smith and Lawson, 2001). However, the mode of entry of both pathogens into immature enterocytes remains unclear. The presence of *L.intracellularis* in loose membrane structures just below the micovillous border in rapidly dividing crypt cells suggested that LI infected mitotically active crypt cells and then spread through the epithelium via host cell division (McOrist *et al*., 1989). However, earlier examination of initial host-pathogen interactions showed that *L.intracellularis* entered villus enterocytes and the LP within 12 hours of infection, and these cells were not associated with markers of cell division (Boutrop *et al*., 2010a), *L.intracellularis* was also observed in close association with crypt cells within 3-6h after inoculation of a ligated ileal loop (Boutrup *et al*., 2010b). The proliferation of immature enterocytes infected with LI could be due to bacterial proteins interfering with host cell division or decreased apoptosis. Machuca *et al*. (2006) found increased apoptosis within two days of hyperplasia in PE affected pigs, indicating that the hyperplasia was not a result of decreased apoptosis. It's more likely that the increased apoptosis was related to clearance of LI and resolution of lesions. Crypt cells are derived from stem cells which undergo a limited number of cell divisions before they differentiate and move up the villus (Quaroni *et al*., 2000). It is possible that *L.intracellularis* delays or prevents differentiation of crypt cells either by inhibiting laminin induction of differentiation in the basement membrane of epithelial cells (Wolpert *et al*., 1999) or by modulating expression of the cyclin kinase inhibitors p21 and p27. Committed crypt cells express p21 which ultimately limits their proliferative potential leading to a growth arrest, increased expression of p27 and differentiation of crypt cells (Quaroni *et al*., 2000). Interference with the expression of either p21 or p27 could lead to the proliferation of crypt cells.

As expected biologically, disease severity is dose-dependent as oral dosing of pigs experimentally with 10^5 LI produces minimal clinical disease (Paradis *et al*., 2005; Collins and Love, 2007). Higher infective doses (10^{10}) result in pronounced lesions and clinical signs (Guedes *et al*. 2003), while 10^9 LI given orally as intestinal homogenate (Collins and Love 2007) or pure culture (Kroll *et al*. 2006) induced mild diarrhoea and reduced weight gains two to three weeks after infection. Protection from re-infection with *L.intracellularis* is observed following a significant primary challenge, with duration of immunity persisting beyond 14 weeks post primary challenge (Collins and Love, *pers. comm*.) The gross pathology comprises adenomatous proliferations of the intestinal mucosa primarily in the lower ileum and proximal colon with mucosal thickening but limited inflammation and paucity of goblet cells (McOrist *et al*. 1994). By analogy, one other enteric pathogen, *Citrobacter rodentium*, the causal bacterium of transmissible murine colonic hyperplasia (TMCH) induces similar distinctive epithelial hyperplasia, loss of goblet cells and minimal inflammation despite being non-invasive (Luperchio and Schauer, 2001). It has been speculated that these two pathogens may manipulate the TLR signalling pathways (Luperchio and Schauer, 2001), but intracellular osteopontin appears to mediate *C.rodentium*-induced cell hyperplasia (Wine *et al*., 2010). Porcine intestinal epithelial cells do not express MHC class II molecules, so that suboptimal brush border development and a lack of MHC class II receptors may contribute to the preferential colonisation of immature crypt cells and colonic epithelial cells by *L.intracellularis* (MacIntyre *et al*. 2003), as may the lack of T-cells in the IPP associated with the generation of the B-cell repertoire (Butler *et al*., 2009). The lack of inflammation during PE is reflected in reduced numbers of $CD8^+CD25^+$ T lymphocytes observed within the epithelium (IELs) and lamina propria, although increased numbers of activated macrophages have been reported in infected crypt cells (MacIntyre *et al*. 2003).

To define potential virulence determinants and vaccine targets, the genome of *L.intercellularis* has been reported to contain coding sequences for LsaA (for Lawsonia surface antigen) associated with attachment to and entry into cells and LscN, -O and -Q, homologues for components of the T3 secretory system (TS33) of *Yersinia spp*. (Pilar Alberdi *et al*., 2009). Type-3 secretion systems are found in intracellular pathogens such as *Shigella sp., Salmonella spp., Yersinia spp.* and *Chlamydia sp*. (Coburn *et al*., 2007), enabling translocation of effector proteins into host cells and contributing to pathogenicity (Cornelius, 2006). During infection with *L.intracellularis*, transcripts for LscN and LscQ were detected by RT-PCR and recombinant rLscQ was recognized by antiserum from infected pigs (Pilar Alberdi *et al*., 2009). The accumulation of IgA in crypt cell cytoplasm appears consistently amongst studies of the pathogenesis of *L.intracellularis*, which could immobilise intracellular antigens or bacterial metabolism (Casadevall, 1998) or prevent re-infection of crypt cells if secreted into the lumen.

Since the pathogenesis of LI infection involves intracellular parasitism and manipulation, activation of both innate and acquired immune effector systems would be expected to provide the most comprehensive protection against re-infection. To this end, a primary infection with reduced severity would activate all components envisaged for mucosal protection, including antibody to block attachment and cell entry, effector cells to block intracellular proliferation, metabolism and assembly, and also induce of host cell apoptosis to prevent LI development. In this process, vaccination against LI has been achieved using LI isolate B3903 with a dose of around 10^5 live attenuated whole organisms grown in McCoy cells and originally isolated from the ileum of a Danish sow with acute PHE (Kroll *et al*. 2004). Oral administration of a single dose ($10^{4.9}$ $TCID_{50}$/dose; Enterisol Ileitis™, Boehringer-Ingelheim GmBH, Ingelhein, Germany) caused a significant reduction ($P<0.05$) in the amount of primary gross and microscopic lesions in the ileum of vaccinates (Kroll *et al*., 2004), and improved grower uniformity and average daily liveweight gains (ADLG) compared to non-vaccinates. Current studies of immune responses report production of LI-specific IgG and IgA to whole cells, LPS (Kroll *et al*., 2004) LsaA (McKluskey *et al*., 2002), 3 outer-membrane proteins (OMPs: 19/21, 37 and 50kDa) (Jacobs *et al*., 2009) and LscQ (Pilar Alberdi *et al*., 2009) as well as induction of IFN-γ during infection (Geudes and Gebhart, 2003) and following antigenic stimulation of sensitised PBMC from vaccinated or immune piglets (Riber *et al*., 2011).

Induction of mucosal immunity has often been considered dependent upon mucosal presentation of antigen due to migratory patterns of activated systemic and mucosal lymphocytes. However, systemic vaccination has been shown to induce high serum titres and ruminant IgG1 can be secreted into the intestinal lumen through concentration gradients between blood and gut tissue (Husband *et al*., 1996). Mucosal and systemic immune responses could be generated in ruminants and rodents by intraperitoneal (IP) inoculation of adjuvanted antigen for which inflammation was essential (Husband *et al*., 1996). Given the success of systemic vaccination against other intracellular, mucosal pathogens such as *Salmonella enteriditis* using OMPs from killed bacteria (see Charles *et al*., 1994), protection against LI at mucosal surfaces should also be feasible. From this theoretical background, Dale *et al*. (1997) reduced faecal LI counts by 98.5% in four pigs vaccinated twice intramuscularly (IM), three weeks apart, with killed LI in incomplete Freunds adjuvant. Pigs inoculated with recombinant GroEL-like protein also showed reduced faecal bacterial counts compared to infected controls and both groups were free of LI and pathology at necropsy 21 days after a challenge infection (Dale *et al*., 1997). More recently, Jacobs *et al*. (2011) induced significant protection against LI challenge after 2 IM inoculations four weeks apart, of 2.8×10^8 killed LI in oil adjuvant and significant reductions in LI shedding (but not pathology) at 21 days after infection by vaccination (IM) with 50μg each of recombinant 19/21 and 37kDa OMPs in adjuvant. Significant protection was also achieved by IM inoculation of piglets at three and 25 days of age with similar adjuvanted vaccines containing 5×10^7 killed LI or putative LPS (carbohydrate) from 2.8×10^8 LI (Jacobs *et al*., 2011). In contrast, a vaccine containing 1.25×10^7 killed LI was not protective (Jacobs *et al*., 2011). These inactivated vaccines indicate that with sufficient antigenic stimulus, antibodies against LI membrane components can protect against infection, presumably by preventing intracellular infection as evidenced the heightened serological titres at challenge and the lower quantities if LI as determined by faecal PCR one week later (Jacobs *et al*., 2011). The lesser performance of the recombinant proteins may be elevated by increased antigenic doses or using different adjuvant formulations, but the bacterins are complicated by the need to use a total of 10^8 killed LI per pig, two vaccinations and the technical problems and costs associated with LI culture. However, the developments highlight research towards an understanding of pathogenesis, protective immunity and successful vaccination against an important economic pathogen for pig production and the possibility of combined vaccines with recombinant antigens to reduce costs.

Conclusion

In the piglet, the first four weeks after birth reveals an amazing interaction between developmental changes in the porcine gut and GALT, establishment of the microbiome and gut ecosystem and functional maturation of the digestive process and immune competence. Also becoming apparent is the variety of mechanisms by which pathogens can subvert or modulate this process, which is important knowledge for generating any integrated control program. An understanding of disease pathogenesis in the context of the gut ecosystem assists with identification of protective mechanisms and not simply involves generic extrapolations from immune responses against various viral, lumenal or intracellular pathogens. Knowledge of protective responses also enables identification of those microbial components which induce protection and most optimistically, new means to enhance the formulation and delivery of vaccines to mucosae in young animals. There is an urgent need to replace antibiotics in feed, but this cannot be achieved unless proven alternative means are available and more effective vaccines are one option with a proven, cost-effective, track record. This has been demonstrated with the approach to vaccination against a variety of pathogens including *E.coli* and *L.intracellularis* in pigs and coccidiosis in chickens.

Alternatively prebiotics may enhance gut function or "chemically" control pathogens. Most prebiotics and probiotics must be given continuously. Whether particular probiotics could establish in the piglet microbiome if given during initial colonisation in the first four weeks postpartum needs investigation as these might modulate immunity to, or establishment of, endogenous pathogens (rather than "lifestyle" or autoimmune diseases in humans) and optimise growth and reproduction in pig production. Alternatively, if probiotics given during late gestation establish amongst the intestinal flora of progeny (Buddington *et al.*, 2010), these might influence the suboptimal performance of gilt progeny, decrease weaning stress or reduce the impact of particular pathogens (such as *L.intracellularis*, *E.coli* or Salmonella) that are endemic in particular industry herds.

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Immune System Stimulation in the Pig: Effect on Performance and Implications for Amino Acid Nutrition

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Abstract

Even in well managed pig production facilities pigs are routinely exposed to disease-causing pathogens that negatively impact animal productivity. It has been estimated that the cost of disease represents a loss of $USD640 million per year for the United States swine industry alone. Immune system stimulation (ISS; disease) alters nutrient metabolism and utilization and, thus, imposes qualitative and quantitative consequences for the development of optimum feeding strategies. ISS alters the animals' physiology and metabolism via a complex system involving innate and adaptive immune response, a number of cytokines and acute phase proteins, as well as the central nervous system. Daily requirement for dietary energy is decreased during ISS, mainly due to reduced energy requirement for growth and activity. During ISS and amongst the amino acids (AA), glutamine, arginine, aromatic amino acids (especially tryptophan), threonine and the sulfur containing AA (SAA; methionine and cysteine) deserve special consideration. For some of the metabolic demands for these amino acids may be increased during ISS, without impacting dietary requirements. However, there is evidence to suggest that the immune system's needs for tryptophan and SAA are increased and impact the optimum dietary amino acid balance during ISS.

Introduction

Even in well managed pig production facilities pigs are routinely exposed to disease-causing pathogens that negatively impact animal productivity. It has been estimated that the cost of disease represents a loss of $USD640 million per year for the United States swine industry alone (USDA, 2008). It is well established that exposure of animals to disease-causing pathogens results in cellular and metabolic events that modifies metabolism and nutrient utilization. In humans and animals, interestingly, there is a common pattern of response to immune system stimulation (ISS; infectious disease; Reeds and Jahoor, 2001; Obled, 2003). Alterations in metabolism are brought about by the pro-inflammatory cytokines, interleukin (IL) 1β, IL-6 and tumor necrosis factor (TNF)-α, which are produced primarily by stimulated mononuclear myeloid cells, adipocytes and myofibers (Jacobi *et al.*, 2006). Major metabolic alterations involve activation of the hypothalamus to induce sickness behaviour, changes in release of hormones (eg. glucocorticoids, leptin, glucagon, insulin, insulin-like growth factor-1 (IGF-1)), reduced protein accretion in skeletal muscle, increased protein synthesis in visceral organs, and increased lypolysis in adipocytes (Johnson, 1997; Klasing and Leshchinsky, 2000; Orellana *et al.*, 2004; Buchanan and Johnson, 2007). The consequence of these alterations is the redirection of nutrients from growth and reproduction toward processes important for an immune response, thereby reducing the animals' productivity. Studies with growing pigs have demonstrated that sub-clinical levels of ISS reduces lean tissue growth by 20 to 35% and feed efficiency by 10 to 20% (Williams *et al.*, 1997a; Williams *et al.*, 1997b; Le Floc'h *et al.*, 2009). In addition, exposure of farm animals to disease causing agents tends to increase on-farm usage of antibiotics that increases the risk of antibiotic residues in products and the development of antibiotic-resistant pathogens (Barton, 2000). It has been shown that by manipulating the immune response of growing pigs during ISS – by blocking receptors to the key inflammatory cytokine IL-1β – the negative impact of disease on carcase protein can be reduced (Table 1; Dionissopoulos *et al.*, 2006). The latter suggests substantial opportunity to reduce the impact of ISS on animal productivity.

Reduced protein gain in skeletal muscle during ISS is the result of reduced protein synthesis, and increased protein degradation which creates an increased flow of amino acids (AA) to visceral organs for gluoconeogenesis and synthesis of proteinaceous and non-proteinaceous immune system metabolites (Reeds and Jahoor, 2001). These disease-induced quantitative and qualitative changes in protein synthesis have consequences for dietary nutrient requirements and for amino acids (AA) in particular (Obled, 2003).

It has been suggested that daily requirements for dietary energy in growing pigs is decreased during disease, largely because of disease-induced reductions in the animals' energy requirement for growth and activity (van Heugten *et al.*, 1994; van Heugten *et al.*, 1996; Spurlock *et al.*, 1997). However, there is evidence to suggest that the immune system's needs for specific AA is increased during disease and that additional dietary intake of specific AA can alleviate the negative impact of disease on protein gain in growing pigs. Disease and ISS do not impact lysine utilization

efficiency, and changes in lysine requirements reflect changes in body protein gain (Williams *et al.*, 1997a; Klasing and Barnes, 1988). In this review, the impact of a mild or sub-clinical level of disease (ISS) on the physiology and productivity of growing pigs is discussed and some recommendations are made about altering AA nutrition of pigs during systemic ISS. Information on the impact of ISS on requirements for vitamins, minerals and essential fatty acids and the potential application of feed additives to manipulate the pig's response to ISS can be found elsewhere (Klasing and Leshchinsky, 2000; Klasing, 2007).

Table 1. *Effect of immune system stimulation and administration of interleukin 1 beta antagonist (IL-1β ra) on carcase characteristics of growing pigs*[1].

	Treatments					
	Healthy control		ISS control		ISS+IL-1ß ra	
	Mean	SE	Mean	SE	Mean	SE
Number of pigs	7		7		6	
Carcase weight (CW; kg)	12.2[b]	0.28	12.2[b]	0.26	13.6[a]	0.30
Protein mass (g/kg CW)	179	2.1	179	1.9	179	1.9
Lipid mass (g/kg CW)	56.1	5.4	55.4	5.4	61.6	6.1
Lipid:protein	0.33	0.03	0.33	0.03	0.37	0.04
Retained carcase protein (g/d)	34.5[b]	2.89	32.4[b]	2.89	44.3[a]	3.27
Retained carcase lipid (g/d)	-1.09	3.13	-1.79	3.13	5.84	3.55

[ab]Means within a row with different superscripts differ significantly (P < 0.05). [2]Internasal inoculation of *Mycoplasma hyopneumoniae* was used to induce ISS. Pigs in ISS+IL-1ra group were infused with 2.0 mg/kg /h IL-1 ra for 28 days. Pigs in healthy control and ISS control groups were infused with saline. SE, standard error. Adapted from Dionissopoulos *et al.* (2006).

Immune System and Immune Response

All vertebrates have an immune system capable of distinguishing endogenous from foreign molecules and inactivating foreign molecules in order to maintain the host's integrity (Colditz, 2002). There are two main aspects of the immune system in vertebrates: i) innate and ii) adaptive (Colditz, 2002). Innate immunity represents a rapid and non-specific first line of defense that can mobilize in minutes to hours, and is based on detection of pathogen-associated molecular patterns (PAMPs) that summon an inflammatory response. Inflammation is one of the first responses of the immune system to infection or injury and includes processes that direct immune system components to the site of infection or injury to ward off noxious influences and initiate the process of healing (Elgert, 2009). Innate immunity involves a number of different cell types, most importantly those of the mononuclear phagocyte lineage. These cells express receptors (eg. toll-like receptors; TLRs) that recognize a wide range of molecular patterns foreign to the mammalian organism (Iwasaki and Medzhitov, 2010). Ligation of TLRs transmits signals that activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and mitogen activated protein kinase (MAPK) pathways and, thus, induces expression of a wide variety of genes like those encoding proteins involved in production of reactive oxygen species and phagocytosis, and in eliciting the production of pro-inflammatory cytokines that augment inflammation (Hansson *et al.*, 2002; Iwasaki and Medzhitov, 2010).

Adaptive (acquired) immunity represents highly specialized systemic cells and processes that prevent or eliminate pathogenic challenges and can mobilize in days to years. This system involves T and B lymphocytes, and employs antigen receptors that are not encoded in the germ line but are generated *de novo* in each organism by somatic rearrangement. Once T cells are exposed to foreign antigens, they initiate adaptive immune responses that are specific against unique antigens (Iwasaki and Medzhitov, 2010).

Various species differentially emphasize innate and adaptive arms of the immune system due to their relative metabolic costs and protective values. For instance, species with short life spans (eg. birds) are considered to have their immune system balanced more towards innate immunity, while species with a longer life span (eg. pigs) have greater tendency towards investing in adaptive immunity (Lee, 2006). In general, upon the exposure to an antigen, the metabolic costs to develop innate immunity are low, but the metabolic costs of using it are relatively high. In contrast, the metabolic costs of developing the adaptive immunity is high but the costs of using it is low (Lee and Klasing, 2004; Lee, 2006).

Mediators of Innate and Adaptive Immunity

The molecular mediators of both innate and adaptive immunity are called cytokines and consist primarily of proteins. Cytokines are not only the core of communication between immune system cells but also induce responses in non-immune system cells (Klasing and Leshchinsky, 2000). Based on their role in inflammation, cytokines can be classified as pro-inflammatory and anti-inflammatory. The former induces and the latter suppresses inflammation. Pro-inflammatory cytokines are pleiotropic because they can mediate different functions in various organ systems (Johnson, 1997). Release of pro-inflammatory cytokines primarily from stimulated mononuclear myeloid cells initiates the immune response (Iwasaki and Medzhitov, 2010).

Acute-Phase Response

An immune response can be described as specific shifts in cellular and metabolic processes as well as behaviour, orchestrated by the immune system in response to variety of stimuli (Klasing and Leshchinsky, 2000). The changes in physiology of the host are most obvious during the initial stages of the immune response, which is usually denoted as the "acute-phase response". When these changes have become persistent, then the immune response is considered "chronic" (Colditz, 2002). The immune response to a pathogen depends on both innate and adaptive immune systems. If stimulation of the immune system is sufficiently large, pro-inflammatory cytokines increase in concentration in the blood circulation and act systemically (Iwasaki and Medzhitov, 2010). Cells in virtually all tissues have receptors for these cytokines and respond to them (Johnson, 1997).

In the central nervous system (CNS), cytokines initiate neural mediated events, either by directly accessing the CNS or by stimulating CNS cells to synthesize cytokines and neurotransmitters that act locally, resulting in stimulation of the hypothalamic-pituitary-adrenal (HPA) axis and induction of sickness-related behaviours such as fever and reduced feed intake (Karrow, 2006; Buchanan and Johnson, 2007; Miguel *et al.*, 2010). The latter occurs via suppressing feeding behaviour and decreasing gastric emptying; the former occurs via increasing metabolic rate (Figure 1; Johnson, 1997; Buchanan and Johnson, 2007).

Altered protein metabolism in skeletal muscle and visceral organs, especially in the liver, are distinctive features of direct effects of pro-inflammatory cytokines on peripheral tissue (Johnson, 1997). It has been shown that pro-inflammatory cytokines stimulate uptake of AA by hepatocytes, while they reduce AA uptake by skeletal muscle in pigs (Luiking and Deutz, 2007). It is noteworthy that many of the metabolic effects of ISS on peripheral tissue are also mediated through the CNS (Figure 1; Johnson, 1997).

Oxidative Immune Response

Another metabolically important response to stress and disease is the production of reactive oxygen and nitrogen species (ROS, RNS), the free radicals, by immune cells as part of the innate immune response. These molecules contain oxygen atoms with an unpaired electron and have strong bactericidal properties. Free radicals are non-selective in their targets, thus they have the potential to cause severe damage to the host's cells. As part of cell's defense during oxidative stress, a peroxidative protection system is activated to react with free radicals (Colditz, 2002). Central to this system is the tripeptide glutathione (GSH), of which synthesis generally increases during an immune response (Breuille and Obled, 2000). Glutathione contains a free and easily oxidizable sulfhydryl (SH) group (Brosnan and Brosnan, 2009). Other important intracellular antioxidants are taurine (TAU) and melatonin (Hardeland, 2005; Stipanuk and Ueki, 2010).

Protein Metabolism and Amino Acid Utilization During the Immune Response

As discussed in the previous section, it is clear that the immune system orchestrates physiological changes that affect nutrient metabolism when pigs are exposed to an antigen. It is apparent that the immune response involves both nutrient mobilization (eg. skeletal muscle protein degradation) and nutrient utilization (eg. acute-phase proteins and GSH synthesis, fever). In this section the impact of ISS on protein metabolism and AA utilization is discussed.

Protein Metabolism During the Systemic Immune Response

The net mobilization or reduced accretion of skeletal muscle proteins and increased protein synthesis in visceral tissues, especially the liver, are hallmarks of systemic ISS (Breuille *et al.*, 1998; Orellana *et al.*, 2004). Mobilization or reduced accretion of muscle protein is generally the result of both enhanced protein degradation and reduced protein synthesis (Lang *et al.*, 2007). The relative contribution of changes in protein synthesis and degradation to muscle protein mobilization or accretion may vary depending on acuity of the disease challenge, age and nutritional status of the animal. For instance, studies with growing pigs demonstrated that during the fed state, when substrate for protein synthesis is available, protein synthesis in muscle is largely maintained during mild-chronic endotoxemia (Orellana *et al.*, 2004; Luiking and Deutz, 2007). However, it has been shown that muscle protein synthesis rate is markedly decreased during severe systemic inflammation in growing pigs (Jahoor *et al.*, 1999b).

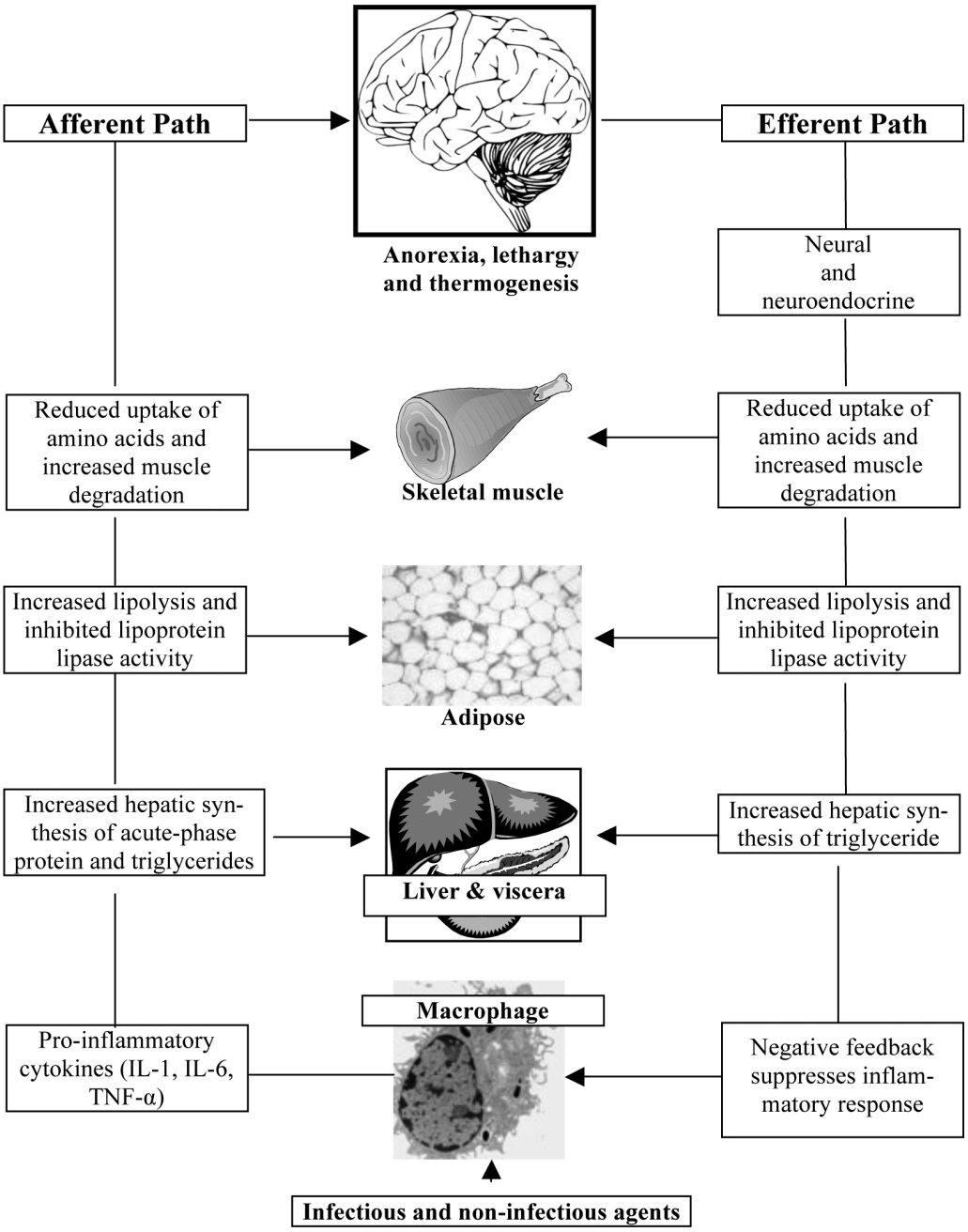

Figure 1. *Mechanisms by which pro-inflammatory cytokines inhibit growth. Cytokines act on peripheral and central targets. Cytokines in the brain reduce appetite, but they also alter the hypothalamic-pituitary axis and increase sympathetic nervous system outflow, which ultimately affect metabolism. Adapted from Johnson 1997.*

Protein Synthesis During Systemic Inflammation

In skeletal muscle, inflammation induces reductions in synthesis of both myofibrillar and sarcoplasmic proteins, primarily via a decrease in translational efficiency (Lang *et al.*, 2007). Of the three phases of mRNA translation, initiation, elongation and termination, the majority regulatory control in muscle occurs during initiation. Disease and inflammation alter the level of phosphorylation of various eukaryotic initiation factors (eIF) and binding proteins, and thus suppress formation of the pre-initiation complex (43S; methinyl-tRNA and 40S ribosomal complex) and assembly of the initiation factor complex of proteins that are involved in loading 43S complex onto activated mRNA (Lang *et al.*, 2007). In growing pigs, specifically, it has been shown that sustained endotoxemia markedly reduces translation initiation by lowering the binding capacity of 43S to mRNA (Orellana *et al.*, 2004). It has been suggested that pro-inflammatory cytokines, especially TNF-α, alter the translation initiation via suppressing activity of protein kinase mammalian target of rapamycin (mTOR) either directly or indirectly through increased release of glucocorticoid hormones. Glucocorticoids have been shown to impair translation initiation both *in vitro* and *in vivo* in skeletal muscle (Lang *et al.*, 2007; Frost and Lang, 2011).

Increased inflammation-induced protein synthesis in visceral organs is the result of the direct pleotropic effect of IL-6 on visceral tissues, and the indirect effect of IL-1β and TNFα on the release of anabolic and catabolic hormones (ie. glucocorticoids, growth hormone, IGF-1), especially in growing animals (Jacobi *et al.*, 2006; van Hall *et al.*, 2008). In addition, it has been suggested that the increased flow of AA from skeletal muscle to visceral tissues stimulates protein synthesis via activating the mTOR signaling pathway (Orellana *et al.*, 2004; Frost and Lang, 2011). Increased protein synthesis in visceral organs is the result of increased synthesis of both structural and secretory proteins. The latter mainly include proteins and non-protein compounds that are involved in innate and cell mediate immune response (Obled, 2003; Kohl and Deutschman, 2008).

Disease and inflammation substantially increase protein synthesis in the liver with a preferential increase in synthesis of inflammation-induced secretory proteins, also referred to as acute-phase proteins (APP; Colditz, 2002). During the acute-phase response, the levels of APP in blood plasma are either increased (ie. the positive APP) or decreased (ie. the negative APP). The major porcine positive APP, whose plasma levels at least double during ISS, include haptoglobin, fibrinogen, α_1-antitripsin, lipopolysaccharide binding protein, C-reactive protein, serum amyloid A and kallikrein-related pig 'major acute phase protein' (pigMAP). The major porcine negative APP includes albumin, transferrin, retinol binding protein, and cortisol binding globulin (Gruys *et al.*, 2005). It has been shown that increases in synthesis rates of positive APP are far greater than changes in plasma concentrations. Moreover, synthesis of negative APP markedly increase, even though plasma concentrations fall during systemic inflammation (Jahoor *et al.*, 1999b).

Protein Degradation During Systemic Inflammation

Increased protein degradation in skeletal muscles serves to maximize the mobilization of AA to support increased gluconeogenesis and synthesis of metabolites involved in the immune response. This confers an adaptive advantage to animals to ward off noxious influences. Skeletal muscle protein degradation occurs as a result of reduced AA intake (Reeds and Jahoor, 2001), direct and indirect effect of pro-inflammatory cytokines, and the need to mobilize body protein to supply specific AA that are required for immune system metabolites (Hasselgren *et al.*, 2005). It is of interest to note that 40-70% of total nitrogen loss in humans, depending on severity of disease challenge, can be ascribed to the effect of reduced nutrient intake during disease (Klasing and Leshchinsky 2000; Reeds and Jahoor, 2001). Pro-inflammatory cytokines, especially TNF-α, as well as glucocorticoids exert their proteolytic effects on muscle proteins mainly by activating caplain and ubiquitin-proteasome-dependent protein breakdown pathway (Hasselgren *et al.*, 2002; Moylan and Reid, 2007). Increases in the synthesis of metabolites involved in an immune response, such as APP, increases the demand for specific AA, according to their AA composition (Table 2). This would require breakdown of skeletal muscle proteins, which serve as a source of AA when AA intake is reduced during an immune response (Reeds and Jahoor, 2001; Obled, 2003). For example, in pigs in the post-absorptive state, the synthesis of 1 g of cysteine (CYS)-rich albumin requires degradation of 6 g muscle protein (Table 2). Synthesis rate of albumin can increase by as much as 100 mg/kg body weight/d in pigs with systemic inflammation (Jahoor *et al.*, 1999b). Taken together, these data suggest that increased synthesis of metabolites involved in an immune response creates an internal AA imbalance, which imply qualitative and quantitative consequences for AA requirements (Obled, 2003).

It has been suggested that provision of additional intake of selected AA may alleviate the negative impact of ISS on muscle protein mobilization or accretion. According to AA composition of immune system metabolites, glutamine (GLN), arginine (ARG), aromatic amino acids (especially tryptophan, TRP), threonine (THR), methionine (MET) and CYS putatively become more critical relative to other AA during an immune response (Reeds and Jahoor, 2001; Obled, 2003).

Table 2. *Amino acid composition of acute-phase proteins and pig skeletal muscle protein (Muscle).*

Amino acid	Fibrinogen[1]	Haptoglobin[1]	C-reactive[1]	α_1-Antitripsin[1]	Amyloid A[1]	Albumin[2]	Muscle[3]
	g amino acid/kg protein						
Phenylalanine	46	30	105	83	103	30	43
Tyrosine	56	70	50	27	67	21	36
Tryptophan	35	32	42	11	45	3	13
Leucine	62	82	91	124	29	65	83
Ileucine	32	47	54	49	29	15	48
Valine	48	84	77	59	18	38	51
Lysine	77	92	71	92	33	61	95
Histidine	27	38	16	37	35	16	44
Methionine	32	16	16	28	22	5	29
Cysteine	15	24	13	6	0	35	6
Threonine	60	54	58	66	30	34	48
Arginine	84	28	36	23	116	26	65
Proline	48	44	44	41	34	28	39
Glycine	59	44	46	33	61	17	43
Serine	91	40	84	49	47	32	42
Alanine	29	54	31	43	106	48	58

[1]Data from Reeds *et al.* (1994). [2]Data form Peters (1985). [3]Data from Dahl (1962).

Metabolism of Key Amino Acids During the Systemic Immune Response

Glutamine

Glutamine is the most abundant free AA in plasma and skeletal muscle. Within the body GLN plays important roles as a source of energy, precursor for glutamate, carrier of N, as well as regulator of gene expression and protein turnover (Lobley *et al.*, 2001; Xi *et al.*, 2011). It has been suggested that GLN is conditionally essential during ISS primarily because of its roles in immune cell proliferation and metabolism of nitrogen (Bongers *et al.*, 2007; Wu *et al.*, 2010). However, reports on responses to supplementation of GLN during ISS are inconsistent (Reeds and Jahoor, 2001; Obled, 2003). In critically ill patients the parenteral supplementation of GLN has been shown to improve whole body nitrogen balance (N-balance), while enteral supplementation of GLN had no beneficial effect, probably due to high rates of first-pass splanchnic GLN metabolism (Bongers *et al.*, 2007). In several studies and when individuals suffered severe intestinal dysfunction, enteral supplementation of GLN has been shown to improve N-balance as well as gut functionality or gut health (Reeds and Jahoor, 2001; Xi *et al.*, 2011). In contrast, during mild systemic inflammation, beneficial effects of enteral GLN supplementation on growth or N-balance could not be detected (Lobley *et al.*, 2001; Bongers *et al.*, 2007). This is probably because additional requirements for GLN during mild ISS can be met from endogenous GLN sources (Reeds and Jahoor, 2001).

Arginine

Arginine is another AA whose metabolism appears to be specifically important during an immune response, as outlined by Wu and co-authors (Wu *et al.*, 2009). Arginine is synthesized from GLN, glutamate, and proline via the intestinal-renal axis in pigs. Increased ARG utilization during disease and inflammation is largely attributed to enhanced synthesis of ornithine, a precursor for polyamines and nitric oxide (NO), as well as enhanced irreversible ARG loss via the urea cycle. However, despite several reports on beneficial effects of dietary ARG supplementation on human obesity and insulin resistance, there is no indication of increased dietary requirement for ARG during moderate ISS (Obled, 2003; Soeters *et al.*, 2004). Dietary supplementation of ARG, above requirement for maximum growth does not improve whole body N-balance in lipopolysaccharide (LPS) challenged broilers (Webel *et al.*, 1998; Kidd *et al.*, 2001). Moreover, partial efficiency of ARG utilization for whole-body protein accretion and maintenance requirement for ARG was not altered in LPS-challenged broilers (Webel *et al.*, 1998). In endotoxemic pigs, L-ARG supplementation did not improve whole body N- balance while enhancing liver NO production (Luiking and Deutz, 2007). It has been shown that feeding high levels of ARG can exacerbate the inflammatory conditions in humans and animals, due to increased production of NO, which is highly destructive to the cell and can act as a pro-inflammatory secondary messenger (Wu *et al.*, 2009). Collectively, these data suggest that increased metabolic demand for ARG can be satisfied from endogenous sources during a mild systemic inflammation.

Aromatic Amino Acids

Increased utilization of phenylalanine (PHE), tyrosine (TYR) and TRP during ISS are often associated with increased turnover of aromatic AA-rich APP (Reeds *et al*., 1994). However, when evaluating the nutritional impact of APP turnover, an increase in protein turnover does not necessarily mean an increase in AA needs, because AA released from proteolysis can be recycled for use in protein synthesis with high efficiency (> 85 %; Waterlow *et al*., 1978). In a few studies, possible beneficial effects of PHE and TYR supplementation on immune system function in critically ill and phenylketonuria human subjects have been reported (Reeds *et al*., 1994; Lykkelund *et al*., 1988; Soeters *et al*. 2004; Spronsen *et al*., 2005). However, no beneficial effects have been observed when providing additional PHE and TYR to humans during mild systemic inflammation (Reeds and Jahoor, 2001; Obled, 2003; Breuille *et al*., 2006). Amongst the aromatic AA, TRP deserves special attention. Decreases in plasma TRP levels have been repeatedly reported in pigs and other species during chronic systemic ISS (Melchior *et al*., 2004). This AA is a precursor for synthesis of kynurenine, serotonin and melatonin, whose synthesis rates increase markedly during ISS. Furthermore, it has been shown that TRP is a potent regulator of appetite in pigs not only via serotonin production but also through modulation of ghrelin, as well as insulin secretion and sensitivity (Le Floc'h and Seve, 2007). Moreover, it has been suggested that TRP participates in an anti-oxidative defense response and has a suppressive effect on pro-inflammatory cytokines (Kim *et al*., 2010; Le Floc'h *et al*., 2010b). Considering the relatively low TRP content in skeletal muscle protein (Table 2), it is reasonable to assume that dietary supplementation of TRP attenuates the adverse effects of ISS on skeletal muscle protein mass. Several studies have reported the beneficial effects of dietary TRP supplementation on immune function and N-balance during systemic ISS (Le Floc'h *et al*., 2010b). Kim *et al*. (2010) reported that TRP supplementation reduced inflammation and enhanced the rate of recovery in experimentally-induced colitis in young pigs (Kim *et al*., 2010). Le Floc'h *et al*. (2010a) reported an increase in dietary TRP requirement relative to lysine in ISS pigs exposed to high pathogenic environmental antigens. However, in a separate study, Le Floc'h *et al*. (2009) demonstrated that dietary TRP supplementation, above established requirements for optimal growth, did not improve pigs' overall performance when they were exposed to a high environmental microbial load (Le Floc'h *et al*., 2009). In a study in our laboratory, using a slope ratio assay, it was demonstrated that at TRP levels below requirement for maximum PD, repeated injection with increasing amounts of LPS (Rakhshandeh and de Lange, 2011) reduced the partial efficiency of TRP utilization for protein deposition (PD) in growing pigs, but had no effect on TRP maintenance requirements (Figure 2; Levesque *et al*., 2011). This suggests that the requirement for TRP may increase during disease. The observed reduction in efficiency of TRP utilization for PD can largely be attributed to increased irreversible loss of TRP during ISS. This is also part of the nutritional immunity mechanism to deprive microorganisms of TRP, and thereby control microbial proliferation (Heseler *et al*., 2008).

Branched-Chain Amino Acids

The branched-chain AA (BCAA) leucine (LEU), isoleucine (ILE) and valine (VAL) are essential AA whose metabolism during ISS is associated with increased protein turnover in leukocytes; they also appear to be essential for proliferation, growth and normal function of lymphocytes (Calder, 2006). Moreover, cell culture studies have shown that mitogen stimulation of lymphocytes increases the activity of branched-chain keto acid dehydrogenase, and hence, increases the catabolism of BCAA (Schauder and Schafer, 1987; Schafer and Schauder, 1988; Koch *et al*., 1990). Studies with *Salmonella typhimurium*-challenged and tumor-bearing rats have shown that severe restriction of dietary BCAA can substantially compromise the activity of killer cells. The latter are involved in the elimination of virally infected or tumor cells (Petro and Bhattacharjee, 1981; Tsukishiro *et al*., 2000). However, reports on beneficial effects of dietary supplementation of BCAA, above daily requirements for optimum growth, on immune function are controversial. An increase in blood lymphocyte count was reported in patients that received, post-surgery, a high BCAA diet (0.7 g/kg/d) compared to those who received the standard diet (0.36 g/kg/d; Cerra *et al*., 1984). Garcia-de-Lorenzo *et al*. (1997) reported that supplementation of BCAA reduces the rate of mortality in septic patients. This was related to higher plasma levels of BCAA and better immune function in survivors. However, another study failed to show the positive effect of parenteral BCAA supplementation on mortality rate related to sepsis or stress (von Meyenfeldt *et al*., 1990). Growing rats subjected to a dietary LEU overload displayed a strong impairment of immunological reactions to sheep red blood cells (Chevalier and Aschkenasy, 1977). A study with broilers fed diets varying in ILE from deficient to surfeit indicated that marginal deficiency of ILE does not compromise immunity during ISS (Hale *et al*., 2004). In addition, dietary supplementation of VAL, above requirement for optimal growth, did not improve the measures of immune function and growth performance of broilers challenged with sheep erythrocytes (Thornton *et al*., 2006). Taken together, these data suggest that despite an increase in metabolic demand for BCAA during ISS, the dietary levels of BCAA that support maximal growth performance appear to be sufficient for adequate immune function.

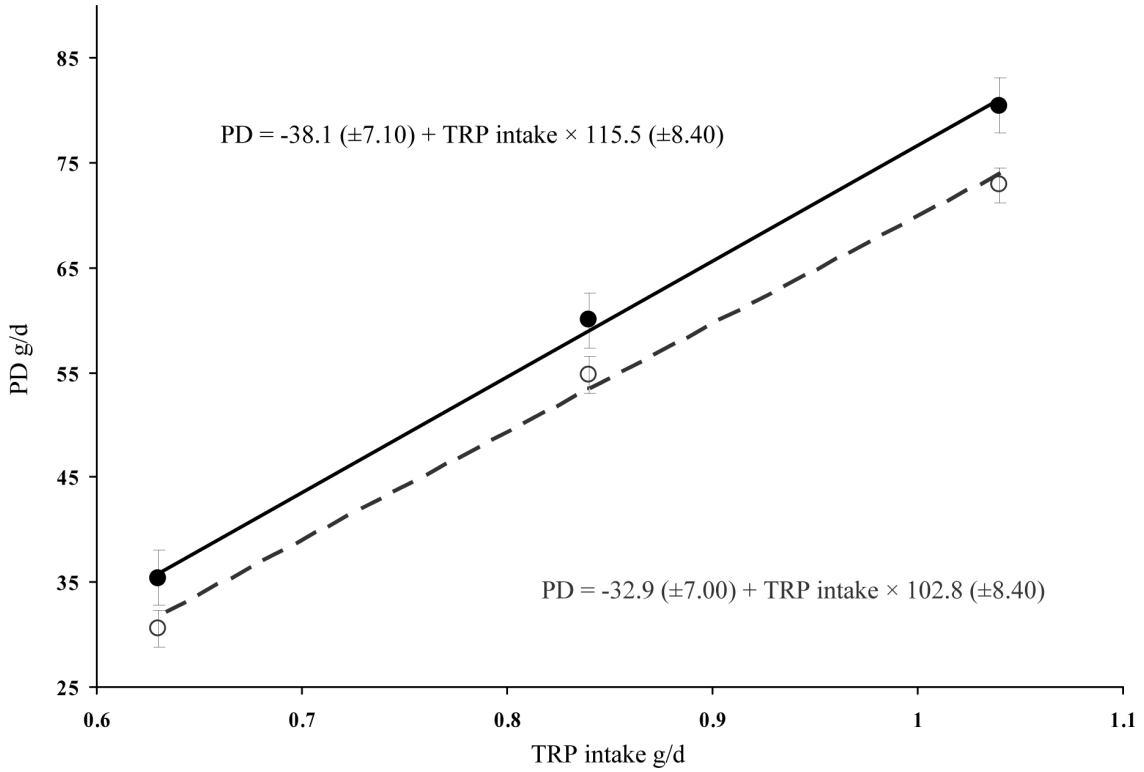

Figure 2. *Linear response of protein deposition (PD) to increasing tryptophan (TRP) intake pre- and post-immune system stimulation (ISS). Thirty-six pigs (initial bodyweight (BW) of 20 ±1.1 kg) were either injected with sterile saline (ISS-, ●) or increasing amounts of bacterial lipopolysaccharide (intramuscularly) every 48 hours for 3 days (ISS+, ○). Whole body nitrogen (N) balance was measured prior to immune system stimulation (Pre-ISS, 5-d) and during ISS (ISS-1, 3-d). Data from Levesque et al. (2011).*

Threonine

Threonine (THR) is an essential AA that is required for synthesis of THR-rich immunoglobins, APP, and mucins among many other proteins (Reeds *et al.*, 1994; Klasing and Leshchinsky, 2000; Faure *et al.*, 2007). During ISS, increased mucin production appears quantitatively the most important pathway contributing to increased THR requirements, as shown in trinitrobenzene sulfonic acid-challenged minipigs (Remond *et al.*, 2009). Despite the few reports on beneficial effects of dietary THR supplementation on gut immune function, there is a lack of solid information on beneficial effects of providing additional THR, above requirements for optimum growth, on animal performance during systemic ISS (Faure *et al.*, 2003; Breuille *et al.*, 2006; Faure *et al.*, 2006; Faure *et al.*, 2007). In broilers, it has been shown that the partial efficiency of THR utilization for protein accretion and dietary THR requirements are not altered during systemic ISS (Webel *et al.*, 1998).

Sulfur Amino Acids

Metabolism of MET and CYS is closely interlinked. In mammals, MET is a nutritionally essential AA, while CYS is considered semi-essential because it can be synthesized from MET (Stipanuk, 2004; Grimble, 2009). During systemic ISS increased utilization of MET has been reported by a number of investigators (Yu *et al.*, 1993; Breuille and Obled, 2000; Malmezat *et al.*, 2000b; Burrin and Stoll, 2007). Malmezat *et al.* (2000b) reported a 16% increase in plasma MET flux in septic rats relative to healthy controls. Yu *et al.* (1993) reported a marked increase in MET transmethylation in patients with systemic inflammation (Yu *et al.*, 1993). Increased MET utilization during ISS has been attributed to increased synthesis of polyamines, carnitine and, especially, CYS (Yu *et al.*, 1993; Malmezat *et al.*, 2000b). Cysteine synthesis from MET and serine is catalyzed by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CTH) (Stipanuk, 2004). In a study in our laboratory, it was observed that systemic ISS substantially up-regulated the expression of CBS and CTH at the transcriptional level in the liver of pigs (Rakhshandeh *et al.*, 2010c). Yu *et al.* (1993) showed a marked increase in biosynthesis of CYS in patients with severe systemic inflammation (Yu *et al.*, 1993). Similarly, and in septic rats, CYS biosynthesis was found to increase by 172% (Malmezat *et al.*, 2000b). However, there is some evidence indicating that biosynthesis of CYS from MET is not sufficient to respond to the increased CYS demand during the systemic ISS (Breuille and Obled, 2000; Malmezat *et al.*, 2000b). It has been suggested that the capacity for biosynthesis of CYS from MET varies among species (Malmezat *et al.*, 2000b).

Cysteine is utilized for synthesis of proteins and several other essential metabolites, including GSH, TAU and sulfate (SO_4; Stipanuk, 1999). Glutathione is the main intracellular anti-oxidant and is involved in numerous detoxification, conjugation and bioreduction reactions; GSH also serves as a reservoir of CYS and as a means for transporting CYS from the liver, the main site of GSH synthesis, to other tissues (Breuille and Obled, 2000). Almost every organ and tissue bed, except for plasma, can synthesize GSH (Lu, 2009). Synthesis of GSH from glutamate, CYS and glycine is catalyzed by glutamate-cysteine ligase (GCL) and GSH synthetase (GSS). Glutamate-cysteine ligase catalyzes the rate limiting step of GSH synthesis, which is regulated by feedback inhibition by GSH level and by transcriptional regulation in response to oxidative stress (eg. ROS). Synthesis of GSH is highly regulated by availability of its AA substrates especially CYS (Lu, 2009). It has been shown that 40% of the plasma CYS flux in healthy subjects in the post-absorptive state can be attributed to the turnover of GSH. The contribution of GSH turnover to plasma CYS flux increases substantially during ISS (Breuille and Obled, 2000; Malmezat *et al*., 2000a).

Metabolism of CYS to TAU and SO_4 via CYS sulfinate-dependent and CYS sulfinate-independent pathways represents an irreversible loss of CYS. Both pathways lead to the production of pyruvate and SO_4, but only the CYS sulfinate-dependent pathway leads to TAU production (Stipanuk, 2004). Taurine and SO_4 are central to a variety of essential functions in the body including bile and retinoic acid conjugation, osmoregulation, development of CNS, anti-oxidative defense, electrolyte balance and synthesis of iron sulfur proteins (Stipanuk, 1999). In mammals, TAU and SO_4 are excreted almost entirely in the urine. Hence, it has been suggested that urinary total sulfur excretion can provide a measure for SAA catabolism during different metabolic state (Hou *et al*., 2003).

Enhanced utilization of CYS during systemic ISS has been suggested by a number of investigators. Malmezat *et al*. (2000b) reported that in restricted fed-rats, sepsis increased plasma CYS flux by 44% without affecting plasma CYS levels. Similarly, Lyons *et al*. (2001) showed that sepsis increased the plasma CYS flux in humans by 40% while it had no effect on plasma CYS levels. In a study in our laboratory, LPS-induced endotoxemia increased plasma CYS flux by 26% and reduced plasma CYS and total SAA levels by 16 and 20%, respectively, in growing pigs, suggesting a substantial increase in CYS utilization in ISS pigs (Rakhshandeh and de Lange, 2011; Rakhshandeh *et al*., 2010a).

Despite the fact that only a few studies have been conducted *in vivo* on GSH synthesis, the enhanced utilization of CYS during ISS has been largely associated with increased GSH synthesis and to lesser extent elevated APP synthesis. In contrast, however, irreversible loss of CYS during ISS has received little attention (Lyons *et al*., 2001; Reeds and Jahoor, 2001). Injection with inflammatory turpentine increased the fractional synthesis rate of GSH in erythrocytes of protein-deficient pigs (Jahoor *et al*., 1995). Hunter and Grimble (1997) reported a 2.6-fold increase in liver GSH synthesis rate in TNFα-treated rats. Using a direct method for measuring GSH synthesis, Malmezat *et al*. (2000a) showed that sepsis caused a three-fold increase in whole-body GSH synthesis, from 251 to 846 μmol/d. Similarly, in a study in our laboratory, LPS-induced ISS increased overall synthesis rate of GSH from 4.7 to 6.7 mmol/d in growing pigs. This was largely as a result of increases in the absolute synthesis rate of GSH in liver (38%), small and large intestine (99 and 89%, respectively), heart (131%) and spleen (53%; Rakhshandeh and de Lange, 2010). We also showed that the expression of GCL, GSS and glutathione reductase (GSR) genes were markedly up-regulated in the liver of LPS-treated pigs (Rakhshandeh *et al*., 2010b). It is worth noting that in all of the above studies, observed increases in GSH synthesis rates are far greater than observed changes in plasma and tissue GSH levels (Reeds and Jahoor, 2001; Obled, 2003). Increases in GSH turnover may have an impact on dietary CYS requirements, and may contribute to muscle protein mobilization during ISS.

Only few estimates are available of the impact of ISS on irreversible loss of CYS to TAU and SO_4. Larson *et al*. (1982) reported a decrease in urinary S excretion in patients with bone fractures and burns when compared to healthy individuals. A decrease in urinary SO_4 excretion has been described following TNFα injection in rats (Hunter *et al*., 1993). Malmezat *et al*. (1998), using ^{35}S labeled CYS, reported a decrease in irreversible loss of CYS to SO_4. These authors concluded that CYS catabolism is decreased due to increased utilization, and thus retention in the body, of CYS during ISS. However, in these studies little attention was given to metabolism of CYS to TAU. Indeed, in the study by Malmezat *et al*. (1998), appearances of ^{35}S in TAU was increased by 54%, while the decrease in appearance of ^{35}S in SO_4 was 30% in septic rats, suggesting an increase in overall irreversible loss of CYS induced by ISS. In a study in our laboratory, using an isotope tracer infusion method (^{35}S-CYS), LPS-induced ISS increased overall irreversible loss of CYS to TAU by 104% while it had no effect on conversion of CYS to SO_4 in growing pigs. In this study, LPS-induced ISS increased total irreversible loss of CYS from 343 to 535μmol/d (Rakhshandeh *et al*., 2011). Interestingly, we found that endotoxemia reduced appearance of $^{35}SO_4$ and total SO_4 excretion in urine by 55 and 57%, respectively, suggesting that urinary total S excretion is not a reliable indicator for short-term evaluation of irreversible loss of SAA during ISS (Rakhshandeh and de Lange, unpublished data). In addition, we have shown that LPS-induced ISS increases the expression of key regulatory genes involve in TAU production (ie. cysteine deoxygenase) but has no effect

on expression of genes involved in SO_4 production (Rakhshandeh *et al*., 2010c). Observed increases in CYS oxidation in septic pediatric patients confirms observations in septic rats and ISS pigs (Lyons *et al*., 2001). Taken together, these results suggest that ISS increases irreversible CYS loss, largely because of enhanced TAU production. Increased TAU production likely contributes to enhanced CYS requirement during ISS. Collectively, these data suggest that ISS substantially increases the metabolic demand for CYS. Therefore, it can be hypothesized that an exogenous supply of CYS improves whole-body protein homeostasis and the immune response during ISS.

Improvements in GSH homeostasis as the result of dietary SAA supplementation, quantified by synthesis rate and tissue levels of GSH, have repeatedly been reported in the literature. Alhamdan and Grimble (2003) reported that SAA intake prevented the fall in tissue GSH levels in rats, which usually occur during endotoxemia. In rats injected with TNFα, a positive correlation was found between tissue CYS levels and synthesis rates of GSH (Hunter and Grimble, 1997). Cysteine supplementation elicited substantial increases in both GSH synthesis and GSH levels in erythrocytes in HIV-infected patients (Jahoor *et al*., 1999a). Moreover, pigs fed protein deficient diets cannot maintain GSH homeostasis during systemic inflammation (Jahoor *et al*., 1995). Depletion of tissue GSH levels exacerbates the adverse effect of an inflammatory response (Reeds and Jahoor, 2001). Recently, it has been shown that dietary L-CYS supplementation improved gut immune function by attenuating inflammatory responses in pigs with chronic gastrointestinal tract (GIT) inflammation (Kim *et al*., 2009). Collectively, these data provide evidence that CYS supplementation has beneficial effects on measures of immune function. However, none of these studies detected any effect of SAA supplementation on whole-body N-balance or provided estimates for SAA requirement. To our knowledge, the effect of dietary supplementation of a mixture of AA, including CYS, on body protein balance, has only been evaluated in one study in septic rats (Breuille *et al*., 2006). The authors reported an improvement in whole body protein balance in response to providing supplemental AA. However, in this experiment the effect of providing additional CYS on body protein balance was confounded with the effect of other AA. In a study in our laboratory, using a slope ratio assay, it was observed that endotoxemia induced by repeated injection of increasing amounts of LPS (Rakhshandeh and de Lange, 2011) decreased dietary SAA requirements for maximum PD due to a reduced overall capacity of endotoxemic pigs for PD (Figure 3). However, the linear relationship between SAA intake and PD at levels below requirement for maximum PD indicates that endotoxemia has no effect on partial efficiency of SAA utilization for PD, but it substantially increases the extrapolated SAA maintenance requirements (~23%; Figure 3; Rakhshandeh *et al*., 2011). The latter suggests an increase in dietary SAA requirements for a given rate of PD in endotoxemic pigs. Furthermore, and in a separate study in our laboratory, we observed that LPS-induced endotoxemia increases the optimal dietary MET to total SAA (MET + CYS) ratio from 57 to 62% for PD in growing pigs, suggesting that MET is the preferred source of dietary SAA during ISS (Litvak *et al*., 2011).

Nutrient Digestibility During the Systemic Immune Response

When assessing an animal's response to nutrient intake, both pre-and post-absorptive aspects of nutrient utilization need to be taken into account. During systemic inflammation, the GIT quickly becomes involved in inflammatory responses. Major physiological alterations in the GIT during systemic inflammation include changes in gut motility, permeability, mucin production, microflora, expression of digestive enzymes, and epithelial transport systems (Yamada and Alpers, 1999; Tappenden, 2008; Turner, 2009).

Suppressive effects of pro-inflammatory cytokines on intestinal contractile activity have been reported in various animal models (Eskandari *et al*., 1997; Gonzalo *et al*., 2011). These cytokines reduce the intestinal motility directly by suppressing circular muscle activity (Gonzalo *et al*., 2011) and indirectly by stimulating production and release of catecholamine, nitric oxide (NO) from smooth muscle or nerves, as well as cyclooxygenase-2 and prostaglandin E_2 from endothelial cells (Eskandari *et al*., 1997; Jiang *et al*., 2004; Gonzalo *et al*., 2011). Moreover, pro-inflammatory cytokines partially exert their suppressive effect on intestinal motility by stimulating release of the gut-derived satiety hormone cholecystokinin (Wong and Pinkney, 2004). Furthermore, pro-inflammatory cytokines, especially TNF-α and interferon (INF-γ), increase the permeability of the mucosal barrier by altering tight junction barrier function and thus initiating extensive apoptosis of the epithelial cells (Turner, 2009; Blikslager, 2010).

Several studies have demonstrated an increase in mucin synthesis aimed at maintaining an effective intestinal barrier during local and systemic inflammation (Faure *et al*., 2007; Dharmani *et al*., 2009). Increased synthesis and release of mucins induced by pro-inflammatory cytokines is a receptor-mediated event that is regulated via stress-activated protein kinase, c-Jun NH2-terminal kinase and janus kinase pathways (Dharmani *et al*., 2009). Increased mucins synthesis requires AA and can reduce availability of AA for other body functions (Faure *et al*., 2007).

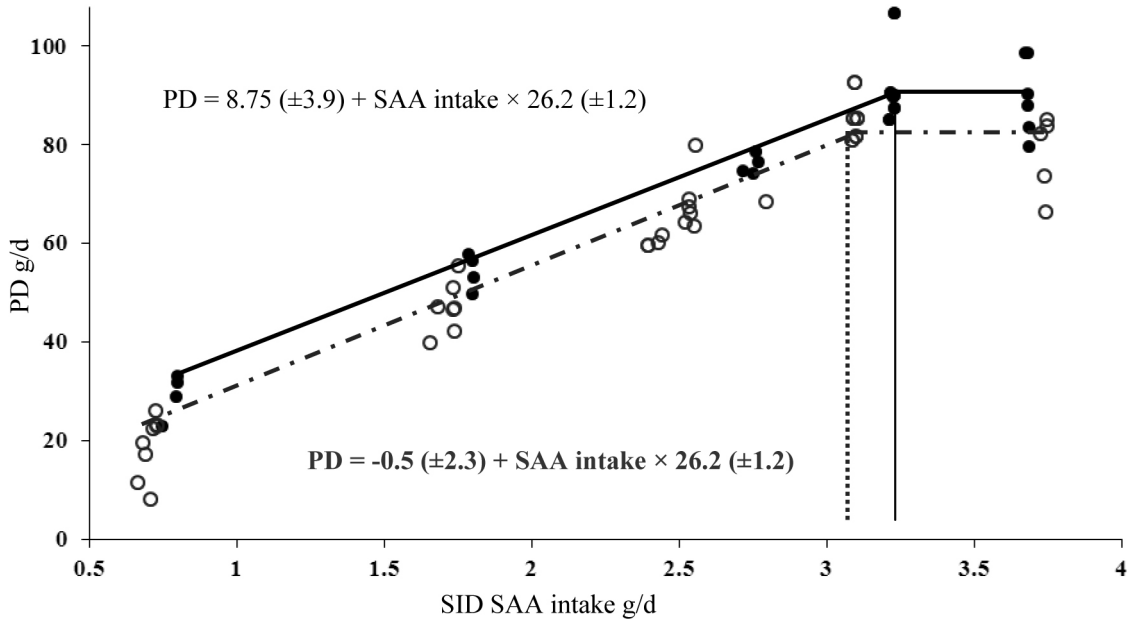

Figure 3. *Impact of immune system stimulation (ISS) and standardized ileal digestible (SID) methionine + cysteine intake (SAA) intake on whole-body protein deposition (PD) in growing pigs. Pigs were fed corn starch and soy protein based diet. Diets were formulated to be iso-caloric and first limiting in SAA (Wang and Fuller 1989). Five levels of SID SAA intake (1.0, 2.4, 3.6, 4.0 and 5.0 g/d) were used. Thirty-six out of 60 pigs were injected (intramuscularly) every 48 hours for 7 days with increasing amounts of lipopolysaccharide (ISS+, ○). The remaining pigs were injected with sterile saline solution (ISS-, ●). Adapted from Rakhshandeh and de Lange (2011).*

Pro-inflammatory cytokines can alter the intestinal microflora by altering intestinal motility, and the capacity for producing antimicrobial peptides (Johnson, 1997; Barbara *et al*., 2005; Willing and Van Kessel 2010). Systemic inflammation-induced decreases in gut motility and reduced absorption of nutrients may lead to intestinal bacterial overgrowth, and even diarrhea. Systemic inflammation also compromises production of antimicrobial peptides (eg. β-defensins) and, thus, increases the risk of intestinal colonization by pathogenic bacteria (eg. *E. coli*). This, in turn, can contribute to intestinal inflammation and deregulation of the mucosal barrier function (Willing and Van Kessel, 2010). Furthermore, it has been suggested that intestinal colonization by pathogenic microbes affects intestinal motility and absorptive capacity (Willing and Van Kessel, 2010). It is unclear, however, whether the change in gut motility is the cause or the consequence of the altered microflora composition (Barbara *et al*., 2005).

Systemic inflammation alters production of digestive enzymes mainly by influencing pancreatic function (Fitzal *et al*., 2003). However, reports on the impact of systemic inflammation on pancreatic production of proteases are inconsistent. Some studies report a mild cytokine-induced pancreatitis, which results in increased secretion of proteases and their corresponding zymogens, while others report a marked impairment in exocrine pancreatic function (Fitzal *et al*., 2003; Tribl *et al*., 2003). These contrasting reports may reflect variation in intensity or duration of systemic inflammation. Nevertheless, these reports indicate that systemic inflammation deregulates normal exocrine function of the pancreas (Leung and Chan, 2009).

Reports on impact of systemic inflammation on intestinal nutrient transport system are also inconsistent. Hang *et al*. (2003) reported a two-fold increase in the expression of nutrient transporters per gram of intestinal tissue in rats with systemic inflammation. Similarly, pro-inflammatory cytokines induced an increase in expression of glucose and proline transporters in the small intestine of rabbits (Tappenden, 2008). Moreto and Perez-Bosque (2009) reported that *Staphylococcus aureus*-induced ISS does not influence intestinal transport of LEU, MET or lysine in rats (Moreto and Perez-Bosque 2009). However, Abad *et al*. (2001) reported a marked reduction in AA absorption in LPS-induced ISS in rabbits. Similarly, this rabbit model of chronic inflammation was associated with impaired function of Na+/K+-ATPase and transporter PEPT1 in the GIT (Sundaram *et al*., 1999). It has been suggested that pro-inflammatory cytokines reduce the AA uptake by epithelial cells mainly by affecting Na-dependent transport systems, through reducing apparent transport capacity (Vmax) and decreasing Na^+/K^+-ATPase activity (Turner, 2009; Abad *et al*., 2001).

Collectively, these observations suggest that digestive and absorptive function of GIT is compromised during a systemic inflammation. However, little solid information is available about the impact of inflammation-induced physiological changes in the GIT on overall digestive capacity. In a study in our laboratory, using a slaughter technique, it was demonstrated that LPS-induced systemic ISS does not affect apparent ileal digestibility of dietary energy, crude protein and key AA (Table 3; Rakhshandeh *et al*., 2010). We concluded that digestibility is not a limiting factor for utilization of dietary energy, protein and AA for various body functions during a systemic ISS. Similarly, in two other well-controlled studies the impact of systemic inflammation on nutrient digestibility has been evaluated. In both these studies apparent faecal digestibility of neither dietary protein nor dry matter was affected by systemic ISS (Williams *et al*., 1997a; Zoric *et al*., 2003).

Table 3. *Main effect of immune system stimulation (ISS) on apparent ileal digestibility (AID; %) of energy, nitrogen and selected amino acids*[1].

	Immune system stimulation			
Item	-	+	SEM	P-value
Energy	84.0	83.2	3.1	0.71
Nitrogen	74.1	71.4	4.9	0.67
Methionine	81.6	78.8	4.1	0.34
Cysteine	69.2	61.1	7.9	0.18
Lysine	82.3	77.6	5.1	0.62
Threonine	71.1	64.3	6.9	0.19
Isoleucine	80.6	76.9	5.2	0.69
Leucine	81.3	77.5	5.0	0.77

[1]Data are least square means ± standard error (SE) and represent data obtained on d 7 after the start of ISS. AID was determined using an indigestible marker (TiO_2) and the slaughter technique. Adapted from Rakhshandeh *et al.* (2010).

Conclusions and Implications

Clearly exposure to disease and ISS can have negative effects on productivity of pigs, and thus dietary nutrient utilization and profitability in pork production. As we are improving our understanding of the pig's immune response to disease and other environmental challenges, opportunities to reduce the negative impact of ISS on pig performance are identified. For example, it has been established that the dietary AA balance for an optimal immune response is different from the optimum dietary AA balance for maximum growth or reproductive efficiency. Recent studies suggest that amongst the AA, the dietary levels that support optimal growth performance appear to be adequate, or even in excess, for lysine, ARG, and BCAA during ISS but SAA and TRP are exceptions, and their ratio to other AA (eg. lysine) needs to be increased to attenuate the effect of ISS on pigs' performance. Supplementation of GLN appears to be beneficial when animals are suffering from intestinal inflammation. Manipulation of the dietary AA balance represents one of the means to reduce the negative effects of disease on pigs and improve gut health.

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The Immune Response to a Ten-Times Oral or Intramuscular Immunisation With *Lawsonia* Vaccine in Weaner Pigs

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Proliferative enteropathy (PE) is an important economic disease of pigs, and is caused by the intracellular Gram-negative bacterium *Lawsonia intracellularis*. PE affects pigs of all ages, causing haemorrhagic enteropathy and death in the acute form or weight loss and diarrhoea in the chronic form. Clinical signs of PE can be controlled with a live oral commercial vaccine (Enterisol® Ileitis, Boehringer Ingelheim Pty Ltd, North Ryde, NSW; Kroll *et al*., 2004) and a *L.intracellularis* bacterin vaccine administered intramuscularly (Dale *et al*., 1997). This research ultimately aims to identify an immune marker for protection against PE following vaccination to ensure life-long immunity to PE. This pilot experiment compared the systemic and mucosal immune responses to a ten times (10x) dose of the Enterisol® Ileitis vaccine given orally or intramuscularly (IM), as previous studies indicated that serum antibodies were not detected following a single vaccine dose ($10^{5.5}TCID_{50}$ *L.intracellularis*; Kroll *et al*., 2004).

Fifteen weaner pigs (Landrace x Large White) were selected from a commercial herd clinically and serologically free of PE and transferred to a controlled environment research facility. At five weeks of age (d 0), pigs weighed 6.0 ± 0.3kg and were randomly allocated to one of three treatment groups housed in separate rooms; four control pigs, six orally vaccinated pigs (10x Oral) and five intramuscularly (IM) vaccinated pigs (10xIM). Blood was collected from each pig at d 0, 9 and 17 and *L.intracellularis* specific antibodies were determined as percent inhibition (PI) for the bioScreen Ileitis ELISA (Synbiotics Corporation, Kansas City, MO) or as a serum titre using a modified direct ELISA for IgG and IgA (Holyoake *et al*., 1994). Seven pigs (two controls, three oral and two IM) were humanely euthanized on d 9 and the remainder on d 17. Mucosal secretions were collected by gently scraping the ileal mucosa with a sterile scalpel and normalised to equivalent protein concentrations. Samples were tested for cytokines (IFN-γ, IL-6, IL-10, TNF-α) using porcine Quantikine ELISA kits (R&D Systems Inc., Minneapolis, MN) and IgG and IgA as above. Differences in results between treatment groups and days post-vaccination were analysed using Restricted Maximum Likelihood (REML) analysis.

Table 1. *Mean cytokines mucosa concentration (pg/ml) to ten times (10x) oral or intramuscular (IM) vaccine.*

Treatment	IFN-γ		IL-6		IL-10		TNF-α	
	Day 9	Day 17	Day 9	Day 17	Day 9	Day 17	Day 9	Day 17
10x Oral	189[a]	770[a]	95[a]	530[a]	18[a]	163[a]	61.2[a]	105.8[a]
10x IM	43[b]	75[b]	58[a]	70[b]	19[a]	40[a]	28.8[a]	73.4[b]
Control	115[ab]	450[ab]	50[a]	155[ab]	20[a]	45[a]	27.4[a]	72[b]

[ab]Means in the same column with different superscripts differ significantly (P<0.05); IFN-γ: Interferon-gamma; IL-6: Interleukin-6; IL-10: Interleukin-10; TNF-α: Tumor Necrosis Factor-α

Cytokine concentrations increased over time for all treatments (Table 1). Significant increases in mucosal TNF-α were observed in pigs orally vaccinated compared to IM vaccinates and control pigs at d 17. IFN-γ and IL-6 concentrations were significantly elevated in oral vaccinates relative to IM vaccinates, but not to control pigs. Increasing serum and mucosal IgG responses were detected between d 9-17 in all vaccinated pigs, with mucosal IgG significantly higher (P<0.05) in pigs vaccinated orally compared with IM, however, no significant differences (P>0.05) were observed between vaccinated and control treatments (results not shown).

These patterns of serum and mucosal response are consistent with the induction of local gut inflammation following oral vaccination. While IM vaccination tended to induce a systemic immune response, it did not induce a local immune response in the intestinal mucosa.

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Chronic Immune System Activation Increases the Growing Pig's Requirement for Sulphur Amino Acids

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A mild disease challenge as commonly occurs in commercial production facilities may significantly decrease performance by redirecting (partitioning) amino acids from body protein synthesis to immune activation. The amino acids that are used by the pig for synthesis of immune molecules may, therefore, be in short supply and hence may limit body protein deposition. Sulphur amino acids (SAA), especially cysteine, are the most abundantly used amino acids for synthesis of immune molecules (Rakhshandeh *et al*., 2010). The experiment reported here was conducted to test the hypothesis that pigs whose immune system has been activated will respond to higher SAA levels than those without chronic immune system activation.

A split-plot experiment without and with immune system activation as a main plot, and four diets containing different amounts of standardised ileal digestible (SID) sulphur amino acids (SAA to SID lysine ratios of 0.45, 0.55, 0.65 and 0.75) as subplots, was conducted with 64 male pigs (Large White x Landrace x Duroc) weighing 52.9kg ± 0.41 (Mean ± standard error of mean). A two-phase feeding program was employed. Phase 1 (50-75kg) and phase 2 (75-95kg) diets were formulated to contain 13.5 MJ digestible energy (DE)/kg and 0.60 and 0.55g SID lysine/MJ DE, respectively. Pigs were intramuscularly injected each Monday and Thursday with either saline or *E. coli* lipopolysaccharides (LPS, serotype 055:B5, Sigma, 60 µg/kg body weight) for six weeks to mimic chronic immune system stimulation (Rakhshandeh *et al*., 2010). Week 1 data were excluded for statistical analysis due to severe immune stimulation with negative performance responses.

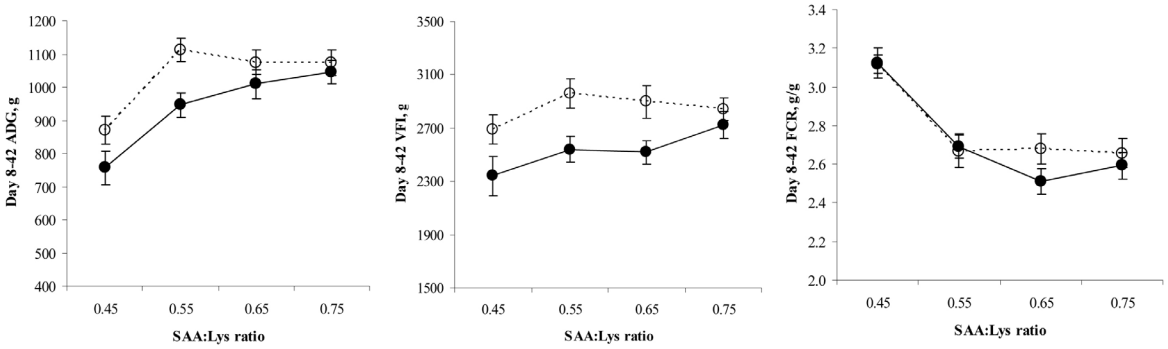

Figure 1. *Performance responses (mean ± standard error of mean) to sulphur amino acid:lysine (SAA:Lys) ratio without (saline injection - - -) and with (LPS injection —) chronic immune system stimulation.*

Measurement of increased daily rectal temperature indicated that the chronic immune system activation was successful over the entire experimental period in LPS-injected pigs (Data not shown). Pigs that received a saline and LPS injection showed a quadratic ($P<0.001$) and linear ($P < 0.001$) response in average daily gain (ADG) to increasing dietary SAA:Lys ratio, with the fastest ADG achieved at SAA:Lys ratios of 0.55, and 0.75, respectively (Figure 1). The SAA:Lys ratio that supported minimal feed:gain ratios were 0.55 and 0.65 for saline and LPS treated pigs, respectively. The results indicate that chronic immune system activation increases the level of SAA needed to support maximum performance. Under the model used, chronic immune system activation increased SAA:SID lysine ratio to support maximum performance from 0.55 to 0.75.

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Colostrum and Milk Immunoglobulins Increase in Sows Fed Live *Saccharomyces cerevisiae boulardii*

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The acquisition of passive immunity by the newborn piglet is necessary because it provides a high level of immune protection, therefore improving the development of piglet's active immunity during lactation and its level of systemic immunity at weaning (Le Dividich *et al.*, 2005). The acquisition of passive immunity depends mainly on the amount of colostrum consumed, and on its immunoglobulin G (IgG) content. Live yeast fed to the sow has been shown to improve the IgG plasma level in their 24h old piglets in a dose dependent manner (Jang *et al.*, 2010), suggesting an increased colostral IgG concentration and /or colostrum production of the sows. This experiment tests the hypothesis that sows fed live *Saccharomyces cerevisiae boulardii* CNCM I-1079 (SCB) produce more immunoglobulins in colostrum and milk.

This experiment was performed in a commercial farm located in Brittany, France. Sows (n=66; Large White×Landrace), housed individually in stalls, were blocked by parity and body condition three weeks before expected parturition. They were divided into a control group (C, n=33) fed the regular feeding program of the farm (gestation diet before moving the sow to the farrowing room, followed by peri-partum diet until the week-end after farrowing, then lactation diet until weaning), a group fed 25 g/d of SCB concentrate (10% SCB on dextrose carrier equivalent to 5×10^{10} CFU per day), from the start of the experiment to weaning (21d post-partum; SB, n=33). On each sow, colostrum was sampled randomly from most teats all along the udder just after birth of the first piglet (0), and 12 and 24h later. With the same procedure, a sample of milk was collected during natural letdown on d 19 of lactation. Samples were immediately filtered on gauze and stored (-20°C). Colostral Ig (A, G and M) and milk IgA were analyzed using a commercial kit (Pig Ig ELISA Quantitation kit, Bethyl Laboratories, Montgomery, USA). For the colostrum data, area under the Ig (G, A and M) curves (AUC) was calculated using the trapezoidal rule to represent the mean value over 24h. Colostrum Ig AUC and milk Ig contents were compared using Student's t test.

Table 1. *Influence of* Saccharomyces cerevisiae boulardii *CNCM I-1079 on immunoglobulins in sow colostrum (mg/mL).*

	IgA			IgG			IgM		
	C	SB	SEM	C	SB	SEM	C	SB	SEM
0h	14.5	17.5	0.72	105.2	127.7	4.81	7.1	7.7	0.35
12h	11.2	12.7	0.64	72.4	83.1	3.68	5.7	6.0	0.30
24h	6.8	8.9	0.56	30.9	50.7	3.54	4.4	4.8	0.29
AUC (mg.h/mL)	263	317	NS	1684	2016	P<0.05	138	141	NS

SB, *Saccharomyces cerevisiae boulsrdii*; C, Control; SEM, standard error of mean; Ig, immunoglobulin; NS, not significant; AUC, area under Ig curves

IgG represented the major share of total immunoglobulins (73 to 84%, depending on sampling time), followed by IgA (11 to 16%) and IgM (5 to 10%; Table 1). Feeding SB increased significantly the IgG concentration of colostrum (P<0.05), but did not significantly (P>0.05) improve IgA or IgM concentration in both colostrum and milk (milk IgA: 5.78 *vs*. 4.90 mg/mL in SB *vs*. Control, respectively). These data support previous findings of an increase in piglet plasma IgG concentration following live yeast supplementation (Jang *et al.*, 2010). However, to what extent feeding SCB improves the colostrum produced by sows and consumed by piglets remains to be determined. Feeding the probiotic *Saccharomyces cerevisiae boulardii* CNCM I-1079 to the sow should be considered when designing strategies to improve piglet's immune status.

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Intramuscular Administration of Porcine Plasma Does Not Improve Performance of Neonatal Pigs

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Piglets are immunocompetent at birth but they are immunologically underdeveloped from lack of exposure to antigen (Rooke and Bland, 2002). The piglets rely on colostral intake from their dams for protection against pathogens in the first few weeks of life. Within 24 h of birth, gut closure occurs in the newborn pig intestine which leads to a decline in the absorption of maternal immunoglobulins. Hence, there is a very narrow window in the life of a neonatal pig to achieve maximal immune protection from the dam. There is evidence that giving plasma to neonatal piglets intramuscularly (IM) can reduce the mortality (Normantiene *et al.*, 2000). The objective of this experiment was to evaluate whether the IM administration of porcine plasma to neonatal piglets enhances growth performance and reduces mortality. The hypothesis for the experiment was that IM administration of porcine plasma can improve piglet health.

One hundred and sixty-four piglets (Large White x Landrace, PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) were selected within 24 h of birth (total 15 litters, average sow parity 1.4) in a 5300 sow farm in NSW, Australia. All fosterings were conducted within 24 h of birth to ensure that all piglets had access to a functional teat. Within each litter, piglets were randomly selected to either the control or treatment group. Control piglets were left untreated, while the treatment piglets received 5 ml of porcine plasma at d 1 and d 3 via intramuscular injection into the neck. The porcine plasma was collected from culled sows from the same farm. The blood was collected into containers containing dissolved Ethylenediaminetetraacetic acid (EDTA). Following collection, blood was processed and the plasma harvested and subsequently stored frozen (-20°C) until use. Piglets were individually weighed at d 7 and 14. Any deaths occurring within the two week period were recorded. Differences in piglet performance due to plasma treatment were determined using an analysis of variance (ANOVA). The individual piglet was considered the experimental unit for all analyses, while the sow rearing the litter was included as a blocking factor resulting in a paired-treatment comparison experimental design. Differences in piglet mortality were determined using chi-squared analysis.

Table 1. *Effect of intramuscular injection of porcine plasma at d 1 and d 3 after birth on piglet growth performance.*

	Untreated Control	Plasma Treatment	SED	P-value
Weight d 0 (kg)	1.43	1.41	0.029	0.50
Weight d 14 (kg)	3.80	3.65	0.157	0.34
Growth rate d 0-14 (g/d)	165.0	158.0	0.01	0.49

SED, standard error of difference; Mortality - Controls, 16.8%; Plasma treatment, 14.1%, $\chi^2 = 0.67$, P = 0.41.

The administration of porcine plasma to piglets during the first week of life had no effect on growth performance or mortality rate (Table 1).

No negative effects were observed in plasma-treated piglets. A higher dose of porcine plasma may be needed to further enhance piglet health. A larger sample size could be used in future experiments for a more robust experimental design. It would be useful to also assess whether porcine plasma treatment has any efficacy in older piglets or early weaned piglets. Further investigations are also being conducted on the impact of plasma treatment on low birth weight neonatal piglets which may not have had sufficient colostrum intake.

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Probiotics Limit the Severity of Post-Weaning Diarrhoea

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Post-weaning diarrhoea (PWD), especially that caused by enterotoxigenic *Escherichia coli* (ETEC), is a significant source of loss for pig producers due to costs of treatment, animal death and suboptimal production. In the past, PWD has often been managed with the use of antimicrobials. Protracted use of antimicrobials has encouraged development of antimicrobial resistance increasingly expressed as multi-drug resistance amongst ETEC (Smith *et al.*, 2010). Ineffectiveness of common antimicrobials against ETEC has raised considerable concern about how this pathogen will be managed in the future. Probiotics have been suggested as an approach to prevention of PWD that could reduce the reliance on antimicrobials. This experiment aimed to assess if an *E. coli* probiotic containing three commensal strains could reduce PWD in an ETEC challenge model.

Twelve gilts (Large White x Landrace) from a commercial farm were housed in pig research facilities six weeks prior to farrowing. Gilts were randomly allocated to either a control (placebo supplement) or probiotic (probiotic supplemented) treatment with the latter receiving daily probiotics (1x109 colony forming units {CFU}) *per-os* as a drench from two weeks prior to farrowing until weaning. Piglets received a corresponding oral dose at birth, at five days old and weaning. All piglets (n=60) were weaned and immediately challenged with $5x10^9$ CFU of ETEC in a 3mL suspension administered *per-os* once daily for three consecutive days. Faecal consistency scores were recorded daily from weaning until four days post weaning using a scale of 1 to 5 (1 and 2 were considered within the normal range and 3 – 5 as scouring) and the mean faecal consistency score per piglet over these four days used for analysis. Mean faecal consistency scores were assessed in a mixed effects linear model with solutions obtained by restricted maximum likelihood. To account for the effect of piglets nested within litters, the effect of "gilts" was treated as a random effect. Fixed effects were "probiotic treatment", "ETEC challenge" and the interaction of these two.

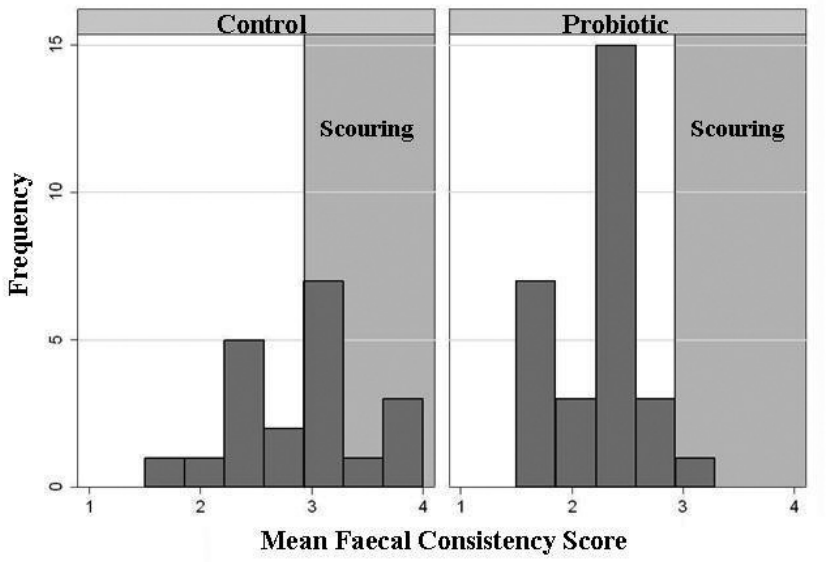

Figure 1. *Histograms of observed mean faecal consistency score over four days post-weaning for probiotic treated (n=33) and control piglets (n=27).*

Only the probiotic treatment had a significant effect (P<0.05) with the mixed model estimating that probiotic treatment reduced mean consistency scores by 0.70 compared to controls. The work here needs to be extended to an industry setting to understand if the promising benefits of probiotic supplementation can be realised with natural exposure to ETEC and the other factors that cause PWD. Extending this research to an industry setting is crucial for understanding if the promising results shown here can be realised under conditions of natural exposure to ETEC and the other factors that cause PWD.

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The Effect of Feed Intake Pattern on the Microflora in the Gastro Intestinal Tract of Piglets in the Period after Weaning

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At weaning, young piglets are subjected to stress factors that result in low feed intakes, diarrhoea and even mortality (Pluske *et al.*, 1997). Low feed intakes lead to reduced enteral stimulation and compromise the mucosal integrity during the first four days after weaning (Vente-Spreeuwenberg *et al.*, 2001). This acute phase is followed by a transition phase where the piglets start eating and their gut subsequently adapts to solid feed. Kamphues (1987) showed that a too high feed intake in young piglets affects stomach function and intestinal health. We hypothesized that, too high or spiking feed intakes in the transition phase may lead to an overload of the stomach and microbial disturbances in the small intestine, which could subsequently lead to a drop in feed intake. To test this hypothesis, we studied the effect of feed intake level and the access time to feed on pH and dry matter in the stomach contents and on the number of selected micro-organisms in the jejunum and colon of weaned piglets.

Sixty (Hypor x Topigs, Top Pi, Topigs, Helvoirt, The Netherlands) piglets were weaned at day 21±1 (body weight 8.2±0.5kg) and housed in individual cages. All animals received a commercial cereal based weaning diet (174 g crude protein/kg; 10.8 MJ net energy/kg; 13.5 g lysine/kg). Littermates and genders were equally assigned to one of the following treatments: Treatment A (Control) – *ad libitum* access to feed during the whole day; Treatment B- *ad libitum* access to feed only from 0730–0900 h; Treatment C – restricted feeding based on a linear feed curve (FI=40*D-25 where FI=feed offered (g/d); D=day number post-weaning) and fed in three portions equally distributed over the day. On d 8 post–weaning, five barrows and on d 9 five gilts per treatment group were sacrificed 4h after the moment of introduction of fresh feed in the morning. Digesta samples from stomach, mid jejunum and colon were collected for chemical analyses and conventional microbiology. Data were analysed using a generalised linear model (GLM) procedure (SAS Institute Inc., 2004) with feeding regime as an independent variable,

Table 1. *Least squares means for growth and feed intake during the first week after weaning, pH and dry matter in the stomach and E. Coli (EC) and lactic acid bacteria (LAB) counts in mid jejunum and Clostridium perfringens (CF) in the colon.*

			Stomach		Mid jejunum		Colon
Treatments	Growth (g/d)	Feed intake (g/d)	% DM	pH	EC log CFU/g	LAB log CFU/g	CF log CFU/g
A	192^{a}	196^{a}	21.9^{b}	4.3^{b}	6.34	6.87^{a}	3.14^{ab}
B	73^{b}	67^{c}	31.2^{a}	4.9^{a}	5.65	6.48^{ab}	4.43^{a}
C	100^{b}	105^{b}	18.3^{b}	3.6^{c}	5.05	5.72^{b}	2.80^{b}
SEM	11.4	8.1	1.8	0.2	0.5	0.3	0.5
P value	**	**	**	**	NS	*	*

Treatment A, *ad libitum* access to feed during the whole day. Treatment B, *ad libitum* access to feed only from 0730–0900 h. Treatment C, restricted feeding based on a linear feed curve. CFU, colony forming units. SEM, standard error of the mean; DM, dry matter; ^{abc}Means within a column with different superscripts differ significantly * P<0.05, ** P<0.01.

Animals on restricted feeding (Treatment C) showed a significant lower pH in the stomach compared to piglets fed *ad libitum* (Treatment A) or fed during a short period of the day (Treatment B). They also showed significantly lower lactic acid bacteria counts in the jejunum and *Clostridium perfringens* counts in the colon as compared with piglets fed during a short period of the day (Treatment B). From these results it can be concluded that a low and regular feed intake supports gastric function and influences bacterial counts in the intestines.

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CHAPTER 2

General Production

Physi-Trace™: A Demonstration of Rapid Traceability for Australian Pork

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Physi-Trace™ provides a simple, low-cost, rapid and robust traceability validation tool for the Australian pork supply chain and product integrity system by using trace element profiles to represent the farm of origin of fresh pork meat. The developed system would facilitate rapid re-entry of unaffected product into the export market in the event of an embargo following a food safety related incident. Physi-Trace™ requires regular sampling and trace elemental analysis of fresh meat samples from participating abattoirs to establish a database against which data associated with questioned samples may be compared. Following several years of development in association with export abattoirs and regulatory authorities, Physi Trace™ is now approaching implementation. A recent trace-back request from a participating export abattoir provided a valuable opportunity to demonstrate the effectiveness of the Physi-Trace™ system.

A sample of fresh meat, with questioned origin, was delivered to the analytical facility and dissolved alongside other fresh meat samples of known origin using a mixed acid digestion. The resulting solutions were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), generating trace elemental data. Data were normalised to the existing Physi-Trace™ database using quality control measures (Certified Reference Materials, in-house standards and cross over samples from previous batches). Assessment and interpretation of the data was undertaken using a data pre screening technique followed by linear discriminant analysis (LDA).

Interrogation of the data confirmed that the questioned sample was Australian in origin and had been processed at the abattoir that had initiated the trace-back enquiry. The data pre-screening technique was then used to identify the ten most likely matches within the database to the sample, which provided the seven most likely farms of origin. An iterative forward stepwise LDA process was undertaken on all data associated with these farms, ultimately identifying the most likely farm of origin for the sample in question. A discriminant plot associated with this classification is illustrated in Figure 1.

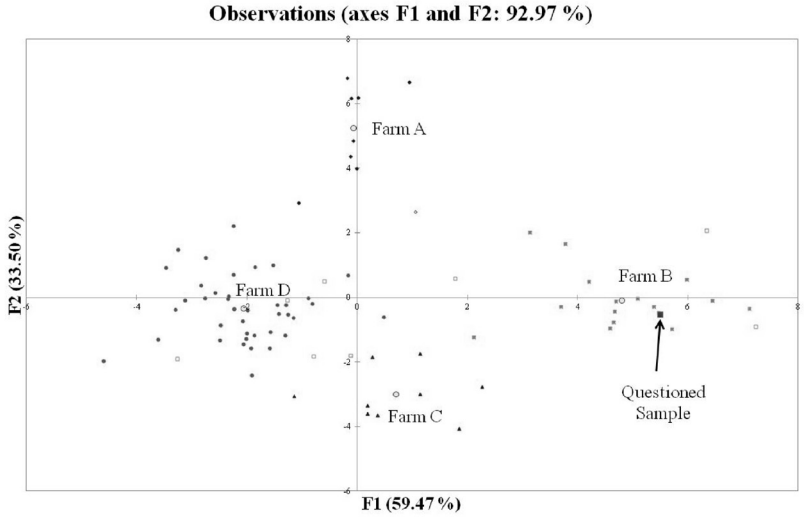

Figure 1. *Discriminant plot detailing the classification of a questioned sample to the farm of origin (Farm B).*

The trace-back result was reported to the abattoir within 52 hours of receipt of sample, with representatives from the abattoir confirming that animals from the identified farm had been processed at the abattoir on the day the questioned sample was collected. Furthermore, the farm had been identified by the abattoir as one of four most likely sources for the questioned sample, based upon carcase throughput and the location from which the sample was collected within the abattoir. As such, the farm of origin of the questioned sample was successfully identified utilising the Physi-Trace™ technology.

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Inclusion of Lupin Hulls or Lucerne in Finisher Pig Diets Can Increase Percent Drip Loss in Fresh Pork

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Adjusting fibre levels within pig diets can be used as a tool to manipulate carcase composition as increasing the level of dietary fibre in the finisher diet can reduce carcase fatness (Håkanssan *et al*., 2000). Compared to ruminants, pigs do not utilise fibre efficiently and the energy obtained through digestion varies between fibre sources. Fibre from forage sources, such as pulses, is better utilised by pigs and therefore provides more nutrients than fibre from cereal sources (Lindberg and Andersson, 1988). The pig's total energy and nutrient intake affects pork quality therefore it is necessary to investigate the impact of dietary interventions on fresh pork. The aim of this experiment was to determine if objective pork quality differed between pigs that had been fed diets containing different fibre sources during the finisher phase.

At approximately 13 weeks of age (48 ± 6.2 kg), 64 Large White x Landrace female pigs were blocked by live weight (LW) into groups of four. Pigs within each block were allocated to adjacent individual pens within an insulated naturally-ventilated grower-finisher facility. Treatments were randomly allocated within blocks and were 1) Control, commercial finisher diet (13.6 MJ digestible energy (DE)/kg; 145g/kg neutral detergent fibre (NDF)), 2) 10% cereal straw (13.4 MJ DE/kg; 194 g/kg NDF), 3) 10% lupin hull (13.6 MJ DE/kg; 170g/kg NDF) and 4) 10% lucerne (13.7 MJ DE/kg; 182g/kg NDF). All diets were formulated to contain 0.55g available lysine/MJ DE and were pelleted (9 mm diameter). The fibrous ingredients were ground in a tub grinder and passed through a series of screens with the final screen being a mesh of 5mm diameter. Additional tallow was used to adjust DE level. Pigs were fed the control diet for one week before starting treatment diets *ad libitum* at 54 ± 6.8 kg LW. Pigs were slaughtered at a commercial abattoir (91 ± 4.9 kg). Twenty-four hours after slaughter, approximately 1 kg of the *Longissimus dorsi* was collected and pH_u (24h), muscle colour (L*, a*, b*), drip loss, cook loss and Warner-Bratzler peak shear force measures were determined. Data were blocked by group and analysed using general ANOVA procedures.

Table 1. *Objective pork quality measures from the Longissimus dorsi of pigs fed a commercial finisher diet or finisher diets containing 10% straw, 10% lupin hulls or 10% lucerne.*

Treatment diet	Control	10% Straw	10% Lupin hulls	10% Lucerne	SEM	P value
pH_u	5.46	5.53	5.48	5.48	0.018	0.09
Shear force (kg)	5.55	5.24	5.49	5.01	0.363	0.70
Colour - L*	58.8	52.8	54.4	53.3	0.81	0.56
Colour - a*	5.77	5.71	6.22	6.40	0.236	0.12
Colour - b*	3.57	3.40	3.93	3.94	0.217	0.22
Drip loss (%)	5.92[a]	6.37[ab]	7.36[b]	7.59[b]	0.426	0.02
Cook loss (%)	32.5	32.2	35.6	32.6	0.44	0.93

[ab]Means in a row with different superscripts differ significantly (P<0.05); SEM, standard error of mean

Treatment diet did not significantly affect pork colour, percent cook loss or tenderness (Table 1.). Pork from pigs fed the lucerne and lupin hull diets had significantly higher percentage drip loss compared to pork from pigs fed the control diet (P<0.05). Higher drip loss can result in tougher, drier cooked meat and can have a negative impact upon the appearance of fresh product. The results for shear force indicated that the treatment differences in percent drip loss did not affect pork tenderness, however sensory analyses would be required to determine if pork dryness was affected. Increasing fibre in the finisher diet by adding lucerne or lupin hulls altered pork quality, however, when comparing fibre sources, cereal versus pulses, there was no effect.

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Higher Heritability Estimates for Fat and Muscle Depth Obtained Using the Porkscan™ System

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Fat and muscle depth are important characteristics that determine the market value of a pig carcase. Pig breeding programs often consider these traits using ultrasound information recorded on the live animal, since information on selection candidates prior to selection is beneficial for genetic gain. However, as payment systems are based on carcase measures, estimates of genetic relationships between selection criteria recorded on the live pig and carcase traits are required. The PorkScan™ (PorkScan Pty Ltd, Canberra, ACT) system, developed in Australia, provides accurate ultrasound information about carcase fat and muscle depth and to date genetic parameters have not been available for these traits. It was hypothesized that fat and muscle depth recorded with PorkScan™ on the carcase are heritable and are genetically associated with fat and muscle depth recorded on the live pig with real time ultrasound.

Data were recorded from February 2010 until January 2011 on 2442 pigs from two terminal sire lines at a hot carcase weight of 69.9 (± 7.95) kg (TRIM 13, Aus-Meat Ltd, South Brisbane, QLD) and an age of 151.9 (± 5.4) days. Fat (LFD) and muscle (LMD) depth on the live animal were recorded at the P2 site, 65 mm from the midline of the carcase at the last thoracic rib, eight days before slaughter using real time ultrasound. These traits were then also recorded on the hot carcase (CFD, CMD) using the PorkScan™ system, which provides ultrasound measurements of fat and muscle depth on the slaughter floor. Significant fixed effects included line, date of recording, sex (LMD, LFD only) and testing system (LFD, CFD only). The weight of the animal (LFD, LMD) or the carcase (CFD, CMD) was fitted as a linear covariable. An animal model was used to estimate genetic parameters with ASReml (Gilmour *et al*., 2006) fitting additive genetic effect as the only random effect.

Table 1. *Number of records (N), means, standard deviations (SD), phenotypic variances (VP), heritabilities (underlined) as well as genetic (above diagonal) and phenotypic (below diagonal) correlations with standard errors (in brackets) for muscle and fat depth recorded on the carcase (CFD, CMD) and live pig (LFD, LMD).*

	Data statistics				Genetic Parameters			
	N	Mean	SD	VP	LFD	CFD	LMD	CMD
LFD	2276	7.70	1.20	1.10	0.13 (0.04)	0.85 (0.09)	0.11 (0.21)	-0.23 (0.19)
CFD	2423	7.04	1.37	1.49	0.42 (0.02)	0.34 (0.05)	0.15 (0.14)	0.10 (0.12)
LMD	2377	42.7	5.12	17.5	0.06 (0.02)	0.07 (0.02)	0.21 (0.05)	0.87 (0.08)
CMD	2400	48.8	5.87	25.0	0.02 (0.02)	0.09 (0.02)	0.29 (0.02)	0.40 (0.06)

LFD, Live fat depth; CFD, carcase fat depth; LMD, live muscle depth; CMD, carcase muscle depth.

Due to scaling effects, a higher mean is often associated with a higher standard deviation. This was observed for muscle depth measurements (Table 1). In contrast, fat depth recorded on the carcase was more variable than the live-animal measure despite a lower mean, since it included records below 5 mm, which were not distinguished for the live-animal trait. Therefore, variation in backfat was not fully expressed on the live pig resulting in a lower heritability estimate of 0.13 for LFD compared to 0.34 for CFD. The heritability estimate was also considerably higher for muscle depth obtained using PorkScan™ (CMD: 0.40) compared to the live-pig measure (LMD: 0.21). The larger and more variable fat depth measure recorded on the carcase also had a higher heritability than the live-animal trait in the study by Mérour *et al*. (2009), while there were no differences in means, variability and heritabilities for muscle depth traits recorded on the live pig or the carcase.

Higher heritability estimates for fat and muscle depth using Porkscan™ in comparison to live-pig measures suggest a higher accuracy of this technology even at low fat levels, possibly due to more standardised measurement conditions in the abattoir. The high genetic correlations of 0.85 and 0.87 between the same measures recorded on the live pig and the carcase are valuable for pig breeding programs and provide good opportunities for genetic gain in these economically important carcase traits.

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Erythropoietin Increases Iron in Pork

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The average iron (Fe) content (0.6mg/100g) of Australian pork is below 10% of recommended daily intake. This leads to reduced consumer perception of pork's nutritional status, thereby negatively influencing product value and sales. The aim of this experiment was to determine if muscle Fe could be increased in pigs by injection of erythropoietin (EPO), which has been shown to stimulate erythropoiesis and increase the Fe content in human muscle (Robach *et al*., 2009). We hypothesised that injection of human recombinant EPO would increase muscle iron content in pigs.

Twelve gilt pigs (Large White x Landrace, 63.4 ± 2.7 kg) were offered *ad libitum* access to water and pelleted grower feed with an Fe content of 228 ± 16.5 mg/kg. Pigs (n = 6) received either 300 µL of EPO (4000U) or saline (C) on days 0, 2, 4, and 6 and were slaughtered on d 8. Prior to ear vein injection, and on d 8, blood was collected by venapuncture for haematology and serum iron. Immediately post mortem, heart, spleen, liver and muscle samples (*Triceps brachii* (TB); *m. longissimus dorsi* (LD); *m. semitendinosus* (ST); *m semimembranosus* (SM); the diaphragm (thick skirt, TSK)) were collected for Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) estimation of Fe (mg/kg). Haematology data was analysed using the General Linear Model procedure in SAS (v9.1, SAS institute Inc., Cary, NC, USA) with treatment and sampling day as fixed effects in the model. Muscle iron was analysed in a model consisting of treatment and muscle as fixed effects, a first order interaction of treatment x muscle and liveweight-d 8 and serum Fe-d 8 as covariates.

Table 1. *Effect of EPO treatment on haematology variables and Fe content (mg/kg) in pig muscle on d 8.*

	Haematology (Day 8)			Muscle Fe					
Treatment	Erythrocytes ($x10^{12}$)	Haematocrit (%)	Haemaglobin (g/L)	TB	LD	ST	SM	TSK	Heart
Control	6.2	35.3	115.5	7.7	5.8	7.2	6.4	15.3	25.1
EPO	6.6	37.5	123.8	10.6	7.2	7.1	8.7	18.1	28.8
Significance	NS	0.05	0.01	NS	NS	NS	NS	NS	NS

EPO, Erythropoetin; TB, Triceps brachii; LD, *m. Longissimus dorsi*; ST, *m. semitendinosus*; SM, *m. semimembranosus*; TSK, diaphragm; NS, not significant.

EPO treated pigs had more red blood cells and a significantly (P<0.05) higher haematocrit and haemoglobin concentration than C pigs (Table 1), indicating that EPO had stimulated erythropoiesis. There was a significant (P<0.05) 35% reduction in Fe in the spleen (from 318.9 to 208.4 mg/kg) and 50% reduction in the liver (from 89.0 to 45.5 mg/kg) of EPO-treated pigs. This suggests that dietary Fe was not sufficient to meet the EPO-stimulated erythrocytic demand for Fe. Muscle and EPO treatment were significant (F=51.4 and F=4.72, respectively) factors in the model that indicated that Fe content was 20% higher in EPO-treated muscles taken as a whole compared to C. On an individual muscle to muscle basis, and despite the Fe content being higher in five of six muscles of EPO-treated pigs (Table 1), the observed differences were not significant at P<0.05.

This experiment is the first in pigs to show that EPO caused the mobilisation of Fe from the spleen and liver and increased Fe storage by muscles, but not to the fullest extent. EPO therapy in humans is known to cause a rapid reduction in Fe stores (Bhandari *et al*., 1998) that can lead to a sub–optimal response to EPO. We postulate Fe storage in muscle was retarded by additional growth requirements for Fe during EPO challenge and erythrocytic response.

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Predicting Threshold Sensory Scores Required for Pork to Achieve Positive Consumer Re-Purchase Intention Ratings

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Consistent availability of pork that meets eating quality expectations is anticipated to drive consumer demand as recipes containing pork increasingly became part of their weekly meal repertoire. However, the threshold consumer sensory scores that need to be attained to result in positive re-purchase intention are not understood. It is hypothesised that the implementation of several production and processing quality interventions will improve pork eating quality consistency. This experiment was conducted to establish consumer sensory scores of pork eating quality required to achieve positive re-purchase intention ratings for pork and determine which of the production and processing factors investigated had the greatest influence on improving pork eating quality consistency.

A total of 144 female pigs (Large White x Landrace) were slaughtered at liveweight ranges of 75-85 kg and 100-110 kg, respectively, over two slaughter days at three different abattoirs. A total of 24 pigs were slaughtered on each slaughter day. Following slaughter and during dressing, carcases were either hung from the aitchbone or from the Achilles tendon. *M. longissimus lumborum* (loin) muscles were obtained from each carcase, aged for either two or seven days post-slaughter and prepared into 2.5 cm thick steaks for sensory evaluation. This experiment was therefore a 3 (processors) x 2 (carcase weight groups) x 2 (hanging method) x 2 (ageing period) factorial design. Pork steaks were cooked to an internal temperature of 75°C and assessed for eating quality as described by Channon *et al*. (2004) and graded into one of five quality/re-purchase intention categories, including 1: unsatisfactory; 2: below average; 3: average (may buy on some occasions); 4: above average (probably would buy on some occasions) and 5: premium (definitely would buy). Regression analyses were undertaken to determine the threshold consumer sensory scores required for pork that may indicate positive re-purchase intention (grades of 4 and 5).

Table 1. *Average sensory scores (± standard deviation (SD)), degree of fit (R^2) and predicted threshold sensory scores (± 95% prediction interval) for tenderness, flavour, juiciness and overall liking of pork loin steaks required to achieve a positive intention of consumers to re-purchase pork (quality score of 4 and 5).*

			Predicted threshold sensory score	
Sensory attribute	Mean ± SD	R^2	Probably would buy on some occasions (Quality Score 4)	Definitely would buy (Quality Score 5)
Tenderness	55.3±11.43	77.7	72.4 ± 10.64	96.4 ± 10.88
Flavour	61.0±6.99	72.6	71.1 ± 7.22	85.3 ± 7.39
Juiciness	59.3±8.89	46.0	69.5 ± 12.91	83.9 ± 13.21
Overall liking	59.7±8.57	85.4	73.1 ± 6.55	91.9 ± 6.60

Threshold sensory scores required to achieve positive consumer re-purchase intention for pork were considerably higher than the average sensory scores for all four sensory attributes of pork loin steaks. This suggests that eating quality performance needs to shift upwards for pork to be re-purchased. Although the final internal temperature of 75°C may have impacted on pork eating quality, Australian pork consumers typically cook pork to between medium to well done and a well done degree of doneness. Whilst no interactions between hanging method and ageing for any sensory quality parameters were found, 50% of loin steaks aged for seven days from aitchbone hung carcases achieved quality grade scores of 4 or 5 compared with 32% of loin steaks aged for two days from Achilles hung carcases. This indicates that the implementation of different pathway parameters could lead to improved consumer appreciation of pork. This research provides useful background to support current initiatives being undertaken to develop an eating quality predictive model for Australian pork.

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Eating Quality of Australian Pork is Variable

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Eating quality variability remains a significant issue for the Australian pork industry and needs to be addressed as this impacts consumer confidence, demand and satisfaction (D'Souza *et al*. 2003). This study was conducted as part of the Australian Pork Limited's Pork Eating Quality Assurance Program (1998-2002) to identify the production, processing and post-slaughter factors that influence eating quality of Australian pork. The objective of this study was to ascertain the variability in pork eating quality.

Pig carcases (n=300) were selected on the slaughter floor of five abattoirs according to sex (entire male or female), vendor, hot carcase weight (Trim 1, AusMeat Ltd, South Brisbane; 60-75 kg) and P2 fat depth (8-13 mm). The *Longissimus lumborum* (loin) muscles (n=60 per abattoir) were aged for two days post-slaughter and prepared into six 2.5 cm thick steaks for consumer sensory evaluation (where 0=dislike extremely, tough, dry and 100=extremely acceptable, very tender, very juicy). Pork steaks were cooked and assessed as described by Channon *et al*. (2001). Skatole and androstenone in adipose tissue was analysed using reverse phase high-pressure liquid chromatography (HPLC) with fluorescence detection following extraction of intramuscular fat of the loin muscle using diethyl ether. Data was analysed using REML (Genstat 5.4, Lawes Agricultural Trust, Rothamsted, UK) with abattoir, vendor, day, sex, animal and pack included in the model.

Table 1. *Consumer sensory scores for eating quality attributes and intramuscular fat content (IM fat%) of pork loins collected from five Australian abattoirs.*

	Abattoir site						
	A	B	C	D	E	SED	P value
Flavour	63.4	58.9	58.7	58.3	63.2	0.90	P<0.05
Juiciness	63.4	55.4	54.2	54.0	59.7	0.90	P<0.05
Tenderness	60.8	52.5	52.1	48.9	59.7	3.15	P<0.05
Overall liking	64.6	56.4	56.9	56.2	63.3	1.55	P<0.05
IM fat%	1.09	0.70	0.93	1.14	1.11	0.30	NS

SED, standard error of difference; NS, not significant.

No effect of sex on aroma, tenderness, juiciness, flavour and overall acceptability of pork loin steaks was identified. Overall, pork eating quality was inconsistent, with only 35% of pork steaks rated by consumers to be slightly acceptable or higher (ie. scores above 60) for tenderness. Furthermore, 76%, 54%, 42% and 50% of pork loins obtained average sensory scores of 60 or higher for aroma, flavour, juiciness and overall liking, respectively.

The average intramuscular fat content of pork loins in this study was 0.98 ± 0.50%, with 74% of loins having intramuscular fat levels ranging from 0.5–1.4%. Neither abattoir nor sex influenced intramuscular fat content. Correlations between intramuscular fat content of pork loin muscle and tenderness, flavour and overall liking were low (0.14, 0.18 and 0.22, respectively). The incidence of pork with high concentrations of androstenone (>1.0 μg/g) and skatole (>0.2 μg/g) was 14% and 10%, respectively. Levels of both androstenone and skatole higher than the threshold limits were found in 6% of entire male pigs. In comparison to females, entire males had higher (P<0.001) skatole levels (0.06 and 0.16 μg/g, respectively). Low correlations were found between skatole and flavour, odour and overall liking of pork loin steaks (R=-0.019, R=-0.022 and R=-0.022, respectively). Similarly, correlations between androstenone and flavour, aroma and overall liking of pork loin steaks were also very weak (R=-0.019, R=-0.022 and R=-0.022, respectively). This suggests that Australian consumers may have been insensitive to boar taint and/or the relatively low levels of intramuscular fat in loin muscles may have limited consumer detection of boar taint. A large variability in eating quality of the *longissimus* muscle from 'generically produced' pork was found, but it was not possible to precisely determine those factors contributing to this eating quality variability.

CHANNON, H.A., REYNOLDS, J. BAUD, S.R. (2001). Identifying pathways to ensure acceptable eating quality of pork. Final Report DV 163/1385. Australian Pork Limited:Canberra.

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Effect of Dietary Nano-Sized Chromium Picolinate on Growth and Carcase Traits in Finisher Gilts During Summer

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Chromium (Cr) is an essential mineral element and has been used in pig diets in the past decades to improve growth performance, insulin sensitivity, immune response, carcase traits and to reduce heat or other stress responses (Hung *et al.*, 2010). However, dietary Cr^{3+} is considered to be poorly absorbed and utilised by the pig, even when supplemented in the organic form. Despite this, recent studies have shown benefits of Cr inclusion in finisher diets when the Cr is ground to a smaller particle size. For example, Hung *et al.* (2009) reported that carcase P2 was improved when gilts fed a high fat finisher diet were supplemented with 400ppb of 100nm particle size Cr picolinate (CrPic) for 42 days. Sahin *et al.* (2002) demonstrated that dietary Cr supplementation can alleviate negative effects of heat stress in poultry but to date, there is lack of information on the effects of dietary Cr in pigs under heat stress. Therefore, the aims of this experiment were to determine the impact of nano-sized CrPic (nCrPic) on growth performance, feed efficiency and carcase characteristics of finisher gilts during the summer period.

A total of 60 finisher Large White x Landrace (PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) gilts were stratified on liveweight (initial weight 67.7±0.46 kg, mean±standard error (SE), kg) and then within strata randomly allocated into two treatment groups in three replicates during mid-summer (January–February, 2011). All pigs were housed in individual pens and had *ad libitum* access to feed and water. Pigs were fed either a control finisher diet (wheat based diet containing 13.8 MJ digestible energy (DE)/kg and 0.56 g available lysine/MJ DE) or a diet containing 400 ppb Cr as nCrPic. nCrPic was processed by a dry polish method in a dry cryo-nanonization grinding system integrated with a size separator (Hsin-Fang Nanotech. Co. Ltd. Tainan, Taiwan). Briefly, the raw CrPic material was ground and then passed through appropriate sized end-plates sieves to collect nano-sized particles of CrPic. Feed intake and liveweight were recorded weekly. At the end of the experiment, pigs were slaughtered at a commercial abattoir to determine hot standard carcase weight (HCWT), carcase P2 and dressing percentage. Data were analysed by analysis of variance using GENSTAT Release 11.1 (VSN International Ltd. UK). Initial weight was used as a covariate for average daily gain (ADG), final weight and HCWT and initial P2 were used as covariates for final P2.

Table 1. *Effect of dietary nCrPic on growth performance and carcase characteristics of finisher gilts (n=30).*

	Control	nCrPic	SED	P-value
ADG (0-14 d; g)[1]	877	951	4.67	0.12
ADG (0-28 d; g)[1]	937	985	2.95	0.09
FCR (0-14 d)	2.72	2.55	0.127	0.20
FCR (0-28 d)	2.61	2.62	0.08	0.96
Final weight (kg)[1]	94.0	95.4	0.83	0.09
HCWT (kg)[1]	70.2	71.1	0.69	0.14
Dressing percentage (%)	74.4	74.6	0.44	0.70
P2 (mm)[2]	8.0	8.0	0.19	0.94

[1]Initial weight used as a covariate; [2]Initial P2 and HCWT used as covariates; SED, standard error of difference; FCR, feed conversion ratio; nCrPic, nano-sized chromium picolinate; ADG, average daily gain; HCWT, hot standard carcase weight; .

Dietary nCrPic had no significant effect (P>0.05) on ADG, FCR, final weight, HCWT, P2 depth or dressing percentage (Table 1). This data demonstrates that if these finisher pigs were stressed as a result of elevated summer temperatures, then nCrPic was not effective at alleviating heat stress as measured by growth response or carcase parameters.

HUNG, T. Y., LEURY, B. J., LIEN, T. F., LU, J.J. and DUNSHEA, F.R. (2010). Proceedings of the 14th Asian-Australasian Association for Animal Production Volume 1: 108.

HUNG, T.Y., LEURY, B.J., SABIN, M.A., LIEN, T.F. and DUNSHEA, F.R (2009). In "Manipulating Pig Production XII", p.183, ed. R. J. van Barneveld. (Australasian Pig Science Association: Werribee).

SAHIN, K., OZBEY, O., ONDERCI, M., CIKIN, G., AYSONDU, M. H. (2002). *Journal of Nutrition*. **132**:1265.

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Dietary Lecithin Improves the Ratio of Polyunsaturated to Saturated Fatty Acids in Pork

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Previous research has shown that dietary lecithin may improve the fatty acid profile of pork (D'Souza *et al.*, 2005). The aim of this experiment was to test the hypothesis that dietary lecithin would improve the fatty acid profile of pork and to extend the range of fatty acids analysed.

Thirty-six Large White x Landrace (PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa) gilts were randomly allocated at 16 weeks of age (62.9±0.56 kg, mean±standard error; SE) to finisher diets containing 0, 4, 20 or 80 g/kg soybean lecithin (ADM Australia Pty Ltd, Bondi Junction). Pigs were housed individually and had *ad libitum* access to feed and water for six weeks prior to slaughter at 103.2±1.67 kg. Twenty-four hours after slaughter, the *L. thoracis* was removed and frozen prior to fatty acid analysis by capillary gas chromatography and cholesterol analysis using a kit (Sigma-Aldrich Pty Ltd, Sydney, Australia). Data were analysed for linear and quadratic effects of dietary lecithin.

Table 1. *Effect of dietary lecithin on skeletal muscle fatty acids, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), total fat and cholesterol concentrations (mg/100g fresh muscle).*

	Dietary lecithin (g/kg)					P-value	
	0	4	20	80	SED	Linear	Quadratic
C14:0	28	18	18	21	5.1	0.037	0.780
C16:0	515	355	370	415	79.6	0.045	0.740
C18:0	270	191	201	218	43.0	0.068	0.820
C18:1n-9c+t	837	587	596	635	127.1	0.033	0.930
C18:2n-6t	1.05	0.91	0.82	0.39	0.110	<.001	<.001
C18:2n-6c	208	189	209	289	26.9	0.35	0.002
C18:3n-6	1.73	1.93	1.82	1.70	0.138	0.440	0.260
C18:3n-3	11.1	9.2	10.7	18.0	2.62	0.470	0.005
C20:2n-6	6.16	4.98	5.54	8.76	1.233	0.790	0.009
C20:4n-6	43.0	46.5	44.8	44.9	1.79	0.100	0.570
C20:3n-3	1.76	1.36	1.63	2.74	0.430	0.680	0.007
C20:5n-3	2.62	2.74	2.86	2.61	0.136	0.310	0.210
C22:4n-6	7.34	7.05	6.54	6.69	0.372	0.065	0.380
PUFA	221	199	220	308	29.6	0.370	0.002
SFA	545	374	389	438	84.9	0.045	0.740
PUFA:SFA	0.456	0.556	0.601	0.729	0.0597	0.001	0.019
n6:n3	13.79	14.02	13.76	13.40	0.620	0.894	0.606
Total muscle fat	2060	1509	1560	1763	294.2	0.070	0.660
Cholesterol	468	472	456	427	38.2	0.590	0.490

SED, standard error of difference.

Lecithin increased polyunsaturated fatty acids (PUFA) such as C18:2n-6t, C18:2n-6c, C18:3n-3, C20:2n-6 and C20:3n-3 and the ratio of PUFA to saturated fatty acid (SFA) ratio in pork (Table 1). However, there was no significant (P>0.05) difference in n-6:n-3 ratio in pork between the dietary treatments. Pigs fed dietary lecithin had lower C10:0, C12:0, C14:0, C16:0, C20:0, C21:0 and consequently lower total SFA composition in pork compared with pigs fed the control diet. There was no effect of dietary lecithin on pork cholesterol or total intramuscular fat. These data indicate that dietary lecithin supplementation can improve the fatty acid composition of pork.

D'SOUZA, D.N., EDMUNDS, B.L., WILLIAMS, I.H., MCGLEISH, J., MULLAN, B.P., PETHICK, D.W. and DUNSHEA, F.R. (2009). In "Manipulating Pig Production XII", p10, ed. R. J. van Barneveld. (Australasian Pig Science Association: Werribee).

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Dietary Lecithin Alters the Expression of Genes Involved in Skeletal Muscle Collagen Synthesis and Degradation

H. Akit[1], C.L. Collins[2], F.T. Fahri[1], T.Y. Hung[1], D.N. D'Souza[3], B.J. Leury[1] and F.R. Dunshea[1]

[1]University of Melbourne, Parkville, VIC 3010. [2]Rivalea (Australia) Pty Ltd, Corowa, NSW 2646. [3]Australian Pork Limited, Deakin West, ACT 2600.

Previous research has shown that dietary lecithin may improve eating quality of pork by reducing chewiness and hardness (D'Souza *et al*., 2011). Although the exact mechanism is unknown, it is hypothesized that dietary lecithin may decrease skeletal muscle collagen content or the extent of collagen cross-linking. The aim of this experiment was to test the hypothesis that dietary lecithin would improve meat tenderness by reducing collagen content and collagen cross-linking, inferred by measuring the gene expression of collagen precursors and enzymes involved in collagen synthesis and degradation.

Thirty-six Large White x Landrace gilts were randomly allocated at 16 weeks of age (62.9±0.56 kg) to finisher diets containing either 0, 4, 20 or 80 g/kg soybean lecithin (ADM Australia Pty Ltd, Bondi Junction). The pigs were housed individually and had *ad libitum* access to feed and water for six weeks prior to reaching a final slaughter liveweight of 103.2±1.67 kg. Muscle samples from the abdomen were collected 25 minutes post-slaughter and frozen in liquid nitrogen for gene expression using real time polymerase chain reaction (PCR). Gene expressions measured were Type I (α1) and Type III (α1) procollagens, matrix metalloproteinases-1 and 13 (MMP-1 and MMP-13), tissue inhibitor matrix metalloproteinases-1 and 3 (TIMP-1 and TIMP-3), prolyl-4 hydroxylase and lysyl oxidase. All samples had a C_T value for both the gene of interest and the housekeeper gene (ribosomal 18S) with the difference between the two C_T values evaluated as the ΔC_T. All expression data were reported as ΔC_T. A difference in ΔC_T of -1.0 is associated with a doubling and +1.0 a halving of expression. The magnitude of the ΔC_T of a gene indicates the level of gene expression, with the lower the ΔC_T indicating a higher gene expression, and the higher the ΔC_T indicating less gene expression. Since there were no significant linear or quadratic dose effects, the contrasts assessed by analysis of variance were for control versus pooled lecithin treatment and within pooled lecithin treatment.

Table 1. *Effect of dietary lecithin on skeletal muscle gene expression (data are presented as ΔC_T relative to the housekeeper gene ribosomal 18S).*

	Dietary lecithin (g/kg)					P-value	
	0	4	20	80	SED	Lecithin	Within Lecithin[1]
Type I α1 procollagen	19.83	21.18	22.23	20.94	0.708	0.029	0.30
Type III α1 procollagen	18.09	19.66	19.08	19.09	0.523	0.031	0.59
MMP-1	26.31	31.24	28.26	30.60	1.693	0.035	0.33
MMP-13	32.76	35.88	34.76	30.95	1.741	0.53	0.067
TIMP-1	36.08	37.09	34.04	36.78	1.578	0.95	0.24
TIMP-3	50.50	42.70	46.10	43.30	3.450	0.071	0.70
Lysyl-oxidase	37.65	37.88	39.73	36.77	2.042	0.82	0.50
Prolyl-4-hydroxylase	26.57	27.23	28.01	27.43	0.500	0.056	0.43

SED, standard error of difference; MMP, matrix metalloproteinase; TIMP, tissue inhibitor matrix metalloproteinases. [1]Lethicin multiplied by 1.22

Dietary lecithin decreased Type I (α1) and Type III (α1) procollagen gene expression by 67 and 46%, respectively (Table 1), indicating a decrease in the precursor for collagen synthesis. Lecithin decreased MMP-1 expression by 92% but had no significant effect on MMP-13. Skeletal muscle TIMP-3 expression was low in control pigs (ΔC_T=50.5) and was increased 90-fold by lecithin, possibly because of decreased collagen. There was no effect of lecithin on TIMP-1 or lysyl-oxidase gene expression whereas prolyl-4-hydroxylase expression was decreased by 50%. These data show that dietary lecithin can decrease procollagen gene expression and alter the expression of genes involved in synthesis and degradation of collagen.

D'SOUZA, D.N., MULLAN, B.P., PETHICK, D.W., PLUSKE, J.R. and DUNSHEA, F.R. (2011). *Animal Production Science*. **51**: (in press).

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Semi-Moist Extruded Creep Feed Improves Intake of Pigs Post-Weaning

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Inadequate piglet feed intake post-weaning contributes to poor growth and renders the piglet vulnerable to a host of gastro-intestinal challenges (Pluske *et al.*, 1997). Strategies that promote intake of creep feed immediately before and after weaning, without separation of the piglets from the sow, have potential to enhance the lifetime productivity of the piglet. The aim of this experiment was to test the hypothesis that semi-moist (<80% dry matter) extruded creep feeds, based on high and low-cost ingredients, promote feed intake and subsequent piglet performance post-weaning compared with pelleted creep feed.

Three base diets were formulated to contain 14.8 MJ digestible energy (DE)/kg and 0.85 g available lysine/MJ DE. The first base diet included maize, rice, wheat, wheat gluten, soybean meal, meat and bone meal, fish meal, skim milk powder, whey powder, synthetic amino acids, vitamins and minerals. This diet was offered as a mash (Mash (Low)), or as a semi-moist extruded creep feed with the addition of preservatives including propylene glycol, potassium sorbate and a mould inhibitor (SMEC (Low)). The second base diet was similar to the first, but also included barley, a variety of medium and long-chain fatty acid sources and emulsifiers and was also offered as a mash (Mash (High)) and semi-moist-extruded creep (SMEC (High)). The third base diet included wheat, soybean meal, fish meal, meat and bone meal, skim milk powder, synthetic amino acids, vitamins and minerals and was offered as a steam-pressed pellet (4 mm). Semi-moist feeds were produced using a Wenger X-85 extruder fitted with a 3 mm die and contained approximately 200 g/kg moisture at the time of feeding. Diets were offered *ad libitum* to individually housed male piglets (20 pigs/treatment) from weaning (26 d) for 28 days in a randomised block design, blocked on weaning weight. Data were subjected to an analysis of variance and means separated by least significant differences (P<0.05).

Table 1. *Feed intake (g/d), average daily gain (g/d) and feed conversion ratio of weaner pigs fed high or low-cost ingredient semi-moist extruded and mash creep feeds or steam-pelleted creep feed for 28 d.*

Diet	Start weight (kg)	End weight (kg)	Feed intake (g/d)	Average daily gain (g/d)	FCR (kg/kg)
SMEC (Low)	5.89	11.34^{a}	360^{a}	195^{a}	2.01^{c}
SMEC (High)	5.88	15.67^{b}	510^{c}	350^{c}	1.50^{ab}
Mash (Low)	5.89	14.69^{bc}	430^{b}	314^{bc}	1.39^{a}
Mash (High)	5.89	13.91^{b}	410^{ab}	286^{b}	1.43^{a}
Steam Pellet	5.92	12.20^{a}	360^{a}	224^{a}	1.68^{b}
SED	0.170	0.701	30.0	23.1	0.106
P value (diet)	1.000	<0.001	<0.001	<0.001	<0.001

^{a,b,c}Means in a column with different superscripts differ significantly (P<0.05); FCR, Feed conversion ratio; SMEC, Semi-moist extruded creep; SED, Standard error of difference.

SMEC (High) significantly increased (P<0.001) the feed intake of piglets in the first 28 d post-weaning compared to the same diet offered as a mash (Mash (High)) and the steam-pellet (Table 1). SMEC (Low) became contaminated with mould by d 10 of the experiment which compromised intake, however, comparative performance of pigs fed Mash (Low) and Mash (High) suggests limited benefit from inclusion of higher-cost ingredients. If mould contamination can be contained, semi-moist extruded creep feeds may offer a practical means of promoting piglet feed intake post-weaning.

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

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Sourcing Cereal Feed Grains for Pigs: What are the Views of the Supply Chain?

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Fell (2010) estimated that feed costs accounted for approximately 55% of total production costs in a pig enterprise. He also suggested that between 2010/11 and 2014/15, the pig to feed grain price ratio, which is an indicator of returns from pig production, is expected to remain largely unchanged. Hence, reliable supply and price of feed grains are important considerations for pig producers and the entire pork supply chain. In an effort to increase feed grain production specific for the major pig producing areas of Australia, Australian research has been directed towards development of new cereal grain varieties that can compete, in terms of profit per hectare, with current food grain production, and also provide increased nutritive value to pigs. This paper reports findings from a socioeconomic study where the aim, in part, was to assess attitudes to growing and sourcing feed grains as well as price premiums associated with these grains. Such findings can be used by relevant supply chain participants to aid management decisions.

In line with Bardsley and Thomas (2005), a semi-structured, interactive, open-ended interview approach was used to record respondents' perceptions; questions were common to all interviews but allowed for secondary in-depth questions. A small representative sample of stakeholders (grain, pig and feed producers; feed buyers and sellers; feed and grain information providers) from WA, SA, Victoria and NSW were selected using dimensional sampling techniques. All interviews were conducted at a location convenient to the interviewees with two interviewers present. Ethics approval was granted from the Murdoch University Research Ethics Office. Following Monette *et al.* (1986), the interview summaries recorded were written to minimise bias and hence ensure validity of results. Care was taken with coding to enhance reliability of data.

Of the 25 respondents, 52% indicated that when selecting a variety to grow, its capacity to be dual purpose (eg. grazing and grain) and/or profit were the most important drivers for grain growers. When considering triticale, agronomic reasons, in combination with either profit or the market, were believed to be the most important influences over variety selection by the majority of respondents (56%).

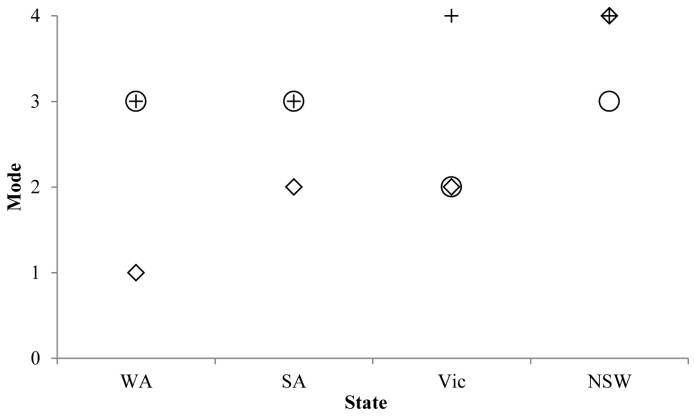

Figure 1. *The mode for ordinal data (Monette* et al. *1986) reflecting survey participants' attitudes to: sourcing feed grains ○ (1= difficult; 2= quite difficult; 3= not very difficult; 4= not at all difficult); premiums paid to grain growers ◇ (1= yes; 2= yes but difficult to implement; 3= maybe; 4= no); and premiums paid by pig producers + (1= yes; 2= yes but difficult to implement; 3= maybe; 4= no).*

Interview results suggested that it can be difficult to source feed grains from Victoria, obtaining local feed grains in WA, SA and NSW was generally not as difficult, and grain premiums may be an option when buying and selling feed grains in WA and SA but less likely to be so in Victoria and NSW (Figure 1). In addition, 80% of respondents did not believe grain growers would enter into formal contracts with end users. As there are difficulties in procuring grains and market tools such as premiums and contracts are not widely favoured, at least in the short term, communicating to growers that a reliable market for high yielding, high energy grain exists will be important.

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FELL, J. (2010). *Australian Commodities*. **17**:76-78.

MONETTE, D.R., SULLIVAN, T.J. AND DEJONG, C.R. (1986). "Applied Social Research" (Holt, Rinehart Winston USA: Florida).

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The Responses of Finishing Pigs to Ractopamine are Enhanced in Higher Energy Diets

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Ractopamine hyrdochloride (Paylean®, Elanco Animal Health, West Ryde, NSW) has been shown to improve the lean content of pigs (Dunshea *et al*., 2005), yet responses to the commercial application of Ractopamine in Australia have been variable. Genetic selection in commercial pigs in recent years has resulted in a marked decrease in backfat levels of market pigs. However there is still widespread conservatism with regard to the energy density of finishing diets, to avoid excessive backfat levels. The hypothesis explored in this experiment was that these lower energy finisher diets could be constraining the potential benefits of Ractopamine supplementation.

Commercial female grower pigs (n=960; [(Landrace x Large White) x terminal sire]) were grown out to a start weight of 75.50 ± 3.05 kg liveweight in conventional grower facilities (48 pens of 20 pigs) utilising a common feeder between each two pens. A 3 x 2 factorial design was used with the factors being energy density (13.4, 14.2 or 15.0 MJ digestible energy (DE)/kg) and Ractopamine inclusion level (0 or 7.5ppm). All diets were formulated to contain 0.58g available lysine/MJ DE. The experimental diets were offered *ad libitum* for 28 days. Growth performance and feed intake were recorded from d 0 to d 28. At completion of the experiment all pigs were processed in a commercial abattoir yielding carcase weight and backfat (at the P2 position) values for each individual pig. The pen was used as the experimental unit for the daily gain and carcase data analysis, whilst the feeder was used as the experimental unit for feed intake and feed conversion analysis. All statistical analysis was conducted using the multifactorial analysis of variance procedure in Statsgraphics Plus 5.1 (Statpoint Technology Inc., Warrenton, VA).

Table 1. *The effects of dietary energy density (D) and Ractopamine (P) on the growth performance and carcase characteristics and income over feed costs of female pigs from 75 to 100 kg liveweight.*

Energy (MJ DE/kg)	13.4		14.2		15.0			Significance		
Paylean (ppm)	0	7.5	0	7.5	0	7.5	SEM	D	P	DxP
ADG (kg/d)#	0.855	0.897	0.864	0.923	0.871	0.952	0.025	NS	0.007	NS
ADFI (kg/d)	2.66	2.59	2.53	2.57	2.51	2.52	0.068	NS	NS	NS
FCR	3.12^{c}	2.89^{b}	2.93^{b}	2.79^{ab}	2.88^{b}	2.65^{a}	0.059	0.003	0.001	NS
Δ Car. wt (kg)#	17.55^{a}	19.29^{bc}	18.16^{ab}	20.18^{cd}	19.01^{abc}	21.43^{d}	0.54	0.007	0.001	NS
BF (P2, mm)#	10.36	10.23	9.98	10.34	10.91	10.51	0.238	0.062	NS	NS
IMFC ($/pig)		3.30	0.63	3.61	0.85	5.10				

^{abcd}Means in a row with different superscripts differ significantly (P<0.05); ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; Δ Car. Wt, carcase weight – (initial liveweight x 0.76); BF, backfat; IMFC, income minus feed cost; D, dietary energy density; P, Paylean; # pen used as the experimental unit; NS, not significant (P>0.05).

As the energy density increased from 13.4 to 15.0 MJ DE/kg there was no shift in growth rate but there was a trend for reduced feed intake resulting in an improvement in feed conversion (FCR, P<0.01). The application of Ractopamine had no effect on feed intake but improved growth rate (P<0.01), FCR (P<0.01) and carcase gain (P<0.01). Neither dietary energy density nor Ractopamine supplementation influenced backfat (P>0.05). The magnitude of the net economic benefit of Ractopamine supplementation increased with increasing energy density, indicating the full benefit of Ractopamine will only be realised when applied in high energy diets.

DUNSHEA, F.R., RIKARD-BELL, C., CURTIS, M.A., EDWARDS, A.C., GANNON, N.J., HENMAN, D.J., MULLAN, B.P. and VAN BARNEVELD, R.J. (2005). In "Manipulating Pig Production X". p.152, eds J.E. Paterson (Australasian Pig Science Association: Werribee).

Supported in part by the Pork CRC Ltd

How do Commercial Processing Conditions Affect the Quality of Pig Grower Diets?- Process Conditions and Pellet Quality

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The nutritional quality of pellets as judged by the rate of starch digestion *in vitro*, degree of gelatinisation and proportion of damaged starch is affected by grain particle size and the proportion of sorghum in the diet (Sopade *et al.*, 2008). Based on this data, we hypothesise that the extent of conditioning and the retention time during processing are key variables influencing the physical and nutritional quality of the final product.

This experiment was undertaken to examine the effects of specific conditioning temperatures and extended conditioning on diet mixtures containing different proportions of sorghum:wheat using a 2x2x2 factorial design. Diets formulated to a typical pig grower specification (0.68 g available lysine/MJ digestible energy (DE), 13.8MJ DE/kg) were manufactured in a commercial feedmill at a nominal throughput of 10 tonnes per hour and steam pressure of 200kPa. Sorghum was included in the diet at 0 and 600g/kg, steam temperature at the mixer conditioner was set to 80ºC or 95ºC and retention time was varied by by-passing the expander to simulate a conventional steam conditioning method (Conventional) or by utilizing an expander with 11 bar pressure at the cone (Extended). Damaged starch (DS), as measured by an enzymatic test was used as an indicator of the extent of conditioning. Statistical analyses were undertaken using a GLM procedure (Minitab Version 11; Minitab Pty Ltd, Sydney, NSW).

Table 1. *Influence of processing conditions on pellet quality.*

	Sorghum Inclusion		Temperature		Conditioning			
	0	600g/kg	80°C	95°C	C	E	SED	P value
SMEp (kWh/tonne)	7.4	7.0	7.2	7.2	6.9[a]	7.6[b]	0.22	P<0.05
Retention time (seconds)	95	95	93	97	85[a]	105[b]	7.2	P<0.001
Particle size mash (µm)[1]	683	684	683	684	680	686	22	NS
Bulk density (kg/m^3)	661	666	656	671	659	658	0.06	NS
Durability (%)	95.9	96.5	96.7	95.6	95.6	96.8	0.55	NS
Length (mm)	14.2	14.1	14.8	13.7	14.1	14.3	0.39	NS
Hardness (kg)	6.7[a]	7.2[b]	6.9	6.9	6.5[a]	7.4[b]	0.23	P<0.01
Starch (g/kg)	477[a]	497[b]	465[a]	509[b]	483	490	3.7	P<0.05
DS mash (g/kg starch)	52	55	43[a]	64[b]	55	52	3.2	P<0.001
DS pellet (g/kg starch)	78[a]	104[b]	79[a]	103[b]	74[a]	109[b]	3.67	P<0.001

C, conventional; E, extended; SMEp specific mechanical energy at the press; [1]Geometric mean particle size; SED, Standard error of the difference; [ab]Main effects with different superscripts differ significantly (P<0.05); NS, not significant. DS, damaged starch.

Processing conditions had no significant effect on the physical characteristics (pellet length, durability and bulk density) of the pellets produced but markedly increased measures of damaged starch. Increasing sorghum inclusion to 600g/kg of the diet, increasing the temperature at the conditioner and extending the conditioning process each produced a highly significant increase in damaged starch content of the final product. Significant interactions were identified between conditioning and both sorghum inclusion (P<0.01) and temperature (P<0.05) for damaged starch content with extended conditioning producing greater increases in starch damage as sorghum content and processing increased. Extending conditioning time caused an increase (P<0.05) in energy use at the press and an increase in pellet hardness but produced little appreciable change in pellet quality or the degree of conditioning as measured by damaged starch.

SOPADE, PA, MAHASUKONTHACHAT, K & GIDLEY, MJ 2008, pp176-179; in "Proceedings of the 58th Australian Cereal Chemistry Conference", eds, J. F. Panozzo and C. K. Black, Surfers Paradise, Gold Coast, Australia, 31 August - 4 September, 2008.

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Development of New Wheat Varieties For the Pig Industry: Digestible Energy Yield of Candidate Wheats

J.C. Kim[1], J.R. Pluske[2], B.P. Mullan[1], R.H. King[3], R.H. Wilson[3], D.J. Mullan[4], T.G. Walmsley[4] and J. M. Pluske[5]

[1]Department of Agriculture and Food WA, South Perth, WA 6151. [2]Murdoch University, Murdoch, WA 6150. [3]RHK Consulting Pty Ltd, Essendon, VIC 3040. [4]Rob Wilson Consulting Pty Ltd, Perth, WA 6012. [5]InterGrain, Kensington, WA 6151. [6]SciEcons Consulting, Subiaco, WA 6904.

Securing more reliable and consistent energy supplies for pig diets is one of the major challenges facing Australian pig production due to volatility of the international grain price and variable grain yields between seasons. A more reliable and consistent energy supply will reduce variation in the annual cost of pig feed and total cost of pig feed. The aim of this experiment was to identify specific lines of wheat that are high yielding and contain high levels of digestible energy (DE) from the InterGrain (Kensington, WA) gene pool for potential commercial release.

Pre-screened, potentially high yielding and high DE wheats from 25 genetic lines were grown in the 2010/2011 season and included released varieties. The 25 lines were grown in 35 sites across Australia (NSW 454 plots, SA 568 plots, Vic 511 plots and WA 618 plots), and a total of 2151 wheat samples were collected at harvest and scanned through an established NIRS calibration (AusScan Calibrations, Pork CRC Ltd, Roseworthy, SA). To account for the variation within plot and across variety, as well as to compare candidate varieties to currently grown varieties, the data were transformed to a selection index ((DE x Yield)/1000) and expressed as total DE production/ha (Gigajoules (GJ)/ha). Data were pooled across state and analysis of variance was used to test the effects of variety on DE yield per hectare. Fishers-protected least significant difference test was used to separate difference between varieties.

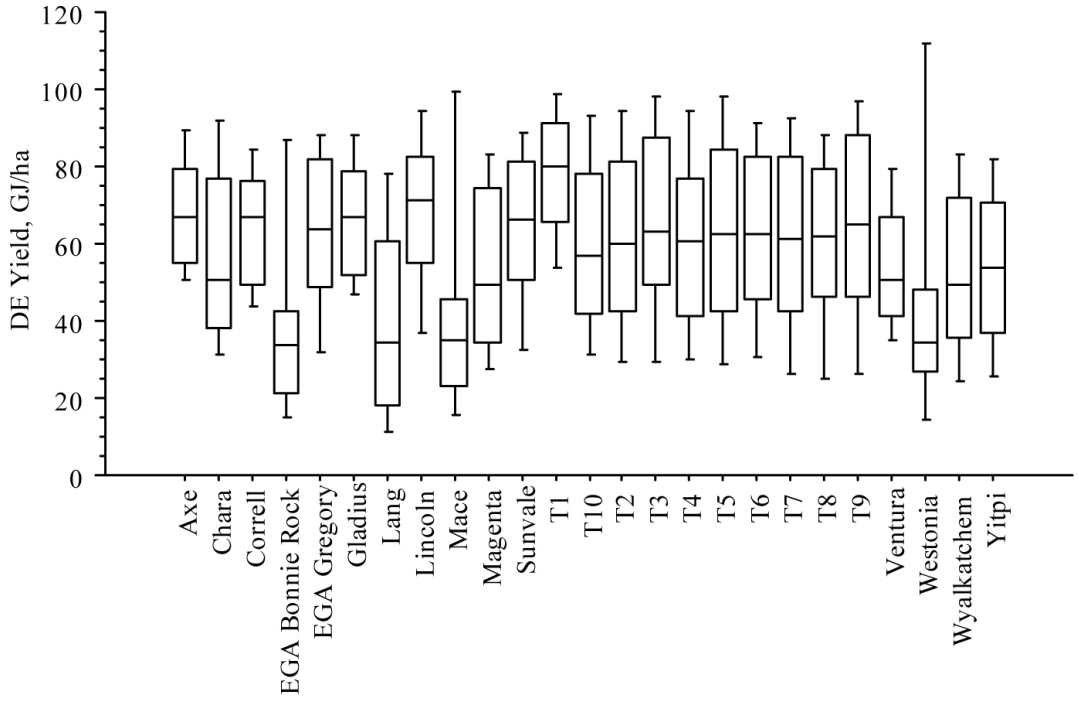

Figure 1. *The digestible energy (DE) yield (expressed as GJ/ha) of candidate wheats (T1–T10) and selected benchmark wheat varieties grown across Australia in the 2010/2011 season.*

Results showed that of the test varieties T1 outperformed selected benchmark wheat varieties ($P<0.01$) in terms of the DE yield/ha, showing the potential of new lines of wheat that could be used in the Australian pig industry to improve overall production efficiency.

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Variability in The Total and Reactive Lysine Content of Soybean Meal

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[1]Department of Agriculture and Food, South Perth, WA 6151. [2]Barneveld Nutrition Pty Ltd, Loganholme, QLD 4129. [3]John L Black Consulting, Warrimoo, NSW 2744. [4]Rob Wilson Consulting, Perth, WA 6012. [5]JCS Solutions Pty Ltd, Berwick, VIC 3806.

During heat processing and prolonged storage of feedstuffs, the ε-amino group of lysine can react with other compounds, specifically reducing sugars, and form biologically unavailable lysine derivatives (eg. fructoselysine). This form of lysine is known to be unavailable for body protein deposition and is excreted largely in the form of urinary nitrogen even though this form of lysine can be absorbed through the small intestinal epithelium (van Barneveld *et al.*, 1995). However, some of this unreactive lysine can revert to lysine through the process of acid hydrolysis during conventional amino acid analysis, which causes inaccuracy in the quantification of biologically available lysine content (Rutherfurd and Moughan, 2007). Only the lysine with a free ε-NH_3 group is considered as biologically available lysine for body protein deposition (Rutherfurd and Moughan, 2007). Soybean meal is a common amino acid source in pig diets and there is a need to quantify the variation in total and reactive lysine content for the Australian pig industry to improve the precision of diet formulation, utilisation efficiency of amino acids and hence production efficiency of Australian pork per unit of nutrient fed. The hypothesis tested in this study was that reactive lysine can not be accurately predicted from total lysine content in soybean meal.

A total of 209 soybean meal samples from the major soybean meal producing countries such as USA, Brazil, Argentina, China and India were collected over 12 months. Samples were immediately stored at 4°C and analysed for total and reactive lysine content using the method described in Rutherfurd and Moughan (2007). For reactive lysine content, the within batch and between batch variations (coefficient of variation) were less than 5 % and 10%, respectively. Data were analysed using a regression analysis.

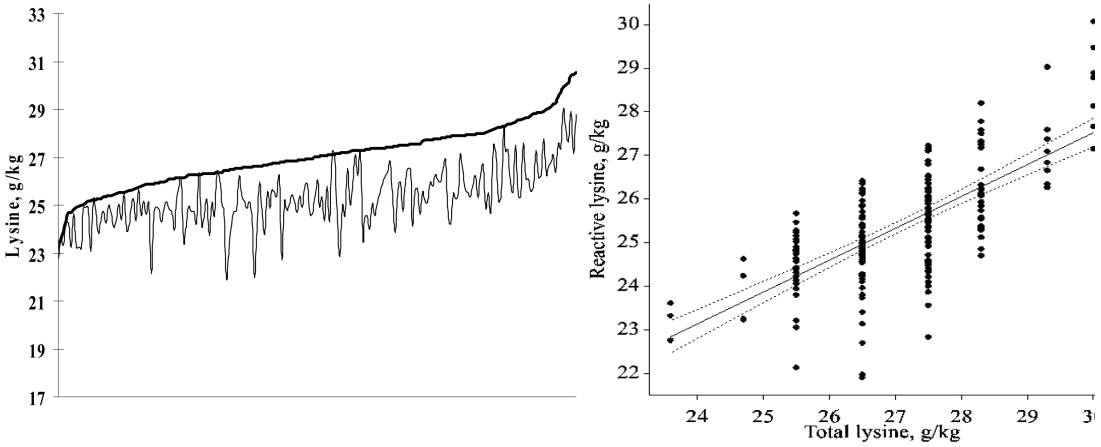

Figure 1. *Variation in (a) reactive lysine content (▬ total lysine,— reactive lysine) and (b) relationship between total and reactive lysine with a 95% confidence interval.*

The results demonstrate that absolute reactive lysine content varied by 27% (ranged from 21.9 to 30.1 g/kg SBM) and the reactive/total lysine ratio varied by 17% (Figure 1). A significant relationship between total and reactive lysine was observed (R^2 = 0.52, P < 0.001) indicating that the amount of heat damaged lysine was partly dependent on the total amount of lysine in a soybean meal. However, weak predictability of reactive lysine from total lysine (R^2 = 0.52) highlights the importance of developing rapid screening tools such as near infra-red reflectance (NIR) calibrations for quantitative screening of protein quality in soybean meal.

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Supported in part by the Pork CRC Ltd.

Weight Loss During First Lactation Does Not Influence Subsequent Reproductive Performance

R.J.E. Hewitt[1], S.K.J. Peucker[1] and R.J. van Barneveld[2]

[1]CHM Alliance Pty Ltd, Millmerran, QLD 4357. [2]Barneveld Nutrition Pty Ltd, Loganholme, QLD 4129.

The loss of live weight during lactation is a relatively normal response to the high milk yield and reduced appetite seen in modern sows (Aherne and Williams, 1992). However, when excessive weight loss occurs, subsequent wean to oestrus intervals increase and farrowing rates and total born litter sizes fall (Thaker and Bilkei, 2005). Thus, to maintain optimal reproductive performance, feeding and management practices should be designed to reduce live weight losses during lactation. We hypothesized that the reproductive capacity of gilts that lose a large percentage of weight during their first lactation will be reduced.

As part of an experiment into methods to increase reproductive performance and longevity in the herd (Hewitt *et al*., 2009), first-litter sows were offered a standard (0.56 g available lysine/MJ digestible energy; DE) or high-lysine (0.90 g available lysine/MJ DE) lactation diet and assigned to one of two suckling regimes, seven or twelve piglets, maintained for the whole lactation (21.2±0.07 d). Changes in maternal body weight were assessed by weighing sows at entry (d 112 of gestation) and exit from the farrowing room. Empty maternal weight at entry was calculated by subtracting the weight of uterine gain (foetal and placental tissue, fluid and increased uterine weight), based on the product of the total number of pigs born and piglet birthweight and an adjusting factor of 0.61 (Noblet *et al*., 1990). Relationships between measures were determined by Pearson's correlation.

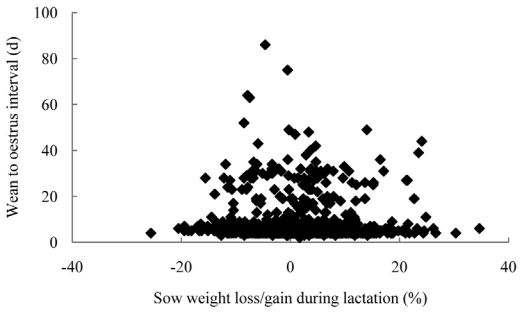

Figure 1. *The effect of sow weight loss or gain during first lactation on subsequent wean to oestrus interval.*

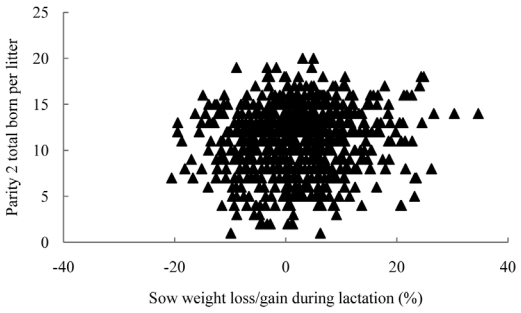

Figure 2. *The effect of sow weight loss or gain during first lactation on subsequent total born in the second parity.*

The impact of lactational demand, as well as the natural variation in feed intake, resulted in a wide range of body weight changes during lactation (Figure 1), with losses of up to 25% of maternal body weight through to a gain of almost 35% of body weight, although 95% of gilts fell between a 14% loss and an 18% gain of post-farrowing live weight. The relationship between sow weight loss or gain during lactation and wean to oestrus interval was poor (r^2=0.02, P>0.05, Figure 1) as it was with the total number of piglets born in the subsequent litter (r^2=0.10, P<0.01, Figure 2), with gilts returning to service (8.2±0.29) and having normal litter sizes (11.1±0.12) despite large losses in live weight, and no apparent advantage in gaining weight in lactation. Similar to Clowes *et al*. (2003), who hypothesized that the lactating sow is able to sustain a degree of protein loss without loss of reproductive function, this data suggests that the modern commercial sow's genetic propensity for reproduction outweighs significant weight loss in lactation.

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Supported in part by the Pork CRC Ltd.

Finisher Gilt Performance Responds Linearly to Ractopamine Dose

R.J.E. Hewitt[1], S.K.J. Peucker[1] and R.J. van Barneveld[2]

[1]CHM Alliance Pty Ltd, Millmerran, QLD 4357. [2]Barneveld Nutrition Pty Ltd, Loganholme, QLD 4129.

Ractopamine hydrochloride (Paylean®, RAC, Elanco Animal Health Pty Ltd, West Ryde, NSW) is an approved ingredient for pigs that repartitions nutrients towards lean tissue growth and thus improves production efficiency and carcase quality (Dunshea *et al*., 2005). To date, research has focussed on RAC inclusion in 5ppm increments two to four weeks prior to market. The additional cost of a 10 ppm dose and the corresponding improvement in growth performance may not be justified; however, there may be benefits in an intermediate dose for a two week period. The aim of this experiment was to evaluate changes in production efficiency in response to a 7.5 ppm RAC dose relative to the more commonly applied 5 and 10 ppm RAC doses, with the hypothesis that production efficiency will be improved with an increasing dose of RAC.

Twelve pens of gilts (42 pigs/pen), were allocated via a randomised block (blocked on weight) to one of four treatments fed for 14 d prior to sale. Two base diets (14.0 MJ digestible energy (DE)/kg, 0.70 g available lysine/MJ DE) with or without RAC (10 ppm) were blended using the FEEDPro system (Feedlogic Corporation, Wilmar, MN) to deliver four blends ranging in RAC content (0, 5, 7.5 and 10 ppm). Data was subjected to an analysis of variance and means separated by least significant differences (P<0.05).

Table 1. *Mean performance of finisher gilts offered Ractopamine hydrochloride at 0, 5, 7.5 or 10 ppm for the final two weeks prior to slaughter.*

	Ractopamine Dose (ppm)					
	0 ppm	5 ppm	7.5 ppm	10 ppm	SED	P value
Start weight (kg)	89.6	91.5	91.6	87.0	2.95	0.411
ADG (kg/d)	0.604[a]	0.817[b]	0.861[bc]	0.987[c]	0.069	0.003
ADFI (kg/d)	2.47	2.53	2.60	2.47	0.13	0.720
FCR	4.10[a]	3.12[b]	3.04[b]	2.50[c]	0.21	<0.001

[abc]Means in a row with different superscripts differ significantly (P<0.05); ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SED, standard error of difference.

Average daily gain (ADG) was significantly increased by the inclusion of RAC in the diet (P=0.003), with a significant response to increasing dose. Increasing the inclusion rate of RAC did not affect average daily feed intake (ADFI), but had a significant effect on feed conversion (FCR, P<0.001), with a response to increasing dose being observed.

The relatively poor performance of the control treatment is likely a reflection of high summer temperatures during the experimental period, with reduced ADFI in summer most likely a result of reduced feeder access in the cooler time of the day when all pigs are looking to feed simultaneously. Routine monitoring of feed intakes in this facility shows a 0.3 kg/d difference in ADFI between summer and winter. It is likely that a similar response to environment is seen in other conventionally housed finisher pigs during summer. The response of finisher gilts to RAC observed in this experiment suggests supplementation with dietary RAC may offset the effects of reduced feed intake associated with summer temperatures.

The inclusion of RAC in the diet of finisher gilts improved production efficiency, however, it was not strictly dose dependent with the intermediate dose of 7.5 ppm not resulting in improved performance over the standard inclusion rate of 5 ppm. The inclusion of RAC at 10 ppm resulted in the best performance and despite the increased cost of the diet, the improved feed usage resulted in a further 60% saving in feed costs compared to the standard 5 ppm inclusion rate. These results suggest that increasing RAC inclusion to 10 ppm will improve production efficiency.

DUNSHEA, F.R., D'SOUZA, D.N., PETHICK, D.W., HARPER, G.S. and WARNER, R.D.(2005). *Meat Science*. **71**:8-38.

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Influence of Pelleting and Supplementing Sodium Metabisulphite in Nursery Pig Diets Contaminated with Deoxynivalenol

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[1]University of Melbourne, Parkville, VIC 3052. [2]Kansas State University, Manhattan, KS, USA 66502. [3]Hubbard Feeds, Mankato, MN, USA 56001. [4]New Fashion Pork, Jackson, MN, USA 56143.

Deoxynivalenol (DON) develops in crops when moisture is too high during the flowering period. Pigs are particularly susceptible to DON, which can cause reductions in performance, sub-clinical immune suppression and feed refusal. Previous studies found that combinations of hydrothermal treatment and sodium metabisulphite (SMB) can convert DON to a non-toxic 10-sulfonate adduct (Young *et al*., 1987; Danicke *et al*., 2005). Thus, this experiment aimed to determine the effect of pelleting and supplementing SMB in naturally DON-contaminated distiller's dried grains with solubles (DDGS) from corn on nursery pig performance.

A total of 1180 mixed sex (gilt and barrow) pigs (PIC 337×1050; initially 11.1 ± 0.32 kg) were used in a 21 d experiment conducted concurrently at two locations in order to replicate results in university (seven pigs per pen) and commercial (28 pigs per pen) conditions. At weaning, pigs were weighed and allotted to one of seven treatments (five replicate pens; repeated at each location) in a 2×3+1 factorial arrangement with the respective factors being 1) Diet form: meal (M) or pellet (P), 2) DDGS source: positive control (PC; final diet <0.5 ppm DON), negative control (NC; final diet 4.0 ppm DON), or NC DDGS pelleted and crumbled before mixing into the final diet. A seventh treatment, fed in meal form, included 25 g/kg SMB prior to pelleting DDGS (Final diet 0.77% SMB). Diets were manufactured simultaneously and contained 300 g/kg DDGS. Pens of pigs were weighed and feed disappearance was recorded weekly. Data were analyzed as a completely randomized design with the pen as the experimental unit and research location as a random effect. Analysis of variance used the MIXED procedure in SAS (SAS Institute Inc., Cary, NC, USA).

Table 1. *Effects of pelleting, DDGS source and sodium metabisulphite on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of pigs fed deoxynivalenol(DON)-containing diets[1].*

				NC+	NC+ DDGS		Significance			
	Form	PC	NC	DDGS	/SMB	SEM	PvM	D	DDG	SM
ADG (g/d)	M	584	520	543	577	10.5	***	***	***	**
	P	628	582	581						
ADFI (g/d)	M	881	791	799	848	18	NS	***	***	**
	P	875	801	807						
FCR	M	1.51	1.53	1.47	1.47	0.043	***	NS	NS	NS
	P	1.40	1.38	1.39						

[1]Growth and intake data collected on a pen basis; PC, positive control; NC, negative control; DDGS, dried distillers grains with solubles; SMB, sodium metabisulphite, SEM, pooled standard error of mean; NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001; PvM contrast compared diet form: Meal vs Pellets; D, contrast compared DON content; DDG, contrast compared main effect means of the three DDGS sources; SM, contrast compared effect of SMB.

Pelleting the final diet increased (P<0.001) ADG, FCR and final weight but did not alter ADFI. Pigs fed diets containing DON had reduced (P<0.001) ADG, ADFI and final weight, but there were no differences in FCR. Furthermore, DDGS source influenced (P<0.001) ADG, ADFI, and final weight. Finally, adding SMB to DDGS prior to pelleting and crumbling into a meal diet improved (P<0.01) ADG, ADFI and increased (P<0.05) final weight. However, FCR was not affected. These results suggest that when feeding diets containing DON, pig producers may be able to recover reductions in performance by pelleting the final diet. Although pelleting the DDGS before presenting in the final diet had no effect, adding SMB prior to pelleting appears to impact DON concentrations in the final diet and may be a way for producers to offset performance losses associated with feeding diets containing DON.

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The Response of Male Pigs Immunised Against Gonadotrophin Releasing Hormone to Dietary Available Lysine

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[1]Department of Agriculture and Food WA, South Perth, WA 6151. [2]University of Melbourne, Parkville, VIC 3052.

Immunisation of entire males against gonadotrophin releasing hormone (GnRH) is effective in eliminating boar taint, however, it may be associated with an increase in backfat and reduced feed conversion compared with the production of entire males. In order to minimise these negative effects, it is important that the level of amino acids in diets fed to immunised males are appropriate (Dunshea, 2009). However, there is no published work on the lysine requirements of immunised males. The hypothesis of this experiment was that male pigs immunised against GnRH will respond differently to increasing levels of available lysine per MJ digestible energy (Av Lys/MJ DE) than entire male pigs.

Four hundred and twenty pigs (Large White x Landrace x Duroc) were randomly allocated to a 2x5 factorial experiment comprising sex (S; entire males and males immunised against GnRH) and available lysine (L; 0.32, 0.43, 0.54, 0.64 and 0.75 g av lys/MJ DE). The experimental diets were formulated to contain 13.5 MJ DE/kg. The diets were offered *ad libitum* for six weeks from 60.1±1.8 kg liveweight (LW). Pigs were housed in group pens of seven pigs of the same sex. The first immunisation occurred at 10 weeks of age and the second immunisation when the experimental diets were implemented. The pigs were weighed and voluntary feed intake was recorded weekly. Data were analysed by analysis of variance (ANOVA) with pen as the experimental unit.

Table 1. *Average daily gain (ADG), feed conversion ratio (FCR), carcase weight (CW) and backfat (P2) for entire male pigs (E) and male pigs immunised against GnRH (I) fed varying levels of available lysine per MJ digestible energy (Av Lys/MJ DE) over 42 days from 60.1 to 107 kgs liveweight (LW; n=6).*

		g Av Lys/MJ DE						Significance		
	Sex	0.32	0.43	0.54	0.64	0.75	SED	S	L	SxL
ADG (kg)	E	0.85^{a}	1.02^{b}	1.15^{c}	1.20^{d}	1.21^{d}	0.031	<0.001	<0.001	<0.001
	I	1.06^{a}	1.24^{c}	1.19 ^{b}	1.25^{c}	1.24^{c}				
FCR	E	3.05^{a}	2.59^{b}	2.33^{c}	2.33^{c}	2.28^{c}	0.060	0.019	<0.001	<0.001
	I	2.84^{a}	2.53^{b}	2.50^{b}	2.52^{b}	2.51^{b}				
CW (kg)	E	63.2^{a}	69.2^{b}	73.2^{c}	74.7^{d}	73.9^{cd}	0.959	<0.001	<0.001	<0.001
	I	68.4^{a}	73.8^{b}	74.3^{b}	75.0^{b}	74.3^{b}				
P2 (mm)[1]	E	13.0^{a}	12.9^{a}	11.1^{b}	10.4^{b}	9.86^{b}	0.757	<0.001	<0.001	0.298
	I	15.1^{a}	13.2^{b}	12.6^{bc}	11.8^{cd}	11.2^{d}				

SED, Standard error of difference of means for Sex x Lysine; [1]Carcase weight used as a covariate.; ^{abcd}Means in the same row with different superscripts differ significantly (P<0.05).

On average, the immunised males had a higher average daily gain (ADG; P<0.001), lower feed conversion ratio (FCR; P=0.019), heavier carcase weight (CW; P<0.001),) and a higher P2 (P<0.001) compared to the entire males (Table 1). Increasing the level of lysine increased ADG and improved FCR in a linear and quadratic manner (P<0.001 and P<0.001, respectively). The immunised males had a higher ADG at lower lysine levels compared to entire males (P<0.001). At higher levels of lysine immunised males had a higher FCR compared to entire males (P<0.001). P2 decreased linearly (P<0.001) as the lysine level increased (P<0.001). These results suggest that the lysine requirement for males immunised against GnRH to maximise growth performance and minimise carcase fatness over the LW range and time period investigated is lower than for entire males.

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Juncea (*Brassica juncea*) Meal Can be Utilised as an Alternative Vegetable Protein Source in Growing Pig Diets

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Juncea (*Brassica juncea*) has been bred as a crop for low rainfall regions in Australia producing a seed with similar properties to that of traditional canola (*Brassica napus*). There is currently commercial interest in the use of juncea oil for bio-diesel production, and as such the remaining meal may become available as a source of vegetable protein for animal feed. Juncea meal, like canola meal, contains glucosinolates which are secondary plant metabolites that can reduce feed intake when consumed in high concentrations. As such, there is a need to determine the growth response of growing pigs to increasing concentrations of juncea meal in the diet. This experiment tested the hypothesis that expeller extracted juncea meal could be included in the diet of growing pigs at up to 240 g/kg without negatively affecting feed intake or growth performance.

Seventy entire male pigs (Large White x Landrace, PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) were identified at 13 weeks of age (weight 40.4 kg ± 0.41 kg, mean ± standard error) and transferred to individual grower accommodation. At 14 weeks of age pigs were individually weighed and randomly allocated to one of five experimental diets containing 0, 60, 120, 180 or 240 g/kg juncea meal. The nutritional composition of the expeller extracted juncea meal and canola meal used in this experiment were determined using the methods of AOAC (2004), while the amino acid profile of the meals were determined by high performance liquid chromatography. Representative samples of juncea meal were analysed for total glucosinolate concentration by Mailer and Wratten (1985). Diets were formulated to contain 14.0 MJ digestible energy (DE)/kg and 0.62 g available lysine/MJ DE, with juncea meal replacing canola meal at increasing concentrations in the test diets. The DE and amino acid contents of the diets were maintained by slightly altering the inclusion levels of tallow and synthetic amino acids. Pigs were offered their test diets *ad libitum* for 35 days with average daily feed intake (ADFI) and rate of gain measured during this time. Data were subjected to an analysis of variance for treatment effects in a randomised design. The response to dietary juncea concentration was tested for linear and quadratic effects using the polynomial function in Genstat Version 8 (VSN International, Oxford UK). The experimental unit for all analyses was the individual animal.

Table 1. *Effect of juncea meal concentration on the growth performance of growing pigs (d0-35).*

	Concentration of juncea meal (g/kg)						Significance	
	0	60	120	180	240	SED	Linear	Quadratic
ADG (kg/d)	1.07	1.07	1.03	1.08	0.87	0.063	0.009	0.063
ADFI (kg/d)	2.55	2.46	2.46	2.37	2.09	0.120	<0.001	0.14
FCR (kg/kg)	2.38	2.35	2.40	2.20	2.45	0.134	0.99	0.38

ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SED, standard error of the difference

Average glucosinolate concentration in the juncea meal was 15.9 μmoles/g (as received). Diet significantly influenced feed intake ($P<0.01$) and daily gain ($P<0.01$), but had no influence on feed efficiency ($P>0.05$). Feed intake declined linearly with increasing juncea meal (Table 1), resulting in reduced growth rates at the highest inclusion level. These data indicate that expeller-extracted juncea meal can be included in the diet for growing pigs at concentrations up to 180 g/kg without adversely affecting growth rate. Based upon the results of this and other studies with *Brassica* meals (eg. Corino *et al*., 1991; Schone *et al*., 1997), it is recommended that the maximum inclusion level of juncea meal in commercial grower/ finisher diets be limited to levels in which the total glucosinolate concentration of the complete diet does not exceed 2.0 mmol/kg diet. It is also recommended that testing of individual batches of juncea meal be undertaken prior to animal feeding.

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In Vitro Digestion of the Sorghum Kafirin Fraction with Commerical Feed Enzymes

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One reason for poorer performance of pigs fed sorghum-based diets compared with wheat-based diets may be due to the poor nutritional quality of its main storage protein, kafirin, which has been attributed to its low solubility and deficiencies in essential amino acids (Sastry *et al.*, 1986). It is hypothesised that there will be an improvement in sorghum kafirin digestion through addition of exogenous enzymes. To determine efficacy of exogenous enzymes in the digestion of sorghum kafirins, five commercial exogenous enzymes (Avizyme 1210 (xylanase, ß-glucanase, cellulose), 1310 (xylanase), 1512 (protease), 1514 (amylase) and Phyzyme XP (phytase); Danisco Animal Nutrition, Marlborough, UK) were used in combination and individually when added to either the yellow-seeded sorghum (Liberty) or red sorghum (Red1).

Enzymes were added to hammer milled whole grain at rates equivalent to manufacturer's recommendations (1210 and 1310 at 300ppm; 1512, 1514, Phyzyme XP at 100ppm) in various combinations (Table 1) then the mixture was subjected to protein extraction and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Total protein was extracted from the sorghum samples for the SDS-PAGE analysis as described by Bean *et al.*, (1998) while the kafirins were further analysed using SDS PAGE as described by Bean (2003).

Table 1. *Combinations of commercial exogenous enzymes applied to sorghum samples.*

No.	Enzyme	No.	Enzyme	No.	Enzyme	No.	Enzyme	No.	Enzyme
1	None	4	1512	7	1514,1210,1512	10	1512,1210	13	1514,XP
2	1210	5	1514	8	1514,1512	11	1210, XP	14	1512, 1210
3	1310	6	XP	9	1512, XP	12	1512, XP		

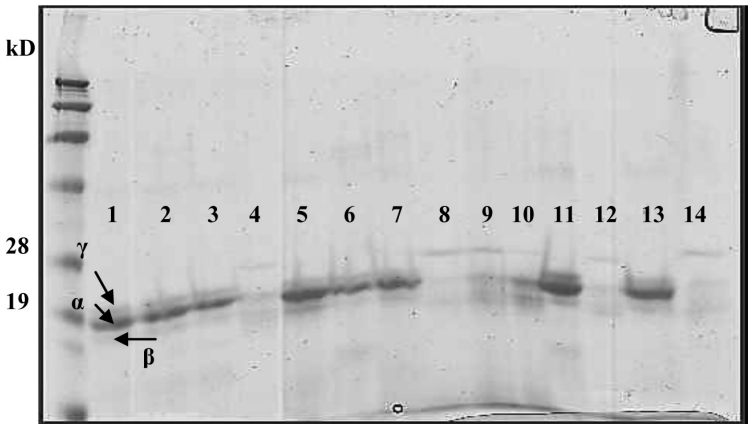

Figure 1. *Sodium dodecyl sulphate polyacrylimide gel electrophoresis (SDS PAGE) separation of proteins for yellow-seeded sorghum (Liberty) grain with exogenous enzyme combinations applied as per Table 1.*

SDS-PAGE of processed samples of Liberty and Red1 showed that digestion of sorghum α-, β- and γ-kafirins was improved by the addition of Avizyme 1512 when it was on its own or in combination with several other exogenous enzymes. However, in combination with Avizyme 1514 and 1210 it appeared to lose its efficacy. This *in vitro* data demonstrates potential for protease-based exogenous enzymes to digest the kafirin fraction of sorghum, especially on the normally more protease resistant β- and γ- kafirins.

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Buserelin and Single Fixed Time Insemination In Gilts Achieves Reproductive Performance Comparable to Standard Practice

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Buserelin (Receptal, Intervet UK Ltd (trading as MSD Animal Health, Milton Keynes, UK) a synthetic gonadotrophin releasing hormone (GnRH), synchronises ovulation in about 90% of gilts over a 24 h period if it is given 120 h after the last treatment of an 18 d altrenogest (Regumate, Intervet UK Ltd (trading as MSD Animal Health, Milton Keynes, UK) program. It permits pigs to be inseminated at a fixed time and, if this can be successfully applied on farms, provides benefits in terms of the skill required to detect sows in oestrus and time savings. The approach has been demonstrated successfully in the laboratory by Martinat-Botté *et al.*, (2010) with two fixed time inseminations. The purpose of this experiment was to satisfy the hypothesis that a GnRH-altrenogest treatment schedule combined with single fixed time insemination could deliver on-farm reproductive performance similar to using oestrous detection and inseminating twice during that oestrus.

The experiment was designed fit with production schedules on an outdoor farm mating 20-30 crossbred gilts each week. At approximately 27 weeks of age and 120 kg, all the gilts were treated with altrenogest (20mg daily) for 18 d. They had earlier been exposed to a mature boar and had shown oestrus. Each week for four weeks, the gilts, having completed their altrenogest schedule and ready for mating, were assigned to the treatment (GnRH) group or the control group. Control and treatment groups were kept in adjacent pens in a straw bedded shelter. Control gilts were exposed to a boar daily and checked for oestrus once each day. They were inseminated once each day while they were on heat. Treatment groups were given 10 μg of GnRH 120 h after the last altrenogest treatment and inseminated once 30 h later. Data were recorded in farm diaries. Total born and the number of sows farrowed were analysed in a Generalised Linear Model with normal errors (GenStat for Windows (2007), 10th Edition, VSN International Ltd., Hemel Hempstead, UK).

Table 1. *Farrowing rate and total born in gilts synchronised with altrenogest and gonadotrophin releasing hormone (GnRH) and inseminated once 30 h after GnRH.*

Week	Treatment	Number	Farrowing Rate (%)	Total Born	Standard Deviation
1	GnRH	10	80	10.7	2.7
	Control	10	70	11.6	1.4
2	GnRH	15	80	10.5	2.3
	Control	15	93	11.6	4.3
3	GnRH	15	80	9.9	3.1
	Control	15	79	8.8	4.2
4	GnRH	15	93	10.6	3.3
	Control	15	93	11.7	2.5
TOTAL GnRH	55	83.3	10.4		
TOTAL Control	54	83.8	10.9		

There was no difference between the four weekly groups so the results were pooled. There is no difference between the farrowing rate or litter size in gilts synchronised with GnRH and inseminated once 30 h later compared with control gilts inseminated twice.

This experiment demonstrates that an 18 d altrenogest program, combined with Buserelin given 120 h after the last altrenogest treatment, enables producers to inseminate gilts one at a fixed time 150 h after the last altrenogest treatment and achieve reproductive performance broadly comparable to standard farm practice. The experiment needs to be repeated with larger sample sizes to provide full confidence in the technology.

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Pregnancy Outcomes of Porcine Embryo Transfer are Not Affected by Increasing the Volume of Transfer Medium

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Embryo Transfer allows rapid upgrading of genetic stock with minimal disease risk but due to the costs and logistics involved it is seldom used commercially. The spiral anatomy of a sows cervix means non-surgical trans-vaginal embryo transfer is not repeatedly successful. The results of previous porcine embryo transfer studies suggested that depositing the embryos in small volumes of culture medium (<1.6 ml) increased the efficiency of embryo transfer compared with large volumes (Nakazawa *et al*., 2008). However, culturing embryos in a transfer syringe containing a small volume of medium (0.6 ml) significantly reduced their viability within three hours (Harris and Grupen, 2011). The aim of this experiment was to investigate the effects of the volume of culture medium used to deposit the embryos on pregnancy outcomes following surgical embryo transfer. The hypothesis for this experiment is that increasing the volume of Hepes-buffered Porcine Zygote Medium Version 3 (HPZM-3) expelled into the uterus from 1.4ml to 5ml during embryo transfer will have no negative effects on pregnancy outcomes and piglets born alive.

Ovulation in eighty-one donor sows (PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) synchronised at weaning using a regimen of equine chorionic gonadotropin, Folligon® (Intervet Australia Pty Ltd, Bendigo, VIC) and human chorionic gonadotropin, Chorulon® (Intervet Australia Pty Ltd, Bendigo, VIC), and artificially inseminated on the day of first standing heat. Embryos were surgically recovered three to four days later (4-cell to morula stages of development). Embryos were harvested using a standard surgical harvesting procedure (Cameron *et al*., 1989). Viable embryos were loaded into 1 ml transfer syringes with 0.7 ml HPZM-3 and held for no longer than 30 min before transfer to recipient animals. A total of 76 parity one and parity two recipients (PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) were synchronised at the same time as the donor animals. A mean (± standard error of mean) of 24.1±0.95 embryos was transferred to the recipients. Embryos were deposited in control sows (n=65) using a total of 1.4 ml HPZM-3 (0.7ml containing embryos followed by 0.7ml flush), and in treatment sows (n=11) using a total of 5 ml HPZM-3 (0.7ml containing embryos followed by 4.3ml flush). The influence of PZM-3 volume on born alive, still born and mummified foetuses was determined using a logistic regression analysis. Differences in the number of successful pregnancies were also determined using logistic regression analysis. The experimental unit for all analysis was the individual sow.

Table 1. *Litter characteristics of Control (1.4ml) versus High volume (5ml) media transfer (mean±SEM).*

	Control (n=65)	Treatment (n=11)	P-value
Born alive per litter	9.9±0.02	10.7±0.06	0.3
Still born per litter	0.63±0.01	0.57±0.02	0.95
Mummified per litter	$0.03±4x10^{-4}$	$0±2x10^{-5}$	0.18

SEM, standard error of the mean

Increasing the volume of medium used to deposit the embryos from 1.4 ml to 5 ml did not affect the number of piglets born alive, still born or mummified (Table 1). Farrowing rates also did not differ for the two groups (control 62%; treatment 64%; P>0.05). These results support the null hypothesis that for surgical embryos transfer, depositing embryos in 5 ml of HPZM-3 was as effective as depositing them in 1.4 ml of HPZM-3. Increasing the volume of medium used to culture porcine embryos from 0.6 to 5ml increases the time that embryos remain viable. To be able to implant embryos in 5ml of media has the potential to greatly simplify the embryo transfer procedure as it eliminates the need for an embryologist at the implanting site whilst still allowing embryos to be cultured for long durations and therefore moved over large distances.

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The Influence of Boar Sexual Behaviour at the Time of Exposure on the Induction of Puberty in Gilts

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The most important single stimulus to accelerate the onset of gilt puberty appears to be exposure to mature boars, and in particular the olfactory cues (Hughes *et al*., 1990). However, pheromones alone will not substitute for the presence of the boar and it is likely that sensory cues other than olfactory stimuli, for example boar libido or courtship behaviour, could act synergistically with pheromones. Therefore, this experiment tested the hypothesis that exposure to boars that display high levels of courtship behaviours will result in a greater proportion of gilts reaching puberty and puberty at an earlier age than exposure to boars with low levels of courtship behaviour.

The sexual behaviour of 14 Large White x Landrace boars was observed weekly for three weeks from 12 months of age in 15 minute mating tests and the three highest motivated (HM) and three lowest motivated (LM) ranked boars based on the number of copulations (means of 5.0 v. 2.7 for HM and LM, P<0.01) and time to first mount (36 v. 71 secs, P=0.09) were selected for this experiment. Forty-eight Large White x Landrace pre-pubertal gilts (mean±standard error, 160±1.1 days, 93.4±1.3 kg) were randomly allocated to twelve groups fed 2.5 kg of a gilt developer diet (13.8% crude protein, 14.4 MJ digestible energy/kg) per pig per day. All gilts were group-housed in isolation from mature boars from birth until the treatments commenced. Each of the boars was individually exposed for 15 minutes daily to two groups of pre-pubertal gilts for 40 days from 160 days of age, or until pubertal oestrus was observed. Video records of these exposures were utilized to record the frequency of the behaviours including head-to-head contact (HH), nosing the sides of the gilts (N), anogenital sniffing (AGS) and attempted mounts (M) initiated by boars and gilts. Differences in behaviours between HM and LM boars were analysed by analysis of variance using GENSTAT Release 11.1 (VSN International Ltd., UK). The proportions of gilts reaching puberty were analysed using Chi-squared tests.

Table 1. *Effect of boar motivation (High (HM) or Low (LM)) on boar- and gilt-initiated courtship behaviours between 5 and 40 days of boar exposure. Results are mean ± standard error of the mean[1].*

	Boar-initiated		Gilt-initiated		Total	
Behaviour	HM	LM	HM	LM	HM	LM
Nosing	22.8±1.34	26.6±1.73	0.2±0.07	0.4±0.12	23.0±1.35	27.0±1.72
Anogenital sniffing	6.2^{a}±0.52	11.9^{b}±0.84	5.0±0.54	5.1±0.68	11.2^{e}±0.84	17.0^{f}±0.98
Head-to-head	17.0±1.13	19.5±1.17	36.3^{c}±2.14	25.8^{d}±1.80	53.7^{e}±2.40	45.3^{f}±1.88
Mounting	1.7±0.30	1.8±0.23	0.1±0.46	0.1±0.05	1.77±0.31	1.91±0.24
Total courtship behaviours	46.6^{a}±2.78	59.8^{b}±3.26	41.5^{c}±2.50	31.3^{d}±2.30	89.2±3.87	91.1±3.46

[1]Means within a boar- (a,b) or gilt- (c,d) initiated or total (e,f) behaviour with different superscripts differ significantly (P<0.05). No comparisons were made between boar- and gilt-initiated behaviours.

Boar motivation had no effect on the proportion of gilts achieving puberty by 200 days of age (83 v. 87 %, for HM and LM, $\mathbf{a}^{2}$=0.22, P=0.64). The HM boars displayed fewer (P<0.01) courtship behaviours than LM boars, specifically AGS after 5 days of exposure (Table 1). Conversely, gilts exposed to HM boars initiated more (P<0.05) courtship behaviours than gilts exposed to LM boars, specifically HH. Total boar- and gilt-initiated behaviours did not differ between HM and LM groups, although types of behaviours differed slightly. These data suggest that exposure to HM and LM boars provides a similar frequency of tactile interactions between boars and gilts and that these interactions do not appear to be implicated in the boar-stimulating effect on puberty in gilts. Other boar stimuli such as olfactory stimulation may be more important in stimulating puberty (Hughes *et al*., 1990) than sexual motivation *per se*.

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Increasing Endogenous Progesterone in Early Pregnancy Increases Litter Size in Pigs

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The number of pigs weaned per sow per year is a major driver of profitability and the economic success of commercial pig operations. Reproductive performance is primarily measured by the number of living pigs at birth. Based on ovulation rate, the average litter size of sows is between 40 to 60% less than potential with the majority of loss occurring before d 30 of pregnancy (Pope *et al.*, 1990). One of the major causes of embryo loss is believed to be insufficient progesterone production by the ovary during early pregnancy and almost all of the events important for the maintenance of pregnancy in pigs are associated with adequate levels of progesterone. We investigated the hypothesis that gonadotrophins given during early pregnancy increases endogenous production of progesterone and increases litter size. Treatment with gonadtrophins was given to produce accessorory corpora lutea during early pregnancy to produce additional progesterone during the period of known embryo mortality in the pig.

An intramuscular injection of 1000IU of equine chorionic gonadotrophin (eCG; Folligon®, Merck Animal Health, Summit, NJ) was given on d 10 after first artificial insemination (AI) followed by an intramuscular injection of 650 IU of human chorionic gonadotrophin (hCG; Chorulon®, Merck Animal Health, Summit, NJ) on d 13 in 24 first parity sows. Twenty-two sows received two injections of sterile saline as a control for this experiment. Treatment comparisons were made using an analysis of variance (ANOVA).

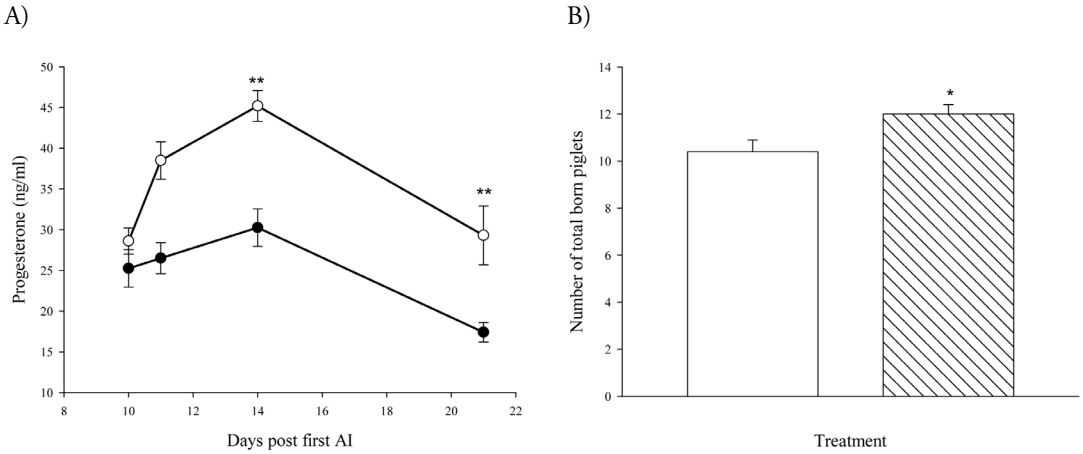

Figure 1. *The effect of treatment with equine chorionic gonadotrophin (eCG) / human chorionic gonadotrophin (hCG) given in early pregnancy to sows (n=22 Control (●); n=24 eCG/hCG (○)) on (A) plasma progesterone content and (B) average number of piglets born per sow. *P<0.05; data are presented as mean ± standard error of the mean (SEM).*

Sows treated with eCG/hCG increased plasma progesterone content by between 30 and 40% compared with controls and had an increase of approximately 1.5 total born piglets (P<0.05; Figure 1). Notwithstanding the relatively small number of sows involved in this study, it supports the hypothesis that increasing progesterone during early pregnancy improves embryo survival leading to an increase in litter size in the pig. Understanding the role of progesterone in early pregnancy and designing treatments to increase endogenous progesterone levels has the potential to increase litter size and profitability for the pig industry.

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Relationship Between Vaginal Conductivity, Onset of Oestrus and Time of Ovulation

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The optimum time to inseminate sows is during the 24-hour period before ovulation. However, there is great variation among sows in the duration of oestrus and in the oestrus-to-ovulation interval (EOI), presenting a challenge in determining optimum time for artificial insemination (AI). A device that measures the electrical resistance (ER) of the mucus in the sow's vagina may offer an option in determining the optimal time for AI. The time of ovulation is controlled by a surge of luteinizing hormone (LH) occurring approximately coincident with onset of oestrus, and a relationship between ER and the preovulatory LH surge has been documented (Dusza *et al.*, 1995). Significant changes in vaginal ER during the oestrous cycle have been observed in pigs with the lowest ER in the follicular phase and highest in the luteal phase of the oestrous cycle. Further, timing AI on the basis of changes in ER has resulted in improved sow performance (Yamauchi *et al.*, 2009), presumably due to improved synchronization between times of sperm deposition and ovulation. The present experiment was undertaken to examine the hypothesis that the time of ovulation can be predicted based on ER changes in vaginal mucus. This was an observational study and sow performance after ovulation was not monitored.

In 113 mixed parity Yorkshire x Landrace sows, ER was measured from the day after weaning until d 7 post-weaning using a Draminski oestrus detector (Draminski – Electronics in Agriculture, Olsztyn, Poland) to determine its usefulness as a predictor time of ovulation. The ER readings were obtained as follows: d 1 to 3, one reading daily; d 4 and 5, three readings daily; d 6, two readings; d 7, one reading. The probe was inserted into the vagina and the reading obtained within 20 seconds. Transrectal real time ultrasound was performed twice daily from d 4 to estimate the time of ovulation, and sows were checked for oestrus in the presence of a boar from d1 to d 7.

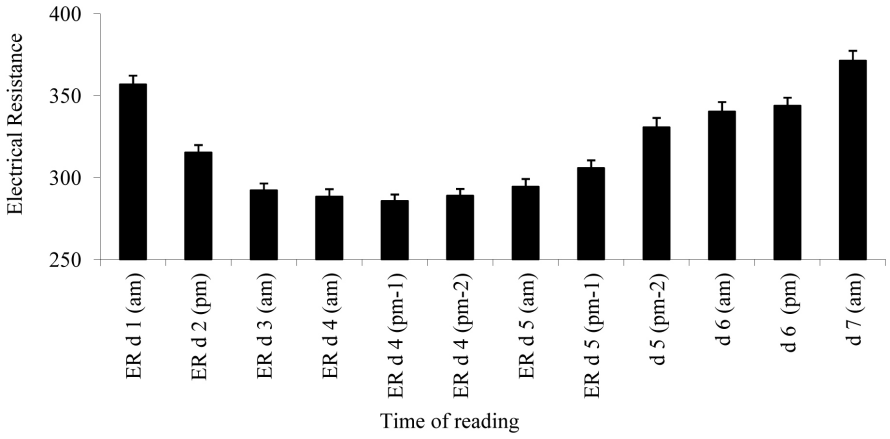

Figure 1. *Sow vaginal electrical resistance from d 1 to d 7 after weaning (ER, electrical resistance).*

Oestrus started from the time of the second reading on d 4 and the end of oestrus was observed at the second reading on d 6. Oestrus was observed after the lowest ER readings were recorded. Ovulation occurred between late d 5 and late d 6, while ER values were still increasing (Figure 1). These data show a progressive decrease in ER during the follicular phase with a nadir coincident with onset of oestrus before increasing to the time of ovulation. If this is confirmed in additional studies, it suggests that if ER measurements are made twice daily, first AI can be at the time of the second increased reading and again 24 hours later. Alternatively, a single AI at 24 h after the onset of ER increases may permit optimal fertility. However, these suggestions require further confirmation before being applied commercially.

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Feeding Level and Dietary Energy Source During Early Pregnancy in First Parity Sows: Effects on Pregnancy and Litter Size

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High feeding levels have been shown to reduce systemic progesterone (P_4) concentrations during early pregnancy, and have been associated with a decrease in embryo survival (Jindal *et al.*, 1996). However, the effects of feeding level on embryo survival have been equivocal with other studies finding no or even positive effects of an increased feeding level on embryo survival and pregnancy rate (Virolainen *et al.*, 2004; Quesnel *et al.*, 2010). This paradox may be due to the previous focus on P_4 concentrations in systemic blood circulation, even though recent studies shows that a high feeding level may actually result in an increase in P_4 secretion at the ovarian level (Athorn *et al.*, 2010). Therefore, it was hypothesised that a high feeding level would not be detrimental to litter size and may even increase it. In addition, it was also hypothesised that specific dietary energy sources may also positively influence P_4 production and thus embryo survival, and therefore diets rich in starch, fat and fibre were tested.

After mating (d 0 of gestation), 225 individually housed first parity sows (Large White x Landrace F1, PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) were randomly assigned to either a starch-based diet (starch, 500g/kg; fat, 20g/kg; crude fibre, 40g/kg) at a low feeding level (26 MJ digestible energy (DE)/d, n=55); a starch-based diet at a high feed level (39 MJ DE/d, n=51); a diet with partial replacement of starch by fat (starch, 420g/kg; fat, 9.5g/kg; crude fibre, 40g/kg) at a high feeding level (39 MJ DE/d, n=49); or a diet with a high fibre content (starch, 270g/kg; fat, 420g/kg; crude fibre 100g/kg) at a high feeding level (39 MJ DE/d, n=59). Oat hulls and millrun were used as the fibre sources in this experiment. Sows were fed treatment diets until d 25 of gestation. At mating sows weighed 208 kg and had a backfat P2 of 18.5 mm on average. Data were analysed with analysis of variance (ANOVA) or a Chi-square test (farrowing rate).

Table 1. *Effect of dietary energy source (starch, fat or fibre) fed at either a low (26 MJ DE/d) or a high (39 MJ DE/d) feeding level from d 0 to 25 of gestation on weight gain and reproductive performance (means ± SEM) in first parity sows.*

Dietary treatment	DE (MJ/d)	Feed Ration (kg/d)	n[1]	Bodyweight gain (g/d)	Backfat P2 gain (mm)	Farrowing rate[1] (%)	Total born	Born alive
Starch	26	2.0	55	320 ± 57[a]	-1.7 ± 0.3[a]	97 (55/57)	13.3 ± 0.4	12.1 ± 0.4
Starch	39	3.0	51	598 ± 41[b]	-0.3 ± 0.6[b]	95 (51/54)	13.2 ± 0.4	12.2 ± 0.4
Fat	39	2.7	49	611 ± 50[b]	-0.3 ± 0.3[b]	89 (49/55)	13.0 ± 0.4	12.0 ± 0.4
Fibre	39	3.6	59	505 ± 46[b]	-1.2 ± 0.4[a,b]	98 (58/59)	13.2 ± 0.4	11.9 ± 0.3

[1]at farrowing, excluding sows that did not farrow for non-reproductive reasons. [ab]Means in a column with different superscripts differ significantly ($P < 0.05$). DE, digestible energy; SEM, standard error of the mean.

A clear difference in weight gain was seen between sows on the low and high feed levels (Table 1). Backfat P2 followed the same trend although not in sows on the high fibre diet. Interestingly, P2 decreased 0.9mm on average despite a positive growth rate. Feed level or energy source did not have any effect on farrowing rate and litter size. However, when pooled across treatments sows with a high growth rate (≥740 g/d (25% highest); n=58) had a 100% farrowing rate compared to 94% for sows with a medium (241-739 g/d (50%) n=108) and 92% for sows with a low growth rate (≤240 g/d (25 % lowest); n=59; P=0.09). In conclusion, in this experiment a high feeding level was not detrimental to litter size. However, there was no difference in litter size between the different dietary energy sources tested.

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Day of Gestation Influences Progesterone Concentrations in Local Utero-Ovarian Blood Circulation

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Progesterone (P_4) plays an important role in embryo survival. Most studies assess plasma P_4 concentration in systemic blood circulation. There is, however, evidence to suggest that P_4 is transferred directly from the ovary to the uterus bypassing the systemic circulation (Krzymowski *et al*., 1990). Indeed, it has been shown that removing one ovary prior to ovulation reduces embryo survival in the horn ipsilateral to the removed ovary (Athorn *et al*., 2011). The aim of this experiment was to characterise the nature and concentration of P_4 in local utero-ovarian blood circulation on d 6 and d 9 of pregnancy in gilts. It was hypothesised that P_4 would be secreted in a pulsatile manner and that P_4 concentration would be higher on d 9 of gestation compared to d 6.

Nineteen Large White gilts had a cannula inserted into the vena cava (VC) draining the uterus just after their second oestrus. The gilts were mated at the first standing response during their second oestrus and this day was determined as d 0 of gestation. Gilts were 28 weeks old and weighed 126±2kg at the time of cannulation. On d 6 and d 9 of gestation VC samples were collected every 15 min over a 6 h period. Possible P_4 pulses were found from the profile after plotting an individual's samples against time. A pulse was defined as at least two consecutive samples that exceeded 100 ng/ml and in which the peak concentration was completed within two subsequent sampling intervals. The basal P_4 concentration was defined as the mean of three consecutive samples preceding each pulse. Data were analysed with an analysis of variance (ANOVA). The number of pulses per 6 h were analysed with a non parametric test (rank test).

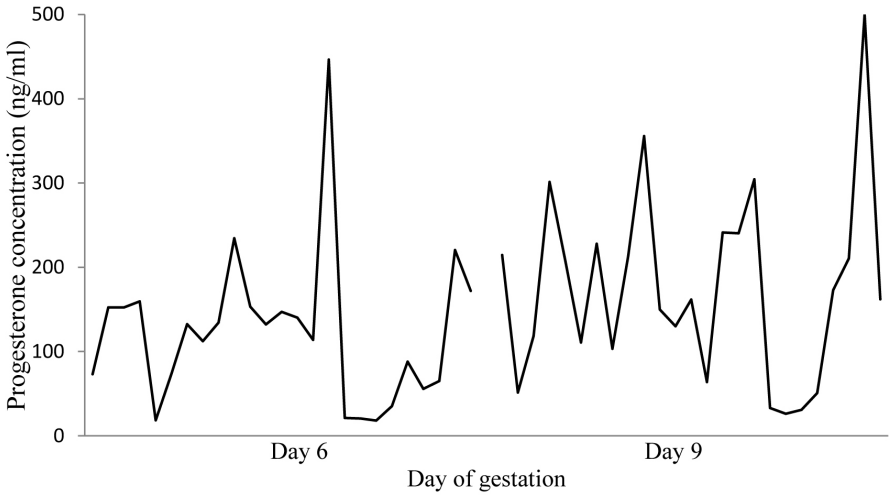

Figure 1. *Vena cava progesterone profiles collected over a 6 h sampling period on both days 6 and 9 of gestation in an individual gilt.*

Concentration of P_4 in the VC was pulsatile (average of 3 pulses on d 6, increasing to an average of four pulses on d 9 ($P<0.05$)). Mean P_4 concentration in the VC was 77±7ng/ml on d 6 and 91±8.7 ng/ml on d 9 ($P<0.05$). Basal level of P_4 in the VC also differed between days with an average of 37±4.3 ng/ml on d 6 and 45±4.9 ng/ml on d 9 ($P<0.05$). However, mean pulse amplitude did not differ between d 6 (160±10.8 ng/ml) and d 10 (176±15 ng/ml). In conclusion, P_4 is clearly secreted in a pulsatile manner and P_4 concentration is significantly higher on d 9 compared to d 6 of gestation.

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Supplementary Betaine: Effects on Growth and Reproductive Performance of Replacement Gilts

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Higher growth rates prior to puberty and breeding herd entry can improve gilt reproductive performance. Adding betaine to finisher pigs increased growth rate (Suster *et al.*, 2005). Betaine supplementation during summer decreased age at puberty and increased ovulation rate when fed to gilts pre-mating, and increased litter size when added to gestating sow diets (van Wettere and Herde, 2009; van Wettere *et al.*, 2009). The current experiment tested the hypothesis that adding betaine to pre-mating and gestating diets during summer/autumn would reduce age at first mating, and increase litter size of gilts reared outdoors.

From 23 weeks of age, 485 Large White x Landrace gilts (109±0.5kg; Mean ± standard error of mean) received either a standard gilt developer diet (13.3 MJ digestible energy (DE)/kg; 0.06 g available lysine/MJ DE; CON; n=143 gilts) or a standard gilt developer diet supplemented with 2g/kg Betaine (BET; n=340 gilts). Gilts were housed outside, in two hectare paddocks, until 28 weeks of age and 141±0.4 kg. Gilts were then moved to indoor group pens (8 gilts/pen) and received daily boar exposure until first detection of oestrus. Artificial insemination (AI) occurred at first observed oestrus, following which gilts were housed in groups. After their second AI, BET supplemented gilts were randomly allocated to receive either a standard gestation diet (BETCON) or the standard gestation diet supplemented with 3g/kg betaine (BETBET) through to farrowing shed entry on d110 of gestation. The three individual treatments were CON (n=143), BETCON (n = 171) and BETBET (n=169). Gilt liveweight (LW) was measured at 23 weeks of age, selection for entry into the breeding herd and at first AI. On d 24 post-AI, gilts were detected for pregnancy using real time ultrasound. At farrowing, total litter size (TB) and number of piglets born alive (BA) were recorded. Treatment effects were determined using a general analysis of variance. A chi-squared test was used to examine treatment effects on pregnancy rates.

Table 1. *Effect of betaine supplementation on gilt liveweight (LW) gain pre-mating, incidences of early pregnancy failure and first litter size.*

Treatment	Pre-AI LW Gain (kg/d)	Early Pregnancy Failures (< d 24)	Total Born	Born Alive
CON	0.76 ± 0.02^{a}	0.04^{ab}	10.6 ± 0.25	9.9 ± 0.25
BETCON	0.83 ± 0.02^{b}	0.06^{b}	10.9 ± 0.24	10.2 ± 0.24
BETBET	0.83 ± 0.02^{b}	0.01^{a}	11.0 ± 0.23	10.4 ± 0.23

Means in a column with different superscripts differ significantly (P<0.05). CON, standard gilt developer diet; BET, betaine; AI, artificial insemination.

Betaine supplementation pre-mating increased (P<0.05) LW gain by 70 g/d (Table 1). First litter size was not improved (P>0.05) by betaine supplementation. Betaine supplementation pre-mating did not affect incidences of early pregnancy failure, however, incidences of early pregnancy failure were significantly (P<0.05) lower for BETBET compared to BETCON gilts (Table 1). Regardless of treatment, first litter size was positively correlated with gilt LW at breeding herd entry (r^2=0.018; P<0.05).

These data demonstrate that dietary betaine increases gilt growth rate. The lack of a significant effect of betaine pre-mating on first litter size was unexpected given previous evidence of a positive effect on ovulation rate (Van Wettere *et al.*, 2009). However, this may reflect insufficient experimental power, or suggest that ovulation rate does not determine litter size in gilts. Importantly, further studies are required to determine the cause of the increased pregnancy losses when betaine is removed from the diet following insemination.

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Seasonal Differences in Circulating Progesterone Concentrations in Pregnant Sows

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A depression in sow fertility is common during summer and early autumn. Low fertility is associated with impaired progesterone production during early pregnancy, with differences in progesterone concentrations affecting embryo survival (van Wettere and Hughes, 2007). Consequently, the current experiment tested the hypothesis that progesterone levels during the first 31 days of pregnancy differ between summer and winter.

Blood samples were collected by jugular venepuncture, from weaned, multiparous sows mated in either summer (January) or winter (August) on d 3, 7, 11, 15, 19, 23, 27 and 31 following first oestrus detection. The complete set of samples from 64 pregnant, parity 3.7 ± 0.3 sows were used in this experiment (n=32/season). All samples were assayed for progesterone (P4) by radioimmunoassay. Samples collected on d 3 and 15 post-mating were assayed for insulin-like growth factor-I (IGF-I) and total IGF-binding proteins (IGFBP) by radioimmunoassay following separation of IGF and IGFBP by HPLC at pH 2.5. A repeated linear measures regression model was used to determine the effects of season on all measures (Genstat 10th Edition; Rothamsted Experimental Station, Harpendon).

Table 1. *Effect of season (winter versus summer) on peripheral progesterone concentrations during early pregnancy (d 3 to 31 post-mating).*

	Progesterone concentrations (ng/ml)							
Season	d3	d7	d11	d15	d19	d23	d27	d31
Winter	3.2[a]	9.5[a]	16.1	18.9	15.4[b]	12.6[b]	12.3	12.1
Summer	4.7[b]	13.8[b]	16.9	17.0	12.9[a]	11.1[a]	11.1	11.4
Pooled SEM	0.51	1.23	1.46	1.09	0.70	0.52	0.50	0.50

[ab]Means in a column with different superscripts differ significantly (P<0.05); SEM, standard of the mean.

Total litter size was similar in summer and winter (12.7±0.8; P>0.05). P4 concentrations were higher in summer than in winter on d 3 and 7, but lower on d 19 and 23 post-mating (P<0.05; Table 1). IGF-I concentrations on d 3 did not differ between summer and winter (128±9 versus 107±8 ng/ml; P>0.05), but the ratio of IGF-I:IGFBP was significantly different between summer and winter (0.14±0.01 versus 0.11±0.01; P<0.05). IGF-I concentrations and the IGF-I:IGFBP ratio were similar at d 15 in both seasons (IGF-I: 115±8 ng/ml; IGF-I:IGFBP: 0.15±0.02; P>0.05). IGFBP concentrations on d 3 (1011±68 ng/ml) and d 15 (888±79 ng/ml) were unaffected by season (P>0.05).

Progesterone rose earlier after mating in sows mated during summer than in those mated during winter. This earlier elevation implies that either luteinisation occurs more rapidly after ovulation, or that ovulation occurs more rapidly after the onset of behavioural oestrus in summer compared to winter. Exposure to high temperatures, as experienced by summer mated sows, stimulates premature luteinisation of follicle cells (Bridges *et al.*, 2005), but effects of seasonal environmental cues on the relative timing of oestrus and ovulation have not been investigated. Luteinisation may also be stimulated by higher IGF-I bioavailability in summer, indicated by a higher IGF-I:IGFBP ratio. Langendijk *et al.* (2008) reported a positive correlation between plasma P4 and IGF-I during the first 12 days post-mating. We suggest that the lower circulating P4 on d 19 and 23 will adversely affect the uterine environment and embryo development and that this contributes to decreased sow fertility in summer.

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Oral Spermine Supplementation Promotes Pre-Weaning Growth and Intestinal Development of Piglets

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[2]Massey University, Palmerston North 4442 New Zealand.

Piglet growth immediately post-weaning is often compromised by failure to adapt to the changing composition of the diet. Milk-born polyamines (putrescine, spermidine, spermine) play an important role in neonatal gut maturation, triggering adaptive changes in the gastrointestinal tract of suckling offspring which aid the progressive transition from a milk-based to a solid dietary regimen (Larque *et al*., 2007). Polyamine supplementation enhances intestinal maturation in suckling rat pups (Larque *et al*., 2007). Therefore, the current experiment tested the hypothesis that oral supplementation of spermine prior to weaning promotes gastrointestinal maturation and liveweight gain of suckling piglets.

Twelve first lactation (P1) and twelve third lactation (P3) Large White x Landrace sows were randomly selected at farrowing. Within 24 h of parturition, litter size was standardised to, and maintained throughout lactation at 10 piglets, with five piglets from each litter allocated to receive 2 ml of 462.7 nmol/ml spermine solution (SP; Sigma-Aldrich Pty Ltd, Sydney, NSW) or 2 ml of water (W). The SP dose provided piglets with an additional 20% above estimated intake via sow's milk (Sabator-Molina *et al*., 2009). Doses of SP and W were delivered using an oral drench gun, with piglets drenched every second day from d 14 to d 22 post-partum. Individual piglet liveweight (LW) was recorded on d 3, 14, and 24 post-parturition, with weaning occurring on d 24. A subset of six piglets/treatment were sacrificed on d 24 post-parturition and gastrointestinal samples collected to measure villus height and crypt depth in the duodenum and jejunum. Treatment effects on all measures were determined using a general analysis of variance with piglet LW on d 3 included as a co-variate. No significant interactions were observed so only main effects are reported.

Table 1. *Main effects of oral supplementation (spermine (SP) versus water (W)) and maternal parity (one (P1) versus three (P3)) on piglet liveweight (LW), piglet LW gain and villus height:crypt depth ratio on d 24.*

Main effects[1]	Piglet LW (kg)			Piglet LW gain (kg/d)		Villus height:Crypt depth	
	d 3	d14	d 24	d 3 - 14	d 14 - 24	Duodenum	Jejenum
Supplement							
SP	2.05[b]	4.45[b]	7.29[b]	0.27	0.25[b]	2.44[b]	2.45[b]
W	1.94[a]	4.11[a]	6.58[a]	0.25	0.21[a]	1.45[a]	1.30[a]
Sow parity							
P1	1.96	4.25	6.64a	0.26	0.21a	1.67a	1.57a
P3	2.04	4.31	7.24b	0.26	0.25b	2.23b	2.18b
Pooled SEM	0.05	0.12	0.21	0.001	0.02	0.10	0.12

[1]Within main effects, values in a column with different superscripts differ significantly (P < 0.05). Piglet LW on d3 included as a covariate in analysis of variance; SEM, standard error of mean.

SP piglets were heavier (P<0.05) than W piglets on d 3, 14 and 24 post-partum, respectively. Piglets suckling P1 and P3 sows were similar weights on d 3 and 14, but 0.57 kg heavier (P<0.05) on d 24 (Table 1). There was no effect of SP or maternal parity on piglet LW gain from d 3 to 14. However, between d 14 and 24, SP supplementation resulted in a 21% increase (P<0.05) in piglet growth rate and piglets suckling P3 sows gained an extra 40g of LW/d compared to those suckling P1 sows. The villus height to crypt depth ratio in the duodenum and jejunum was greater (P<0.05) in SP piglets as well as those suckling P3 sows (Table 1).

Overall, the current data demonstrate a beneficial effect of spermine supplementation on pre-weaning piglet growth and absorptive surface area of the gastrointestinal tract, and support a stimulatory effect of polyamines on neonatal gut maturation (Larque *et al*., 2007). Future studies will determine whether polyamine supplementation improves post-weaning growth, and whether supplementation with polyamines post-weaning is beneficial. It was interesting that LW gain of piglets suckling parity 3 sows was only improved during the last 10 days of lactation, and that piglets suckling parity 1 sows had a smaller intestinal absorptive area compared to those suckling parity 3 sows.

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A Survey of Sow Blood Haemoglobin by Parity in Western Australia

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Piglet iron reserves at birth are determined by sow iron status (Capozzalo *et al.*, 2009). Since the mineral status of sows has been shown to decline after the third parity (Peters and Mahan, 2008), it follows that piglets from older sows have lower stores of iron and may be at risk of anaemia in spite of routine supplementation at birth. The purpose of this study was to assess the iron status of a cross section of sow herds in Western Australia by parity, by measuring blood haemoglobin (HGB) using an Advia® 120 Haematology Analyser (Siemens Healthcare Diagnostics Inc., Deerfield, IL). It was hypothesised that sow HGB levels would decline with increasing parity.

In phase one, approximately 128 crossbred sows (Large White x Landrace, even distribution of parities 0 to 6+) were sampled from a large commercial piggery, hereafter referred to as the Index Herd. The distribution and variance of HGB values within and between parities in the Index Herd were used to determine a simplified sampling strategy to assess herd HGB status by parity. The second phase surveyed sows on five diverse commercial herds representing outdoor and conventional indoor (stalled and grouped) production systems, and major genotypes commonly found in WA. Using the devised sampling strategy, five sows from each of parities 0 to 6+ totalling 35 sows per farm, were sampled between d 50 and d 90 of gestation when HGB levels are relatively stable and sows could be handled with low risk to the maintenance of pregnancy.

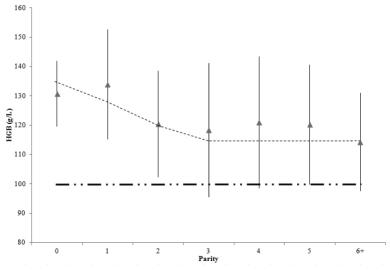

Figure 1. *Index Herd 95% confidence interval for parity mean (Δ) haemoglobin (HGB)*.*

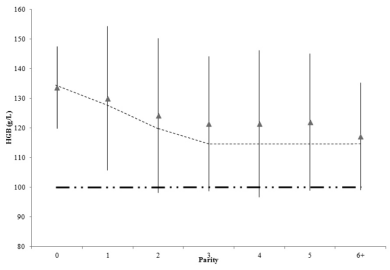

Figure 2. *Pooled data, all farms 95% confidence interval for parity mean (Δ) HGB*.*

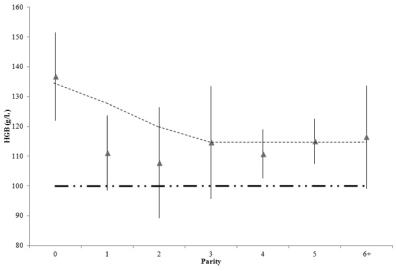

Figure 3. *Farm B, 95% confidence interval for parity mean (Δ) HGB*.*

*Danish recommended mean by parity (Poulsen, 2006, – – – – – – –); Lower limit of normal HGB range (– ·· – ·· – ·· – ·· –).

The Index Herd (Figure 1) and pooled data from all herds (Figure 2) show a gradual reduction in mean HGB levels with increasing parity with mean values at or above levels recommended by Danish research (Poulsen, 2006). The lower limits of the 95% confidence intervals of Parity 3 and older of the Index Herd are near or below the lower normal range (100g/L). This trend is also evident in the pooled data from parity two and above (Figure 2). This suggests that approximately 2.5% of sows on farms surveyed fall below 100g/L HGB after their second litter, and a similar proportion of sows surviving to higher parities are likewise marginal. Figure 3 shows a sharp reduction in mean HGB on Farm B (a selected farm from phase two) in first litter sows compared to gilts, with similarly marginal results recorded for parity two. This may reflect issues with lactation feed and intake. The lower 95% confidence interval limit for Parity 1, 2, 3 and 6+ sows show that at least 2.5% of the parity is below the 100g/L lower limit.

This survey confirms that blood HGB levels fall with increasing parity of sows. Furthermore, the variance of HGB values within parities is such that approximately 2.5% of sows on farms surveyed fall below the clinical threshold for anaemia by Parity 2. This leaves progeny of sows with marginal HGB levels at risk of iron deficiency and highlights the need for iron supplementation at birth.

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A Method to Evaluate Piglet Preferences Specific to Taste

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The manipulation of feed intake through palatability requires specific knowledge of factors influencing taste. Electrophysiological recordings from pig tongue show that citric acid (sour) induces the highest response compared to a wide range of tastants, while sugars are sensed to a similar extent to that observed in humans (Danilova *et al*., 1999). Electrophysiological recording is a valuable and accurate technique for mammalian taste research but is invasive and the procedure is time-consuming. As an alternative, studies of pig taste using a double choice (DC) feeding system are long (minimum 48 hours) and do not differentiate between peripheral and postprandial effects (Torrallardona and Sola-Oriol, 2009). Modified DC models using water solutions have been widely used in laboratory rodents and can produce rapid and reliable results in pigs. Given the threshold for primary tastes in humans is between 1-10mM but varies with sex, we hypothesise that pigs will show similar thresholds. The aim of this experiment was to study dose (1 and 10mM) and gender related preference responses in pigs to the non-bitter primary tastes (sweet, sour, salty and umami) using a modified DC model based on water solutions.

Ninety-six piglets were selected by initial body weight (average 9.0±2 kg) and housed in 48 pairs of males or females in two environmentally controlled rooms. Feed (14.75 MJ/kg digestible energy (DE); 203.9g/kg crude protein; 0.79 g available lysine/MJ DE) and water were offered *ad libitum*. On d 12, a four day DC training session started by simultaneously offering two stainless steel bowls containing approximately 500g each of either plain water or a 200mM sugar solution. The training sessions (twice daily) lasted ten (d 1 and 2), five (d 3) and two (d 4) minutes. After the training, experimental solutions were tested in two minute DC sessions twice daily. The DC consisted of offering water in one bowl and 1 or 10mM solutions of sugar, monosodium glutamate (MSG), citric acid or NaCl in the second bowl. Treatments were distributed following an incomplete block design with 24 replicates each. Test solution intake (intake) and test solution preference (preference; measured as the ratio of test solution consumed over total (test solution plus water), in %) were analysed using a linear mixed model where primary taste, concentration and gender and their interactions were fitted as fixed effects. A covariate for average pig weight per pen was fitted and found to be statistically significant for intake (P<0.01).

Table 1. *Intake[1] (g) of and preference (%) for sugar, monosodium glutamate (MSG), citric acid and NaCl solutions at 1 or 10 mM in a double choice to plain water.*

Conc.	Sugar		MSG		Citric acid		NaCl	
	Pref.	Intake	Pref.	Intake	Pref.	Intake	Pref.	Intake
1mM	50[a]	53.5	45[c]	42.1[c]	55	55.7	43[c]	34.5[a]
10mM	62[b]	66.0	66[d]	85.6[d]	64	56.3	59[d]	55.2[b]

[1]Consumption values were transformed to a log scale for statistical analysis; [ab]Means in a column with different superscripts differ significantly (P<0.05); [cd]Means in a column with different superscripts differ significantly (P<0.01); Pref, Preference; Concent, Concentration.

The interaction of taste and concentration was statistically significant for both parameters (P<0.01). The preference values for the 200mM sugar solution over water increased during the training days and averaged 59%, 60%, 72% and 78% for each of the four consecutive daily morning sessions showing that pigs had learned to make choices. During the test sessions, it was observed that gender did not have a significant effect on preferences regardless of taste and concentration. None of the tastes at 1mM had a preference over water significantly higher than the neutral value of 50%. However, at 10mM sugar (P<0.05), MSG and NaCl (P<0.01) had significantly higher preferences than at 1mM (Table 1). In addition, preferences for MSG, citric acid and sugar at 10mM were significantly higher than 50%. It is concluded that compared to water pigs prefer 10 mM solutions of MSG, citric acid and sugar but not NaCl. No taste preferences were observed at 1mM.

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Insulin Secretion and Pig Performance are Altered by Feeding Pattern

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Previous studies have suggested that feeding pigs *ad libitum* may be metabolically inefficient as insulin secretion remains unresponsive to this feeding strategy, whereas, when pigs are fed bi-phasically, consisting of two one hourly intervals of feeding, insulin secretion occurs in a distinct spike that follows each feeding period (Scrimgeour, 2008). A more recent small study has shown an 8% improvement in feed efficiency in pigs fed bi-phasically when compared to feeding *ad libitum* (Newman *et al*. 2008). The current experiment sought to confirm the results of the previous experiments using a larger cohort of animals to test the hypothesis that feeding pigs at two succinct intervals aligns insulin secretion more closely to the time of feeding resulting in improved productivity.

One hundred female pigs (Large White x Landrace, PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) weighing 58.8±0.48 kg (mean±standard error) were allocated to single pens. Pigs were acclimatized to the facility and randomly allocated to an *ad libitum* (n=50) or a bi-phasic (n=50) feeding pattern for a period of seven days prior to the start of the test period. The bi-phasic feeding pattern consisted of two 60 min feeding periods (0800 to 0900 h and 1400 to 1500 h) per day. Pigs were offered a commercial grower diet (14 MJ digestible energy (DE)/kg and 0.95% available lysine) to d 14, and a commercial finisher diet (13.8 MJ DE/kg, 0.70% available lysine) thereafter to slaughter. Feed intakes and body weights were measured at two weekly intervals for 42 days (0-14, 14-28 and 28-42). On d 43, a sub sample of pigs from both treatment groups (8/treatment) were randomly selected and the external jugular vein of each pig catheterized via an ear vein. On d 44, blood samples (3 ml) were collected from each pig at hourly intervals for 11 h commencing at 0700 h in tubes containing K_3 ethylenediaminetetraacetic acid (EDTA). Plasma insulin concentrations were determined (Kit # PI-12K; Merck Millipore, Billerica, MA). Linear mixed models were used to analyse all the traits using a REML procedure.

Table 1. *Performance characteristics for female pigs fed* ad libitum *or twice daily (bi-phasic) for 42 days.*

	Ad libitum (mean±SEM)	Bi-Phasic (mean±SEM)	P-value
N	50	50	
Final liveweight (kg)	107.60 ± 1.1	107.04 ± 0.8	0.63
Daily feed intakes (kg/day)	2.91 ± 0.02	2.82 ± 0.01	< 0.001
Total feed consumption (kg)	122.22 ± 0.92	118.68 ± 0.85	0.05
Feed Conversion ratio (kg/kg)	3.05 ± 0.03	2.86 ± 0.05	0.46
Peak insulin (μU/ml)[1]	14.1 0 ± 3.8	89.30 ± 29.4	< 0.001

[1]60 minutes post am feed. SEM, standard error of the mean.

There was no difference (P>0.05) in final liveweight for the two treatment groups (Table 1). However, feed intakes over the experimental period for pigs fed bi-phasically were substantially reduced (P<0.05) when compared to those pigs fed *ad libitum* with no improvement in feed utilization (P>0.05). Plasma insulin for animals fed *ad libitum* remained relatively constant whereas, the post-prandial insulin concentrations for pigs fed bi-phasically were significantly elevated (P<0.001) 1 h following each feeding bout. This altered insulin status was associated with enhanced productivity resulting in substantial reduction in feed intakes without a compromise in body weight. A feeding regimen where pigs are fed over a small number of succinct intervals may indeed be a strategy for improving pig production.

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Pulsatile But Not Basal Maternal Growth Hormone During Late Pregnancy Predicts Piglet Birth Weights

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Growth hormone (GH) plays an important role in maternal adaptation to pregnancy. Maternal GH levels are low in human pregnancies with poor foetal growth (McIntyre *et al.*, 2000). In the pig, administration of exogenous GH increases foetal growth following treatment and increases birth weight when treatment is sustained to d 100 of pregnancy (Gatford *et al.*, 2009). In growing animals, responses to GH depend on the pattern of delivery as well as the dose (Clark *et al.*, 1985). Little is known about how circulating GH profiles change during pregnancy in non-human species, and which characteristics of maternal GH profiles affect foetal growth. We therefore characterised circulating GH profiles in gilts before and during their first pregnancies to test our hypothesis that endogenous circulating GH predicts birth weight in the pig.

Blood samples (10 minute intervals, 6 h) were collected serially in Large White x Landrace gilts before their first mating (n=5), and early (d 30 after mating, n=10), mid (d 65, n=6) and late (d 100, n=7) in their first pregnancies (term ~115 d). Where possible, the same pigs were sampled at multiple stages. Litter size and piglet birth weights were recorded at delivery. Plasma GH levels were measured by radioimmunoassay and pulse characteristics fitted. Effects of pregnancy were analysed by one-way analysis of variance (ANOVA), and relationships with litter outcomes were analysed by Pearson's correlation.

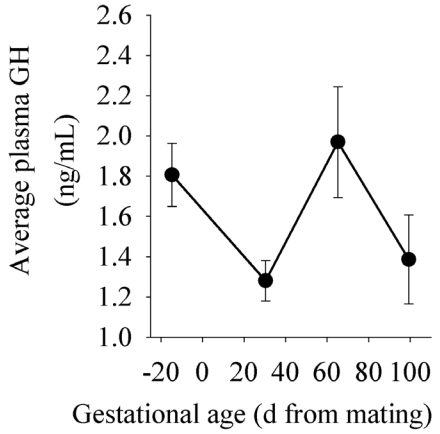

Figure 1. *Mean circulating growth hormone varies before and during pregnancy in gilts (P=0.044).*

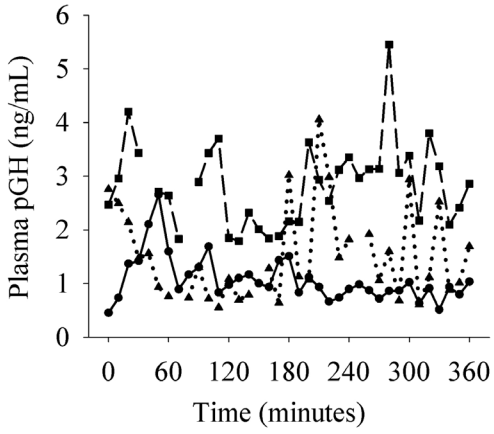

Figure 2. *Representative growth hormone profile in gilt #938 in early (●), mid (■) and late (▲) pregnancy.*

Mean plasma GH (Figure 1) fluctuated through pregnancy (P = 0.044), being highest before mating and at mid-pregnancy, and remained pulsatile (Figure 2), with similar GH pulse frequencies and amplitudes before and throughout pregnancy (P>0.1). Progeny birth weight correlated positively with measures of pulsatile GH secretion in late pregnancy (pulse frequency: r=0.749, P=0.026, n=7; pulse area: r=0.643, P=0.060, n=7; pulse area/h: r=0.701, P=0.040, n=7). Progeny birth weight was not correlated with mean GH concentrations (P>0.2) and tended to correlate negatively with mean basal GH (r=-0.555, P=0.098, n=7). Endogenous pulsatile, but not basal, circulating GH in the pregnant pig positively predicted the average birth weight of her progeny. Further research is warranted into the role of endogenous GH in regulating foetal growth.

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Ractopamine Effects β-1 and β-2 Adrenergic Receptor Gene Expression in Fat and Muscle Tissue of Boars and Gilts

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Dietary ractopamine (RAC) reduces fat mass due to direct activation of β-adrenergic receptors (βAR) in adipocytes promoting triglyceride hydrolysis and decreasing fatty acid and triglyceride synthesis leading to less lipid accumulation (Mills, 2002). Fat accretion in pigs fed RAC is not consistently reduced (Dunshea, 1993) which may result from irregularities in βAR down-regulation. Dietary RAC consistently improves lean deposition in pigs in the first two weeks of treatment after which response begins to decline, this may be because down-regulation of βAR in skeletal muscle is either not significant or delayed (Mills, 2002). It is not clear whether down regulation of the βAR in fat or muscle tissue is affected by dose of RAC and whether there are notable sex differences. The aim of the experiment was to determine the effect of RAC dose, duration of treatment, or sex effect on βAR gene subtype expression in fat or muscle tissue.

The experiment involved three groups (blocked by time) of 36 pigs in a 2 x 3 factorial design with the factors being sex (boars and gilts) and dose (0, 5 and 20ppm Paylean®, Elanco Animal Health, West Ryde, NSW) for 28 days. All 108 pigs were *ad libitum* fed and kept in individual pens. Muscle (*Gluteus maximus*) and subcutaneous fat biopsies were taken from three separate replicates on d 1, 15 and 29 for each treatment. Using real-time polymerase chain reactions (PCR), the mRNA levels for the transcripts of individual β1 and β2-adrenergic receptor (β1AR and β2AR, respectively) were normalized relative to three multiple reference genes TPB, RPL19 and UCHL5. A linear mixed model was fitted to the data using REML procedure in Genstat (Release 11.1, VSN International Ltd, Hemel Hempstead).

Table 1. *The effect of sex and dietary ractopamine dose (RAC) on β1 and β2 adrenergic receptor (AR) gene expression[1] in fat and muscle tissue as a percentage of controls (0 mg/kg RAC) for d 15 and d 29 samples.*

			Day 15 RAC (mg/kg)			Day 29 RAC (mg/kg)				Probability	
Gene	Tissue	Sex(S)	0	5	20	0	5	20	SED	RAC	Sex
β1AR	Fat	Boar	100.0	75.7	71.4	100.0[a]	64.2[b]	60.9[b]	15.19	0.020	0.554
		Gilt	100.0	76.8	88.4	100.0	84.0	96.9	14.36		
	Muscle	Boar	100.0[a]	228.4[b]	44.6[a]	100.0	100.4	83.3	38.3	0.044	0.429
		Gilt	100.0[a]	225.7[ab]	340.4[b]	100.0[a]	298.5[b]	147.7[a]	58.4		
β2AR	Fat	Boar	100.0	92.9	91.3	100.0	65.2	65.5	17.9	0.418	0.226
		Gilt	100.0	94.7	90.9	100.0[ab]	83.6[a]	118.1[b]	16.2		
	Muscle	Boar	100.0	90.8	69.2	100.0	120.8	78.4	16.8	0.069	0.050
		Gilt	100.0	97.1	71.2	100.0[a]	78.8[a]	68.5[b]	15.9		

[1]β1 and β2 adrenergic receptor expression has been normalized to UHCL5, RPL19 and TBP. [ab]Means with different superscripts within each row and day differ significantly (P<0.05); SED, standard error of difference

Significant down regulation of the β1AR gene in fat tissue of boars fed RAC was observed at d 29. The β2AR expression was not affected by RAC (P>0.05) indicating that lipolysis may be mediated through both β1AR and β2AR. In muscle tissue, β1AR expression was increased by 5 mg/kg RAC at d 15 for both sexes and remained elevated in gilts at d 29. Gilts fed 20 mg/kg RAC had increased β1AR expression at d 15 and reduced β2AR expression at d 29. These results suggest that in muscle, dietary RAC stimulates β1AR activity in both sexes and the reduced response to lean gain maybe controlled by β2AR down regulation particularly at the 20 mg/kg dose.

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Dietary Supplementation of L-Arginine HCl Increases Lactating Sow Feed Efficiency and Promotes Male Piglet Growth

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Milk is the major source of nutrition for pre-weaned piglets, with changes in milk production and composition being primary determinants of piglet survival and weight at weaning. Sow milk only provides approximately 40% of the nutrients required for maximal piglet growth during lactation (Boyd *et al.*, 1995), with arginine in particular identified as limiting piglet growth (Wu *et al.*, 2004). Increasing the supply of arginine to piglets through maternal dietary supplementation of lactating gilts increases the growth of their piglets, as does supplementing piglets directly (Mateo *et al.*, 1998; Kim *et al.*, 2004). The effects of supplementing arginine in the lactation diet of multiparous sows on piglet growth, and the mechanisms for this, have not been reported. This experiment was conducted to determine whether supplementing the lactation diets of multiparous sows with arginine would improve piglet growth rate due to increased arginine concentrations in the milk and/or milk production.

Multiparous Large White x Landrace sows (parity 2-4,) were allocated to two different treatment groups (n=75 sows/treatment). One group (CON) received a standard lactation diet (14 MJ digestible energy (DE)/kg, 9.7g/kg lysine) whilst the other group (ARG) received a standard lactation diet supplemented with L-arginine hydrochloride (+9.3 g/kg arginine; 14.03 MJ DE/kg, 9.7g/kg lysine), from d 1 to d 21 of lactation. Sow liveweight (LW) and P2 backfat (P2) were measured on d 1 and d 21 post-farrowing. On d 4, 14 and 21, milk samples were collected for composition analysis, and milk production determined using the Weigh-Suckle-Weigh method (Allen and Lasley, 1960) in a subset of sows (CON n=19, ARG n=20). Piglets were weighed on d 0, 1, 4, 14 and 21 post-farrowing. Weaning to oestrus interval (WOI) and subsequent litter size was recorded following the experimental lactation. Effects of lactation diet on sow measures were analysed by analysis of variance (ANOVA) using PASW Statistics v 17 (SPSS Inc., Chicago, USA). Piglet responses were analysed by repeated measures ANOVA, treating each piglet as a measure on the dam and including piglet sex as a between-animal variable. Litter size was included as a covariate in all models.

Table 1. *Effect of dietary arginine during lactation on sow feed intake, liveweight (LW) and P2 backfat change, daily milk production and weaning to oestrus interval (WOI; mean±SEM).*

Diet	Feed intake d 1-7 (kg/d)	LW change (kg)	P2 backfat change (mm)	Milk production (kg/d)	WOI (days)
CON	5.80 ± 0.11[a]	-7.2 ± 2.2	-2.79 ± 0.74	17.63 ± 1.33	6.9 ± 0.8[a]
ARG	5.53 ± 0.11[b]	-8.9 ± 2.2	-1.94 ± 0.75	17.93 ± 1.37	5.9 ± 0.7[b]

[ab]Means in a column with different superscripts differ significantly (P<0.05). SEM, standard error of mean.

Arginine supplementation did have some effect on sow performance (Table 1), however, milk arginine concentrations were not different between CON and ARG sows (P>0.60). Piglet LW at d 21 did not differ between groups (6.44 ± 0.12 kg; P>0.05). Between d 1 and 21, ARG increased growth rates of male (0.251 ± 0.005 versus 0.236 ± 0.006g/d; P=0.042), but not female piglets (0.234 ± 0.006 versus 0.240 ± 0.005 g/d; P>0.05). Similar changes in liveweight and P2 backfat, despite lower feed intake in ARG sows implies that feed use efficiency was improved by increased dietary arginine concentration. The arginine content of commercial lactation diets may therefore limit sow performance. Increased arginine in lactation diets may also promote follicular development, since the ARG sows displayed post-weaning oestrus one day earlier than CON sows. Increased overall growth rate in male, but not female piglets, from ARG sows suggests that arginine requirements may be higher in growing males. Overall, dietary arginine supplementation during lactation was less effective in promoting the growth of mature sow progeny than previously reported in earlier gilt studies (Mateo *et al.*, 1998; Kim *et al.*, 2004).

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Changes in Faecal Short-Chain Fatty Acid Concentrations in Pigs Infected With *Lawsonia intracellularis*

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Proliferative enteropathy (PE) or ileitis is a common production limiting disease, causing weight loss and diarrhoea in pigs from weaning onwards. *Lawsonia intracellularis* (LI), the aetiologic agent of PE, causes abnormal proliferation of immature ileal crypt cells. Pigs clinically affected with PE have reduced digestive enzyme activity in the ileum (Collins *et al*., 2009), which impacts on nutrient absorption. Undigested carbohydrates and amino acids in the large intestine are fermented by the resident microflora to produce short-chain and branched chain fatty acids (MacFarlane and MacFarlane, 2003). The hypothesis of this experiment was that faecal short-chain fatty acid (SCFA) concentrations would be significantly different between uninfected and LI infected pigs.

Thirty-six male, hybrid weaner pigs (Large White x Landrace) were randomly allocated at 13.8 ± 1.0 kg (mean ± standard deviation) live weight to two treatments: pigs infected with LI and a cohort of uninfected controls. Pigs were housed in individual pens, with treatments housed in separate rooms with strict quarantine between rooms to prevent faecal contamination. Pigs were tested for faecal shedding of LI and serological antibodies to LI from 5 to 9 weeks of age to verify they remained naïve to LI. At 9 weeks of age, the treatment group of 18 pigs was orally inoculated with 5.9 x 10^9 LI extracted from PE-affected mucosa and the control group were inoculated with phosphate buffered saline. Pigs were monitored daily for clinical signs and evidence of LI infection (faecal shedding and serology weekly). In addition, faecal samples were collected at 0, 3, 17, 20 and 24 d post inoculation (PI) to determine the SCFA composition by gas chromatography using the method described by Taylor (2002). The impact of time and disease (PE) on SCFA concentrations was analysed by repeated measures analysis.

L.intracellularis infection was demonstrated by faecal PCR and serology between 14 and 38 days PI in all pigs challenged with LI, with the peak in mean number of LI shed at 3 x 10^6 LI/g faeces between 17 and 28 days PI, and the peak in serum immunoglobulin G (IgG) titres between 28 and 38 days PI. The majority of pigs were sub-clinically affected, but LI infected pigs had a higher probability ($P<0.05$) of diarrhoea than control pigs between 22 and 26 days PI. *L.intracellularis* infection did not affect pig weights or weight gains but did significantly reduce feed intake and increase variation in pig weights between 0 and 21 days PI ($P<0.05$). Feed intake was significantly higher ($P<0.05$) between 22 and 42 days PI relative to the previous period in LI infected pigs.

At the time of LI colonisation (d 3), no significant differences in SCFA concentration were observed between treatments. *L.intracellularis* infected pigs showed reduced branched chain fatty acids (iso-butyric and iso-valeric) at 17 days PI, and increased acetic and butyric concentrations at 20 days PI ($P<0.05$). However, this was reversed at 24 days PI, when the concentration of each SCFA was reduced in LI infected pigs compared with the control pigs ($P<0.05$). The number of LI shed in faeces at 14 days PI correlated positively with butyric acid ($r=0.81$) and negatively with hexanoic and heptanoic acids ($r=-0.975$ and -0.89 respectively) at 17 days PI. We were unable to demonstrate increased microbial fermentation metabolites in LI infected pigs at the time of LI colonisation, but did observe increased SCFA close to the peak in infection, with the exception of amino acid fermentation metabolites. Conversely, reduced microbial fermentation metabolites were demonstrated in LI infected pigs after the peak in infection, as pigs began to recover. The strong correlations (both positive and negative) between individual SCFA and PE severity (increasing numbers of LI) suggest that replication of LI in the ileum leads to changes in the intestinal microflora as evidenced by changes in fermentation metabolites measured in the faeces. Future analysis of faecal SCFA concentrations at 7 and 10 days PI and in more severely affected pigs might highlight more clearly the effect of LI infection on digestion, absorption and substrate availability for microbial fermentation.

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Evaluation of a *Mycoplasma hyopneumoniae* Culture-Based Challenge Regime for Assessing Protective Efficacy of Experimental Vaccines

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Differences in *Mycoplasma hyopneumoniae* strain virulence and infection patterns are important factors to consider when utilising experimental challenge systems to evaluate the protective efficacy of *M. hyopnuemoniae* vaccines (Villarreal *et al.*, 2011). To identify an appropriate *M. hyopneumoniae* strain for use in future vaccine/challenge systems, this experiment examined two *M. hyopneumoniae* strains (Hillcrest and Beaufort) for their ability to induce clinical and pathological responses following experimental pig challenge. We hypothesised that virulence varies between the two strains and both clinical signs and lung lesion severity will correlate with proinflammatory cytokines IL-1β and 1L-6 in tracheobronchiolar washings (TBW).

Male pigs (n =19; 8-9 weeks old; mean weight 15.5 kg) from a commercial herd historically free of *M. hyopneumoniae* were assigned to three treatment groups balanced by mean weight (range 15-17 kg per pig per treatment pen). After anaesthesia with a mixture of xylazine (IIium Xylazil®, Troy Laboratories, Smithfield NSW) and ketamine (Ketamil®, Troy Laboratories, Smithfield NSW), TBW were collected using phosphate buffered saline (pH 7.2-7.3) immediately prior to intratracheal instillation of 10 mL Friis medium containing Hillcrest (Group HC, n = 8), Beaufort (Group BC, n = 8) or no organisms (Group UC, n = 3). Animals were monitored twice daily for coughing episodes (over 15 min) and TBW collection was repeated at three and four weeks post-challenge. Cytokine levels were measured in all pigs prior to challenge (Pre-HC and Pre-BC) and at three weeks post-challenge, but were not determined in pigs at four weeks post-challenge. Pigs inoculated with Friis medium containing no organisms (UC) served as a negative control group. The pigs were euthanased at three (n = 9) or four (n = 10) weeks post-challenge and gross lung pathology was assessed by Goodwin lung score. Statistical comparison between effects of challenge strain on coughing, severity of pneumonic lesions and cytokine responses were performed using analysis of variance at a 95% confidence interval.

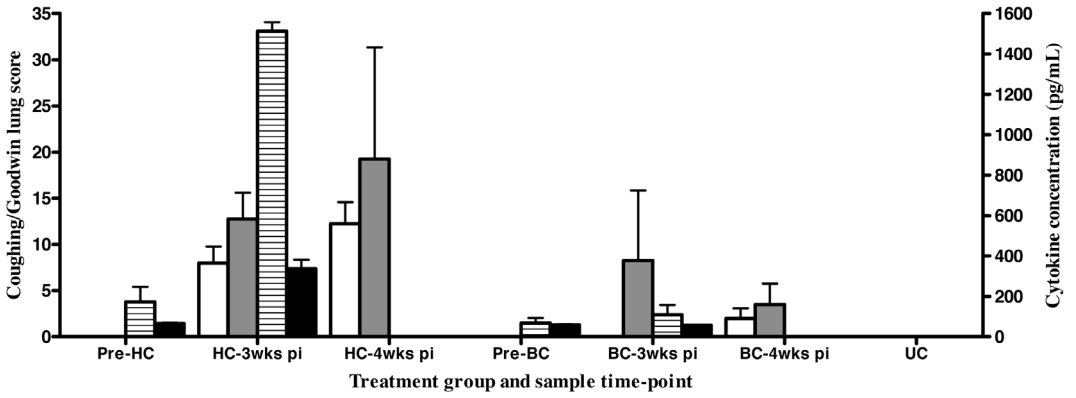

Figure 1. *Association between coughing episodes (□) , Goodwin lung score (▩), and cytokine responses of IL-1*β *(▤) or IL-6 (■) in* M. hyopneumoniae *Hillcrest (HC) and Beaufort (BC) strain challenged pigs slaughtered three or four weeks (wks) post challenge (pi). (Mean ± standard error of the mean).*

Hillcrest strain was superior to Beaufort in its ability to induce both coughing (P< 0.001) and pneumonic lesions (P<0.05) in challenged pigs. Overall, IL-1β concentrations in TBW were greater than IL-6 in challenged pigs, and Hillcrest-challenged pigs had much higher concentrations of both cytokines than Beaufort pigs (P<0.001), reflecting their greater degree of coughing and lung pathology (Figure 1). This experiment demonstrates the potential value of cytokine assays, coupled with suitable challenge strains and lung pathology at various timepoints, in assessing disease severity and thus protective efficacy of vaccines.

VILLARREAL, I., MAES, D., VRANCKX, K., CALUS, D., PASMANS, F and HAESEBROUCK, F. (2011). *Vaccine*. **29**:1731-1735.

Supported in part by the McGarvie Smith Institute.

Post-Weaning Diarrhoea and Performance of Pigs Fed a Low Protein Diet Without Essential Amino Acid Supplementation

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Previous experiments have shown that feeding a low protein (LP) diet within the first two weeks post-weaning reduces protein fermentation in the gastrointestinal tract and the clinical expression of post-weaning diarrhoea (PWD: Heo *et al*., 2009). However, growth of young pigs receiving a LP diet without crystalline essential amino acid (CEAA) supplementation is depressed after weaning. The hypothesis tested in this experiment was that the short-term reduction in performance caused by feeding a LP diet for two weeks immediately after weaning would not affect the lifetime performance of pigs because of compensatory growth.

Two hundred individually-housed pigs weaned at 21 d of age (Large White x Landrace, 5.5±0.05 kg liveweight, mean±SEM) were stratified to one of four dietary treatments (n=50) – 1) High protein plus antimicrobial compound: 230 g crude protein (CP) with 2.5 g lincospectin and 3 g zinc oxide per kg feed (HP+AMC), 2) 230g CP/kg (HP), 3) Low protein plus amino acids: 185 g CP/kg with added CEAA up to HP level (LP+AA), and 4) 185 g CP/kg without CEAA supplementation (LP). Incidence of PWD was visually assessed for first 14 days and blood samples were collected on d 14. With equal numbers of castrates and females per treatment group, pigs were fed the experimental diets for two weeks and then all pigs were fed the same series of commercial diets until slaughter. All pigs were experimentally infected with an enterotoxigenic strain of *E. coli* given orally (6 and 10 mL of 1.9 x 10^9 CFU/mL, serogroup O149:K91:K88) at 72 and 96 h after weaning. A one-way analysis of variance was used for statistical analysis.

Table 1. *Effect of dietary treatments on post-weaning diarrhoea (PWD) and short- and long-term performance in pigs challenged with enterotoxigenic* E.coli *at 72 and 96 h after weaning.*

Treatment	HP+AMC	HP	LP+AA	LP	SEM	Significance
Number of pigs with PWD	8/50	26/50	8/50	9/50		
Diarrhoea index (%)[1]	1.1[a]	8.1[b]	1.7[a]	2.0[a]	0.95	0.001
Number of antibiotic treatments	0.2[a]	1.1[b]	0.2[a]	0.3[a]	0.07	0.001
Plasma urea nitrogen (mmol/mL)	3.9[a]	4.1[a]	2.1[b]	3.6[a]	0.21	0.001
Post-weaning performance (1-3 weeks post-weaning)						
Daily gain (g)	215[a]	207[a]	210[a]	169[b]	12.4	0.035
Daily feed intake (g)	326[a]	317[ab]	327[a]	276[b]	14.9	0.056
Feed conversion ratio	1.56	1.58	1.60	1.72	0.052	0.145
Lifetime performance (4-15 weeks post-weaning)						
Daily gain (g)	852	840	846	839	9.2	0.733
Days to 90 (kg)	133.9	136.4	136.4	137.7	1.14	0.138

[1]The mean proportion of days with diarrhoea in the 14 days after weaning; [abc]Means in a row with different superscripts differ significantly (P<0.05). HP, high protein; AMC, antimicrobial compound; LP, low protein; AA, amino acid; SEM, standard error of the mean.

Pigs fed the HP diet showed an increased diarrhoea index (P<0.001) and had a greater number of therapeutic antibiotic treatments (P<0.001) compared to pigs fed other diets. Post-weaning growth (P<0.05) and feed intake (P=0.056) were decreased in pigs fed the LP diet compared to pigs fed other diets (Table 1). The performance reduction occurred mainly in the second week as pigs fed the LP diet grew less (P<0.001), and utilised the feed less efficiently (P<0.001) in this time. When pigs were fed an identical commercial diet in week 3, however, performance indices were not different between treatments. Lifetime performance was not affected by the dietary treatment after weaning (P>0.05), and carcase characteristics were similarly unaffected by the treatments (data not shown). The results indicate that although feeding a LP diet without CEAA supplementation decreased performance immediately after weaning while it was being fed, it did not influence lifetime performance or carcase characteristics and reduced the clinical expression of PWD.

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.

Supported in part by WA Agricultural Produce Commission Pork Producers' Committee.

Micro-Encapsulated Zinc Oxide as a Means of Controlling Post-Weaning Diarrhoea in Pigs

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The pharmacological use of zinc oxide (2,500-3,000 ppm ZnO) is widely accepted as an efficient means of controlling post-weaning diarrhoea (PWD) in piglets and is being used worldwide as an alternative to antibiotics (Mullan *et al*. 1995). However, the strategy is criticized because high levels of zinc are excreted into the environment through the effluent system. Recently, a microencapsulated zinc oxide (ME-ZnO) product (Shield Zn®, CTC Bio Inc., South Korea) claimed to reduce PWD while at a reduced concentration of ZnO of 100ppm. The ME-ZnO was evaluated relative to ZnO to assess its efficacy in controlling PWD. The hypothesis tested was that inclusion of ME-ZnO will efficiently control PWD and reduce faecal zinc excretion.

An experiment with a split-plot design for which the whole plots were arranged in randomised blocks was conducted. Challenge versus no-challenge with enterotoxigenic *E. coli* (at 72 h, ETEC, serogroup O149: K91:K88, 10 mL of 10^8 CFU) were the factors in the whole plot, and the three dietary treatments (control, 3000 ppm ZnO and 100 ppm ME-ZnO) formulated to 15 MJ digestible energy (DE)/kg and 0.88 g standardised ileal digestible lysine/MJ DE were used as subplots (n=12). Vitamin mineral premix used for all diets supplied 100 ppm zinc as ZnO. A total of 72 individually housed weaner pigs (Landrace x Large White x Duroc cross-bred, castrate and female, 1:1) weighing 5.6±0.11 kg (mean ± standard error of mean) were used in a three week feeding experiment immediately after weaning. Incidence of PWD was assessed for 14 days after weaning, Blood and faecal samples were collected on d 14 to assess plasma zinc and faecal zinc excretion.

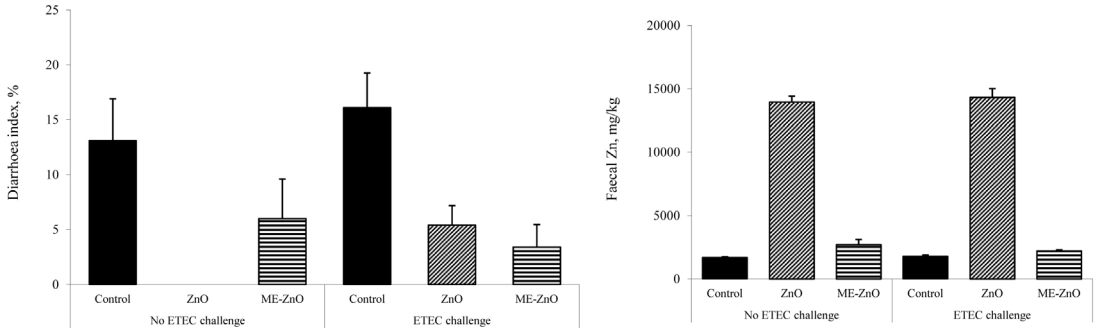

Figure 1. *Effect of diet (■ control, ▨ ZnO, ▤ ME-ZnO) on the diarrhoea index (% days with diarrhoea for 14 days after weaning) and faecal zinc excretion (mean ± standard error of mean). The diet effect was significant (P<0.001), while ETEC challenge and the interaction between diet and ETEC challenge was not (P>0.05).*

The results showed that 100 ppm ME-ZnO suppressed the expression of PWD in both ETEC-challenged and non-challenged pigs, and kept the faecal zinc levels to the levels of that found in the pigs fed a control diet without additional ZnO supplementation. The results suggest that under the experimental conditions the expression of PWD could be reduced by supplementing 100 ppm ME-ZnO in the diets for weaner pigs without increasing faecal zinc excretion levels. A combination of good hygiene and management with use of microencapsulated zinc may reduce faecal zinc excretion and reduce the impact of *E.Coli* scouring but not impact on antibiotic removal except when used for *E.Coli* scouring.

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Development of Methodologies for Future Surveillance of Methicillin-Resistant *Staphylococcus aureus* in Australian Pigs

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a newly recognised agent of zoonotic disease. Its presence in pigs overseas represents a potential hazard for those routinely exposed to pig production and processing systems. Pig-adapted strains of MRSA were first reported in Europe in 2005 (Smith and Pearson, 2011). Since then, the geographical distribution of the known occurrences of these strains has greatly expanded. They are now present in pig herds across many regions of Europe, North America and Asia (Smith and Pearson, 2011). To date, MRSA has not been reported in pigs in Australia and a recent study investigating MRSA carriage in veterinarians in Australia did not implicate pigs as a potential source of zoonotic MRSA infection (Jordan *et al*., 2011). Nevertheless, the potential for emergence of pig-adapted MRSA strains in Australia is very real and this has prompted the pork industry to support the development of methods allowing the efficient conduct of a national baseline study if deemed necessary. The aims of this experiment were to 1) develop and validate a PCR approach for the rapid screening of pooled porcine samples for the presence of MRSA, and 2) to validate the application of culture-dependent methods for the isolation and identification of MRSA colonies. We hypothesised that our methodologies would be suitable for the screening, isolation and identification of MRSA from Australian porcine nasal samples.

Porcine nasal samples from 30 pigs at three farms (ten per farm) were analysed using a previously published method (Broens *et al*., 2011). Individual nasal samples and MRSA positive controls were grown overnight in a selective enrichment broth (Mueller Hinton broth, with 6.5% NaCl, Oxoid Ltd, Cambridge, UK), followed by inoculation into a selective-differential broth (MRSA broth, Edwards Instrument Co., Sydney, Australia). Cultures were then streaked onto selective-differential agar plates (Brilliance MRSA agar, Oxoid Ltd, Cambridge, UK). Presumptive MRSA colonies (denim-blue in colour) were isolated and analysed with two routine diagnostic tests (catalase and Staphaurex-plus, Oxoid Ltd, Cambridge, UK), before being analysed by PCR for the presence of the *S. aureus* specific gene *femB* and methicillin-resistance gene *mecA*. No colonies from these porcine samples were identified as MRSA. Based on these results, we created four sample pools (in Mueller Hinton broth) with each containing four negative samples spiked with a MRSA positive control. These served as a tool for validating the use of PCR to screen for MRSA in pooled porcine nasal samples. Pig samples included those from two geographically distinct farms. Following incubation, DNA was extracted from the sample pools and the *femB/mecA* PCR was performed.

In all four sample pools, both the *femB* and *mecA* genes were able to be amplified and the MRSA used to spike the samples were able to be re-isolated. This result indicates that that the PCR assay is not inhibited by the contents of porcine nasal samples or the selective-enrichment broth, and that it is suitable for the rapid screening of multiple porcine nasal samples. The results also presumptively validate the ability of these methods to isolate and identify MRSA in pigs in Australia. These are currently being used to pre-screen and attempt to isolate and identify MRSA from pigs in a pilot study. If successful, this may open the way for the efficient conduct of national baseline studies assessing the epidemiology of MRSA in Australian livestock.

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BROENS, E., et al. (2011). *Veterinary Microbiology*. **147**:440-444.

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The Relationships Between Agonistic Behaviour, Injuries and Stress in Group-Housed Sows

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Although there is increasing housing of gestating sows in groups, there are few rigorous recommendations in the scientific literature on reducing sow aggression. A better understanding of social behaviour of individuals may assist in reducing sow aggression. This study examined the relationship between agonistic behaviour, stress and injuries in gestating sows housed in groups.

Within a larger experiment studying the effects of floor space allowance and group size in sows, the agonistic behaviour of 90 crossbred mixed parity sows (Landrace x Large White) was studied during the first two days after mixing, which occurred within 1-7 days of insemination. Sows were randomly allocated to nine groups of ten in indoor concrete-floored pens of varying space allowances (1.4, 1.8, 2.0, 2.2 and 2.4 m^2/sow). Feed was delivered to the pen floor on the day after mixing (d2) via overhead hoppers four times at hourly-intervals from 0700 h. From digital video records, continuous observations were conducted to measure the number of bouts of aggressive behaviour in the 90 min after mixing (d1) and in the 30 min following each feed drop on d2. Bouts of agonistic behaviour (bites, pushes, lunges and chasing) displayed or received by each sow were recorded. On the afternoon of d2, injuries were measured (Karlen *et al.*, 2007) and blood samples were collected via venipuncture from the jugular vein for analyses of cortisol. Live weights were recorded on entry to treatment and prior to farrowing, and reproductive performance (litter size, born alive, mummies, still births) was also recorded. Agonistic behaviour delivered and received was positively correlated at both d1 ($r=0.92$, $n=90$, $P<0.001$, data transformed square root) and at feeding d2 ($r=0.50$, $n=90$, $P<0.001$, data transformed square root), indicating considerable reciprocal agonistic behaviour on both days. Agonistic behaviour was also correlated with injuries, cortisol concentration, parity and live weight gain during gestation. Linear regression (backward method) was used to examine the relationships between these variables (PASW Statistics 18.0, SPSS Inc., Chicago, Illinois, USA).

Table 1. *Models developed to predict skin injuries, cortisol concentration and live weight gain.*

Dependent variable	Independent variable	Adjusted R Square	*i*	Standard Error (*i*)	F	P value
Injuries[1]	Space	0.365	2.030	0.498	$F_{3,84}=16.10$	P<0.001
	Agonistic delivered d1[1]		-0.224	0.129		
	Agonistic received d1[1]		0.570	0.151		
Cortisol[2]	Space	0.152	-0.298	0.116	$F_{2,85}=8.79$	P<0.001
	Agonistic received d2[1]		0.064	0.032		
Weight gain	Space	0.431	-27.74	9.020	$F_{4,51}=9.43$	P<0.001
	Agonistic delivered d1		8,402	2.333		
	Agonistic received d1		-6.322	2.759		
	Total injuries d2		3.422	1.856		

[1,2] Data transformed square root and log10 transformed, respectively, prior to statistical analysis.

As indicated by the standardized regression coefficient (*i*), increased floor space, increased agonistic behaviour received, and decreased agonistic behaviour delivered at mixing were associated with increased skin lesions, while reduced floor space and increased agonistic behaviour received at feeding on d2 were associated with increased cortisol concentrations (Table 1). Reduced floor space, increased agonistic behaviour delivered and received at mixing and increased skin lesions were associated with increased live weight gain over gestation. The negative relationships between space and cortisol and live weight gain, and those between agonistic behaviour and weight gain, are expected. However, the positive relationships between space and injuries and between injuries and weight gain and the lack of a relationship between injuries and cortisol are surprising and illustrate the importance of a better understanding of the social behaviour of individual sows in groups.

KARLEN, G.M., HEMSWORTH, P.H., GONYOU, H.W. and SMITS, R.J. (2007). *Applied Animal Behaviour Science*. **85**(1-3): 87-101.

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A Phenotypic and Genetic Analysis of Still Born Piglets

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Still born piglets (SB) are a major economic cost to the pig industry. Not only is there a cost associated with the loss of a potential finished pig, there are also costs associated with the gestation of that animal to the sow. These are opportunity costs to the other piglets in the litter, such as a higher chance of mortality and sub-optimal growth rates (Boulot *et al*., 2008). Bunter (2009) suggested that heritability (h^2) estimates for preweaning mortality may have increased over time. A similar comparison of h^2 estimates was not available for SB. The aim of this study was to derive phenotypic trends and obtain variance components for SB.

Data were sourced from the National Pig Improvement Program (NPIP; Animal Genetics and Breeding Unit, Armidale, NSW). Large White, Landrace and Duroc records were extracted from eight herds over a 15 year period from 1996 until 2010. Data consisted of 76,851 reproductive records for SB from 19,121 sows. The model corrected for month, year, herd, breed, parity, litter type (pure versus crossbred litter), and the linear covariates of total born (TB: overall mean=11.8, 1996-2000 mean=11.7, and 2006-2010 mean=11.9) and gestation length (mean=115.2). Trends were expressed as least squares means (weighted by year) from the full model and standard deviations of residuals by year from a model where year was removed. Genetic analysis on the first five years of data (1996-2000) and the last five years of data (2006-2010) used an animal model applying ASReml (Gilmour *et al*. 2006). Variances due to additive genetic and permanent environment of the sow were estimated.

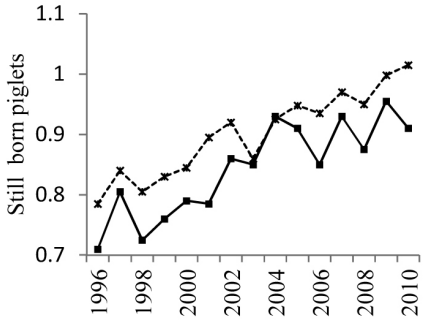

Figure 1. *Least squared means for year effects (*) and standard deviation (■) of residuals by year for still born piglets*

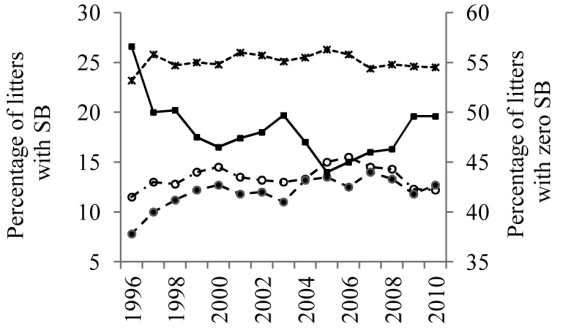

Figure 2. *Percentage of litters with zero (■), a single (*), two (○) or multiple (●) still born piglets (SB)*

The overall mean (standard deviation) for SB was 0.996 (1.29). The regression coefficient for TB was 0.12±0.002. Yearly least squared means and SD of the residuals increased over time (Figure 1). The increase in SD illustrates that there are now more bouts on farms where SB piglets become a greater issue. This increase in SB is driven by more litters with two or more SB piglets (Figure 2). Genetic analyses showed that in the early dataset the heritability (h^2) estimate for SB was 0.09(±0.02) with a permanent environment (pe^2) of 0.05(±0.02). The analysis of the more recent data, h^2 was basically unchanged at 0.06(±0.01) with a pe^2 of 0.05(±0.01). Without correcting for TB, h^2 estimate went from 0.09(±0.01) in early data to 0.07(±0.01). Although the mean and variation of SB adjusted to constant TB had increased, the heritability of SB remained unchanged. The majority of increased phenotypic variation of SB was partitioned into the error variance which suggests that the increase in mean and variation was caused by unrecorded environmental factors on farm. On farm management factors should be examined, first focusing on avoiding multiple SB per litter.

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Sow Health Influences Piglet Survival

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Identifying factors which affect piglet survival can help producers develop interventions and management strategies to reduce pre-weaning mortality. While puerperal disease affects subsequent fertility and litter size of sows (Hoy, 2006), Gerjets and Kemper (2009) suggested that the primary economic impact of post-parturient problems is via increased pre-weaning mortality of piglets. We hypothesise that both pre- and post-farrowing sow characteristics can be identified which are indicative risk factors for their piglets' survival until weaning and examine a range of potentially important sow level factors in a multivariate context.

Individual piglets (n=9133) born to mixed parity sows representing four sow lines were recorded from 847 litters in two separate time periods at a single location. Sows were recorded pre-farrowing for teat count, weight and fat depth, and scored for their reaction to being approached by a human, overall body condition, quality of locomotion, udder development and the presence or absence of colostrum. These measurements were recorded at or shortly after sow groups were transferred to farrowing accommodation. After farrowing, litter size traits, the sow's response to piglet handling, sow rectal temperature, and daily feed intake were also recorded. Data on sow rectal temperature was classified as a binary score indicating post-partum fever (temperature >39.7°C) or not. Daily intake was summed over the first three days post-partum. All piglets were weighed individually at birth. Piglet gender, fostering status (fostered or not), the length of gestation and the interval between transfer and farrowing were also known and considered. Sow parity group was concatenated into a single descriptor with fostering status for model testing. Logistic regression (Proc LOGISTIC, SAS Institute, Cary NC) was used to establish whether the binary trait of piglet survival until weaning was associated with scores for sow attributes, or quintile rank for continuous traits such as piglet birth weight. Significant factors ($P<0.05$) were identified by fitting a full model containing all the above effects, with automated sequential backwards elimination of non-significant effects based on their Wald test statistic to obtain the final model. The relative survival rates are indicated by the Odds-Ratio (OR) for each factor level, which is expressed relative to the reference (lowest) level within each factor, with an OR of 1.0.

Date of sow transfer, which represents both seasonal and management effects, piglet gender, birth weight quintile and gestation length were significantly associated with piglet survival, consistent with previous research. The relative survival rate of females was 38% better than males (OR: 1.38). Survival rates of heavier piglets relative to the bottom quintile (piglets <1.25 kg) for birth weight were around two to seven fold higher (OR: 2.50-6.70), increasing with weight. Longer gestation lengths and an increased number of sow teats were significantly associated with improved piglet survival; 10% per day and 6% per additional teat. These factors potentially reflect increased physiological maturity of piglets at birth and an increased access to colostrum at birth. Piglets born to sows with evidence of poor locomotion pre-farrowing, post-partum fever, and a P2 fat depth exceeding 22mm had reduced survival rates (OR: 0.85, 0.75 and 0.82). Conversely, sows with a less developed udder scored post-farrowing had higher piglet survival (OR: 1.24). Since pre- and post-farrowing udder scores are only moderately correlated (data not presented), this outcome probably represents evidence for piglet suckling (reducing udder distension), lack of udder oedema, or better access to teats, rather than lack of udder development *per se*. Voluntary sow feed intake of less than nine kilograms consumed in total over the first three days post-partum was associated with reduced piglet survival. Sows with poor locomotion also had a higher incidence of low feed intake post-partum. All other factors were not significantly ($P>0.10$) associated with piglet survival, although some confounding between descriptive factors exists.

Results demonstrate that some sow health attributes pre- and post-partum are associated with piglet survival. Establishing management, housing and health care systems to ensure good locomotion pre-farrowing, detection and treatment of sows with puerperal fever, and remedying factors that limit adequate sow feed intake in the first three days after farrowing should assist in improving survival outcomes for piglets.

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GERJETS, and KEMPER, (2009). *Journal of Swine Health and Production*. **17**:97-105.

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Strategies for the Early Detection of Sick and Injured Stall-Housed Gestating Sows

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Early detection and appropriate treatment of sick and injured sows aids in managing health and welfare and minimizes productivity losses from uncorrected problems. Although there is a voluntary phase-out of sow stalls by 2017, a survey conducted in 2004 found that approximately 64% of pig producers in Australia housed pregnant sows individually in stalls for at least part of their gestation (Pearson, 2004). Stalls may be used to house individual sows that need special attention to restore their health or body condition, particularly group-housed sows that may have been subjected to persistent bullying.The aim of this experiment was to develop a method for detecting sick or injured sows housed in stalls. The null hypothesis was that inspection method had no effect on the proportion of physical abnormalities detected in sows housed in stalls on a commercial farm.

Approximately 350 pregnant sows housed in stalls for the first six weeks and the last three weeks of gestation on a 1000-sow commercial farm were inspected over four days in a cross-sectional epidemiological study. Five inspection methods were tested, (front of stall, back of stall; front of stall at feeding, back of stall at feeding, back of stall at feeding and standing the sows up). The route of inspection and the person conducting inspections were the same each day. The number and description of physical abnormalities detected in sows using each inspection method were recorded and classified as leg lesions, claw lesions, low body condition (<3), lameness, fight wounds, pressure sores and vulval discharge or damage. The proportions of abnormalities detected using the different inspection methods were analysed using a generalized linear model, with inspection site and feeding time included in the model.

Table 1. *Proportion of abnormalities detected and time taken to inspect stall-housed gestating sows using five different inspection methods on a 1000-sow commercial herd.*

Aisle	Feeding	Abnormalities	Time taken
Front	No	27/363 (7%)	33 min (9.1 min/100 sows)
Back	No	27/361 (7%)	28 min (7.7 min/100 sows)
Front	Yes	27/339 (8%)	38 min (11.2 min/100 sows)
Back	Yes	45/339 (13%)	73 min (21.5 min/100 sows)
Back	Yes+Standing	42/347 (12%)	65 min (18.7 min/100 sows)

A higher proportion of abnormalities were detected when stall-housed sows were inspected at feeding time compared to non-feeding time ($P<0.05$; Table 1). While the highest proportion of abnormalities was detected in sows inspected from the back at feeding time, inspection from the rear alone did not detect a significantly higher proportion of abnormalities than inspecting from the front ($P>0.05$). Encouraging sows to stand when examining them from the back during feeding time did not increase detection sensitivity ($P>0.05$). Low body condition was the most common abnormality detected (38% of abnormalities), with approximately half of these sows identified by farm staff to receive extra feed. These results suggest that stall-housed sows should be inspected at feeding time. Body lesions accounted for a lower proportion (18%) of total abnormalities among stall-housed sows compared with group-housed sows observed in a previous study (Kelk, 2011). Stall housing may be a useful management tool to reduce the prevalence of body lesions resulting from inter-sow aggression. Stockpersons must be provided with clear instructions and with sufficient time to properly examine the sows under their care.

PEARSON, A.B. (2004) Sow housing and animal welfare survey. Report commissioned by Australian Pork Limited., Prime Consulting International Ltd. New Zealand.

KELK, F., CUTLER, R.S. and HOLYOAKE, P.K. (2011). In "Manipulating Pig Production XIII", p 243, ed R.J. van Barneveld, (Australasian Pig Science Association: Werribee, Australia).

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A Study of the Relationships Between Social and Feeding Behaviour and Injuries in Group-Housed Gestating Gilts

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Rising public concern for animal welfare, particularly confinement of farm animals, has led to stall housing for sows being phased out and replaced by group housing during gestation in Australia. Group housing systems offer sows opportunities for more movement, exploration and social interaction, but aggression, particularly at mixing, may limit sow welfare (Velarde, 2007). This aggression, especially if intense and prolonged, may lead to injuries and stress. Agonistic behaviour is defined as any behaviour associated with threat, attack or defence and includes features of behaviour involving escape or passivity, as well as aggression (Fraser and Broom, 1997). The aim of this study was to examine the relationship between agonistic behaviour, feeding behaviour and injuries in group housed gilts.

One hundred and twenty pregnant Landrace x Large White gilts were housed in three groups that had been mixed within a week of insemination. Each pen was fitted with a single electronic feeder and each pig was offered 2.4 kg/d of feed commencing at midnight over one or more meals. Agonistic behaviour around the feeder entrance and electronic records on feeder entries and feeding times were studied over two consecutive days in weeks 2 or 3 post-mixing. Displacements around the feeder, where one animal withdraws in the presence of another, were studied and two types of displacements, were considered: displacements involving aggression (ie. bites, slashes, butts and levering) and displacements in which there was no aggressive interaction. It is recognized that in the latter situation, displacement may occur not only as a result of a threat but also for other reasons apart from a social interaction. Furthermore, with each classification method, we identified gilts under three classes: submissive, in which the gilt did not displace another gilt during the observations; sub-dominant, in which the gilts were displaced more times than they displaced others; and dominant, in which the gilt displaced others more times than they were displaced. Skin lesions were measured in weeks 3 or 4 post mixing (Karlen *et al.*, 2007). Relationships between the classifications of displacement, feeding behaviour and injures were examined using Kruskal-Wallis tests (PASW Statistics 18.0, SPSS Inc., Chicago, Illinois, USA).

Table 1. *Relationships between classifications based on non-aggressive displacements and latency to first feed, latency to consume ration and injuries (Means ± standard deviation).*

	Classification			
	Submissive	Sub-dominant	Dominant	P value
Latency to first feed (min)	353.3 (±48.7)	493.9 (±43.7)	537.0 (±37.5)	0.01
Latency to consume ration (min)	375.9 (±48.6)	518.9 (±43.6)	566.0 (±37.4)	0.008
Skin Injuries	3.2 (±0.3)	4.2 (±0.2)	3.9 (±0.2)	0.02

There were no significant relationships between the classifications of submissive, sub-dominant or dominant based on aggressive displacements and feeding behaviour and injures. In contrast, when gilts were classified as submissive, sub-dominant or dominant based on non-aggressive displacements, this classification was related to the latency to first feed, the latency to consume the ration and skin injuries (Table 1).

Hierarchies are used by pigs as a means of minimizing aggression and by the time that the observations were conducted in this study a stable hierarchy may have been established. Since non-aggressive displacements may be more commonly used during social interactions in a stable social hierarchy, these results show that the influence of social behaviour on feeding behaviour and injuries may be best appreciated through an understanding of agonistic behaviour, not just aggressive behaviour.

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VERLARDE, A. 2007. In "On Farm Monitoring of Pig Welfare", pp.53-56, eds A. Verlarde, and R. Geers, (Wageningen Academic Press, Wageningen).

KARLEN, G. M.; HEMSWORTH, P .H.; GONYOU, H. W ; SMITS, R. J. (2007). *Applied Animal Behaviour Science*. **85**: (1-3) 87-101.

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Nutrient and Heavy Metal Contents of Spent Bedding From Straw-Based Pig Housing

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As part of a project investigating the utilisation of spent bedding from straw-based pig housing in broadacre cropping systems, a product survey was conducted. The aim of this was to determine nutrient (macro and trace elements) and heavy metal contents of spent beddings in order to assess its potential value as an alternative source of nutrients for crops, and potential risks in relation to heavy metal accumulation in treated soils. The samples were collected by taking thirty random grab samples from spent bedding piles, then combined in a bucket and a subsample selected for analysis. Thirty three samples, which were predominately cereal straw based, were sourced from commercial pork farms in South Australia and New South Wales.

Table 1. *Moisture, macro and micro nutrient contents of 33 spent bedding samples.*

	Moisture (%)	N (% DM)	P (% DM)	K (% DM)	S (% DM)	Zn (mg/kg)	Cu (mg/kg)	Mg (mg/kg)
Average	48.4	2.9	1.24	1.97	0.6	1133	100	367
Minimum	6.4	1.73	0.52	0.39	0.35	320	<0.05	191
Maximum	73.7	4.54	2.63	3.84	1.00	4289	474	585
SD	19.45	0.63	0.39	0.72	0.16	866	135	83

SD, standard deviation; DM, dry matter.

Useful quantities of plant nutrients were contained in spent bedding from straw-based pig housing, indicating good potential for use as a fertiliser alternative in broadacre cropping systems. On average, macro nutrients (N, P, K and S) occurred in quantities comparable to other organic by products (chicken litter and reclaimed biosolids) currently utilised to supply nutrients to broadacre crops. Micro nutrients (Zn, Cu and Mn) were also contained in spent bedding samples indicating potential to supply these nutrients to broadacre crops. The survey indicated a high degree of variation in nutrient and moisture contents between product batches. (Table 1). This has implications for valuing product based on nutrient content, and for calculating appropriate rates of application by broadacre users. It highlights the importance for users to obtain an analysis of the product intended for use to refine nutrient application decisions.

Table 2. *Heavy metal (mg/kg) content and biosolids standards (mg/kg)[1] for spent bedding.*

	As	Cd	Cr	Cu	Pb	Ni	Zn
Average	1.1	0.25	8.2	100	2.7	6.8	1133
Minimum	0.3	0.05	1.1	<0.05	0.4	2.1	320
Maximum	2.4	0.60	23.0	474	6.4	14.6	4289
Standard[4]	20	1	400	150	300	60	300

[1]EPA Victoria Biosolids Grade C1 Standard, EPA Victoria (2004).

When compared with biosolids contaminant grade standards (EPA Victoria, 2004) the results indicate that spent bedding contained low levels of heavy metals with respect to As, Cd, Cr, Pb and Ni (Table 2). Although Zn and Mn are regarded as essential micro nutrients for crops, these elements are also heavy metals with potential to accumulate in soils. All batches contained moderate levels of Zn, with three testing at high levels. Fifty percent of samples contained moderate levels of Cu, with the remainder containing levels below detection limits. As a result, soil accumulation of Zn, and potentially Cu, need to be monitored with long term use or high application rates.

EPA Victoria (2004). Guidelines For Environmental Management - Biosolids Land Application. State Government of Victoria; Melbourne.

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CHAPTER 3

Feed Processing

Symposium: Voluntary Feed Intake of the Modern Pig: Mechanisms for Regulation

Symposium Introduction

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It is generally acknowledged that pigs regulate their food intake according to the most limiting nutrient that will allow the animal to grow to their genetic potential (Ferguson and Gous, 1997). Intensive genetic selection to maximize growth, improve feed efficiency and reduce subcutaneous fat, however, has substantially changed the pig over the past 10 years, in particular, reducing voluntary feed intake. There is also recent evidence that the regulation of the modern animals feed intake is less influenced by the energy density of the diet, and governed by other dietary factors.

The major focus on improving feed efficiency of pigs often means the benefits of maintaining or increasing voluntary intake to maximise protein deposition and live weight gain are neglected. Therefore, the knowledge of what influences feed intake, especially how it can be increased, is more critical more, than in the past.

The first paper of the symposium (Roura, 2011) will describe what is known about chemosensing (taste and smell) and its importance for determining preference for feed ingredients and its influence on metabolic processes affecting overall animal metabolism and intake. The paper will detail some individual nutritional components and anti-nutritional factors that can influence feed intake, which are not associated with dietary energy or protein concentration.

The second paper (Black *et al*. 2011) will review the physiological and metabolic regulation of feed intake, showing that animals attempt to eat to fulfil their metabolic demand for energy and how this is monitored and regulated at a metabolic level by the animal. The capacity of the pig to consume sufficient energy for optimal energy metabolism is limited by various dietary, climatic, disease and social constraints. The impact of these constraints and possible mechanisms through which they operate are described

The final paper (Hazzledine, 2011) will present some conflicting evidence on whether energy density does, or does not, affect feed consumption of modern genotypes. Once the effects on daily energy intake are discussed, the author documents how least-cost linear programming can be used to adjust the energy level and bulk or volume of the diet to effectively optimize diet costs.

The overall aim of this symposium is to educate and inform the attendees how voluntary feed intake is controlled in the modern pig, which will hopefully provide scientists with direction for future research and give commercial nutritionists and associates some practical outcomes.

Taste Beyond Taste

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Abstract

Some of the highest positively selected genes in vertebrates are part of the taste system suggesting that taste plays a critical role in the survival of species. Beyond the classical concept of taste in sensorial food evaluation, it is now clear that taste sensors procure an identification of dietary nutrients in the oral cavity. Genomic advances of the last decade have allowed the identification of the two main families of taste receptors (TR), the G-protein coupled receptor families T1R (for sweet and umami tastes) and T2R (for bitter taste). The scientific community is considering if the number of primary tastes is bigger than the classical five. Perceptions of fat, complex carbohydrates, water, calcium or carbonation are being studied and some might be included as novel taste types in the future. However, the fact that nutrient levels are finely monitored in order to guarantee homeostasis implies the existence of a network of nutrient sensing that orchestrates the control of nutrient intake, digestion, absorption and metabolism. In recent years it has become apparent that the taste system is involved in that network. Taste receptors and other genes related to the intracellular taste transduction mechanisms, such as α-Gustducin, are expressed away from the oral cavity in tissues such as the respiratory system or the gastrointestinal tract (GIT). Recent progress is summarized in relation to how TR sense the luminal content of nutrients and toxicants in the GIT and the endocrine-related events that they may trigger. Unfortunately, the advances related to pig science are still scarce since the pig genome has only been available only since 2009. The current findings in pigs emphasize the adaptation of the TR system to the nutritional status of the animal presumably geared to ensure energy and protein homeostasis. Practical implications related to feed intake of bitter feedstuffs, protein sources, cereals and feed additives (mainly sweeteners, organic and amino acids) in pigs are discussed.

Abbreviations

CC, chemosensory cell; **CCK**, cholecystokinin; **ENaC**, epithelial amiloride-sensitive sodium channel; **FFA**, free fatty acids; **GIP**, glucose-dependent insulinotropic peptide; **GIT**, gastrointestinal tract; **Gβ3**, gustducin β subunit protein 3; **Gγ13**, gustducin γ subunit protein 13; **GLP1**, glucagon-like peptide protein 1; **GLUT2**, glucose transporter protein 2; **GPCR**, guanine-coupled-nucleotide-binding protein-coupled receptors; **GPR(41,43)**, G-protein coupled receptor 41 or 43; **L-AA**, L amino acid; **MSG**, monosodium glutamate; **NEO**, neohesperidin; **PLCβ2**, phospholipase β subunit protein 2; **PYY**, peptide YY; **REEP2**, receptor accessory protein 2; **RTP3**, receptor transporter protein 3; **SAC**, saccharin; **SCFA**, short chain fatty acids; **SGLT1**, sodium-glucose co-transporter protein 1; **TASR**, taste receptor gene; **TR**, taste receptor; **T1R(1,2,3)**, taste receptor family 1 proteins 1, 2 or 3; **hT1R(1,2,3)**, human taste receptor family1 proteins 1, 2 or 3; **pT1r(1,2,3)**, porcine taste receptor family 1 proteins 1, 2 or 3; **Tas1R (1,2,3)**, taste receptor gene family 1 proteins 1, 2 or 3; **Tas1r**, non-human taste receptor gene family 1 proteins 1, 2 or 3; **T2R**, taste receptor family 2; **Tas2R**, taste receptor gene family 2; **Tas2r**, non-human taste receptor gene family 2; **TRP (M5,A1)**, transient receptor potential; **TSC**, taste sensory cell

Introduction

The taste system is one of the most preserved in vertebrates. Positive Darwinian selection plays a major role in evolutionary innovation and between species divergence. Currently evolutionary biologists are focusing their interest in identifying the genes that positive selection has affected and the functional consequences (Kosiol *et al.*, 2008). Eight of the highest positively selected genes are involved in "sensory perception of taste" including five taste receptors. Understanding why taste has been playing such a critical role in allowing living organisms to adapt to ecological niches and survive is a task well beyond the scope of this review but some aspects are discussed.

Voluntary feed intake in farm animals does not seem to be controlled by any single nutrient, rather by an overall balance where energy and protein play a central role (Black *et al.*, 2011; Simpson *et al.*, 2003). In addition, voluntary feed intake might also be driven in part by levels of micronutrients such as calcium, phosphorus and sodium, among others (Roura *et al.* 2008a). Furthermore, under certain circumstances, availability of food rather than nutrient balance, may overrule the mandates of the energy homeostasis laws (Tordoff, 2002). Overall, the fact that nutrient balance is finely monitored implies the existence of a network of nutrient sensing mechanisms that orchestrate the control of nutrient intake, digestion, absorption, storage, metabolism and excretion. This review paper explains the role of taste receptors as (part of) a major nutrient sensing system in and outside the oral cavity in mammals with emphasis in pigs.

The Mammalian Nutrient Sensing System Called "Taste".

There are five different tastes widely accepted by the scientific community and these are: sweet, umami, salty, sour and bitter. Sweet taste is mainly triggered by carbohydrates such as sugars. Umami taste (mostly recognized as the monosodium glutamate taste in humans) is related to dietary protein and senses some L-amino acids (L-AA), such as glutamic acid (or its sodium salt monosodium glutamate; MSG) and peptides. Salty and sour respond to sodium and protons (or acids). Finally, bitter taste identifies anti-nutritional compounds and other potentially toxic molecules present in the diet. In general, the mammalian gustatory system is capable of providing a nutritional assessment of the quality of ingested foods and has been defined as the peripheral nutrient sensing system in mammals (Bachmanov and Beauchamp 2007; Roura *et al*. 2008a).

Why Five Tastes?

Historically taste science has recognised four "basic" qualities: sweet, sour, salty and bitter. After more than a century of reports, the discovery of specific receptors has given support to a consensus that considers umami a fifth basic taste quality. However, technological advances have continuously challenged that basic taste number to be bigger. Today the discovery of a wide array of receptors on the tongue has raised the prospect of additional taste qualities to a point that the whole taste concept seems to be under scrutiny (AChemS, 2011; Montmayeur *et al*. 2011). For example, the three lines of evidence that the scientific community currently requires to confirm "*bona fide*" a specific receptor in the genome, are not met for sour and salt tastes (Klasing and Humphreys, 2009). In turn, novel chemosensing pathways have been discovered that define an intricate web of senses taking place in the oral cavity. It includes a taste sense linked to fat (primarily free fatty acids; FFA) and also other candidate taste modalities related to water perception, starch-derived glucose polymers, calcium and carbonation among others. How these tastes mingle with other oral but non-taste food flavour attributes such as pungency, texture or temperature, is a matter of profound dissertation.

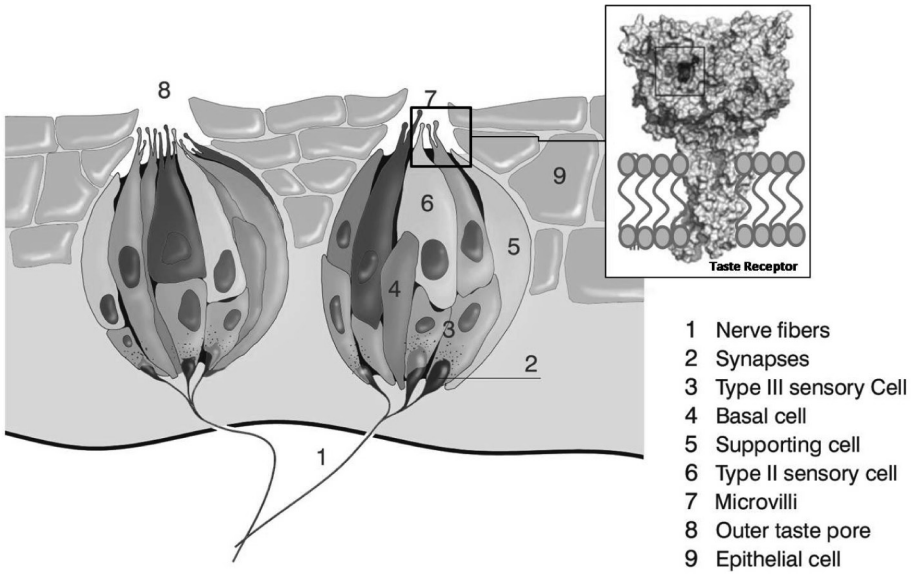

Figure 1. *Taste bud structure. The taste buds are clusters of sensory cells present in the oral cavity generally grouped in organelle-like structures referred to as taste papillae. A bud consists of groups of ca 100 sensory and supporting cells. There are at least three different taste sensory cell types: type I are sour-sensing, type II are sweet, umami and bitter sensing and type III cells play an intermediate signalling role with sensory neurons. In addition, basal cells are believed to be precursor taste sensory cells. Transmembrane taste receptors (TR) mediate the signal by recognizing ligands from food and are located in the apical cell membrane of the microvilli in type I and II sensory cells near the outer taste pore (drawing by Joaquim Roura).*

The Taste Machinery

The taste buds present in the oral papillae consist of groups of sensory cells (roughly around 100 cells per bud; Figure 1). There are at least three different functional taste sensory cell (TSC) types. Type I are sour-sensing, type II are sweet, umami and bitter sensing and type III cells play an intermediate signalling role between the true TSC (type I and II) and the sensory neurons (DeFazio *et al*., 2006). Stimulation of TSC is mediated through transmembrane taste receptors (TR). Some of the TR are part of the G protein coupled receptors (GPCR) super-family characterized by 7 transmembrane domains. The TR have been divided into two classes: T1R and T2R. The T1R is a family of three genes that code for the two heterodimeric receptors the umami composed of T1R1 and T1R3, and the sweet composed of T1R2 and T1R3 (Li *et al*., 2002). The T2R is a big family related to bitter taste sensing (Adler *et al*., 2000; Meyerhoff *et al*., 2010). The size of the T2R family differs among mammals and may range from 16 (dog) up to 37 (rat) different functional genes (Shi and Zang, 2006). The number of pig T2R is currently unknown but access to the pig genome facilitates initial estimates (http://www.ensembl.org/Sus_scrofa). For example, at least 10 different Tas2Rs sequences can be currently identified from the porcine genome databases (Table 1) but these most likely represent only a fraction of the true repertoire.

The binding of sugars and other nutrients that stimulate the G-protein TRs activate an intracellular transduction pathway (Figure 2). The transmembrane receptor is coupled with the heterotrimeric gustducin (α-gustducin, β-gustducin (Gβ3), and γ-gustducin (Gγ13)) that, in turn, activates phosphodiesterase to decrease cAMP (mediated by α-gustducin) and phospholipase Cβ2 (PLCβ2) to generate inositol trisphosphate and diacylglycerol (mediated by transducin; Gβ3/Gγ13). As a result, the extracellular Ca2+ influx and the release from internal stores activates the transient receptor potential channel TRPM5 which leads to taste cell depolarization and signalling to afferent nerves (reviewed by Margolskee, 2002). In addition, two more proteins seem to be involved in taste-related machinery: RTP3 and REEP2. The RTP3 is a transmembrane protein involved in the transport of TR to the apical membrane of sensory cells and potentially also involved in TasR gene expression (Kosiol *et al*., 2008). REEP2 is an integral membrane protein expressed in taste cells that physically associates TR dimmers and alters their spatial organization promoting receptor access to tastants (Ilegems *et al*., 2010). The existence of a repertoire of proteins specifically related to taste perception, is a very important tool to identify cell types that function as taste sensory cells. Thus, in addition to the repertoire of TR themselves, taste proteins α-gustducin, transducin, PLCβ2, TRPM5, RTP3 and REEP2 form a group of seven proteins used by the scientific community to identify taste-related sensory cells.

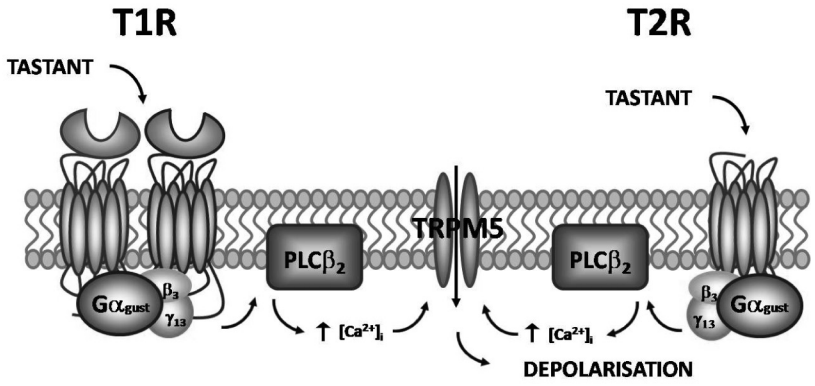

Figure 2. *Transduction mechanism in taste receptor cells for sweet and umami (T1R) and bitter (T2R) taste qualities. Sweet and umami tastants activate T1R heterodimers linked to the G-protein heterotrimer Gustducin. The activated α subunit of Gustducin via PLCβ2 (and IP3 and/or cAMP) determines the release of Ca^2+ from intracellular stores, which in turn activates TRPM5 ultimately resulting in depolarization of the cell. Similarly bitter compounds stimulate a specific receptor of the big T2R receptor family, which activate Gustducin heterotrimers (drawing by Simon Foster - School of Biomedical Sciences, The University of Queensland).*

Initial estimates from the pig genome show predicted sequences for the full repertoire of Tas1r, some of the Tas2r and most of the novel taste candidates, sour and salt receptors as well as six out of seven taste-related intracellular transduction pathway proteins. Overall, it is estimated that the family of mammalian genes documented to date to be related to taste sensing and that includes TR and TR candidates, TR pseudogenes and intracellular transduction pathway proteins, consists of more than 70 members (Table 1).

Taste in Non-Taste Tissues.

The peripheral sensing system is responsible for the identification and decoding of chemical signals from the environment that are important for survival. For example, taste and smell receptors sense the nutritional quality of foods. However, the monitoring of external substances increases once they are about to cross the boundaries of the internal milieu. Nutrients and toxicants present in food need to be scrutinized as they advance through the GIT tract undergoing digestion and absorption. Furthermore, once absorbed, nutrient levels need to be carefully assessed and information sent to the brain that, in turn, controls the hunger-satiety cycle. Luckily, the chemosensing system may have evolved to use the same nutrient sensors all the way through. Several molecules implicated in taste signalling have been found in the GIT, some of them expressed in enteroendocrine cells, providing evidence that the taste-related transduction mechanisms identified in the tongue have also a role in the gut.

Table 1. *Human taste gene repertoire[(a)], predicted pig repertoire[(b)] and their ligands.*

Taste Type	Human Gene repertoire	Pig Gene Repertoire	Ligands
Umami	hTas1R1/hTas1R3	pTas1r1/pTas1r3 (sequenced)	L-Amino acids and peptides, nucleotides
Sweet	hTas1R2/hTas1R3	pTas1r2/pTas1r3	Simple carbohydrate, D-Amino acids
Bitter	hTas2R (25 functional genes and 11 Pseudogenes)	pTas2r (10 genes)	Toxicants, drugs, anti-nutritional plant composition
Salty	ENaC (4 genes)	ENaC (3 genes)	Na
Sour	PKD1L3/PKD2L1 HCN1/HCN4	PKD2L1 HCN1	Ions
Fat	CD36, GPRs 40, 41, 43, and 120	CD36, GPRs 40, 41, 43, and 120 (predicted)	Fatty acids
Calcium	CaR	CaR	Ca and some amino acids
Amino acid	GPRC6A	GPRC6A	Amino acids
Starchy	Unknown	Unknown	Starch
Carbonation	PKD2L1	PKD2L1	CO2
Other taste candidates	GPRs 34, 84, 92 and 93	GPRs 92 and 93	Several
A-Gustducin (3 genes)	α-GNAT (3 genes) β-GNB (5 genes) γ-GNG (12 genes)	α-GNAT (2 genes) β-GNB (3 genes) γ-GNG (7 genes)	Intracellular pathway
Transporter	RTP3 and 4	RTP4	Intracellular transporter: Taste Receptors
Membrane protein	REEP2	Not predicted	
Phospholipase	PLC β2	PLC β2	Intracellular pathway: ATP/ADP
Number of genes	83	42	

[a]Human gene repertoire includes taste receptor and candidate taste receptor genes and intracellular taste transduction and transporter proteins (for a glossary of terms and nomenclature see reviews by Bachmanov and Beauchamp, 2007 and Wellendorph *et al.*, 2010).

[b]From the pig repertoire only pTas1r1 and pTas1r3 have been sequenced. The other genes have been predicted by bioinformatic tools at: http://www.ensembl.org/Sus_scrofa/Info/Index

The Diffuse Chemosensory System

All internal organs require a chemosensing system by which to test their environment. It has become clear that the TSC present in tongue's taste buds are part of a family of chemosensory cells (CCs) found also in non-lingual epithelia of endodermal origin (ie. respiratory and digestive epithelia). That system of CCs has been referred to as the diffuse chemosensory system. The CCs are characterized by a set of signal transduction components typically found in TSCs (reviewed by Sbarbati *et al*., 2009). A significant body of literature has been published particularly since 2006 uncovering some of the principles of the diffuse chemosensory system.

The CCs are widely distributed in the body suggesting that they are involved in important processes both in respiratory (Gulbransen *et al*., 2008; Deshpande *et al* 2010) and digestive systems (Rozengurt, 2006; Salmon *et al*., 2007; Mace *et al*., 2007). Within the GIT, CCs are located predominantly at the interface among different microenvironments such as the glandular ducts and the boundary between the fundic and the oxyntic mucosa of the mouse stomach (Luciano and Reale, 1992; Haas *et al*., 2007) and have been related mostly to absorptive and secretory processes (Salmon *et al*., 2007; Mace *et al*., 2007; Sclafani, 2007, Kerllett *et al*., 2008), but also to the control of the microbial population (Sbarbati and Osculati, 2006; Merigo *et al*., 2008) and the detection of irritants (Finger *et al*, 2003; Gulbransen *et al*., 2008a; Osculati *et al*., 2007).

Taste-related proteins T1R2, T1R3, Gustducin and TRPM5 were preferentially expressed in cells along the proximal small intestine (mostly jejunum and duodenum), including Paneth cells, CCs and enterocytes (Mace *et al*., 2007; Young *et al*., 2009). However, a Gustducin-positive large cluster of cells have been found in the stomach in close association with ghrelin-secreting enteroendocrine cells (Hass *et al*., 2007). In addition, α-Gustducin expressing cells have been found in human colon and pancreatic duct (Sternini *et al*., 2008).

Taste Sensors and the Enteroendocrine System

Around 1% of the cells in the GIT are specialized cells with endocrine functions generically grouped under the enteroendocrine system representing the largest endocrine organ in mammals. They produce a wide array of hormonal compounds many relevant to the control of food intake including gastrin, ghrelin, cholecystokinin (CCK), serotonin, glucose-dependent insulinotropic peptide (GIP), glucagon-like peptides (GLPs) and peptide YY (PYY; Starder and Woods, 2005). These cells are responsive to changes in the luminal contents of the gastrointestinal tract but the sensing mechanisms involved are still poorly understood. Gustducin was the first taste-related protein identified in brush border cells of the GIT (Hofer *et al*., 1996). Hass *et al*. (2007) found a large cluster of gustducin-positive cells in the stomach mucosa in close association with two populations of enteroendocrine cells: one population containing the satiety regulating hormone ghrelin, the other population comprising serotonin-secreting cells.

In recent years, the number of taste-related proteins shown to be expressed in enteroendocrine cells has increased and includes the three subunits of Gustducin, T1R1, T1R2, T1R3, TRPM5 and PLCβ2 (Sternini *et al*., 2008; Kokrashvili *et al*., 2009). In the intestinal mucosa, there are two commonly studied enteroendocrine cell lineages involved in peptide gut hormone secretion: the K (secreting GIP) and the L cells (producing GLP1). Gut K cells are sparse and difficult to study while L cells are abundant in all the gut but predominantly the ileum and colon (Jang *et al*., 2007). The two incretin peptides (GIP and GLP1) are secreted from the K and L cells after oral intake of glucose and result in an increase of insulin secretion from the pancreas. Incretins may also be involved in gastric motility (Theodorakis *et al*., 2006). Luminal glucose stimulates the taste sensors (T1r3 is involved) of the enteroendocrine system (L-cells) initiating a paracrine response involving GLP1 (Jang *et al*., 2007; Margolskee *et al*., 2007). The incretin effect of GLP1 explains why oral glucose ingestion is more effective in raising insulin levels than intravenous administration of glucose (McIntyre *et al*., 1964). T1r3 and α-Gustducin knockout mice failed to release GLP1 in response to glucose within the gut lumen and that led to an abnormal insulin response and prolonged elevation of postprandial blood glucose (Jang *et al*., 2007). The luminal sensing of non-caloric sweeteners resulted in similar incretin responses. In fact, Mace *et al*. (2007) proved that in both an heterologous cell system and rat intestine, simple sugars and artificial sweeteners act synergistically through a T1R2+T1R3–α-gustducin–PLCβ2 pathway to activate glucose transport. There are two pathways of intestinal glucose absorption. At low concentrations the predominant pathway is the active absorption mediated by the Na+ glucose cotransporter (SGLT1). At high concentrations, such as after a meal, glucose transporter GLUT2 provides additional glucose transport capacity and becomes the major pathway of absorption. In addition, taste-receptor (T1R1) mediated responses to L-AA (umami) in the gut is integrated in an energy network involving both sugar and amino acids sensors and transporters (Mace *et al*. 2009) as a function of the nutritional status.

The scope of TR involvement in the enteroendocrine system goes beyond the T1R family and the small intestine. Complex carbohydrates, such as fibre and resistant starch, are known to be fermented in the colon in humans where they are metabolized into short-chain fatty acids (SCFA) that, in turn, contribute to an additional energy supply. Karaki

et al. (2008) showed that the SCFA receptor (and candidate fat taste receptor) GPR43 is expressed in rat intestine and in enterocytes and enteroendocrine cells in the human colon. Novel TR candidates for fatty acids (GPR41 and GPR43) have been reported in enteroendocrine L-cells of human colon apparently responding to increased availability of the SCFA butyric (Al-Rammahi *et al*., 2011). Other TR or candidate TR reported to be expressed in GIT include amino acid, fatty acid and calcium sensors (reviewed by Wellendorph *et al*., 2010). In addition, T2Rs (bitter) have also been observed in non-taste tissues.

Bitter Taste Sensing in the Gut

Bitter taste identifies anti-nutritional compounds, drugs and toxins present in the diet. The immediate result is a decrease in the food ingested. However, many of the undesired substances make it through the oral cavity and into the GIT where chemosensors will trigger gastrointestinal defense mechanisms such as increased neutralizing secretions (eg. saliva), gut motility and regulation of blood flow. In addition, vomiting and food aversive behaviours might be developed as protective responses to highly deleterious compounds. The enteroendocrine cells of the GIT seem to play a pivotal role on these defence mechanisms that may involve bitter sensors (Sternini *et al*., 2008).

Functionality of bitter-taste receptors outside the tongue was first provided by bitter agonists inducing an increase in intracellular Ca2+ in enteroendocrine cell lines expressing bitter-taste receptors and G α-proteins (Wu *et al*., 2002). Chen *et al*. (2006) observed how bitter agonists induce release of CCK (an anorexic hormone) in intestinal endocrine cell lines. In addition, multiple T2R and α-Gustducin transcripts have been detected in the mucosa of the mouse and rat gastrointestinal tract and human colon in some cases colocalized with PYY, GLP1 or CCK (Rozengurt *et al*., 2006).

Glendinning *et al*. (2008) reported that intragastric infusions of a known bitter agonist (denatonium benzoate) delayed gastric emptying in rats. Furthermore the inhibition of gastric emptying by bitter agonists might be directly mediated by T2R as opposed to what had been previously speculated regarding a mediation of CKK and/or GLP1 (Janssen *et al*. 2011). In addition, bitter TR in human and rat large intestine evoke anion secretion (Cl- and HCO3) following bitter agonist stimulation (Kaji *et al*., 2009), a mechanism that might be important for host defence mechanisms.

Overall, the physiology behind T2R in the GIT shows promising implications related to modulation of food intake.

Changes on Taste Sensing Due to Nutritional Status

Hedonic responses to taste stimuli decrease as food is ingested following a progressive decrease in the sensitivity of the brain reward circuit (Morton *et al*. 2006). The chemosensory system is subjected to adaptive regulation to the nutritional status of the animal. Taste sensory cells in taste buds are responsive to anorectic (leptin and CCK) and orexigenic (orexins) hormones (Hoppe *et al*. 2006). For example, sweet taste sensitivity is mediated by leptin levels. Gene expression level of the sweet taste receptor Tas1r3 was significantly decreased in high fat diet-induced obese rats confirmed by a low saccharine intake. In contrast, diet-restricted rats had decreased serum leptin concentration and showed enhanced consumption and preference for saccharin (Chen *et al*., 2010). However, the gene expression of the leptin receptor was markedly increased in the taste buds. It is interesting to note that an acute fasting event in rats caused a marked decrease in the gustatory threshold possibly related to the effects of hunger on taste receptors (Zverev, 2004).

The expression of the taste system in non-taste tissues is also affected by nutritional status and nutrient availability. Glucose and amino acids in the luminal content determine the trafficking of taste receptors (T1Rs) and sugar transport proteins (SGLT1 and GLUT2) to/from the apical cell membrane (Mace *et al*., 2009). The expression of T1R2, T1R3, TRPM5 and α-Gustducin in the upper gastrointestinal tract were inversely correlated with blood glucose concentration in Type-2 diabetes subjects. Transcript levels of T1R2 were reduced by 84% following jejunal glucose perfusion in mice (Young *et al*., 2009). It appears that the expression of the nutrient sensing machinery mediated by the taste receptors is responsive to the nutritional status and the availability of nutrients in the gut lumen. That is part of an integrated network that modulates absorption of sugars, peptides and amino acids with the final aim of efficiently preserving energy homeostasis (Mace *et al*., 2009).

The Sense of Taste in Pigs

Pigs and cows with around 20,000 taste buds (Figure 1) in the oral cavity have the highest known number of these organelles among mammals, at least three times more than humans (Roura *et al*., 2008a). Assuming that the number of taste buds is positively correlated with taste acuity (Miller and Reedy, 1990) we might consider pigs and cows as the highest tasters in the mammalian family.

Sweet and Umami Taste in Pigs

Sweet and umami sensors in the pig are similar to the other known mammalian species in that they involve heterodimeric receptors one specific umami (pT1r1) and one specific sweet (pT1r2) with a common coupling unit (pT1r3) for both tastes (Humphrey *et al*. 2009; Roura *et al*. 2008a,b). Simple carbohydrates stimulate sweet taste in pigs. However, high intensity sweeteners known to humans and that are often used in piglet diets (Sodium Saccharin, Thaumatin and Neohespiridine dehydrocalcone) only trigger minor sweet taste responses in the pig tongue (Danilova *et al*., 1999; Glaser *et al*., 2000). Even though they do not seem to be effective in increasing feed intake they may however play a key role in carbohydrate digestion/absorption by stimulating the sweet taste receptors (TR) present in the gastrointestinal tract (GIT) as will be discussed later.

In humans sweet perception has become a paradigm of "good taste" easy to sense and identify while umami remains largely as an unknown taste modality to the general public possibly because it may not be easy to distinguish from other tastes (ie. salty) or flavours (meaty). However, humans have a detection threshold for MSG that is around 10 fold lower than for sugar (around 1mM and 10 mM, respectively). Several L-AA, such as glutamic acid (L-Glu) or monosodium glutamate (MSG), trigger umami taste and they seem to enhance voluntary feed intake in pigs (Roura and Tedo, 2009). Preliminary results from our group show that pig preference threshold for MSG is around one to 10 mM and for sugar is around 10mM (Roura *et al*., 2011b). Furthermore, pigs show a positive preference not only to MSG (and L-Glu) but also to other amino acids not perceived as umami by humans such as glutamine, alanine and asparagine (Table 2). Similarly, the umami TR in laboratory rodents is widely tuned and identifies almost the full repertoire of L-AA (Nelson *et al*. 2002). In general these observations suggest that mammals have a higher acuity of taste in detecting amino acids than sugars and a potential higher appetite for dietary protein compared to carbohydrates.

Table 2. *Gustatory responsiveness of pigs to L-amino acids and predominant hedonic response in humans (adapted from Roura and Tedo, 2009).*

L-Amino Acid	Human Taste	Pig Response[1]
Alanine	Sweet	Umami
Arginine	Bitter	Umami
Asparagine	Bitter	Umami
Aspartic acid	Umami, sour	Umami
Cysteine	Sulphur	NA
Glutamic acid	Umami, salty	Umami
Glutamine	Sweet, umami	Umami
Glycine	Sweet	Yes
Histidine	Bitter	No
Hydroxyproline	Sweet	Yes
Isoleucine	Bitter	No
Leucine	Bitter	No
Lysine	Bitter, salty, sweet	Yes
Methionine	Bitter, Sulphur, umami	No
Phenylalanine	Bitter	No
Proline	Sweet, salty	Umami
Serine	Sweet	Yes
Threonine	Sweet	Umami
Tryptophan	Bitter	Bitter
Tyrosine	Bitter	NA
Valine	Bitter	No

[1]Pig response: NA, not available; Yes, means that there is a response but the type of taste has not been identified; No, means no response.

Characterization of the porcine heterodimer umami TR (pT1r1/pT1r3) showed that the pT1r1 protein contains 844 amino acid residues, the first 571 accounting for the extracellular domain including the ligand binding pocket (Humphrey *et al*. 2009; Roura *et al*. 2008b). Ten different amino acid residues are critically involved in agonist recognition all conserved, both type and location, between pig and human (Figure 3) but only eight of the amino acids are conserved in mouse and rat sequences. The results of the computer model comparing the T1R1 ligand binding domains in humans, pig and rat offer a good mechanistic explanation regarding the differences with *in vivo* L-AA preferences (Roura *et al*., 2011a).

The existence of umami TR in TSC as part of the taste buds is geared towards sensing the protein quality and content of the diet. Under protein or general malnutrition (such as at weaning) these taste receptors are over-expressed as part of the response that increases the craving for protein rich diets in piglets (Tedo *et al*., 2011). During these critical periods, therefore, stimulating the umami taste may result in significant increases in feed intake.

The Bitter Taste in Pigs

The mammalian bitter gene family (Tas2r) show a strong adaptive response to the environment through gene inactivation or pseudogenization. The size of the family ranges from 16 (dog) up to 37 (rat) different functional genes among the reviewed mammalian genomes (Shi and Zang, 2006).

The number of pig Tas2r genes is currently unknown but is estimated to be within that same range. It has been reported that pigs elicit avoidance responses to antibiotics and quinine HCl, denatonium benzoate and caffeine among other compounds (Blair and FitzSimons 1970; Danilova *et al*. 1999; Nelson and Sanregret, 1997). However, the sensitivity of pigs to denatonium (in the milimolar range) seems much lower than that of humans (in the nanomolar range). In contrast, relative to ruminants, pigs might be four times more sensitive to dietary glucosinolates from rapeseed meal (du Toit *et al*. 1991). Such large variation in number and functionality of bitter receptors might be linked to the adaptation to different diets and digestive strategies.

The Sour and Salty Tastes in Pigs

Sodium (salty) and protons (sour) may penetrate cell membranes through ion or ligand gated protein channels or other mechanisms that include potential intracellular targets. The epithelial sodium channel ENaC is a trimeric receptor, composed of three subunits (α,β,γ), highly selective towards sodium (Lin *et al*. 1999) and can be identified from the porcine genome database (Table 1). Sour taste is known for acid perception and is sensed by hydrogen gated channels (recently PKD1L3 and PKD2L1 have gained support as candidate sour receptors -Ishimaru *et al*. 2006). However, only a moderate correlation exists between the hydrogen ion concentration of a food and its perceived sourness. Weak organic acids such as acetic, propionic, formic and lactic are able to penetrate cell membranes in an undissociated form and then dissociate inside the taste cell. In contrast, acidifying the neuronal cytosol of the trigeminal nerve in the oral cavity causes the stimulation of the nociceptor TRPA1 leading to pungency and pain. However, strong acids such as Citric and Tartaric, were inert at a trigeminal level resulting in no or insignificant pungency responses (Wang *et al*., 2011). Similarly, in electrophysiological studies in pig taste nerves performed by Danilova *et al*. (1999) citric acid elicited the largest response among a wide group of taste agonists.

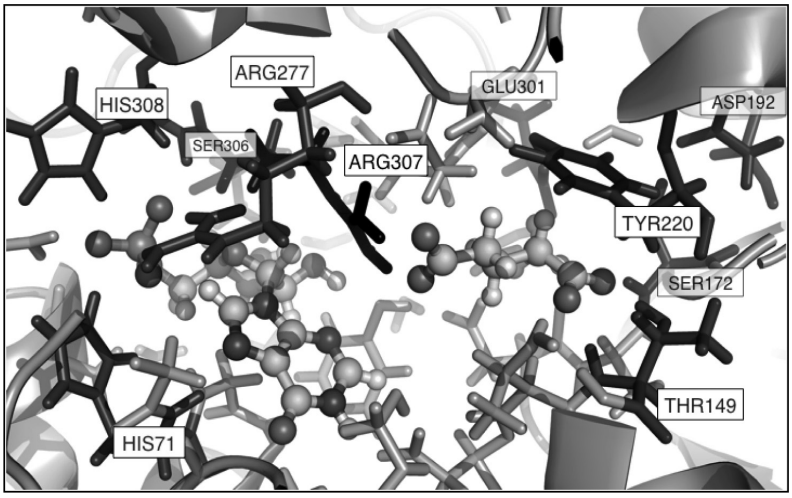

Figure 3. *Computational model indicating the 10 amino acid residues believed to be directly inolved in the ligand binding domain of the pig pT1r1. The compounds represented with spheres represent the two ligands Glutamic acid (interacting with amino acids residues of ARG307, GLU301, ASP192, TYR220, SER172 and THR149) and the nucleotide GMP (interacting with HIS71, HIS308, SER306, ARG277 and ARG307). Source: Roura et al. (2011).*

Taste in Non-Taste Tissues in Pigs

Our understanding of the peripheral chemosensing systems and the identification of the first TR came along with the availability of animal (and human) genomes at around the turn of the millennium. Unfortunately, the pig genome only became available in 2009. In the subsequent two years there has been limited progress in identifying pig TR repertoire and functions. However, some advances have been made that illustrate some of the potential implications of the taste machinery in the porcine GIT.

In a first publication related to porcine TR, high expressions of pTas1r3 were reported in tongue circumvallate papillae, fungiform papillae, mucosal epithelium, lymphocytes and spermatogenic cells by polymerase chain reaction and *in situ* hybridization (Kiuchi *et al*., 2006). In recent years, additional evidence related to the expression of pTas1r3 in the GIT has been presented by others (Moran *et al*., 2010a,b). Moran and co-workers additionally, showed that T1R2, T1R3 and α-Gustducin were co-expressed in the enteroendocrine cells of piglet intestine together with the gut incretin hormones GLP1 and GIP (from L and K cells). The authors suggested that the increased expression of glucose transporter SGLT1, which resulted in an increase in glucose absorption, was mediated by the T1Rs. It is interesting to note, however, that phytic acid reduced the SGLT1 gene expression in the duodenum, jejunum, and ileum in young pigs (Woyengo *et al*., 2011) but the mechanism is unknown.

The heterodimeric porcine umami TR (pT1r1/pT1r3) has been the first porcine TR to be sequenced, cloned and fully characterized (Humphrey *et al*. 2009; Roura *et al*. 2008b). A computational model of the ligand binding domain of the pig pT1r1 has been developed (Figure 3) and compared to the broadly tuned rat T1r1 and the narrowly tuned human T1R1 (Roura *et al*., 2011a). In addition, Tedo *et al*. (2011a,b,c) reported that the three porcine Tas1r genes are significantly expressed in tongue papillae but also in stomach, ileum, jejunum, duodenum and liver. The degree of expression (measured as mRNA abundance) of the three genes was age dependent but there were no differences due to gender. In addition, under protein or general malnutrition (such as at weaning) these taste receptors are over-expressed as what is believed to be part of the response that increases the craving for protein rich diets in piglets. Similarly, α-Gustducin in pigs was identified by immunohistochemical analyses and was present in tongue papillae and several GIT tissues. The density of α-Gustducin-positive taste buds have been reported to increase under low dietary protein (Suarez *et al*., 2011a). Lastly fatty acid sensors, and candidates for fat taste receptors GPR41 and GPR43, have been reported in enteroendocrine L-cells of pig colon apparently responding to increased availability of short chain organic acids (eg. butyric) released after fibre and resistant starch fermentation (Al-Rammahi *et al*., 2011).

The role of TR in non-taste tissues in mediating the hunger-satiety cycle is gaining momentum. The knowledge related to pig science is far behind that of other species and warrants further investigation given the potential implications related to nutrient digestion, absorption, optimization of essential nutrient requirements and above everything else feed intake.

Taste Receptor Biology and Feed Intake in Pigs

All domestic ungulate animals are ruminant or non-ruminant herbivores except pigs. Pigs in the wild have adapted to an omnivorous diet eating fruits, tubers, grass, leaves, roots, grains but also insects, worms, frogs, toads, mice, eggs, chicks and animal remains. The number and functionality of taste receptors is likely to have evolved to adapt to different diets and digestive strategies that are important to applied pig feeding practices.

Bitter Feedstuffs and Feed Intake

The family of bitter taste receptors in some herbivores, particularly ruminants, have a high degree of pseudogenization meaning that they have lost functionality (Shi and Zhang, 2006). A high fermentation capacity in their GIT (ie. rumen) may help herbivores deal with toxic plant derived compounds so that they are no longer a health threat (du Toit *et al*. 1991; Hill and Tamminga 1998). In contrast, omnivores such as laboratory rodents, opossums or humans, have the highest number of functional bitter taste receptors among mammals. Pigs are four times more sensitive to dietary glucosinolates from rapeseed meal than cows (du Toit *et al*. 1991). However they seem to adapt rapidly to non-harmful bitter tastants as reported with denatonium benzoate by Blair and FitzSimons (1970). When pigs were offered a diet supplemented with denatonium, the feed intake dropped significantly but pigs recovered their level of consumption only after a few days. Bitter tasting feed additives commonly used in pig diets may include some plant extracts (Windisch *et al*., 2008) and drugs (Nelson and Sanregret, 1997). Under practical circumstances there are many feed ingredients that contain plant derived anti-nutritional factors that are known to taste bitter (Table 3). Tannins, alkaloids, glucosinolates and saponins reduce feed palatability and intake in pigs. However, other anti-nutritional compounds such as trypsin inhibitors, lectins and polysaccharides have a direct impact on gut functions and growth but not necessarily affecting intake (Clasadonte and van der Poel, 2009). How these compounds might be seen by the taste machinery in the GIT and the implications on gut motility and digestive secretions are subjects for future research.

Taste of Protein Sources and Feed Intake

Sola-Oriol *et al*. (2011) recently published a systematic study showing that the protein sources from animal origin had the highest preferences (preference over a reference feed is expressed as a per cent ratio of the consumption of the test feed over the total consumption). Every 1% change in the inclusion of high quality proteins resulted in an average increase of 5.3% over the 50% neutral preference value. In contrast, 1% changes in inclusion of highly preferred cereals, fibers or fats resulted in increases of preference values of 1.5, 1.5 and 0.6% respectively, indicating that protein sources may have a much higher relative impact on feed preference per unit of feed ingredient than cereals, fats, or fiber sources. In addition, Tokach *et al*. (2003) reviewed the ingredients showing a direct positive impact on feed intake in piglets finding dried whey and other lactose and carbohydrate sources, whey protein concentrate, spray-dried animal plasma and blood meals, dried porcine solubles and high quality fish meal. All these ingredients are void of plant derived anti-nutritional factors and contain a significant amount of sweet and umami active compounds (Table 3). Overall, pigs seem to have higher taste acuity for amino acids and peptides than sugars and a potential higher appetite for dietary protein compared to carbohydrates.

Taste of Cereals and Feed Intake

Cereals account for more than 60% of porcine diets and play a fundamental part in dietary appetite. Feed preferences in pigs have been directly related to the starch content and *in vitro* glucose release of the main cereal (Sola-Oriol *et al*., 2008a). Glucose release is higher in small starch granules (Tester *et al*., 2006) such of rice starch, and may result in stimulation of the sweet taste receptor both in the oral cavity and further down the GIT. Abundance of glucose in the luminal content of the intestines stimulates glucose absorption through the T1R2-SGLT1-GLUT2 system (Moran *et al*., 2010b; Mace *et al*., 2007) and results in activation of the enteroendocrine axis involving the release of incretins GLP-1 and GIP (Jang *et al*., 2007; Margolskee *et al*., 2007). For example, Sola-Oriol *et al*. (2008b) reported that pig preference for rice was higher than for corn. Recently, pigs fed rice had higher glycemic index (GI), increased glucose absorption and a greater and longer serum insulin response than pigs fed corn (Menoyo *et al*., 2011). Cereals resulting in high GI may increase insulinemia causing a faster clearance of glucose in the blood and a more rapid return to a hunger state, which in turn might result in an increase in feed intake (Aston 2006; Menoyo *et al*., 2011). In contrast, van Kempen *et al*. (2007) reported conflicting results showing that a low GI might be beneficial for weanling pigs in the long term. Feed intake and feed efficiency increased up to 14% in pigs fed a diet with slow degrading starch compared to a diet with fast degrading starch.

Table 3. *Preference values (%) of different protein sources at 50, 100 or 200 g/kg of inclusion (Sola-Oriol et al., 2011) in pigs and their taste active compounds (adapted from Roura et al., 2008).*

	Inclusion rate			Taste Active Compounds	
	50[1] or 100 g·kg-[1]	200 g·kg-[1]	Pooled SE	Unpleasant	Pleasant
***Dried porcine hydrolysed protein**[*1]*	*76.3**/61.2[ab]	**32.0[bcd] ***	7.46	Very high Na content (6.0%)	Free amino acids (AAs)
*Fishmeal**	*72.5[d] **	66.2[a]	8.67	-	AAs and oligopeptides
*Raw lupin**	*69.9[a] **	46.9[ab]	6.47	Alkaloids	Simple CHO
*Soybean meal-44**	*69.4[a] **	55.3[ab]	7.99	Saponins, glicinine, conglicinine.	Simple CHO
Extruded soybeans	67.9[a]	53.0[ab]	8.05	Saponins, glicinine, conglicinine.	Simple CHO, AAs and oligopeptides.
Soybean meal-48	61.9[ab]	54.1[ab]	6.44	Saponins, glicinine, conglicinine.	Simple CHO
*Dried skimmed milk**	*58.2[abc] **	49.1[ab]	3.84	-	High lactose and oligopeptides
Spray-dried porcine plasma	57.1[abc]	43.1[abc]	10.33	-	AAs, oligopeptides and simple CHO
Sweet milk whey[1]	54.2/41.2[abcd]	39.0[abcd]	9.84	-	High Lactose
Soybean protein[1]	52.5/41.5a[bcd]	**32.0[bcd] ***	6.09	Saponins, glicinine, conglicinine.	Simple CHO, AAs and oligopeptides
Sunflower meal*	40.1[abcd]	**9.0[d] ***	5.93	Chlorogenic acid	Simple CHO
Rapeseed meal*	**30.2[bcd] ***	**10.2[cd] ***	4.00	Glucosin, Sinapine, Tannins	Simple CHO
Acid milk whey*	**22.6[cd] ***	**24.2[bcd] ***	11.07	Lactic, Sulphur, Chloride acids	High Lactose
Potato protein*	**16.5[d] ***	**7.6[d] ***	2.55	Alkaloids (solanin)	-

[abcd]Values in the same column with different letters are significantly different (P < 0.05); n = 9. [1]Preference values are expressed as the per cent (%) contribution of the tested diet to total feed intake. [2]Protein based diets were prepared by replacing variable amounts of SBM-56 from the reference diet. [3]Two-way choice of pure protein sources were performed using pure SBM-56 as reference. *Asterisk and Italics underlined*: indicates the ingredients with preference values significantly (P<0.05) higher than 50%. **Asterisk and Bold**: indicates the ingredients with preference values significantly (P<0.05) lower than 50%; SE, standard error; CHO, carbohydrate.

Taste of Feed Additives and Feed Intake

Low inclusion ingredients (ie. feed additives) have often strong effects on feed intake that may involve taste. It has been mentioned earlier in this paper that plant extracts and drugs (eg. antibiotics) are bitter to pigs (Nelson and Sanregret, 1997; Windisch *et al*., 2008). But more common is the use of sweeteners and acidifiers as feed additives in piglet diets. Pigs perceive the taste of simple carbohydrates (ie. sugars) to a similar extent than humans do (Danilova, 1999, Glaser *et al*. 2000). Sucrose, lactose, or D-glucose, have been known to increased feed intake and weight gain in post-weaned piglets at levels of around 5% or more (Lewis *et al*., 1955; Salmon-Legagneur and Fevrier, 1956; Munro, 2000). In contrast, sweeteners defined as high intensity in humans may not be equally intense to pigs (Danilova, 1999, Glaser *et al*. 2000). Dietary addition of stevia and saccharin (SAC) and neohesperidin (NEO) did not result in significant performance increase in weanling piglets (Munro *et al*., 2000; Sterk *et al*., 2008). No positive evidence has been reported either in the scientific literature regarding other commonly used sweeteners in piglets such as thaumatin. However, porcine sweet TRs have been involved in up-regulating SGLT1 and glucose uptake after stimulation with sucrose, SAC and NEO in piglet intestinal mucosa (Moran *et al*. 2010b). If these findings were confirmed we could speculate that HIS is involved in improving glucose uptake but also to initiate an orexigenic response through GLP1, thus potentially enhancing feed intake in piglets through post-ingestive events. The use of SAC, thaumatin and/or NEO maybe justified in the light of their potential post-ingestion effects.

It may not be a coincidence that citric acid is the most common of the acids found in fruits, tubers and vegetables followed by malic and tartaric (Table 4). Recently, Suarez *et al*. (2010) reviewed in a systematic manner, how the addition of organic acids and some of their salts to feed affected pig preferences. The results showed that, at 1% inclusion level, citric and tartaric acids significantly improved feed preferences while all the other acids tested either did not have an effect or elicited avoidance responses. For example, acetic, phosphoric and formic acids resulted in aversive responses. In trials performed under more practical conditions, formic acid and combinations of formic and propionic acids have consistently been reported to decrease feed intake (Eisemann and van Heugten 2007; Ettle *et al*. 2004). In contrast, the potassium salt of formic acid did not show any sign of aversion what may partially explain the increasing market use of these salts. Based on palatability issues, weak acids such as acetic, phosphoric and formic should be avoided in piglet diets unless presented in the form of salts or coated, while the use of citric acid poses no appetite threats.

Among other acids relevant to mammalian nutrition, ascorbic acid deserves some attention from a chemosensing perspective. In an electrophysiological study performed using the taste sensing Chorda Tympani nerve in pigs, a 40mM solution of ascorbic acid gave one of the strongest responses only second to citric acid (Danilova *et al*., 1999). It has been inferred, but never proven, that ascorbic acid stimulates sour sensors. However, some amino acids such as L-Glu and D-Tryptophan (D-Trp) do not activate the sour receptor but the umami and sweet taste receptors respectively. All pig diets in modern husbandry are balanced with the addition of synthetic essential amino acids mainly Lysine (Lys), Methionine (Met), Threonine (Thr) and Tryptophan (Trp).

Table 4. *Organic acids in fruits and vegetables (sources: Belitz* et al.*, 2009; Baillot* et al.*, 1987; Adel et al., 2005).*

Citric	Fruits	Apple, ***Apricot, Bananas,*** Bilberry, ***Blueberries, Boysenberries,*** Cherries, ***Cranberries, Currants, Elderberries, Figs, Gooseberries, Grapefruit,*** Grapes, Kiwifruit, Kumquat, ***Lemons, Limes,*** Loganberry, Orange Peel, Orange, Peaches, Pears, ***Pineapples, Raspberry, Strawberries,*** Tangerine, ***Youngberries, Guava,*** Kiwi, Papaya, Nectarine
	Vegetables	***Beans, Broccoli, Carrots, Potatoes, Rhubarb, Tomatoes***
Malic	Fruits	***Apples, Apricots, Bananas, Blackberries,*** Blueberries, Boysenberries, Cherries, Cranberries, Currants, Elderberries, Figs, Gooseberries, Grapefruit, ***Grapes,*** Lemons, ***Limes,*** Loganberry, ***Nectarine, Orange peel,*** Orange, ***Passion fruit, Peaches, Pears, Pineapples,*** Rosehip, ***Quinces,*** Strawberries, Youngberries, ***Plum, Kiwi,*** Papaya
	Vegetables	***Beans, Broccoli, Carrots, Peas, Potatoes, Rhubarb, Tomatoes***
Tartaric	Fruits	***Avocados,*** Bananas, Cherries, ***Currants,*** Grapefruit, ***Grapes,*** Lemons, Limes, Pears, Plum, Mango
Ascorbic	Fruits	Guava, Pawpaw, Strawberry, Mango, Pineapple, Cantaloup, Banana, Peach, Apple, Grape, Plum, Kiwi
	Vegetables	Rhubarb
Fumaric	Fruits	Apples
	Vegetables	Bean, Carrots, Mushrooms, Tomatoes
Oxalic	Fruits	Blackberries, Grapefruit, Strawberries, Grapes, Lemons, Limes, Orange Peel, Orange, Apples, Plum, Banana, Pear, Cantaloup, Pineapple
	Vegetables	Beans, Broccoli, Potatoes, Rhubarb, Tomatoes
Succinic	Fruits	Apples, Blueberries, Cherries, Currants, Strawberries
	Vegetables	Beans, Carrots, Tomatoes
Acetic	Fruits	Banana
Formic acid	Fruits	Banana
Lactic	Fruits	Apple

Fruits and vegetables with significant quantities of the acid are shown in ***bold italics***. Fruits and vegetables in plain format only contain traces of the acid. The relative amount of each acid may change widely with the fruit variety, degree of ripeness and seasonal influences

Pigs are able to distinguish and prefer diets better balanced for Lys, Met, Thr and Trp to the same diet deficient in the corresponding amino acid (Ettle *et al*., 2006; Ettle and Roth, 2004, 2005, 2009). Arguably, as proposed by the researchers, the driver for preference might have been a non-taste dependent mechanism of craving for a nutritionally balanced diet. In addition, the same group found evidence for Met preference above optimal growth requirements and linked to the Met-source type where DL-Met was preferred over Methionine Hydroxyl Analog (MHA). In spite of a subtle denial from the authors, taste perception seems to be a plausible explanation of the outcome of their research. For example, in two minute double choice tests, 50mM solutions of Lys and Met were preferred over plain water in piglets (Tedo *et al*., 2010) while Trp was significantly rejected proving that the taste component in some amino acids is strong. In the same experiments, MSG, L-Glu, L-Glutamine (Gln) and L-Alanine (Ala) resulted in high preferences as well. The results on Gln and Ala are consistent with previous findings (Tinti *et al*., 2000). In addition, Tinti and co-workers also identified positive preferences for L-Asparagine, L-Hydrorxyproline, L-Serine and Thr. Preferences in young pigs for Met, Thr and Trp have also been recently reported by Suarez *et al*. (2011b,c,d) as a function of a nutritional status (deficient, adequate or in excess) of the amino acid being assessed. Results indicate that the preference for Met and avoidance of Trp where independent of the nutritional status again demonstrating that taste responses are not necessarily driven by nutritional needs. However, the preference for Thr developed only after the consumption of the excess treatment, evidence that more than one mechanism co-exist related to amino acid preferences in pigs. The multiple mechanisms related to amino acid linked appetite might be related to the different receptors known to be involved in mammals (Table 1).

Conclusions

There are five primary tastes recognized today in mammalian species but candidate taste receptors for fat, calcium or water are being studied and some might be included as novel taste types in the future. Taste receptors are nutrient sensors present in a diffuse system throughout the body that contributes in guaranteeing homeostasis. Umami and sweet (T1r1/T1r2/T1r3) receptors are present in the porcine GIT and seem to be involved in nutrient digestion and absorption. Taste receptors in pigs are relevant to feed intake through nutrient sensing of feedstuffs. Feed ingredients rich in tannins, alkaloids, glucosinolates and saponins reduce feed palatability and intake in pigs. In contrast, ingredients that are void of plant derived anti-nutritional factors and contain a significant amount of sweet and umami active compounds have the highest rates of preference. Among commonly used feed additives in pig diets, sugars (but not HIS), citric acid and some amino acids exert a positive impact on feed appetite that nutritionists should be aware of in order to optimize feed intake in critical phases such as the weaning. Overall, pigs seem to have high taste acuity and the impact on feed intake is a combination of peripheral (oral cavity) and GIT luminal sensing as part of the enteroendocrine system that, in turn, orchestrates the hunger-satiety cycle.

Summary of Potential Outcomes

Emerging evidences of the relevance of the taste sensing mechanisms in the digestive system have been outlined. Current knowledge warrants further research in a number of topics that may significantly modulate/change the classical view on several issues relevant to pig nutrition:

a) Amino acids. Do we understand the interactions between dietary amino acids and their multiple sensors in the GIT? Dietary implications?

b) Energy. The sensors for simple carbohydrates and non-essential amino acids seem to cross talk at intestinal level. Can we decode their talks? Is the enteroendocrine system listening?

c) Is umami (amino acids) prevalent over sweet (carbohydrates) in driving pig appetite?

d) Bitter sensors: a big family of genes with potentially big implications in feed intake.

e) Fat. What and how sensors read dietary fat and how they integrate with the rest of nutrients?

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Physiological and Metabolic Regulation of Feed Intake

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Abstract

Evidence is presented showing there is a close relationship between voluntary energy intake and energy expenditure in animals. The reduction in feed intake in modern pig genotypes has been argued by some people to be limiting the rate of protein deposition, whereas others believe the fall in intake is a consequence of a reduction in energy deposition as pigs were bred to be leaner. Until recently, it has not been possible to distinguish cause from effect in the association between energy intake and energy use. However, increased knowledge over the past decade shows that animals closely monitor their short term energy status and the degree of adiposity. Two systems (adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR)) continuously monitor the immediate energy status of an animal through the ratio of adenosine monophosphate (AMP):adenosine triphosphate (ATP). In addition, insulin and leptin monitor the lipid content of the body and ghrelin the leanness of the animal. These hormones interact with AMPK and mTOR, which stimulate responses in peripheral tissues and in the melanocortin system of the hypothalamus. Within the melanocortin system, AMPK controls malonyl-CoA concentrations, which regulates the expression of orexigenic or anorexigenic peptides that act through higher centres of the brain to stimulate a sensation of hunger or satiety.

In many situations experienced by pigs raised commercially, the capacity of the individual to consume sufficient energy for optimal energy metabolism is limited by various dietary, climatic, disease and social constraints. The impact of these constraints and possible mechanisms through which they operate are described. One important constraint requiring greater understanding is how variation in the physical nature of indigestible fibre and the source of other nutrients interact to alter rate of passage of digesta to increase intake with the negative effects of the intestinal brake that reduces intake.

Introduction

Strong evidence indicates that animals eat to fulfill their metabolic capacity to utilise energy. However, in many situations experienced by animals, this drive is overridden or changed by other physiological, metabolic and/or psychological constraints. These constraints can be related to the physical and chemical characteristics of a diet; climatic conditions including temperature, humidity, air speed and day length; genetic or exogenous manipulation of the propensity to deposit fat and protein; infectious diseases and immune responses; and social interactions between animals.

This paper first provides evidence showing that the capacity of an animal to utilise energy is the primary regulator of feed intake. The mechanisms through which this regulation occurs are briefly outlined. Examples are then given illustrating how various dietary, environmental, social and disease factors modify either the propensity to utilise energy or the ability of the animal to meet its metabolic energy demand and therefore voluntary feed intake. Finally discussed are the mechanisms through which some of these modifications to feed intake occur, whereas others require further experimentation and understanding before the mechanisms can be fully elucidated.

Intake Determined by Energy Utilisation

Energy intake for a range of animal types from rats to humans and pigs is closely related to total energy demand as determined by rate of energy deposition and energy required for maintenance of basal body functions (Figure1; R^2=0.999 for the relationship between energy intake and energy utilised). Similarly, energy intake by juvenile Baltic salmon is closely related to changes in water temperature which are associated with differences in metabolic rate and are reflected in changes in total energy deposition (Table 1). The results are from two different experiments (Koskela *et al*., 1997a, 1997b). The optimum temperature for growth was predicted to be approximately 18°C with intake and performance of the fish falling in warmer water. The efficiency of energy utilisation for growth also fell dramatically at the highest water temperature, suggesting that energy was being used for non growth functions (R^2=0.98 for the relationship between energy intake and energy deposited by Baltic salmon up to their optimum temperature).

Further evidence showing that energy intake of animals is closely related to their capacity to utilise energy comes from the experiment of Campbell *et al*. (1990) where exogenous porcine growth hormone was administered to pigs of different sex and genotype growing from 60 kg to 90 kg (Table 2). Over all eight treatments with and without growth hormone administration ($^{R}2$=0.93 for the regression relating energy intake to energy deposition). Administration of growth hormone stimulated protein deposition and growth rate of the pigs, but reduced fat deposition and total deposition of energy. When the effects of growth hormone administration were averaged, energy deposition across the four pig types was reduced by 5.6 MJ/d and energy intake reduced by 6.0 MJ/d.

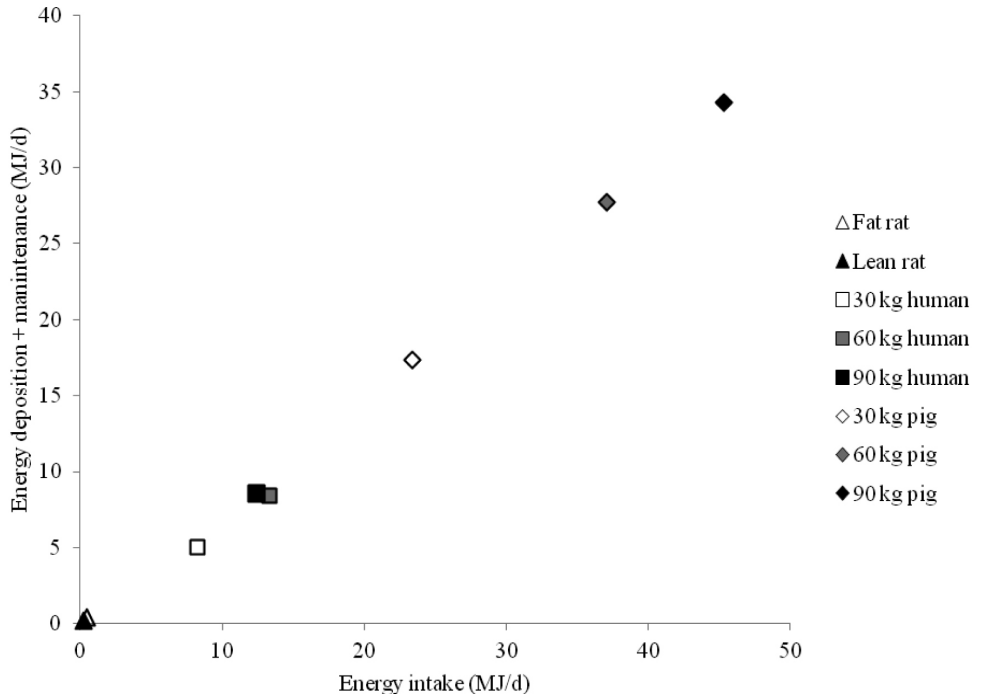

Figure 1. *Relationship across rats (Radcliffe and Webster, 1976), humans (FAO, 2001) and pigs (NRC, 1998) between energy intake and total energy demand as determined by rate of energy deposition plus basal energy requirements either as given in the references or calculated, $0.444W^{0.75}$, where W is live weight in kg (Baldwin, 1995). R^2 = 0.999 for the relationship.*

Table 1. *Effect of water temperature and size of salmon on energy intake and energy deposition during growth. Calculated from Koskela et al. (1997a,b).*

	Initial weight	Energy intake	Energy deposition	
Water temperature (°C)	(g)	(kJ/kg/d)	(kJ/kg/d)	Energy gain: intake
		Experiment - Koskela *et al*. (1997b)		
2	140.5	20.4	12.3	0.60
4	140.5	34.9	22.0	0.63
6	138.9	63.8	42.8	0.67
		Experiment - Koskela *et al*. (1997a)		
11	40.1	174.6	100.9	0.57
15	40.3	246.0	128.1	0.50
17	40.0	262.8	121.6	0.44
19	42.3	258.0	111.9	0.45
23	38.9	225.9	52.8	0.22

Table 2. *Effect of administration of porcine growth hormone on feed intake, growth performance and the deposition of protein, fat and energy for entire male and female pigs of a fast growing lean genotype (A) and a slower growing fatter genotype (B). Derived from Campbell et al. (1990). R^2=0.93 for the relationship between ME intake and energy deposition.*

Treatment	*Ad libitum* Feed intake	ME[a] intake	Live weight gain	Feed gain	Protein deposition	Fat deposition	Energy[b] deposition
	(kg/d)	(MJ/d)	(g/d)		(g/d)	(g/d)	(MJ/d)
Genotype A							
Male control	3.14	44.1	1177	2.67	162	340	17.1
Male pGHc	2.76	38.8	1519	1.82	273	134	11.6
Female control	3.05	42.9	894	3.41	119	344	16.3
Female pGH	2.63	37.0	1250	2.10	220	134	10.3
Genotype B							
Male control	3.24	45.5	992	3.27	127	375	17.7
Male pGH	2.61	36.7	1292	2.02	225	165	11.7
Female control	2.77	38.7	734	3.77	92	309	14.3
Female pGH	2.48	34.8	1080	2.30	189	126	9.3

[a] Metabolisable energy calculated from the digestible energy (DE) content of the diet assuming the conversion of DE to ME = 0.96. [b]Energy deposition was calculated from protein and fat deposition assuming the energy content of protein and fat was 23.1 MJ/kg and 39.9 MJ.kg, respectively. cPorcine growth hormone.

A final example of energy intake in pigs being closely related to energy expenditure is seen in the fall in feed intake of many pig genotypes as they have been bred over the last 30 years for increased growth rate and lean deposition and reduced fat deposition (Smith *et al*. 1991). Knapp (2009) illustrates these changes in the Swiss Landrace and Swiss Large White breeds where feed intake for growing pigs fell from approximately 2.5 kg/d in 1980 to approximately 2.2 kg/d in 2007. The greatest decline of 30-35 g/d per year in feed intake for many breeds occurred between 1980 and 1990 when the genetic changes were greatest (Knapp, 2009). The trend for increased lean deposition and reduced feed intake has continued in most pig genotypes around the world, but at a substantially slower rate than earlier.

The examples given above show only that there is an association between voluntary energy intake and the capacity of an animal to utilise energy and not that voluntary energy intake is caused by the capacity of an animal to utilise energy. Ellis *et al*. (1983), Webb (1989) and others have argued that the depression in feed intake of modern pig genotypes is a major constraint on lean growth rate and that genetic selection should be focused on increasing feed intake so the 'genetic potential' for growth can be expressed. Knapp (2009) examined this proposal and could not find convincing evidence to show that lean tissue growth in pigs reared in good environments was limited by feed intake. Alternatively, others (Black *et al*., 1986; Emmans and Fisher, 1986; Poppi *et al*., 1994; Whittemore *et al*., 1995; Nyachoti *et al*., 2004) have argued that voluntary intake is determined by the 'genetic potential' of an animal to metabolise energy, but may be limited by many environmental factors. This concept has been termed the 'potential-constraint' approach and has been used in numerous computer models simulating voluntary intake of pigs (Black, 2009).

Until recently, it has not been possible to distinguish cause from effect in the association between energy intake and energy utilisation because the mechanisms an animal uses to monitor its energy status were unclear. However, recent evidence suggests that animals continuously monitor their energy status with a high degree of accuracy and that associated biochemical mechanisms are activated within the hypothalamus to closely control both short term and long term intake (Black *et al*., 2009).

Monitoring Energy Status, Adiposity and Intake Control

There are two opposing energy-sensing serine/threonine kinase systems within the body that continuously monitor the energy status of an animal. These are the adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR), which act both peripherally and centrally within the hypothalamus, where they control feed intake. Similarly, the fat content of animals is monitored by two hormones, leptin and insulin, which also act on peripheral tissues and via the hypothalamus to alter feed intake. The body lean status of animals also is monitored by the hormone ghrelin, which influences short and long term feed intake.

Energy Monitoring Systems

Change in the energy status of the cell and the whole body of an animal causes alterations in AMPK concentrations that ensure there is adequate adenosine triphosphate (ATP) for essential metabolic pathways. AMPK is activated by changes in its phosphorylation state in response to a low ratio of adenosine monophosphate (AMP) to ATP concentration in the cell. AMPK activation is extremely sensitive to small changes in AMP, because there are three distinct biochemical pathways through which AMP promotes its activation (Hardie *et al*., 2006). AMPK is therefore activated whenever there is a decline in ATP and a rise in AMP status of a cell caused by either lack of nutrients or oxygen supply, or by excessive use of ATP such as during exercise or cold exposure (van Thuijl *et al*., 2008). Activation of AMPK inhibits anabolic ATP-consuming pathways within a cell such as gluconeogenesis and fatty acid synthesis and activates catabolic pathways that generate ATP such as glycolysis and fatty acid oxidation. AMPK stimulates glucose uptake by skeletal muscle during exercise, while it limits lipogenesis and enhances lipid oxidation.

AMPK controls lipogenesis and lipolysis through the inhibition of one enzyme, acetyl-CoA carboxylase (ACC), and the activation of another enzyme, malonyl-CoA decarboxylase (MDC), in lipid metabolic pathways. ACC is responsible for catalysing the formation of melanyl-CoA from acetyl-CoA. Malonyl-CoA becomes a substrate for fatty acid synthase (FAS) which is responsible for carbon-chain elongation during synthesis of long-chain fatty acids. Alternatively, MDC converts malonyl-CoA back to acetyl-CoA. Thus, when the energy status of an animal is low, there is a high AMP:ATP ratio and AMPK in tissues, including the hypothalamus, is activated. This activation of AMPK inhibits ACC, stimulates MDC and results in a depression in malonyl-CoA concentration. Conversely, when the energy status of an animal is high, the AMP:ATP ratio is low, AMPK concentration is low, ACC is activated, MDC is inhibited and malonyl-CoA concentrations rise. The high concentration of malonyl-CoA also results in an increase in energy expenditure through increased expression of skeletal uncoupling protein 3 (Cha *et al*., 2003).

Malonyl-CoA has its effects on feed intake by changing the expression of neuropeptides produced by the hypothalamic melanocortin system. Low concentrations of malonyl-CoA in the hypothalamus stimulate the expression of the orexigenic peptides, neuropeptide tyrosine (NPY) and agouti-related peptide (AgRP), and decrease expression of the anorexigenic peptides, pro-opiomelanocortin (POMC), its derivative α-melanocyte-stimulating hormone (α-MSH) and cocaine- and amphetamine-related transcript (CART), thereby increasing intake and decreasing energy expenditure (Cha *et al*., 2004; Hu *et al*., 2003). Conversely, a high concentration of malonyl-CoA reduces the expression of the orexigenic peptides and increases the expression of the anorexigenic peptides, with a resultant reduction in feed intake and increase in energy expenditure.

In contrast to AMPK, mTOR is activated by high nutrient and energy availability, particularly by a high ATP:AMP ratio and by amino acids, especially leucine (Wullschleger *et al*., 2006). mTOR senses the energy status of cells through AMPK. When AMPK is highly activated, mTOR activation is low and vice versa. mTOR is highly conserved across species and has a major role in the control of cell growth and proliferation. Cota *et al*. (2006) found, by using antibodies to mTOR, that it was present particularly in regions of the hypothalamus associated with the melanocortin system neuropeptides controlling feed intake. It was found to be 90% associated with NPY/AgRP neurons and 45% with POMC/CART neurons. Fasting causes a marked depression in mTOR expression in the hypothalamus, reflecting low activity when the energy status of the animal is low, whereas the reverse occurs in recently fed, satiated animals (Cota *et al*., 2006). The importance of mTOR in controlling feed intake has been demonstrated by the intracerebroventricular administration of the mTOR inhibitor, rapamycin, which significantly stimulated feed intake in pre-satiated rats (Cota *et al*., 2006). Administration of excess leucine is known to be anorectic in animals (Anderson and Moore 2004). When leucine, but not valine, is administered into the third ventricle of rats, feed intake was significantly reduced (Cota *et al*., 2006). The negative effect of leucine on feed intake was overcome by co-administration of rapamycin, suggesting strongly that the effect of leucine on intake is via the mTOR pathway.

Adiposity and Lean Monitoring Systems

Basal insulin concentration is a direct reflection of the amount of visceral fat stored by an animal (Woods *et al*., 2006). Plasma insulin concentration provides a signal to the hypothalamus about the adiposity status of the animal and exerts control over feed intake. Insulin administered near the hypothalamus of animals has been found to reduce the amount of feed consumed and body weight in a dose dependent response (Woods *et al*., 1979). Conversely, administration of insulin antibodies near the hypothalamus or reducing insulin receptor activity by various means increases feed intake and body weight (Woods *et al*., 2006). Circulating insulin enters the brain via an active transport mechanism, where the concentrations convey to the brain the stored energy status of the animal and either lead to a long term increase or decrease in feed intake.

Leptin is released primarily from white adipose tissue in proportion to the number and activity of adipocytes (Considine *et al.*, 1996; Havel, 2004). Leptin concentrations in blood directly reflect the adiposity status of an animal and particularly the amount of subcutaneous adipose tissue (Woods *et al.*, 2006). Leptin, like insulin, also acts on the hypothalamus to reduce intake and body weight. Animals that have a genetic mutation resulting in the loss of either the leptin gene or the leptin receptor gene have elevated feed intake, reduced energy expenditure and are obese (Clement *et al.*, 1998; Montague *et al.*, 1997). Leptin replacement in animals with genetic loss of the leptin gene leads to substantial reduction in feed intake and body weight (Farooqi *et al.*, 1999; Halaas *et al.*, 1995). However, long-term administration of leptin does not induce weight loss because of development of 'leptin resistance' (Roth *et al.*, 2008).

Insulin, through its responsiveness to blood glucose concentrations, reflects the ongoing, short term energy status of an animal as well as the long term energy status through its relationship with the amount of adipose tissue. Alternatively, leptin concentrations provide only a reflection of the long term energy status. Short term changes in glucose concentrations lead to immediate changes in AMPK activity through the rapid effects on hypothalamic AMP:ATP ratio (Wolfgang *et al.* 2007). However, adiposity has its effect on intake through insulin and leptin, which are believed to increase mTOR activity and decrease AMPK activity in the hypothalamus (Minokoshi *et al.*, 2004; Shan *et al.*, 2008; Woods *et al.*, 2008).

Although ghrelin is released primarily from the stomach and has a major role in regulating hunger during the postprandial period, it also plays an important role in monitoring the energy or weight status of animals (Chaudhri *et al.*, 2006). Plasma concentrations of ghrelin are negatively correlated with body weight in humans and increase after weight loss (Cummings, 2006). Peripheral administration of ghrelin increases feed intake in several animal species and long-term treatment induces obesity, whereas administration of ghrelin antibodies or ghrelin receptor antagonists causes a reduction in feed intake and body weight (van Thuijl *et al.*, 2008). Thus, ghrelin action counterbalances those of leptin and insulin by increasing concentration in lean, lightweight animals. Ghrelin also acts in the hypothalamus and has been shown to increase the expression of melanocortin system orexigenic peptide, NPY (van Thuijl *et al.*, 2008).

Changes in expression of the orexigenic and anorexigenic peptides within the melanocortin system of the hypothalamus are transmitted to higher centres of the brain and, depending on the balance of these peptides, result in a sensation of hunger or satiety to control eating behaviour and intake of animals in associated with short term energy status and long term adiposity and lean content.

Modifiers of Energy Metabolism and Intake

Although animals closely monitor their energy and adiposity status and have metabolic and neural pathways through the hypothalamus and higher brain centres to regulate intake, many dietary, environmental and psychological constraints are known to alter both short term and long term intake (Poppi *et al.*, 1994; Nyachoti *et al.*, 2004; Black, 2009). Several of these factors constraining the potential metabolic regulation of feed intake are discussed.

Palatability and Feed Preference

Smell, taste and somatic sensing by an animal can influence whether a specific feed is selected, the initiation of feeding, length of a meal and long term intake (Roura *et al.*, 2008). Animals are innately cautious when presented with novel feeds. This neophobic behaviour is associated with reduced feed intake and is highly conserved across animal species (Roura *et al.*, 2008). The strongest neophobic responses have been reported with unfamiliar feed ingredients that are high in anti-nutritional factors such as lectins, tannins, alkaloids and glucosinolates (Clasadonte and van der Poel, 2009). Anti-nutritional factors can be divided into two groups; those which have a direct impact on gut function (trypsin inhibitors, lectins, tannins, saponins and specific polysaccharides), and those which do not affect gut function (alkaloids and glucosinolates). The influence of the former group of anti-nutritional factors on feed intake could be associated, at least partially, with changes in the extent and site of digestion of feed components in the intestines, which may be responsible for the depression in feed intake. The mechanism associated with site of nutrient digestion affecting intake are described in the following section.

The second group of anti-nutritional factors, although not affecting digestion, also affects metabolism within the animal. For example, plant alkaloids are known to alter the metabolism of copper and amino acids in animals and to have direct effects on the nervous system (Clasadonte and van der Poel, 2009). Similarly, glucosinolates inhibit the uptake of iodine by the thyroid gland and lower plasma concentrations of the thyroid hormones (Clasadonte and van der Poel, 2009). Part of the influence of these anti-nutritional factors therefore could be through their effect on metabolic processes and capacity of the animal to utilise energy.

Nevertheless, there is evidence that bitter compounds, in particular, affect preference for feeds by animals. Kleuss *et al.* (2007) observed that the inclusion of 5% *Phaseolus* bean, which provided 0.94 g/day of phytohaemagglutinin

lectin, into the diet of 33 day old pigs reduced the intake from 329 g/d for the control to 251 g/day. Huisman (1990) had previously shown in rats that inhibition of protein utilisation from the presence of lectin was not the cause of the depression in intake. He showed that the inclusion of 4% *Phaseolous* bean in the diet of rats depressed intake from 234 g/day in the control diet to 135 g/day. The addition of casein to the latter diet did not significantly improve intake (146 g/day). However, toasting the beans reduced the lectin intake from 1.2 to 0.0 g/day and resulted in an intake in rats similar to the control diet of 225 g/day.

Liu (1994) showed that the inclusion of 15.5% untreated rapeseed meal into the diet of 20 kg pigs reduced intake from 1510 g/day for the control treatment to 800 g/day. Washing the rapeseed meal removed most, but not all of the glucosinolate compounds, and resulted in a significant increase in intake to 1230 g/day. There is strong evidence that pigs have a low preference for diets containing rapeseed meal. Substitution of soybean meal with rapeseed meal in diets used in a double choice preference test with pigs showed only an 11% preference for the diet containing rapeseed meal (Roura *et al.*, 2007). Subsequently, Torrallardona and Solà-Oriol (2009) found that rapeseed meal, sunflower meal and potato protein when included at 20% of the diet were significantly less preferred by young pigs than a diet containing soybean meal.

Stimulation of a range of senses has been shown to alter intake and preference of feeds by pigs. For example, oxidation of choice white grease to produce rancidity has been shown to significantly reduce feed intake in young pigs without changing nutrient utilisation (DeRouchey *et al.*, 2004). Similarly, many spices, oleoresins and plant extracts seem to be perceived as noxious through the somatic senses causing feed rejection in mammals. Feed supplemented with capsicum oleoresin resulted in decreased feed intake but feed intake depression was prevented after the use of a cherry-honey flavour proving that palatability is an important issue when using spices in pig diets (Roura *et al.*, 2008). In addition, the somatic sensing accounts for the food rheological characteristics such as feed hardness and energy required for chewing have a negative correlation with preference values (Sola-Oriol *et al.*, 2009).

Animals experiencing a digestive disorder, toxicoses or nausea following the consumption of a feed develop long term aversions to that feed or other feeds with similar odour and taste characteristics (Olsen and Ralphs, 1986; Povenza *et al.*, 1994). These aversions are frequently maintained in the long term (Day *et al.*, 1998). Evidence from mice indicates that taste aversion is related to the release of the peptide PYY which, when administered to animals results in long term depression of feed intake (Halatchev and Cone, 2005). Alternatively, maternal transmission of flavour cues through prenatal exposure from amniotic fluids to the flavours of the diet of their mother, reduces the innate caution of a young animal to novel food sources containing the same flavours, provided they have been consumed by the mother. For example, prenatal exposure to garlic and anise flavours through the maternal diet has reduced weaning associated problems in piglets and resulted in higher feed intake and growth rate when offered diets containing these flavours (Oostindjer *et al.*, 2010).

Digestive Tract Constraints

The chemical composition and physical form of a diet can have a major influence on feed intake and growth rate of pigs. For example, Cadogan *et al.* (1999) incorporated ten samples of wheat from different sources into the diets of young pigs and observed a 50% range in feed intake and growth rate, despite digestibility of the samples with extremes in intake being the same. Changes in the physical form of a diet for pigs or other monogastric animals by: grinding and pelleting (Wondra *et al.* 1995); removing large particles (Henman *et al.*, 2011); adding different types of soluble (Fleming and Lee 1983) and insoluble fibre (Kyriazakis and Emmens 1995); providing excess protein (L'Heureux-Bouron *et al* 2003) or fat (Meyer *et al.* 1998) have been shown to change the intake of available energy, efficiency of feed use and growth rates of animals.

The likely mechanisms by which chemical and physical characteristics of diets affect feed intake in pigs have been reviewed by Black *et al.* (2009). Feed intake is stimulated in monogastric animals as rate of passage of digesta, particularly through the stomach and small intestine increases. Physical characteristics of the diet that cause mild distension stimulate the rate of stomach emptying and propagative peristaltic contractions in the gastrointestinal tract and increase feed intake. However, excessive distension inhibits these responses, slows rate of passage and induces satiety via the vagus nerve-hindbrain-forebrain reflex. Increasing the amount of indigestible fibre, or other indigestible particles, in a diet for pigs can increase or decrease rate of passage and feed intake depending on the amount of fibre and size of particles. Small indigestible fibre particles are less effective for stimulating rate of passage than medium sized particles, which in turn, are more effective than very large particles (Ehle *et al.* 1982; Gidenne, 1992). The optimum size and amount of fibre needed to stimulate rate of passage of digesta in pigs is not known. Excessive distension of the gastrointestinal tract and reduced rate of passage can be caused by excessive amounts of large indigestible particles and also by diets with high digesta viscosity or air volume. Soluble fibres and other compounds can markedly increase viscosity, slow rate of passage and intake in monogastric animals.

Nutrients and their metabolites present in the gastrointestinal tract and absorbed from the tract control feed intake over the short and long term through the direct or indirect release of peptide hormones and their interaction with local and central neural processes. Consumed carbohydrates, fats, proteins and their products from both mammalian and microbial enzymic digestion directly affect the release of hormones from the gastrointestinal tract and pancreas. The quantitative and temporal release of these hormones depends on the specific composition of nutrients consumed, their site of digestion and products released within the gastrointestinal tract. These nutrient-released hormones act to slow the rate of stomach emptying, reduce the frequency, pressure and progressive distance of peristaltic contractions in the small intestine and reduce feed intake. The action of these hormones has been defined as the jejunal, ileal and/or colonic brakes, depending on the site of nutrient stimulation and release of the hormones, specifically peptide tyrosine tyrosine (PYY), glucagon-like peptide-1 (GLP-1), oxyntomodulin (OMX) and apolipoprotein A-IV (apo A-IV). Long-chain fatty acids and volatile fatty acids are particularly potent at stimulating the intestinal brake and slowing rate of digesta passage, but partially hydrolysed proteins, undigested starch and soluble fibrous substances also stimulate the intestinal brake (Black *et al*., 2009). Many of these nutrient-stimulated hormones also act through the vagal nervous system or directly on specific regions of the brain to have longer-term effects on reducing feed intake.

Hence, rate of passage can be stimulated by mild distension, but inhibited by excessive distension and the presence of undigested nutrients near the terminal ileum and colon that stimulate the intestinal brake. The potential interaction between these positive and negative effects of the diet on rate of passage and feed intake, provide a perfect axis for manipulating feed intake of pigs through modifying the physical and chemical characteristics of a diet and changing rate of passage and site of digestion of individual nutrients.

Dietary Fibre-Intestinal Brake Interactions

The large effect of cereal grain sample on intake of pigs observed by Cadogan *et al*. (1999) and others could be explained by differences in undigested dietary material reaching the distal ileum and colon, with a resultant long term increase in the release of intestinal brake peptides including PYY, GLP-1 and OMX. The effect of release of these peptides would be both short term through slowing of digesta transport through the intestines, and long term through the impact of the peptides on the hypothalamic melanocortin neuropeptides. However, the negative effect of the ileal brake on feed intake may be counterbalanced by a high proportion of indigestible fibre in the diet stimulating digesta flow rates and intake. These concepts are supported by studies that show cooked rice with a higher digestibility than cooked maize results in 25% greater intake of feed and 29% faster growth rate in young pigs (Vicente *et al*. 2008). Addition of 20 or 40 g/kg oat hulls significantly decreased feed intake of pigs offered a maize-based diet, but increased intake of pigs offered a rice-based diet (Mateos *et al*. 2007). This observation could be explained by more undigested maize starch passing to the distal intestine with increasing fibre content of the diet, increasing rate of passage of digesta, and stimulating the ileal/colonic brake. However, with the more highly digested starch in rice than in maize, little more starch would have reached the distal intestine with increasing dietary fibre content and the ileal/colonic brake would not have been activated. Conversely, intake of the rice-based diet would be increased through the positive effect of added indigestible fibre on rate of passage of digesta.

Effect of Dietary Fat

The depression in energy intake observed when animals are initially offered diets high in fat could be explained by an increase in chylomicron absorption and release of apo A-IV, augmented by the other intestinal brake peptides that will slow digesta transport (Black *et al*., 2009) The increase in absorption of long-chain fatty acids is known to increase fatty acyl-CoA in the hypothalamus and decrease feed intake, while increasing energy expenditure through activation of the melanocortin system. Apo A-IV is also a potent anorexigenic peptide in the hypothalamus. However, prolonged feeding of diets high in fat can lead to down-regulation of the processes within the hypothalamus that reduce feed intake and result in enhanced consumption and diet-induced obesity.

Excess or Deficiency in Protein and Other Nutrients

Because branched-chain amino acids are not metabolised in the liver, excess dietary protein is expressed as high concentrations of these amino acids in plasma, specifically leucine. Although a considerable amount of leucine entering the brain is used in the formation of glutamate, it also has a major function activating mTOR, which will lead to a depression in intake and increase in energy expenditure. A similar situation would occur when feeding a diet with a severely imbalanced in amino acid pattern, where excess leucine and a low AMP:ATP ratio would stimulate activity of mTOR. Morrison *et al*. (2007) has shown that intracerebroventricular administration of either a mixture of amino acids or leucine alone decreased the expression of AgRP via the mTOR signaling system. Alternatively, diets inadequate in protein, but with a balanced amino acid pattern, often result in an increase in feed intake. Such diets have been shown to increase in the expression of AgRP (Morrison *et al*. 2007), which would provoke an increase in feed intake.

However, excessive intake of a low protein diet would be prevented by the decrease in AMP:ATP ratio caused by an inability of the animal to use energy for protein synthesis, a decrease in activation of AMPK and an increase in hypothalamic malonyl-CoA.

Deficiencies in other essential nutrients such as phosphorus (Mahan, 1982), sodium (Alcantara *et al*., 1980) and potassium (Jensen *et al*., 1961) reduce feed intake in pigs. Deficiencies in these nutrients are likely to impair energy utilisation within the animal, decrease the ratio of AMP:ATP, decrease the activation of AMPK, increase the concentration of malonyl-CoA within the hypothalamus, thus stimulating the release of anorexogenic peptides and inducing satiety earlier than in animals receiving a nutrient balanced diet.

Climatic Constraints

Climatic conditions can have a profound effect on the intake of pigs (Black *et al*., 1999). Energy expenditure of a pig increases to maintain body temperature when ambient temperature falls below the lower critical temperature. Voluntary intake tends to increase, often after a delay of one or two days, to compensate for the additional energy consumed. Conversely, feed intake declines when ambient temperature exceeds the evaporative critical temperature, which is the temperature when energy expenditure is first increased through panting as ambient temperature rises. The magnitude of the rise in feed intake at low temperatures and the fall in intake at high temperatures is influenced by characteristics of the diet, stocking arrangements and weight of the pig.

Pigs exposed to cold will not always increase feed intake to maintain the rate of energy deposition that would occur under thermoneutral conditions. Close (1989) showed that feed intake in pigs weighing 90 kg continued to increase as ambient temperature fell below the lower critical temperature, but intake increased little for pigs weighing 18 kg. The inability of lighter weight pigs to increase feed intake when exposed to cold is due primarily to limitations imposed by gastrointestinal tract capacity, and the small increase observed can be attributed to an increase in rate of passage of digesta through the gastrointestinal tract in pigs exposed to cold conditions (Phillips *et al*., 1982). Conversely, gastrointestinal tract capacity is unlikely to limit the increase in feed intake of pigs weighing 90 kg needed to compensate for the increasing energy lost to the environment when ambient temperature declines below the lower critical temperature.

Group housed pigs spend more time huddling and less time eating when exposed to cold conditions than pigs in a thermoneutral environment (Parker *et al*., 1980). This observation suggests that feed intake of pigs exposed to cold conditions may be influenced by the number of pigs in a pen. Nienaber *et al*. (1990, 1991) subjected pigs either penned individually or in groups of four to ambient temperatures 12°C below the estimated lower critical temperature. Feed intake increased over that obtained under thermoneutral conditions by 30% for pigs penned individually compared with only 11% for pigs penned in groups of four. The pigs penned individually spent approximately the same time eating as pigs in thermoneutral conditions, whereas the cold, group-penned pigs spent only half the time eating. These observations suggest that pigs prefer to remain warm and huddled in a group when exposed to cold conditions and eat only when the hunger drive is high, and then for a short period of time compared with pigs penned individually. This inclination to remain warm rather than eat, is an example of a psychological constraint on feed intake in the pig.

In hot conditions, feed intake and activity are modified to allow total heat production from the pig and heat input from the environment to coincide with that lost to the environment through convection, conduction and evaporation (Black *et al*., 1986, Knapp, 1999). The pig ceases non-feeding associated activity once the ambient temperature exceeds the evaporative critical temperature to reduce body heat production (Giles, 1992). In addition, Giles (1992) showed that once ambient temperature rose above the evaporative critical temperature, where respiration rate commenced to increase due to heat load, the pig begins to store some of the extra heat input from the environment as an increase in body temperature. Collation of results from many experiments (Giles *et al*., 1998) showed that feed intake of pigs under various high temperature regimes relative to intake under thermoneutral conditions fell linearly with increasing body temperature from 39.2°C with intake ceasing at 41.3°C.

Cold exposure and exercise both result in increases in feed intake that could be explained by a reduction in available energy and a high AMP:ATP ratio in cells. This would lead to high AMPK activity and low hypothalamic malonyl-CoA, which will stimulate expression of orexigenic peptides while depressing expression of anorexigenic peptides of the hypothalamic melanocortin system.

Number of Pigs per Pen and Stocking Density

Placing growing pigs in groups has a detrimental effect on feed intake and performance. The negative effect on feed intake of placing pigs in groups is exacerbated by reductions in floor space per pig, and when the air volume per pig becomes too small. Chapple (1993) showed that simply by increasing the number of pigs in a pen from one to

three or from one to five caused a significant depression in feed intake of 5% and 9% respectively. Similarly, Gonyou *et al*. (1992) found that increasing the number of pigs in a pen from one to five depressed feed intake by 8% despite there being adequate space for each pig. There appears to be little additional effect on performance from increasing the number of pigs in a pen beyond five, but the published results are equivocal. Chapple (1993) found that the intake of pigs was similar irrespective of group size ranging from five to 15 pigs per pen. Similarly, Nielsen and Lawrence (1993) observed no difference in feed intake for pigs housed in groups of five, 10, 15 or 20. Gonyou and Stricklin (1998) examined the effect of group size ranging from three to 15 pigs per pen and found the greatest depression in feed intake occurred between group sizes of three and six pigs, but a small depression in intake continued to 15 pigs per pen. Petherick *et al*. (1989) reported that the intake of pigs housed in groups of 36 was significantly less than that for pigs in groups of either six or 18 per pen. Although McGlone and Newby (1994) observed no difference in the performance of pigs housed in groups of 10, 20 or 40 per pen, animals in the pen with 40 pigs showed the greatest rates of injury and morbidity. Turner *et al*. (2000) found that the growth rate was greater for pigs housed in a group of 20 than for groups of 80 pigs. However, neither Schmolke and Gonyou (2000) nor Wolter *et al*. (2001) observed any differences in the performance of pigs housed in groups of 10, 20, 40 or 80 and 25, 50 and 100, respectively.

Reduction in the intake and performance of pigs when placed into groups occurs even when the animals have been acquainted previously with one another (Black *et al*., 2001b; Gomez *et al*., 2000). There appears to be little difference in the negative effect of grouping pigs that have been acquainted or are unfamiliar with one another (Kerr *et al*., 2005). However, the effects on feed intake and performance of grouping pigs are reversible when pigs are sequentially placed individually or in groups within pens (Bornett *et al*. 2000; Kerr *et al*., 2005).

Two factors appear largely responsible for the reduced feed intake by pigs when placed in groups. There is the stress of competing with other pigs in a group. Kerr *et al*. (2005) found that plasma cortisol concentrations increased significantly every time pigs were moved from single to group pens. The second reason appears to be related to the behaviour of the dominant pigs. The dominant animals largely control the feeding patterns of the subordinate pigs and also control access to the feeders. Subordinate pigs frequently commence feeding activities in response to the dominant pig actively seeking feed. In addition, dominant pigs frequently 'guard' the feeders or preferred feeder when not eating to prevent the less dominant animals from eating (Black *et al*., 2001b).

The full mechanisms through which stress affects feed intake are still to be elucidated. Physical and psychological stressors are known to stimulate neurotransmitters in the locus-ceruleus region of the brain and result in the release of adrenalin and nor-adrenalin from the adrenal medulla. These catecholamines stimulate the release of corticotrophin releasing hormone (CRH), which stimulates the release of adrenocorticotropin and cortisol (Black *et al*., 2001b). CRH also stimulates the release of several proinflammatory cytokines, which as described in the next section, are known to have direct effects on the melanocortin system of the hypothalamus and stimulate the release anorexogenic peptides within the brain to reduce feed intake.

Health Constraints

A reduction in feed intake generally occurs when animals are exposed to infectious diseases and other microbial or dietary factors that stimulate an immune response (Exton, 1997). Diseases affect several physiological functions of animals including feed intake, oxygen exchange rate, efficiency of energy utilisation, body protein synthesis and catabolism, body temperature control and tolerance to heat stress. The pattern of changes in feed intake following an infection has been described by Kyriazakis and Doeschl-Wilson (2009). There is an initial lag phase while the pathogen or agent is being recognised by the innate immune system. This is followed by a marked depression in feed intake that may include a complete cessation of feeding depending on the nature of the pathogen and severity of the infection. The depression in intake in pigs commonly lasts for several days to weeks depending on the specific infection.

Feed intake appears to fully recover only when the pig has acquired full immunity to the pathogen (Zaralis *et al*., 2008). Kyriazakis and Doeschl-Wilson (2009) suggest that the length of the lag period before feed intake is depressed and the period of intake depression are related to the ability of the host to recognise the pathogen and build an effective immune response. Consequently, bacteria and viruses that replicate rapidly and are quickly recognised by the host immune system cause a short lag period before intake declines and the time of intake depression is relatively short because frequently complete immunity is acquired. By comparison, macroparasites, such as intestinal worms, require a longer time to become established within the host, are frequently not well recognised by the immune system and never invoke full immunity. Thus, with these macroparasites the lag period from infection is long, feed intake depression is prolonged and frequently never recovers to levels of uninfected animals.

Airborne pollutants within pig buildings can have a significant negative effect on pig health and performance (Holyoake, 2005). The concentration of viable bacteria appears to be the most important single component of air

quality affecting pig performance (Black, 2003). High concentrations of endotoxins and respirable dust can also depress performance. Although high concentrations of ammonia at 50 ppm and hydrogen sulphide of 8.5 ppm do not appear to affect pig performance when present alone, a concentration of only 5 ppm ammonia can significantly exacerbate the prevalence of respiratory disease. There is strong evidence that the concentrations of airborne viable bacteria, ammonia, respirable dust and endotoxins exacerbate the severity of a range of diseases including enzootic pneumonia, pleuropneumonia, rhinitis, porcine reproductive and respiratory syndrome (PRRS) and roundworm infestations (Black, 2003). Non-respiratory disease causing viable bacteria from pig buildings in association with increased ammonia concentrations can induce lung infections and reduce pig performance. Poor air quality through induced inflammatory responses appears to reduce the capacity of a pig to mount a normal challenge against pathogenic organisms and resistance to disease is diminished.

The depression in feed intake associated with infection is believed to be caused by the release of proinflammatory cytokines, particularly interleukin 1 (IL-1), IL-6, IL-8, tumour necrosis factor (TNF-α) and interferon gamma (IFN-γ: Klasing and Johnstone, 1991; Baarsch, 1995; Exton, 1997). Treatment with IL-1 receptor antagonist (IL-1ra) of pigs experimentally challenged with Actinobacillus pleuropneumoniae has been shown to increase gain in the week following the challenge by 74% over the control receiving a treatment of saline and substantially reduce lung lesion score (Black *et al*., 2001a). There is evidence suggesting that the inflammatory cytokines, or at least IL-1, has its effect by influencing the melanocortin system of the hypothalamus. Catania *et al*. (1991) showed a marked rise in α-MSH, which is derived from the anoerexogenic POMC in the melanocortin region of the brain. Intracerebroventricular administration of α-MSH has been found to significantly increase the reduction in feed intake and activity associated with lipopolysaccharide (LPS) administration to rats (Huang *et al*. 1999). However, administration of antagonists, either natural (AgRP) or synthetic (SHU-9119), to the α-MSH receptors, melanocortin receptor 3 and 4 (MC3-R/MC4-R), reversed the symptoms caused by LPS (Huang *et al*., 1999, Marks *et al*., 2001). Similarly, the symptoms did not appear in MC4-R knockout mice following administration of LPS. These results show a central role for the hypothalamic melanocortin system in fever and disease and that a blockade of MC4-R signalling normalises feed intake and activity without an increase in morbidity or mortality (Marks *et al*., 2001; Catania, 2007).

Other Examples of Constraints to Feed Intake

The male deer during the mating period is known virtually stop eating, with intake decreasing by 94% for three weeks, and largely maintain body protein content, but losing large amounts of fat (Newman *et al*., 1998). Similarly, the black bear (*Ursus americanus*) during winter sleep gives birth to its young, lactates, but does not eat even if food is offered (Nelson, 1980). The black bear also during this period of 3 to 5 months does not lose body protein, except for that excreted in milk, and has the capacity to reabsorb urea from the bladder to synthesise non essential amino acids, while preserving essential amino acids. Bears starved during summer do not elicit the same response. They catabolise body protein and lose as much weight in three weeks as other during the whole of their winter sleep. These changes in metabolism of the black bear during winter sleep appear to be associated with a substantial increase in testosterone production (Nelson, 1980). Newman *et al*. (1998) showed that the administration of testosterone to bucks for 28 days during a period of at least six weeks prior to the expected rut, resulted in a decline in feed intake comparable to that observed during the rut. Thus, increases in testosterone concentrations will substantially reduce feed intake in animals and may be partially responsible for the depression in intake and performance of young boars that reach the so called 'boar wall' (Dunshea *et al*., 2011)

Conclusions

A great deal of progress has been made over recent years in understanding how characteristics of a diet in association with products from digestion and metabolism control meal eating and long-term intake in animals. An important discovery has been elucidation of the way an animal monitors its immediate and long term energy status and through these monitoring systems alters orexigenic and anorexigenic peptides within the brains to alter the sensation for hunger or satiety. These systems largely allow animals to control feed intake to be equivalent to the energy they expend. However, equally important is the recognition that within commercial piggeries there are many dietary, climatic, disease and social factors that constrain intake to below the potential of the pig to metabolise nutrients. Concepts have been developed that allow possible explanations of the fundamental mechanisms that underlie a wide range of dietary and non-dietary factors that are known to alter feed intake in pigs. An understanding of these mechanisms also provides an opportunity to develop strategies for either increasing or decreasing feed intake by animals, through manipulating the physical and/or chemical composition of diets, or by use of a range of pharmaceutical products being investigated for humans.

So Do Finishing Pigs "Eat to Energy" and if so What are the Commercial Implications?

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Abstract

With both an increasing world population, and rapidly expanding biofuel production, pig feed raw material prices are changing rapidly. In 2001 barley, wheatfeed (millrun) and extracted rapeseed meal in the UK were comparatively inexpensive compared to wheat and fat/oil. Furthermore the supply of dried distillers grains and solubles was increasing. The cost and availability of these more fibrous materials focused attention on minimal energy levels for pig diets and in particular if they should or could be reduced and the consequences of such an action. In eight recently published energy response trials in finishing pigs, feed intake fell with increasing energy density, but in most cases this was insufficient to prevent an increase in energy intake, particularly at lighter weights. The higher energy intake resulted in increased growth rate, carcase fatness, and a higher killing-out percentage. At lighter weights the growth response to increased energy intake was greater, presumably as energy was limiting lean growth. The economics of these responses was determined assuming a growth response of 23g/MJ digestible energy (DE). For a feed offered *ad libitum* from 65-105kg feed costs on a live weight basis were minimised, using 2010 costs, at 13.6 MJ DE/kg. Using 2011 costs this fell to 12.75 MJ DE/kg. Energy and bulk unit cost, which are outputs from least-cost feed formulation programmes, can both be useful indicators of feed cost. The removal of the bulk constraint when formulating feeds gives a simple, and quick, initial estimate of optimum nutrient density. Nutrient density can be refined depending upon the relative importance of growth and grading and other farm circumstances. However more research is required on the pig's responses to dietary energy concentrations below 13 MJ DE/kg. The influence of straw bedding on optimum energy concentration also requires study.

Introduction

Thirty years ago, commercial pig production in Northern Europe utilised a wide range of feedstuffs from all over the globe. These included cassava, rice bran, maize gluten feed, sunflower meal and soybean meal from the USA. In recent years increased domestic demand, and increased transport costs, has resulted in less imported feedstuffs, with feeds being more based upon domestic supplies of wheat, barley, wheatfeed, biscuit and rapeseed meal.

In 2010, the UK pig industry faced a new challenge with major increases in both protein and cereal prices, and their volatility, due to low levels of stocks worldwide. The reasons for this are well documented and include increases in commodity demand from countries such as China, increased bioethanol production, and adverse weather patterns. During 2011, in the UK, barley, wheatfeed, and extracted rapeseed meal have been relatively inexpensive compared to wheat and soybean meal. Fat and oil remain very expensive. The first of the major UK bioethanol plants, producing 350 thousand tonnes of wheat DDGS, is now in operation and a second plant of similar size is due to enter production in 2012. These plants will potentially use two million tonnes of wheat, out of an average exportable surplus of 2.4 million tonnes, putting more pressure on domestic wheat supply. Rapeseed production in the UK continues to expand. As a consequence of these changes in commodity prices maximum fibre levels and the costs of energy constraints within UK pig feed formulations are increasing. The changes raise questions on the pig's ability to maintain energy intake on lower energy diets and whether lower energy/higher fibre diets can be made cost-effective.

This paper examines how well finishing pigs maintain their energy intake at varying energy densities, and how this knowledge might be incorporated into commercial feed formulations.

The Energy Content of Feed

For over 10 years many EU countries have used net energy (NE) for the formulation of pig feeds and it is gradually replacing digestible (DE) or metabolisable energy (ME) as the system of choice in many other major pig producing countries. A major reason for the development of the NE system is that at times in the last 20 years high fibre and high protein feedstuffs have appeared relatively inexpensive (on a DE basis) but feeds formulated with high levels of these materials have performed poorly. It is now well understood that DE and ME both overvalue feeds and feedstuffs that are high in fibre and protein.

The choice of energy system is pertinent when we consider the question "do pigs eat to energy?" If animals do eat to energy, then it might be reasonable to expect that they would do so at the "net" level in that this is the energy remaining for productive purposes. There may, however, be interactions to consider in that feeds with a relatively high DE/NE will have a high heat increment which might influence appetite if pigs are not in a thermo-neutral environment.

In this paper DE is the major energy system used, partly because many of the papers reviewed were DE (or ME) based, and partly because DE is currently the energy system most widely used in Australia. Net energy is though referred to in some areas where it adds to the interpretation of the data.

So How Well do Finishing Pigs "Eat to Energy"?

If pigs are offered feeds differing in dietary energy concentration do they recognise the fact and adjust their feed intake in order to regulate their energy intake? If they do, how accurate are they at maintaining their energy intake?

Beaulieu *et al*. (2009) fed 300 finishing pigs (gilts and castrates) feeds ranging in determined digestible energy from 12.9 to 14.9 MJ DE/kg. The study was conducted at the Prairie Swine Centre with pens containing five pigs. The increase in nutrient density was achieved by replacing barley with wheat, soybean meal, and rapeseed oil. The lysine to energy ratio of the feeds was kept constant; amino acids were specified so that they did not limit pig performance. The pigs were offered the feeds as a meal between 31 and 155 kg. As the digestible energy concentration of the feeds increased the pigs significantly reduced their feed intake. However, compensation was incomplete so that energy intake significantly increased (Table 1). Feed conversion ratio (FCR) improved with increasing energy density ($P<0.001$) but there was no consistent effect on growth rate. Backfat level increased from 16.8 to 19.4mm ($P<0.001$). Dressing percentage was significantly increased with increasing energy density ($P<0.001$).

Table 1. *The influence of feed digestible energy (DE) content on the performance of pigs (gilt/castrate) fed amino acid adequate feeds from 31-115kg, in a research environment (Beaulieu et al, 2009).*

DE (MJ/kg)	12.9	13.6	14.0	14.3	14.9	P -value
Start weight (kg)	31.2	31.1	31.5	31.2	31.1	
End weight (kg)	115.1	115.5	115.3	115.0	115.6	
Feed intake (kg)	2.66	2.62	2.62	2.52	2.44	<0.001
Growth rate (g/day)	1.00	1.02	1.04	1.02	1.03	0.03
Feed conversion rate	2.56	2.5	2.44	2.39	2.27	<0.001
Energy intake (MJ DE/day)	34.4	35.5	36.7	36.0	36.4	0.003
Dressing (%)	78.1	78	78.6	78.8	79	0.001
Lean yield (%)	61.5	61.2	60.9	61.1	60.7	0.01
Fat depth (mm)	16.8	17.8	18.3	18.6	19.4	<0.001

A second trial was conducted on a single site 600-sow farrow-to-finish operation, utilizing 720 pigs (gilts and castrates). Three finisher and three grower rooms, with 12 pens each, were used. Pigs were fed feeds of three differing nutrient densities (determined DE levels of 13.1, 13.8 and 14.4 MJ DE/kg from 37-115kg bodyweight). The amino acid to energy ratio was kept constant in the feeds. As energy density increased the pigs significantly reduced their feed intake (Table 2).

When considered over the whole study energy intake increased with increasing energy density but this was not statistically significant. There was no significant effect, overall, of energy density on growth rate, backfat or lean yield. There was a tendency ($P<0.10$) for a poorer killing-out percentage on the lowest nutrient density feed. However when the study was split into time periods it is interesting to note that energy intake was significantly increased from day 0-21 and from day 22-42 (Table 3), and that this resulted in a significant increase in growth rate in these periods (up to 75-80kg bodyweight).

Stein *et al*. (1996) fed 150 PIC barrows (approximately 54-113kg) five feeds ranging in formulated energy from 11.8 to 15.3 MJ DE/kg. The feeds were corn and soya based with lower energy levels being achieved by the addition of up to 30% wheat bran, 15% corn gluten feed and 9% alfalfa. The higher energy feeds contained 0.6g lysine/MJ DE whilst this fell slightly to 0.57g/MJ DE at the lowest energy level. However this decline was more dramatic if digestible lysine was considered (0.48, 0.48, 0.45, 0.43 and 0.39g/MJ DE as the energy of the feeds decreased). Daily feed consumption was not significantly different except at the highest energy level (15.3 MJ DE/kg), where it was considerably reduced (Table 4). Energy intake was not significantly different in feeds from 13.5-15.3 MJ DE/kg but was significantly lower in the feeds with 11.8 and 12.6 MJ DE/kg.

Table 2. *The influence of feed digestible energy (DE) content on the overall performance of pigs (gilt/castrate), fed amino acid adequate feeds, from 37-115kg, in a commercial environment (Beaulieu et al, 2009).*

DE (MJ/kg)	13.05	13.81	14.35	P-value
Start weight (kg)	37.40	36.60	36.50	
End weight (kg)	118.60	118.00	119.00	
Feed intake (kg/day)	2.94	2.85	2.77	0.01
Growth rate (kg/day)	0.99	0.98	1.00	0.31
Feed conversion rate	2.94	2.94	2.78	0.002
Energy intake (MJ DE/day)	38.24	39.08	40.08	0.14
Dressing (%)	78.50	79.30	79.00	0.07
Lean yield (%)	62.00	61.80	62.00	0.55
Fat depth (mm)	15.80	16.20	16.00	0.53
Loin muscle (mm)	61.80	62.50	63.50	0.08

Table 3. *The influence of feed digestible energy (DE) content on the interim performance of pigs (gilt/castrate), fed amino acid adequate feeds, from 37-115kg, in a commercial environment (Beaulieu et al, 2009).*

DE (MJ/kg)	13.05	13.81	14.35	P-value
Energy Intake (MJ DE/day)				
0-21 days	27.1	28.8	30.1	0.001
22-42 days	35.6	37.3	38.8	0.001
43-57 days	44.0	45.8	45.9	0.53
57-market	47.0	46.7	45.9	0.72
Overall	38.2	39.1	40.1	0.14
Growth rate (g/day)				
0-21days	910	960	1000	0.02
22-42 days	960	1010	1070	0.02
43-57 days	1080	1080	1050	0.41
57-market	980	910	940	0.08
Overall	990	980	1000	0.31

Table 4. *The influence of feed digestible energy (DE) content on the performance of pigs (gilt/castrate) fed from 54 to approximately 133kg bodyweight (Stein et al. 1996).*

DE (MJ/kg)	11.8	12.6	13.5	14.4	15.3
Start weight (kg)	54.1	53.6	54.4	54.7	53.9
End weight (kg)	111.2	112.9	111.8	113.9	113.8
Feed intake (kg/day)	3.31^{b}	3.23^{b}	3.36^{b}	3.28^{b}	2.91^{a}
Energy intake (MJ DE/day)	39.0^{c}	40.8^{bc}	45.4^{a}	47.2^{a}	44.4^{ab}
Growth rate (g/day)	872^{c}	931^{bc}	1006^{ab}	1038^{a}	1017^{a}
Feed conversion	3.85^{d}	3.45^{c}	3.33^{bc}	3.12^{b}	2.86^{a}
Dressing (%)	73.5^{c}	74.0^{c}	74.6^{bc}	74.9^{ab}	76.0^{a}
Carcase weight (kg)	81.7	83.5	83.4	85.3	86.5
10th rib fat (mm)	17.5^{b}	17.8^{b}	19.8^{ab}	21.8^{a}	21.6^{a}
Carcase lean (%)	52.0^{ab}	52.3^{a}	51.7^{ab}	50.4^{b}	50.8^{ab}
Digestible lysine intake (g/day)	15	17	21	23	21

*within rows values with different superscriptss are significantly different (P<0.05).

There was a marked deterioration in gain on the lower energy feeds. Also these feeds had a lower digestible lysine/MJ DE which resulted in the pigs having a lower digestible lysine intake. There was a significant increase in rib fat as energy density increased, and a significant increase in dressing percentage. These feeds contained a wide range of fibre (estimated as 7.9-26.1% NDF).

Zier-Rush *et al*. (2009) used 2571 gilts and castrates in 100 pens to examine the influence on three energy levels and three sire lines on performance from 35.8kg to slaughter after 84 days. Feeds were offered as meals. The feed treatments included a maize/corn/DDGS control, a second lower energy treatment containing 16% wheat middlings, and a third based upon the control but with 5% added choice white grease. There were four feeds within each treatment group and mean formulated energy level was 14.11, 14.17, 15.04 MJ DE/kg (assuming ME/DE at 0.96). Analysed crude fibre varied from 2.2-4.1% and NDF from 7.2-14.7%. Adding fat increased the formulated DE density by 6.1% from that of the control feed, but DE intake was only increased by 0.6% so that energy compensation was nearly complete (Table 5).

Table 5. *The influence of feed digestible energy (DE) content on pigs (gilts/castrates) fed from 36kg for 80 days (Zier-Rush et al. 2009).*

	Middlings	Control	5% fat	P-value
Formulated digestible energy (MJ/kg)	14.11	14.17	15.04	
Start weight (kg)	36.30	35.60	35.70	NS
End weight (kg)	122.2[a]	126.0[b]	127.2[c]	<0.0001
Feed intake (kg/day)	2.51[a]	2.56[b]	2.43[c]	<0.0001
Energy intake (MJ DE/day)	35.4	36.3	36.5	
Growth rate (g/day)	917[a]	948[b]	984[c]	<0.0001
Feed conversion ratio	2.74[a]	2.69[b]	2.47[c]	<0.0001
Interim feed intake (kg/day)				
Day 0-43	2.40[a]	2.45[b]	2.32[c]	<0.0001
Day 43-64	2.91[a]	2.94[a]	2.80[b]	<0.0001
Day 64-84	3.05[a]	3.10[a]	2.93[b]	<0.001
Interim growth rate (g/day)				
Day 0-43	939[a]	966[b]	1012[c]	<0.0001
Day 43-64	962[a]	943[b]	1071[c]	<0.0001
Day 64-84	907[a]	957[b]	953[b]	0.028

NS, not significant. [abc]Within rows values with different superscripts are significantly different (<0.001).

Adding middlings to the control reduced formulated DE concentration by 0.4%, but reduced energy intake by 2.5%. Overall growth rate was significantly improved by the inclusion of fat, and deteriorated with that of middlings. In the first two growth periods the addition of fat significantly increased growth rate but this was not apparent in the final period. Pigs fed middlings showed significantly poorer growth rates in the first and final growth periods. Campbell (1997) fed 150 female pigs, from 65kg bodyweight, five feeds with energy levels ranging from 12.1 to 14.6 MJ DE/kg. Differences in energy level were obtained largely by replacing barley with wheat and tallow. The feeds were formulated to contain 0.77g available lysine per MJ DE and the experiment was terminated when the first pen reached 105kg (49 days). There were no significant effects of energy concentration on feed intake (Table 6). Growth rate, feed conversion and dressing percentage were all significantly improved with increasing energy density. Back fat was significantly lower at 12.1 MJ DE/kg than when feeds of over 13.4 MJ DE/kg were fed although the carcase was lighter.

Magowan *et al*. (2010) fed 960 Landrace cross - Large White pigs (boars and gilts) in a commercial herd, housed in groups of 20 from 14-24 weeks of age. Pigs were in groups of 20 in fully slatted concrete pens, allowing 0.65m2/pig, with automatically controlled natural ventilation. Pigs were offered feed from wet and dry single space feeders with two feeders and two bowl drinkers per pen. Pigs commenced the trial at 45.6kg and were fed *ad libitum* for 70 days. Two basal pelleted feeds were offered. The first feed was formulated to contain 13.0 MJ DE/kg and was composed largely of barley, wheat and soya bean meal. The second feed was formulated to a lower energy, 12.5 MJ DE/kg, and contained more by-products including maize gluten meal, maize gluten feed, pollard and rapeseed meal. To these feeds was added 4% vegetable fat (formulated 13.4 and 13.9 MJ DE/kg respectively), either all in the mixer or with 1% in the mixer and 3% sprayed. All feeds were formulated to the same SID lysine level of 0.87%. The DE of the experimental feeds was determined.

Table 6. *Influence of feed digestible energy (DE) content on the performance of female pigs fed from 65kg bodyweight (BW; Campbell, 1997).*

Digestible energy (MJ/kg)	12.1	13.0	13.4	14.2	14.6
Feed intake (kg/day)	2.33	2.58	2.57	2.46	2.49
Energy intake (MJ DE/day)	28.7	33.3	34.7	34.9	36.6
Growth rate (g/day)	676^{b}	782^{ab}	826^{a}	825^{a}	876^{a}
Feed conversion ratio	3.45^{c}	3.27^{bc}	3.11^{b}	2.96^{b}	2.84^{a}
Final weight (kg)	96.6^{a}	99.9^{b}	102.4^{ab}	105.8^{ab}	105.2^{a}
Carcase weight (kg)	76.5^{b}	79.8^{b}	82.4^{ab}	84.7^{ab}	85.8^{a}
Dressing (%)	79.6^{cd}	79.8^{c}	80.5^{bc}	81^{b}	82.0^{a}
Fat depth P2 (mm)	11.6^{a}	12.8^{ab}	14.3^{b}	13.9^{b}	14.1^{b}

[ab]Means within rows with different superscripts differ significantly (P<0.05).

Table 7. *Influence of feed digestible energy (DE) content on the performance of pigs (boar/gilt) from 46kg to approximately 104kg (Magowan et al , 2010).*

	By-product based		Cereal based		P-value	
	Basal	+4% fat	Basal	+4% fat	Diet	Oil addition
DE formulated (MJ/kg)	12.5	13.4	13.0	13.9		
DE actual (MJ/kg)	12.7	13.2	13.0	13.9		
Start weight (kg)	46.1	45.2	44.7	46.2		
Feed intake (kg/day)	2.20	2.15	2.22	2.15	NS	NS
End weight (kg)	102.7	102.7	105	106		
Energy intake (MJ DE/day)	28.0	28.4	28.8	29.9		
Growth rate (g/day))	731	765	808	825	<0.001	NS
Feed conversion ratio	3.02	2.81	2.75	2.61	<0.001	<0.001
Killing out (%)	74.4	74.6	74.4	74.4	NS	NS
Carcase weight (kg)	76.1	76.2	78.0	79.2	<0.001	NS
Backfat (mm)	11.2	12.1	11.8	12.2	NS	<0.01
Lean meat (%)	62.1	61.3	61.5	61.1	NS	<0.01

NS, not significant.

Adding 4% oil reduced feed intake, although this was not significant, and energy intake increased (Table 7). Growth rate improved with added fat, but not significantly so. Feed conversion was significantly improved by the addition of fat, and back fat increased.

Boyd and Johnson (1997) examined the performance of 240 castrates and females offered feeds containing either 14.4 or 15.2 MJ DE/kg from 25-127kg. Feeds were corn and soya based with the lower energy feed containing 7.5-10% wheat middlings and the high energy feed 3.5% added fat. There was no significant effect of energy density on feed intake (Table 8). The pigs fed the higher energy density feed grew significantly faster (885 vs. 844g/day, P<0.01), were significantly fatter, and had a tendency towards a higher carcass yield (P<0.07)

Lopez-Bote *et al*. (1997) fed a control feed, and a feed with additional 4.2% lard, to boars and gilts from 30-90kg bodyweight. The lysine:energy ratio of the feeds was constant. Feed intake was low in this experiment and was significantly reduced on the higher energy feed (Table 9). Growth rates were similar on the two feeds, so that feed conversion was significantly improved. Carcase yield was significantly higher on the higher energy feed, and rib fat was increased from 13.6 to 14.6mm although this was not statistically significant.

Table 8. *Influence of feed digestible energy (DE) content on the performance of pigs of pigs (castrates and females) from 25-127kg (Boyd and Johnson, 1997).*

Digestible energy (MJ/kg)	14.38	15.25	P-value
Start weight (kg)	24	25	
End weight (kg)	128	129	
Intake (kg/day)	2.66	2.64	
Growth rate (g/day)	844	885	<0.01
Feed conversion ratio	3.15	2.98	<0.05
Energy intake (MJ DE/day)	38.3	40.3	
Fat depth P2 (mm)	19.3	21.6	<0.01
Carcase lean (%)	54.3	52.5	<0.01
Carcass yield (%)	75.2	76.6	0.07

Table 9. *Effect of feed digestible energy (DE) content on performance of pigs (boars/gilts) from 30-90kg bodyweight (Lopez-Bote et al., 1997).*

Digestible energy (MJ/kg)	13.1	14.0
Start weight (kg)	30.4	30.5
End weight (kg)	89.1	90.1
Intake (kg/day)	1.78[a]	1.73[b]
Energy intake (MJ DE/day)	23.4	24.2
Growth rate (kg/day)	0.7	0.71
Feed conversion rate	2.55[a]	2.44[b]
Carcase weight (kg)	72.1[a]	73.8[b]
Carcase yield (%)	78.8[a]	79.5[b]
Last rib fat (mm)	13.6	14.6
Carcase lean (%)	55.7	55

[ab]Means within rows with different superscripts differ significantly (P<0.05).

Summarizing the Responses to Energy Concentration

Comparing the above eight papers is complicated by a number of factors, including:

1. In some papers the energy is determined whilst in others it is formulated, and may, or may not, be accurate.
2. In all of these papers ME or DE was used for feed formulation which overvalues the true energy content of the lower energy feeds compared to the higher energy feeds.
3. In some papers amino acid supply is suboptimal, and in one, total amino acids were used for feed formulation. As lower nutrient density feeds generally contain more fibre, amino acid digestibility will generally decline.
4. Some experiments are based on a final weight whilst some are time based so that there can be major differences in finishing weight. Weight ranges are different between experiments as is sex.
5. Many of the experiments report significant changes to carcass yield and but few report gain and conversion on a deadweight basis.
6. There is inconsistency in the measurement of carcase lean and fatness.

In five of the eight papers reviewed feed intake fell significantly with increasing energy concentration. Ferguson *et al.* (1999) reviewed ten papers published between 1967 and 1995, with pigs in varying weight groups and also found that feed intake decreased with increasing DE concentration. He concluded that there were no significant differences in the slopes between the experiments and that it could be assumed that the feed intake responses to DE share a single slope value (-0.0567 kg d^{-1}/MJ DE kg^{-1}). The current review suggests that whilst feed intake is reduced with increasing energy concentration it is, in most cases, insufficient to prevent an increase in energy intake (Figure 1).

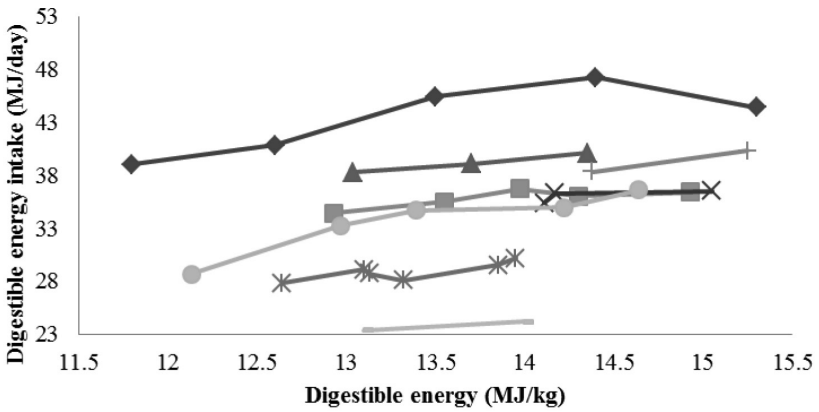

Figure 1. *Relationship between feed digestible energy concentration and digestible energy intake.*
(✳, Magowan et al., 2010; ■, Beaulieu et al., 2009; ▲, Beaulieu et al., 2009; ×, Zier-Rush et al., +, 2009; Boyd and Johnston, 1997; ●, Campbell, 1997; −, Lopez-Bote et al., 1997; ♦, Stein et al., 1996)

A visual examination of this data might suggest a curvilinear response, perhaps with pigs offered feeds with less than 13 MJ DE/kg compensating poorly, and those over 14 MJ DE/kg showing greater compensation. However, energy compensation appears poorer at lighter weights (Beaulieu, 2009; Boyd, 2009) so such an interpretation will be compromised by the weight range of the trials. For example, Stein *et al.* (1996) grew pigs from approximately 50-112kg, whereas Boyd and Johnson (1997), where less compensation was evident, started at 25kg. The relationship between growth rate and energy concentration (Figure 2) suggests only a modest increase in growth rate at higher energy densities when the entire growth period is considered.

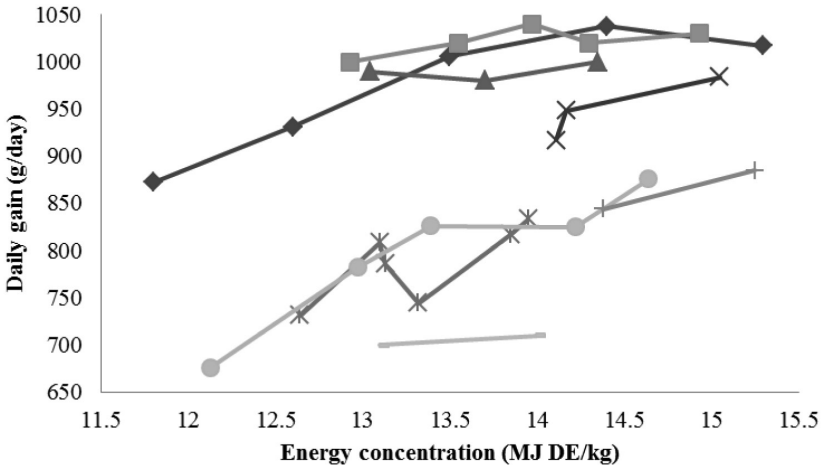

Figure 2. *Relationship between feed digestible energy concentration and daily gain.*
(✳, Magowan et al., 2010; ■, Beaulieu et al., 2009; ▲, Beaulieu et al., 2009; ×, Zier-Rush et al., +, 2009; Boyd and Johnston, 1997; ●, Campbell, 1997; −, Lopez-Bote et al., 1997; ♦, Stein et al., 1996)

Increasing energy intake increases growth rate (Figure 3) but the rate of increase varies between trials. The Campbell (1997) data gives a slope of 25g gain/MJ DE ($P<0.001$), the Prairie Research Centre trial of Beaulieu *et al.* (2009), 16g gain/MJ DE ($P<0.01$), whilst that of Stein *et al.* (1996), 20g gain/MJ DE ($P<0.01$), although this latter trial contains two feeding treatments where amino acid intake was low. In marked contrast a much higher growth increase of 46g gain/MJ DE was apparent in the research of Magowan *et al.* (2010).

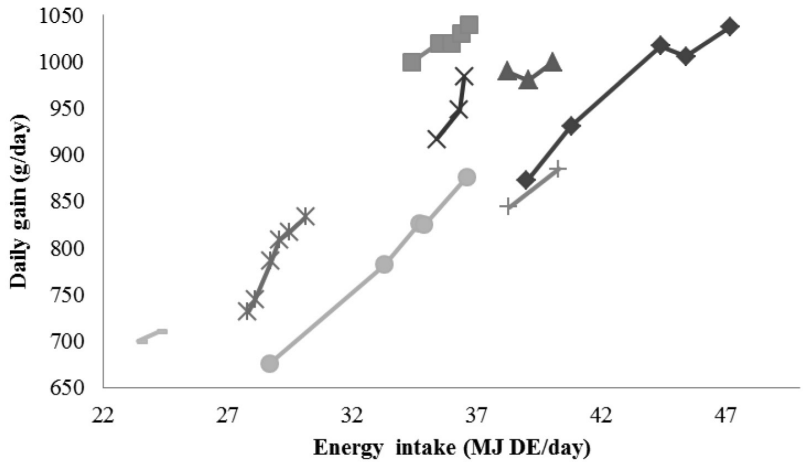

Figure 3. *Relationship between feed digestible energy intake and growth rate.*
(✳, Magowan et al., 2010; ■, Beaulieu et al., 2009; ▲, Beaulieu et al., 2009; ×, Zier-Rush et al., +, 2009; Boyd and Johnston, 1997; ●, Campbell, 1997; −, Lopez-Bote et al., 1997; ♦, Stein et al., 1996)

It is suggested that there are two factors contributing to this variation. The first is the extent to which energy intake is limiting lean gain. The lighter the pigs initially on trial, and the higher the genetic merit, the greater the likely response to additional energy providing that amino acid intake is not limiting. Thus for the first 21 days of the commercial trial conducted by Beaulieu *et al.* (2009), from approximately 37-50kg bodyweight, the growth response was 30g gain/MJ DE/day (P<0.001), whilst in the second period, to approximately 71kg) it was 27g gain/MJ DE (P<0.05). ARC (1981) suggested a lipid deposition rate of 17.9g gain/MJ DE.

A second factor to consider is net energy. It is well documented that digestible energy overvalues the energy content of protein and fibre and undervalues that of fat. Thus, for example, in the case of Stein *et al.* (1996) the NE/DE of the lowest energy feed is estimated at 0.688 whilst that of the highest feed is 0.742. The growth response of 20g gain/MJ DE is only marginally higher on a net energy basis, 21.8g gain/MJ NE. Similarly with the trial of Campbell (1997) 25g gain/MJ DE becomes 28.7g gain/MJ NE. Whittemore *et al.* (2003) suggests 25g gain/MJ NE. As energy concentration increases, feed conversion falls, with an average improvement of around 0.2 for each 1MJ DE/kg in the feed (Figure 4).

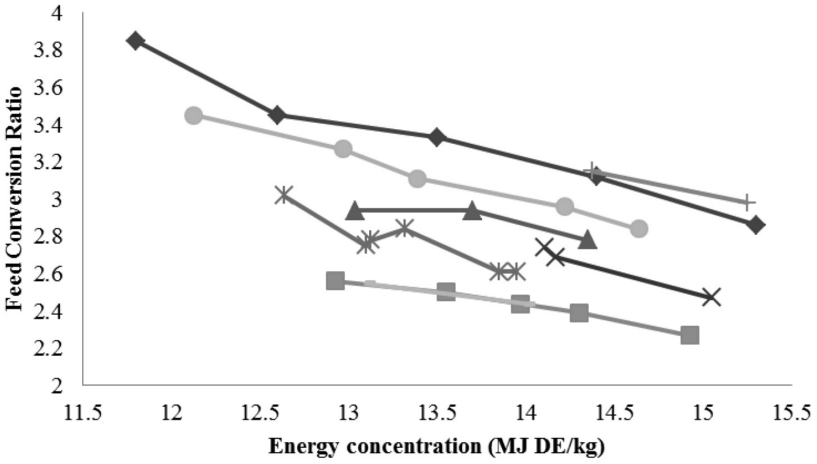

Figure 4. *Relationship between feed digestible energy concentration and feed conversion.*
(✳, Magowan et al., 2010; ■, Beaulieu et al., 2009; ▲, Beaulieu et al., 2009; ×, Zier-Rush et al., +, 2009; Boyd and Johnston, 1997; ●, Campbell, 1997; −, Lopez-Bote et al., 1997; ♦, Stein et al., 1996)

In the majority of papers an increase in both killing-out percentage (Figure 5) and carcase fatness was evident with increasing energy concentration. Again consolidation of this data should be viewed with caution due to differences in slaughter weight, whether the carcase is "head on" or "head off", and the position, and method, of carcase fatness determination. The correlation between killing-out percentage and digestible energy density in the data sets of Stein *et al.* (1997), with a slope of 0.67, Beaulieu *et al.* (2009) with a slope of 0.52, and Campbell (1997) with a slope of 0.92, were significant (P<0.05). It is suggested that improvements in carcase yield with increasing nutrient density is a function both of the pigs being fatter, and of being fed lower fibre feeds resulting in reduced weight of digestive tract.

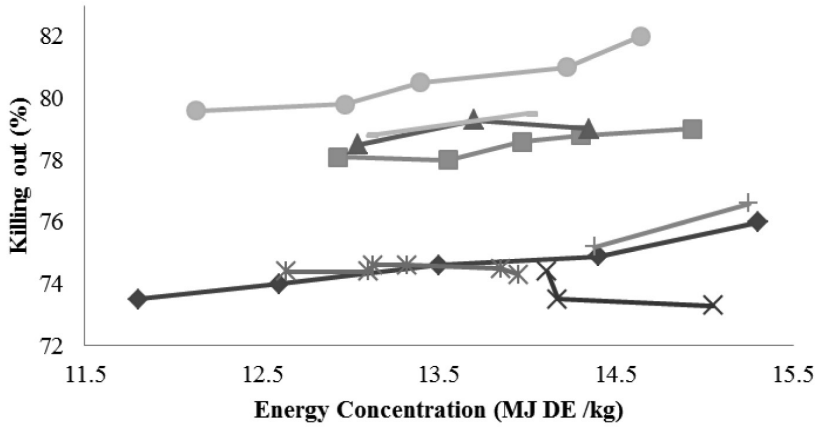

Figure 5. *Relationship between feed digestible energy concentration and killing-out percentage. (✳, Magowan et al., 2010; ■, Beaulieu et al., 2009; ▲, Beaulieu et al., 2009; ×, Zier-Rush et al., +, 2009; Boyd and Johnston, 1997; ●, Campbell, 1997; −, Lopez-Bote et al., 1997; ♦, Stein et al., 1996)*

Factors That May Influence Energy Compensation

It is evident that there is considerable variation noted in the literature in the ability of pigs to maintain their energy intake when fed feeds of varying energy density. Some of these factors (pig weight range, range in feed energy concentration) have already been commented upon. This paper does not attempt to look at other factors in any detail but the following are just some of those that are likely to be of importance when considering energy compensation.

1. Feed availability – hopper numbers, design, adjustment, number of feeds, feeding time.
2. Bulk – higher fibre feeds (including intake of straw on bedded systems).
3. Feed form – meal versus pellets, grist.
4. Feed palatability – eg. glucosinolates.
5. Water availability and flow rates.
6. Stocking density, group size and social effects.
7. Environment – temperature, noxious gasses, humidity.
8. Genetics.
9. Disease.
10. Amino acid balance.
11. Previous nutrition, adaptation.

Unfortunately in a practical farm situation the influence of many of the above factors are almost impossible to quantify. Where feed intakes look relatively high (after considering the genotype, health, buildings etc) there may be logic in suggesting there is a better opportunity for energy compensation than when intakes appear low. However, it is interesting that the pigs in the research of Lopez-Bote *et al* (1997), which had a very low feed intake, still showed a significant decrease in feed intake with increasing energy concentration.

Commercial Interpretation – How Best Do We Use This Information in Routine Feed Formulations?

There are various levels of sophistication that we can apply to dose response data in order to develop finishing feed specifications. Comprehensive pig growth models can be useful, particularly when setting up feed specifications for the first time, or when there is a major change to economic or physical data. However these can be very time consuming, need to be run by a nutritionist (many feed formulations are routinely run by feed formulators rather than nutritionists), and often the input data required for the model is not available. There is consequently a requirement for simpler "rules of thumb" or methodologies that can be used on a day-to-day basis when advising pig producers and formulating feeds.

Table 10. *Calculated growth rate and feed conversion of finishing pigs (65-105kg), showing either no or full energy compensation, assuming a growth increase of 23g/day per MJ digestible energy (DE).*

		Digestible Energy (MJ/kg)					
No energy compensation*		12.75	13.00	13.25	13.50	13.75	14.00
Feed intake	kg/day	2.6	2.6	2.6	2.6	2.6	2.6
Energy intake	MJ DE/day	33.15	33.8	34.45	35.1	35.75	36.4
Growth rate	g/day	805	820	835	850	865	880
Feed conversion rate		3.23	3.17	3.11	3.06	3.01	2.95
Full energy compensation*							
Feed intake	kg/day	2.75	2.70	2.65	2.60	2.55	2.51
Energy intake	MJ DE/day	35.1	35.1	35.1	35.1	35.1	35.1
Growth rate	g/day	850	850	850	850	850	850
Feed conversion rate		3.24	3.18	3.12	3.06	3.00	2.95

* With no energy compensation feed intake remains constant; with full energy compensation energy intake remains static

A simple starting point is to estimate growth and feed conversion, across a range of energy concentrations, and from this determine feed cost/kg live weight gain. An example is given for pigs from 65-105kg live-weight using a growth response of 23g gain/MJ DE (Table 10). Interestingly in this example the degree of energy compensation has little influence on feed conversion at a particular energy concentration.

In order to examine the economics of energy density, two series of feeds were formulated using differing raw material cost-sets. In the first (Table 11) barley is £7/T less expensive and wheat feed £15/T less expensive, than wheat. Extracted rape meal is £100/T less expensive than soya meal, and soya oil is £850/T. These price differentials are typical of those in the UK in 2010. Increasing energy density initially leads to a replacement of wheat feed by barley and then the barley by wheat. At higher energy levels rape seed meal is replaced by soya bean meal and fat addition is increased. In this example, using the feed conversation calculated in Table 10, feed cost per kg live weight gain is minimized at around 13.6 MJ DE/kg, irrespective of the degree of energy compensation.

Using Financial information From Least-Cost Feed Formulations

There are two "unit cost" outputs produced when a least-cost feed formulation is generated that are useful when considering the economics of energy concentration. Firstly, energy unit cost shows that at 12.75 MJ DE/kg there is no cost to energy (Table 12), as it is "floating" above the minimum, but the cost increases to almost £27/MJ DE in the feed with the highest energy density, when the additional energy is from expensive soybean oil.

Previously it has been estimated that feed cost/kg live weight was minimized at around 13.6 MJ DE/kg (Table 11) assuming a daily growth response of 23g/MJ DE. At this energy concentration the unit cost of energy is about £11.51/MJ DE (Table 12); this is the value of DE and can be used on a routine basis as a simple check when formulating feeds. In other words if, having formulated a feed, the unit cost of energy is say £5/MJ DE then there are savings in feed costs by using a feed of higher energy concentration. The value of energy determined in this example is of course specific to this specification and the costs used, assumes that the intake of other nutrients is none limiting, and that the daily response in growth rate averages 23g/MJ DE.

Secondly, bulk or volume unit cost provides useful information. The bulk constraint indicates optimum nutrient concentration In other words are the nutrients specified best supplied in 1000kg of feed, or, for example, could the same nutrients be supplied more cost effectively in 950kg of feed (a higher nutrient density)? Returning to the example formulations (Table 11) formulating the feed at 12.75MJ DE/kg the bulk sensitivity is £200/unit and indicates that bulk needs to "drop", indicating the feed should be higher in nutrient density (Table 12). Bulk unit cost changes from "drop" to "rise" between 13.6 and 13.75 MJ DE/kg so that the optimum nutrient density lies in this range. Note that this is a similar result to that when energy unit cost was considered previously (13.6 MJ DE/kg).

Table 11. *Feed formulations at a range of dietary energy concentrations and estimated feed cost/kg live-weight gain assuming no, and full, energy compensation.*

		Digestible Energy (MJ/kg)						
	£/T	12.75	13	13.25	13.5	13.6	13.75	14
Wheat	205	18.3	25.2	45.2	50	50	50	50
Barley	198	35	35	15	16.5	19.8	22.8	25.5
Wheatfeed	190	21.8	13.8	14	6.3	2.5	0	0
Rape meal	190	12.5	12.5	12.5	12.5	12.5	9.6	0.9
Soya 48	290	9.7	10.8	11	12.1	12.7	14.9	20.5
Soya oil	850	0.5	0.5	0.5	0.7	0.8	0.9	1.1
Mins/vits/aminos		to 100	to 100	to 100	to 100	to 100	to 100	to 100
Raw material cost	£/t	214.6	216.5	218.9	221.66	222.81	225.81	232.43
Delivered feed cost*	£/t	239.6	241.5	243.9	246.66	247.81	250.81	257.43
Cost/kg live weight (no energy compensation)	p/kg	77.4	76.6	75.9	75.4	75.3	75.4	76.1
Cost/kg live weight (full energy compensation)	p/kg	77.6	76.7	76.0	75.4	75.2	75.3	75.9

*Delivered feed cost includes £25/t for manufacture and delivery. Exchange rate 1.56 US$/£ sterling; 1.46A$/£ sterling, July 2011

Table 12. *Energy and bulk unit costs outputs from a least cost formulation program for the formulations shown in Table 11.*

		Digestible Energy (MJ/kg)						
	£/t	12.75	13	13.25	13.5	13.6	13.75	14
DE unit cost	£/MJ	0	7.93	10.95	11.51	11.51	26.22	26.8
Bulk unit cost	£/unit	200_{drop*}	82.8_{drop}	32.3_{drop}	27.1_{drop}	27.1_{drop}	26.2_{rise*}	153_{rise}

* "drop" indicates a feed where the nutrient cost would be less should they be supplied in a lower bulk, that is higher concentration.

In practice is not necessary to run a range of feeds to determine the optimum nutrient concentration as the bulk constraint in the feed specification, normally set to one or 100, can be removed. Formulating the feed without a bulk constraint effectively gives a least-cost solution for the nutrients specified, and will indicate at what bulk this occurs. For example, if the 12.75 MJ DE/kg feed is formulated without a bulk constraint, a bulk of 925kg is indicated suggesting that nutrient density is minimized at 13.78 MJ DE/kg (ie. 12.75/0.925). Additionally cost savings are readily determined. At 12.75 MJ DE/kg the feed cost was £214.60/T and after removal of the bulk constraint it was £211.00/T. Thus there is a saving of £3.60/T for the same amount of nutrients; the higher energy feed is of course more expensive per tonne at £228.11/T (£211/0.925). Additionally there are further savings as less feed has to be manufactured and delivered.

Determining optimum bulk by removing the bulk constraint is very simple and quick. It does not take account of feed manufacturing and delivery costs, and so will underestimate optimum nutrient concentration. Further it also results in a change in raw material and nutrient minimum and maximum constraints. For example in this example the feed having 12.75 MJ DE/kg had 12.5% of rapeseed meal, the maximum constraint. By removing the bulk constraint, 125kg of rapeseed meal is still being used, but in 925kg of feed, so effectively an increased inclusion rate of 13.5%. Of course if pigs maintain their energy intake by eating less of the higher density feed then daily rapeseed meal intake won't change.

In the spring of 2011 the UK experienced atypical raw material costs in that wheat was £205/T, but barley and wheat feed were relatively inexpensive at £175/T and £140/T. Repeating the above formulation exercise using these costs resulted in feed cost/kg live weight being minimized at a much lower 12.8 MJ DE/kg with a DE unit cost of £12/MJ. Formulating the 12.75 MJ DE/kg feed without a bulk constraint suggested that the optimum bulk was 996kg, in other words this was already very close to the optimum nutrient density, and again is in good agreement with the value derived from the examination of energy unit cost.

The example discussed to date relates to a finisher feed offered to pigs from 65kg.. At lighter weights it has been shown that both the degree of energy compensation is reduced and the growth response to the additional energy consumed is greater. A further exercise was therefore conducted using a feed specified for 30-65 kg pigs and using a higher value of 30g daily gain/MJ DE. Using 2010 raw material costs, feed cost/kg live weight gain was minimized with an energy concentration of 14 MJ DE/kg, and an energy unit cost of £13/MJ DE. Formulating by removing the bulk constraint revealed that the optimum nutrient density also occurred at 14 MJ DE/kg.

Using the 2011 costs, it was estimated by both methods that feed cost was minimized in the 30-65kg period with an energy concentration of 13.2 MJ DE/kg. This is a low energy density compared to normal commercial practice and raises further issues. If energy compensation is poor, which is likely, growth rate may decline noticeably. To what extent changes in growth rate in one weight period influences that in the next, is a question of much debate. In the research of Beaulieu *et al*. (2009), there was no significant influence on energy density on growth rate from 37-115kg, despite an advantage in growth rate of 90g/day in the first 21 days, and 110g/day in the following 21days. However in that of Zier-Rush *et al*. (2009) pigs fed feed containing 5% additional fat achieved higher early growth rates, and these were maintained until slaughter.

Bulk unit constraint is then a simple initial step in the determination of optimum nutrient density and the potential savings in feed cost (on a live weight basis). Obviously it is important to challenge some the existing constraints within the feed specification. Fibre constraints in particular can become costly and will limit nutrient density reductions unless relaxed. However relaxation on the fibre constraints will at some ill-defined point limit feed intake, and reduce the ability of pigs to maintain energy intake.

The above calculations and formulations allow the commercial nutritionist to assess the likely impact of changes to energy or nutrient density on growth, feed conversion and feed cost per unit of live weight gain. To complete the financial picture killing-out percentage and carcase fatness needs to be included. Killing out percentage, and carcase fatness, both increase with increasing energy density. However, the extent of the increase will depend upon the length of time that the feeds are offered. Thus simple averaging of the regression coefficients for carcase fatness, or killing-out percentage against energy concentration from the above trials is compromised by time. Further final pig weight in a number of the trials varies which further complicates the interpretation of the killing-out and carcass fatness data. Dynamic growth models should be used to predict changes in carcass fatness and killing-out percentage enabling the overall economic impact of energy concentration to be determined.

Finally, as a word of caution, the majority of the data in the papers reviewed contained feeds over 13 MJ DE/kg. Only three papers contained feeds of lower energy density. Of these that of Magowan *et al*. (2010) had only one treatment below 13 MJ DE/kg, which was at 12.7 MJ DE/kg (and performed relatively poorly), and the low energy feeds used by Stein *et al*. (1996) supplied lower daily intakes of digestible lysine. The data from Campbell (1997) indicated a marked reduction in growth rate with a feed of 12.1 MJ DE/kg compared to 13 MJ DE/kg, despite being fed from 60kg, and even if the data is corrected for final weight. A number of factors may combine that result in feeds below (a nominal) 13 MJ DE/kg performing poorer than anticipated. Such feeds are relatively low in net energy and they are higher in fibre and water holding capacity, which will increasingly limit energy intake. Further our assumed amino acid digestibility values may not be valid in high fibre feeds and amino acid balance may need to change because, for example, of increased mucin production. Finally in none of the reviewed experiments were pigs housed in straw based systems. As pigs eat substantial quantities of straw it is possible that the optimum nutrient density is higher on such a system than when bedding is not available. Further research on low energy feeds would be welcome and is needed

Conclusions

Whilst many factors influence feed intake, in the majority of data reviewed, feed intake fell with increasing energy density. In most cases this was insufficient to prevent an increase in energy intake, particularly at lighter live weights. The higher energy intake resulted in increased growth rate, carcase fatness, and a higher killing-out percentage. At lighter weights the growth response to increased energy intake was greater, presumably as energy was limiting lean growth. The use of energy and bulk unit cost from least-cost formulations can both be used effectively to indicate optimal feed cost, and generally gave similar results. The removal of the bulk constraint is recommended as a simple and quick initial estimation of optimum nutrient concentration which can be refined by additionally considering feed manufacturing and delivery costs, killing-out percentage and carcase fatness. However more research is required on the responses of pigs to feeds with energy levels of less than 13 MJ DE/kg.

Symposium Conclusions

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There is always a large focus on feed conversion in pig research and commercial production, however, little focus is placed on the factors that affect voluntary feed intake. One reason for this is that feed intake is difficult to measure commercially. Additionally, the genetic gains in recent times means the modern pig is potentially more sensitive to different dietary factors, whether it has a negative or positive effect on daily feed intake. This is particularly important for pigs between 35 and 110kg live weight, as 65 to 70% of the total feed consumption occurs during this period of growth, and it is critical that the dietary components such as energy and fibre are fully optimized to insure cost effective growth. Hazzeldine (2011) demonstrated this particularly well, with the knowledge of how daily energy intake is influenced by energy density and "bulk", using linear programming to set the optimal energy density to maximize profitability in commercial operations.

Black *et al*. (2011) highlighted that the capacity of the individual animals to consume sufficient energy for optimal energy metabolism is limited by various dietary, climatic, disease and social constraints. Black *et al*. (2011) also pointed out that a greater understanding is required on how the variation in the physical nature of indigestible fibre, and the source of other influential nutrients, interact to alter rate of passage of digesta to increase feed intake with the negative effects of the intestinal brake that reduces intake.

The impact of taste and flavour in the pig is much more than the simple definition of the five different tastes accepted by the scientific community. Roura (2011) explained the term chemosensing (taste and smell) and its importance for determining preference for feed ingredients and its influence on metabolic processes affecting overall animal metabolism and intake. The paper explained that taste is also influenced by "taste receptors" along the whole of the gastro-intestinal tract, and how certain tastes and smells can influence the intake of important nutrients like glucose, as well as affect voluntary feed intake. This new information helps explain past positive intake responses to particular amino acids and carbohydrates, and opens up another great area for further research.

We hope this symposium will set a new platform for future discussion and research into the manipulation of voluntary feed intake in modern genotypes and help improve the efficiency of the Australian pork Industry.

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Kinetics of Digestion in Grower Pigs Fed Sorghum and Barley Diets

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An important factor limiting the use of sorghum by pigs is its slower rate of starch digestion relative to other cereal grains (Al-Rabadi *et al*., 2009). It is hypothesised that the starch granule/protein matrix and other factors in sorghum limit the rate and extent of digestion of starch in the small intestine which potentially influences the site and extent of starch fermentation in the digestive tract as a whole. This experiment was undertaken to examine the kinetics of digestion in cannulated grower pigs fed sorghum and barley-based diets.

Three grains, a white sorghum variety (Liberty), a red sorghum variety (Bonus) and a barley variety (Gardiner) were included in grower diets as described by Cadogan *et al*. (2011). Quantities of each diet were sprayed with a solution containing liquid chromium ethylenediaminetetraacetic acid (CrEDTA) and a solid phase marker (ytterbium chloride, Yb). The marked mixture was incorporated into each diet to provide approximately 50mg Cr and 50 mg Yb per kg of feed. Known weights (~200g) of the marked diets were then fed to three ileal cannulated pigs (55±1.2kg initial live weight) per diet prior to their normal feed, which was offered *ad libitum*. Samples of ileal and faecal material were obtained at intervals over a 72h period to estimate marker passage rate (Grovum and Williams, 1973). Treatments were allocated to individual pigs according to a randomised block design. Sample site and grain variety effects were assessed using a generalised linear model procedure.

Table 1. *Intake and marker clearance parameters for grower pigs fed diets containing sorghum varieties (Liberty or Bonus) or barley (Gardiner).*

	Liberty	Bonus	Gardiner	Liberty	Bonus	Gardiner	SEM	P value
N	3	3	3*					
Live weight (kg)	59.8	58.9	60.9				1.02	0.75
Intake (kg/day)	1.75	1.80	1.84				0.02	0.39
Sample		Ileum			Faeces			Variety
k (Cr/h)	-0.147	-0.210	-0.202	-0.060	-0.068	-0.073	0.015	0.346
k (Yb/h)	-0.179	-0.228	-0.178	-0.058	-0.086	-0.099	0.020	0.594
pt (Cr/h)	3.3	3.7	4.3	56	58	43	2.1	0.100
pt (Yb/h)	4.0	4.7	3.0	56	58	42	2.1	0.076

SEM, Pooled Standard Error of the Mean; k, clearance rate. proportion of dose per hour; Cr, Chromium; Yb, Ytterbium; pt, peak time; time to maximum marker concentration; *no faecal data for animal 3.

Both markers reached peak concentrations in ileal digesta within hours of feeding and showed clearance rates (k) from the ileum roughly three times ($P<0.001$) that found in faecal samples (Table 1). Appearance of Cr and Yb markers in the faeces showed numerical differences ($0.076<P<0.1$) between the two sorghum diets (Liberty and Bonus) and the barley diet (Gardiner) that were consistent with a reduced rate of passage for both markers through the hindgut of sorghum-fed animals. Differences in the appearance of marker in the faeces of sorghum and barley-fed pigs would support the view that there may be quantitative differences in the site as well as extent of digestion of sorghum relative to other cereal grains.

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Variation in Wheat Digestible Energy and Crude Protein Content Measured Online by Near Infrared Spectroscopy

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Near Infrared Spectroscopy (NIRS) is used to routinely measure the nutritional composition of raw materials and manufactured diets during stockfeed production. The Australian pork industry has invested heavily in the development of NIRS calibrations for the nutritional composition of cereals including wheat, barley, triticale, sorghum and maize (Black *et al*., 2010). Commercial calibrations (AusScan Calibrations, Pork CRC Ltd, Roseworthy, SA) are now available to rapidly measure over 15 different cereal nutrients, including digestible energy (DE) to within ±0.27 MJ/kg based on the calibration standard error of cross validation. NIRS technology has also improved, with robust on-line units capable of taking a reading every 6 seconds now available that can be located on grain conveyors and at other strategic locations within the mill. A survey was conducted using AusScan Calibrations and online NIRS technology to test the hypothesis that weekly and monthly variation in wheat DE and crude protein (CP) is significant and needs to be routinely monitored if mill and livestock production efficiency is to be optimised.

To complete the analysis for this survey, an online NIRS system (NIR-Online® GmbH, Walldorf, Germany), complete with AusScan Calibrations for DE and CP, was installed in a commercial feedmill on an air assisted grain conveyor just before the hammermill. The NIRS took a spectral scan of whole wheat every 0.04 seconds, and recorded the mean of these spectra over each milling batch. The recorded data was then filtered to exclude any batch under 5 minutes, and analysed statistically over the whole 10 month period to determine variation in DE and CP.

Table 1. *Variation in wheat digestible energy* (DE, MJ/kg) and crude protein* (CP, %) content in a commercial Feedmill over a 10 month survey period determined using online near infrared spectroscopy.*

			Period of Highest Variation				Period of Lowest Variation			
	Total Period		Month		Week		Month		Week	
	DE	CP	DE	CP	DE	CP	DE	CP	DE	CP
Mean	13.1	13.1	13.0	12.7	12.8	13.2	13.1	13.7	13.1	13.6
Minimum	8.6	4.0	10.0	4.0	10.5	8.3	10.9	9.5	12.4	11.3
Maximum	14.1	16.4	14.1	15.8	13.6	14.3	13.7	15.6	13.5	14.7
Std Dev	0.46	1.25	0.64	1.57	0.77	1.30	0.35	0.94	0.20	0.68
CV (%)	3.54	9.51	4.91	12.4	6.05	9.88	2.66	6.84	1.50	5.2

Std Dev, standard deviation; CV, coefficient of variation; *All data is reported on an as received basis.

Prior to the development of the AusScan Calibrations, *in vitro* analysis of DE content using data derived from *in vivo* experimentation was not possible, nor was real time analysis without NIRS. Data derived from this survey confirms that variation in DE and CP content is significant and unless real-time analysis can be incorporated into mills, stockfeed and livestock production efficiency will be compromised (Table 1). Variations in DE content of 5.5 MJ/kg means that diet formulations will not be optimised for cost per unit of DE, and pigs fed these diets will experience periods where the diet composition does not match their specific nutrient requirements. Further, most Australian nutritionists would apply a wheat DE value of between 14.0 and 14.2 MJ/kg in diet formulations (King, 1999), so in this case diet DE contents would be below expectations for the 10 month survey period. It is also concerning to note that variations of up to 3.1 MJ/kg can occur over a seven day period and up to 4.1 MJ/kg over a month. Based on this data, the single most effective way for Australian pork producers to improve production efficiency would be to develop ways to account for this variation in DE and CP prior to diet manufacture.

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CHAPTER 4

Feed Additives and Grains

The Use of Sodium Bromide to Influence Feed Intake of Finisher Pigs During Summer

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Feed intake of growing pigs declines during the hot summer months typical of Southern Australia. As a direct result, growth rates also reduce and pigs take longer to reach target slaughter weights. Summer is also a period of peak pork sales in the lead up to Christmas so strategies to maintain growth performance during this time are worthy of investigation. Previous studies have shown that the addition of bromide to the finisher diet can enhance feed consumption in group housed entire boars (Dunshea *et al*., 2000). The aim of this experiment was to determine if sodium bromide (NaBr) could be used as a strategy during summer to improve feed intake and therefore growth rates of finisher pigs. The experiment tested the hypothesis that NaBr increases feed intake of finisher pigs (female, entire male and immunocastrated (Improvac®, Pfizer Animal Health Pty Ltd, West Ryde, NSW) male pigs) when included in the diet offered during summer.

A total of 792 pigs (Large White x Landrace, PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) were selected at 17 weeks of age (mean weight 64.6kg ± 0.24 kg) and housed in pens of nine pigs of the same sex. Pens were randomly allocated to a 2 x 3 factorial experiment with the respective factors being dietary NaBr (0 or 0.04 g/kg) and sex (female, entire male and Improvac® vaccinated males). The experiment was conducted from late November 2010 to February 2011. Diets were formulated to contain 0.52 g available lysine/ MJ digestible energy (DE) and 13.8 MJ DE/kg. Pen weights were recorded at d 0, 14 and 35 of the experiment and pen feed intake (FI) measured by feed disappearance during these periods. All deaths and removals were taken into account when calculating FI and feed efficiency by the adjustment of the number of days that pigs were on trial. Pigs were slaughtered in a commercial abattoir at the conclusion of the 35 d test period. Data were analysed using a residual maximum likelihood (REML) mixed model analysis. The model included the random effect of replicate and the fixed effects of the 2 x 3 factorial for NaBr and sex. The experimental unit for all analyses was the pen of pigs.

Table 1. *Effect of dietary sodium bromide (NaBr) on feed intake and growth performance of finisher pigs (d119-154).*

Dietary treatment	ADG (g/d)	ADFI (kg/d)	FCR (kg/kg)	HSCW (kg)
Control	852.0	2.39	2.81	72.2
NaBr	872.0	2.47	2.85	72.6
SED	17.50	0.036	0.037	0.48
P-value	0.27	0.020	0.37	0.38

ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; HSCW, hot standard carcase weight; SED, standard error of difference.

The response to NaBr supplementation was similar across the sexes, with no significant interactions between sex and NaBr. As such, only the main effects of NaBr supplementation are displayed in Table 1. Pigs offered the NaBr diet consumed more feed, however the magnitude of the response was insufficient to increase growth rate. There was no impact of NaBr supplementation on carcase weight, P2 or dressing percentage. Climatic conditions during the test period were mild, with only six days in which the maximum temperature exceeded 35°C. It is unclear if a greater response may have been obtained under more 'normal' climatic conditions. The results from this experiment support the hypothesis that NaBr can enhance feed intake of group housed finisher pigs. However, as the improvement in feed intake was modest and did not translate into enhanced growth, the use of NaBr at the dose tested here to maintain the performance of finisher pigs during summer is not supported by this experiment.

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Phytase Improves the Digestibility of Minerals and Essential Amino Acids in Barley and Sorghum-Based Diets

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Most phytase research has been conducted on maize and wheat-based diets in pigs, demonstrating significant improvements in Phosphorus (P), Calcium (Ca) and essential amino acid (EAA) digestibility (Selle and Ravindran, 2008) but there is very limited information on pig performance when phytase is included in barley or sorghum-based diets. The aim of this experiment was to test the hypothesis that exogenous phytase increases the digestibility of minerals and EAA's in both barley and sorghum-based diets.

Three grains, barley (Gairdner), red sorghum (Buster) and yellow sorghum (Liberty) were incorporated at 800g/kg into grower diets, providing 13.6 MJ digestible energy (DE)/kg and 0.65g available lysine/MJ DE. Each diet contained two levels of phytase (0 or 500 FTU/kg Phyzyme™, Danisco Animal Nutrition, Marlborough, UK) producing a 3x2 factorial design. Six pigs, surgically fitted with a T-cannula, were housed in individual metabolism crates and each received each experimental diet over a six week period (3 x maintenance; five day adaptation plus two day collection; van Barneveld, 1999). Collected ileal digesta and faeces were freezed dried, and analysed for the digestibility marker (Celite), minerals, amino acids and gross energy.

Table 1. *The effects of grain type and phytase (FTU/kg) on the ileal digestibility coefficients (%) of minerals, essential amino acids and digestible energy (DE).*

Grain	Phytase	P	Ca	Lysine	Met	Cys	Thr	Iso	Try	DE
Gairdner (B)	0	49.1	52.2	87.2	88.9	73.1	73.7	77.4	76.6	71.0
Gairdner (B)	500	63.4	59.3	88.5	89.8	75.7	79.1	81.7	77.7	71.0
Buster (S)	0	47.2	47.0	87.3	87.1	68.0	73.9	80.3	76.5	74.0
Buster (S)	500	58.1	59.4	89.7	89.7	68.8	77.0	81.3	77.8	76.3
Liberty (S)	0	45.9	43.0	88.0	91.7	74.7	78.4	84.0	80.2	76.2
Liberty (S)	500	51.4	54.1	88.0	91.7	73.8	78.7	84.0	81.4	77.4
Grain (G)		<0.001	0.027	<0.001	0.001	<0.001	0.035	0.001	0.004	<0.001
Phytase (P)		<0.001	<0.001	0.017	0.004	0.212	<0.001	0.006	NS	NS
G x P		NS	NS	NS	0.042	NS	0.015	0.007	NS	NS

Met, Methionine; Cys, Cystine; Thr, Threonine; Iso, Isoleucine; Try, Tryptophan; NS, not significant; B, barley; S, sorghum

There was a significant effect of grain type on ileal digestibility of P, Ca, all the EAA's and DE. The yellow sorghum produced lower mineral digestibility than the other two grains, and the red sorghum had inferior ileal EAA digestibility compared to the barley and yellow sorghum. The presence of phytase improved P, Ca and EAA digestibility ($P<0.05$) except for cystine and tryptophan, and had no effect on ileal DE ($P>0.05$). Barley and red sorghum were the most responsive to phytase in terms of ileal EAA digestibility, whereas the yellow sorghum showed little to no response, with interactions on methionine, threonine and isoleucine ($P<0.05$). There was also an interaction between grain type and phytase on faecal P and Ca digestibility ($P<0.05$), with phytase improving barley P and Ca by 28% and 22%, respectively, whereas phytase had a more pronounced effect on Ca (23%), compared to P (17%), in both sorghums (data not shown). Phytase improves mineral and EAA digestibility, although grain type may influence the magnitude of the response.

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SELLE P.H., RAVINDRAN V. (2008). *Livestock Science*. **113**:99–122.

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Protease Supplementation of Sorghum Improves Growth Performance of Young Pigs

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Few studies have demonstrated the use of protease in sorghum-based diets for pigs, however, protease has been used in combination with other enzymes to improve performance of poultry fed sorghum (Selle *et al.*, 2010). The aim of this experiment was to test the hypothesis that protease will improve endogenous amylase access to sorghum starch granules by demonstrating that graded addition of protease to sorghum-based diets will improve pig performance relative to those fed a wheat-based diet.

One hundred and twenty-six, individually housed, entire male pigs (Primegro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) were selected at 22 d of age (7.0±1.1 kg). A total of nine experimental diets were offered (14 pigs/treatment) for a period of 21 d in 2x4 factorial arrangement with the factors being a) sorghum variety (yellow sorghum (Liberty) or red sorghum (Buster)), and b) protease (Subtilisin, Danisco Animal Nutrition, Marlborough, UK) dose (0, 50, 100 or 500 ppm)), plus a control dietary treatment based on wheat. Base diets were formulated to be iso-energetic (14.6 MJ digestible energy (DE)/kg; 0.90 g available lysine/MJ DE) and included 650 g/kg of the test grains, respectively. All diets contained titanium dioxide as an indigestible marker so that dietary DE content could be measured following analysis of pooled faeces samples collected on d 14 and 16.

Table 1. *Growth response of weaned pigs (7±1.1kg) and digestible energy (DE) content of a wheat-based diet and sorghum-based diets containing graded levels of protease (0, 50, 100, or 500 ppm) and fed to for 21 d.*

Grain type	Protease (ppm)	ROG (kg/d)	FCR	ADI (kg/d)	DE (MJ/kg)
Wheat (Control)	0	0.48^{a}	1.30^{b}	0.62^{a}	14.87^{a}
Yellow Sorghum (Liberty)	0	0.40^{b}	1.56^{a}	0.61^{ab}	14.87^{a}
	50	0.43^{ab}	1.37^{b}	0.60^{ab}	14.82^{a}
	100	0.39^{b}	1.43^{ab}	0.60^{ab}	14.77^{a}
	500	0.44^{ab}	1.31^{b}	0.57^{ab}	14.73^{a}
Red sorghum (Buster)	0	0.38^{b}	1.43^{ab}	0.54^{b}	14.28^{b}
	50	0.41^{ab}	1.49^{ab}	0.63^{a}	14.29^{b}
	100	0.38^{b}	1.39^{ab}	0.53^{b}	14.62^{a}
	500	0.41^{ab}	1.33^{b}	0.54^{b}	14.70^{a}
SEM		9.2	0.023	11.6	0.043
P values					
Protease (P)		0.329	0.046	0.159	0.187
Grain (G)		0.013	0.043	0.296	<0.001
G x P		0.993	0.359	0.453	0.035

^{ab}Values in a column with different superscripts differ significantly (P<0.05). ROG, rate of gain; FCR, feed conversion ratio; ADI, average daily intake; SEM, standard error of mean.

Diets containing red sorghum (Buster) had a significantly (P<0.05) lower DE than diets containing wheat or yellow sorghum (Liberty), with this DE content significantly (P<0.05) enhanced through the addition of 100 or 500 ppm of protease (Table 1). Enhanced DE content did no translate into improved rate of gain (ROG) or feed conversion ratio (FCR) of the pigs fed the red sorghum diets. Protease addition did not affect ROG or average daily intake (ADI) of pigs fed yellow sorghum, but FCR of pigs fed diets containing 50, 100 or 500 ppm of protease was significantly (P<0.05) improved to a level equivalent to that observed in the pigs fed the wheat-based diet. These data suggest that the influence of protein matrices on digestible energy yield differs between sorghum varieties, and that protein matrices may be affecting FCR in pigs fed yellow sorghums without negatively influencing DE yield.

SELLE, P. H., CADOGAN, D. J., RU, Y. J. & PARTRIDGE, G. G. (2010). *International Journal of Poultry Science*. **9**:53-58.

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Feed Conversion Ratio is Improved in Growing Pigs Fed Wheat/Barley-Based Diets Supplemented With Bacterial Xylanase

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Carbohydrase enzymes such as xylanase are used widely in young pig diets (ie <20 kg) to enhance gut health through manipulation of soluble and insoluble non-starch polysaccharides and to improve the digestibility of energy and other nutrients (Partridge, 2001). In older pigs, cost-effective application of carbohydrase enzymes is dependent upon the capacity of the enzyme to improve energy yield from key ingredients and to be able to account for this in the diet formulation. The objective of this experiment was to test the hypothesis that grower pig (25-70 kg) performance can be maintained when fed wheat/barley-based diets reformulated to account for potential digestible energy (DE) uplifts when supplemented with a bacterial xylanase enzyme.

Three wheat/barley-based diets were formulated to assess the efficacy of added bacterial xylanase (Nutrase Xyla, Nutrex, Belgium). All diets contained equivalent amounts of barley (150 g/kg), soybean meal (50 g/kg), sunflower meal (20 g/kg), meat and bone meal (50 g/kg), synthetic amino acids, vitamins and minerals. A control diet (Treatment 1) was formulated to contain 0.7 g available lysine/MJ DE and 14.0 MJ DE/kg with the balance made up of wheat, canola meal, blood meal, tallow, limestone and monocalcium phosphate. The DE content of ingredients containing soluble and insoluble arabinoxylans (barley, wheat, canola meal, soybean meal, sunflower meal) was re-calculated using a proprietary equation (Nutrex, Belgium; Adjusted DE (MJ/kg) = DE + (0.09 x Soluble Aarabinoxylan) + (0.05 x Insoluble Arabinoxylan)) to account for energy yield increases achieved through addition of xylanase at 0.1 g/kg and the diet reformulated to an adjusted DE of 14.0 MJ/kg (unadjusted DE=13.7 MJ/kg; Treatment 2).

Treatment 3 comprised the same diet as Treatment 2 without the addition of xylanase. Diets were offered to 30 individually-housed pigs (10 pigs/treatment) from 25-70 kg based on a randomized complete-block design. Pigs were weighed weekly and their feeding rate adjusted to 3 x maintenance ((3 x $0.5W^{0.75}$)/Diet DE). Rejected feed was collected twice a week and dried in a forced-air oven to facilitate feed intake calculations. Data was subjected to an analysis of variance and means separated by least significant differences (P<0.05).

Table 1: *Average daily gain (g/d) and feed conversion ratio of grower pigs (25-70 kg) fed wheat-barley-based diets with and without xylanase (Nutrase Xyla, Nutrex, Belgium).*

Treatment	Description	Average daily gain (g/d)	Feed conversion ratio
1	Control	913	1.96b
2	Up-specified + xylanase	953	1.82a
3	Up-specified – xylanase	939	1.93b
	Standard error of difference	34.5	0.111
	Probability (diet)	0.502	0.040

[a,b]Means in a column with different superscripts differ significantly (P<0.05).

Feed conversion ratio was significantly improved (P<0.05) in the reformulated diet containing 0.1 g/kg xylanase (Treatment 2; Table 1). Treatments 1 and 2 had the same theoretical DE content and hence should have induced similar growth responses suggesting that we overestimated the DE contribution from these ingredients in the first instance and that the equation used to up-specify the DE content of ingredients was conservative. Overall, addition of xylanase to growing pig diets containing arabinoxylans can facilitate a significant increase in energy yield and reduce diet costs if accounted for at the time of formulation.

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Hydrothermal Treatment of Corn Dried Distiller's Grains with Solubles Reduces Vomitoxin Levels in the Presence of Sodium Metabisulfite

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Mycotoxins are toxic secondary metabolites derived from fungi that can be found in many varieties of grain and forage produced for feed. Deoxynivalenol (DON), also known as vomitoxin, is one of the most frequent contaminants of wheat, corn and barley worldwide. Corn dried distiller's grains with solubles (DDGS), an ethanol by-product, also presents significant problems as mycotoxin levels are more concentrated than in the original corn source. The use of adsorbent feed additives to bind mycotoxins in the digestive tract has shown promise for some mycotoxins, but their efficacy against DON has until now proven ineffective. However, increasing moisture and temperature when aqueous sodium bisulfite is added to DON-contaminated corn has been shown to convert DON to a non-toxic 10-sulfonate adduct (Young *et al*., 1987). Therefore, the aim of this experiment was to examine the effects of varying hydrothermal treatments of DON-contaminated DDGS batches in the presence of sodium metabisulfite (SMB), using a commercial pellet conditioner.

The pellet mill was set to a production rate of 454 kg/h so that temperature and retention rate could be manipulated within each batch. Batches were prepared from a uniform source of DDGS homogenized prior to subsampling with a known DON concentration (23.4 ppm). The four 100% DDGS batches (treatments) included: control, 1.0, 2.5 and 5.0% SMB. Within each batch, pelleted samples were collected at conditioning temperatures of 66 and 82°C and retention times of 30 and 60 seconds within each temperature. Samples were ground, homogenized and sent to North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND, USA) for mycotoxin assays involving a variety of mass spectrometry, ELISA and high-pressure liquid chromatography methods. Data were analyzed for linear and quadratic effects of SMB and interactions with temperature and retention time using GENSTAT (VSN International Ltd., United Kingdom). Since there were no significant effects of retention time, these data have not been presented.

Table 1. *Effect of conditioning temperature (Temp) and dose of sodium metabisulfite (SMB) on deoxynivalenol (DON) and acetyl DON in diets containing naturally DON-contaminated corn distiller's dried grains.*

		Sodium metabisulfite (%)					P-value[1]		
	Temp (°C)	0	1.0	2.5	5.0	SED	Temp	Linear[3]	Quad[3]
Total DON, ppm[2]	66	23.2	12.8	8.2	6.0	1.52	0.25	<0.001	<0.001
	82	21.5	11.5	7.0	6.5				
DON, ppm	66	20.5	10.2	5.6	3.3	1.29	0.15	<0.001	<0.001
	82	18.7	9.0	4.2	3.6				
Acetyl DON, ppm	66	2.7	2.6	2.7	2.8	0.42	0.74	0.45	0.64
	82	2.8	2.5	2.8	3.0				

[1]No significant interactions (P>0.69) between Temp x SMB so P-values not reported;[2]Total DON reported as a combination of DON and 15-Acetyl DON levels; [3]Linear and quadratic effects of SMB; SED, Standard error of the difference for the Temp x SMB interaction. For SED for effect of Temp and SMB multiply by 0.50 and 0.71, respectively; Quad, Quadratic.

Conditioning temperature had no effect on total DON, DON or Acetyl DON levels. However, pelleting DDGS reduced (quadratic; P<0.001) DON and total DON levels as SMB inclusion increased. This indicates that structural modification of the DON-molecule occurs and the resulting DON-sulfonate adduct is not detected by traditional DON assays. Based on these results, it appears that the reduction in DON and total DON levels plateau somewhere between SMB levels of 2.5 and 5.0%. These results imply that hydrothermal treatment in combination with SMB may allow pork producers to more effectively utilize DON-contaminated DDGS, although it is unknown what effect SMB inclusion in pig diets may have on performance.

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Field Pea Phenology: The Effect of Planting Time on Grain Yield of Field Pea (*Pisum sativum L.*) Varieties in Northern New South Wales

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Pork producers in northern New South Wales and south-east Queensland currently lack access to a reliable locally produced source of vegetable protein. Adapted field pea varieties offer pork producers in the north the potential to fill this gap. Crop phenology studies including time of planting experiments have long been recognised as a valuable tool in achieving optimal yield performance in crops (Fletcher *et al.*, 1966). In northern New South Wales, with widely variable seasonal conditions, such experiments are commonplace (Moore, 1999). The objective of this experiment was to determine the genotypic response of grain yield to planting time and identify appropriate planting times for varieties in the region.

Field pea varieties were planted in late April/early May (P1), mid May (P2), mid June (P3) and early July (P4) at Narrabri, NSW in 2009 and 2010. Sixteen lines of field peas, including some commercial varieties, were planted as replicated experiments in a Nearest Neighbours design (a design used to reduce the effect of spatial variation on experimental error variance), in both years. In-crop data was collected on days to 30% flowering (DF30). Detailed meteorological data was also obtained for mean daily temperature (MDT °C) and daily incident solar radiation (ISR Mjm^{-2}/d). . All plots were mechanically harvested at different dates appropriate to maturity, the grain cleaned and weighed and an analysis of variance performed on each experiment. A representative subset of northern region varieties, Maki (mean DF30; 80 d), Yarrum (mean DF30; 96 d) and CRC Walana (mean DF30; 70 d) at each planting time in 2009 and 2010 were examined to determine the effect of genotype/planting interactions on grain yield.

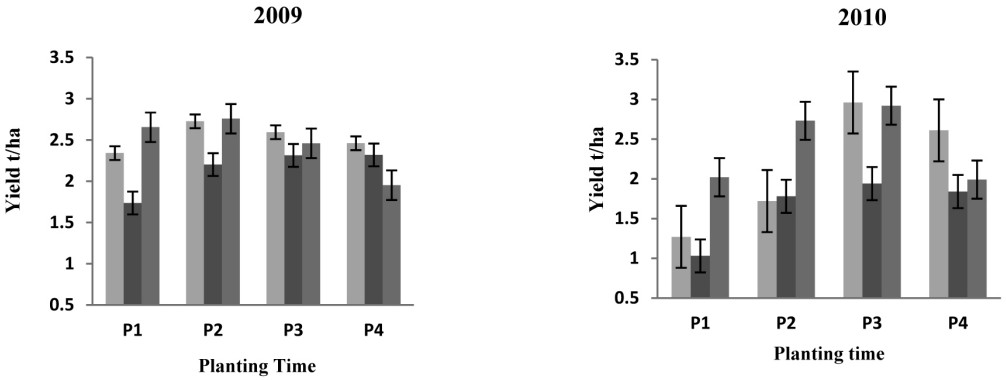

Figure 1. *Grain yield (t/ha) of selected field pea varieties (CRC Walana, Maki, Yarrum) at four planting times (P1 (late April/early May), P2 (mid May), P3(mid June) and P4 (early July)) in 2009 and 2010.*

Inherent grain yield differences were evident and the data suggests that genotypic response in grain yield to planting time occurred in both 2009 and 2010 (Figure 1). The highest yielding planting times common to all varieties were P2 and/or P3 in both years (mean yield 2.32 and 2.53 t/ha respectively). Grain yield at other planting times varied and appeared to be associated with DF30 in both years. The higher DF30 variety (Yarrum) was lowest yielding at P4 in both years. The medium and lower DF30 varieties Maki and CRC Walana were lowest yielding at P1in both years. Both of these varieties exhibited differential responses across years at P4. Examination of the MDT for both 2009 and 2010 may go some way in explaining the differences between varieties in their response to planting time. The impact of variable seasons (particularly frost and temperature) necessitates a closer study of meteorological factors, particularly MDT and ISR and their interaction with planting time and grain yield. However, the current data suggests that the appropriate planting time for medium and longer season varieties is mid May to June (P1 and P2), while shorter season varieties such as CRC Walana may benefit from later planting towards early to mid June (P3 and P4). For northern pork producers, the selection of pea varieties with appropriate phenology will ensure success as a reliable and commercially viable source of vegetable protein for the industry.

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The Effects of Endo-Xylanase Application to an Energy Reduced Diet on Performance of Grower-Finisher Pigs

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Non-starch polysaccharide (NSP) structures in feed raw materials have potential to reduce nutrient availability in pigs. Addition of xylanases to grain based diets can improve nutrient digestibility of such diets (Babinszky *et al*., 2010). The aim of this experiment was to evaluate the effect of a thermo tolerant endo-xylanase produced by *Trichoderma reesei* (Econase® XT, AB Vista, Marlborough, Wiltshire UK) on the performance of pigs fed an energy reduced diets compared to pigs fed similar diets with higher energy content.

Three hundred pigs of 33.5±3.5 kg initial body weight (BW) were allocated to three treatments (Hungarian Large White, 50% barrows and 50% females, 10 pigs/pen, 10 pens/treatment). Wheat-barley-soybean meal based diets with adequate (positive control, PC) or reduced (negative control, NC) energy contents were applied in two feeding periods from 33 to 60 kg BW (13.5(PC) or 12.5(NC) MJ metabolisable energy (ME)/kg, 168g crude protein (CP)/kg; 8.6 g standardised ileal digestible (SID) lysine/kg) and 61 to 110 kg BW (13.5(PC) or 12.5(NC) MJ ME/kg; 148g CP/kg; 7.6g SID lysine/kg). To evaluate the effect of xylanase (Xyl) pigs were fed NC diets with added endo-xylanase (NC+Xyl, 16000 Birch Xylan Units (BXU)/kg). Diets were offered in mash form *ad libitum*. The trial period ended after 96 days when first animals achieved a body weight of 110 kg. Trial data were analysed with analysis of variance (ANOVA) and significant differences determiend by least significant difference (LSD).

Table 1. *Performance of grower-finisher pigs fed diets with adequate (PC) or reduced (NC) energy level compared to pigs fed NC diets plus xylanase (NC+Xyl).*

	Treatments			
Parameters	PC	NC	NC-Xyl	RMSE
Initial body weight (kg)	32.6	32.4	32.1	4.2
Final body weight (kg)	107.1[a]	104.5[b]	106.9[a]	8.6
Weight gain d 1-96 (kg)	777[a]	751[b]	780[a]	78
Feed intake (g/d)	2470[a]	2502[a]	2495[a]	92
Feed conversion ratio (kg/kg)	3.19[a]	3.33[b]	3.20[a]	0.08

RMSE, Root Mean Square Error; [a b]Means within a row with different superscripts differ significantly (P<0.05)

Pigs fed NC+Xyl improved BW by 2.4kg and average daily gain (ADG) by 29 g/d compared to pigs fed NC diets, and had a improved feed conversion ratio of 0.13 kg/kg (Table 1). Similar improvements compared to the NC were achieved in pigs fed diets with the higher energy content (PC). The average daily feed intake of the pigs was identical over the entire experimental period (P>0.05).

The results from this experiment demonstrate that the reduction in performance of grower-finisher pigs fed energy reduced wheat-barley-soybean meal diets can be restored to that of pigs receiving adequate energy diets through the addition of a xylanase.

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Effect of Grain Type, Particle Size and Processing Conditions on Growth Performance Characteristics in Pigs

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In Australia, various *in vivo* pig digestibility studies have revealed that 1-4 MJ/kg dietary gross energy is not available at the end of the small intestines (Black, 2006), and this is a significant economic issue for the pork industry. In barley and sorghum, large particles (>1.0 mm for barley and >0.75 mm for sorghum) were resistant to starch digestion *in vitro* (Al-Rabadi *et al.*, 2009) and we hypothesized that this, along with reduced protein digestion, is the main factor that results in incomplete energy digestion in pigs. The objective of this experiment was to demonstrate that the reduced digestibility of starch in large particles obtained *in vitro* also apply *in vivo*.

This experiment was a 2x2x2 factorial design with the factors being grain type (sorghum or barley), feed form (mash or pelleted) and milling process (normal Ground or Recycle - separating particles >1.7mm barley; >0.9 mm sorghum and remilling through a 3mm screen). Male pigs (n=176, Primegro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) were selected at 28 days of age to enter the 6 d pre-experimental period and fed a 15.5 MJ digestible energy (DE)/kg and 1.5% lysine creep diet. One hundred and sixty pigs were randomly allocated to the eight treatment diets at an average live weight of 5.9±1.0kg. All pigs were fed *ad libitum* their respective diets and had access to water via nipple drinkers. The diets were designed for grower pigs from 20-40 kg live weight (14 MJ DE/kg and 1.0% lysine) due to the use of the diets across multiple experiments. Pigs were fed daily with individual weights recorded at the beginning of the experiment and each week for the three week duration of the experiment. Feed intake was calculated weekly. The data was analysed using an analysis of variance (ANOVA) for the main effects of grain type, processing type and particle size.

Table 1. *The main effects of grain type, processing type and particle size on average daily intake (ADI), rate of gain (ROG) and feed conversion ratio (FCR) of pigs from 0-21 d.*

							P Value		
Measurement	Grain type		Processing Type		Particle Size		Grain	Process	Particle Size
ROG (kg/day)	Barley	0.263	Mash	0.265	Ground	0.256			
	Sorghum	0.255	Pellet	0.253	Recycle	0.262	0.409	0.201	0.514
FCR	Barley	1.82	Mash	2.05	Ground	2.16			
	Sorghum	2.19	Pellet	1.96	Recycle	1.85	<0.001	0.344	0.001
ADI (kg/day)	Barley	0.471	Mash	0.530	Ground	0.539			
	Sorghum	0.542	Pellet	0.482	Recycle	0.473	0.002	0.032	0.003

The growth rate of the piglets was not affected by any of the dietary treatments (Table 1). Piglets fed barley-based diets had a lower ADI (P<0.01) resulting in a better FCR (P<0.001). Piglets fed mash feeds had a higher ADI (P<0.05). Piglets fed the diets containing the recycled grain had a lower ADI (P<0.01) which was greater in sorghum diets (P<0.05). Recycled grain diets improved FCR in mash diets and not pelleted diets (P<0.05). The results show that there is an improvement in feed efficiency predominately through a reduction in ADI when the larger particles of grain in a diet are reground. This effect is greater in diets based on sorghum than barley. The improvement in feed efficiency supports the hypothesis that there was a significant depression in digestibility of grain particles over 1mm in diameter in weaner pigs.

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CHAPTER 5

Reproduction

Nutritional Manipulation of Sows in Late Gestation to Reduce Stillbirth and Improve Pre-Weaning Mortality Rates

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In the Australian pork industry, the combination of stillbirths and pre-weaning mortality usually reduces the size of the litter born by approximately 20% (or 2.2 piglets) by the time weaning occurs. For an individual piglet the major risk factors for being stillborn or dying pre-weaning are low birthweight and low liver glycogen stores (Knol, 2008). To improve mean piglet birthweight, higher feeding levels in late gestation have been commonly adopted. Theoretically, liver glycogen stores at birth would also be maximised by increased maternal feeding from approximately d 80 of gestation. This experiment tested the hypothesis that a high feeding strategy in late gestation will reduce the stillbirth and pre-weaning mortality rates in a modern genotype and considered the longer-term consequences of such a strategy.

The experiment was conducted in two replicates, each of 90 parity 2-6 sows (30 sows/treatment), at a large commercial piggery. All sows were fed a diet containing 13.0 MJ digestible energy (DE)/kg, 14.5% crude protein and 0.42g available lysine/MJ DE at 2.3kg/d for the first 72d of gestation. For the last 0, 3 or 6 weeks of gestation, sows were either fed 2.3kg/d (Control), or 3.0kg/d for 3 weeks (High 3) or for 6 weeks (High 6) of the same diet. No farrowing induction was used, all piglets were weighed at birth and cross-fostering was delayed until 72 h post-farrowing. Litter size was recorded at farrowing (alive and dead), 72 h post-farrowing and at weaning. The subsequent reproductive performance of all sows was recorded up to and including the next farrowing. Data were analysed using an analysis of variance with blocks built in.

Table 1. *The effects of sow gestation feeding regimen on piglet performance and sow subsequent reproduction.*

	Control (n=58)	High 3 (n=58)	High 6 (n=56)
Litter size – total born	13.0 (± 0.44)	13.3 (± 0.42)	12.5 (± 0.38)
Stillbirths (%)	4.9	4.1	4.7
Mean piglet birthweight (kg)	1.48 (± 0.03)	1.51 (± 0.03)	1.54 (± 0.03)
Pre-weaning mortality to d3 (%)	8.1	7.7	7.8
Mean wean-to-oestrus interval (d)	5.3 (± 0.61)	4.8 (± 0.58)	5.8 (± 0.52)
Subsequent farrowing rate (%)	86.0	93.2	96.2
Subsequent litter size (total born)	12.5[a] (± 0.38)	12.8[a] (± 0.36)	11.5[b] (± 0.32)

[ab]Values in a row with different superscripts differ significantly (P<0.05). Values in brackets are standard errors of the mean.

There was no clear indication that either stillbirth rate or early pre-weaning mortality were affected by gestation feeding regimen despite the fact that increasing maternal feed intake in late gestation did slightly raise mean piglet birthweight (Table 1). More importantly, higher maternal gestation feeding regimens failed to reduce the proportion of the piglets with a birthweight less than 1.0kg (8.6% vs. 7.8% vs. 8.1% for Control, High 3 and High 6 treatments, respectively). While subsequent farrowing rate may be slightly enhanced in sows fed high levels in the previous gestation this difference was not significant (P>0.05). The subsequent litter size data shows no positive effects of the higher gestation feeding regimens. Indeed, they suggest that excessive high feeding in the previous gestation (treatment High 6) may adversely affect the size of the next litter. If this effect is substantiated in future work, we speculate that it may reflect sows gaining greater body condition during gestation, this reducing lactation feed intake and thus increasing lactation body condition loss. The negative effects of excessive body condition loss on subsequent litter size have been extensively reported (eg. Thaker and Bilkei, 2005).

These results suggest that, at least within the genotype, facilities and management of the herd used, high feeding for the last 3-6 weeks of gestation may be unnecessary. However, care must be taken in interpreting these data as subsequent farrowing rate did numerically increase in the high-fed (High 3 and High 6) sows vs. Control sows while the High 6 treatment significantly reduced subsequent litter size. This possible longer-term effect requires further study.

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Feeding Level and Dietary Fibre Content During Early Pregnancy in Gilts and Effects on Pregnancy and Litter Size

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High feeding levels have been shown to reduce systemic progesterone concentrations during early pregnancy, and therefore have been associated with a decrease in embryo survival (Jindal *et al.*,1996). However, the effects of feeding level on embryo survival have been equivocal with other studies finding no or even positive effects of an increased feeding level on embryo survival and pregnancy rate (Virolainen *et al.*, 2004; Quesnel *et al.*, 2010). This paradox may be due to the focus on progesterone concentrations in the systemic blood circulation. Recent studies (Athorn *et al.*, 2010) show that a high feeding level may actually result in an increase in progesterone secretion at the ovarian level, despite a decrease in the systemic circulation. The increase in secretion of progesterone may be beneficial at the uterine level due to direct ovarian-uterine transfer. We hypothesised that a high feed level does not reduce litter size and may even increase it. The aim of this experiment was to compare three different feed levels of a standard diet (30 g/kg crude fibre, 13.0 MJ digestible energy (DE)/kg), and a fibrous diet (100 g/kg crude fibre, 10.9 MJ DE/kg), in their effects on pregnancy rate and litter size.

Gilts (Large White x Landrace F1 gilts, PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW, n=233) were allocated to one of four treatments at mating and maintained on the treatments until d 25 of pregnancy, the treatments being 1) Low (standard diet at 21 MJ DE/d), 2) Medium (standard diet at 31 MJ DE/d), 3) High (standard diet at 41 MJ DE/d) or 4) Fibre diet at 31 MJ DE/d. A high fibre diet may increase the removal of progesterone from systemic circulation but nevertheless result in increased progesterone secretion by the ovary and as a consequence improve embryo survival. Data were analysed by analysis of variance or a Chi-square test (pregnancy rate).

Table 1. *Effects of dietary treatments during d 0-25 of gestation on weight gain and reproductive performance.*

	N	Weight Gain (g/d)	P2 gain (mm)	Pregnancy rate d28 (%)	Total born	Born alive
Low (21 MJ DE/d)	46	421 ± 41^{a}	1.7 ± 0.3^{a,b}	83 (50/60)	12.5 ± 0.4	11.5 ± 0.4
Medium (31 MJ DE/d)	39	495 ± 45^{a}	0.6 ± 0.4^{a}	81 (44/54)	12.2 ± 0.4	11.3 ± 0.4
High (41 MJ DE/d)	45	912 ± 40^{b}	2.6 ± 0.4^{b}	91 (53/58)	11.8 ± 0.4	11.2 ± 0.4
Fibre diet (31 MJ DE/d)*	42	569 ± 34^{a}	1.8 ± 0.5^{a,b}	82 (50/61)	12.3 ± 0.4	11.3 ± 0.4

^{ab}Values in a column with different superscripts differ significantly (P < 0.05); N, number at farrowing; *100 g/kg crude fibre versus 30 g/kg in the other three treatments; DE, digestible energy;

There was a clear difference in weight gain between low versus high feed levels, although medium sows did not differ from low sows (Table 1). Pregnancy rate and litter size were not affected by feed level or inclusion of fibre. Interestingly, when pooled across treatments, the 25% gilts with the highest growth rate (1003 g/d) had a higher pregnancy rate (92 %) than gilts with the 25% lowest growth rate (216 g/d; 85 % pregnant), and medium growth rate (573 g/d; 80 % pregnant; P<0.09). This experiment shows that a high feeding level during early pregnancy in gilts is not at all detrimental to pregnancy and litter size, and that the fibrous diet used in this experiment can be used without impacting pregnancy or litter size.

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B-Vitamin and Methyl Donor Supplementation of Gestating Sow Diets: Effects on Pregnancy Outcomes and Prolificacy

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Dietary supplementation with B-vitamins during gestation can improve sow prolificacy (Matte *et al*., 2006), and supplementary betaine during gestation increases litter size of summer-mated sows (van Wettere *et al*., 2009). This experiment tested the hypothesis that supplementing sow gestation diets with B-vitamins (Folic acid plus vitamin B12) and betaine would increase litter size of sows mated during autumn/winter.

A total of 1079 Large White/Landrace sows mated between the March 27 and May 5, 2010 were used in this experiment. The experimental design was a 2x2 factorial, incorporating two levels of betaine supplementation (0 versus 3 g added betaine/ kg feed (0BET and SBET, respectively)) and two levels of B-vitamin supplementation (0 versus 20 mg/kg Folate plus 150 µg/kg vitamin B12 (DSM Nutritional Products, Wagga Wagga, NSW) added as a supplement (0BVIT and SBVIT, respectively)). Diets were fed from d 1 post artificial insemination (AI) until d 112 of gestation. All sows were fed a base diet (13.0 MJ/kg digestible energy (DE), 0.64% lysine; 5 mg/kg folate; 20 µg/kg vitamin B12) at the same level during gestation: 2.5 kg/d; 2.7 kg/d and 3.0 kg/d on d 1 to 42, d 43 to 84 and d 85 to 112 of gestation, respectively. On d 18–24 post-AI, sows were detected for oestrus by fenceline boar exposure. Preprandial blood samples were collected from 20 sows/treatment on d 3, 30 and 107 of gestation, and assayed for homocysteine (HCY) using an enzyme immunoassay. On d 30, sows were detected for pregnancy used real time ultrasound, with non-pregnant sows re-tested 7 d later. At farrowing, total litter size (TB) and number of piglets born alive (BA) were recorded. Within treatment, sows were blocked according to two parity groups (parities 2 and 3 (P2/3) versus parities 4 plus (P4+)), and a general analysis of variance model, with block built-in, was used to study the main effects of betaine and B-vitamin supplementation and parity group on all measures (Genstat 10th Edition, Rothamsted Experimental Station, Harpendon). A chi-squared test analysed treatment effects on pregnancy rates.

Table 1. *Effect of Betaine and B-vitamin supplementation during gestation and sow parity at mating on total litter size, born alive, still born and mummified foetuses.*

	SBET		0BET		SBVIT		0BVIT	
	P2/3	P4+	P2/3	P4+	P2/3	P4+	P2/3	P4+
TB	11.9±0.3^{bc}	11.6±1.2^{b}	12.2±0.3^{c}	11.1±0.2^{a}	12.3±0.3^{b}	11.2±0.2^{a}	11.7±0.3^{a}	11.4±0.2^{a}
BA	11.0±0.3^{bc}	10.6±0.2^{b}	11.4±0.3^{c}	10.1±0.2^{a}	11.4±0.3^{b}	10.2± 0.2^{a}	11.0± 0.3^{b}	10.4± 0.2^{a}

0BET, no betaine; SBET, betaine; 0BVIT, No B Vitamins; SBVIT, B Vitamin; P2/3, parity 2 and 3 sows; P4+, parity 4+ sows; TB, total piglets born; BA, piglets born alive. ^{abc}Means within a row and main effect with different superscripts differ significantly (P<0.05).

B-vitamin supplementation reduced (P<0.05) incidences of pregnancy failure prior to d 35 of gestation compared to unsupplemented sows (0.03 versus 0.07, respectively). Plasma HCY was lower (P<0.05) for SBVIT compared to OBVIT sows (12.9±0.23 versus 14.7±0.22 µM), but was similar for OBET and SBET sows (13.9±0.24 and 13.8±0.24 µM). Litter size of P4+ sows was increased (P<0.05) by betaine supplementation, however, the litter size of P 2/3 sows was unaffected. Supplementary B-vitamins increased (P<0.05) litter size of P2/3 sows, but did not affect P4+ sows (Table 1). The positive effect of betaine on the prolificacy of older parity sows is consistent with our previous findings in summer-mated sows (van Wettere *et al*., 2009). The increased litter size in B-vitamin supplemented P2/3 sows suggests that dietary levels are insufficient to optimise prolificacy, possibly reflecting the continued growth, and hence increased requirement for vitamin B12 (Matte *et al*., 2006). The decreased incidence of early pregnancy failures in B-vitamin supplemented sows occurred in conjunction with a decline in peripheral homocysteine (HCY) concentrations. Elevated homocysteine impairs maternal recognition of pregnancy, and is associated with early pregnancy failure in sows (van Wettere *et al*., unpublished), suggesting a B-vitamin induced reduction in (HCY) may have caused the decrease in pregnancy losses.

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Optimal Timing of Oestrus Induction During Lactation to Achieve Normal Farrowing Performance

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Previous research demonstrated that oestrus can be achieved during lactation in 87% of sows using an injection of gonadotrophins at 20 days after parturition, combined with boar exposure and piglet separation overnight until mating (Downing *et al*., 2009). A practical strategy for induction of oestrus during lactation will uncouple weaning from mating, allowing for increased weaning age without limiting the number of litters per sow per year. The objective of this experiment was to test the hypothesis that induction of oestrus can be achieved as early as d 14 of lactation without affecting subsequent litter size or farrowing percentage.

One hundred and fifteen F1 multiparous sows (PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW), maintained in conventional farrowing crates were allocated at random to four treatments on the basis of parity and suckling litter size. The experiment was replicated in time on three successive weeks to provide 28-29 sows per treatment. The first treatment included boar exposure only. Treatments 2, 3 and 4 included an intramuscular injection with 400 IU of pregnant mare serum gonadotropin plus 200 IU of human chorionic gonadotrophin (PG600; Merck Animal Health, Summit NJ) at either d 14, 16 or 18, respectively, after parturition, combined with boar exposure and piglet separation from 1530 to 0730 hours each day until mating by artificial insemination (AI). The boar was held in front of the sow crates sufficiently long enough for nose to nose contact and oestrus detection. Average litter size was 9.0 ± 1.75 (mean ± standard deviation). Sows had *ad libitum* access to water and a diet formulated to contain 14.9 MJ of digestible energy (DE)/kg and 0.50 g available lysine/MJ DE. Piglets on an induced sow were separated to one side of the farrowing crate using a wooden partition and were provided with supplementary heating, a nipple drinker and unlimited access to a creep diet (15.5 MJ DE/kg and 21.7% crude protein). Piglet separation ceased after AI and then remained on each sow until weaning on the same day, at a mean of 26 d after parturition. Piglets were weighed on d 14 and 23 of age. All mated sows were housed as a group and pregnancy was confirmed at d 40 by ultrasound. Pregnant sows were farrowed as a group. Proportional sow measures were analysed using Fisher's Exact Test. Mating day in lactation was analysed by analysis of variance (ANOVA), while piglets born alive and piglet growth rate were assessed using a linear mixed model (Genstat v.13; VSN International Ltd, Hemel Hempstead, UK).

Table 1. *Mating and subsequent farrowing performance of 115 multiparous sows with boar exposure only or induced[1] during lactation at either d 14, 16 or 18 after parturition and mated by artificial insemination (AI).*

Measurement	Boar Only	Day 14	Day 16[2]	Day 18	P
Sows mated during lactation (%)	20.7[a]	79.3[b]	92.9[b]	89.7[b]	<0.0001
Mean (±SE) mating day in lactation	22.8±0.9[a]	19.2±0.2c	20.9±0.3[b]	22.5±0.2[a]	<0.0001
Sows pregnant of those mated (%)	50.0 [a]	73.9[b]	88.0[b]	73.1 [b]	<0.0001
Sows farrowed of those mated (%)	50.0[a]	65.2[b]	73.1 [b]	69.2[b]	<0.0001
Mean (±SE) piglets born alive	12.4 (0.8)	10.2 (0.9)	10.9 (0.8)	11.7 (0.9)	0.23
Piglet growth rate d 14-23 (g/d)	230±12[a]	122±14[b]	112 ±12[b]	127±15[b]	<0.001

[1]Injection PG 600 combined with boar exposure and piglet separation (1530-0730 h) each day until mating. [2] 28 sows treated and 29 for all other treatments. [ab]Within a row means with different superscripts differ significantly (P<0.001); SE, standard error.

Mating, pregnancy and farrowing performance for the d 16 and d 18 treatments were similar to the previous findings of Downing *et al*. (2009). However, there was a non-significant trend for a reduction in mating, pregnancy and farrowing performance when induction occurred at d 14. Hence, a recommended mating strategy during lactation would be to limit oestrus induction to d 16 or later after parturition. Oestrus behaviour in 20.7% of sows with boar exposure only warrants further study.

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Effect of Supplemental Human Chorionic Gonadotrophin on Ovulation in Hormone-Treated Gilts

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Injection of 200 IU human chorionic gonadotrophin (hCG; Chorulon®, Merck Animal Health, Summit, NJ) at 24 or 48 h after injection of 400 IU equine chorionic gonadotrophin combined with 200 IU hCG (PG600®, Merck Animal Health, Summit, NJ) increased early luteal blood progesterone concentrations in gilts (Manjarin *et al*. 2010a). Interestingly, injecting sows with PG600 at weaning followed by a supplemental 200 IU hCG at 24 hours reduced the farrowing rate (Manjarin *et al*. 2010b) but, in contrast, we have shown improved reproductive performance of anoestrus sows treated at seven days after weaning. The objective of this experiment was to test the hypothesis that an additional injection of the LH analogue hCG will improve the ovarian responses of gonadotrophin-stimulated prepubertal gilts resulting in an increased ovulation rate.

Prepubertal gilts (106±2.6 kg) received an injection of PG600 (PG600; n=8), or PG600 followed by either 100 IU hCG at 24 h (hCG-100; n=8) or by 200 IU hCG at 24 h (hCG-200; n=10). Gilts were slaughtered 10 to 15 days after PG600 and their ovaries recovered for determination of incidence of ovulation, numbers of corpora lutea and incidence and numbers of follicular cysts (>12 mm). Data were analysed by analysis of variance (ANOVA).

Table 1. *Effect of human chorionic gonadotrophin (hCG) supplementation on oestrus and ovulation in PG600-treated gilts.*

	PG600	hCG-100[1]	hCG-200[1]
Number of gilts	8	8	10
Number ovulating[2]	6	7	10
Number of corpora lutea	19.8±4.3[a]	23.6±4.3[a]	38.4±3.9[b]
Number of gilts with follicular cysts[3]	0	4	8
Number of cysts per gilt	0[a]	1.25±0.25[a]	17.5±2.8[b]

[1] 100 and 200 refer to dose (IU) of human chorionic gonadotrophin (hCG) administered 24 h after PG600 injection. [2] Determined at slaughter, 10 to 15 d after PG600 injection. [3] Follicles >12 mm. [ab]Means in a row with different superscripts differ significantly (P<0.01).

Numbers of corpora lutea were 26.3±3.6, 27.0±3.6, and 38.4±3.8 for PG600, hCG-100 and hCG-200 gilts, respectively (P<0.01). No follicular cysts were noted on the ovaries of PG600 gilts but four hCG-100 gilts and eight hCG-200 gilts had cysts (P<0.01; Table 1). Gilts with follicle cysts did ovulate as indicated by the presence of corpora lutea.

The contrasting effects of supplemental hCG in sows treated with PG600 at weaning or in anoestrus sows treated at seven days after weaning, and the current data for treatment of prepubertal gilts may be a reflection of endogenous circulating LH concentrations although, in the absence of hormone determinations, this suggestion remains speculative. However, in pigs the primary driver of ovarian follicle development beyond 4 mm is LH (Driancourt *et al*., 1995). If LH is limiting, such as is likely in seasonally anoestrus sows or in relatively immature gilts, the supplemental LH activity provided by hCG may facilitate follicle development. However, if LH is not limiting, such as may have been the case in the relatively mature gilts employed here, a down-regulation of LH receptors may have resulted when using the highest dose of hCG and, consequently, some follicles failed to respond to the endogenous LH surge prior to ovulation resulting in cystic follicles. Therefore, based on the present data, we suggest that in the absence of conditions likely to reduce endogenous circulating LH concentrations, that the administration of hCG will adversely affect ovarian function.

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Stress During Gestation Effects the Growth, but not the Stress Response of the Offspring

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Elevated levels of maternal glucocorticoids during gestation can affect maternal reproductive performance and the development and behaviour of the progeny, including increased percentage of piglets born alive (Kranendonk *et al*. 2006b) and altered functioning of the hypothalamic-pituitary-adrenal (HPA) axis (Kranendonk *et al*. 2006a) and reduced body weights (Kranendonk *et al*. 2006b) in the offspring of sows. We hypothesised that repeatedly stimulating the HPA axis of sows during late gestation would affect the reproductive performance of the sow and the development of the offspring. The objectives of this experiment were to determine 1) if sow reproductive performance would be affected by repeated administration of adrenocorticotropic hormone (ACTH) during late gestation, and 2) if this would affect the offspring's stress response to processing.

Third parity, commercial white line (Pig Improvement Company, North America, Hendersonville, TN, USA.) sows individually housed in standard gestation stalls (0.6 x 2.1 m) were used in this experiment. During late gestation (d 76 until 115) sows were either given an injection of ACTH (100 IU) 3 times a week (n=10) or control handled only (n=10). Sow reproductive performance (eg. number born alive, stillborn, mummies, weaned and percent mortality) and body weights of the offspring at birth, 6 and 24 weeks of age were recorded. Within the first 5 days of age, the offspring of the ACTH (n=34) and control (CON; n=42) handled sows were processed (eg. teeth clipped, tail docked and castrated) and the stress response elicited by this process was assessed using an automatic stress call monitoring system software (STREMODO, Dummerstorf, Germany). Data were analysed as a randomised design using the MIXED and NPAR1WAY procedures of SAS version 9.1 (SAS Inst., Inc., Cary, NC).

Table 1. *Reproductive performance of sows control handled (CON) or exposed to repeated stimulation of the hypothalamic-pituitary-adrenal axis (ACTH) during late gestation.*

Treatment	Total born	Number born alive	Number of stillborns	Number of mummies	Number of deaths	Number weaned	Survival (%)
CON	11.6	11.0	0.4	0.1	0.9	10.7	93.5
ACTH	13.0	12.9	0.0	0.1	2.5	10.5	84.2
SEM	1.21	1.21	0.12	0.12	0.96	0.89	5.34
P value	0.421	0.287	0.027	0.803	0.248	0.867	0.336

SEM, Standard error of mean.

Reproductive performance of ACTH and CON sows did not differ (P>0.05), except for the number of stillborns which was greater (P<0.05) in CON than ACTH sows (Table 1). Body weight did not differ (P>0.05) between CON and ACTH piglets at birth or 6 weeks of age, but was greater (P<0.05) in CON compared with ACTH piglets at 24 weeks of age [Birth: CON: 1.7±0.09, ACTH: 1.8±0.10; 6 weeks: CON: 8.2±2.10, ACTH: 7.8±2.04; 24 weeks: CON: 116.2±2.16, ACTH: 108.4±2.23 kg]. The percentage of stress vocalisations did not differ (P>0.05) between CON and ACTH piglets [CON: 75.5±2.82, ACTH: 78.3±3.01 %].

Stress, simulated by repeatedly administering ACTH during late gestation in the present study, did not markedly affect reproductive performance in the sow but did reduce body weight gain at finishing by 8 kg in the offspring. This has clear ramification for production, in that stress experienced by the sow during gestation could impair subsequent performance of the litter. However, prenatal stress did not appear to affect the behavioural stress response of the offspring to processing.

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Effect of Embryo Transfer Transport Conditions on the Viability of *In Vitro*-Produced Porcine Embryos

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Embryo transfer (ET) is an important tool for introducing new genetic material into closed herds with minimal risk of introducing disease, and is recognised as the ideal way of moving pig genetic material from one country to another (Cameron *et al*., 1989). However, the efficiency of porcine ET remains poor compared with other livestock species. High embryonic losses (about 75% of transferred embryos) have been observed in field trials after exposure of freshly collected embryos to standard transport conditions (G. Tuckett, personal communication), suggesting that the transport conditions have a profound effect on the efficiency of ET. The objective of this experiment was to test the hypothesis that holding time and transport vessel type can influence the subsequent development of *in vitro*-produced embryos.

Ovaries of prepubertal gilts were collected at a local abattoir and the recovered oocytes were matured *in vitro*. Matured oocytes were then fertilized *in vitro* using frozen-thawed boar semen. The resulting embryos were cultured in Porcine Zygote Medium (PZM-3; Yoshioka *et al*., 2002) for five days at 38°C in 6% CO_2, 5% O_2 and 89% N_2. Hepes-buffered PZM-3 was used as the transport medium. To assess the influence of holding time, embryos were loaded into 1 ml Norm-Ject embryo transfer syringes (Henke Sass Wolf, Tuttlingen, Germany) in 0.6 ml of medium (20-40 embryos/syringe/replicate) and held for 0 min, 30 min, 3 h or 18 h. The syringes were carefully maintained at 38°C (in air). Influence of vessel type was determined by loading embryos into either a 1 ml syringe (in 0.6 ml of medium), a 5 ml Falcon® tube (BD Biosciences, North Ryde, NSW; in 5 ml of medium) or a modified tom cat catheter (submerged in 5 ml of medium in a 5 ml tube), and maintained at 38°C for 3 h (in air). Following the simulated transport period, all groups of embryos were transferred to PZM-3 containing 10% foetal calf serum, and incubated at 38°C in 6% CO_2, 5% O_2 and 89% N_2. Blastocyst formation was assessed at d 7. Arcsine-transformed data were subjected to analysis of variance and means separated using a Tukey's post-hoc test.

Table 1. *Effect of holding time and vessel type on blastocyst formation rate (mean ± standard error of mean).*

Treatment	Total embryos	Day 7 blastocysts	Blastocyst rate (%)
Holding time			
Control (0 min)	161	39	23.3 ± 11.2[a]
30 min	143	26	19.7 ± 13.4[a]
3 h	138	1	0.5 ± 0.5[b]
18 h	115	1	1.0 ± 1.0[b]
Vessel type			
None (control)	90	19	19.4 ± 4.7[a]
Culture tube (5 ml)	80	21	25.8 ± 1.9[a]
Tom cat catheter (5 ml)	79	16	20.0 ± 3.3[a]
Transfer syringe (0.6 ml)	91	1	1.1 ± 1.1[b]

[ab]Values within holding time and vessel type with different superscripts differ significantly ($P<0.05$).

Embryos held in 1 ml syringes for 3 and 18 h subsequently developed to the blastocyst stage at significantly reduced rates compared with those held for 0 and 30 min (Table 1). In contrast, holding embryos for 3 h in 5 ml of medium (tube or tom cat catheter) did not reduce their developmental potential (Table 1). The results indicate that using a small volume of medium and/or a 1 ml syringe to transport porcine embryos for ET does not maintain embryo viability. While transporting embryos in a 1 ml transfer syringe may greatly simplify the procedure, transport in a vessel containing 5 ml of medium is highly recommended.

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Feeding Gilts High Fibre Diets Prior to Mating Improves Oocyte Quality

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In female pigs, approximately 20 - 30% of embryos are lost during the first 35 days of gestation, with a large proportion of this loss related to impaired developmental competence (or quality) of the oocytes shed at ovulation (Ferguson *et al.*, 2006). Including high levels of sugar beet pulp in pre-mating diets has been demonstrated to improve oocyte quality (Ferguson *et al.*, 2007). However, sugar beet pulp is not readily available in Australia and therefore the objective of this experiment was to determine whether feeding gilts either a wheat bran or lupin-based high fibre diet would improve oocyte developmental competence.

A total of 54 Large White cross terminal line pre-pubertal gilts were used in this experiment. Gilts were randomly allocated to receive one of three diets from 154 days of age until d 19 of the first oestrous cycle: control (13.25 MJ digestible energy (DE)/kg, 153g/kg crude protein (CP), 39 g/kg crude fibre (CF)); bran (500 g/kg wheat bran, 13.2 MJ DE/kg, 154 g/kg CP, 65 g/kg CF); lupin (350 g/kg lupin, 13.22 MJ DE/kg, 186 g/kg CP, 118 g/kg CF). Gilts received 3 kg/d of their respective diet, and puberty was stimulated at 175 d of age using PG600 (400 iu equine chorionic gonadotrophin and 200 iu chorionic gonadotrophin; Intervet Australia Pty Ltd, Bendigo East, VIC) and daily, physical boar contact. Gilts were sacrificed on d 19 of the first oestrous cycle. For each gilt, ovaries were collected and the 15 largest follicles (presumed ovulatory pool) were aspirated, and the oocytes matured *in vitro* for 44 hours, and stained to assess the stage of nuclear maturation. The pooled oocytes per gilt were classed as the experimental unit and a general analysis of variance model was used to determine the effect of treatment on all measures recorded (Genstat, 10th Edition, Rothamsted Experimental Station, Harpenden).

Table 1. *Preovulatory follicle size and percentage of oocytes at the different stages of nuclear maturation from gilts fed either control, bran or lupin diets.*

	Treatment diet		
	Control (n=15 gilts)	Bran (n=14 gilts)	Lupin (n=13 gilts)
Mean follicle diameter (mm)	7.5 ± 0.3	7.0 ± 0.3	7.1 ± 0.3
Stage of nuclear maturation			
Germinal vesicle (%)	10.2 ± 2.3[a]	6.3 ± 2.4[b]	1.3 ± 2.5[b]
Germinal vesicle breakdown (%)	8.7 ± 3.0[a]	17.3 ± 3.1[b]	2.5 ± 3.2[a]
Metaphase I (%)	10.3 ± 3.0[a]	7.2 ± 3.1[a]	7.3 ± 3.3[a]
Anaphase – Telophase (%)	0.5 ± 1.0[a]	3.1 ± 1.0[a]	0.0 ± 1.1[a]
Metaphase II (%)	71.5 ± 4.6[a]	65.4 ± 4.8[a]	88.9 ± 4.8[b]

[ab]Means in a row with different superscripts differ significantly (P<0.05)

Liveweight at 175 d of age was not significantly different for control, bran and lupin fed gilts while P2 back fat was significantly higher in controls compared to bran and lupin (14.3±7, 11.9±7 and 11.6±7 mm respectively). There was no effect of diet on the mean diameter of the follicles within the presumed ovulatory pool (Table 1). However, the lupin diet resulted in a 24% and 35% increase (P<0.05) in the percentage of oocytes that reached metaphase II *in vitro* compared to those collected from control and bran gilts, respectively (Table 1). Although the lupin diet provided approximately 3% more crude protein than the bran and control diets, Ferguson *et al.* (2006) demonstrated that a 15% increase in protein intake prior to mating did not alter ovulation rate or embryo survival. Therefore, these data support previous reports of a beneficial effect of high crude fibre diets on oocyte quality (Ferguson *et al.*, 2007), but also demonstrate the effect of fibre on oocyte quality in gilts differs between fibre sources. Lupin and sugar beet pulp are both rich in non-starch polysaccharides, while bran has high levels of non-fermentable fibre which may explain the differences observed in both studies.

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Nutritional Manipulation to Improve Lactation Performance and Ovarian Function of Primiparous Sows

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Altering the energy and lysine content, as well as the quantity, of the diet consumed during different periods prior to mating can significantly alter oocyte developmental competence and sow reproductive performance (Foxcroft *et al*., 2007). This experiment tested the hypothesis that providing primiparous sows with a high energy, protein rich supplement in the last week of lactation would improve oocyte developmental competence (blastocyst formation *in vitro*) of sows with a low feed intake during the first 21 days of lactation.

Thirty-six Large White x Landrace primiparous sows received one of three different lactation dietary regimens (n=12 sows/treatment): HIGH, 6 kg/d throughout lactation (85 MJ digestible energy (DE)/d, 1186g crude protein (CP)/d, 71g lysine (Lys)/d); REST, 4 kg/d throughout lactation (57 MJ DE/d, 791g CP/d, 48 g Lys/d); SUPP, 4 kg/d throughout lactation plus 1 kg per day of a specially designed supplement for the last 7 d of lactation to provide a total of 74 MJ DE/d, 1009g CP/d and 62 g Lys/d during the last 7 d of lactation. The base diet was the same for all treatments. Sow liveweight (LW) and P2 backfat (P2) were measured on d 1 and 19 of lactation and at weaning (d 26.7 ± 0.2). On d 3 post-weaning, sow reproductive tracts were collected, and the number of ovarian follicles >4 mm in diameter were counted and aspirated. Aspirated oocytes were matured and fertilized *in vitro* (Kelly *et al*., 2010). Cleavage rate was recorded 48 hours post-fertilization and stage of embryonic development assessed on d 6 post fertilisation. Prior to analysis, oocyte cleavage and blastoscyt formation data was transformed by arcsin square root. Data was analysed using a two-way analysis of variance, with sow as the experimental unit (Genstat 10th Edition; Rothamsted Experimental Station, Harpendon).

Table 1. *Effect of three lactation feeding regimens on sow lactation liveweight (LW) loss, the number of follicles >4 mm present on d 3 post-weaning, oocyte cleavage and blastocyst development in vitro.*

	REST	HIGH	SUPP
Sow LW loss d1-19 (%)	8.4 ± 1.9^{a}	3.6 ± 1.9^{b}	7.8 ± 1.9^{a}
Sow LW loss d19-26 (%)	4.7 ± 0.9^{a}	2.7 ± 0.9^{b}	1.3 ± 0.9^{b}
Sow LW loss d1-26 (%)	14.4 ± 1.1^{a}	6.0 ± 0.1^{b}	9.1 ± 0.1^{c}
Mean number of follicles >4 mm in diameter per sow	28.0 ± 1.3	27.6 ± 1.9	30.9 ± 3.2
% cleaved	61.6 ± 6.6	51.8 ± 6.9	66.3 ± 6.8
% blastocysts from cleaved embryos	71.7 ± 8.0^{xy}	50.9 ± 8.3^{x}	78.5 ± 8.2^{y}

Means within a row with different superscripts differ significantly, ^{abc}P<0.05; ^{xy}P<0.10; HIGH, 6 kg/d throughout lactation; REST, 4 kg/d throughout lactation; SUPP, 4 kg/d throughout lactation plus 1 kg/d supplement for the last 7 days.

On d 1of lactation, sow LW (200.7±4.5kg) and P2 (22.7±0.2 mm) was similar for all treatments. LW loss from d 1-19 was lower (P<0.05) for HIGH compared to REST and SUPP sows, while between d19 and weaning REST sows lost more weight (P<0.05) than HIGH and SUPP sows (Table 1). There was no effect of treatment on P2 loss during lactation (5.5±0.1mm) or the number of follicles >4 mm on d 3 post-weaning. However, blastocyst formation rates were 35% higher (P<0.1) for SUPP compared to HIGH sows (Table 1). Overall, feeding the dietary supplement during the last week of lactation tended to improve blastocyst formation compared to HIGH feeding throughout. Interestingly, the current data suggest that moderate energy and lysine (33%) restriction during the last week of lactation does not impair oocyte developmental competence. In contrast to the available literature, restrictive as opposed to high feeding resulted in higher blastocyst formation rates *in vitro. In vivo* experiments are being conducted to confirm the positive effects of the supplement on ovulation rate, embryo development and early function of the corpora lutea.

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CHAPTER 6

Health Management and Product Integrity

Symposium: Modern Health Management of Pigs

Symposium Introduction

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Disease can have a major impact on the productivity, profitability and welfare of pig farms. In particular, enteric and respiratory disease-causing pathogens decrease profitability by reducing feed conversion efficiency, decreasing growth, increasing mortality, increasing medication costs and increasing variations in carcase weight. Control of disease is more difficult where there are large numbers of pigs of different ages on one site, where housing facilities are aging and the pigs' environment is compromised, and on farms with clinical disease associated with Porcine Circovirus Type 2 (PCV2).

There is increasing concern among health professionals regarding the development of resistance to antimicrobial drugs in the community. Antimicrobial use is associated with amplification of resistant strains of bacteria. The contribution that non-human antimicrobial use has to antimicrobial resistance in the human health sector has not been defined. However, the JETACAR report (1999) details the potential for resistant bacteria/genes to transfer from animals to humans. It is likely that the animal industries (veterinary and agriculture sectors) will come under increasing pressure to undertake surveillance on antimicrobial use and monitor resistance trends. There will also be increasing pressure to develop interventions to reduce non-human antimicrobial use.

The three papers in this symposium discuss risks of antimicrobial overuse in pigs and how to minimise these risks through prudent antimicrobial use and through application of nutritional alternatives to antibiotics. The first paper (Trott, 2011) focuses on two major bacterial pathogens; multidrug-resistant, extraintestinal pathogenic *E. coli* and methicillin-resistant *Staphylococcus* which cause both hospital and community-acquired opportunistic infections in humans. Identical pathogens have been isolated from pets, poultry and other livestock species, leading to speculation regarding the potential for transmission to humans. In the second paper, Holyoake (2011) discusses the current challenges facing veterinarians and producers managing the health of pig herds. Antimicrobials are an integral component of preventing and controlling disease. The author discusses principles of prudently using antimicrobials to minimise the risk of resistance amplification. Both Trott (2011) and Holyoake (2011) identify the need for a united effort to monitor antimicrobial use and resistance to support methods to minimize resistance amplification. In the final paper, Edwards and Edwards (2011) report on nutritional supplements that have been identified to have physiological activities to support pig health.

Public Health Risks of Using Antimicrobials in Pigs

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Abstract

Use of antimicrobials in intensive livestock industries has been under increased scrutiny since avoparcin was first linked to increased carriage of vancomycin resistant enterococci (VRE) in both poultry and pigs. In some countries, total bans and/or increased regulation pertaining to the use of certain antibiotics as growth promoters resulted in significant decreases in the prevalence of VRE faecal carriage but concomitant increases in the use of additional classes of drug for therapeutic purposes. The recent emergence of animal-associated strains of highly virulent, multidrug resistant extraintestinal pathogenic *Escherichia coli* (MDR-ExPEC) and methicillin-resistant *Staphylococcus aureus* (MRSA) has placed further scrutiny on the use of therapeutic antibiotics in livestock and the need to provide viable alternative control measures for diseases such as post-weaning colibacillosis in pigs. In particular, off-label use of third and fourth generation cephalosporins needs to be carefully managed. Australia has history of conservative antimicrobial registration in livestock and innovation regarding viable alternatives to antibiotics for disease control in pigs. The local industry should be well placed to capitalise on future investments in this area. There will always be a need to prescribe antibiotics but the public health impact of antimicrobial usage in pigs can be limited by accurate estimation of the true prevalence of resistance in key bacterial organisms, careful and judicious antimicrobial choices by veterinarians and producers and workable alternative strategies.

Introduction

The WHO identified antibiotic resistance as one of three major future threats to global health (Infectious Diseases Society of America, 2010) and recently dedicated World Health Day (7th April 2011) to the theme of antibiotic resistance, "*no action today, no cure tomorrow*". The medical costs, in the USA alone, associated with treating and managing an antibiotic-resistant infection, are estimated to be US$18,588 to US$29,069, resulting in an annual cost to the US health system of over US$20 billion. The annual cost to US households in terms of lost productivity is estimated at over US$35 billion (BioMérieux 2009; Roberts *et al*., 2009). A total of 25,000 patients in the European Union (EU) die annually from infection with Multiple Drug Resistant (MDR) bacteria, despite many EU countries having world's best practice hospital surveillance and infection control strategies (Anon, 2009). Understanding the epidemiology of resistance development, including the contribution of animal reservoirs is essential to developing effective mitigation strategies. Medical/veterinary practitioners are now encouraged through the One World One Health concept to work in unison to preserve the lifespan and efficacy of existing drug classes. This paper will focus on two significant, antibiotic-resistant bacterial pathogens of humans that have recently emerged in animals, multidrug-resistant, extraintestinal pathogenic *E. coli* (MDR-ExPEC) and methicillin-resistant *Staphylococcus* (MRS).

Multi-Drug Resistant *Escherichia coli* and Methicillin-Resistant *Staphylococcus* in Humans and Animals

Multidrug-resistant, extraintestinal pathogenic *E. coli* and MRS are two major bacterial pathogens causing both hospital and community-acquired opportunistic infections in humans that result in significant morbidity and mortality (Gottlieb and Nimmo, 2011). Health costs attributed to MDR-ExPEC and MRS infections are significant. In Western Australia alone, 31 nosocomial MRSA bloodstream infections (2008-2009) cost WA healthcare facilities $907,723 and contributed an additional 279 days of hospitalization (McCann *et al*., 2009). Fundamental changes in MDR-ExPEC and MRSA ecology occurred recently with some strains becoming host-adapted in several animal species (Weese 2010; Platell *et al*., 2011) and cases of human-to-animal and animal-to-human transfer of resistant strains have been documented (Johnson *et al*., 2009). This has important but currently unquantified consequences to both the epidemiology and public health significance of these pathogens, as well as financial and emotional costs to animal producers and owners and welfare implications to animals themselves.

Globally, there is currently much conjecture concerning antimicrobial usage in animals and the relative impact to public health of resistance selection and dissemination in livestock produced for food (pigs, poultry and ruminants) compared to companion animals that share close contact with humans (dogs, cats and horses). Recent research (described below) suggests that frequent exchange of MDR-ExPEC and MRSA between humans and animals may be much more significant than previously realised. In the case of MDR-ExPEC, companion animals and poultry appear to be significant reservoirs, whereas for MRS, both companion animals and livestock are implicated.

MDR-ExPEC O25b:ST131 in Humans and Animals

Prior to 2007, we isolated identical MDR-ExPEC strains from canine nosocomial infections, rectal swabs from hospitalised dogs and veterinary hospital personnel (Sidjabat *et al*., 2006a; Sidjabat *et al*., 2006b). The extraintestinal virulence gene profile of these isolates and their reduced capacity to cause disease in septicaemia and urinary tract infection (UTI) models (Sidjabat *et al*., 2009) supported concurrent research demonstrating that MDR-ExPEC strains isolated from humans had reduced virulence potential and belonged to phylogenetic groups A, B1 and D. By comparison, antibiotic sensitive strains with greater virulence potential were more likely to belong to phylogenetic group B2 (Johnson *et al*., 2003).

In 2007, emergence and pandemic spread of a virulent, phylogenetic group B2 MDR-ExPEC clonal group belonging to O-type O25b and multilocus sequence type 131 (O25b:ST131) challenged the paradigm and confirmed that ExPEC strains can concurrently evolve both virulence and resistance (Rogers *et al*., 2011). MDR-ExPEC O25b:ST131 is now a major cause of UTI and bloodstream infections in humans. We were among the first to report O25b:ST131 as a cause of extraintestinal opportunistic infections in companion animals (Platell *et al*., 2010). Internationally, O25b:ST131 strains have now been identified in several additional animal species, including poultry, rats and pigs (Platell *et al*., 2011). We showed that 10% of a large collection of fluoroquinolone-resistant (FQ^r) *E. coli* isolates from extraintestinal infections in companion animals (n=9) belonged to O25b:ST131 in comparison to >35% of FQ^r *E. coli* isolated from similar infections in humans (n=205) over a similar time period (Platell *et al*., 2010). Furthermore, the majority of Australian companion animal and human O25b:ST131 isolates shared many genetic and pathotypic similarities and belonged to an internationally distributed major human ST131 subtype (pulsotype 968). Some of the FQ^r *E. coli* O25b:ST131 strains also showed resistance to ß-lactams via carriage of a plasmid expressing CTX-M-15. Many of the ST131 strains isolated from animals in Europe carry this enzyme and it remains to be determined whether usage of the fourth generation cephalosporin, cefquinome (registered only in Europe) in livestock may be selecting for this particular resistance gene.

Emergence and Spread of MRS in Animals and Veterinary Personnel

Echoing the recent epidemiology of *E. coli* ST131 in animals, Methicillin-resistant *S. aureus* (MRSA) has now emerged internationally as a significant problem in companion animal hospitals and livestock facilities (Weese, 2010). It has generally been accepted that the strains colonizing and causing infections in dogs and cats such as clonal-complex (CC) 22 may have originated in humans (Morgan, 2008; Weese and van Duijkeren, 2010). By contrast MRSA strains isolated from horses and livestock such as CC8 and ST398 appear to be animal adapted. Strains of *Staphylococcus* normally associated with animals such as *S. pseudintermedius* are also becoming resistant to methicillin, with horizontal movement of *SCCmec* gene cassettes containing *mecA* into susceptible strains (van Duijkeren *et al*., 2008). Veterinary personnel and professions with significant animal contact have much higher rates of MRSA nasal carriage compared to the general population and several cases of MRSA infection in humans have been attributed to close animal contact (Jordan *et al*., 2011). MRS infections have been reported in companion animals in Australia, with the majority of strains also belonging to clonal-complex 22 (Malik *et al*., 2006).

In 2009, we surveyed 771 veterinarians for MRSA nasal carriage (Jordan *et al*., 2011). Among the respondents, non-clinical veterinarians (controls) had the lowest prevalence (0.9%). Veterinarians in mixed practice with horses as a major area of work emphasis had a prevalence of 11.8% (13x the controls) and those who indicated that their major emphasis was only horses had a prevalence of 21.4% (23x the controls). Veterinarians with dogs and cats as a major activity had a 4.9% prevalence (5x the controls). These results suggest that animal contact in a clinical setting may be an important risk factor for MRSA nasal carriage. The CC identities of 45 MRSA strains isolated from Australian veterinarians were determined utilizing real-time PCR high resolution melt curve analysis (HRMCA; Lilliebridge *et al*., 2011). A high proportion of strains from companion animal veterinarians belonged to CC22 (76.9%) and showed resistance to ciprofloxacin whereas strains from equine practitioners belonged to CC8 (62.5%) and were resistant to gentamicin and rifampicin. Two of the strains could not be typed by HRMCA and one of these strains was isolated from a pig veterinarian. This isolate has since been determined to belong to ST398 (G. Coombs, D. J. Trott and M.D. Barton, unpublished data). Whilst this result indicates that the major international animal-associated MRSA subtype ST398 does not appear to be prominent in Australia, the single ST398 strain identified was isolated in 2009 and more up to date studies are urgently required to determine how widespread this strain has become. Other major subtypes (CC22 and CC8) appear to be well established. The resistance profile of the isolates closely matching antibiotic usage patterns in each sector may indicate that the physical handling of antibiotics and administration to animals could be a significant risk factor for nasal carriage, The prevalence of resistance to fluoroquinolones (used mainly in companion animals) was close to 100% in isolates sourced from veterinarians who worked with dogs and cats, but zero in isolates

sourced from vets who worked exclusively with horses. Similarly, the prevalence of resistance to gentamicin and rifampin (used almost exclusively in horses) was much higher in isolates sourced from equine veterinarians compared to those who worked with dog and cats

Conclusion

These changes in the epidemiology of key MDR pathogens confirm that antibiotic resistance is no longer just an issue for public hospitals and the medical system. It is now an ecological issue, with selection pressure applied by the use of antimicrobial agents in many diverse environments including animal health. Every veterinarian must therefore make an evidence-based decision when prescribing an antibiotic to an individual animal or a group of animals and animal industries must be proactive in continuing to fund alternative control measures for bacterial diseases of livestock. There will always be a need to prescribe, but what to prescribe is currently a very grey area and the widespread availability of prudent use guidelines for each industry will be an important mitigation strategy. A major problem in defining the development of antimicrobial resistance and the emergence and spread of MDR pathogens such as MDR-ExPEC and MRS in veterinary settings in Australia is that the majority of clinical isolates obtained by veterinary diagnostic laboratories (VDLs), particularly private veterinary laboratories, are not stored for future surveillance purposes. A second problem is the current lack of co-ordinated and multi-disciplinary efforts to combat the emergence of resistance between University and Government veterinary teaching and research organizations, the veterinary profession and the major pharmaceutical companies supplying anti-infectives for the animal market. There is still much debate regarding the contribution of agricultural versus medical use of antibiotics to the development of pan-resistance of public health significance. In 2012, a proposed, multi-centre Australian Research Council (ARC) Linkage grant will take an Australia-wide approach to resistance surveillance in clinical isolates from all animal species and will provide much needed, objective data for future policy development and anti-infective product stewardship. By building an interconnected network of veterinary professionals and VDLs contributing to a single research programme, this project will attempt to encompass the recently outlined strategic vision for animal antimicrobial resistance surveillance in Australia (Gottlieb and Nimmo, 2011).

Prudent Use of Antimicrobials

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Abstract

Inappropriate or over-use of antibiotics risks selection and amplification of strains of bacteria that are resistant to antimicrobials. The challenges facing veterinarians and producers in modern commercial pig production include the emergence of Porcine Circovirus Associated Disease (PCVAD) and haemolytic *Escherichia coli* (*E. coli*) in pigs pre-weaning (10 days+). Unfortunately, antimicrobials remain a necessary tool for controlling disease caused by *Mycoplasma hyopneumoniae, Lawsonia intracellularis* and *Haemophilus parasuis*. Prudent antimicrobial use to minimize resistance development hinges on accurate disease diagnosis, followed by appropriate prescribing, dispensing and administration of effective drugs to target animals. There is no on-going national surveillance of antimicrobial use and resistance in Australia, unlike the situation in developed countries overseas. Antimicrobial stewardship programs, implemented in hospitals in Australia, are a potential model for prudent antimicrobial use on large-scale pig farms.

Introduction

Health management on pig farms is becoming increasing complex as new diseases emerge and average herd size increases. The emergence of PCVAD brought about challenges for disease control on even "high health" status farms prior to the availability of commercial vaccines. Antimicrobials are no longer the first line of defence for many diseases as commercial alternatives become available. This paper focuses on how individual farms and the pig industry as a whole are working toward minimal use of antimicrobials as a means of preserving their efficacy for animal and human use into the future.

Major Health Challenges

Respiratory and enteric diseases have the greatest impact on the biological performance of pigs on the farms where they exist, resulting in decreased weight gain, reduced feed conversion efficiency, increased mortalities, increased variation and increased control costs. Respiratory disease complex initiated by *Mycoplasma hyopneumoniae* continues to be a major contributor to poor herd performance, despite the availability of effective commercial *M. hyopneumoniae* vaccines. This disease has its greatest impact on pigs at the optimal growing period between ten weeks of age to slaughter (Thacker, 2006). The financial losses due to *M. hyopneumoniae* are likely to vary considerably between herds depending on environmental conditions and the presence of secondary infections (Maes *et al*., 1998).

Haemophilus parasuis is a commensal organism of the upper respiratory tract of pigs, but under appropriate conditions can invade and cause acute severe systemic disease (Glassers disease), characterized by fibrinous polyserositis, arthritis and meningitis (Oliveira and Pijoan, 2003). *H. parasuis* can also be a secondary invader to *M. hyopneumoniae*-induced respiratory disease complex. Control of *H. parasuis* infections can be achieved with commercial or autogenous vaccines, supplemented with judicious antibiotic use. However, serovar diversity and the substantial proportion of non-typable isolates often complicate the development of efficacious vaccination programs for disease control (Bak and Riising, 2002).

Pre-weaning diarrhoea due to *E. coli* continues to plague many pig herds, resulting in economic losses due to mortality, morbidity, decreased growth rate and cost of medication (Fairbrother *et al*., 2005). There is multiple bacterial resistance among *E. coli* isolates to a wide range of commonly-used antibiotics on many farms (V.A. Fahy, personal communication). Two commercial vaccines are available in Australia to give parenterally to sows before farrowing to prevent disease in piglets. These contain either whole cell bacterins (Ecovac®, Intervet Australia Pty Ltd, Bendigo East, VIC) or purified fimbrial antigens (Neovac®, Pfizer Australia Pty Ltd, West Ryde, NSW). These vaccines are very effective in controlling diarrhoea due to non-haemolytic enterotoxigenic *E. coli* in the first five days of life. They are however not effective at controlling haemolytic *E. coli* (HETEC) after that age. This requires the presence of lactogenic immunity in the sow and mucosal immunity in weaners. In the past 15 years, HETEC have been increasingly responsible for disease in older piglets (seven days of age to 14 days post-weaning). Piglets are often first seen in a state of shock with sub-normal temperatures. Control of this disease is difficult as it is hard to predict and the HETEC responsible are often resistant to registered antimicrobials so prophylactic medication is often unrewarding (Fahy *et al*., 2003). Oral vaccination of sows to produce lactogenic antibodies, and of piglets one week before weaning (Autovac®, Department of Primary Industries, Bendigo, VIC) have proven to be highly efficacious on many farms.

However, on some farms in the weaner house, the disease is not completely controlled, despite using oral vaccination (V.A. Fahy, personal communication; A Lee, personal communication). This is most likely due to inadequate expression of virulence antigens (fimbri) during vaccine manufacture.

Proliferative enteropathy (PE) caused by *Lawsonia intracellularis* affects young pigs (six to 20 weeks of age) resulting in decreased growth rate, reduced feed conversion efficiency and increased variation in liveweight among batches of pigs of the same age. In pre-sale pigs or selected breeding stock, the haemorrhagic form of the disease causes pigs to lose blood through their intestine, resulting in anaemia, dysentery and death. PE is endemic in pig herds in Australia, with a reported within-herd prevalence of 84.2% (Holyoake *et al*., 2010). Recent estimates of the economic impact of PE using the AUSPIG growth simulation model found that subclinical disease reduced net revenue by $8.33AUD, whilst clinical disease reduced net revenue by $13.00AUD, relative to a non-infected "herd" (Holyoake *et al*., 2011). These figures are independent of control costs. Much of the cost of PE is due to the widespread use of antibiotics in the pigs' diets to treat and prevent the disease, and veterinarians and producers may be reluctant to remove or change the medication. Availability of a commercial vaccine (Enterisol Ileitis®, Boehringer Ingelheim Pty Ltd, North Ryde NSW) in Australia in 2006 has provided an alternative to reliance on in-feed medications to control PE. Barriers to adopting vaccination in Australia include perceived lack of efficacy, particularly in bedded systems, high cost relative to medication ('medication is more effective/reliable'), difficulties in drenching weaner pigs and pigs housed outdoors and the requirement to have a 'medication-free window' around the time of vaccination (Holyoake *et al*., 2009).

PCVAD is a relative newcomer to the list of diseases affecting pigs in Australia, despite the widespread prevalence of Porcine Circovirus Type 2 (PCV2) in this country (Finlaison *et al*., 2007). Overseas, PCV2 has been associated with a number of disease syndromes in pigs, including post-weaning multi-systemic wasting syndrome (Clark, 1997), porcine dermatitis and nephropathy syndrome (Wellenberg *et al*., 2004), reproductive disorders (Mateusen *et al*., 2007), enteritis (Jensen, 2006) and respiratory disease (Kim et. al., 2003). Few outbreaks of PCVAD have been reported in Australia (Cameron, 1995; O'Dea *et al*., 2011). Prior to the availability of commercial vaccines, attempts to control PCVAD relied largely on management of the environment (Madec *et al*., 2000), often with little improvement in pig health.

Prudent Antimicrobial Use

Prudent use of antimicrobials is an integral part of sustainable livestock production to maximise therapeutic efficacy and minimise selection of resistant micro-organisms. Prudent use principles are a guide for optimal use of antibiotics and exist in most developed countries. The Australian Veterinary Association in 2005 published prescribing and dispensing guidelines targeted at veterinarians, and these include guidelines for the use of veterinary medicines in the pig industry (Anon, 2005). These guidelines include legal and ethical requirements for veterinarians prescribing Schedule 4 (S4) restricted substances, valid vet-client relationships and duty of care and specify the documentation required with prescribing S4s. The World Veterinary Association released a draft position on the responsible use of antimicrobials in 2011 (Jorna and Vogel, 2011). This document outlines 12 basic principles on the use of antimicrobials, and not on the governmental measures such as licensing and controls.

Included in the draft WVA principles are the requirement that antimicrobials be used for disease prevention, control and treatment. It is recommended in this document that use of antimicrobials to enhance production through growth promotion and feed efficiency should be subjected to risk analysis, including animal and human health benefit assessments, to determine if risk management measures are needed. Denmark began a voluntary removal of antibiotics as growth promoters for finishing pigs in 1998. In 2000, the use of antibiotics as growth promoters was withdrawn for all swine. As of January 1, 2006, the European Union, of which Denmark is a member, has prohibited the use of antibiotics as growth promoters in swine, cattle, poultry and rabbits. There are currently no legislated restrictions on the use of antimicrobials to promote growth and feed efficiency in Australia.

The choice of antimicrobial should be based on an accurate diagnosis, approved use for the species and indication, proven efficacy, known or predictable sensitivity of the micro-organisms involved and pharmacokinetics/tissue distribution. An accurate diagnosis is crucial to determine whether antimicrobials are appropriate, and should be based on clinical and pathological evaluation of the animals under the care of the prescribing veterinary surgeon and on the judgement that antibiotic therapy will have a beneficial effect. When it is not possible to make a direct clinical evaluation, the diagnosis should be based on past experience, on knowledge of the farm epidemiological status and on ongoing diagnostics (eg. abattoir monitoring of carcases) and sensitivity testing.

No medicinal product can be placed on the market unless its quality, safety and efficacy have been demonstrated. All agricultural and veterinary chemical products with a therapeutic claim in the Australian marketplace must be registered by the Australian Pesticides and Veterinary Medicines Authority (APVMA). The first antimicrobial choice should be based on the products approved for the species and the indication concerned. Use of antimicrobials that are registered for food-producing animals may also be prescribed by veterinarians ("off-label") unless there is a label restraint ("do not use"). An example of this is Excenel® Powder for Injection (Pfizer Australia Pty Ltd, North Ryde, NSW), which is registered to treat respiratory disease in cattle and horses but has a label restraint preventing "mass medication". The use of this product in food-producing animals is contentious, as the active ingredient (ceftiofur) is classed as a "last resort" antimicrobial in human medicine (JETACAR, 1999).

Antimicrobials should only be used as the first line of treatment when it is known or suspected that an infectious agent, that will be susceptible to the therapy, is present. The exceptions to this would be to institute antimicrobials prior to reaching a definitive diagnosis in disease outbreaks involving high case mortality rates or where there are signs of rapid transmission of disease among contact animals. When treating a disease, the sensitivity of the causal organism should be ascertained before therapy is started where treatment fails. Antibiotic sensitivity trends should be monitored over time, and used to guide clinical judgement on antibiotic usage. Susceptibility testing can only give an indication of what the clinical activity of the drug will be and may be subject to errors with regard to methodology, quality control, appropriate interpretive criteria and calculation of minimum inhibitory concentrations (MICs; Schwartz *et al*., 2010). Breakpoints of "sensitive", "intermediate" or "resistant" are then interpreted with a specific MIC. One must be aware that validated breakpoints are specific for an antimicrobial, a specific regime of the antimicrobial (dose, route, duration, frequency), an animal species (this may include age or another sub-classification), a specific pathogen and a specific disease entity. Much of these data are only available for human pathogens. Different application for a drug may have different breakpoints. At best, breakpoints reported from veterinary laboratories for non-validated antimicrobials/pathogens may be used as a guide only to the efficacy of the product (Apley, 2001).

The efficacy of an antimicrobial in the field depends on its ability to reach the site of infection in a high enough concentration, the nature of the pathological process and the immune response of the host. The choice of the right antibiotic also needs to take into account pharmacokinetic parameters, such as bioavailability, tissue distribution, half-life, tissue kinetics to ensure the selected therapeutic agent reaches the site of infection.

WVA guidelines (2011) state that "antimicrobials used for therapy should be used for as long as needed and over as short a dosage period as possible". Insufficient duration of administration can lead to recrudescent of the infection. This may lead to increased likelihood of selecting micro-organisms with reduced antimicrobial sensitivity. Limiting the duration of use to only that required for therapeutic effect will minimize the exposure of the bacterial population to the antimicrobial. The adverse effects on the surviving commensal microflora are minimized and the medication impact of the remaining zoonotic organisms is minimised/reduced. Theoretically, antimicrobial use should be stopped as soon as the animal's own defence system can control the infection itself.

Monitoring of Antimicrobial Use and Resistance

There are currently no on-going formal systems for monitoring antimicrobial use or resistance in pigs in Australia. A number of systems for monitoring antimicrobial use on pig farms and/or resistance of pig pathogens have been in place in a number of overseas countries including Finland (FINRES-VET), Denmark (DANMAP), Canada (CIPARS), France (French Antimicrobial Resistance Monitoring in Bacteria of Animal Origin), Italy (ITAVARM), Netherlands (MARAN), the USA (NARMS), Norway (NORM-VET), Sweden (SVARM), Spain (VAV) and the UK. The most recent Australian study of antimicrobial resistance in bacteria found in pigs was commissioned by the Department of Agriculture, Fisheries and Forestry in 2003-4 (DAFF, 2007). In this survey, 200 faecal samples were collected at slaughter from pigs and *E. coli* and *Enterococcus spp*. isolated tested for antimicrobial resistance. This study reported a high percentage of tetracycline resistance among isolates. Surprisingly, 30-40% of isolates were also resistant to antimicrobials not used in pigs (florfenicol, chloramphenicol). A more recent survey of bacteria on retail pork (Barlow and Gobius, 2008) produced similar results. The results of these studies suggest there may be little correlation between antimicrobial use and presence of resistant bacterial isolates.

Jordan *et al*., (2009) reported on prescribing practices of pig veterinarians in Australia based on responses from an internet-based survey. The results of this study indicated that most antimicrobials used were drugs of low importance to public health (eg. tetracyclines, penicillins and sulfonamides). Infections attributed to *L. intracellularis*, *M. hyopneumoniae* and *E. coli* motivated the most use of antimicrobials. In Denmark, the majority of antimicrobial agents prescribed from 2002 to 2008 were for the control of gastrointestinal and respiratory disease in weaner and finisher pigs. The most commonly used class of antibiotics was tetracyclines for all age-groups of pigs (Jensen *et al*., 2011).

In Canada in 2007-2008, the most common route of antimicrobial administration among finisher pigs was via feed, predominantly macrolides/lincosamides (Deckert *et al.*, 2010). The primary reasons given for macrolide/lincosamide use were disease prevention, growth promotion and treatment of enteric disease. Exposure via feed can be a concern for antimicrobial development since antimicrobials in-feed typically involve exposure to larger numbers of animals over a prolonged period of time, whereas antimicrobial exposure by injection generally involves a smaller defined number of animals (Dunlop *et al.*, 1998).

Information on the factors motivating veterinarians to prescribe antimicrobials would assist with development of policy making and could be used to specify farm advice and investigation. An epidemiological study conducted using 300 farm-year records during 2004-2007 in the Netherlands reported that the use of antibiotics varied between individual farms (van der Fels-Klerx *et al.*, 2011). "Heavy users" (20% of farms) used 45% of the antimicrobials, and this use was relatively stable over the four-year study period. The authors speculate that this may be due to differences between farms in hygiene status, prophylactic use and treatment decisions made by the farmer or the veterinarian or both. Antimicrobial use was mainly influenced by the farm system (fewer finishers were medicated on farrow-to-finish farms than finish-only farms) and the number of pigs present on the farms (greater number of pigs present on the farm may result in a greater probability of infection). For sow farms only, antibiotic use was affected by the population density in the region of the farm.

In the study conducted in Australia by Jordan *et al.* (2009), it is speculated that antimicrobial choice was largely driven by the availability of forms allowing efficient dosing in feed or water, advantages in pharmacodynamics and the affordability and advantages in the spectrum of activity of the drugs used. An example cited was tylosin, which is suitable for use as a feed or water additive for managing *M. hyopneumonae* –induced respiratory disease in pigs, but also has efficacy against other major pathogens such as *L. intracellularis*. A recent survey of veterinarians working with pigs in Australia (Holyoake *et al.*, 2009) revealed that "efficacy" ranked third behind "cost" and "wide spectrum of activity" as features of an antimicrobial that favoured its use to control ileitis. A short withholding period became more important for older pigs in this study.

Non-Antimicrobials

There is clear evidence that environment and management impact on the prevalence and severity of diseases in pig herds. Environmental factors including air quality, hygiene, temperature, stocking rate and density and pig flow (all-in/all-out (AIAO) versus continuous flow) all impact on pig health and growth performance. Pigs housed in dirty environments under commercial conditions grow slower and have poorer respiratory health than pigs housed in clean or isolated environments (Crowe *et al.*, 1996; Jolie *et al.*, 1999). Pigs housed in AIAO sheds that were cleaned thoroughly between batches grew 39 g/d faster and had significantly less lung damage and pleurisy at slaughter than pigs housed in adjacent AIAO sections that were not cleaned (Cargill and Banhazi, 1998). There are positive correlations between the number of pigs in the shed and pleurisy prevalence, pneumonia prevalence and coughing rate (Cargill *et al.*, 1996). Assuming that the volume (kg/m^3 of floor space/year) of pigs can be maintained, improvements in hygiene, stocking density and ventilation that do not rely on antibiotics to lift pig productivity are profitable and sustainable.

Commercial vaccines are available for the majority of economically-significant pig diseases in Australia. The last 20 years has seen the development and availability of vaccines for *M. hyopneumoniae, Actinobacillus pleuropneumoniae, H. parasuis, L. intracellularis, E. coli* and PCV2. Where vaccine-induced protection is serovar-specific (*A. pleuropneumoniae, H. parasuis, E. coli*), the challenge for veterinarians in recommending these vaccines is ensuring that the pathogenic serotype harvested during a diagnostic investigation is included in the vaccine used on-farm. The availability of live vaccines, where protection afforded crosses all serovars holds promise in overcoming this issue. Optimal vaccine efficacy will only be achieved if vaccines are administered at the appropriate time (particularly to avoid blocking by maternal antibody interference) and according to recommended scheduling by trained stockpersons.

Research on the efficacy of a number of antibiotic alternatives has been on-going. Examples of these include acids, probiotics, prebiotics, bacteriophages and metals (zinc and copper). The addition of organic and/or inorganic acids to the weaner pig's diet or drinking water has been shown to improve feed efficiency and enhance growth performance (Easter, 1993; Partanen and Mroz, 1999). Strong organic acids such as lactic and citric appear to be consistently beneficial (Easter, 1993). Organic acids have been shown to be less successful in the growing finishing stage of pig production (Giesting and Easter, 1985), possibly due to the advanced development of the digestive tract, resulting in a lower gastric pH. Diet acidification is considered the underlying mechanism for fermented liquid feeding (Mikkelson and Jensenm, 1997). The practice of diet acidification and/or liquid feeding is becoming increasingly popular as growth promotants are removed. Probiotics offer the potential to increase normal microflora population by selectively excluding specific pathogenic bacteria (eg. Salmonella, *E. coli*) in the intestine (Gibson and Fuller, 2000). The use of these on commercial pig farms in Australia is sporadic, despite long-term research and development efforts.

Conclusions

The availability and use of a variety of antimicrobials for pigs is essential to assure animal health and welfare. There is a risk that inappropriate use can result in resistance which negatively affects public and animal health. The recently-funded Cooperative Research Centre for High Integrity Australian Pork seeks to halve current antibiotic costs over its lifetime. This is to be achieved through improved diagnostic and health monitoring systems to control disease, new pig genotypes and genetic technologies and antibiotic-free integrated health management strategies.

An effective approach to improving antimicrobial use in hospitals is an organized antimicrobial management program, known as antimicrobial stewardship (AMS; Duguid and Cruickshank, 2011). AMS involves a systematic approach to optimising the use of antimicrobials. It is currently used by healthcare institutions to reduce inappropriate antimicrobial use, improve patient outcomes and reduce adverse consequences of antimicrobial use (including antimicrobial resistance, toxicity and unnecessary costs (Macdougall and Polk, 2005). Opportunities to apply the principles of AMS to the pig industry in Australia should be considered, along with adoption of WVA antimicrobial use guidelines.

It is often quoted that "you can't manage what you don't monitor". With this in mind, it is imperative that antimicrobial use and resistance within the pig industry is monitored, in-line with many developed countries overseas.

Nutritional Alternatives for Antimicrobial Control in Pigs

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Introduction

Antimicrobials have played a significant role in the modern health management of pigs. Traditionally in-feed antibiotics and chemotherapeutics have been used for two main reasons; 1) to stimulate growth, and 2) for control of disease (Bhandari *et al.*, 2008). Whilst it is unlikely that antibiotics and/or chemotherapeutics will disappear from main stream animal production, changing legislative and consumer pressures both domestically and internationally are encouraging the industries to limit there use in animal production where possible. Ultimately these pressures are encouraging producers to find ways to maintain animal production performance whilst reducing the reliance on antibiotics. It is obvious that optimal welfare standards, animal husbandry, hygiene and animal production management are critical to maintaining production performance. However, the search for nutritional antimicrobial alternatives has so far produced some promising contributors, though their success is very much tied to a complimentary input from the above factors.

To be able to evaluate the benefits and limitations of nutritional antimicrobial alternatives those involved in the industry must first appreciate the role antibiotics and chemotherapeutics play in both growth stimulation and disease management. The aim of this review is to discuss firstly the role of antimicrobials in animal production and secondly to evaluate a range of known nutritional alternatives to antimicrobials. The review will focus on favourable regulation and manipulation of gut microflora, favourable regulation of inflammation, influence on immunity and finally the subsequent influences on animal performance.

Antimicrobials collectively are described as substances which either destroy microbes (bacteria, viruses, protozoa) prevent their development or inhibit their pathogenic actions. Antimicrobials can have both antibacterial and anti-inflammatory effects which ultimately can effect growth, feed intake, milk production, reproduction and metabolic health (Buret, 2010).

Alternatives to antibiotics may use a range or combination of mechanisms to enhance growth performance and/or limit the colonisation of intestinal pathogens. These mechanisms include; altering gut pH, maintaining protective mucins, bacterial selection for beneficial organisms or against harmful pathogens, maintaining gut structure, enhanced production of fermentation acids, enhanced nutrient uptake, and altering immune response.

Altering Gut pH

Antibiotics do not directly affect the pH of the intestinal environment. They may in some cases indirectly alter the pH by manipulating the microflora populations. As many bacteria are not acid tolerant, nutritional alternatives which can directly or indirectly alter the intestinal pH can have favourable antimicrobial effects.

Acids

The pH of the gastrointestinal tract can be altered directly by the additional of acidifiers administered either through feed or water. Like antibiotics and chemotherapeutics, organic acids have antimicrobial activity, however, this activity is pH dependent. Short chain organic acids are particularly effective against acid intolerant bacteria including *E. coli*, salmonella and campylobacter. Like antibiotics, the effects of organic acids on intestinal microflora lead to a lower incidence of subclinical infection, decreased secretion of immune mediators, reduced production of ammonia and reduced production of other growth depression microbial metabolites (Dibner and Buttin, 2002). Organic acids also have some advantages over antibiotics which include; reducing digesta pH, increasing pancreatic excretion, trophic effects on the gut, and no risk of bacterial resistance developing. However, like all feed additives the efficiency of organic acids is dependent on external factors which include; buffering capacity of the diet, presence of other antimicrobials, dietary fibre content, endogenous short-chain fatty acid (SCFA) production, vaccine programs, genetic factors, hygiene, and homogeneity of intestinal microbiota (Dibner and Buttin, 2002; Mroz, 2005). The spectrum of antibacterial protection from organic acids has been broadened by using a blend of acids which have a range of pKa values and bacteriocidal anions.

Carbohydrates

Altering the pH in the large intestine is most likely to be a result of changes in the volatile fatty acid production and microbial fermentation (Namkung *et al*., 2004). Feed ingredients including non-digestible fibres such as mannanoligosaccharides and fructo-oligosaccharides have been shown to lower the pH of the large intestine through the production of short chain fatty acids as a result of bacterial fermentation. Offering piglets diets which contained inulin was also shown to lower the caecal pH in pigs (Williams *et al*., 2001).

Protein

Increasing the plant protein sources in weaner pig diets (at the expense of animal proteins) was shown to increase the fermentation activity in the large intestine and subsequently lower the pH (Montagne *et al*., 2004).

Maintaining Protective Mucins

The protective mucus layer is predominantly composed of mucins and glycoproteins, both of which are rich in threonine (Law *et al*., 2007). Whilst threonine is an essential amino acid for lean growth the gastrointestinal tract preferentially utilises up to 90% of the dietary threonine. Threonine deficiency is associated with decreased nitrogen retention, compromised innate immunity, increased scouring and increased maintenance costs in weaned pigs (Law *et al*., 2007). Lactobacillus probiotics, oligosaccharides and galactans are known to mediate mucin production and enhance the epithelial barrier against pathogenic organisms; however mucin production is energetically costly to the young pig (Collier *et al*., 2003; Ferket, 2003).

Bacterial Selection

The philosophy that as microbial diversity increases, the opportunities for pathogenic invasion decreases, is generally accepted. Antibiotics are generally associated with decreased microbial counts along the intestine (Niewold, 2007). Whilst some argue antibiotic administration promotes homogeneity within the microbial populations (Niewold, 2007; Willing, 2011) others suggest species richness may be enhanced and/or maintained at the expense of species diversity through the administration of antibiotics (Bhandari *et al*., 2008). Establishing and maintaining a beneficial gut microbiota is an essential part of the pig's health and well being, as the gut microbes are known to modulate the expression of metabolites not only locally but also systemically (Willing, 2011). It is unlikely that nutritional alternatives to antibiotics and chemotherapeutics can achieve identical manipulation of intestinal microbiota. The majority of nutritional alternatives to antibiotics will likely focus on promoting the growth of beneficial commensal bacteria whilst suppressing those that are deleterious (Collier *et al*., 2003). While having a more favourable balance of microflora should benefit the pig's immune competence, it is unlikely to induce the same level of growth promotion due to the increased metabolic costs associated with maintaining dense microbial populations in the gut.

The dominant phyla of bacteria in the intestine include the bacteroidetes and the firmicutes. Antibiotics have been shown to increase the presence of bacteroidetes at the expense of firmicutes (Bhandari *et al*., 2008). This shift has been proposed as one contributing mechanism by which antibiotics provide protection and subsequently promote growth. Interestingly, diets containing either 5% spray dried porcine plasma or 0.5% prebiotic (*Bacillus subtilis*, $1.2x10^9$ CFU/kg) have been shown to promote a similar shift in the intestinal microflora (Bhandari *et al*., 2008).

Other feed additives/raw materials which have been shown to promote species richness predominately in the colon of pigs include non-digestible fibres like inulin, lactulose, wheat starch and sugar beet pulp (Konstantinov *et al*., 2004). The success of such prebiotics is dependent on factors including water solubility, the degree of polymerization, and lignification of the carbohydrates (Willing, 2011).

Both short chain and medium chain fatty acids have antimicrobial activity. Whilst short chain fatty acids are particularly effective at penetrating the phospholipid membrane of most Gram negative bacteria, the medium chain fatty acids (in their undissociated form) are capable of penetrating both the phospholipid membrane of Gram negative bacteria and the peptidoglycan membrane of Gram positive bacteria via passive diffusion (Decuypere and Dierick, 2003). Interestingly sows milk and most pig feeds are low in medium chain fatty acids, so supplemental medium chain fatty acids in combination with exogenous lipolytic enzymes present another nutritional alternative to antimicrobials (Decuypere and Dierick, 2003).

Dietary nucleotides have been shown to modulate the microflora in the ileum (but not jejunum) of newly weaned pigs, with a decreased rate of microflora differentiation (Andreas-Elias *et al*., 2007). In human infants provided with milk formula, dietary nucleotide supplementation was shown to have a prebiotic effect on the colon microbiology (Singhal *et al*., 2008). Dietary nucleotides are suggested to prevent the endotoxin induced mucosal damage by limiting bacterial translocation of harmful bacteria (Gil, 2002). These results suggest nucleotide supplementation may assist in

the proliferation of beneficial bacteria and therefore aid in maintaining microbial and immunological homeostasis in the intestine of the newly weaned pig.

Phenolic compounds found in phytobiotics (especially thyme, oregano and sage) are known to have antimicrobial properties, which are believed to intrude the bacterial cell membrane, causing structural disintegration and ion leakage (Windisch *et al*., 2008). A blend of essential oils (Crina®, DSM Nutritional Products, Wagga Wagga, NSW) was shown to decrease the shedding of haemolytic *E. coli* in commercial housed piglets (Valientes *et al*., 2011). Plant extracts (containing 5% carvacrol, 3% cinnamaldehyde, and 2% capsicum oleoresin) have been shown to favourable increase the lactobacilli to enterobacteria ratio in the distal jejunum and caecum of early weaned pigs (Castillo *et al*., 2006; Manzanilla *et al*., 2004). This increase is suggested as an index of intestinal equilibrium; however the exact mode of action remains unclear. The inclusion of this plant extract appeared to extend the retention time of feed in the stomach which may have improved nutrient digestion (Manzanilla *et al*., 2004). An improvement in protein digestion in particular may limit the risk of pathogenic bacterial challenges. Use of plant extracts is also believed to have a direct antimicrobial effect which reduces the microbial load in the ileal digesta of early weaned pigs (Manzanilla *et al*., 2004).

The mode of action commonly assigned to probiotics is that of competitive exclusion (Willing, 2011). Probiotics are able to suppress the growth of pathogenic bacteria firstly by, actively competing for limiting nutrients and secondly via the production of multiple bacteriocins (Collier *et al*., 2003).

Dietary zinc oxide included at pharmacological levels is also known to influence the intestinal microflora. Zinc oxide is associated with reduced bacterial translocation from the small intestine to the ileal mesenteric lymph nodes and increased homogeneity of coliform populations (Mavromichalis *et al*., 2000). The antibacterial activity of zinc oxide is greater for Gram positive bacteria (excluding *Streptococcus*) rather than Gram negative bacteria and *Streptococcus*. This bacterial selectiveness may have a similar effect on shifting the balance of bacteroidetes and firmicutes as antibiotics, however the growth promoting benefits of zinc oxide are known to be additive with carbadox, suggesting they have different modes of action (Mavromichalis *et al*.,2000).

Maintaining Gut Structure

The efficiency of nutrient absorption is regulated in part by the structural integrity of the intestinal mucosa including villus height. Weaning is commonly associated with villous atrophy and in some instances, crypt hyperplasia (Pluske *et al*., 1997). Antibiotics and other functional feed additives including spray dried porcine plasma, egg yolk antibodies, organic acids and minerals (ZnO) have been shown to aid in maintaining or promoting villus height in newly weaned pigs (Owusu-Asiedu *et al*., 2003). These benefits to the gut structure are commonly associated with avoidance of anorexia or suppressed feed intake. With appetite being regulated by cytokines (Dunshea *et al*., 2002), it is likely that these feed additives directly or indirectly influence the inflammatory responses of the newly weaned pig.

Enhancing Production of Fermentation Acids

Hind gut fermentation (primarily of non-starch polysaccharides) can contribute up to 16 or 30% of the maintenance energy in growing pigs and sows respectively (Choct and Kocher, 2000; Shi and Noblet, 1993; Varel, 1987). However, the net efficiency of energy utilisation via hind gut fermentation in pigs is low (Choct and Kocher, 2000). Antibiotics are known to reduce hind gut fermentation by up to 50% (Zhu *et al*., 1993). This would suggest that the growth promoting benefits achieved by the use of antibiotics are not directly related to hind gut fermentation. However, alternative strategies which exclude antibiotics may consider the contribution of hind gut fermentation on gut health and optimising nutrient utilisation. The production of short chain fatty acids in the large intestine is known to enhance sodium absorption (limiting the risk of diarrhoea), stimulate blood flow and regulate nutrient absorption (Choct and Kocher, 2000).

Fermentation of nutrients primarily in the hind gut results in the production of short chain volatile fatty acids. The main short chain fatty acids produced as a result of hind gut fermentation include; acetate, propionate and butyrate, which predominately provide an energy source for the periphery, liver and colonocytes respectively (Choct and Kocher, 2000). Increasing the production of these fatty acids is known to reduce the risk of digestive disorders, control microbial proteolysis, and to improve the partitioning of nutrients towards growth (Awati *et al*., 2006, Shen *et al*., 2009).

Dietary prebiotics including oligosaccharides can be used to stimulate hind gut fermentation as they act as a nutrient source for some strains of beneficial bifidobacteria. Oligosaccharide digestion in the hind gut is generally associated with an increase in lactobacilli and bifidobacteria and a decrease in clostridia and enterobacteria as a result of the decreased pH (Choct and Kocher, 2000; Williams *et al*., 2001). The application of dietary prebiotics, however, is

somewhat limited as excessive amounts of oligosaccharides within the small intestine, interferes with nutrient digestion and absorption (Choct and Kocher, 2000).

Micro-encapsulation of organic acids has now made it possible for the slow release of organic acids along the gastro-intestinal tract, allowing the organic acids to have a more direct effect on the microbial metabolism in the large intestine (Piva *et al*., 2002). Encapsulated organic acids have been shown to limit microbial proteolysis, and to stimulate the proliferation of cellulolytic bacteria and fibre digestion in the hindgut (Piva *et al*., 2002).

Enhancing Nutrient Utilisation

The intestinal microflora are involved in regulating nutrient absorption and growth performance. Probiotics containing some lactobacilli strains have been shown to down-regulate lipid absorption through the increased production of short chain fatty acids (Pereira and Gibson, 2002) and the production of bile salt hydrolase (Kalavathy *et al*., 2003). In poultry, probiotics containing 12 strains of lactobacilli were shown to increase growth performance, and carcase yield, whilst decreasing low density lipoprotein cholesterol, triglycerides and abdominal fat deposition (Kalavathy *et al*., 2003). Lactobacilli are often the unintended victim of antibiotic administration (Niewold, 2007). Antibiotic growth promotion is partially credited to the increased lipid absorption achieved through the inhibition of lactobacilli and subsequently bile salt hydrolase. This inhibition of bile salt deconjugation by antibiotics would also improve bile salt recycling via the enterohepatic cycle. However, the same inhibition limits the natural antimicrobial activity of deconjugated bile acids which are responsible for regulating bacterial growth in the small intestine (Midtvedt, 1974).

Supplemental acids can be used to improve protein utilisation in young pigs. A diet containing 0.5% benzoic acid was shown to improve ileal digestible nitrogen by 7% (Halas *et al*., 2010). The improved protein utilisation achieved by acidification has been related to the low pH necessary for the optimal conversion of pepsinogen to the important proteolytic enzyme pepsin; however another explanation may be the improved villus height demonstrated in pigs offered acidified diets.

Essential oils curcumin, capsaicin, piperine have been shown to enhance the pancreatic excretion of amylase in rats. The same essential oils and ginger were also able to increase amylase excretion in the intestinal mucosa (Platel and Srinivasan, 2000). This improved enzyme activity can be very beneficial in maximising starch digestion in young animals including pigs.

Altering Immune Response

A recent and seemingly plausible explanation for the growth promoting benefits of antibiotics was suggested by Niewold (2007) where antibiotics actually regulate intestinal inflammation by inhibiting the production and excretion of catabolic mediators in immune cells. This offers a plausible explanation for the reproducible effects of antibiotics on growth performance. When considering nutritional antimicrobial alternatives there are two main classes; 1) nutrients which support immune competence, especially during challenge, or 2) raw materials which also have the ability to modulate inflammation in the pig.

Nutrition and Immune Competence

Selenium is an essential nutrient in maximising pig immune function (Stein, 2007). Organic selenium has a higher bioavailability than inorganic forms, and is often included in both sow and piglet feeds to support optimal immune function and development. Providing supplemental Vitamin E and/or organic selenium has been shown to improve antibody synthesis and increase resistance to infection (Shurson *et al*., 2007). Chromium requirements have been shown to increase in newly weaned calves which are stressed. Chromium is known to be involved in the immune response of animals.

Pigs under immune challenge have a greater requirement for the antioxidant vitamins (vitamins A, C, E) and B vitamins (niacin, pantothenic acid, riboflavin, B12 and folacin). The increased antioxidant requirement is associated with the increased production of free radicals and cytokines which occur during increased exposure to antigens (Shurson *et al*., 2007).

Nucleotide requirements are known to increase during events which increase cell turn over rates such as inflammation, disease challenge, periods of rapid growth and recovery from malnutrition (Borda *et al*., 2003; Gil, 2002). Nucleotides have important roles in both cellular and humoral immunity. During an immune response in which activation of T lymphocytes occurs, a rapid increase in nucleotide synthesis is required to provide both a metabolic energy source and precursors of nucleic acids (Carver, 1994). Nucleotides contribute to cellular immunity by increasing the nucleotides available to leukocytes, as well as supporting optimal lymphocyte, natural killer cell,

enterocyte and macrophage function (Carver *et al.*, 1991; Carver, 1994; Jyonouchi *et al.*, 1994; Romano *et al.*, 2007). When pigs have an adequate nucleotide pool, immune responsiveness is optimised to support antigen presentation and lymphocyte proliferation (Carver, 1994; Romano *et al.*, 2007) thus suppressing the uncommitted T lymphocyte response (Gil, 2002). Nucleotides are also involved in cytokine expression of interleukins (IL) including; IL-2, IL-6 and IL-8 (Carver, 1994; Gil, 2002) which are involved in regulating; growth factor for lymphocytes, fever, acute phase response, inflammation, antigen presentation, and neutrophil recruitment (Bosi *et al.*, 1994; Carver *et al.*, 1991; Lyoumi *et al.*, 1998). Nucleotides are also involved in regulating humoral immunity. Nucleotide supplementation has been shown to increase plasma immunoglobulins; IgA, IgG and IgM (Lee *et al.*, 2007; Romano *et al.*, 2007). These responses are supported by the findings of Navarro *et al.* (1996) where AMP, UMP and GMP were involved in IgG regulation and GMP also involved in IgM response in mice.

Immune Modulation

Some commensal anaerobic bacteria belonging to the bacteroides phyla are known to secrete effector molecules which attenuate pro-inflammatory cytokine expression and aid in immunological tolerance and homeostatis in humans (Kelly *et al.*, 2004). Enhancing our understanding of the regulator role of specific commensal bacteria in gastrointestinal health and their influence on inflammation is necessary to overcome the challenges associated with chronic inflammation. Perhaps the next generation of probiotics will not only focus on competitive exclusion but will also consider bacterial ability to regulate cytokine expression.

Spray dried porcine plasma has been identified as one raw material which can actively improve the immune response of animals even when challenge is limited. Bio-active components within the product have been shown to successfully modulate the degree of activation of the gut-associated lymphoid tissue, which subsequently allows for the partial prevention of increased intestinal permeability and decreased nutrient absorption and helps to maintain intestinal function and structure (Perez-Bosque *et al.*, 2010). Plasma protein has also been shown to positively influence cytokine expression, resulting in the down-regulation of pro-inflammatory cytokines and an increased regulation of anti-inflammatory cytokines (Bosi *et al.*, 2004; Perez-Bosque *et al.*, 2010). The reduction in inflammation is also associated with a sparing effect on IgA secretion (Bosi *et al.*, 2004), lymphocyte, T cell and NK cell proliferation (Perez-Bosque *et al.*, 2008), and leukocyte infiltration (Jiang *et al.*, 2000). Spray-dried plasma also aids in maintaining mucosal permeability to limit pathogen translocation (Perez-Bosque *et al.*, 2008; Perez-Bosque *et al.*, 2010). Diets supplemented with plasma proteins have also been shown to assist in maintaining the production of mucosal defensins (natural innate antibacterial agents) which also indicates that spray dried plasma products aid in maintaining intestinal immune homeostasis. By maintaining production of mucosal defensins, together with the immunoglobulin and glycoprotein fractions providing alternative binding sites for pathogenic bacteria, plasma proteins may limit the secretions of endotoxins responsible for the onset of watery diarrhoea and increase the pigs resistance to microbial dysbiosis (Coffey and Cromwell, 1995; Owusu-Asiedu *et al.*, 2002; Bosi *et al.*, 2004; Garriga *et al.*, 2005; Pierce *et al.*, 2005). IGF-I from plasma products is speculated to have a localised effect on gut growth and function (de Rodas *et al.*, 1995). The reduced amino acid catabolism (Jiang *et al.*, 2000) and the superior growth performance of pigs offered spray-dried plasma suggests endocrine regulation is likely involved.

Oligosaccharides and mannobiose are nutritional immunomodulators which have been indentified as potential alternatives to antimicrobials (Agunos *et al.*, 2007). Manno-oligosaccharides have been shown to improve vaccine responses and disease resistance in poultry (Agunos *et al.*, 2007). In piglets, manno-oligosacharide supplementation has been shown to enhance disease resistance through leukocyte support, and to ameliorate fever during immune challenge (Che *et al.*, 2011). Mannobiose is a relatively new addition to the list of nutritional antimicrobial alternatives, and have been shown to beneficially modulate cell mediated immune responses. Like nucleotide supplementation, the benefits of mannobiose are time-dependent and continue after the supplementation period has ended (Agunos *et al.*, 2007).

Yeast cell wall components including ß-glucans, mannans and yeast extract/cultures have all been shown to improve macrophage function in a range of species (Shen *et al.*, 2009). This improved efficiency in immune response is associated with the animal's ability to preserve systemic ignorance and limit the migration of the response beyond the mucosal immune system (Shen *et al.*, 2009). Again this mechanism has a favourable effect of the partitioning of nutrients towards growth.

Quillaja saponaria is a plant extract which has been widely used as a vaccine adjuvant. It has been considered as a potential feed additive, with the active component being the saponin fraction. Yucca extract is another source of saponins. Saponins have been suggested as a microflora manipulator. A study by Turner *et al.* (2002) examined whether dietary inclusions (0-500 mg/kg) of Quillaja saponaria would influence the immune function of weaned pigs. The study found marginal effects of Quillaja saponaria on immune function, but also found no detrimental effects at the concentrations tested. Another plant extract (containing 5% carvacrol, 3% cinnamaldehyde, and 2%

capsicum oleoresin) was shown to stimulate the immune system of newly weaned pigs (Nofrarias *et al.*, 2006). This stimulation was evident by a reduction in intraepithelial lymphocytes in the jejunum, a decrease in the percentage of lymphocyte subsets in blood and ileocolic lymph nodes, combined with an increase in blood monocytes and lamina propria lymphocyte-like cell densities in the colon (Nofrarias *et al.*, 2006).

Ammonia Production

Raw material selection and dietary design which promotes maximal utilisation of protein in the small intestine, and results in limited protein entering the large intestine limits ammonia production. This reduction is favourable as it reduces the energy invested in the metabolic and immune processes associated with toxic impacts of the ammonia. Feed additives which are known to limit ammonia production in the large intestine include antibiotics, yucca extract, and prebiotics.

Fermented carbohydrates (prebiotics) are also able to decrease ammonia excretion (Williams *et al.*, 2001) which subsequently helps prolong the lifespan of colonocytes and reduce the maintenance costs associated with cell renewal (Willing, 2011). The inclusion of fermentable carbohydrates for 10 days was shown to decrease ammonia concentrations in the caecum, colon and faeces of pigs (Awati *et al.*, 2004).

Promoting Growth Performance

The growth promoting properties of sub-therapeutic antibiotics have been exploited to enhance the efficiency of livestock production. Antibiotics are known to be most effective in promoting growth in unsanitary and/or stressful environments. Whilst management practices should adopt strategies to limit environmental, physiological and immune stresses, nutritional strategies may assist in improving the growth performance of pigs in the absence of sub-therapeutic antibiotics. Table 1 outlines some of the growth performance benefits achieved in young pigs supplemented with nutritional growth promotants or antibiotics.

Organic Acids

Growth performance benefits of supplemental organic acids have been observed in newly weaned pigs under commercial conditions. In a study where individual organic acids (propionic acid, lactic acid, formic acid, malic acid, fumaric acid) were compared to Lincospectin (44ppm lincomycin and 44ppm spectinomycin) diets containing lactic acid at 1.6% were most effective at maintaining growth performance and health in newly weaned pigs (Tsiloyiannis *et al.*, 2001). Although all of the organic acids tested improved performance relative to a negative control group, none of the individual acids tested were as effective as the antibiotic treatment.

Minerals

Minerals like zinc oxide and copper sulphate have been used at pharmacological levels to improve growth performance of pigs. Zinc oxide is more effective than copper sulphate in the newly weaned pig (Hojberg *et al.*, 2005). Pharmacological levels of copper sulphate are more commonly associated with increased antimicrobial resistance issues (Shelton *et al.*, 2009). These differences highlight that zinc oxide and copper sulphate achieve growth promotion via different modes of action. Whilst the exact mode of action behind the success of zinc oxide remains elusive, dominant possibilities focus on an external effect within the gut. However, there is evidence of systemic effects of dietary zinc oxide included at pharmacological levels (Wilt and Carlson, 2009) and therefore some of the growth promoting benefits may be related to factors other than microflora manipulation.

Essential Oils

Feed intake of barrows was improved (P=0.02) by 9.6% with the dietary inclusion of 75ppm of Crina® (a commercial blend of essential oils) for five weeks prior to slaughter (Campbell *et al.*, 2001). Weight gain was also improved (P=0.03) by 11% in both gilts and barrows. Another study showed an improvement (P=0.05) in the feed conversion efficiency of gilts fed diets containing 75 ppm Crina® when the dietary energy and amino acid balance was optimised (Cadogan *et al.*, 2001). The proposed mechanism for the improved performance is that essential oils increase the availability of energy rather than protein.

Essential oils have also been shown to increase feed intake in lactating sows by 6-13.5% (Valientes, 2011). This improvement resulted in increased weaning weights by 3.2% and decreased body weight losses by 15.3% in sows (Valientes, 2011).

Plasma Proteins

Spray dried animal plasma proteins are presently one of the most promising raw materials available for improving the growth performance of newly weaned pigs. There are a range of spray dried animal proteins on the market including

bovine plasma, porcine plasma and fish plasma peptides, however, the most effective plasma protein for pigs is spray dried porcine plasma (Pierce *et al.*, 2005; Torrallardona, 2010). The superior properties of porcine plasma are believed to be related to specific antibodies against porcine pathogens (Torrallardona, 2010), like anti-K88 antibodies which prevent the colonisation and proliferation of ETEC (Owusu-Asiedu *et al.*, 2002) the bacteria commonly associated with post-weaning diarrhoea. Like antimicrobials, the efficiency of dietary plasma proteins is dependent on the pathogen load of the pigs (Coffey and Cromwell, 1995; Bergstrom *et al.*, 1997; Frank *et al.*, 2003; Zhao *et al.*, 2007). A large review covering 75 trials and 12000 weaners showed significant improvements in both average daily gain and average daily feed intake (Torrallardona, 2010). Improvements in feed conversion are also found but less consistently. The mechanisms which enhance feed intake in pigs offered spray dried porcine plasma are effective but transient (Edwards, 2011). In pigs offered spray-dried plasma products, the significant body weight advantage obtained in the initial week post-weaning can be maintained (Coffey and Cromwell, 1995; Pierce *et al.*, 2005) however growth checks are commonly observed when plasma is removed from the diet (Hansen *et al.*, 1993; Carlson *et al.*, 2005).

Diet Formulation

The gut is a major site of immune activity and this organ is at the forefront of immune competence. Consequently any event or material which irritates or stresses the gut can have far reaching consequences for general health well beyond localised irritation. Care in formulation to avoid dietetic stress or microbial disturbance is particularly important in newly weaned pigs due to their immunological vulnerability, but also has relevance to all other classes of pigs. Feed formulation and feeding management need to be coordinated if gastrointestinal accidents are to be avoided (eg. bowel tympani, twisted bowel, enterotoxaemia, prolapses, ulcers etc). Our reliance on sub-therapeutic antibiotics may be reduced if we can limit nutritional related stresses which disturb intestinal microflora and the intestinal immune system. The significance of dietary regulation will become increasing influential as we optimise the other contributing factors including; hygiene, herd management, genetic selection, welfare, and housing.

Dietary Factors

There are a range of dietary factors, some of which have been discussed above which may induce or limit gastrointestinal stress and/or microbial disturbances. These dietary factors will be discussed below in relation to establishing recommended safeguards in diet design and raw material selection.

Grain Base

The level of starch and the rate of starch fermentation can have an influence on the intestinal environment. When selecting grains for diets, the risks associated with the level of starch and the rate of starch fermentation can be spread by using multiple grain types. Using a range of grains can be beneficial in manipulating grind size and starch granule size and shape to regulate the site and rate of digestion and fermentation. A recent study showed that starch digestibility in pigs varies between grains with maize having the highest digestibility followed by wheat, barley and lastly potatoes (Lee *et al.*, 2011). A range of processing methods (ie. disc mill, hammer mill, roller mill, extruder, pelleting) can be used to improve the digestibility of grain based starch and other nutrients.

The levels of non-starch polysaccharides in the diet can also alter the nutrient availability of the diet, intestinal microflora and site of digestion/fermentation. These detrimental effects can be negated by the use of substrate specific enzymes. The inclusion of non-starch polysaccharide (NSP) enzymes in the diets of grower pigs have been shown to improve nutrient digestibility including energy, protein and amino acids (Ao *et al.*, 2010; O'Connell *et al.*, 2005). The inclusion of NSP enzymes in barley-based diets was shown to have a significant effect on caecal and colonic microflora; whilst the same NSP enzyme in a wheat-based diet have minimal effect on gut microflora (O'Connell *et al.*, 2005). NSP enzymes also influenced the ammonia production and excretion in pigs offered barley based diets (O'Connell *et al.*, 2005).

The grain base is also a potential reservoir for potentially harmful contaminants including mycotoxins, chemicals and alkaloids. Up to 25% of the worlds grain is believed to contain mycotoxins (Akande *et al.*, 2006). Grain inspection and quality assurance testing are essential safe guards in limiting the risk of contaminants. Beyond these initial safeguards, feed additives like mycotoxin binders, mould inhibitors, and feed preservatives (ie. organic acids and antioxidants) are important tools available to limit the detrimental effects (eg. immunosupression, neurotoxicity, reproductive failures) of contaminants in the grain. The cost of contaminants on performance can have long term implications including permanent organ damage, significant immune suppression, and impaired reproductive capacity (Akande *et al.*, 2006). All these implications can impact heavily on animal production and its profitability. Grain contaminated with mould and subsequent mycotoxins can also degrade the nutritional value of the raw material. The degradation will be proportional to both the level of contamination and the period of storage. This degradation effects energy, protein and especially fat contents of grains (Akande *et al.*, 2006).

Table 1. *Improvements in growth performance of young pigs fed nutritional or antimicrobial growth promotants.*

Growth Performance	Acidifier	Inclusion rate	Δ ADG	Δ ADFI	Δ FCR	Reference
Acidifiers	Propionic acid	1%	8.50%	3.2%	-4.7%	Tsiloyiannis *et al.*, 2001
	Lactic acid	1.60%	21.60%	8.9%	-10.4%	
	Formic acid	1.20%	14.60%	6.5%	-7.2%	
	Malic acid	1.20%	10.30%	4.5%	-5.4%	
	Citric acid	1.50%	10.80%	5.5%	-4.8%	
	Fumaric acid	1.50%	13.10%	6.2%	-6.2%	
	Gluconic acid	0.60%	13.90%	10.7%	-2.4%	Biagi *et al.*, 2006
	Sodium butyrate	1000 ppm	5.80%	4.1%	-1.3%	Biagi *et al.*, 2007
		4000 ppm	10.00%	8.0%	-2.3%	
	Benzoic acid	1%	15%	9.0%	-6.0%	Kluge *et al.*, 2006
		0.50%	11.40%	15.0%	3.2%	Halas *et al.*, 2010
	Acid Blend*	0.40%	0%	-3.8%	-1.4%	Walsh *et al.*, 2007
		0.40%	2.90%	-0.8%	-3.5%	
	Fumaric acid	2%	54%	50.0%		Owusu-Asiedu *et al.*, 2003
Antibiotics	Carbadox	55ppm	4.90%	-2.4%	-7.0%	Walsh *et al.*, 2007
		55ppm	11.80%	8.8%	-2.5%	
		55ppm	51.20%	57.7%	4.2%	Owusu-Asiedu *et al.*, 2003
	CTC+Tilmicosin	400+400ppm	5.40%	7.1%	-26.0%	Edwards, 2011
		400+400ppm	6.90%	-1.6%	0.5%	Edwards, 2011
Feed supplements	SDPP	10%	55.20%	51.2%	-2.7%	Owusu-Asiedu *et al.*, 2003
		5%	93%	28.0%	-40.0%	Edwards, 2011
		5%	44%	7.9%	-27.0%	Edwards, 2011
		6%	28.10%	19.0%	-8.1%	Zhao *et al.*, 2007
		8%	62.40%	48.6%	-10.6%	Pierce *et al.*, 2005
Minerals	Zinc Oxide	0.40%	57.50%	52.20%	3.4%	Owusu-Asiedu *et al.*, 2003
		3000ppm	24.60%	0.40%	-13.4%	Smith *et al.*, 1997
		3000ppm	18.70%	15.90%	-2.3%	Hill *et al.*, 2001
	Copper	200ppm	20%	10.70%	-8.10%	Zhao *et al.*, 2007
		250ppm	-2%	-6.40%	-7.30%	Smith *et al.*, 1997

*Acid Lac® (containing fumaric acid, lactic acid, benzoic acid, citric acid, propionic acid and ethyl butyrate) Kemin Industries Inc. USA, CTC, chlortetracycline; SDPP,Spray dried porcine plasma; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

Protein Meals

The selection and combination of protein meals used within a pig diet should be appropriate relative to the digestibility of the raw material and the digestive maturity of the pig. Careful selection and inclusion of raw materials with high digestibility will aid in maximising feed conversion efficiency and minimising intestinal irritation. There are dietary advantages in using a range of protein sources. Diets should aim to minimise the total protein content by using high biological value proteins and complimentary synthetic amino acids. Protein selection should aim to avoid the production of toxic degradation products such as biogenic amines and ammonia both prior to ingestion and in the hindgut.

Protein meals can also be a source of allergens (eg. trypsin inhibitors, glycinin and ß-conglycinin) which may present permanent or transient challenges including hypersensitivity to the pig depending on their developmental stage. Processing methods are often applied to raw material to limit these allergenic compounds in feed, particularly in specialised raw material for young pigs. Limiting the allergens within feed is a means of limiting villous atrophy, malabsorption, localised inflammation, and cell mediated immune responses in pigs (Lenehan *et al.*, 2007; Li *et al.*, 1990; McCracken *et al.*, 1999). Alcohol treatment, extrusion and heat treatment of soya bean meal has been shown to reduce the anti-nutritional effects of soya bean flour in young pigs (Dreau *et al.*, 1994; Lenehan *et al.*, 2007).

Fats and Oils

Like protein meals, the selection and combination of fats and oils used in pig diets should be appropriate relative to the digestive maturity of the pig. Feed formulation needs to consider not only digestibility of the dietary lipids but also chain length and degree of saturation of the lipids. The fatty acid balance of the diet also needs to be considered in the formulation. The ratio between omega-6 and omega-3 fatty acids should remain below 10 to avoid intestinal

irritation, growth suppression and immunosuppression. High levels of omega-6 fatty acids (from safflower oil) were shown to severely suppress growth performance of weaner and grower pigs (Wilkinson *et al*., 2009). High dietary levels of omega-6 were also shown to be detrimental to litter performance in first parity sows (Newman *et al*., 2009). To limit the risk of rancidity and oxidation in fats and oils, which can have negative effects on feed palatability, the use of antioxidants in both stored oils/fats and in finished feeds is encouraged.

Minerals

Dietary macro-mineral balance can be expressed as (Na+K+Ca+Mg)-(Cl+P+S) but is more commonly calculated as a more simplified electrolyte balance (Na+K-Cl) and is an expression of the deficiency or excess of metabolizable anions (Patience *et al*., 1987). The dietary formulation influences the acid base status of most livestock species (Patience *et al*., 1987). Diet formulations which have an electrolyte balance within the range of 180-240 meq/kg should avoid impaired growth performance. The acid binding capacity of the diet is another factor which can be manipulated through raw material selection to optimise performance. Raw materials which have a strong affinity to bind acid in the stomach are best omitted from young pig diets where gastric acid needs to be promoted (Lawlor *et al*., 2005). In diets offered to vulnerable pigs an acid binding capacity below 700 meq HCl/kg (pH 3 scale) is favourable.

Minerals can also be involved in regulation of oxidative stress in pigs. Minerals including selenium, zinc, copper and manganese are involved in protecting cells from oxidative stress by their antioxidant functions (Stoyanchev *et al*., 2006). Minerals should be selected based on their high bio-availability (i.e. in a chelated form) and low oxidative stress (e.g. sulphates rather than oxides).

Feeding Management

Feed presentation is a vital component of pig nutrition. Feeds needs to be presented in a wholesome, palatable form (fresh, limited dust and spoilage) and should be provided in a manner which promotes even distribution and access.

Erratic feed delivery can increase the risk of microbial dysbiosis and lead to diseased related challenges like ulcers (Brumm *et al*., 2005). It can also negatively impact on the overall growth performance of pigs. Management strategies to avoid the incidence and severity of out-of-feed events are critical. Feed integrity should be monitored using stringent quality assurance of feed delivered to silos and feeder hygiene. Contaminated or compromised (spoiled) feed can again be a source of immune challenge for pigs.

To maximise performance it is important that the right feed (in terms of nutrient specifications) is offered to the right pig. Changing feeds by pig weight rather than age can assist in optimising growth performance.

Formulation Continuity

The composition of the microbial community within the gut is partially dependent on the diet composition. Formulations should avoid marked changes in raw material selection in the progression between diets and within the one diet overtime.

Dietary Insurances

The raw material market is continually evolving and providing nutritionists with a broad range of tools to limit gastrointestinal stress and optimise performance. Products which are known to provide dietetic insurance include; acids, enzymes, antioxidants, emulsifiers, mould inhibitors and mycotoxin binders.

Conclusions

Commercial livestock production often operates below full productive efficiency. Part of the shortfall is due to the health x nutrition interaction which involves the immune defence mechanisms drawing nutrients that would have otherwise been available for growth or reproduction. Much of this compromise takes place in the gut and it is of some significance that the gut is the largest immune function organ in the body. The microbial suppression by antibiotics is commonly referred to as 'growth promoting' but some have named it more as 'growth permitting' (ie. a return to unimpeded growth rather than something beyond normal).

Some of the compounds and technologies cited in this paper do have a similar direct action on microbial pathogens or elicit a similar control over inflammatory responses as antibiotics, however, their efficacy may not be of the same order. The growth response to antibiotics added to feed seems best in unsanitary conditions (Turner *et al*., 2002), but as we create a more favourable environment via hygiene and management the potential for alternative approaches improves. Many of the alternative products or systems don't involve direct antigen elimination but rather promote eubiosis by controlling the balance of gut microflora, limiting damage by curtailing inflammatory responses, and deal with pathogens by promoting specific humoral immunity competence. There will always be a need for antibacterial intervention in the face of acute systemic infection but there appears to be adequate nutritional tools available to replace 'growth promotional' antibiotic use.

Symposium Conclusions

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The aim of all pig producers is to rear healthy pigs with minimal reliance on antimicrobials. In the first paper, Trott (2011) demonstrated the emerging issues around over-use of antimicrobials by focussing on two significant, antibiotic-resistant bacterial pathogens of humans that have recently emerged in animals, multidrug-resistant, extraintestinal pathogenic *E. coli* (MDR-*E. coli*) and methicillin-resistant *Staphylococcus* (MRS).

Veterinarians have a number of non-antimicrobial tools to assist producers control endemic diseases in their herds. Despite this, strategic use of antimicrobials continues to play an important role in modern health management of pigs in Australia. In the second paper, Holyoake (2011) highlighted some of the challenges facing veterinarians and producers seeking to control and prevent diseases in pig herds. Prudent use principals for antimicrobial use were discussed, including diagnostic accuracy, antibiotic sensitivity testing, routes of antimicrobial administration and duration of use. Systems for monitoring antimicrobial use and resistance overseas were discussed, together with studies undertaken in Australia describing patterns of antimicrobial prescribing and the drivers of this prescribing by veterinarians. A number of antimicrobial alternatives were discussed, and some challenges for implementing effective vaccine strategies highlighted. Options for reducing antimicrobial use, including antimicrobial stewardship and stockperson training, were discussed.

The third paper (Edwards and Edwards, 2011) presented information on the role of antimicrobials in animal production and discussed a range of known nutritional alternatives to antimicrobials. The review focussed on favourable regulation and manipulation of gut microflora, favourable regulation of inflammation, influence on immunity and the subsequent influences on animal performance. The use of nutritional alternatives for therapeutic purposes on commercial farms requires registration with the Australian Pesticides and Veterinary Medicines Authority (APVMA). There is a requirement to link research and development into the therapeutic potential of nutritional therapeutics with commercial registration with the relevant authorities to allow wider adoption of these substances.

This symposium highlights the need for nationally-co-ordinated surveillance of antimicrobial use and resistance, both in the human and animal sectors. The new Cooperative Research Centre for High Integrity Australian Pork (HIAP CRC) includes "Replacement of Antibiotics with Effective Integrated Health Strategies". The broad objective of this program is to reduce expenditure on therapeutics whilst maintaining or enhancing production efficiency. This approach requires systems for monitoring antimicrobial use before the HIAP CRC commences and after it concludes to measure its effectiveness in meeting this objective.

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Traceability of Offal in the Australian Pork Industry

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Chemical traceability in the form of multi-elemental trace analysis has been demonstrated as a rapid and accurate provenancing methodology for a significant number of food products (Watling *et al.*, 2010). Following extensive research into the development of both analytical protocols and a supporting database to be used for the traceability of Australian Pork, a chemical database (Physi-Trace™) has been developed that facilitates a rapid and robust trace back of any pork muscle product implicated in a food contamination related incident (Australian Pork Limited, 2011). However, this database does not include pork offal, a significant export product of the Australian pork industry, and consequently an equivalent system for the traceability of Australian pork offal products is desirable. The study described in this paper details the results of an investigation into the potential use of trace element data for the traceability of Australian pork offal. This procedure uses the already established pork muscle database, by investigating the relationship between trace elemental profiles of the various pork offal types and the related muscle tissue. Muscle from the brisket, tongue, heart, stomach, liver and kidney were collected for pigs from six different tattoo codes. Subsamples of all tissue types were dried and digested initially in HNO_3 acid and then subsequently in a 4:1 HNO_3:$HClO_4$ acid mixture. The solutions were then analysed using inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7700x ICP-MS in standard and helium collision cell mode and inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a Thermo Scientific iCAP 6500 Duo ICP-AES.

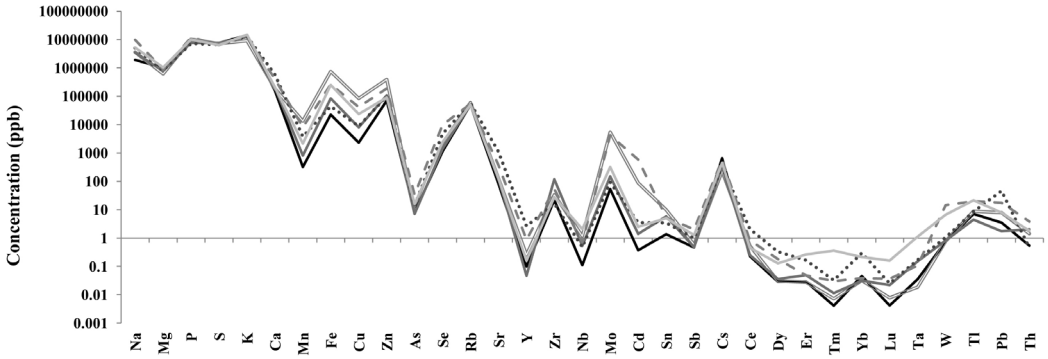

Figure 1. *Trace elemental profiles for muscle, liver, tongue, stomach, kidney and heart tissue sampled from an individual pig. Tissues are indicated as follows, muscle (-), liver (=), tongue (-), stomach (···), kidney (- -) and heart (-).*

The trace elemental profile varied between each tissue type (Figure 1), with metals accumulating in the offal tissues to a greater degree than in the muscle tissue. This accumulation was demonstrated by all offal types for Mn, Fe and Cu concentrations and additionally for liver and kidney for Mo, Cd and Sn, and for stomach and heart for the rare earth metals. Results demonstrate that for many elements of interest elemental profiles for offal are not directly comparable to the Physi-Trace™ muscle data. However, this study has identified several elements including As, Rb, Se and Sr that were comparable across all tissue types. Consequently, these identified elements have demonstrated potential for classifying offal elemental signatures' back to the respective muscle. Not only do the offal types show some similarities between their chemical relationships to muscle, but also the observed patterns were replicated across five other pigs of different origin. The occurrence of these elemental inter-comparisons highlights the potential for the development of a mathematical model to facilitate a comparison of offal signatures back to their respective muscle signatures, thus potentially enabling the use of the Physi-Trace™ database for the provenancing of pork offal.

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Breed and Slaughter Day Affects Carcase and Pork Quality

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In general, there is no direct market incentive to improve the technological, nutritional and sensory quality of pork. However, in order for the pork industry to maintain or increase its market share at a competitive price, pork has to be of consistently high nutritional value. That is why novel measures of nutritional quality are being assessed to ensure that breeding programs can continue to improve pork quality. Pork nutritional quality is affected by many genetic and non-genetic factors such as breeds and slaughter day. There is currently no recent Australian assessment of the impact of breed and slaughter day on pork nutritional quality, including the iron content of pork. It was hypothesized that both breed and slaughter day have a significant effect on the technological and nutritional quality of pork.

During 2010 and 2011, data were recorded on 2442 pigs from two terminal sire breeds (Duroc (Db) and Large White-based (LWb), PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW). Pigs were housed in group pens at a commercial piggery. At 21 weeks of age, blood samples (5 ml) were obtained from all animals via jugular venipuncture to measure haemoglobin content (HEM21) using a HemoCue® blood haemoglobin photometer (HemoCue® Australia Pty Ltd, Wamberal, NSW). Pigs were slaughtered at a hot carcase weight (HCW) of 69.9 (±7.95) kg (AUSMEAT Trim 13 with head and fore trotters off, hind trotters on) and carcase fat depth was obtained at the P2 site, 65 mm from the midline of the carcase at the last thoracic rib. Twenty-four hours post slaughter, a measure of pH was obtained between the tenth and eleventh rib on each carcase from the *longissimus dorsi (LD)* muscle using an MPI pH meat probe (Meat Probes Inc., Topeka, Kansas). Meat colour (L*, a*, b*) was measured using a Minolta Chromameter CR-400 (Thermo Fisher Scientific Pty Ltd, Scoresby, VIC). A sample of around 1000 mg wet weight of the *LD* muscle was used to measure total iron content by flame atomic absorption spectrometry (Haswell, 1991). A general linear model was used including the fixed effects of slaughter day (or collection day for HEM21) and breed (except L*) for all traits. Sex was significant for HEM21 and HCW was fitted as a linear covariable for fat depth and pH_{24}.

Table 1. *Effect of breed and slaughter date on meat quality traits from two terminal breeds.*

		Mean (db)	Mean (Lwb)	Proportion of Variance Explained	
	N	(SE)	(SE)	Breed	Slaughter day
HEM 21 (g/l)[1]	2405	107.1 (0.66)[a]	108.2(0.70)[b]	0.00	-
Iron content(mg/kg)	2367	2.87 (0.01)[a]	2.94 (0.01)[b]	0.00	0.36
P2 fat depth (mm)	2417	7.36 (0.09)[a]	7.10 (0.09)[b]	0.01	0.09
pH_{24}[2]	2430	5.64 (0.00)[a]	5.62 (0.00)[b]	0.01	0.54
Colour L*	2419	47.8 (0.07)	47.9 (0.08)	NS	0.28
Colour a*	2412	5.46 (0.03)[a]	5.82 (0.03)[b]	0.03	0.16
Colour b*	2419	3.82 (0.02)[a]	4.08 (0.02)[b]	0.02	0.24

[1]HEM21, Haemaglobin at 21 weeks of age; [2]pH_{24}, pH at 24 hours post slaughter; Db, Duroc-Based; LWb, Large White-based; SE, standard error; NS, not significant; [ab] Means in a row with different superscripts differ significantly ($P<0.05$).

The LWb breed had a 0.36 higher value for colour a* (measure of redness in the meat) which correlated with a 1.10 g/l higher hemoglobin in the blood at 21 weeks of age and greater iron content in the muscle (0.07 mg/kg; Table 1). The Db breed displayed a 0.26 mm higher P2 fat depth and a 0.02 higher pH at 24h post slaughter also seen in Gjerlaug-Enger *et al.* (2010). There was, however, no significant breed effect on colour L* or plasma HEM21. Slaughter day explained a much larger proportion of the variation (9 to 54%) than breed. Although breed was significant for most traits, due to the size of the experiment, this effect explained only up to 3% of the variation. In conclusion, breeding programs need to be supplemented with good husbandry and slaughter day procedures, so that pork has consistently good technological and nutritional quality characteristics.

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Dietary Lecithin Improves Eating Quality, Dressing Percentage and Meat Colour in Finisher Gilts

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Previous research has shown that dietary lecithin may improve eating quality of pork by reducing chewiness and hardness (D'Souza *et al*., 2011). However, little is known about the effects of dietary lecithin on other aspects of pork eating quality. The aim of this experiment was to test the hypothesis that dietary lecithin would improve pork eating quality by reducing chewiness and hardness and in doing so may improve other aspects of carcase and meat quality.

Thirty-six Large White x Landrace (PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) gilts were randomly allocated at 16 weeks of age (62.9±0.56 kg, mean±standard error) to finisher diets containing either 0, 4, 20 or 80 g/kg soybean lecithin (ADM Australia Pty Ltd, Bondi Junction, NSW). The pigs were housed individually and had *ad libitum* access to treatments and water for six weeks prior to slaughter at 103.2±1.67 kg. The pH of the *Longissimus thoracis* was measured at 45 min and 24h post-slaughter. Twenty-four hours after slaughter, the *L. thoracis* was cut and exposed to air for 30 minutes. Following that, a Minolta Chromameter CR-400 (Minolta Australia Pty Ltd, Macquarie Park, NSW) was used to measure relative lightness (L), relative redness (a*) and relative yellowness (b*) using D_{65} lighting, a 2° standard observer and an 8 mm aperture, standardised to a white tile. Drip loss was measured using the suspension method (Honikel *et al*., 1986). Additional muscle was removed and frozen prior to subsequent hydroxyproline analyses using method of Kolar (1990). Warner-Bratzler shear force and compression were also determined as described by (Bouton and Harris, 1972) using a Lloyd Instrument, LF Plus (Fereham, Hants., England). Data were analysed for linear and quadratic dose effects using GENSTAT Release 11.1. (VSN International Ltd, Hemel Hempstead). Where there were no linear or quadratic effects (all data except surface a* colour) the contrasts assessed were for control versus pooled lecithin treatment and within pooled lecithin treatment.

Table 1. *Effect of dietary lecithin on pork eating quality.*

	Dietary lecithin (g/kg)					P-value	
	0	4	20	80	SED	Lecithin	Within Lecithin[1]
Dressing percentage (%)	73.7	75.5	75.6	75.5	0.81	0.01	0.980
Chewiness	1.51	1.39	1.21	1.24	0.137	0.047	0.130
Hardness (kg)	3.84	3.71	3.26	3.37	0.308	0.130	0.200
Cohesiveness	0.39	0.38	0.37	0.37	0.014	0.069	0.320
Shear force (kg)	3.21	3.91	3.55	3.44	0.382	0.180	0.450
Hydroxyproline (µg/ml)	1.63	1.22	1.17	1.15	0.186	0.043	0.930
Colour L	51.2	49.9	49.6	49.3	0.60	0.01	0.770
Colour a*[2]	5.20	5.42	5.97	6.22	0.254	0.010	0.037
Colour b*	2.63	2.74	2.69	2.94	0.247	0.530	0.680

SED, standard error of the difference for effect of lecithin; [1]For within lecithin multiply by 1.22. [2]Significant linear (P<0.001) and quadratic (P<0.05) effects of lecithin.

Lecithin increased dressing percentage by 2%. However, lecithin had no effect on growth performance, P2 backfat, loin muscle depth as well as pH, drip loss or cooking loss (data not shown). Collagen maybe responsible in improving compression characteristics in pork fed dietary lecithin (D'Souza *et al*., 2011). Our results showed lecithin decreased pork chewiness (1.51 vs. 1.28, P<0.05) and this change is associated with decreased collagen hydroxyproline (1.63 vs. 1.18 µg/ml, P<0.05). However, there were no significant effects of lecithin on shear force, cohesiveness or hardness. Dietary lecithin increased pork surface lightness (L; 51.2 vs. 49.6, P<0.01) and increased surface redness (a*) in a dose-dependent manner. These data show that dietary lecithin increases dressing percentage and results in improved pork tenderness and less pale pork.

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Adipose Tissue Gene Expression in Response to Dietary Nano-Chromium and Fat Supplementation in Finisher Gilts

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Chromium (Cr) is an essential mineral element and has been investigated as metabolic modifier for farm animals, particularly nano-particles of Cr (Hung *et al*., 2010). Over the last three decades, Cr has been used to manipulate fat deposition in farm animals. Hung *et al*. (2009) reported that dietary nano-Cr can reduce P2 back fat and insulin sensitivity in finisher pigs. The transcriptional factors, peroxisome proliferator-activated receptors (PPARs), CCAAT-enhancer-binding protein (C/EPB) and sterol regulatory element-binding proteins (SREBPs) have been found expressed during adipocyte differentiation. Adipose tissue is now recognized as an endocrine organ which produces adipokines such as leptin, adiponectin and tumour necrosis factors-α all of which play important metabolic roles. Therefore, the aim of this experiment was to investigate the interactive effects of dietary Cr picolinate (CrPic) and dietary fat on adipogenesis transcriptional factors and adipokines gene expression in adipose tissue of pigs.

A total of 48 Large White x Landrace (PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) gilts (initial weight 52.2±7.3 kg, mean±standard deviation) were stratified on weight into four blocks of four pens of three pigs and then within each block each pen was randomly allocated to four treatment groups in a 2×2 factorial design. The respective factors were dietary fat (22 or 57 g/kg) and dietary Cr (0 or 400 ppb CrPic ground to 100 nm particles (nCrPic)) fed for six weeks). Subcutaneous adipose tissue samples from above the *Longissimus thoracis* were collected 25 minutes post-slaughter and frozen in liquid nitrogen for gene expression using real time polymerase chain reaction (PCR). The adipose tissues RNA extraction was undertaken using the TRIzol reagent and PureLink™ Micro-to-Midi Total RNA Purification System (Invitrogen, California, USA). The extracted RNA samples were then converted to cDNA by using Superscript™ III First-strand Synthesis System for RT-PCR kit (Invitrogen, California, USA). All gene mRNA expressions were then undertaken in a BioRad MyIQ Single Colour Real Time PCR Detection System (BioRad Laboratories Inc., California USA). All gene expression data were analysed as $2^{-\Delta CT}$ and assessed for main and interactive effects of dietary fat and nCrPic by analysis of variance (ANOVA).

Table 1: *Effect of dietary nano chromium picolinate (nCrPic) on adipose tissue gene expression. Data are expressed relative to tissue from gilts fed the control diet without supplemental fat or nCrPic (n=4).*

	Low Fat		High Fat		P-value		
	Control	nCrPic	Control	nCrPic	nCrPic	Fat	nCrPic x Fat
Akt	100	167	106	206	0.03	0.56	0.75
UCP3	100	384	283	479	0.003	0.02	0.10
Adiponectin	100	109	60	90	0.06	0.01	0.21
Leptin	100	320	554	327	0.38	0.04	0.04
TNFα	100	74	167	115	0.23	0.10	0.90

Akt, serine/threonine protein kinase; UCP3, uncoupling protein-3; TNFα, tumour necrosis factor-α

Dietary nCrPic increased the expression of the insulin signalling pathway gene Akt (P=0.02; Table 1) but had no effect on expression of genes for insulin receptors, phosphoinositide 3-kinase (P=0.58) and glucose transporter-4 (P=0.83) or genes involved in adipocyte differentiation such as PPARγ, C\EBPα, SREBP and FAS (data not shown), despite a reduction in P2 (Hung *et al*. 2009). The UCP3 gene which improves insulin sensitivity was increased by nCrPic (P<0.01) and decreased by fat (P=0.02) supplementation. The adipokines leptin (P=0.04) and TNF-α (P=0.1) were increased with the high fat diet. Also, adiponectin was decreased by high fat (P=0.01) and increased by nCrPic (P=0.06). In conclusion, dietary nCrPic had no effect on transcription factors gene expression in the finishing stage of pig's adipose tissue. However, dietary nCrPic can improve insulin sensitivity and this is improvement is possibly via altering the expression of adiponectin and UCP3 genes.

HUNG, T.Y., LEURY, B.J., LIEN, T.F., LU, J.J. and DUNSHEA, F.R. (2010). Proceedings of the 14th Asian-Australasian Association for Animal Production. Volume 1:108-112.

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Skeletal Muscle Gene Expression in Response to Dietary Nano-Chromium and Fat in Finishing Gilts

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[1]University of Melbourne, Parkville, VIC 3010. [2]Chiayi University, Taiwan.

Chromium (Cr) is an essential mineral element with trivalent Cr being the most stable form occurring in nature. Chromium functions as a cofactor for the hormone insulin and enhances the ability of insulin to regulate glucose, protein and fat metabolism. Dietary Cr has been investigated as a metabolic modifier for farm animals, particularly nano-particles of Cr (Hung *et al*., 2010). Qiao *et al*. (2009) indicated that Cr improve glucose uptake and insulin signalling pathway gene insulin receptor (IR) and glucose transporter 4 (GLUT4) gene expression. Skeletal muscle is particularly important for the action of insulin because skeletal muscle is normally responsible for over 75% of all insulin-mediated glucose utilisation. Therefore, the aim of this experiment was to investigate the interactive effects of dietary Cr picolinate (CrPic) and dietary fat on genes relative to insulin signalling in skeletal muscle of pigs.

A total of 48 Large White x Landrace (PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) gilts (initial weight 52.2±7.3 kg, mean±standard error) were stratified on weight into four blocks of four pens of three pigs and then within each block each pen was randomly allocated to four treatment groups in a 2×2 factorial design. The respective factors were dietary fat (22 or 57 g/kg) and dietary Cr (0 or 400 ppb CrPic ground to 100 nm particles (nCrPic)) fed for six weeks. The nCrPic particles were made through grinding CrPic through appropriate sized sieve end plates. Samples of the *Longissimus thoracis* were collected 25 minutes post-slaughter and frozen in liquid nitrogen for gene expression using real time polymerase chain reaction (PCR). Genes measured included phosphoinositide 3-kinase (PI3K), insulin receptor (IR), glucose transporter type-4 (GLUT4), uncoupling protein-3 (UCP3), interleukin-15 (IL-15), suppressor of cytokine signaling-3 (SOCS3) and serine/threonine protein kinase (Akt). Changes in gene expression were calculated as 2-ΔCt, where Ct represents the cycle in which fluorescence threshold is reached and $\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{housekeeping gene}}$, with ß-actin utilized as a standard housekeeping gene. All gene expression data were assessed for main and interactive effects of dietary fat and nCrPic by analysis of variance (ANOVA). A difference in ΔCt of -1.0 is associated with a doubling (200%) and +1.0 a halving (50%) of expression and for ease of presentation data are presented as % relative to expression in tissue from gilts fed the low fat diet without supplemental nCrPic. This method of presentation prevents the presentation of the standard error of difference (SED).

Table 1. *Effect of dietary nano-chromium picolinate (nCrPic) on skeletal muscle gene expression. Data are expressed relative to tissue from gilts fed the control diet without supplemental fat or nCrPic (n=4).*

	Low Fat		High Fat		P-value		
	Control	nCrPic	Control	nCrPic	nCrPic	Fat	nCrPic x Fat
IR	100	94	74	91	0.71	0.38	0.47
PI3K	100	174	135	212	<0.001	0.02	0.56
Akt	100	114	84	129	0.07	0.85	0.33
SOCS3	100	90	110	88	0.02	0.54	0.32
UCP3	100	130	80	117	0.09	0.36	0.75
IL-15	100	86	117	151	0.10	0.78	0.30

IR, insulin receptor; PI3K, phosphoinositide 3-kinase; Akt, serine/threonine protein kinase; SOCS3, suppressor of cytokine signalling-3; UCP3, uncoupling protein-3; IL-15, interleukin-15.

Dietary nCrPic increased the expression of insulin signalling pathway genes PI3K (P<0.01) and Akt (P=0.07) but had no effect on IR or GLUT4 (Table 1). Expression of SOCS3, which can aggravate insulin resistance, was reduced (P=0.02) by nCrPic. Dietary nCrPic tended to improve UCP3 (P=0.09; Table 1) and IL-15 (P=0.10) gene expression (data not shown), both of which facilitate glucose metabolism. These results were generally consistent with Cr upregulating insulin signalling and UCP3gene expression in skeletal muscle (Qiao et al., 2009). In conclusion, the improvement of insulin signalling pathway gene PI3K and Akt by nCrPic may be via decreased SOCS3 and increased UCP3 and IL-15.

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CHAPTER 7

Health and Production

Piglet Birthweight Influences Slaughter Weight but Not Rate of Gain During the Growing-Finishing Stage

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The birth weight of the piglet can be influenced by many factors (Le Dividich, 1999). Those that can be influenced by the producer include genotype and nutrition, whilst factors like position in the uterine horn is unavoidable and requires management post birth. The increase in litter sizes being obtained by breeders across the world has resulted in a larger proportion of low birth weight piglets, which are associated with higher mortality and slower growth rates (Quiniou *et al.*, 2002), creating challenges for the growing herd. The aim of this experiment was to test the hypothesis that birth weight will affect the rate of gain of pigs during the growing-finishing stage when they are group housed within a commercial environment.

Female piglets born from gilt (F2 commercial hybrid) litters over a two week period were individually identified, weighed (range, 1.22 - 2.73 kg) the morning after their birth. Piglets were cross-fostered at this time to a common litter size. Very light piglets received assistance on this site and were thus excluded from this experiment. Piglets were subjected to normal commercial practices; housed in groups according to birth weight post-weaning and received common diets. Piglets were classified into three birth weight categories being Low (<1.5 kg; μ=1.41, σ=0.06), High (>2 kg; 2.16, 0.15) and Intermediate (1.72, 0.13). Pigs were weighed at various intervals throughout their life, at weaning (~24 d), at transfer to the growing-finishing facility (70 d), at diet changes within this facility (105 d and 126 d) and immediately prior to slaughter (168 d). Data was subjected to an analysis of variance, with pen as the experimental unit, and means separated by least significant differences (P<0.05). Piglets that survived through to slaughter were only included in the analysis.

Table 1. *Mean weight of low (<1.5 kg), intermediate (1.5 to 2.0 kg) and high (>2.0 kg) birth weight piglets at various ages and mean average daily gain (ADG) during the growing-finishing period.*

	Low	Intermediate	High	SED	P value
Birth weight (kg)	1.41[a]	1.72[b]	2.16[c]	0.022	<0.001
Wean weight[#] (kg)	6.06[a]	6.73[b]	7.15[c]	0.165	<0.001
70 d weight (kg)	26.7[a]	29.0[b]	31.9[c]	0.71	<0.001
105 d weight (kg)	56.4[a]	58.8[b]	62.4[c]	0.99	<0.001
126 d weight (kg)	71.6[a]	74.4[b]	76.6[b]	1.28	0.002
168 d weight (kg)	94.2[a]	99.2[b]	100.0[b]	1.86	0.003
ADG 70-168 d (kg/d)	0.709	0.709	0.722	0.060	0.97

[abc]Means in a row with different superscripts differ significantly (P<0.05); [#]Wean weight, weaning weight standardised to the average weaning age (24.3 d); SED, standard error of difference

The weight of each treatment group differed significantly from birth through to 105 days of age (P<0.001, Table 1), with high birth weight pigs maintaining their advantage over the low birth weight piglets, throughout, while intermediate pigs reached similar weights to high birth weight pigs at 126 days of age and beyond. Growth rate during the growing-finishing phase did not differ significantly between treatments (P>0.05).

These results demonstrate that the actual growth of pigs during the growing-finishing phase within this commercial environment is not significantly compromised by the birth weight of the pig. However, the difference in final weight is in agreement with other published studies (Beaulieu *et al.*, 2010) with a 0.75 kg difference at birth resulting in a 5.8 kg difference at final weighing. Segregation of low birth weight piglets (<1.5 kg) and a change to their management within this commercial environment would appear warranted given the influence of low birth weight on growing-finishing performance.

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Influence of Nutrition During Early Follicle Development on Follicle Recruitment and Ovulation

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A severe catabolic state during lactation in primiparous sows is associated with post-weaning reproductive performance. A catabolic state reduces luteinising hormone (LH) secretion which affects early follicle recruitment during lactation and pre-ovulatory follicle development after weaning (van den Brand *et al.*, 2000). It has been reported that under-nutrition during the luteal phase has similar impacts in cyclic gilt models (Hazeleger *et al.*, 2005). We hypothesised that a low feed level reduces (early antral) follicle development during the luteal phase and reduces subsequent ovulation rate.

Crossbred gilts, previously fed 2 kg/d of a standard diet (13.2 MJ/kg digestible energy (DE)/kg; 150 g crude protein (CP)/kg), were randomly assigned to a low (L, 1 x Maintenance; n= 24) or high (H, 1 x Maintenance+1.5 kg/d; n= 27) allowance of the standard diet during the luteal phase (early follicle development). From one day after luteolysis onward, follicles were monitored using transcutaneous ultrasonography. Pre-prandial blood samples were collected by jugular venipuncture on d 3 after luteolysis to assess oestradiol levels. Gilts were slaughtered to obtain ovaries after ovulation. Normally distributed data (eg. follicle size) were analysed with a t-test, and binomially distributed data using Chi-square analysis.

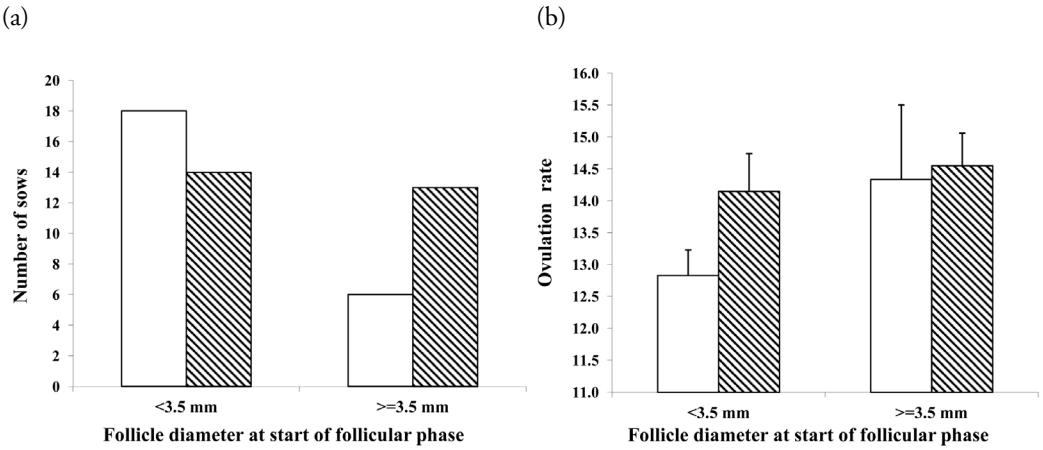

Figure 1. *Follicle diameter at the start of the follicular phase in relation to (a) number of sows and (b) ovulation rate (Mean ± standard error of mean), for gilts fed a Low (□; n=24) or a High (■; n=27) feed level.*

The diameter of antral follicles after luteolysis ($P<0.05$) and ovulation rate ($P<0.05$) were both affected by feed level during the luteal phase (Figure 1). There were a greater number of gilts on the High feed level that had follicles >3.5mm compared to those gilts on the Low feed level (13/27 verses 6/24; $P<0.05$). For gilts on the High feed level, there was no difference in ovulation rate between follicles <3.5mm or >3.5mm, regardless of the follicle diameter at the beginning of the follicular phase. Of the gilts on the Low feed level only those gilts that had follicles >3.5mm achieved similar ovulation rates to those gilts on the High feed level ($P>0.05$). In conclusion, a low feed level during luteal phase decreases ovulation rate due to the impacted early antral follicle dynamics.

HAZELEGER, W., SOEDE, N. M., and KEMP, B. (2005). *Domestic Animal Endocrinology.* **29**: 362-370

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Phenotypic Trends in Means and Variation for Backfat and Growth Rate of the Growing Pig

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Uniform performance is required for optimal use of resources within piggeries. Improving production flow benefits pig health, feed and housing efficiency, and overall management ease. A reduction in variability could assist in providing a high quality, consistent product to markets. Beneficial trends for production traits have been demonstrated (eg. Hermesch and Jones, 2010). However, there has been no examination of how the variability amongst animals for traits has changed over time. It was hypothesized that the mean and variation for backfat (BF, mm) and lifetime growth rate (LADG, g/day) have changed over time.

Data in this study were sourced from the National Pig Improvement Program (NPIP; Animal Genetics and Breeding Unit, Armidale, NSW) database. These data consisted of three breeds from 11 herds recorded over a 15 year period from 1996 until 2010, with 384,194 records for BF and 384,883 for LADG after deletion of outliers. Data were analysed using general linear models in SAS (SAS Institute Inc., 2005). To obtain trends across years, a model that corrected for month of data collection to remove the within-year seasonal effect was used. Other factors included herd, sex and breed while BF was also corrected for the weight of the animal at testing. Overall means (standard deviations, SD) were 10.7(2.21) for BF, 643(74.8) for LADG, 93.0(13.2) kg for weight and 144(17.1) days for age at test.

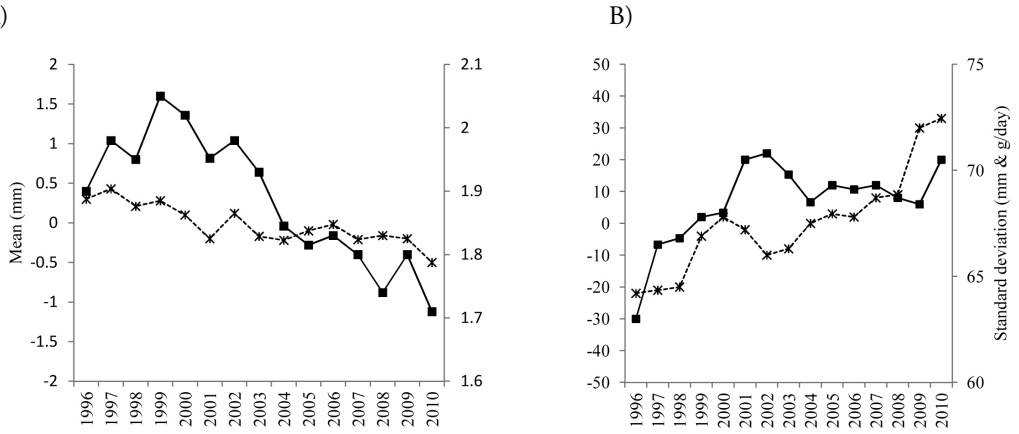

Figure 1. *Across yearly mean (⁎) and standard deviation (■) trends for backfat (A) and growth (B).*

Over the course of 15 years mean BF has reduced on average by one mm and growth has increased by 55 g/day (Figure 1), similar to the phenotypic trends previously presented by Hermesch and Jones (2010) using data from 33 populations. These trends represent improvement from both genetic and non-genetic factors that contribute to leaner and faster growing animals. The SD for BF has reduced from 2.0 mm to 1.7 mm. Due to scaling, a lower mean is associated with a lower SD, however, the reduction in SD is more than expected due to scaling alone, leading to a lower coefficient of variation (CV) of 16.7 in 2010 compared to a CV of 18.5 in 1999. Conversely, the expected SD increase due to a higher mean for LADG was not observed.

Overall these trends suggest that mean performance and uniformity have both improved for BF and LADG. For both traits the improvement in uniformity exceeded what is expected from the scaling effect alone, demonstrating that genetic and non-genetic improvements are transferred into productivity and uniformity.

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Supported in part by Australian Pork Limited.

Improved Response to Ractopamine in Finisher Gilts as Dietary Lysine is Increased

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Ractopamine (RAC; Paylean, Elanco Animal Health, West Ryde, NSW) is a β-agonist that, when included in the diet for the last four weeks pre-slaughter, increases protein deposition and average daily gain (ADG), and improves feed conversion ratio (FCR; Dunshea *et al.*, 2005). Since a major effect of RAC is to increase protein deposition, it is important that the supply of essential amino acids in the diet is sufficient to meet this additional requirement. The accepted commercial recommendation for diets containing RAC in Australia is 0.56 g available lysine (Av Lys)/MJ digestible energy (DE). The hypothesis for this experiment was that the inclusion of RAC in the diet of finisher gilts will enhance performance and the level of lysine required to support maximum performance.

A total of 420 [Large White × (Landrace × Duroc)] gilts (PIC Australia Pty Ltd, Grong Grong, NSW) were used in a 2 x 5 factorial design (7 pigs/pen and 6 replicate pens/treatment) with the main treatments being ractopamine dose (control (C; 0 ppm RAC) and 7.5ppm RAC) and five levels of available lysine per MJ DE (0.40, 0.48, 0.56, 0.64 and 0.72 g). All pigs were fed a standard commercial diet (14.0 MJ DE/kg and 0.75 g available lysine per MJ DE) for three weeks, followed by the experimental diets formulated to contain 13.5 MJ DE/kg and based on an ideal pattern of amino acids. The experimental diets were offered *ad libitum* for four weeks commencing at 73.4 kg ± 0.39 kg live weight. The pigs were weighed and voluntary feed intake was recorded weekly. Two-way analysis of variance (ANOVA) was used for statistical analysis.

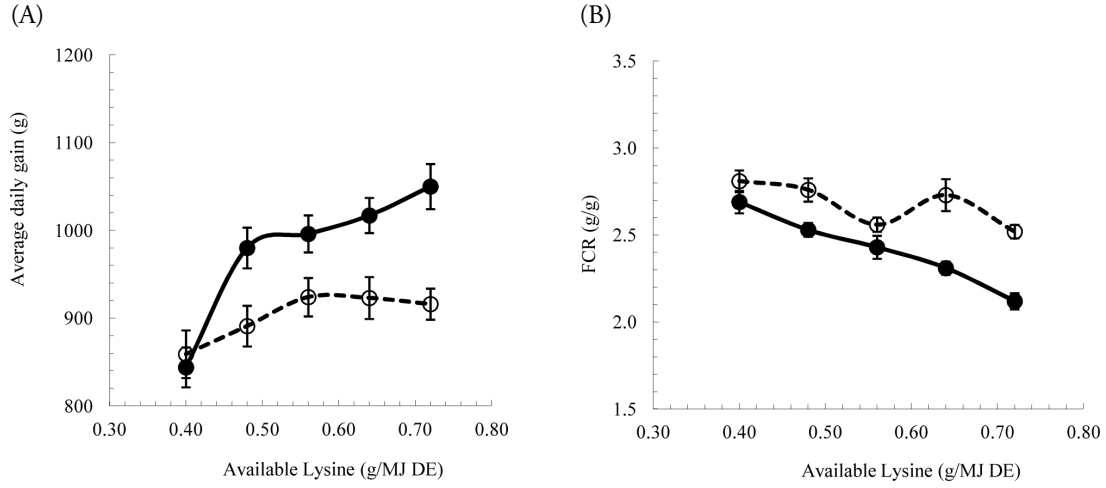

Figure 1. *Mean (±standard error) average daily gain (ADG; A) and feed conversion ratio (FCR; B) of gilts fed either a control diet (○) or a diet containing 7.5ppm Ractopamine(●) in response to increasing levels of dietary lysine per MJ digestible energy (DE).*

Inclusion of RAC increased ADG (P<0.001) compared to gilts fed the C diet at all levels of lysine except the lowest. The ADG for gilts fed the C diet plateaued at 0.56 g Av Lys/MJ DE, whereas the RAC diet continued to respond to the highest level of dietary lysine investigated. FCR declined as the level of lysine in the diet increased, with the response greater (P<0.001) when the diet contained RAC. These results indicate that the current recommendation for lysine may not provide the optimal response to RAC in finisher gilts.

DUNSHEA, F.R., RIKARD-BELL, C., CURTIS, M.A., EDWARDS, A.C., GANNON, N.J., HENMAN, D.J. MULLAN, B.P., and VAN BARNEVELD, R.J. (2005). In "Manipulting Pig Production X" p. 152. ed J.A. Paterson. (Australasian Pig Science Association: Werribee).

Supported in part by the Pork CRC Ltd.

The Effect of Split Weaning on Piglet Growth

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Increasing the length of the suckling period improves piglet growth pre-and post-weaning (Cabrera *et al*., 2010), while early removal of the heaviest piglets in a litter (split weaning) can improve the growth of light weight piglets. Split weaning also reduces the suckling intensity on the sow, enabling lactation oestrus (Terry *et al*., 2011), thus allowing longer lactations to occur without reducing farrowing frequency. This experiment tested the hypothesis that removing the heavier piglets in the litter at d18 and allowing the lightest piglets to continue to suckle to d 30 would improve the growth of the lighter piglets pre-and post-weaning.

Seventy-two litters of 10 piglets nursed by multiparous sows (parity 2.9±0.17; range 2 – 6), were used in this experiment. From d 2 post-partum, litter size was standardised to 10 piglets per sow . On d 18 post-partum, 0, 3, 5 or 7 of the heaviest piglets in each litter were weaned (n=18 litters/treatment). The rest of the litter remained on the sow until d 30 post-partum, at which point they were also weaned. Piglets were weighed at 3, 17, 30 and 40 days of age. Data was analysed using a general analysis of variance (ANOVA) model, with replicate and piglet weight at d 3 built in (Genstat, 10th Edition. Rothamsted Experimental Station, Harpenden).

Table 1. *Effect of weaning age (d 18 vs 30) and suckled litter size (10 versus 7 versus 5 versus 3) from d 18 – 30 post-partum on piglet live weight gain and live weight.*

Age at weaning…	d 30				d 18	Pooled Weight		Pooled SEM
Suckled litter size[1]…	10	7	5	3	N/A	d 30	d 18	
LWG: d 17-30 (kg)	3.9^{a}	4.2^{a}	4.2^{a}	3.8^{a}	2.0^{b}	4.0^{d}	2.0^{e}	0.13
LWG: d 17-40 (kg)	5.2	5.2	5.9	5.2	5.0	5.4	5.0	0.13
LWG: d 30-40 (kg)	1.4^{a}	1.1^{a}	1.6^{a}	1.4^{a}	3.1^{b}	1.4^{d}	3.1^{e}	0.18
LWG: d 3-40 (kg)	8.9^{ab}	8.8^{ab}	9.1^{a}	7.9^{b}	9.3^{a}	8.7^{d}	9.3^{e}	0.14
LW: d 17 (kg)	5.7^{a}	5.6^{a}	5.2^{a}	4.5^{b}	6.4^{c}	5.2^{d}	6.5^{e}	0.10
LW: d 30 (kg)	9.5^{a}	9.7^{a}	9.5^{a}	8.9^{ab}	8.4^{b}	9.4^{d}	8.4^{e}	0.13
LW: d 40 (kg)	11.0^{a}	10.8^{a}	11.0^{a}	10.4^{a}	11.4^{b}	10.8^{d}	11.4^{e}	0.13

Means within a row with different superscripts differ significantly; ^{abc}(P<0.001), ^{de}(P<0.001); [1]Number of piglets suckling from d18 – 30 post-partum; LWG, live weight gain; LW, live weight; SEM, standard error of the mean.

Regardless of piglet age, weaning resulted in a reduction in piglet growth rate post-weaning (P<0.05). Liveweight change from d 17-30 was lower (P<0.05) for weaned compared to unweaned piglets (Table 1). However, piglets weaned on d 18 post-partum grew faster (P<0.05) between d 30 and 40 than those weaned on d 30 (Table 1). Although by d 40 the LW gains made by the lighter piglets weaned at d 30 had declined.

Similar to the findings of Pluske and Williams (1996), extending the suckling period of light-weight piglets improved their weight at d 30. However, our data also indicate that the nutritional and social stressors exerted on the piglets at weaning results in a transient reduction in growth immediately post-weaning regardless of weaning age. As a consequence, overall LW gain from 3 to 40 days of age was greater for piglets that were weaned on d 18 of lactation.

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.

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The Effect of Split Weaning and Boar Contact on the Incidence of Sow Lactational Oestrus

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Maintaining sow reproductive efficiency while increasing lactation length can be achieved by stimulating a behavioural oestrus and ovulation during lactation. This experiment tested the hypothesis that removing a portion of the litter (split weaning) on d 18 post-parturition coupled with boar exposure would enable ovulation and oestrus to occur prior to full weaning.

Seventy-two multiparous (parity 2.9 ± 0.17; range 2 – 6) sows were used in this experiment, which was conducted in four replicates. On d 2 of lactation litter size was standardised to 10 piglets per sow. Sows were weighed on days 1, 17 and 30 of lactation. On d 17 of lactation sows were weighed and allocated based on liveweight (LW) change to one of four treatments; 0 (SPW0), 3 (SPW3), 5 (SPW5), or 7 (SPW7) of their heaviest piglets completely removed for the remainder of lactation (n=18 sows/treatment). From d 18 of lactation until complete weaning on d 30 post-partum, sows were taken daily to a detection mating area, where they received 20 minutes of fenceline boar exposure. The interval from split weaning to expression of behavioural oestrus, the duration of oestrus, and the proportion of sows expressing oestrus was recorded. A general analysis of variance model, with replicate and parity built in, was used to study the effects of treatment on all measures recorded (Genstat, 10th Edition, Rothamsted Experimental Station, Harpenden).

Table 1. *Days to lactational oestrus, duration of oestrus and cumulative proportion of sows expressing lactational oestrus between days 18 and 30 post-partum.*

Treatment[1]	Days to Oestrus[2]	Duration of Oestrus[2]	Cumulative % of Sows Expressing Lactational Oestrus		LW Change (kg)	
			Day 18-24	Day 18-30	Day 1-18	Day 18-30
SPW0	5.6 ± 0.61	2.3 ± 0.24	33.3	55.6	-12.9 ± 2.0	-4.8 ± 1.4[a]
SPW3	4.7 ± 0.48	3.0 ± 0.20	77.8	83.3	-14.3 ± 2.0	-3.2 ± 1.4[a]
SPW5	3.9 ± 0.45	3.1 ± 0.18	88.9	88.9	-10.9 ± 1.9	4.6 ± 1.3[b]
SPW7	4.7 ± 0.45	3.1 ± 0.19	88.9	94.4	-11.6 ± 2.0	1.9 ± 1.3[b]

[1]Number pertains to how many piglets were removed on d 18 of lactation; [2]Only sows expressing oestrus during lactation; [ab]Means within a column with different superscripts are significantly different (P<0.05); LW, liveweight; SPW, split weaning.

There was no effect of treatment on the interval from split-weaning to lactational oestrus, or the proportion of sows expressing oestrus during lactation (Table 1). Sow LW loss from d 1–18 was similar for all treatments, however, sows suckling 10 or 7 piglets from d 18 to 30 lost significantly more weight then those suckling 5 or 3 (Table 1). These data suggest that, if provided boar contact, a reduction in suckling load is not required for sows to express oestrus during lactation. However, although not significantly lower, the decreased proportion of SPW0 sows displaying lactational oestrus suggests a reduction in suckling load increases the number of sows capable of ovulating whilst lactating.

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Dietary Acidifiers Enhance Growth Rate of Weaned Pigs

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BIOMIN Holding GmbH, Herzogenburg, Austria, 3130.

The post weaning lag is a complex phenomenon and evidence suggests that it may be related in part to a limited capacity to maintain proper gastric pH (Cranwell and Moughan, 1989). Insufficient hydrochloric acid secretion impairs the enzyme inactivation and together with the stress of weaning and sudden change in feed consistency, may also disturb the balance of intestinal flora allowing the proliferation of coliforms resulting in scours and poor performance. Dietary supplementation with acidifiers is known to decrease in the occurrence of pathogenic bacteria in the gastrointestinal (GI) tract thus improving growth performance and health status, but responses vary depending on the source of the acid. The aim of the present experiment was to test the hypothesis that a combination of formic and propionic acids would enhance the growth of weaned pigs and reduce the concurrent incidence of diarrhoea.

Four week old, weaned pigs (n=96; [Landrace x Large White x Pietrain] were randomly assigned to two treatments with six pen replicates per treatment and eight pigs per replicate according to sex and body weight (BW; 8.16±0.15 standard error). The treatments were 1) standard diet with no antibiotics or dietary acidifiers, and 2) standard diet supplemented with 3 kg/tonne of a dietary acidifier consisting of a blend of formic and propionic acids (Biotronic® SE, BIOMIN Holding GmbH, Austria). Feed and water were provided *ad libitum*. The standard cereal-based starter and grower diets were formulated to contain 13.70 MJ/kg metabolisable energy (ME), 17.27% crude protein (CP) and 1.37% lysine (Lys), and 12.47 MJ/kg ME, 17.96% CP and 1.14% Lys, respectively. The experimental diets were fed for 56 days. The animals were weighed at the beginning and at the end of the experiment. Feed intake (FI) and mortality were recorded daily. Average daily gain (ADG) and feed conversion ratio (FCR) were calculated. The severity of diarrhoea was assessed from 1 to 3 (soft faeces, fluid faeces, severe diarrhoea) and the duration of diarrhoea was recorded. A one-way analysis of variance (ANOVA) was performed. The significance among the individual means was identified using a t-test (SPSS Science Inc., Chicago, IL, USA).

Table1. *The effect of acidifier on growth performance, mortality and diarrhoea score in weaned pigs.*

Item	Control (-acidifier)	Treatment (+acidifier)	SEM
Number of pigs	48	48	
Initial body weight (kg)	8.2	8.1	0.15
Final body weight (kg)	35.7[a]	38.0[b]	0.58
Feed intake (g/animal)	871	957	32.7
Average daily gain (g)	491[a]	534[b]	9.1
Feed conversion ratio	1.77	1.79	0.033
Mortality (%)	6.3	4.2	0.54
Diarrhoea score[1]	160	16	72.0

[ab]Means within a row with different superscripts differ significantly (P<0.05); [1]Diarrhoea score = days of diarrhoea x severity of scours x number of animals; SEM, standard error of mean.

The results of the present study showed that dietary supplementation with acidifier improved the daily gain in weaned pigs (Table 1). In the treatment group the final BW and ADG were significantly improved (P<0.05) compared to the control group. It might be assumed that acidification of the GI tract contributed to a lower incidence of diarrhoea, which resulted in a higher feed intake and enhanced growth performance.

CRANWELL, P.D. and MOUGHAN, P.J. (1989). In "Manipulating Pig Production II", pp. 140-159, eds J.L. Barnett and D.P. Hennessy. (Australasian Pig Science Association: Werribee).

Efficacy of Benzoic Acid and Sodium Benzoate in Diets for Weaned Piglets (From Pigs to Piglets)

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Previous studies have shown that supplementation of piglet diets with benzoic acid has beneficial effects on post-weaning performance and nitrogen retention (Kluge *et al.*, 2006; Torrallardona *et al.*, 2007). In contrast to other organic acids, benzoic acid is metabolized in the liver to hippuric acid, which is excreted in the urine, reducing urinary pH. This experiment tested the hypothesis that sodium benzoate at 0.4% has the same influence on piglet growth and urinary pH as benzoic acid at 0.35% or 0.5%.

A total of 120, three week old, weaned piglets [(German Landrace x German Edelschwein) x Pietrain] of average weight 6.5 kg were divided into 60 replicate groups of two animals (one castrated male and one female), which were kept in flat-deck pens. The piglets received typical pre-starter diet for two weeks and starter diets for four weeks, both based on wheat, barley, maize, soybean meal and dried whey as the main feed ingredients. The pre-starter diet was formulated to contain 13.9 MJ metabolizable energy (ME)/kg, 180g/kg crude protein (CP) and 12.1g/kg standard ileal digestible lysine (SID). The starter diet was formulated to contain 13.8 MJ ME/kg, 175g/kg CP and 10.1 g/kg SID. Pens were allocated to one of four dietary treatments: negative control without organic acids (NC), benzoic acid (BA, VevoVitall®, DSM Nutritional Products Ltd, Basel, Switzerland) at 5 and 3.5 g/kg, or Na-benzoate (NaB) at 4g/kg. During the six week test period, feed intake and piglet weight were monitored each week. At days 28-33, urinary pH was measured daily in five selected piglets per treatment with a pH meter. The results were subjected to a one-way analysis of variance (ANOVA). In case of significance ($P<0.05$) treatment means were compared by Tukey's HSD test.

Table 1. *Performance of piglets fed either benzoic acid or sodium benzoate for six weeks post-weaning.*

Treatment	Initial weight (kg)	Final weight (kg)	Weight gain (g/day)	Feed intake (g/day)	Feed conversion ratio (FCR)	Urinary pH
NC	6.46	21.04	347[a]	504	1.46	6.64[a]
BA (0.5 %)	6.44	22.96	392[b]	550	1.41	5.96[b]
BA (0.35%)	6.52	23.01	392[b]	559	1.42	6.11[b]
NaB (0.4%)	6.56	22.13	371a[b]	538	1.45	6.78[a]
SEM	0.08	0.30	6.24	8.06	0.01	0.09
P value	0.954	0.072	0.028	0.080	0.136	0.0001

NC, negative control; BA, benzoic acid; NaB, sodium benzoate; SEM, standard error of the mean; [ab]Means in a column with different superscripts differ significantly ($P<0.05$)

Dietary addition of benzoic acid showed beneficial effects on growth performance of weaned piglets (Table 1). Piglets offered diets containing BA at either 0.35% or 0.5% gained weight faster than the negative control but there was no effect on feed intake or feed conversion ratio. The addition of either 0.35 or 0.50% BA significantly reduced the mean urinary pH from 6.64 (NC) to 6.11 and 5.96, respectively. In contrast, the feeding of NaB at 0.4% did not affect this parameter. It is concluded that dietary addition of BA significantly increased the mean daily weight gain of piglets and significantly reduced urinary pH. In contrast, dietary NaB did not significantly improve rate of gain, nor did it acidify the urine.

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TORRALLARDONA, D., BADIOLA, I. and BROZ, J. (2007). *Livestock Science*. **108**:210-213.

New, Relevant Diagnostic Technology for Pig Respiratory Pathogens - Dangers and Pitfalls

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Glässer's disease of pigs, caused by *Haemophilus parasuis*, is recognized as a significant disease in the pork industry worldwide (Ferri *et al*. 2000). Due to its fastidious nature it is difficult to isolate the bacterium. Several polymerase chain reaction (PCR) tests, among them a real time PCR, have been developed to enhance diagnosis of the disease. However, these tests rely on sophisticated equipment, which is lacking in some parts of the world. The alternative to conventional PCR is the loop-mediated isothermal amplification (LAMP), which can amplify nucleic acids under isothermal conditions rapidly with high specificity and sensitivity without the need for specialised equipment (Mori *et al*. 2001). This method uses four specific designed primers and performs auto cycling strand displacement DNA synthesis catalysed by the large fragment *Bst* DNA polymerse (Notomi et al. 2000). Fluorescence dyes like SYBR Green and EvaGreen are added after the reaction to monitor results via ultraviolet (UV) illumination. In recent years, many LAMP assays have been developed including one for *H. parasuis* (Chen et al. 2010). However, some of these methods developed for respiratory bacterial species do not seem to use the relevant related non-target species to validate the specificity of the assay. Therefore the aim of this experiment was to validate the assay with the relevant related non-target species to establish whether it is species specific and a useful alternative to the existing *H. parasuis* PCR assays.

DNA was extracted from *H. parasuis* grown on blood agar supplemented with serum and NADH (BA/SN) overnight. A 1 µl loopful of growth was suspended in 200 µl of PrepMan Ultra (Applied Biosystems, Foster City, CA) and processed according to the manufacturer's instructions. The LAMP assay used the following four primers:

F3 5'– CTGAGAAATTCGGTGGTGATG -3';

B3 5'- CCTTTATCGAGGTAAGATTCG -3';

FIP 5'- ACTTAATTCTAATACTTCCGA+TTTT+TTACTTGAAGCCATTCTTCTT -3';

BIP 5'- GTATTAGAATTAAGTGCAGTG +TTTT+CACCGCTTGCCATACCCTCTT -3'

The primers were used at 40 pmol (FIP 5 and BIP 5) and 5 pmol (F3 and B3). The reaction mix consisted of deoxynucleotide triphospates at 2 mM and 8 U of *Bst* DNA polymerase (New England Biolabs, Hitchin, Hertfordshire UK), 1.0x the supplied buffer and 1 ul template. The mixture was incubated at 65°C for 45 min and the reaction terminated at 80°C for 2 min. EvaGreen was added (1 µl for 25 µl reaction mix) and the tubes placed under UV light and photographed. Closely related non-target species tested were: *Actinobacillus equuli, A. indolicus, A. minor, A. porcinus, A. rossi, A. suis, Haemophilus parainfluenzae, Mannheimia haemolytica, M. varigena, Pasteurella aerogenes, P. canis, P. langaaenis, P. mairii, P. multocida, P. stomatis, P. species* B as well as non- related non-target species - *Erysipelothrix rhusiopathiae, Salmonella enterica* and *E. coli*. All 15 reference serovar strains of *H. parasuis* were used. Seventeen out of the 19 non-target species came up positive in the LAMP assay. All reference strains for *H. parasuis* were positive.

The results highlight the pitfalls with published LAMP assays that have not been validated properly. As this is an isothermal reaction the lack of specificity might be associated with the primers (not specific or excess amount), the buffer concentration (magnesium) and the concentration of template. In its current form this assay can not replace the conventional PCRs, which are species specific.

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Effects of Mycoplasmal Pneumonia During the First Weeks After Challenge

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Experimental challenge systems for *Mycoplasma hyopneumoniae* are critical in the assessment of vaccine efficacy but most are evaluated for only a few weeks after mycoplasmal challenge. To assess whether such challenge systems show any significant production effects and to examine possible relationships between growth, clinical expression of disease and lung pathology at slaughter 3-4 weeks post-challenge, we examined data derived from a pilot study based on two different *M. hyopneumoniae* strains. We hypothesised that a system commonly used to assess pneumonia severity at slaughter by scoring affected lung area (Goodwin and Whittlestone, 1973; Jackowiak, 2010) will correlate with data based on affected lung weight, and that the severity of pneumonia may be reflected in reduced growth and increased coughing scores.

Nineteen male weaner pigs aged nine weeks were challenged by endotracheal instillation of cultures containing either *M. hyopneumoniae* strain Hillcrest or Beaufort, or no organisms. Pigs were weighed 1-2 d before challenge (mean weight 18.3 ± 0.7 kg; range 14-27 kg) and 20-21 d later. Half of each group were euthanased at three weeks and half at four weeks post-challenge, at which time gross lung pathology was assessed by Goodwin lung score, and by comparative weight of pneumonic lung to total lung. Coughing episodes per pig were measured in all pigs during periods of 15 minutes twice daily until three weeks post-challenge. Pigs had *ad libitum* access via Maximat feeders (Skiold Echberg A/S, Denmark) to a commercial pelleted diet (13.7 MJ digestible energy/kg) free of antibiotics and were housed in groups of four. Pens had half solid sides (marine ply with kennel roof) with stockboard flooring and infrared heating, and half open wire mesh sides with rubber mesh over concrete slats.

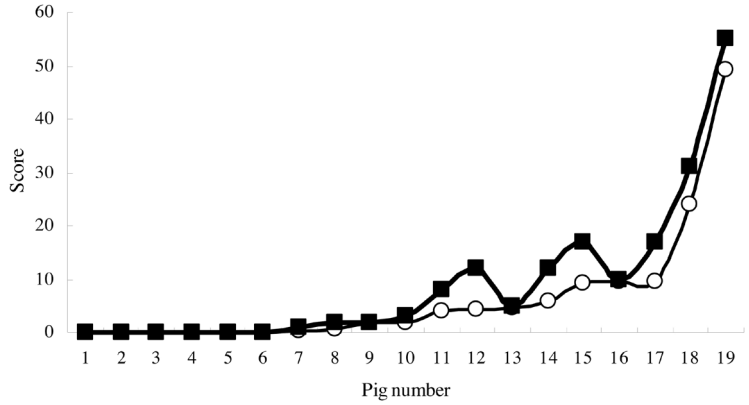

Figure 1. *Comparison of Goodwin lung scores (■) and proportion of pneumonic lung by weight (○) among 16 pigs challenged with* M. hyopneumoniae *and 3 unchallenged controls.*

In the three weeks after challenge, pigs showed a mean (± standard error) body weight of 32.8±1.2 kg range 24-46 kg) and an average daily gain of 0.707±0.029 kg (range 0.495–0.960 kg), with coughing episodes ranging from 0 to 13, Goodwin lung scores between 0 and 55, and affected lung by weight ranging from 0 to 49%. All except two pigs had pneumonia severities of less than 10% affected lung by weight, and both of these severely affected animals were the only pigs with concurrent pleurisy, suggesting secondary bacterial pathogens were also involved. While trends were apparent between ADG or coughing score and pneumonia severity, no significant correlation was evident up to three weeks post-challenge. However, there was strong agreement (r = 0.981) between the two measures of pneumonia severity based on arbitrary lung volume score and affected lung weight (Figure 1).

These findings indicate that initial growth and clinical signs during the first three weeks after challenge are not sufficiently reliable estimators of disease severity, whereas estimations of lung area by Goodwin lung scores agree with quantitative measurements of pneumonic lung by weight.

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Detection of *Clostridium difficile* After Treatment in a Two-Stage Pond System

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Clostridium difficile is a Gram positive anaerobic sporeforming bacterium that causes enteric disease in humans and animals. It is associated with pre-weaning scour in neonatal piglets (Songer and Anderson, 2006). Infection is likely transmitted by contamination of the environment with spores, which are shed in the faeces of both symptomatic and asymptomatic piglets (Hopman *et al*., 2010). Widespread contamination with *C.difficile* occurs rapidly in the farrowing shed environment. Toxigenic *C.difficile* has previously been isolated from 61% (71/116) of crates in a new farrowing shed after being occupied for only one month, where baseline sampling was negative, and scouring was minimal (M.M. Squire, unpublished data). This degree of contamination is unlikely to be explained by scouring alone, so effluent re-use was suggested as a potential source of *C.difficile* spores. In Australia, piggery effluent is often treated in anaerobic ponds to remove pathogens, and re-used to wash sheds or applied to agricultural land. Survival of non-spore forming pathogens in treated effluent and effluent-irrigated soils has been reported (Chinivasagam *et al*., 2004) but there are no data on the survival of *C.difficile*. It is hypothesized that *C.difficile* would survive in effluent throughout the treatment process due to the resistant nature of its spores, thereby contributing to contamination of the farrowing shed. To test this, a pilot study was conducted to ascertain the presence, number and molecular type of *C.difficile* at all stages of piggery effluent treatment and storage.

One litre samples (n=10) of effluent representing pre-treatment and each treatment step in a two-stage pond system were collected from a large farrow-to-finish facility during a single sampling trip in April, 2011. Samples were taken at a depth of at least 30cm except for storage tank samples which were taken from the surface of filled tanks. One ml of effluent from each sampling point was cultured by the direct spread plate method on a chromogenic medium specific for *C.difficile*. Black colonies typical of *C.difficile* on this medium were counted and a selection of colonies from each plate was subcultured onto blood agar and confirmed as *C.difficile* by their colony morphology and characteristic odour. Molecular characterisation was performed by polymerase chain reaction (PCR) ribotyping and PCR detection of toxin genes.

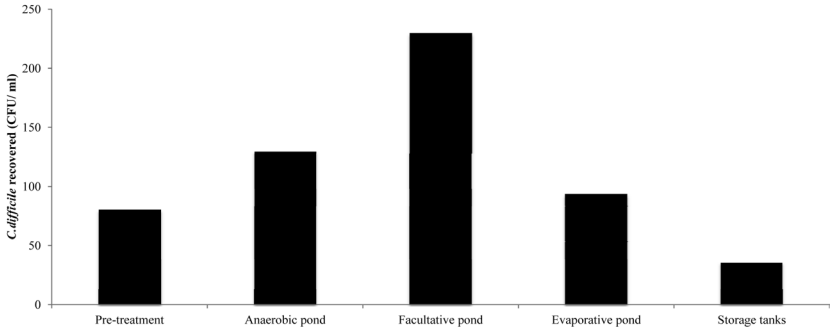

Figure 1. *Viable toxigenic Clostridium difficile (Colony forming units (CFU)/ml) survives in effluent sampled at all stages of treatment in a two-stage pond system (n=10).*

Overall, *C.difficile* was isolated from 100% (10/10) of samples, representing all stages of effluent treatment (Figure 1). All *C.difficile* isolates were PCR ribotype UK 237, a toxigenic strain that predominates in piglets at this farm. Although *C.difficile* numbers increased during anaerobic phases of treatment and decreased during aerobic phases as we expected, the small sample size makes it impossible to ascertain if this variation is significant. We can, however, confirm our hypothesis that viable *C.difficile* spores survive in effluent treated in a two-stage pond system. These spores have the potential to contaminate the piggery environment if treated effluent is re-used. The results also confirm that the methodology is rigorous enough to warrant a broader study that includes a larger sample size from several piggeries. A similar study to investigate the survival of *C.difficile* on effluent irrigated soils and pasture is also warranted.

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CHAPTER 8

Welfare, Behaviour and Piglet Survival

Sow Housing in Australia - Current Australian Welfare Research and Future Directions

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Abstract

The housing of sows during gestation, farrowing and lactation is one of the most controversial animal welfare issues in livestock production. From 2017, the *Australian Model Code of Practice for the Welfare of Animals – Pigs* will limit the duration of housing of gestating sows in gestation stalls to six weeks of gestation and for a maximum of six weeks in farrowing crates to assist farrowing and lactation. Furthermore, the Australian pork industry has voted to pursue the voluntary phasing out of gestation stalls by 2017. Past industry experience has indicated that the opportunity for group housing to improve sow welfare is limited by the high levels of aggression that are commonly observed in newly formed groups of sows after mixing. Therefore, recently, there has been a significant amount of industry-funded welfare research conducted in the area of gestation sow housing to equip the industry to move towards group housing of gestating sows. This research in the area of gestation sow housing has focused on understanding the effects of floor space allowance, group size, time of mixing and provision of feeding stalls on sow welfare and performance, and developing strategies to reliably reduce aggression and stress in group-housed sows. In the future, the newly funded Cooperative Research Centre for High Integrity Australian Pork (CRC HIAP) will meet the challenge of developing Australian confinement-free gestation, farrowing and lactation housing systems that are commercially-viable. This review will encompass the results from recent research that has been conducted in Australia in the area of gestation sow housing and welfare and the future direction for welfare research applicable for commercially-viable, confinement-free gestation, farrowing and lactation housing systems.

Introduction

Gestation Sow Housing

The housing of dry (non-lactating) sows in individual gestation stalls has become one of the most controversial animal welfare issues in livestock production. The main welfare concerns are the general lack of social contact, the inability to exercise and the restricted choice of stimuli for interaction (ie. other pigs and features of the physical environment; Barnett *et al*., 2001). Therefore, there has been significant movement worldwide to eliminate gestation stalls for either part or all of gestation. In the European Union (EU), gestation stalls will be banned between four weeks after mating and one week prior to farrowing, from January 2013 (Council Directive 2001/88/EC; EU, 2001; Ministeries van Justitie en van LNV, 1998, 2003). There are countries within the EU that are more stringent in the use of gestation stalls, and some countries have banned the use of gestation stalls entirely (eg. United Kingdom and Sweden). In New Zealand, the *Animal Welfare (Pigs) Code of Welfare 2005* will limit from 2015 the optional use of dry sow stalls to a maximum of four weeks after mating. In Australia, the *National Model Code of Practice for the Welfare of Animals - Pigs* will limit from 2017 the use of gestation stalls for a maximum of six weeks (Primary Industries Ministerial Council, 2007). Furthermore, the Australian pork industry recently voted to pursue the voluntary phasing out of gestation stalls by 2017. The newly funded CRC HIAP will support these changes to the industry, "through innovative, collaborative, whole value chain research and development programs, by facilitating production that is efficient and ethical without the need for sow confinement in stalls or crates or widespread use of antibiotic medications..." (Pork CRC, 2011).

There is enormous variation in the design and management of group housing systems for gestating sows, and there is not a single representative group housing system. Therefore, few statements can be made that are applicable to all group housing systems for gestating sows (Gonyou, 2003). Regardless of the housing system used, the ultimate goals of any gestation sow housing system are to:

1. Keep sows protected from the environment;
2. Keep sows protected from other sows;
3. Feed sows at levels to avoid obesity/starvation;
4. Maintain pregnancy and health and ensure well-being;
5. Provide a safe and enjoyable workplace for employees;
6. Ensure that the system is robust to errors in management;
7. To meet these aims within the financial constraint of current income levels.

There is a range of group housing systems used in the Australian pig industry. In conventional group housing systems, sows are housed indoors and have automated and natural ventilation systems, fully- or partially-slatted floors and liquid manure handling systems. Pens usually house five to 50 sows with a floor space allowance of 1.4–2.0 m^2/sow. Other alternative group housing options include deep-litter, large group housing and outdoor "free-range" systems. Deep-litter systems are naturally ventilated, have a floor base of deep litter (eg. rice hulls or straw), consist of larger group sizes (ranging from 15 to 200 sows per pen) and provide a greater space allowance of approximately 2.5–3.5 m^2/sow. Other integrated large group housing systems have also been developed in Europe which involve the use of some straw for enrichment (not deep-litter), larger groups (50-100 sows), partly slatted areas for dunging, hiding areas and barriers to reduce aggression (Morrison, 2005).

Morrison (2005) conducted a review on group housing of gestating sows, and outlined strategies for good welfare and reproductive performance in group housing. The review concluded that "the move towards group housing of sows for a significant part of their gestation is on the horizon for many pork producers...however further research needs to be conducted to assess the spatial requirements for gestating sows...and further management strategies to reduce aggression between group housed sows to ensure that good welfare and reproductive performance can be attained". The Australian pork industry has made significant advances in group housing since that review was published working towards implementing group housing options to replace stalls. Furthermore, there has been considerable research conducted within the last five years that will assist producers to adopt group housing of gestating sows.

Farrowing Crates

The use of farrowing crates is still permitted around the world (except Sweden, Switzerland and Norway). In Australia, the *National Model Code of Practice for the Welfare of Animals - Pigs* allows the use of farrowing crates for a maximum of six weeks (Primary Industries Ministerial Council, 2007). The use of farrowing crates can improve the welfare of neonatal pigs by providing warmth in a restricted space and limit the risk that neonates wander away from the sow (or the source of warmth), become chilled and die from either starvation or overlying. Farrowing crates and the concomitant lack of (straw) bedding however, restrict the ability of sows to perform "normal" pre-farrowing behaviours such as nest-site selection, nest-building activity and bonding with the piglets (Baxter *et al*., 2011a), and thus have been criticized on welfare grounds (viz. RSPCA Five Freedoms Concept). The removal of the farrowing crate structure from farrowing pens may result in higher piglet mortality, including increased piglet deaths due to crushing by the sow (see Tables 1-3). Over the last 30 years, researchers have attempted to develop non-crate farrowing systems that 1) address the perceived behavioural deficiencies experienced by sows in the peripartum period, 2) promote piglet survival, and 3) are economically viable and practical to operate for pig producers (Cronin, 2010a). However universal acceptance and commercial adoption of non-crated farrowing systems has not occurred (Baxter *et al*., 2011b).

The Australian pork industry recognizes that alternatives to farrowing crates need to be identified and evaluated, since the community, markets and/or regulators/legislators in the future may not support the continued use of farrowing crates. Australian Pork Limited, the Animal Welfare Science Centre, the Royal Society for the Prevention of Cruelty to Animals (RSPCA) and pork producers from the Australian pork industry recently supported a seminar and workshop titled "Alternative farrowing Systems-Identifying the gaps in the knowledge", where Australian and international researchers presented research on alternative farrowing systems that have been developed. The main objective of that workshop was to identify the gaps in knowledge as science and industry strive towards the development of commercially-viable, welfare friendly alternative farrowing systems. Furthermore, Cronin (2010a) has recently completed an extensive literature review which evaluated sow and litter performance in farrowing crates compared to non-crate systems, and identified gaps in knowledge which need to be addressed as the Australian pork industry investigates non-crated farrowing and lactation systems. This present review extends from the work of Cronin (2010a), with a focus on future research required as the industry investigates and develops non-crated farrowing and lactation systems.

The aim of this present literature review is to provide an update of the current research investigating group size, floor space allowance, time of mixing and the use of feeding stalls for gestating sows housed in groups. This review also outlines the research required in the future to investigate commercially-viable, confinement-free gestation, farrowing and lactation housing systems.

Gestation Sow Housing

Group Size and Floor Space Allowance

Aggression among recently-grouped unfamiliar gilts and sows has both production and welfare implications. In spite of the research to date on minimising aggression when grouping unfamiliar pigs, there are few rigorous recommendations (Barnett *et al*., 2001; Spoolder *et al*., 2009) and this subject clearly required further research to allow industry to successfully manage group housing and minimize risks to welfare and reproduction. In particular, a better understanding of the effects of, and interactions between, factors such as floor space allowance and group size on sow welfare and performance was required. Therefore, Hemsworth and Morrison (unpublished data) examined the effects of space allowance and group size on aggression, stress, injury and reproduction in sows housed in groups during gestation. Three thousand, one hundred and twenty sows were used in the 3 x 6 factorial designed experiment with the respective factors being group size (10, 30 and 80 sows per pen) and floor space allowance (1.4m^2 , 1.8m^2 , 2.0m^2 , 2.2m^2 , 2.4m^2 and 3.0m^2 per sow). The sows were artificially inseminated twice and mixed into their group-housing treatments within seven days of insemination. Pens had concrete floors with a 50% slatted area at the rear of the pens. Drop feeders (two per 10 sows) distributed pelleted feed evenly across the pen, four times per day.

Sow welfare was assessed using a broad examination of the behavioural, physiological, health and fitness responses of sows to assess biological functioning of the animals. Blood samples were collected via jugular venipuncture and cortisol concentrations were measured at d 2, 9 and 51 of treatment. Extensive behavioural observations were conducted during feeding at d 2 after mixing to study aggression, while skin injuries were also assessed at d 2, 9, 23 and 51 of treatment (as described by Karlen *et al*., 2007). Live weight and reproductive performance of the sows were also measured. The results from Hemsworth and Morrison (unpublished data) indicated that there were no significant interactions between group size and floor space allowance. The experiment showed that floor space allowance affected aggression, stress physiology and farrowing rate in sows. A key finding was the effects of floor space on aggression at feeding at d 2 of treatment, both total and free cortisol concentrations at d 2 of treatment and farrowing rate. For all four variables, there was a general decline in aggression and cortisol concentrations with increasing space, while there was general increase in farrowing rate with increasing space. Aggression was not measured later in the treatment period, but there was no evidence that space affected cortisol concentrations at d 9 and 51 of treatment. It should be emphasized that the relationship between space and cortisol can be interpreted in two ways: the results are in accord with either a linear decline in cortisol concentrations early post-mixing from 1.4 m^2/sow to 3 m^2/sow or a decline in cortisol from 1.4 m^2/sow to 1.8 m^2/sow and no further decline above 1.8 m^2/sow. The size of the experiment was insufficient to be able to determine which of these scenarios is more biologically correct, and it was difficult to determine the exact floor space requirements from the experiment. Thus in terms of animal welfare at mixing, it was not possible to give guidance on an adequate space allowance, other than a space allowance of 1.4 m^2/sow is likely to be too small. While the effects of floor space on stress physiology were found early in the treatment, the effects of floor space on farrowing rate highlight that stress might be biologically important in the period shortly after the formation of a static group of sows that have been recently inseminated. Furthermore, the results indicate that the effects of floor space are most pronounced early after grouping. Indeed, it appeared that sows in static groups may adapt to reduced floor space over time. Furthermore, there is evidence, that as gestation proceeds and during lactation there is a dampening of the hypothalamo-pituitary-adrenal axis' response to stressors (Lightman *et al*., 2001). Nevertheless, in terms of risks to both welfare and productivity, these results highlight the importance of sufficient floor space in order to reduce aggression and stress at mixing and that the sow's requirement for space appears to be less once the group is well established.

Smaller studies in the scientific literature have also highlighted the importance of floor space on sow welfare. Hemsworth *et al*. (1986) examined the effects of floor space on the sexual behaviour and stress physiology of non-pregnant gilts. The sexual behaviour of gilts with a space allowance of 1.0 m^2/animal was reduced in comparison to that of the gilts with either 2.0 or 3.0 m^2 animal. After 9-11 days of treatment, cortisol concentrations were higher in the 1.0 m^2 treatment than the 3.0 m^2 treatment. In one of the two replicates, the cortisol concentrations were higher in the 2.0m^2 treatment compared to the 3.0 m^2 treatment. Similar treatment effects on cortisol concentrations were apparent at 12 weeks of gestation. In a factorial experiment examining the effects of floor space and feeding stalls on gestating gilts, Barnett *et al*. (1992) found that plasma cortisol concentrations at 28-29 and 49-51 days of treatment were elevated, immunological responsiveness, assessed on the basis of a cell-mediated response (skin-fold thickness) to a mitogen injection, at the 50-57 days of treatment was reduced, and aggression around feeding was increased in gilts housed in groups of four at a floor space of 0.98 m^2/animal than 1.97 m^2/animal. The number of skin lesions was not affected by floor space. In another factorial experiment examining the effects of floor space and feeding stalls on gestating gilts, Barnett (1997) found that plasma cortisol concentrations at 36 and 53 days of treatment were higher in

gestating gilts housed in groups of five with a floor space of 1.0 m^2/animal than 1.4 or 2.0 m^2 per gilt. Cortisol response to an adrenocorticotrophic hormone (ACTH) challenge and aggression around feeding were also higher in the floor space of 1.0 m^2 than 1.4 or 2.0 m^2 per gilt.

In Hemsworth and Morrison (unpublished data) there were no effects of floor space on fresh or total injuries at d 2, 9, 23 and 51 of treatment. There have been some effects of floor space allowance on aggression injuries reported in the literature. Weng *et al*. (1998) reported increased aggression and injuries with decreasing space allowance and recommended a space allowance between 2.4 and 3.6 m^2/animal for groups of six pregnant sows. Salak-Johnson *et al*. (2007) showed that groups of five sows with 1.4 m^2/sow had higher injury scores than their counterparts at 2.3 or 3.3m^2/animal. Taylor (1997) also reported increased aggression when pen space with groups of 10 sows was reduced from 2.0 to 1.2 m^2/animal. Furthermore, there were no differences in reproductive performance between the treatments in any of these studies, however sows in all studies were introduced to their treatments at least 25 days post-insemination which is the period when reproductive failure is less likely (Ashwood and Pickard, 1998) and aggression may be reduced (Hemsworth *et al*., 2006).

Hemsworth and Morrison (unpublished data) concluded that further research is recommended to examine the effects of floor space allowance in the range of 1.8 to 2.4m^2/sow in more detail, and particular attention should be given to the effects of space and time of mixing relative to mating (d 1 to 4 post-mixing), since this is the period when aggression and stress are likely to be most pronounced and the adverse effects on reproductive performance. The authors also concluded that there is a clear need to examine the effects of varying floor space during gestation (ie. 'staged-gestation penning' in order to provide increased space immediately after insemination) as well as the use of and design features of a dedicated mixing pen. The research on pen design should also include an examination of the effects of pen features such as feeding stalls and feeding systems, since these may have an impact on aggression and stress physiology of group-housed sows (Edwards *et al*., 1993). Barnett *et al*. (1992) found that full body length feeding stalls reduced plasma free cortisol concentrations, and improved immunological responsiveness, assessed on the basis of a cell-mediated response to a mitogen injection, in gestating gilts housed in groups of four at floor space allowances of 0.98 m^2 to 1.97 m^2. While aggression around feeding was reduced, feeding stalls did not affect skin lesions. In another factorial experiment, Barnett (1997) found that feeding stalls, particularly full body stalls rather than shoulder stalls, reduced aggression around feeding over 30 days of treatment and plasma total cortisol concentrations at 36 and 53 days of treatment in gestating gilts housed in groups of five with a floor space of 1.0 to 2.0 m^2.

Hemsworth and Morrison (unpublished data) found that group size did not affect cortisol concentrations, aggression or farrowing rate. However, skin injuries were affected by group size, with groups of 10 sows having consistently low injuries throughout the experiment. Although this is difficult to explain, Taylor *et al*. (1997) reported an increase in aggression as group size increased (group sizes of 5, 10, 20 and 40 sows with a space allowance of 2.0 m^2/animal) although the number of lesions during gestation was similar across treatments. Turner *et al*. (2006) concluded that other factors such as pen design, feeding system and flooring may also affect the incidence of injuries.

Time of Mixing after Insemination

There are numerous options available to the pork producer in terms of when to group sows in relation to breeding. Sows can be grouped from weaning, immediately after breeding, or five to six weeks into gestation when confirmed pregnant. The sow is particularly vulnerable to injuries in the immediate post-weaning period, since her body condition may be reduced during lactation, her vulva may be sensitive as she returns to oestrous and, if natural mating is being performed, she is prone to injuries from the boar. Therefore, management strategies should be implemented in the post-weaning period to ensure that the welfare of the sow is not at risk during this time (Morrison, 2005).

The process of embryo implantation begins 12–13 days post-conception and is usually complete by d 16 (Dantzer and Winther, 2001). The general consensus in the scientific literature is that most embryonic deaths occurs within the first 30 days of gestation and during this period, a range of environmental, social, genetic, nutritional, hormonal and biochemical factors can either alone or in combination affect conception rate and ultimate litter size (Ashworth and Pickard, 1998). Group housing may exacerbate the problem since environmental and nutritional factors may be more difficult to control in groups than in individual stall housing. Therefore, in the past, the safest option in terms of embryo survival was to house sows in gestation stalls for at least five weeks post-mating to ensure that stressors associated with mixing (ie. aggression, change in environment etc.) did not adversely affect embryo implantation (Barnett *et al*., 2001; Monogastric Research Centre, 2001; Nielsen *et al*. 1997; Fisker,1995).

Research by Hemsworth *et al*. (2006) provided evidence that the practice of housing sows in stalls immediately after mating and delaying mixing in large groups on deep litter until pregnancy is confirmed, by reducing aggression at mixing,

may provide some distinct welfare advantages over housing sows either in stalls or in large groups on deep-litter for the entire gestation. For example, mixing in groups at 35 days post-insemination rather than mixing at d 1 post-insemination resulted in less aggression. Furthermore, while sows mixed in groups at 35 days post-insemination had similar cortisol concentrations to those in stalls, sows mixed in groups 1 day post-insemination had higher salivary cortisol concentrations than sows in stalls at both 1 and 35 days post-insemination. A possible mechanism for reduced aggression when sows are mixed later in pregnancy may, be due to increasing progesterone concentrations as pregnancy is established. Progesterone has been shown in a number of rodent experiments to reduce aggression (De Jonge *et al*.,1986; , Payne *et al* 1972).

The scientific literature is deficient in information on the impact of the time of mixing post-mating on the welfare and reproductive performance of sows. Therefore, Hemsworth and Morrison (unpublished data) examined the effects of time of mixing in groups on aggression, stress, injury and reproduction in sows. Five hundred and forty sows were used to study the effects of three housing treatments: 'Gestation stall', in which sows were housed in individual gestation stalls for their entire gestation; "Group", in which sows were housed in groups of 10 with 1.5 m^2 of floor space with individual shoulder feeding stalls; and "Stall followed by Group", in which sows were housed in gestation stalls for the first 36-38 days of gestation then mixed into groups of 10 with 1.5 m^2 floor space and individual shoulder feeding stalls. The sows were inseminated twice and mixed into their housing treatments within three days of insemination. Each individual gestation stall and individual shoulder feeding stall (group pens) had an automatic drop feeder that distributed feed into a feeding trough once per day.

There were no effects of housing treatment on farrowing rate or live weight and backfat gain of the sow. In relation to stress, cortisol concentrations at d 2 and 50 of treatment were higher in sows housed in groups with shoulder feeding stalls throughout gestation than sows housed in gestation stalls or those initially housed in gestation stalls and then housed in groups with shoulder feeding stalls from 36-38 days after mating. Furthermore, sows housed in group pens with shoulder feeding stalls at the time of injury assessment (d 2, 36 and 50 days of treatment) had a higher incidence of injuries. The results indicate that while stress was higher in sows mixed in groups after mating, these effects were not severe enough to adversely affect reproductive performance or growth characteristics of the sow during pregnancy. Turner *et al*. (2005) concluded that the female pig is resistant to the effects of acute stress and elevated cortisol or repeated acute stress and acute and repeated elevation of cortisol. In contrast, the authors also concluded the prolonged stress and sustained elevation of cortisol can disrupt the reproductive process or biological fitness of the animal. Regardless, from a welfare perspective, the authors concluded that attention should be given to the design aspects of these group pens, such as the design of the stalls in the group pens and the pen space available in these group pens, in order to reduce stress and injuries in sows mixed in groups shortly after mating. In terms of the impact of housing treatment on reproductive performance of the sow, results from Hemsworth and Morrison (unpublished data) suggest that mixing of sows at either 1-3 days or 36-38 days post-insemination was conducive to high reproductive performance. Kirkwood and Zanella (2005) found that sows grouped at d 2 post-insemination in pens with floor feeding had a higher farrowing rate than those grouped at about d 14 post-insemination. Spoolder *et al*. (2009), using data extended from Geudeke (2008), found that farms in which sows were grouped 1-2 weeks after insemination had a higher percentage of rebreeding than farms in which sows were grouped later. In a small experiment, van Wettere *et al*. (2008) compared gilts in gestation stalls, gilts in previously existing groups, and gilts mixed on d 3/4 or 8/9 of gestation. In terms of reproductive performance there were no adverse effects on embryo development or survival when group-housed mated gilts were re-mixed in the first 10 days of gestation.

With the Australian industry moving towards group housing, research was also required to determine how existing shed infrastructure could be modified to accommodate a similar number of gestating sows as has been the case in the past, albeit now housed in groups. Hemsworth and Morrison (unpublished data) examined the concept of converting existing gestation stalls into group pens (housing 10 sows with 1.5m^2 pen space allowance per sow). This modification was achieved by using part of the existing gestation stall as a shoulder feeding stall, and the back aisle space as pen space. This concept enabled an existing gestation shed of gestation stalls to be converted to group housing without reducing the number of sows that were housed in the shed (ie. 100% of sows previously housed in stalls converted to 100% of sows housed in groups). Housing sows in this group housing system allowed sows to achieve similar reproductive performance and growth characteristics of sows housed in gestation stalls.

In summary, it is generally accepted that the most suitable time to group sows to avoid reproductive failure is prior to embryo implantation at 12 days or wait until after 30 days of gestation (Ashworth and Pickard, 1998; Dantzer and Winther, 2001). Further research is required to examine the most appropriate time to mix sows into groups after their second insemination, especially as the Australian pork industry moves towards elimination of gestation stalls entirely. The timing of when sows are mixed post-insemination is critical. Furthermore, the investigation of strategies to mix sows into groups prior to insemination, thus perhaps reducing aggression post-insemination requires investigation.

From a welfare perspective, it is recommended that further research is conducted on the design aspects of these group pens, such as the design of the feeding stalls and the pen space available, in order to reduce stress and injuries in sows mixed in groups shortly after insemination. Further studies are also required to further develop methods of converting existing gestation shed infrastructure into pens for group housed sows, addressing the needs of both the sow and producer.

Strategies to Reduce Aggression

Improvements in our knowledge of sow aggression and the principles of mixing sows to reduce aggression and stress are required to develop practical strategies for a commercial environment. Irrespective of the design features of the pen, understanding the effects of individual sow characteristics and group composition is important in reducing sow aggression and stress. In the wild, a presumed adaptive function of intraspecific aggression is dispersion of animals and therefore unfamiliarity or "social strangeness" is likely to be a major factor responsible for this intra-specific aggression in sows (Zayan, 1990). Aggressive behaviour is strongly influenced by experience and there is limited evidence in pigs and other species that level of participation in social interactions may affect stress. For example, there is limited evidence that animals that engage in aggression, either winning more or less of their fights, and animals that avoid social interaction may differ in the stress that they experience and thus their fitness in the group.

Therefore, research on the effects of aggressive behaviour of individual sows on how well individual sows and the group as a whole performs is presently underway in Australia (APL project 2009/2303 - Effects of aggressive characteristics of individual sows and mixing strategies on the productivity and welfare or group-housed gestating sows). There is some evidence that the aggressive behaviour of individual sows is important in terms of how well those sows perform in a group. Mendl *et al*. (1992) was able to classify pigs into three different categories based upon their ability to displace others in agonistic interactions. Using the categories of High Success (displaced other pigs more often than was displaced), Low Success (displaced more often than displaced other pigs) and No Success (displaced no other pigs) pigs, Mendl *et al*. (1992) found distinct differences between the categories in regard to behavioural and physiological reactions when exposed to social stress. No Success sows had the highest cortisol concentrations and produced low birth weight piglets. Verdon and Hemsworth (2011) categorized sows in groups of 10 as Dominant, Sub-dominant and Submissive sows. Dominant and Sub-dominant sows which were categorized as sows that engage in aggression at feeding, had fewer skin injuries on d 9 post-mixing and spent more time feeding than submissive sows that did not engage in aggression at mixing. This research is currently being expanded to examine the influence of group composition, in terms of social strategy, on the welfare and reproductive performance of both individuals and the group as a whole. As part of the larger current APL project 2009/2303, Chow (2010) developed a series of individual characteristic tests, and examined whether behaviours of gestating sows in these tests were predictive of their aggression when mixed into groups. One hundred and twenty pregnant sows were selected randomly post-insemination, and were allocated into one of the three tests developed, which were conducted in individual gestation stalls away from their home stall - 1) aggression of individual sows in stalls when exposed to an unfamiliar older stimulus sow, 2) withdrawal response test to a novel object, and 3) withdrawal response test to a human. At approximately 40 days post-insemination, the sows were divided into 12 groups of 10, with their aggressive behaviours recorded at time of mixing, at feeding on d 2 to day 4 post-mixing, and at resting on d 2 to d 3 post-mixing. The behaviours of sows observed when exposed to unfamiliar older stimulus sows in stalls, particularly the latency to interact with the stimulus sows, the frequencies of foot lifting and bites delivered, were significantly correlated with their aggressive behaviours observed at mixing and at feeding in groups. Chow (2010) concluded that behaviours of individual sows observed when exposed to unfamiliar older stimulus sows may predict their aggression when mixed in groups. This knowledge could be integrated into current strategies aimed at reducing aggression at mixing

Further research is also required to assess the effectiveness of management tools that can be used to reduce aggression between sows at mixing. Some possible strategies include the use of mixing pens with greater pen space allowance, provision of straw enrichment and the use of boars. The effect of straw on reducing sow aggression in groups of sows is equivocal, with some authors reporting benefits of supplementary feeding of straw (Durrel *et al*., 1997) and provision of straw bedding on reducing aggression (Meyer *et al*., 1984), whilst Whittaker *et al*. (1999) reported sows bedded on straw may exhibit increased aggression. The addition of straw to group housing systems may have a two-fold effect in improving overall welfare and performance of the sow. Firstly, feeding additional fibre (ie. straw) may reduce boredom and hunger (promoting satiety), and secondly it may enrich the sow's environment and encourage foraging (Fraser, 1975;). This redirection of behaviour towards the straw may reduce aggression, antisocial and stereotypic behaviour (Beattie *et al*., 1996; 2000a, 2000b), and thus may reduce reproduction loss and improve welfare caused by aggression. The majority of studies in the literature compare the addition of straw to the sow's environment to a barren concrete floor/slatted system. Therefore, depending on the feeding system, pen space allowance, and frequency

of addition of straw material the use of straw has variable effects on aggression. In some studies aggression was reduced with the addition of straw, while in other studies aggression increased because of competition for the straw. However in the latter studies straw was only supplied to their environment in small amounts at daily intervals (Anderson and Boe, 1999; Whittaker *et al.*, 1999). From the scientific literature, it appears that *ad libitum* forage material should be provided to ensure sows do not fight over the limited resource.

Anecdotal evidence suggests that housing boars with sows at the point of mixing in group systems reduces aggression between sows. While this is a common industry practice, the scientific literature is deficient in information on how this management practice influences aggression. It is thought that the presence of boars (ie. pheromones, visual stimulation and tactile contact) encourages the sows to redirect their aggressive behaviour towards the boar and/or the boar pheromones may quieten the sows. Androsterone present in the saliva of boars may also have a sedative effect on the sows (McGlone *et al.*, 1986; McGlone and Morrow, 1988). Grandin and Bruning (1991) showed that the presence of mature boars significantly reduced fighting in newly mixed finisher pigs, however, Seguin *et al.*, (2006) showed that the presence of a boar did not reduce aggression in groups of sows. The review by Barnett *et al.* (2001) mentioned numerous other options that may be useful in reducing aggression between sows such as using masking odours and mood altering drugs, grouping sows after dark, providing *ad libitum* feed to sows. However, the authors concluded that these methods may postpone rather than reduce aggression.

Non-Crated Farrowing Systems - The Future

Cronin (2010a) has recently completed an extensive literature review which evaluated sow and litter performance in farrowing crates compared to non-crate systems, and identified gaps in knowledge which need to be addressed as the Australian pork industry investigates non-crated farrowing and lactation systems. This review highlights the major points from Cronin (2010b), with a focus on future research required as the industry investigates and develops non-crated farrowing and lactation systems. Baxter *et al.* (2011b) have also recently reviewed alternative farrowing systems and have reviewed the welfare and economic aspects of existing farrowing and lactation systems.

Farrowing crates are the most widely used housing system for sows during the farrowing process and lactation. In Australia, the *National Model Code of Practice for the Welfare of Animals - Pigs* allows the use of farrowing crates for a maximum of six weeks (Primary Industries Ministerial Council, 2007). Before the development of farrowing crates, loose pens were widely used for farrowing sows (Harris, 1906). Piglet mortality was high and pig producers often supervised farrowing to improve piglet survival. In the 1940's the farrowing crate concept began to gain recognition and producers found that mortality could be reduced with the use of crates (Phillips and Fraser, 1993). By the mid-1970s, the farrowing crate had become the predominant form of housing for farrowing/lactating sows.

What are the Biological Needs of the Sow and Piglet?

Baxter *et al.* (2011a) have extensively reviewed the literature describing the biological needs and the maternal behaviour of the sow and have presented the design criteria for non-crated farrowing systems based on the biological needs of the sow and piglets. The maternal behaviour is defined as that behaviour, exhibited by mothers towards their young, that is presumed to aid the young in their survival, growth and development, both physically and behaviourally (Cronin, 2010). The level of restriction placed on sows in farrowing crates has been considered a risk to sow welfare by animal ethologists, welfare scientists and increasingly, by consumers. Sows are motivated to perform innate, species-specific behaviour prior to farrowing, which is thwarted through the restriction of movement imposed by the farrowing crate (0.5–0.6 m wide x 1.8–2.0 m long sow stall) and the lack of bedding material provided. In an unrestricted environment, the pre-partum sow leaves her herd, and may travel up to 6 km while she seeks a protected, isolated site for farrowing. At the site she digs a shallow hole through rooting and pawing actions. Vegetation is gathered and placed in the hole to line the earth base of the nest. Additional material is gathered and added to the nest. In colder conditions, more vegetation is added (Jensen 1986, 1989, 1993). The sow then burrows into the nest for parturition. Nest-building is at least partly under the control of endogenous hormones (Lawrence *et al.*, 1992) in the pig. More recently, Algers and Uvnäs-Moberg (2007) published a review of maternal behaviour in pigs indicating that the onset of nest-building behaviour in sows is triggered by a rise in prolactin concentrations, which is itself related to decreased progesterone and increased prostaglandin concentrations. Some nest-building activities such as carrying and depositing straw seem to be related to changes in somatostatin and progesterone concentrations. During the nesting phase the sow will turn around in circles (which may give some indication of the size of the nest area required in the development of farrowing pens; Baxter *et al.*, 2011a). The nest building ends when oxytocin concentrations begin to rise. The nest building behaviour abates approximately four hours prior to parturition. Once the sow has farrowed she remains with her litter for approximately nine days then she rejoins the herd and the litter remains as an intact social unit. There are individual differences between sows in maternal behaviour which is interpreted as genetic variation (Jensen, 1986).

One outcome of the animal welfare criticisms has been the development of a number of non-crated farrowing systems.

Types of Non-Crated Farrowing Systems

The focus of this review is on the investigation of farrowing and lactation systems that do not constrain the sow prior to, during or after the farrowing process and the lactation period. There are farrowing systems currently being used whereby the sow is housed in a farrowing crate prior to farrowing and up to 7 to 10 days post-farrowing to protect piglets from being overlain. After the 7 to 10 days post-farrowing the sow and litter are mixed onto a group 'multi-suckling' environment (described by Wattanakul *et al.*1997). There are also modified farrowing crates where the sow can turn around with the option of still restraining the sow during farrowing (Weber, 2000). These system may provide an interim step in terms of moving toward confinement-free pork production, however, these systems restrict the sow's ability to perform her maternal behaviour prior to, during and after the farrowing process, therefore are not regarded as truly confinement-free.

A significant amount of research must be conducted in the future if the Australian pork industry is to move towards non-crated farrowing and lactation systems for sows. Non-crated farrowing systems must be "commercially viable", in that systems must be able to achieve similar piglet survivability to farrowing crate systems, and must be able to fit into existing "footprints" of buildings or into low-capital cost facilities. The removal of the crate component of the farrowing pen environment increases the opportunity for sow movement in farrowing pens compared to farrowing crates. The opportunity for sows to move about, however, has signalled a number of potentially important pig and human welfare and production problems that also need to be considered before commercial pig producers replace existing farrowing crates with an alternative farrowing/lactation system. Parameters that are typically considered when comparing conventional farrowing crates with alternatives are the survival, health and growth of piglets, since the number of piglets weaned and the efficiency of growth (feed conversion) are important factors determining farm profitability. Other factors must also be considered, such as sow health, productivity and retention in the herd. By modifying the method of housing the sow, to one in which the sow is not restrained, requires reassessment of the human safety issues, stockperson skills and competencies. By changing the type of floor surface, increasing pen size and altering the structural design of the space and potentially introducing bedding, labour inputs for cleaning and maintenance may increase. These parameters need to be measured in terms of work load and ease of management by stockpeople. Handling of manure and effluent also needs to be considered in the development of non-crated farrowing systems. Finally, pork producers need to know the capital investment required to establish any new housing system, and the cost of operating the housing system (Cronin, 2010a).

A number of reviews have been published which describe the various types of farrowing accommodation developed over the years by the pig industry (Thornton, 1990; Phillips and Fraser, 1993; Edwards and Fraser, 1997; Marchant, 1997; Halverson *et al.*, 1997; McGlone, 1997; Baxter *et al.*, 2011). It is not within the scope of this review to provide detailed descriptions of the different alternatives to farrowing crates. Figure 1 represents the main combinations of farrowing accommodation types for sows: Housing may be either indoors, outdoors or a combination of these such as a straw-yard, in which the sow and litter have access to both indoor and outdoor components of a pen. Sows may be accommodated either singly or in a group. Sows may be "confined" in a farrowing crate or "loose-housed" in a pen or paddock. "Loose housing" was defined by Phillips and Fraser (1993) as an enclosure in which the sow can turn around freely.

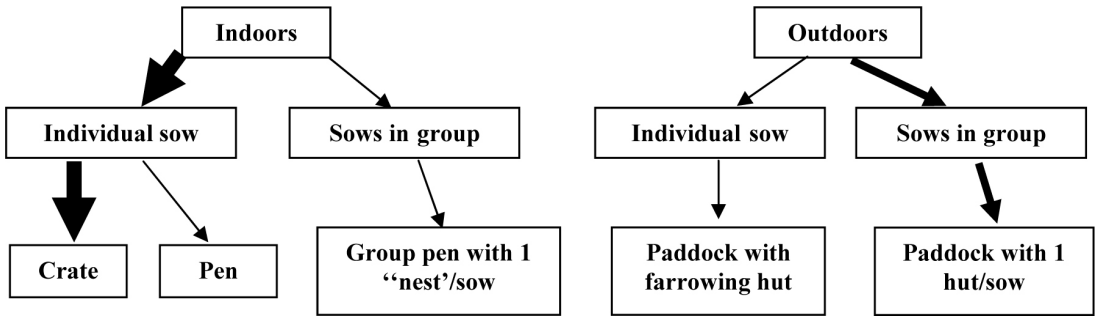

Figure 1. *Farrowing accommodation types for sows and litters. The thickness of arrows suggests the relative occurrence of the different combinations (Cronin, 2010).*

Piglet Mortality in Indoor Farrowing Pen Systems

There are numerous types of farrowing pens that have been developed which occupy a similar footprint to farrowing crates, but do not constrain the sow. These pens normally include pen fixtures that protect the piglet and assist the sow during postural changes and enable provision of straw for nest-building. More detailed farrowing pens include specialised areas for feeding, nesting and dunging (Baxter *et al*., 2011). For example, the Werribee farrowing pen (Cronin, 1997a,b,c), Norwegian farrowing pen (Cronin *et al*. 2010b) and the PigSAFE system (Piglet and Sow Alternative Farrowing Environment; Edwards *et al*., 2010).

The results of experiments reporting piglet mortality in litters of sows housed individually indoors, comparing farrowing crates and farrowing pens, were collated from the literature (Table 1) by Cronin (2010a). Of the 35 comparisons listed in the Table 1, mortality was lower in farrowing crates than farrowing pens for 64% of comparisons. An alternative perspective on the data is that, for 36% of comparisons, piglet survival was better in farrowing pens than crates.

Recently, Edwards *et al*. (2010) reported that piglet mortality in the new PigSAFE farrowing system was 14.9% of born alive (based on 152 litters farrowed). Although comparative data for piglet mortality in farrowing crates was not presented by Edwards *et al*. (2010), the performance of the farrowing pen system is being evaluated against the UK/EU benchmark of 12.8% pre-weaning mortality. Survey data comparing piglet mortality in farrowing crates and farrowing pens have also been reported. Edwards and Fraser (1997) reported four farm surveys published between 1979-1982 and mortality was higher for litters in pens than crates in three of the four surveys; in the fourth survey by Gustaffson (1982; cited by Edwards and Fraser, 1997) involving data from 72,507 litters, piglet mortality of 18.7% was reported for both crate and pen systems. Recently, Weber *et al*. (2007) reported the findings of data mining of the Swiss UFA2000 sow recording scheme, in which records of piglet mortality in farrowing crates were compared to "loose" farrowing pens. Piglet mortality was the same in the two systems, at about 12.8% of live born. Subsequently, Weber (2009) surveyed the records for piglet mortality on Swiss pig farms and reported that piglet mortality was 12.1% for farms with farrowing crates (482 farms) and "loose" farrowing pens (173 farms). The proportions of piglet deaths due to crushing by the sow in the two systems were 37% in crates and 45% in "loose" pens. Weber (2009) identified three important factors influencing piglet mortality that required further investigation: (1) litter size at birth, (2) birth weight and (3) farrowing pen size.

Piglet Mortality in Indoor Group Farrowing Pen Systems

Indoor group farrowing and lactation systems enable sows and litters to mix and include greater floor space allowance compared to farrowing pens. These systems usually include a farrowing "nest box" where the sow can farrow in isolation from other sows and are often bedded with deep-straw. These systems have been developed due to their low capital cost (Västgötmodellen system) and perceived welfare benefits. The "family pen system" for pigs was developed by Stolba and Wood-Gush (1984) after many years of behavioural observations in a semi-natural environment. Other group farrowing systems were also developed around the same time. For example, two Dutch systems were developed which were labelled "integrated" (Buré and Houwers, 1990) and "multi-phase" systems (van Putten and van de Burgwal, 1990), respectively. In both systems the sows remained in groups throughout gestation and farrowing/lactation. Sows wore transponder collars which gave them access to feed from an electronic feeding station, and in the "integrated" system, access to different compartments in the pig shed. Since then, a number of other group farrowing systems have been developed in Sweden which keep the sows in farrowing pens for 7 to 10 days then move into a group lactation system (ie. Ljungstrom). The results of experiments reporting piglet mortality in litters of sows housed individually indoors, comparing farrowing crates and indoor group farrowing pens, were collated from the literature (Table 2) by Cronin (2010a).

Table 1. *A summary of piglet mortality in litters from farrowing crates and farrowing pens. Unless otherwise indicated, percentage values shown are for deaths of live born piglets (from Cronin, 2010a).*

Source	Number of Litters	Mortality Parameter		Notes/comments (Pre-weaning mortality (PWM) with range of values across pen treatments)
		Crates	Pens	
Robertson *et al*. (1966)	150	15.5%	21.3%	Mortality to 3 weeks; outdoors during gestation
Devilat *et al*. (1971)	46	10.2%	13.5%	Mortality to 2 weeks; no bedding in pens
Svendsen & Andréasson (1980)	211	15.0%	12.6%	Mortality to 3 days, includes stillbirths
Svendsen *et al*. (1986)	702	4.4%	6.5%	Mortality due to crushing
Gravås (1982)	76	16.8%	13.3%	Mortality to 6-7 weeks
Gravås (1982)	84	16.1%	15.3%	Mortality to 4 weeks
McGlone & Morrow-Tesch (1990)	20	10.8%	27.1%	Horizontal floors in crates and pens
McGlone & Morrow-Tesch (1990)	20	17.2%	9.1%	Sloped floors in crates and pens
Cronin & Smith (1992a)	64	10.7%	16.8%	Half the sows had straw bedding
Cronin & Smith (1992b)	18	19.8%	8.2%	The pen treatment had straw
Rudd *et al*. (1993)	20	14.0%	37.0%	Summer farrowings
Rudd *et al*. (1993)	20	14.0%	13.0%	Winter farrowings
Blackshaw *et al*. (1994)	16	14.0%	32.0%	Three pigs/litter more in pens than crates
Lou & Hurnik (1994)	64	15.0%	15.4%	The 'pens' were ellipsoid farrowing crates
Cronin *et al*. (1996)	96	9.9%	8.8%	Primiparous sows only
Hesse *et al*. (1996)	310†	16.6%	11.0%	† Piglets born rather than sows or litters
Cronin (1997)	60	8.5%	14.3%	Two pen designs (PWM: 12.5%-16.2%)
Weber (1997)	217	15.7%	14.4%	Two pen designs (PWM: 13.5%-15.2%)
Cronin (1998)	89	13.4%	16.7%	Four pen designs (PWM: 11.2%-24.4%)
Bradshaw and Broom (1999)	18	0.5‡	2.0‡	‡ Data reported as median deaths/litter
Cronin *et al*. (2000)	146	17.5%	15.3%	Multiparous sows
Marchant *et al*. (2000)	28	15.2%	24.8%	
Jones *et al*. (2003)	830	13.5%	20.1%	
Jarvis *et al* (2005)	122	5.6%	12.2%	Deaths due to crushing by sow
Moustsen (2006)	453	11.7%	11.4%	
Moustsen (2006)	339	11.3%	11.6%	
Cronin (2007)	85	16.5%	19.7%	Multiparous sows
Cronin (2007)	66	16.6%	15.7%	Primiparous sows, select for non-crushing
Verhouvsek *et al* (2007)	22	10.1€	9.1€	€: number of piglets weaned/litter
Loudon (2008)	312	6.2%	10.2%	All seasons
Loudon (2008)	234	5.9%	7.9%	Summer data omitted
Pedersen & Jensen (2008)	17	6.0%	19.0%	Primiparous sows; placed in treatments on d 114
Pedersen & Jensen (2008)	20	14.0%	18.0%	Multiparous sows; placed in treatments on d 114
Kutzer *et al*. (2009)	113	1.49❖	1.29❖	❖ Deaths/litter to day 10 of lactation
Edwards *et al*. (2010)	152	12.8%	14.9%	PIGSafe system

† Total mortality reported, therefore includes stillbirths

Table 2. *A summary of piglet mortality in litters from indoor, group farrowing systems. Unless otherwise indicated, percentage values shown are for deaths of live born piglets (from Cronin, 2010a).*

Source	No. of Litters or Herds	Indoor Group; Piglet Mortality	Comparison(s)	Notes/Comments Group system name
Arey and Sancha (1996)	48	28.5%	25.2% in crate	Edinburgh family
Baxter (1991)	40	12%		Freedom farrowing
Nash (1993)	34	25%		Freedom farrowing
Bøe (1994)	15†	16.3%	Norway herd average: 14.4%	Commercial farms using integrated systems
Kavanagh (1995)	>500	19.2%		Free-access farrowing nest system
Halverson *et al.* (1997)	49	14.5%		Västgötmodellen
Jungclaus and Jungclaus (1997)	1†	31.4%	Crates in local region:11.2%	Västgötmodellen
Honeyman and Kent (1997)	28	18.4-24.2%		Multiple commercial farms; Västgötmodellen
Marchant *et al.* (2000)		25%	Crates: 13%	Ljungstrom
Dybkjaer *et al.* (2001)	60	14.1%	Crates: 9.4%	to day 11 of lactation
Dybkjaer *et al.* (2003)	72	10.9%		to day 11 of lactation
Kutzer *et al.* (2009)	230	1.58 deaths	Individual pen: 1.29 Crate: 1.49 deaths	Deaths/litter to day 10
Li *et al.* (2010)	421	22.6%		Range 18.6-30.0%
Payne & Cronin (2010)	1†	28.4%		Västgötmodellen in an Ecoshelter

† Total mortality reported, therefore includes stillbirths

Piglet Mortality in Outdoor Farrowing Systems

There has been a resurgence of interest in the commercial farming of sows in outdoor or free-range systems, due to their low capital cost and niche marketing opportunities. Table 3 presents piglet mortality data from reports investigating piglet mortality in outdoor systems. The majority of systems involve managing sows in groups with one farrowing hut provided per sow in each farrowing paddock. There have been a few investigations of outdoor systems in which the sows are kept singly in paddocks.

Future Research and Directions for Non-Crated Farrowing Systems-Gaps in Knowledge

Whilst outdoor or free-range farrowing systems have a place in the Australian pork industry, the major focus for future research will be the development of commercially-viable farrowing systems that can be retrofitted into the "footprint" of existing conventional accommodation that holds farrowing crates, or into low capital cost structures. It is speculated that many of the outcomes of research will be applicable to outdoor housing systems. New indoor, individual farrowing pen systems for sows and litters require extra floor space and construction complexity compared to farrowing crates. Thus, greater capital investment will be required, so it is important that the number weaned is increased or that higher weaning weight, re-breeding performance or longevity are achieved to ensure that returns on investment are maintained. Further research is clearly needed on low-cost, farrowing pen systems, both for systems involving individual pens as well as group pens. "Multi-step" farrowing systems require investigation whereby sows are housed in a "high capital cost" non-crated farrowing system for approximately the first seven to 10 days post-parturition, then the sow and her litter are moved into a lower capital cost group housing "multi-lactation" system.

The literature indicates that comparable levels of piglet survival, one of the main economic and welfare parameters for evaluating alternatives to farrowing crates, have been achieved in some non-crate farrowing systems (Cronin, 2010a; Baxter *et al.*, 2011b). The lack of consistency in reporting "good" findings suggests we lack understanding of the factor(s) within the "successful" farrowing systems that contributed to their success. A key difference between farrowing sows in a confined, crate environment compared to a "loose" pen with bedding, is that the combination of space and bedding stimulate the sow's natural pre-farrowing behaviour. If we provide the sow with a stimulating (enriched) environment, then it is essential to provide the sow with an appropriate space in which to farrow, that also contains design features to promote piglet survival. Furthermore, the sow and litter need to be managed correctly to identify problems and to rectify them as soon as possible (Cronin, 2010a).

Table 3. *A summary of piglet mortality in litters from outdoor farrowing systems. Unless otherwise indicated, percentage values shown are for deaths of live born piglets (from Cronin, 2010a).*

Source	No. of Litters or Herds	Outdoor Group	Outdoor Single	Indoor	Notes / comments
Andersen (1993)	321		12.2%	9.7%	
Edwards *et al.* (1994)	105	20.0%‡			17.9% of deaths were stillbirths
Berger *et al.* (1995)	64†	20.4%			
Edwards *et al.* (2010)	#	10.5%		12%	MLC 2000-2007 data
Edwards & Zanella (1996)	293†	17.8%		19.1% ‡	MLC 1995 data
Berger (1996)	102,814	16.8%		12.2%	1995 data
Edwards & Zanella (1996)	412†	18.6%		17.7% ‡	Easicare 1995 data
Higgins & Edwards (1996)	47	23.1%	14.9%		
Berger *et al.* (1997)	747,548	21.1% ‡		17.4% ‡	1990-1994 data
Herskin *et al.* (1998)	36	10.3%			
Petrocelli & Burgueno (1998)		17.3%			Individual producers
Petrocelli & Burgueno (1998)	1174	22.2%			Co-operative farm
Kongsted & Larsen (1999)	54†	18.3% ‡		18.7% ‡	1998 data
McGlone & Hicks (2000)	96	19.7%			American hut design
McGlone & Hicks (2000)	29	11.2%			English hut design
Honeyman & Roush (2002)	206	6.0%			USA; primiparous sows only, farrowed in Sept over 4 years
Wülburs-Mindermann *et al.* (2002)	99	1.5		1.3	Reported as piglet deaths/litter
Johnson & McGlone (2003)	206	19.9%			Experiment 1
Johnson & McGlone (2003)	331	24.3%			Experiment 2
Echevarria *et al.* (2005)	#	14.8%		20.4%	Indoor=open front pens
Johnson *et al.* (2008)	128	31.8%			
Wallenbeck & Rydhmer (2008)	40	30.1%		23.3%	Parity 1 (outdoor) v 2 (indoor) €
Wallenbeck & Rydhmer (2008)	40	31.2%		20.5%	Parity 3 (outdoor) v 4 (indoor) €

† Total mortality reported, therefore includes stillbirths. €: organic production, 7 week weaning. #: not stated

The Design and Management of Farrowing Pen Systems for Commercial Use in Australian Climates

Cronin (1997c) and Baxter *et al.* (2011a) have listed the design criteria and recommendations that are essential for increasing piglet survivability in non-crated farrowing systems. Many of the farrowing pen systems reported in the literature have been designed for use in cool climates (ie. the northern hemisphere). The systems rely, at least in part, on controlling the behaviour and resting location of the sow and piglets through manipulating differentials in temperature in different parts of the pen. A very important knowledge gap for Australian conditions is development of farrowing pen systems for the Australian environment.

Pen size

Farrowing crates are criticized for prohibiting the ability of sows to turn around (eg. to perform nest-building behaviour), and a small number of experiments have been published investigating turning around by sows in modified farrowing crates. Heckt *et al.* (1988) compared the prepartum behaviour of gilts in a conventional farrowing crate, a turn-around crate and an open pen. As expected, the gilts were unable to turn around in the farrowing crate, but turned through 90 degrees twice as often in the turn-around crate compared to the open pen. The open pen treatment measured 2.1 m x 1.5 m. One innovative farrowing crate designed to enable the sow to turn around was the 'Ottawa' farrowing crate (Fraser *et al.*, 1988). The crate width was 0.75 m at the bottom (between opposing inward-positioned prongs) and 1.15 m at the top, enabling the sow to "turn around with little apparent difficulty". No difference in stillbirth rate was found between the Ottawa crate and conventional crates (Fraser *et al.*, 1997). The results reinforce the point that there is likely to be a minimum space (and dimensions) required for sows in the pre-farrowing period for the increased pre-parturient activity to benefit piglet survival, including reduction in stillbirths. Cronin *et al.* (1991) found that towards the end of the fourth week of lactation, primiparous sows in both farrowing crates and pens showed

evidence of elevated stress response, probably from not being able to avoid their piglets. Devilliers and Farmer (2008) compared sow behaviour on d 4 and 18 of lactation in conventional farrowing crates, and the same design crates that could be opened at the rear to allow the sow access to an additional 2.4 m^2. Sows in this treatment were given access to the additional space from d 4 of lactation. Sows utilized the extra space by spending more than 85% of the time there, including 70% of suckling bouts.

While limiting space could interfere with pre-farrowing activity of sows, providing too much space by increasing farrowing pen size could adversely affect newborn pig survival. Cronin and Smith (1992a) and Cronin *et al*. (1994) concluded that piglet mortality was higher in open pens than crates due to the extra space available to litters in the pens. Factors associated with the extra space that may contribute to piglet mortality include lower ambient temperature and draughts which increase the risk of chilling and thus starvation and overlying (eg. English and Morrison, 1984).

Changing the dimensions of the nest area in the Werribee farrowing pen was investigated by Cronin *et al*. (1998a,b). The experiment had compared the main effects of nest area orientation (wide versus narrow) and space (large versus small). The small nest area treatment adversely affected pre-farrowing (nesting) behaviour, resulting in more posture changes by sows and poorer suckling behaviour, compared to the other treatments. Piglet mortality was 7.3% in full-sized nests and 13.3% in reduced-size nests. Another experiment by Cronin (1997a,b) found that piglet mortality was higher in farrowing pens than crates. Most of the sows included in this experiment were multiparous, and it was thought that the farrowing nest dimensions (1.8 m) were too narrow for multiparous sows. The literature suggests there is a minimum size for farrowing pens (and huts in outdoor systems). If farrowing pens are too small or too large, piglet survival seems to be adversely affected. Furthermore in group housed farrowing and lactation systems the pen space recommended varies in the literature (Halverson *et al*., 1997). Thus, the optimum floor area and pen dimensions for farrowing pen and group farrowing lactation systems need to be identified under Australian environments.

Causes of Piglet Mortality in Non-Crated Farrowing Systems

High levels of piglet mortality has been an historical problem for pig producers (eg. Harris, 1906). A major reason why farrowing crates were developed was as an attempt to reduce piglet deaths before weaning (Thomson *et al*., 1960). Based on the records from a large number of UK pig herds, Thomson *et al*. (1960) reported that piglet mortality was 27% (range 22-34%) and that most deaths occurred soon after parturition. Many studies of the causes and timing of piglet losses have since been reported (eg. Veterinary Investigation Service, 1960; Sharpe, 1966; Fahmy and Bernard, 1971; Glastonbury, 1976, 1977; Spicer *et al*., 1986; Svendsen *et al*., 1986; Edwards *et al*., 1994) and there have been many reviews of the literature (eg. Edwards and Fraser, 1977; English and Morrison, 1984; Cronin *et al*., 1989; Edwards, 2002; Cutler *et al*., 2006). In summary, the majority of piglets die within the first three days of life (including stillbirths) and the main causes of neonatal loss are intra-partum stillbirth/weak/non-viable, crushing by the sow and chilling/starvation. According to Weber (2007), piglets that are crushed are on average lighter weight at birth than piglets that survive to weaning (crushed: 1.17 kg vs survived: 1.42 kg), therefore underweight piglets may be less viable. Baxter *et al*. (2009) identified behavioural and physiological survival indicators that are influential in outdoor systems that could provide additional information for use when selecting for piglet survival. The most important survival indices with respect to prenatal mortality (surviving versus stillborn) were high ponderal index or body mass index in conjunction with being born earlier in the farrowing birth order. The birth weight and rectal temperature 1 h after birth were the most significant postnatal survival indicators.

Thus the major focus of research has been on perinatal mortality in the pig. A detailed review by Edwards (2002) examined perinatal mortality under commercial conditions and identified solutions which have been, or might be, implemented to improve piglet survival. Edwards (2002), and earlier researchers in this area such as English and Morrison (1984), emphasized the importance of piglet vitality in the immediate post-partum period as a key factor to address for reducing piglet losses. A number of other reviews and papers have also been published on this topic in the past decade (Grandison *et al*., 2002; Herpin *et al*., 2002; Quiniou *et al*., 2002; Knol *et al*., 2002; Mesa *et al*., 2006; Baxter *et al*., 2009). Recent information in the literature suggests that lower weight piglets are more likely to be overlain in non-crated farrowing systems. Further research is required to elucidate the interactions between foetal development, viability, behaviour and survival in non-crated farrowing systems. Furthermore, a major risk to piglets in the neonatal period is their tendency to remain at the sow's udder, thus risking crushing and chilling. This is despite providing a "safe", heated creep zone for the piglets. However, the biology of the pig is such that the neonates are strongly attracted to the udder. There is a gap in knowledge in non-crate systems on how to encourage neonates to move away from the udder to a "safer" location within the pen to prevent piglets from being overlain by the sow.

Provision of Straw Bedding and Nesting Material

The importance of bedding material for parturient sows has long been recognized (eg. Harris, 1906). However, with the move to intensive farrowing crates with perforated floors, the use of straw has reduced to avoid blocking of under floor drainage systems. Recently, there has been increased interest in the importance of straw bedding for sows around parturition. In the older literature, there are some reports indicating the potential benefits of straw for piglet survival (Metz and Oosterlee, 1980). The influence of straw on sow behaviour and piglet survival around parturition has been investigated in a number of studies. Vellenga *et al*. (1983) compared the effects of straw versus no straw in the farrowing crate on mortality, morbidity and injuries of piglets in 375 litters. The provision of straw reduced stillbirths (5.90% versus 8.05%), preweaning deaths (11.97% versus 15.44%), morbidity (33% versus 44% of piglets) and injuries amongst piglets compared to litters born in crates without straw bedding. In a small experiment by Edwards and Furniss (1988), farrowing behaviour of sows and piglet survival in crates with and without chopped straw were compared. The addition of straw resulted in fewer major postural changes by sows early in parturition (eg. first four piglets born), a lower proportion of stillbirths (5.8% versus 7.9%) but no difference in preweaning deaths (12.3% versus 12.1%). Cronin and van Amerongen (1991) performed a similar experiment and reported reduced piglet mortality in the straw-added treatment in farrowing crates. The provision of straw for sows was also found to positively modify suckling behaviour, a finding that was subsequently reported in other experiments (Cronin and Smith, 1992a,b; Cronin *et al*., 1994), and Schouten (1991) indicated that suckling behaviour did not establish as easily in the crate- than straw pen-reared gilts.

The relationship between pre-partum nest-building behaviour and the duration of parturition is not well researched. Limited information suggests an inverse relationship, that is, as the amount of nest-building behaviour performed by the sow increases (eg. through stimulation with nesting material), the farrowing process proceeds faster and with fewer complications. The potential benefits of faster farrowing time include reduced intra-partum stillbirths and possibly a lower incidence of unviable live born piglets. A gap in knowledge therefore concerns enhancing the purported positive relationship between farrowing behaviour and piglet viability. Furthermore, the relationship between pre-partum nest-building behaviour and the level of care taken by sows when changing posture in the days post-partum is not well researched. Limited information suggests that sows which perform more nest-building behaviour are less likely to crush piglets (Anderson *et al*., 2005; Pedersen *et al*., 2006). Furthermore, sows that continue the nest-building behaviour during farrowing are at risk of crushing piglets and have a longer duration of farrowing and higher risk of stillborn piglets (Baxter *et al*., 2008). Further research is therefore required to investigate the purported relationship between pre-farrowing nest-building behaviour and reduced incidence of piglet crushing.

Straw seems to provide both behavioural and nutritional benefits for the sow. These effects also appear to benefit piglets indirectly, through shorter parturition time, reduced incidence of crushing by the sow and better suckling behaviour. An important issue for Australia associated with use of straw bedding is the risk of contributing to heat stress on sows in summer. A gap in knowledge therefore is the optimum quantity of straw provided for nesting and bedding under Australian conditions in non-crated farrowing systems.

Genetic Selection for Survival in Non-Crate Systems?

The genetic selection for piglet survival at birth and weaning shows promise in non-crate systems. Grandinson *et al*. (2002) analysed the farrowing records of 1,046 primiparous sows that had farrowed in farrowing pens. Heritabilities for crushing, stillbirth and total mortality were estimated and found to be low (0.01-0.15). Nevertheless, there were some interesting associations reported: The relationship between crushing and birth weight was negative for both direct and maternal effects, indicating that sows with low-weight piglets were more likely to crush piglets. Recently, Roehe *et al*. (2010) estimated heritabilities for outdoor herds in the UK and reported the direct and maternal heritability of piglet survival at birth to be 0.21 and 0.15, and piglet survival during the nursing period to be 0.24 and 0.14, respectively. Further research is required to establish whether genetic selection can be utilised to improve piglet survivability in non-crated farrowing systems under Australian conditions.

The Selection and Training of Stockpeople

The stockperson's level of understanding of how sows and piglets behave in the particular non-crate system, and the stockperson's ability to recognize and correct problems, is essential for the success of a system. Thus, while specialist training and support information for stockpeople working with farrowing sows will be initially required, a program to identify and select stockpeople with appropriate qualities may be needed.

Conclusion

In conclusion, confinement free sow housing is on the horizon for Australian pork producers. In the near future, gestating sows will be housed in groups for the majority of their gestation. Current Australian welfare research has shown that floor space affects aggression, stress physiology and reproduction in group housed gestating sows. A floor space allowance of 1.4 m^2/sow is likely to be too small for group housed gestating sows. Further research is required to examine the effects of floor space allowance in the range of 1.8 to 2.4m^2/sow in more detail, and the effects of pen features such as feeding stalls and feeding systems, since these are likely to affect aggression and stress. The time of mixing into group pens with shoulder feeding stalls either immediately post-mating or approximately 35 days post mating did not impact reproductive performance. However, stress and injuries were higher in sows recently mixed in groups with shoulder feeding stalls compared to sows housed in gestation stalls. From a welfare perspective, attention should be given to the design aspects of these group pens, such as the design of the stalls in the group pens and the pen space available in these group pens, in order to reduce stress and injuries in sows mixed in groups shortly after mating. Further research is required to determine the most suitable time for mixing into groups pre- or post insemination and strategies to reduce aggression at mixing as the Australian pork industry eliminates gestation stalls.

Future research should investigate non-crated farrowing systems that meet the biological needs of the sow and piglets and are commercially-viable. Gaps in current knowledge include design of non-crated farrowing systems for use in the southern hemisphere, the optimum amount of straw bedding required for sow maternal behaviour, relationships between sow maternal behaviour and piglet survival post-birth, strategies to improve piglet survival, genetic selection and training of stockpeople in non-crated farrowing systems.

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The Influence of Space and Temperature on Sow Farrowing Location and Piglet Survival in an Indoor Free-Farrowing System

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Designing suitable farrowing and lactation environments that maximise both sow and piglet welfare, whilst maintaining economically efficient and sustainable enterprises, is a continuing challenge. When developing alternative systems, an important design consideration is how to encourage sows to farrow in a location suitable and safe for piglets. A prototype, free-farrowing pen (PigSAFE – Piglet and Sow Alternative Farrowing Environment) was developed, which considered these design challenges and included different areas for different functions (ie. nesting area separate to dunging area). We hypothesised that more space would result in better maternal behaviour and therefore improved piglet survival and that a warmer nest might be more attractive to farrowing sows and improve piglet survival (Pedersen *et al.*, 2007). In this experiment, two sizes of pen and two under-floor temperatures (individually controlled in the solid nest areas) were tested with the aim to determine the influence of space and floor heating on farrowing location and piglet survival.

The experiment utilised one pen type measuring 9.7m^2 in total (Large), with a nest area of 4.0m^2, and another that was the same design but smaller, measuring 7.9m^2 in total (Small) with a nest area of 3.3m^2. The floor was heated to either; High at 30°C or Low at 20°C (from 48h before until 24h after farrowing). A 2x2 factorial design saw 89 Large White x Landrace sows and gilts (hereafter sows) randomly assigned to space and temperature treatments. Final numbers in each treatment were unbalanced (Small=47, Large=42, High=45, Low=44) warranting data being fitted to linear mixed models (REML) for statistical analysis. Production information (ie. piglet mortality) was collected from all litters and preliminary behavioural data (via video observations), particularly on farrowing location, have so far been analysed for a subset of animals (n=56). For farrowing location analysis, the pen was divided into seven areas (L1-L7), with L1 deemed the safest area for the piglets to be born (in the nest, furthest from dunging area and closest to creep) and L7 the least protected (being in the dunging area).

Table 1. *Percentage of piglets per litter being born in each location and mortality in Large (9.7m^2) or Small (7.9m^2) PigSAFE farrowing pens with High (30°C) or Low (20°C) floor heating temperatures.*

	Space				Temperature			
Location	Small	Large	F-statistic	P-value	Low	High	F-statistic	P-value
L1 (nest)	47	52	0.03	0.853	55	44	0.64	0.428
L2	14	15	0.07	0.791	5	25	13.51	0.001
L3	10	21	0.79	0.379	15	15	0.00	0.985
L4	4	6	0.23	0.992	4	5	0.11	0.741
L5	17	4	3.91	0.053	12	11	0.00	0.958
L6	3	2	0.00	0.994	4	0	2.06	0.157
L7 (dunging area)	5	0	4.65	0.036	5	0	5.44	0.023
Live-born mortality	14	21	4.29	0.041	18	17	0.14	0.710
Total mortality	20	27	3.57	0.062	25	22	0.33	0.565

The majority of sows started farrowing in L1 (66%), with 50% of the remaining piglets being born in this position (Table 1). Temperature had no effect on piglet mortality but space influenced live-born mortality, with significantly more piglets dying when sows were afforded larger farrowing space. There were no significant interactions between space and temperature treatments. The overall design was successful in promoting farrowing in the desired location, irrespective of nest size and floor temperature. The higher piglet mortality, supported by behavioural data on piglet crushing events, suggest that the larger nest size was less protective for the piglets and thus a smaller nest would be recommended.

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PigFare: An Economic Model for Comparing Sow Housing Systems

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There is discussion in the Australian pig industry regarding alternative sow housing systems. To assist decision makers evaluate options, PigFare, a cost-benefit, spreadsheet-based simulation model, was designed to provide an indication of the possible net benefits of different sow housing systems. The purpose of this paper is to reveal PigFare and present results that provide an indication of how the model can be used to generate outcomes and how parameter values can be altered to indicate the sensitivity of the results to such changes.

The model can accommodate up to nine housing alternatives for sows in each of three stages: farrowing, mating or gestation. Where possible, variable and fixed costs and benefits associated with normal activities as well as welfare and reproductive efficiency are expressed as dollar values. To generate an example of the model output, biological data, industry-based figures or a proxy, if data was not available, was used to populate the model. Being a simulation model, there are many scenarios that could be generated to answer specific questions. Hence it is important to interpret the results presented in this paper as being relevant to just two scenarios.

The two examples of alternative sow-housing systems were: System 1, sows in stalls for each state of the piglet production cycle; System 2, sows in a mating stall, followed by a purpose built pen for 10 sows during gestation and then a Werribee farrowing pen (Cronin *et al*., 2000). Most of the production data were taken from Jones *et al*. (2003) with data for the Werribee farrowing pen from Cronin *et al*. (2000). As facility is not provided in this model for piglets after weaning, it was assumed that they were then sold. It was also assumed that the number of piglets weaned/sow/year was 24 for both Systems and for the purpose of this analysis, the time period was assumed to be 20 years and the discount rate, 6%.

Table 1. *Economic criteria values generated by PigFare that allow comparison of two alternative sow housing systems, and the implications when price and weaning weight benefits are applied to System 2.*

Criteria	System 1[a]	System 2 [b]	System 2 + 10% price premium	System 2 + 10% gain in weaning weight
Piglet weaning weight (kg)	6.36	5.62	5.62	6.21
Cost/weaned piglet/yr ($/kg)	5.77	6.68	6.68	6.04
Internal rate of return (%)	12.28	4.82	12.28	12.34
Benefit:cost ratio	1.09	0.99	1.09	1.09

[a]System 1, sows in stalls or crates for each phase of the piglet production cycle; [b]System 2, sows in a mating stall, followed by a purpose built pen for 10 sows during gestation and then a Werribee farrowing pen.

Results contingent upon the input data suggested that System 1 was an economically viable option because the internal rate of return (IRR) was greater than the average medium-term bank interest rate and the benefit cost ratio (BCR) was greater than 1 (Table 1). The variable cost/weaned piglet/year for System 2 was around $1/kg more than that for System 1 and the IRR and BCR both indicated that System 2 was not economically viable. The economic viability of System 2 equated to that of System 1 if, for example, there was a price premium of 10% on pigs produced in the "welfare friendly" system or a 10% gain in weaning weight (from 5.6 to 6.2kg; Table 1). These results provide a snapshot of the potential output from the model: weaning weights were comparable to those of Jones *et al*. (2003); costs were in line with Dhuyvetter *et al*. (2011). However, they should be used with caution as a change in the assumptions (eg. daily piglet live weight gain) could alter the outcome. Running PigFare with specific data to answer specific questions will generate output that could be used to help decision makers in the Australian Pig Industry adjust from confined to loose housing systems for sows.

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The Behaviour of Piglets and Lactating Sows During Overnight Separation

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Previous research has demonstrated that oestrus can be induced during lactation with an injection of gonadotrophins, boar exposure and piglet separation overnight (Downing *et al.* 2009). Using separation in this manner could be a welfare issue for the piglets and sows. The objective of the present experiment was to investigate the behavioural patterns exhibited by sows and piglets during overnight separation. The hypothesis being that piglets separated from the sow overnight will display behaviours indicative of being distressed.

Eight Large White x Landrace multiparous sows and litters (6-10 piglets, 2-3 weeks of age) were housed in individual farrowing crates. Video cameras (Signet model QV3063, Electus Pty Ltd., Rydalmere, NSW) were set up for each crate. Piglets were separated from their dam at 1600 h and rejoined at 800 h for five consecutive days. Two methods of separation were used, 1) within pen separation (WP), which was achieved by placing a wooden partition into the farrowing crate beside the sow, and 2) communal separation, where piglets were confined to a communal area in the laneway behind the farrowing crates. Piglet behaviours measured were belly-nosing (repeated massaging movements with the snout on another piglets belly), body manipulation (non-aggressive chewing, sniffing, biting and sucking behaviour directed towards other piglets), lying passively (lying ventrally or laterally without any other behaviour), general activity, nudging the sow or other piglets and escape attempts. Sow behaviours determined were lying on her side, standing, lying on the udder, sitting and nosing (repeatedly pushing against an object with the bridge of the snout). Each day, observational data were recorded hourly between 1800-0000 h, 0300-0800 h and 0900-1500 h; every 20 minutes between 0000-0300 h, 0830-0900 h and 1500-1600 h; and every 5 minutes between 0800-0830 h and 1600-1630 h. Data were analysed using a generalised linear mixed model and included any interactions between sow and piglet activities. Significance in behavioural changes across the five days was determined using Wald tests.

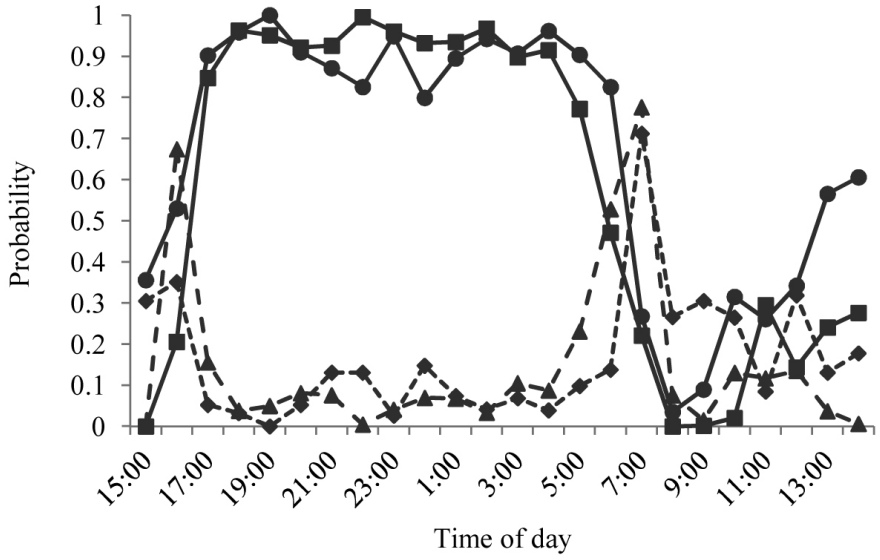

Figure 1. *The probability of piglets displaying general activity or lying passively during separation: Communal general activity (▲), Communal passively lying (■), WP general activity (◆), WP passively lying (●).*

For both separation methods, only three of the six piglet behaviours were observed, lying passive, nudging other piglets and general activity. During the period of separation (1600 h to 0800 h), there was a high probability (range 0.82 to 1.00) of piglets spending their time lying passively and low probability of general activity. There was no relationship between the sow activity and piglets lying passively during the period of separation (P = 0.358). If lying passively is not indicative of distress, our hypothesis is rejected.

DOWNING, J.A., BROEK, D., SMITS, R.J. and GILES, L.R. (2009). In "Manipulating Pig Production XII", p. 144, ed R.J. van Barneveld (Australasian Pig Science Association: Werribee).

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Surface and Core Body Temperature Measurements for the Assessment of Welfare in Weaner Pigs

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Sick and injured pigs lose weight as a result of decreased feed intake which leads to a reduction in body condition score (BCS). Objective measures of BCS would assist producers to decide when medical intervention or humane euthanasia is required in animals that have lost weight as a result of chronic illness or injury. The objectives of this experiment were to determine if core body temperature (measured rectally) varied with BCS in weaner pigs, and to assess the correlation between core body temperature and body surface temperature. The null hypotheses were that core body temperature does not vary with BCS and that there is no relationship between core and surface body temperatures.

Eighty-six weaner pigs (5-9 weeks of age) housed in groups on a commercial piggery in New South Wales were selected in a cross-sectional, epidemiological experiment and classed into three BCS categories (Anon, 2008; BCS=1; 30 pigs; BCS=2; 26 pigs; BCS=3+; 30 pigs). An equal number of pigs from each category were represented in each age group, gender, room and pen. The skin temperature of each pig was taken immediately after it was selected using an infrared thermometer (Wahl® Heat Spy®, Wahl Instruments Inc., Asheville, USA) aimed between the shoulder blades at a distance of 30 cm from the skin surface. The core body temperature was then measured rectally using a digital thermometer. The rectal temperature data were plotted against surface temperature data and the correlation tested for differences from zero using R for Windows (Gentleman and Ihaka, 1997).

Table 1. *Number (%)* of pigs in each rectal temperature category, by body condition score.*

	Body Condition Score			
Temperature	1	2	3	Total
Low (<37.5)	17 (57)	0 (0)	0 (0)	17
Normal (37.5– 38.5)	5 (16)	4 (15)	2 (7)	11
High (>38.5)	8 (27)	22 (85)	28 (93)	58
Total	30	26	30	86
Mean	37.59	39.35	40.70	

* Percentage of pigs with rectal temperatures within the stated range from each category.

There were significant differences in the rectal temperatures of pigs in the three BCS categories (low (<37.5°C) versus within/above reference range (37.5 – 38.5; P<0.01), and within versus outside of the reference range (P<0.05)). Sixty-eight percent of the rectal temperatures outside the reference range among BCS1 pigs were below the reference range. Half of the BCS1 pigs with rectal temperatures above the reference range had temperatures above 40°C. Mean rectal temperatures increased with increasing body condition (Table 1). The mean rectal temperatures of pigs with BCS2 and BCS3 were above the reference range, possibly as a result of handling stress. Only seven different skin temperature measurements were displayed by the infrared thermometer, suggesting some inaccuracy with the instrument. Skin temperature had a weak correlation with rectal temperature (R=0.34, P<0.01), and there were large variations in rectal temperature for each skin temperature reading.

The results of this experiment indicate that core temperatures below the reference range are a good indicator of extremely low BCS, which could reflect chronically-compromised welfare. High rectal temperatures over 40°C may be valuable to identify weaner pigs with compromised health, assuming handling stress is minimal. Under the conditions of this experiment, there was a weak but significant correlation between body surface temperature and core body temperature. However, these results should be viewed with caution given the low number of readings displayed by the instrument.

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Strategies for the Early Detection of Sick and Injured Group-Housed Gestating Sows

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Strategies for the early detection of sick and injured sows must have high detection sensitivity so that appropriate treatments or interventions can be made. Ideally, they should be cost-effective in terms of labour requirements. The aim of the experiment was to compare the frequencies of physical abnormalities detected in group-housed sows using a variety of inspection methods. The null hypothesis was that inspection method had no effect on the proportion of physical abnormalities detected in sows housed in groups on a commercial farm.

Approximately 315 sows (6 to 12 weeks gestation) housed in groups of 12 sows per pen on a 1000-sow farm were inspected over four days in a cross-sectional, epidemiological study design. The sows were inspected pen-side from the aisle, sow-side from within the pen and during feeding/non-feeding times. The route of inspection and the person conducting inspections were the same each day. The number and description of physical abnormalities detected using each inspection method were recorded and classified as leg lesions, claw lesions, low body condition score (<3), lameness, fight wounds, pressure sores and vulval discharge or damage. The proportions of abnormalities detected were analysed using a generalized linear model, with inspection site and feeding time included in the model.

Table 1. *Proportion of abnormalities detected and time taken to inspect group-housed gestating sows using four different inspection methods on a 1000-sow commercial herd.*

Walk	Feeding	Abnormalities	Time taken
Aisle	Yes	24/328 (7%)	40 min (12.2 min/100 sows)
Aisle	No	7/328 (2%)	50 min (15.2 min/100 sows)
Pen	No	39/307 (13%)	45 min (14.7 min/100 sows)
Pen	Yes+standing	66/305 (22%)	85 min (28 min/100 sows)

The highest proportion of physical abnormalities was detected by inspecting sows from within the pen at feeding time whilst encouraging them to stand (Table 1). Inspecting sows at feeding time significantly increased the proportion of abnormalities detected compared to non-feeding time inspections ($P<0.001$). Similarly, inspecting pigs from within the pen whilst standing them up increased detection sensitivity relative to aisle inspections ($P<0.001$). Lameness was the most common abnormality detected, accounting for 39% of total abnormalities. Skin lesions were present on 23% of sows, mostly as a result of fighting. Inspecting sows from within the pen at feeding time and encouraging them to stand took approximately twice as long as the other inspection methods (Table 1).

These results suggest that group-housed sows should be inspected sow-side, at feeding time with an emphasis on encouraging them to stand. This is particularly important as lameness was the most common physical abnormality detected. Our findings are in agreement with Schemann *et al*., (2010) who reported increased detection sensitivity in group-housed pigs at sale yards and abattoirs from pig-side inspection compared to pen-within inspection. Stockpersons must be adequately trained to detect abnormalities and be provided with sufficient time to examine the sows under their care.

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Changes in Aggression in Groups of Sows Within and Between Days Two and Eight Post-Mating

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Due to increasing public concern for the welfare of gestating sows, confinement stall housing is being phased out internationally. While group-housing allows for increased movement and social interactions, persistently high levels of aggression increases the occurrence of injury and stress compromising sow welfare and productivity. Competitive feeding environments promote the use of aggression by sows as they attempt to gain priority access to the feeding area. In avoiding aggressive encounters, subordinate sows may sacrifice the opportunity to feed. Increasing feeding frequency can reduce the number of injuries sustained by sows in groups (Schneider *et al.*, 2007). A better understanding of social and feeding behaviour may assist in reducing aggression in group-housed sows. As part of a larger experiment examining the effects of floor space allowance and group size in sows, this study determined the changes in the frequency of aggression between sows housed in groups over four feed drops on the day after mixing (day 2, D2), and between the first feed drop of D2 and day eight (D8) after mixing.

Two replicates of 780 crossbred sows (Landrace x Large White) of mixed parity (1-6) were randomly grouped within seven days of insemination into indoor concrete-floored pens of size 10, 30 or 80 with varying floor space (1.4, 1.8, 2.0, 2.2 and 2.4 m^2/sow). Feed was delivered via an over-head hopper onto the pen floor four times per day (0700, 0800, 0900, 1000 h). From digital video records, continuous observations were conducted to measure the number of bouts of aggressive behaviour (bites, pushes, knocks, lunges) in the 30 minutes after each feed drop on D2, and the first feed drop on D8. The number of animals in the field of view (FOV) of the camera was recorded using instantaneous time sampling with 30 second intervals for 30 minutes after each corresponding feed drop. The animal was said to be in the FOV if the snout and two ears could be seen. The number of aggressive bouts/pig in FOV was then calculated for each pen (with the pen being the experimental unit). To reduce skewness of the residuals, data were square root transformed and repeated measure analysis of variance, with group size and space in the model, was used to test for differences in aggression between the four feed drops on D2 and differences in aggression for the first feed drop between D2 and D8. A paired sample t-test was used to analyse the differences in aggression between feed drops on D2.

Aggression differed between feed drops on D2 (transformed means (+/- standard error; SE) for feed drops 1, 2, 3 and 4, respectively were 2.9 (0.10), 2.8 (0.12), 2.7 (0.11), 2.5 (0.11) aggressive interactions per sow, $F_{3,66}$=5.443, P=0.002), with reduced aggression at drop 4 compared to drops 1 and 2 (P=0.000 and P=0.001, respectively). In addition, there was more aggression during feed drop 1 on D2 compared to D8 (transformed means (+/- SE) for D2 and D8, respectively, were 2.9 (0.10) and 2.2 (0.04) aggressive interactions per sow, $F_{1,30}$=30.011, P=0.000).

Two interpretations of the reduction in aggression by the fourth feed drop on D2 are that aggressive sows have become satiated and left the feeding area, or that by the fourth feed drop a developing dominance hierarchy is ameliorating aggression. The former would provide subordinate sows with the opportunity to feed in later feeding bouts. With the latter condition, dominant sows may continue to monopolise the feeding area using more non-aggressive agonistic behaviours. Thus, in conjunction with increased active avoidance of dominant sows by subordinate sows, the need to use aggression is reduced. By D8, familiarity and a stable hierarchy may regulate access to the feeding area, by allowing more disputes to be resolved using non-aggressive agonistic behaviours such as avoidance.

In conclusion, the number of aggressive encounters between sows housed in groups diminishes modestly with successive feeding bouts on D2 and over time from D2 to D8 post-mixing. These results support those found by Schneider *et al.* (2007) and suggest that feeding groups of sows over multiple bouts could be a useful strategy to reduce aggressive encounters. However, a better understanding of the feeding behaviour of individual sows within a group is required to ensure subordinate sows are not sacrificing the opportunity to feed for safety.

SCHNEIDER, J., TOKACH, M., DRITZ, S., NELSSEN, J., DEROUCHEY, J., GOODBAND, R. (2007). *Journal of Animal Science*. **85**: 3462-3469.

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Physical Characteristics of Surviving Piglets Born in a Loose Farrowing System

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Farrowing crates are employed widely in pig production systems to reduce piglet mortality. However, the system imposes numerous behavioral and physical restrictions on the sow and raises serious welfare concerns. With increasing pressure to abolish the farrowing crate, investigations into improving survival in alternative farrowing systems are essential, yet it cannot be assumed that survival indicators are common between different production systems. Consequently, the aim of the current study was to identify physical survival indicators in a population of piglets born in farrowing pens. The primary hypotheses was that survival would be affected by individual physical characteristics of piglets, defined as birth weight, relative birth weight in the litter, length, body mass index, ponderal index and degree of growth restriction.

The study was conducted as a survey in a 1200 sow piggery where the sows were housed in groups during mating and gestation. During farrowing and lactation the sows were loose housed in individual farrowing pens, meaning that sows were not constrained at all during the farrowing process. The individual farrowing pens measured 2.8 m x 1.8 m and had slatted metal floors in the centre of the pen and slatted plastic floors along the pen separations. The creep area could be accessed from the inspection alley and had an area of 0.9 m^2. An adjustable lid covered 0.5 m^2 of the creep area and a rubber mat was placed on the floor in this area while the floor outside the covered area was slatted. All 3392 piglets in 203 litters from Landrace x Yorkshire sows mated with Duroc semen were used in the study. All piglets were ear tagged at birth and gender, body weight (BW) and crown to rump length (CRL) recorded. Subsequently, body mass index (BMI; BW/CRL^2) and ponderal index (PI; BW/CRL^3) were calculated. In addition, piglets were judged as either normal (1), light intrauterine growth retarded (IUGR; 2) or IUGR (3). Piglets with a) steep forehead, b) bulging eyes and c) wrinkles around the mouth were judged as IUGR whereas piglets with one or two of these three criteria were evaluated as light IUGR. Data was analysed using a general linear models procedure.

Table 1. *Physical characteristics of piglets born in farrowing pens (means ± SD).*

	Still born	Died pre-weaning	Weaned	P value
n	256	534	2612	
Birth weight (kg)	1.12 ± 0.39[a]	1.12 ± 0.38[a]	1.44 ± 0.33[b]	<0.001
CRL (cm)	24.3 ± 3.4[a]	23.3 ± 2.7[b]	24.6 ± 2.1[a]	<0.001
BMI (kg/m^2)	18.3 ± 3.2[a]	20.0 ± 4.0[b]	23.7 ± 3.1[c]	<0.001
PI (kg/m^3)	75.9 ± 12.7[a]	86.3 ± 16.3[b]	96.9 ± 13.4[c]	<0.001
IUGR score[1]	1.3 ± 0.5	1.4 ± 0.6	1.2 ± 0.4	0.009

[abc]Means within a row with different superscripts differ significantly (P<0.05); SD, standard deviation; CRL, crown to rump length; BMI, body mass index; PI, ponderal index; IUGR, intrauterine growth retarded. 1Scored on a scale from 1-3 where (1) normal, (2) light IUGR and (3) IUGR.

In accordance with previous reports (eg. Baxter *et al.*, 2009), piglets surviving the suckling period were heavier at birth compared with piglets dying (P<0.001), had a larger BMI (P<0.001) and higher PI (P<0.001; Table 1). There was no difference in the CRL between still born or alive piglets, however the frequency of piglets with IUGR or light IUGR were less among the weaned piglets. In total 16% of the weaned piglets showed signs of IUGR at birth compared with 36% of the piglets that died pre-weaning and 23% of the still born. The body condition measurements were significantly correlated with each other with birth weight, BMI and PI important survival indicators whereas only 40% of the IUGR pigs survived until weaning. The study has confirmed previous work by Baxter *et al.*, (2009) in an outdoor setting with respect to birth weight, BMI and PI. Further research will be necessary to identify interventions that can improve the survival rate of IUGR and light IUGR pigs.

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Piglet Attributes at Birth Can Influence Survival

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Piglet attributes at birth are important for postnatal survival. Knol *et al*. (2002) demonstrated that some aspects of piglet quality had declined due to prior selection for improved production efficiency. Determining attributes (eg. birth weight, rectal temperature, crown-to-rump length) which define better piglet quality and therefore an improved survival rate may enable producers to identify "at risk" piglets (for intervention) and potentially new selection criteria for breeding programs. We hypothesise that individual piglet attributes evaluated soon after birth in a commercial setting are associated with their survival until weaning.

Data was collected on 9133 piglets, within 12 hours of birth, of known gender from 610 sows (847 litters) of mixed parity. Shivering, meconium staining, and incisor (I_2) eruption were recorded as absent or present. Respiration rate, muscle tone, skin colour and hydration level were classified, for this study, as normal or abnormal. Birth weight, crown to rump length, ponderal index, and rectal temperature were measured and ranked into quintiles. Ponderal index was calculated as birth weight/(crown to rump length)^3, expressed in kg/m^3. Piglet body condition was also subjectively scored into three groups as poor, moderate, and good. Sow parity group was concatenated with fostering status of each piglet for model testing. Due to the binomial distribution of the dependent variable survival (survive=1 or not=0), logistic regression was performed to analyse the data using the GLM procedure of R (R Development Core Team, Vienna) fitting a logit link function. The full model included all the factors noted above and the final model was obtained by applying stepwise elimination of non-significant (P>0.05) effects. The odds-ratio (OR) was used to show the relative survival rate for each factor level compared to the lowest factor level, which has an OR=1.

Increased birth weight, ponderal index, body condition, and rectal temperature were significantly associated with improved piglet survival. Survival rate of heavier piglets relative to the lowest quintile (birth weight <1.25kg) increased up to two fold (OR: 1.4-2.1). However, relating weight to piglet size, via ponderal index and condition scores, provided additional information on survival over birth weight alone. In comparison to Bunter and Tabuaciri (2011), where no other data on piglet attributes was used, the OR for birth weight had a lower range in this study. In addition to birth weight, the survival rate of piglets with ponderal index above 109 was increased up to 60% (OR: 1.3–1.6). Piglets with better body condition scores also had an increased rate of survival (OR: 1.6-2.3). Further, survival rate increased by two to three fold (OR: 2.2-3.0) when rectal temperature was above 37.6°C. These results demonstrated that piglets with higher birth weight, ponderal index, good body condition, and higher rectal temperature were physiologically more mature and better able to survive in their new environment.

Poor piglet colour (OR: 0.58), abnormal respiration (OR: 0.71), muscle tone (OR: 0.73), shivering (OR: 0.79) or hydration levels (OR: 0.84) reduced relative survival rates. These attributes are generally unfavourably correlated with body weight, body condition and rectal temperature (Tabuaciri *et al*. 2011). The presence of an erupted I_2, meconium staining, or variation in crown to rump length, which were also correlated with birth weight and other traits, were not significantly (P<0.05) associated with survival in a multivariate model.

These findings show that piglet attributes recorded at birth are associated with their subsequent survival. Although a considerable number of attributes are at least partially indicated by birth weight, the efficiency of identifying at risk piglets is improved with more data on other attributes. Selection to improve piglet survival might also be improved by using piglet attributes in addition to birth weight as selection criteria. This possibility has been investigated further in Tabuaciri *et al*. (2011).

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KNOL, E.F., LEENHOUWERS, J.I. and VAN DER LENDE, T. (2002). *Livestock Production Science*. **78**:47-55.

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CHAPTER 9

Pig Management

Nutrient Mapping of Free Range Pork Production Using Electromagnetic (EM) Technology

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Free range pig production is often promoted on the basis of improved welfare and environmental performance compared to conventional pork production. However, uneven distribution of nutrients has been identified in free range piggeries (Zadow *et al*. 2010). EM technology can be used to measure soil apparent conductivity (EC_a) in a rapid, non-invasive survey. The technology was originally used for soil salinity research in the US during the 1980's. However, with the uptake of global positioning systems (GPS) in agriculture, additional applications have since been developed. A summary of EM applications in agricultural research has been collated by Corwin and Lesch (2005). This study tested the hypothesis that electromagnetic induction (EM) survey technology can be used to spatially map the distribution of nutrients in free range areas.

Electromagnetic soil surveys were conducted using a Geonics EM38-MK2 (Geonics Limited, Ontario, Canada). Data including EC_a and GPS location was logged whilst towing the EM38-MK2 on a non ferrous sled behind an all terrain vehicle (ATV). Statistically relevant soil sampling locations (6 samples/paddock) were selected using the ESAP (EC_a Sampling, Assessment and Prediction) software package (Lesch *et al*., 2000). Following laboratory analysis, statistical relationships between EC_a and the various soil parameters of interest were determined using multiple linear regressions in ESAP. Soil properties with strong regression relationships to EC_a were mapped to show the predicted distribution and concentration.

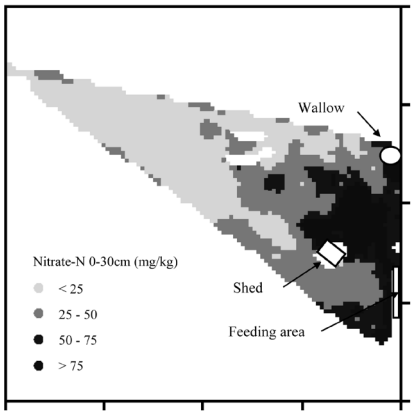

Figure 1. *Predicted Nitrate-N Distribution (0-30cm).*

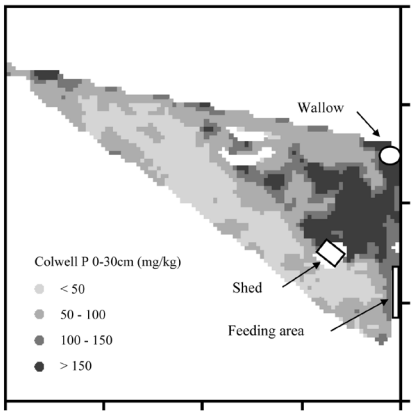

Figure 2. *Predicted Colwell P Distribution (0-30cm).*

The results for Site 1 showed high correlations (r^2 = 0.93, 0.95, and 0.93) with electrical conductivity, nitrate-N (Figure 1) and phosphorus (Figure 2), respectively. The mean soil phosphorus and nitrate-N concentrations for all free range sites were in excess of recommended levels in the National Environmental Guidelines for Piggeries (NGEP). Further, the data indicates that nutrients are not evenly distributed in the free-range area. The EC_a and nutrient distribution maps clearly show that nutrients are concentrated around sheds, feeding areas and wallows. While results from this study are in agreement with similar research in other animal industries, it is noted that the study was limited to four paddocks on two free range farms, with only a small number of soil samples taken from each site. Hence, further research is required to confirm the observed trends.

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Estimation of Greenhouse Gas Emissions from Alternative Piggery Effluent Treatment Systems

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Research by Wiedemann *et al*. (2010) estimated that emissions from effluent treatment in conventional liquid manure flushing systems were >50% of total greenhouse gas (GHG) for pork production. The present study estimated on-farm GHG emissions from three alternative effluent treatment systems, including 1) uncovered anaerobic lagoons (current practice), 2) covered anaerobic pond (CAP) with methane (CH_4) flaring (CAP-Flaring), and 3) CAP with on-farm combined heat and power (CAP-CHP). Results were calculated per kg of pig live weight (LW), but excluded upstream impacts such as grain production.

All effluent treatment system emission estimates were based on production data collected from a farrow-to-finish piggery in Queensland. Inventory methods, including modelling of GHG emissions, was done following methods outlined in Wiedemann *et al*. (2010) for the current practice scenario. The CAP scenarios used the following assumptions; hydraulic retention time (HRT) was 47 days, CH_4 production of 0.28m^3 CH_4/kg volatile solids (VS) added to the pond and VS reduction of 70%. Remaining VS in effluent was assumed to flow to a secondary, uncovered anaerobic pond, resulting in residual CH_4 emissions. Impact assessment and uncertainty analysis were done using SimaPro 7.1.8 (PRé Consultants bv, Amersfoort, The Netherlands). Confidence intervals were determined using a Monte Carlo analysis of uncertainty to produce 95% confidence intervals.

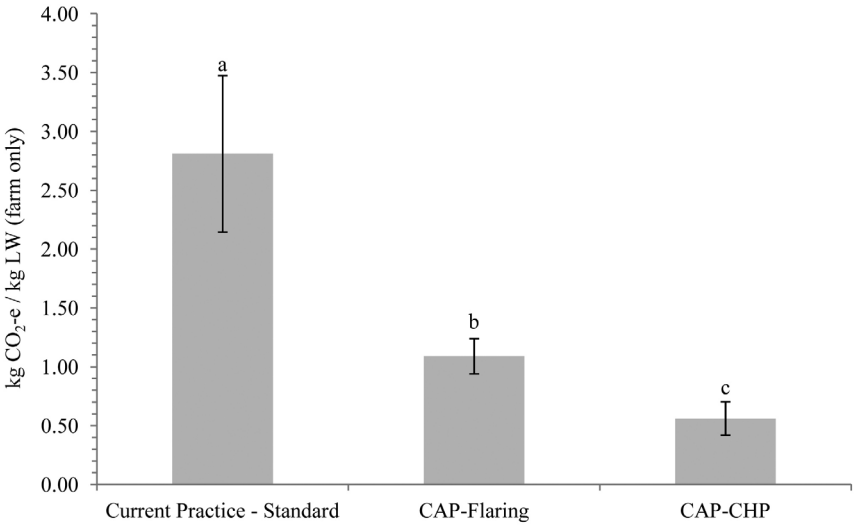

Figure 1. *Estimated greenhouse gas (GHG) emissions per kg of liveweight (LW) from three effluent treatment scenarios in a simplified conventional flushing farrow-to-finish piggery system.*

Significant reductions in GHG were observed for both scenarios where methane was destroyed (Flaring and CHP). The modelled CHP system was estimated to generate sufficient electricity and heat to meet farm requirements, and provide a modest amount of electricity to the grid. Emissions from all systems were dominated by CH_4 from uncovered ponds within the effluent treatment system. For both CAP scenarios, residual CH_4 emissions from secondary ponds contributed the majority of total GHG, amounting to 55% (Flaring) and 75% (CHP) of total GHG. Effluent stream characterisation and methane emissions for uncovered ponds followed assumptions from DCCEE (2010). These assumptions were subject to a large degree of uncertainty, mainly associated with prediction of VS in the effluent stream, CH_4 potential (Bo) of pig manure and the CH_4 conversion factor (MCF). Considering the importance of GHG from piggery waste streams, further research may be warranted to improve effluent stream characterisation and MCF values for a number of treatment systems.

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Effect of Dietary Fibre Content and Feeding Frequency During Late Gestation on Lactation Performance of First Parity Sows

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Current feeding methods for gestating sows, which commonly consists of feeding a small amount of highly digestible carbohydrate in a single ration, may stimulate unnecessarily high levels of insulin resistance in peri-parturient sows. Insulin resistance reduces voluntary feed intake in lactation (Mosnier *et al*., 2010). Increased feeding frequency provides a more regular supply of nutrients, and fermentable fibre stabilises interprandial blood glucose and insulin levels (de Leeuw *et al*., 2004). This experiment determined whether feeding sows once or thrice daily a standard or high fibre diet in late gestation would improve milk production and subsequent reproduction.

Thirty-six, Large White gilts were used in the experiment. The experimental design was a 2 x 2 factorial arrangement (n = 9 gilts/treatment), involving two diets - High Fibre (HF, 7.2% crude fibre (CF), 13.2 MJ digestible energy (DE)/kg) versus Low Fibre (LF, 4.7% CF, 13.2 MJ DE/kg) and two feeding frequencies (once (0800hrs) versus three (0800, 1200, 1600hrs) times daily) from d 80 to 112 of gestation. Lupin hulls were used as the fibre source. Gilts received 3 kg/d of their respective diet, with the daily ration spread equally for the three times daily group. From d 112 of gestation to parturition, gilts were fed 3kg of a standard lactation diet (4.7% CF; 14.2 MJ DE/kg). Post-parturition, feed intake was gradually increased to a maximum of 7kg/d until weaning at d 26. Litter size was maintained at 10 piglets. Lactation feed intake, sow live weight (LW) and P2 backfat (P2) were measured. Piglets were weighed individually on d 1, 21 and 26 of lactation. Data were analysed using a two-way analysis of variance. Sow was considered to be the experimental unit and piglet birthweight (BW) was included in the model for piglet performance.

Table 1. *Effects of dietary fibre content (7.2% (HF) versus 4.7% (LF)) and feeding frequency (1 versus 3 times daily) in late gestation on total litter and individual piglet birthweight (BW), piglet liveweight (LW) gain, sow feed intake and LW loss during lactation.*

Main Effects		Total litter BW (kg)	Piglet BW (kg)	Piglet LW gain (kg/d)		Lactation intake (kg/d)	Sow LW loss (kg)	
				d1-21	d21-26		d1-21	d21-26
Diet	HF	13.7	1.41	0.205	0.266	5.3	6.4	1.1
	LF	13.7	1.44	0.213	0.261	5.4	7.7	1.8
Feeding Frequency	1	14.0	1.44	0.199[a]	0.259	5.4	5.0[a]	1.2
	3	13.5	1.41	0.219[b]	0.268	5.3	9.2[b]	1.8
Pooled SEM		0.51	0.05	0.01	0.02	0.14	2.0	1.3

[ab]Means in a column with different superscripts differ significantly (P<0.05); SEM, standard error of mean; HF, high fibre; LF, low fibre

Treatment did not affect sow LW or P2 on gestation d 80 (200.8±2.5kg; 21.8±0.7mm) or 112 (227.5±1.2kg; 23.0±0.8mm), or d 1 (205.0±1.4kg; 22.2±0.8mm) and 26 (197.1±2.2kg; 18.7±0.8mm) of lactation. Thrice daily feeding significantly (P<0.05) increased sow LW loss and increased piglet LW gain between d1 and 21 of lactation (Table 1). First litter size (Total born, 10.5±0.4; born alive, 9.8±0.4), lactation feed intake (Table 1), WOI (4.9±0.1d), and second litter size (total born, 10.7±0.5; born alive, 9.8±0.6) were unaffected by treatment (P>0.05). Our data demonstrate that feeding sows thrice daily during the last 22 days of gestation improves piglet growth during the first 21 days post-partum, regardless of dietary fibre content. The concurrent increase in maternal LW loss during this period suggests that thrice daily feeding promotes milk production. Although we observed no effect of feeding highly digestible fibre during gestation on sow or piglet performance, bulky, high fibre (20% CF) gestation diets have been shown to increase sow lactation intake (Matte *et al*., 1994).

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Validation of a Sow-Side Test for Measurement of Blood Haemoglobin

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Gains in prolificacy and growth performance of modern genotypes leave producers concerned with the adequacy of pig diets with respect to iron requirements. In addition to transport of oxygen in blood haemoglobin (HGB), iron contributes to immune, liver and thyroid function, and energy metabolism in pigs. Marginal iron status in sows (Mullan *et al*., 2009) can thus predispose them to poor health, productivity and longevity. HGB levels are a useful indicator of individual and herd level iron status since haemoglobin and myoglobin are amongst the last iron-containing compounds in the body to relinquish iron under deficient conditions. The HemoCue blood haemoglobin photometer (Medipac Scientific Pty Ltd, Sydney, NSW), predominantly used in human diagnostics, is a small portable analyser that measures haemoglobin in venous or capillary whole blood. A preliminary trial of a HemoCue in the field demonstrated its promise as a practical sow-side test for iron deficiency. The hypothesis was that there was no significant difference between results obtained via a HemoCue unit versus results from laboratory analysis. The aim of this experiment was to validate the use of the HemoCue photometer by assessing the correlation between data obtained from it in the field with that obtained by standard laboratory analysis.

Samples were collected from 166 commercial crossbred gilts and sows (Large White x Landrace) with approximately five sows being sampled from each of parities 0 to 6+ equalling up to 35 sows per farm. The animals were drawn from five diverse commercial herds, representative of production systems and genotypes commonly found within Western Australia. Samples were taken via jugular venepuncture into ethylenediaminetetraacetic acid (EDTA) tubes. A 10μL volume of blood was subsequently used for HemoCue analysis and the remainder of the sample was sent for analysis using an Advia 120 (Siemens Health Care Diagnostics, Deerfield, IL, USA) haematology analyser. The number of pigs sampled permitted the investigation of a difference in haemoglobin concentration results of 5g/L with 95% certainty, a power of 90%, and considering a standard deviation of 13.0. The agreement and correlation between the laboratory and on farm measurements of haemoglobin was assessed using a paired t-test and Pearson's correlation coefficient. Differences were considered significant at $P<0.05$ (two-sided test).

Table 1. *Mean and standard deviation (SD) from laboratory analysis (Advia) versus HemoCue analysis.*

	Mean	SD
Advia	125.6	13.0
HemoCue	125.4	12.3

The paired t-test supported the hypothesis that there was no significant difference between the two means. The Pearson's correlation coefficient of 0.96 shows the result from both methods are highly correlated. Maes *et al*. (2011) reported lower correlation between HemoCue and laboratory analysis, which they explained by sample collection techniques being variable such as the amount of pressure applied during alcohol rubbing and animal positioning. The importance of correct sample handling has also been stressed in human literature (Mazzachi and Shephard, 2002).

The results support the hypothesis that a HemoCue photometer returns accurate results in comparison to standard laboratory analysis of HGB levels. This confirms its usefulness as a sow-side test for herd screening purposes as well as for testing individuals. Examples of its use could include screening piglets of older sows for anaemia at weaning or screening of sows before farrowing. Compared with conventional blood sampling and processing methods, the HemoCue unit uses less blood (10μL), less invasive sampling techniques (ear prick versus jugular or vena cava venipuncture requiring full restraint) and is significantly lower in cost (approximately $AUD2.00 per sample versus $AUD18.00).

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Semen Factors Associated With Post-Breeding Sow Responses

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This experiment aimed to determine the effect of sperm traits on sows returning to oestrus; a source of significant economic loss in pig production systems in which bred sows are neither gestating nor lactating and therefore not producing a litter. Return to oestrus can be caused by factors such as incorrect oestrus or pregnancy detection, seasonal infertility, age and pathogens. Here it was hypothesized that male factors also contribute to early pregnancy loss in pigs.

Sows of mixed age and parity representing 16 dam lines (n=959; 710 sows farrowed) were artificially inseminated over a 12 month period with single sire semen doses twice during their oestrous period. Extended semen was assessed within 24h after collection for motilities, velocities, clump score and concentration by computer-assisted sperm analyser, morphology (Differential interference contrast (DIC) microscopy), chromatin condensation (Acridine orange), bacteria (DiffQuick, Thermo Fisher Scientific Pty Ltd, Taren Point, NSW) and membrane integrity (eosin/nigrosin). Sow return rate was 26% and farrowing rate was 74% of all sows inseminated. Return categories were defined as: early return (0-18d, n=7); regular (19-23d, n=69); foetal skeletal calcification stage (24-35d, n=67), irregular (36-45d, n=23) and late (>46d, n=83). Sperm traits were analysed using linear mixed models with fixed effects for dam line, parity, insemination season and sire line. The random terms included were inseminator and sire identification. There were no significant interactions (P>0.05), therefore, the main effects only were included in the model. Predicted values and rankings were based on Tukey's pairwise multiple comparison at 5% family significance level.

Table 1. *Predicted semen trait values (means ± standard deviation) determined using a linear mixed model.*

Return Category	Return Time	Abnormal Head %	Abnormal Acrosomes %
Early	0-18d	11.65 ± 1.72^{b}	0.93 ± 0.45^{ab}
Regular	19-23d	6.79 ± 0.77^{a}	0.25 ± 0.18^{a}
Calcification	24-35d	7.67 ± 0.80^{ab}	0.41 ± 0.18^{ac}
Irregular	36-45d	7.27 ± 1.06^{ab}	1.12 ± 0.26^{bc}
Late	>46d	8.32 ± 0.73^{ab}	0.94 ± 0.16^{b}

[abc]Means in a column with different superscripts differ significantly (P<0.05).

Sperm traits significantly associated with return category were abnormal head % (mean =7.3%; range = 0-33%; P<0.01) and abnormal acrosome % (mean=0.5%; range =0-12%; P<0.001), respectively (Table 1). Head abnormalities included diadems/craters, degenerate heads and misshapen heads while acrosome abnormalities included degenerated, knobbed or missing. Dam line also significantly (P< 0.05) influenced return type.

As acrosomes are necessary for successful sperm binding to the ovum, it is logical that abnormal acrosomes would be linked with sow returns, although predictive values in this experiment were not high. In this experiment, the relationship was highest with late returns, possibly reflecting poor indications of an earlier return to oestrus. Similarly, sperm head abnormalities have been associated with both lack of fertilisation and pregnancy loss (Saacke *et al.*, 2000) whereas sperm morphology in general can influence return rates in sows (Alm *et al.*, 2006). In this experiment, abnormal sperm heads were linked with sow returns associated with failure of fertilization (ie. early versus regular return). None of the semen factors studied, however, could be directly linked with early pregnancy loss.

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Failure to Program Piglet Growth Performance in Barrows Through Neonatal Androgenisation

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The potential for male piglets to grow to genetic potential is limited by castration conducted to modulate aggressive behaviour and limit boar taint. This results in the loss of the source of testosterone known to potentiate the secretary patterns of growth hormone (GH) from pituitary somatotropes through the stimulation of the hypothalamic GH releasing hormones. Thus castrates are fatter and convert feed to liveweight less efficiently. It has been shown that physical and endocrine manipulation of neonates can influence the growth efficiency of piglets chronically (Gallagher *et al* 1998). Since the use of anabolic steroids in commercial production is illegal, we have assessed the potential for non-steroidal testosterone mimics (non-steroidal androgen receptor modulators: SARMS) to program the GH secretary capacity of castrate male pigs so that they grow with the efficiency of gonadally intact piglets. Dihydrotestosterone (DHT) was assessed as an androgenic control while testosterone propionate (TP) was included as it is aromatised to oestrogen and provides a comparison for the androgenic molecules being tested.

Newborn male piglets (n=30: Windridge Farms, Young, NSW) were cross-fostered to three multiparous sows (n=10 piglets per litter). All treatments were replicates within sow as sows vary in their lactation performance. Thus two piglets from each litter received injections of either A) TP: 8mg/kg bodyweight (BW); B) DHT which is not aromatised to oestrogen: 8mg/kg BW; C) SARM 1 (CE-284821, Pfizer Inc., West Ryde, NSW, 3mg/kg BW); or D) and E) peanut oil vehicle only: 2ml. At 24 h *post-partum* the animals in treatments A-D were castrated while treatment E remained as an intact control. Animals were weighed on d 1, 13 and at weaning (d 24). A blood sample (5ml) was collected by venipuncture (19G needle) on d 24 for analysis for GH by radioimmunoassay (AL Parlow, Harbor UCLA California, USA) and for IGF1 by ELISA (Immunodiagnostic Systems, Tyne and Wear, UK). The data were analysed using linear mixed models (REML) in GenStat13.1(VSN International, Oxford, UK) to allow for the repeated measures nature of the data. The fixed effects were day, treatment and day x treatment while the random effects were dam, piglet and day.

Table 1. *The effect of neonatal androgens and castration on live weight (kg) change of piglets to weaning. Mean standard error of difference (SED) for day: 0.60. Mean SED for Treatment 0.45.*

Day	A:TP/Cast.	B:DHT/Cast.	C:SARM1/Cast.	D:Vehicle only/Cast.	E:Vehicle only/entire
1	1.68[a]	1.77[a]	1.88[a]	1.83[a]	1.87[a]
13	4.28[b]	3.65[b]	4.81[b]	4.16[b]	4.61[b]
24	6.50[c]	5.68[c]	7.11[c]	6.80[c]	6.53[c]

[abc]Values with different superscripts differ significantly (P< 0.05). TP, testosterone propionate; DHT, dihydrotestosterone; SARM, selective androgen receptor modulator; Cast., castrated.

There was no significant effect of treatment or interaction between treatment and day of weighing on live weight change (Table 1). Circulating GH concentrations were 4.1, 3.7, 4.6, 4.2 and 4.3ng/ml for piglets from treatments A-E, respectively. There was no significant treatment effect (P>0.05). Circulating IGF1 concentrations were 55.7*, 25.1, 32.5, 64.4* and 32.8ng/ml for piglets from treatments A-E, respectively (*P<0.05). Since values for treatments A and D were significantly higher, it would suggest that the direct androgen effect provided by treatments B, C and E are actually suppressing IGF1. This difference is not reflected in the growth data. Neonatal TP treatment has not altered growth potential to weaning acting through the androgen or oestrogen receptor. Similarly DHT has failed to alter growth which would have been directly through the androgen receptor since this androgen is not aromatized. In the case of the SARM operating through the androgen receptor there is also no treatment effect. Thus we have failed to program a superior growth rate to weaning with these neonatal treatments.

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Effect of Pasture Allowance on Pasture Dry Matter Consumption of Grazing European Wild Boar

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Commercial meat production of European wild boar (*Sus scrofa* L.) is increasing both in Chile and internationally, using production systems with access to pasture for grazing and supplementation with concentrated diets. The animals satisfy around 84-142% of their maintenance energy requirements through pasture consumption (Hodgkinson *et al*., 2009, Quijada *et al*., 2011). The effect of pasture allowance on pasture consumption has not been previously investigated but is important to allow an efficient use of grazing areas. The aim of this experiment was to determine whether increasing the pasture allowance increases the pasture consumption (dry matter (DM) basis) of wild boar, even when pasture consumption is not limited by pasture availability.

Paddocks sown with *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover) were used. The treatments involved pasture allowances controlled by allowing pairs of animals access to different sized areas with the pasture cut to 1500 kg DM/ha (actual value (mean±standard error of mean (SEM)) 1485±16.8 kg DM/ha, pasture energy content 18.3 MJ gross energy (GE)/kg DM): no pasture (areas 8 m^2 with all pasture removed), low (5.5 m^2, pasture allowance 825 g DM), medium (8.0 m^2, pasture allowance 1200 g DM) and high (16 m^2, pasture allowance 2400 g DM). Forty-eight purebred nose-ringed European wild boar of 14.4±0.39 kg (mean±SEM) were randomly grouped into pairs (one male and one female), and distributed between the four treatments (six pairs per treatment). The experiment commenced following a 14 d acclimation period. Each day of the 28 d study, pairs of animals entered their pasture area from 0830h until 1630h, after which they had free access to a commercial diet containing 14.1 MJ digestible energy (DE)/kg for 60 min with the diet consumption of each pair measured. The animals then entered pens with concrete floors in a temperature-controlled shed (18±1°C) until the following day. Water was provided *ad libitum*. The animals grazed fresh areas daily, with a rotation of 7 d, which was sufficient time to maintain the desired pasture availability. The DM content of each grazed area was calculated using pasture samples cut to soil level (area 0.25 m^2) taken pre- and post-grazing from each grazed area and the DM consumption per pair of animals was determined (difference between the DM availability pre- and post-grazing). All animals were weighed weekly. Statistically significant differences in overall liveweight gain (LWG), total supplemental diet consumption, pasture apparent DM consumption and total apparent DM intake (supplemental diet and pasture) between treatments were determined using an analysis of variance (ANOVA).

Table 1. *Liveweight gain (LWG), total supplemental diet (SD) consumption, total pasture apparent dry matter (DM) consumption and total apparent DM intake (supplemental diet plus pasture) of wild boar (mean±SEM) during 28 days with different pasture allowances.*

	Pasture allowance			
	No pasture	Low	Medium	High
LWG (kg/animal)	6.3 + 0.51[a]	8.8 + 0.29[b]	8.6 + 0.24[b]	8.3 + 0.19[b]
Total SD consumption (kg DM)[1]	19.8 ± 0.79[a]	21.4 + 0.73	20.5 + 0.58	19.3 + 0.38
Pasture apparent consumption (kg DM)[1]	0	2.9 + 0.19[a]	3.9 + 0.38[b]	6.4 + 0.45[c]
Total DM intake (kg DM)[1]	19.8 ± 0.79	24.3 ± 0.59[b]	24.4 ± 0.28[b]	25.7 ± 0.56[b]

SEM, standard error of the mean; n=6 pairs of animals. [abc]Means in a row with different superscripts differ significantly (P<0.05). No pasture, no access to pasture; Low, 825 g DM; Medium, 1200 g DM; High, 2400 g DM. [1]Data averaged over two animals.

The animals that did not receive pasture had a lower LWG than those that had access to pasture (Table 1) showing the importance of pasture consumption in this production system. There was a tendency towards a decreased consumption of supplemental diet with increased pasture allowance, but any differences did not reach statistical significance (P>0.05), possibly due to the variation between animals. Pasture consumption of European wild boar can be increased by increasing the pasture allowance for grazing, at least within the range used in this experiment, without affecting the LWG of the animals, with the increased pasture consumption being equivalent to the slightly decreased consumption of the supplemental diet.

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CHAPTER 10

Delivering Quality Pork

High Boar Taint Risk in Entire Male Carcases

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Androstenone and skatole are the main compounds responsible for boar taint, an off-odour/flavour in pork from entire male carcases (Hennessy *et al*., 1997). Hennessy *et al*. (1997) reported a high incidence of boars from multiple sites in Australia exceeded the international sensory threshold of 1µg/g for androstenone, and 0.2µg/g for skatole. The aim of this experiment was to measure the androstenone and skatole concentrations in porker and baconer entire male carcases from two major Australian pork supply chains. Anecdotal observations from a number of Australian supply chains also suggest use of lower carcase weights may minimise the boar taint risk. This experiment will determine the correlation between carcase weight and androstenone and skatole concentrations in entire male carcases.

A total of 400 fat tissue samples (10g) were collected at two abattoirs from crossbred entire male carcases to assess the concentration of androstenone and skatole at two carcase weights (HSCWT): porker (60-70kg) and baconer (71-80kg). The fat samples were collected from the belly region at approximately 45 min post-slaughter. Androstenone and skatole was analysed by high performance liquid chromatography/mass spectrometry (HPLC/MS). Analysis of variance was used to analyse the effect of carcase category on androstenone and skatole concentrations. Pearson's correlation was used to determine the variance between androstenone and skatole concentrations and HSCWT.

Table 1. *Mean androstenone and skatole concentrations in fat and percentage of porker and baconer carcases over the sensory thresholds of greater than 1µg/g for androstenone and 0.2µg/g for skatole.*

Site (S)	A		B		
Carcase Category (C)	Porker (64kg±5kg)	Baconer (80kg±10kg)	Porker (60kg±5kg)	Baconer (75kg±5kg)	Significance
Mean androstenone (µg/g)	0.50	0.59	0.95	1.54	S***; C**; SxC**
Androstenone >1 µg/g	11%	20%	33%	50%	
Mean skatole (µg/g)	0.05	0.09	0.13	0.18	S*; C**
Skatole >0.2 µg/g	2%	8%	13	27%	

*P<0.05; **P<0.01; ***P<0.001.

The slightly heavier carcases (porker and baconer) from Site A had significantly lower (P<0.05) androstenone and skatole levels compared Site B carcases (Table 1). Porker carcases had lower (P<0.05) mean androstenone and skatole concentration compared to baconer carcases. The correlations between HSCW and androstenone (Site A, r=0.098; Site B, r=0.125) and skatole (Site A, r=0.194; Site B, r=0.247) concentrations were, however, weak. The incidence of porker and baconer carcases with androstenone >1µg/g from sites A and B was 11% and 20%, and 33% and 50%, respectively. The incidence of porker and baconer carcases with skatole >0.2 µg/g from sites A and B was 2% and 8%, and 13% and 27%, respectively.

These data indicate variable levels of boar taint compounds in entire male carcases across sites and carcase type, and are similar to that reported by Hennessy *et al*. (1997). These data also indicate a poor correlation between androstenone and skatole concentrations in fat and HSCWT. Hence the use of carcase weight selection strategies to minimise the boar taint risk in entire male carcases is not appropriate. To date, there is no commercially available on-line detection system for boar taint compounds in carcases. The elimination of androstenone and skatole compounds using the boar taint vaccine may be a more appropriate strategy to maximise pork quality from entire male carcases.

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Testes Size of Male Pigs Treated With an Anti-GnRF Vaccine is Dependent on Slaughter Age After Second Vaccination

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Anti-gonadotropin-releasing factor (GnRF) vaccine, Improvac® (Pfizer Australia Pty Ltd, West Ryde, NSW) has been promoted as an effective, non-surgical solution to minimize the risk of boar taint (Hennessy, 2009). Boar taint causes off odours during cooking and is caused by androstenone, a non-hormonal testicular steroid, and skatole, produced by microbial activity in the hind gut (Claus *et al*. 1994). During commercial use of the vaccine when male pigs are vaccinated at fixed ages (10 and 17 weeks) an increase in the range in testes size of vaccinated pigs has been observed in older pigs. It is currently recommended to slaughter males between two to five weeks after their second vaccination. Carcases from males sold later than five weeks after their second vaccination may be at higher risk of boar taint due to androstenone if the vaccine efficacy declines. We hypothesized that testes size and androstenone increase when vaccinated males are sold more than five weeks after their second vaccination at 17 weeks of age and slaughter.

One hundred and twenty male pigs (Large White x Landrace, PrimeGro™ Genetics, Rivalea (Australia) Pty Ltd, Corowa, NSW) were vaccinated (intramuscular) with a 2 mL dose at 10 weeks of age (V1) and again at 17 weeks of age (V2). Pigs were allocated based on live weight at 17 weeks of age (63.0 ± 0.7 kg, mean ± Standard error) to 18, 20, 22, 24, 26, or 28 weeks of age at slaughter. Pigs were group housed and fed commercial diets *ad libitum* (13.8 MJ digestible energy (DE)/kg, 151 g crude protein/kg, 0.52 g available lysine/MJ DE) from 17 weeks of age until slaughter. A 10g sample of fat was taken from the belly, stored at -20°C until analyzed for androstenone by Liquid Chromatography Mass Spectrometry. Androstenone values recorded at the minimum detectable level of 0.2µg/g were assigned a value of 0.1µg/g, half way between the limit of detection and zero, consistent with the approach adopted by Dunshea *et al*. (2001). Both testes were weighed and scrotal width and length were measured by digital calliper on the carcase. Data were analyzed by GLM analysis of variance as a one-way comparison and the proportion of carcases with androstenone > 1µg/g threshold for boar taint risk was analysed by chi-square.

Table 1. *Carcase performance and androstenone concentration in fat sampled from male finishers vaccinated with an anti-GnRF vaccine (Improvac®) at 10 and 17 weeks of age and slaughtered at increasing ages.*

	Slaughter age (weeks)							
	18	20	22	24	26	28	SEM	P value
Carcase weight (kg)	54.4^{a}	64.0^{b}	74.6^{c}	85.2^{d}	95.7^{e}	98.8^{e}	1.69	<0.001
Carcase P2 (mm)	7.0^{a}	8.8^{a}	11.4^{b}	12.6^{bc}	13.6^{c}	14.2^{c}	0.39	<0.001
Testes width (mm)	81.4^{a}	79.2^{a}	84.6^{ab}	96.8^{b}	88.8^{ab}	114.5^{c}	2.20	<0.001
Testes length (mm)	88.5^{a}	93.2^{a}	94.0^{a}	104.8^{b}	102.3^{b}	118.0^{c}	1.47	<0.001
Testes weight (g)	335.6^{a}	371.7^{a}	403.3^{a}	547.3^{b}	600.9^{b}	739.0^{c}	22.8	<0.001
Androstenone (µg/g)	0.136^{a}	0.126^{a}	0.120^{a}	0.325^{ab}	0.218^{a}	0.430^{b}	0.033	0.022
Carcases >1µg/g (%)	0/16	0/19	0/19	2/20	0/20	2/20	χ^2 27.8	<0.001

^{abcde}Values within a row with different superscripts differ significantly (P<0.05); GnRF, gonadotropin releasing factor; SEM, standard error of the mean.

Testes size and weight increased when pigs were slaughtered at 24 weeks of age and older (Table 1). Pairwise comparison of average androstenone levels at 22 weeks vs 24 weeks of age tended to differ (P=0.06) whereas the average carcase androstenone was significantly higher at 28 weeks of age. Similarly, a higher proportion of carcases 24 weeks and older exceeded the threshold for boar taint risk compared to younger pigs at slaughter (6.7% vs 0%, χ^2 3.73, P=0.053). Regression coefficients (r^2) of the quadratic model between androstenone and testes width, length and weight were 0.36, 0.41 and 0.52, respectively. The results show that testes growth recommences in pigs five weeks after V2 of Improvac®. Nevertheless, testes size is not an accurate indication of androstenone and hence boar taint. In some genotypes there may be a higher risk of boar taint when Improvac® vaccinated males are slaughtered more than five weeks after V2.

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Comparison of Cooked Pig Ham Aroma Profiles Obtained by Solid Phase Microextraction (SPME) or Stir Bar Sorptive Extraction (SBSE)

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Cooked ham is one of the most consumed ready-to-eat meat products. A good flavour profile is an important parameter in consumer choice in meat products. Several methodologies have been used for analysis of volatile compounds from cooked hams including vacuum distillation, dynamic headspace, simultaneous distillation-extraction and solid-phase micro-extraction (SPME), among others. SPME has a higher sensitivity, is solvent-free and quick, relatively easy to use and avoids formation of artefacts during extraction (Garcia-Esteban *et al.*, 2004). Complementary to SPME, stir-bar sorptive extraction (SBSE) allows improved accuracy in identification of volatile organic compounds from aqueous solutions. The SBSE has been successfully used in trace analysis from biomedical, environmental and food applications (Prieto *et al.*, 2010) but, to date, no studies have been reported for analysis of cooked ham volatiles. The aim of this research was to compare the aroma profiles of cooked pig ham obtained by SPME and SBSE in order to assess the potential benefit of using SBSE in meat analysis.

Pork hams were selected from 3 Large White gilt carcases with pH<6.2 measured on the *semimembranosus* muscle at 24h post mortem (pH_{24}). The hams (n=3) were deboned and subcutaneous and intramuscular fat, connective tissue and rind were removed. Brine was injected to increase ham weight by 20% and to obtain a final ham content of 0.3% pentasodium tripolyphosphate, 0.05% sodium ascorbate, 1.8% NaCl and 0.01% sodium nitrite. Hams were vacuum-tumbled at 4°C and 200 mbar (total 2000 rotations at 4 rpm). After eight days of maturation, the hams were sealed-bag packed into aluminium moulds and cooked in a steam oven to an internal temperature of 68°C (70°C external temperature). After 24h of temperature stabilization, cooked hams were vacuum packaged individually in polyethylene sealed bags and stored at 2°C. Two weeks later, the samples were analyzed by gas chromatography-mass spectrometry (GC-MS) after extraction by either SPME or SBSE.

Table 1. *Number and percent chromatographic area of volatile compounds (VOC) identified in cooked ham by SPME compared to SBSE.*

	SPME		SBSE	
Chemical families	VOC identified	Chrom. Area (%)	VOC identified	Chrom. Area (%)
Alcohols	13	22	6	20
Aldehydes	16	26.4	8	33
Acids	8	11	12	22
Ketones	8	24	3	17
Esters	8	3.5	4	1.4
Furans	2	0.4	0	0.1
Terpenoids	12	2.5	9	2
Lactones	11	7.2	5	3
Nitrogen + Sulphur	6	0.3	3	0.45
Others	5	2.7	6	1.05

SPME, Solid-phase micro-extraction; SBSE, Stir-bar sorptive extraction; VOC, Volatile compounds; Chrom, Chromatographic

Overall, more compounds were identified by SPME than SBSE (89 vs 56, respectively; Table 1). Compared to SPME, the SBSE improved the identification of high molecular weight compounds including long chain fatty acids (12 vs 8, respectively). Using both techniques a higher number of lipid oxidative volatiles (ie. aldehydes, alcohols, ketones and acids) were identified constituting an important proportion of the measured chromatographic profile. In addition, volatile compounds related to the brine injection (eg. hexanitrile) were identified by SBSE but not by SPME.

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Delivering Consistent Quality Australian Pork to Consumers – A Systems Approach

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Abstract

Tenderness, juiciness and flavour are the key eating quality attributes of pork that influence consumer appreciation of pork, but there is no simple 'on-line', low cost tool available to industry to grade carcases for these traits. Previous research has determined and documented the importance of a number of pre- and post- slaughter management factors in determining pork eating quality, but this information has not been integrated into an eating quality system. Previous research has also demonstrated significant issues in pork eating quality. The development of an eating quality assurance program, with clearly defined production pathways from production to consumption, is regarded as the way forward to enable the Australian pork industry to supply domestic and export customers with consistent high quality pork. Previous studies have demonstrated that fail rates for pork, in eating quality terms, far exceed tolerance limits imposed for other food products. The introduction of a non-prescriptive, cost-effective system to reduce the fail rate for consumer acceptability of Australian pork to less than 5% would assist with improving consumer demand and pork's positioning in the marketplace against other meat proteins. This review examines the key pathway factors, from production to consumption, which can influence eating quality attributes of fresh pork. The implementation of eating quality systems developed by the Australian beef and sheepmeat industries will also be discussed, including the success of the program in terms of increased returns for producers, processors and retailers. In addition, current initiatives being undertaken to develop an eating quality system for the Australian pork industry will be presented.

With the limited funds for investment, it is recommended that the pork industry considers adopting a similar eating quality system to Meat Standards Australia (MSA) for sheepmeat. The key factors for the success of MSA for beef and sheepmeat were that it was scientifically based, documentation and training were available, people involved were passionate about assuring quality to the consumer and an effective committee of people met regularly to design and modify the system. The underlying challenge, therefore, is how the Australian pork industry implements an effective, science based-system with scant resources.

Introduction and Industry Context

Australian pork producers aim to produce pork efficiently and sustainably in order to satisfy consumer demand for lean pork and remain competitive, particularly with increasing threats from imported pork. This continuing drive to improve efficiency and meet customer requirements has led to modifications in on-farm management practices to reduce costs of production for Australian pork producers, whilst producing lean carcases, at lighter carcase weights compared with pork produced in USA and Canada. This has reduced intramuscular fat content of Australian pork, negatively affecting flavour and juiciness. The effect of low fat content on eating quality is exacerbated by Australian consumer's often overcooking pork. Consumer knowledge of how to prepare and cook pork that satisfies taste and quality expectations therefore requires on-going effort.

There is clear evidence to show that some consumers are prepared to pay more for meat with superior eating quality. Consumers expect premium value, as well as premium quality, from meat (Pethick *et al.*, 2006) and quality can be influenced by both environmental and genetic factors. Hughes (2008) recently showed that 41% of consumers in the EU are prepared to pay more for high quality food. Furthermore, consumers of beef have been reported to be prepared to pay as much as two to three times more for quality (Lyford *et al.*, 2010).

Hofmeyr (1998) identified considerable variation in tenderness of pork sampled at the retail level, with 54% of pork loins purchased found to be unacceptable to consumers, based on a Warner-Bratzler peak shear force value of 5 kg (49 N) being the maximum for acceptability for lamb (Shorthose *et al.*, 1986). The US Pork Quality Targets for quality attributes in fresh pork loin measured at 24 hours post-slaughter included shear force of < 3.2 kg (Meisinger, 1999). Channon *et al.* (2001) also reported considerable variation in pork eating quality – based on a total of 3528 pork steaks tasted by 1572 consumers. Only 49% of pork loins obtained average scores for sensory overall liking of 60 or higher (on a 0 to 100 scale, where 0=dislike extremely and 100=like extremely) which was required for pork to be rated as slightly acceptable or higher. The implementation of production and processing interventions, including aitchbone hanging (rather than hanging carcases from the Achilles tendon) and ageing period (from two days to seven days), were shown by Channon *et al.* (2001) to reduce the incidence of unsatisfactory pork from an eating quality perspective (based on a consumer having a negative re-purchase intention) from one in four (25%) to one in 20 (4%).

Key Determinants of Pork Eating Quality

Boar Taint

In Australia, male pigs have historically been left entire due to leanness and growth rate advantages compared to surgically castrated male pigs. Castration can result in a reduction in growth performance and excess deposition of fat compared to entire males (Campbell and Taverner, 1988; Dunshea *et al*., 1993). However, boar taint from entire male pigs continues to be a major quality issue facing the Australian pork industry. 'Boar taint' refers to the undesirable, often intense, faecal, urine-like odour and/or flavour of pork. Skatole and androstenone, the two major causative compounds implicated in boar taint, behave synergistically in their contribution to boar taint. Androstenone is lipophilic and hydrophobic and is stored in the lipid component of tissues (Gower, 1972). Skatole is preferentially deposited in fatty tissue and is lipophilic and hydrophilic (Claus *et al*., 1994). Testes development and function is controlled by gonadotropin-releasing factor (GnRH) that triggers the release of luteinising hormone and follicle stimulating hormone from the pituitary gland, which then regulate the secretion of testicular steroids, including testosterone and androstenone. Testicular steroids reduce liver clearance of skatole in entire male pigs, and it therefore accumulates in fat. Skatole is a breakdown product of tryptophan in the hind gut of the pig and exhibits an intense faecal odour (Babol and Squires, 1995).

Untrained consumers have determined threshold concentrations of 1.0 and 0.2 μg/g fat for androstenone and skatole, respectively (Desmoulin and Bonneau, 1982; Bonneau *et al*., 1992; Bonneau, 1998). The percentage of pigs with levels above the thresholds ranged from 6 to 18% in four piggeries in Australia and New Zealand (Hennessy *et al*., 1997). King (1998) showed that sex had a major effect on the levels of both skatole and androstenone, but age at slaughter (90-120 kg liveweight) had no effect on the level of taint compounds in the fat. It is noteworthy that threshold levels for skatole with Singaporean consumers has been determined to be 0.03 μg/g (Leong *et al*., 2011). Although not reported, this finding suggests that the threshold for androstenone may also be lower for Singaporean, and other Asian, consumers. Consistent methods for sensory evaluation of boar taint are needed and a range in threshold levels in fat of 0.2 to 0.25 μg/g for skatole and 0.5 to 1.0μg/g for androstenone are being used in different studies (Lundström *et al*., 2009). Lunde *et al*. (2010) found that consumers who were sensitive to boar taint gave a lower liking score for pork with androstenone levels of 0.3μg/g during frying whilst for skatole, consumers differentiated skatole samples for flavour at 0.15μg/g – lower than the Norwegian threshold level of 0.21μg/g. The large range in threshold values for androstenone may be influenced by intrinsic differences in an individual's ability to detect androstenone depending on their origin, age, sex or sensitivity to androstenone, differences in methodology used to conduct sensory evaluations as well as analytical methods used in different laboratories to determine androstenone.

Intramuscular Fat Content

The eating quality of pork may be influenced by the level of intramuscular fat. Intramuscular fat consists of the lipid present in the perimysial connective tissue surrounding the muscle fibre bundles and influences eating quality by providing a smoother mouth-feel, reducing water loss during cooking, promoting saliva flow and improving flavour. Intramuscular fat can also reduce the force required to shear myofibrils and aid the separation of muscle fibre bundles during eating (Essén-Gustavsson *et al*., 1994).

Intramuscular fat typically constitutes 0.5 to 2.5% of muscle wet weight in the pork *M. longissimus* muscle and is related to carcase fat content. Positive effects on sensory attributes of pork of intramuscular fat content in excess of 2% have been reported in Denmark (Bejerholm and Barton-Gade, 1986; Touraille *et al*., 1989; Fernandez *et al*., 1999) and in France (Touraille *et al*., 1989; Fernandez *et al*., 1999). In the USA, Devol *et al*. (1988) suggested a threshold value for intramuscular fat content of 2.5 - 3.0% for optimum tenderness and reported that intramuscular fat content was most highly related to tenderness and shear force compared with other muscle characteristics evaluated (including flavour, juiciness, firmness, colour and connective tissue content). However, Fernandez *et al*. (1999) stated that intramuscular fat levels above 3.5% were associated with a high rejection score by French consumers due to negative effects of texture, taste and visual appearance. In Australia, Channon *et al*. (2001) found that the average intramuscular fat content of pork sourced from five large abattoirs was 0.98 ± 0.50%, with 74% of pork loins having intramuscular fat levels ranging from 0.5 – 1.4%.

Pale Soft and Exudative (PSE) and Dark, Firm and Dry (DFD) Pork

Irreversible anaerobic glycolysis occurs in the muscle after death due to the removal of oxygen. The concentration of glycogen present in the muscle at slaughter can influence the amount of lactate produced after death as well as the extent of the fall in muscle pH post-slaughter. The pH of living muscle is about 7.0 and declines in well-fed, rested animals to an ultimate pH of about 5.5 - 5.7 at 24 hours post-slaughter. Many studies have investigated the influence of the rate, and extent, of pH decline on PSE and DFD conditions rather than on pork eating quality *per se*. PSE meat results from a rapid rate of pH decline post slaughter whilst muscle temperature is still high (>35°C) causing protein denaturation and cell membrane leakage. Denaturation of myofibrillar proteins results in a loss in protein solubility

and water holding capacity whilst sarcoplasmic protein denaturation causes a loss in intensity of muscle pigment colour (Offer and Knight, 1988). PSE pork is paler and is defined as pork with a muscle lightness (L* value) > 50 and a drip loss exceeding 5% (Warner *et al.*, 1997). The incidence of PSE pork can be minimised by reducing pre-slaughter stress, removing pigs carrying the halothane gene from the herd and incorporating rapid chilling systems into pig processing plants. Aaslyng *et al.* (2007) reported, as part of a study to determine the effect of raw meat quality on consumer preferences for pork eating quality, that the odour and flavour of pork of low pH (<5.4) was rated as more sour and was less juicy compared with pork with a pH of >5.8. Furthermore, as juiciness was shown to have a positive influence on consumer preference, and sour taste had a small negative influence, it can be inferred that quality issues can be discerned from low pH pork.

DFD pork results from low glycogen reserves in the muscle at the time of slaughter resulting in ultimate pH greater than 6.0. Reduced glycogen stores are often associated with long lairage times (> 24 hours), mixing of unfamiliar and/or entire male pigs pre-slaughter, prolonged periods of transportation, extended time off feed, fighting, prolonged stress (D'Souza *et al.*, 1998; D'Souza *et al.*, 1999) and over-activity (Rosenvold *et al.*, 2002). The darker colour of DFD pork can be attributed to a higher proportion of reduced myoglobin because at a higher ultimate pH, oxygen uptake and utilisation is greater and less oxymyoglobin is formed (Fox, 1987; Rosenvold *et al.*, 2002). The dry surface appearance can be attributed to the water being tightly bound to the proteins as the proteins are highly charged since the isoelectric point of myofibrillar protein is lower than the ultimate pH (Judge *et al.*, 1989). DFD pork is defined as a surface lightness <42, a drip loss <5% and an ultimate pH >6.0 (Warner, 1994). DFD pork has a shorter shelf life as high pH meat is more susceptible to microbial spoilage than normal pork (Newton and Gill, 1981), however, DFD pork is generally more tender after cooking (Kauffman and Marsh, 1987).

Production Effects

Production effects including genotype, gender, halothane status, plane of nutrition and use of metabolic modifiers can influence the eating characteristics of pork. Increasingly, on-farm factors such as breed type, animal management and housing system are being stipulated by large supermarket companies around the world as well as being incorporated onto on-pack stickers at retail.

Gender

Ultimate pH - Numerous studies have compared the influence of entire males, surgical castrates and females on ultimate pH. Although no differences in ultimate pH between females and castrated male pigs have been reported (Bendall *et al.*, 1966; Prange *et al.*, 1979; Barton-Gade, 1987; Murray *et al.*, 1989), other studies have found that muscles from castrates had higher ultimate pH (Evans *et al.*, 1978; Eikelenboom *et al.*, 1989). Entire males generally have a higher ultimate pH, irrespective of the muscles considered (Moss and Robb, 1978; Gallwey and Tarrant, 1979; Tarrant *et al.*, 1979; Shorthose *et al.*, 1984; Lundstrom *et al.*, 1987). Differences in ultimate pH and final pork quality between entire male and female pigs could be attributed to pre-slaughter stress and behaviour. Entire male pigs tend to be more aggressive during lairage at the abattoir (Moss and Robb, 1978) and if unfamiliar entire male pigs are mixed, aggressive behaviour and fighting can lead to a greater depletion of muscle glycogen during the pre-slaughter period and thus result in a higher ultimate pH (D'Souza *et al.*, 1999). Under minimal pre-slaughter handling conditions of pigs, Brown *et al.* (1998) reported that carcases from entire males was judged by consumers as leaner, pork fat had a greater abnormal odour and cooked meat was darker in colour, less tender and less acceptable in comparison to females. However, pork from entire male pigs has also been reported not to be tougher than that from females (Malmfors and Lundström, 1983; King, 1998; Channon *et al.*, 2001; Bunter *et al.*, 2008) when compared at similar carcase weights.

Boar taint - European consumer studies have shown that the flavour and odour of pork from entire males is unacceptable to 21.5% and 32.5% of consumers, respectively, compared to 18.5 and 26% of consumers assessing meat from female pigs (Bonneau *et al*,.2000). It was concluded that for small differences in consumer dissatisfaction for flavour and odour between pork from entire male and females to be achieved, reductions in both androstenone and skatole are required. Importantly, it is likely that consumer dissatisfaction with pork odour and flavour from entire male pigs reported by Bonneau *et al.* (2000) may have been underestimated (Matthews *et al.*, 2000) as tests were conducted on pre-cooked samples and therefore consumers were not exposed to volatiles released during cooking. In general, skatole is more highly correlated with taint and overall negative taste than androstenone and more variation in odour score of pork from entire male and female pigs is explained by skatole, rather than androstenone (Lundström *et al.* 1988; Matthews *et al.*, 2000). Although skatole has a greater effect on odour than androstenone, both compounds are found to contribute similarly to flavour. Interestingly, pork from entire males with low levels of androstenone and skatole are less acceptable than pork from castrates, females or immunocastrated males (Font i Furnols *et al.*, 2008) suggesting that other compounds or factors may affect consumer acceptance of pork from entire males .

A consumer study in Canada compared the sensory quality of pork loin chops of Yorkshire entire male pigs divided into three groups based on the level of 16-androstene steroids in salivary glands (high, 56-114µg/g; medium

35-55µg/g; low 6- 26µg/g) with pork from females (Babol *et al*,.2002). Pork from entire male pigs with low levels of 16-androstene steroids was preferred to females, with higher consumer ratings also obtained for both texture and overall liking. Although the reasons for this were not clear, it was suggested that it may be due to improved tenderness and/or improved carcase leanness of pork from low 'boar taint' entire males compared with females. Jeremiah *et al*. (1999b) showed a higher proportion of unpleasant odours and flavours were present from cooked chops from entire males than those from castrates and females, however, texture was not influenced by gender when pigs were slaughtered at an average liveweight of 95.7 kg and 151.5 days of age.

Prevention of boar taint - Improvac®, a vaccine developed in Australia and manufactured by Pfizer Inc., is now being used to immunocastrate entire males in Australia to minimise flavour issues associated with the production of entire males, but this is not widespread. Worldwide interest in Improvac® use is increasing. In the EU, from January 1, 2012, surgical castration of piglets can only be performed with prolonged analgesia and/or approved anaesthetic methods and the procedure will be banned by January 1, 2018. The vaccine contains a modified gonadatropin releasing factor (GnRH) antigen in an aqueous adjuvant system that causes little tissue aggravation when injected. It requires the administration of two injections, one at 10 weeks of age and the other from 4-5 weeks (Dunshea *et al*., 2001) to two weeks (Lealiifano *et al*., 2011) prior to slaughter. A delay in administration of the secondary injection of the vaccine to two weeks pre-slaughter presents economic advantages to producers by both maximising growth rate advantages associated with testicular hormone production of entire males and through minimising feed costs, as immunocastration can result in increased feed intake. Immunocastrated male pigs therefore have lower levels of skatole and androstenone in subcutaneous fat due to the inhibition of GnRH. Immunocastrated males are leaner and grow faster than surgical castrates and entire male pigs, when the second dose is administered at either 15 or 19 weeks of age for pigs slaughtered at 23 weeks of age or at either 18 or 22 weeks of age for pigs slaughtered at 26 weeks of age (Dunshea *et al*., 2001). This was proposed to be due to reduced sexual and aggression activities of immunocastrated male pigs. In general, immunologically castrated males produce pork of equivalent quality to females and surgically castrated males, and is superior to that from entire males (Allison *et al*., 2009). Studies that have determined sensory quality of pork from immunocastrated males are shown in Table 1.

Table 1. *Summary of sensory studies investigating the eating quality of pork from immunocastrated male pigs (adapted from Allison* et al. *2009). All sensory scores have been converted to a 0-100 scale, where 0=dislike extremely (odour, flavour and overall liking), very tough and very dry and 100=like extremely (odour, flavour and overall liking), very tender and very juicy.*

Reference	Gender	Odour	Tenderness	Juiciness	Flavour	Overall liking
D'Souza and Mullan (2002)	SC*	62	65	63	64	64
	IC*	62	56	55	58	60
	F*	62	36	46	49	48
	SC**	58	55	51	54	60
	IC**	61	58	57	57	62
	F**	62	51	50	50	54
Hennessy *et al*. (2006)	IC	72	73	69	66	69
	SC	71	71	69	65	68
	F	75	71	67	66	69
Jeong *et al*. (2008a)	SC	21	74	73	75	75
	IC	20	75	73	74	75
Jeong *et al*. (2008b)	SC	26	79		78	78
	IC	33	77		76	76
	EM	31	76		76	75
	F	26	78		77	77
Silveira *et al*. (2008)	SC	73			75	79
	IC	77			80	83
Font i Furnois *et al*. (2008)	SC	69			71	
	IC	69			71	
	EM	60			63	
	F	69			69	
Font i Furnois *et al*. (2009)	SC			37		
	IC			38		
	EM			34		
	F			38		

SC, surgical castrate; IC, immunocastrate; F, female; EM, entire male; *Genotype A, >50% Duroc; **Genotype B, 25% Duroc

In the Philippines , studies have shown that overall preference and consumer intention to purchase pork from immunocastrated pigs is similar to surgical castrates and females (Hennessy *et al*., 2006). In Brazil, 66% of consumers prefer pork from immunocastrated male pigs compared with surgical castrates (Silveira *et al*., 2008). In Switzerland, Pauly *et al*. (2010) used a trained consumer panel and showed that boar odour and flavour was more intense in pork from entire males compared with surgical castrates and immunocastrated males, although there were no differences in tenderness.

Genotype

In Australia, the majority of commercial pigs produced for slaughter are Large White, Landrace and Large White/Landrace crosses, with a proportion of Duroc included in some terminal sire lines. Australian pig breeders have limited knowledge of the relative merit of individual sires or sire breeds on meat and eating quality traits (Bunter *et al*., 2008), so commercial producers have limited ability to make informed choices of sire selection to improve eating quality characteristics. Unlike tenderness, intramuscular fat content, colour and drip loss which have moderate variability, there is low variability in the eating quality attributes of pork (eg. juiciness, texture and flavour) through line selection (Sosnicki and Newman, 2010). There is less opportunity to improve eating quality traits such as flavour and juiciness through line selection (Barbut *et al*., 2008). PIC North America Inc. have included ultimate pH as a selection criteria to achieve pork with a pH>5.7 to eliminate PSE pork as well as improve quality attributes including drip loss, colour and tenderness (Sosnicki and Newman, 2010).

Durocs - Pork from Duroc pigs has been reported to be more tender (Thornton *et al*., 1968; Candek-Potokar *et al*., 1998; Bunter *et al*., 2008), more palatable (Wood *et al*., 1996; Jeremiah *et al*., 1999a), of better flavour (Martel *et al*., 1988; McGloughlin *et al*., 1988; Candek-Potokar *et al*., 1998), of higher pork flavour intensity (Wood *et al*., 1996; Jeremiah *et al*., 1999a) and juicier (Cameron *et al*., 1990; Cameron and Enser, 1991; Channon *et al*., 2004) than Large White, Landrace and their crosses. However, other studies have found no differences between Duroc, Large White, and Landrace sired pigs for tenderness (Martel *et al*., 1988; McGloughlin *et al*., 1988; Edwards *et al*., 1992; Lo *et al*., 1992a; Channon *et al*., 2004) and juiciness (Martel *et al*., 1988; McGloughlin *et al*., 1988; Purchas *et al*., 1990; Edwards *et al*., 1992; Lo *et al*., 1992b; Candek-Potokar *et al*., 1998). In Australia, the intramuscular fat content of loin muscles from 50 or 100% Duroc pigs is higher (1.6-3.6%) and consumers report it is juicier, compared with 0% Duroc pigs (~1.0% intramuscular fat; Channon *et al*., 2004; D'Souza and Mullan, 2002). This is in spite of the fact that the correlation between sensory juiciness and intramuscular fat content is weak (r=0.253; Channon *et al*., 2004). Bunter *et al*. (2008) reported that 25% Durocs have similar intramuscular fat levels to Large White pigs and lower than 50% Durocs and identified that significant variation exists in sire progeny groups within sire for desirable meat quality characteristics as well as between sire groups. Selection through choice of sire breed may not therefore be accurate due to within breed sire differences, so selection of pigs on the basis of percentage Duroc content alone may not necessarily result in the production of pork of superior eating quality. In general, the higher intramuscular content in Durocs is not phenotypically associated with improved meat quality (Hermesch, 1997).

Hampshires - Pork from the Hampshire breed is reported to be juicier and more tender than pork from other white breeds of pigs (Fjelkner-Modig and Persson, 1986; Purchas *et al*., 1990; Jeremiah *et al*,. 1999a). Intramuscular fat content of Hampshire pigs is 0.3 and 0.7% units higher than Landrace and Yorkshire breeds, respectively, but is fatter, and has the lowest average liveweight gain and highest feed intake (Fjelkner-Modig and Persson, 1986). Hampshire pigs generally have an elevated muscle glycogen compared with other breeds (Sayre *et al*., 1963) and Monin and Sellier (1985) referred to this condition as the 'Hampshire effect', which is thought to explain the superior eating quality. Pigs with the dominant RN– allele possess a mutation in the PRKAG3 gene that encodes the AMP-activated protein kinase (AMPK) γ3 subunit. AMPK is a major energy sensor in skeletal muscle influencing enzyme activity, gene and protein expression, fibre type, and mitochondrial biogenesis. The γ3 subunit is specific to skeletal muscle, is more highly expressed in fast "white" muscles and pigs with this mutation possess a 70% increase in glycogen in muscle, possibly in response to a modified osmolarity in the intracellular medium (Estrade *et al*., 1993), but not in liver and heart (Milan *et al*., 2000). This can detrimentally affect the water holding capacity of both fresh and processed meat. High cooking losses associated with the 'Hampshire effect' is caused by a very low ultimate pH, however this is not accompanied with a rapid fall in muscle pH or protein denaturation, as occurs with the development of PSE pork. The ability of γ3-mutant pigs to prolong pH decline may be related to an improved ability to buffer ATP levels or maintain ATP levels longer during the postmortem period as due to higher phosphocreatine levels compared to wild type (rn^+) pigs at 0 and 30 min post-slaughter (Copenhafer *et al*., 2006). Increased phosphocreatine levels could preserve ATP levels as it can act as an ATP buffer and delay postmortem glycolysis, whilst a lower phosphocreatine:creatine ratio increases AMPK activity and reduces creatine kinase activity (Scheffler *et al*., 2011). Although the Hampshire breed is not common in Australia, any selection programs that incorporate the Hampshire breed should ensure that the wild type allele (rn^+) is present.

Selection for lean carcases - Intramuscular fat is a carcase characteristic that is highly heritable (h^2=0.26-0.86, mean of 0.50; Hocquette *et al*., 2010). Selection and production of pigs with reduced levels of subcutaneous fat is likely to have negative effects on pork eating quality, particularly flavour and juiciness, due to a reduction in intramuscular fat content (Dikeman, 1996; Cameron *et al*., 1990). Also, with increased carcase leanness and reduced intramuscular fat content, the fatty acid composition of intramuscular fat becomes more unsaturated (Cameron *et al*., 1990). Saturated and monounsaturated fatty acids are positively correlated with flavour, juiciness, and to a lesser extent tenderness, whilst negative correlations are obtained between polyunsaturated fatty acids and eating quality traits (Cameron and Enser, 1991). A negative correlation between growth rate and intramuscular fat content has been found in Landrace pigs (Hermesch *et al*., 2000) although Lo *et al*. (1992a) and Suzuki *et al*. (2005) reported a positive correlation in Duroc and Landrace crossbred pigs .

The intramuscular fat content of loin muscles from European, British commercial, MLC and Danish pigs of four genotypes is reported to be 1.5 to 2.2% (Wood *et al*., 1979), 1.0%, 1.3% (Wood *et al*., 1995) and 1.44 to 1.93% (Støier *et al*., 1998), respectively. In Denmark, the highest intramuscular fat content is produced from progeny sired by Duroc boars with a high index for intramuscular fat (Støier *et al*., 1998). In Canada, the average intramuscular fat content in pork loins is 3.0% (on a wet weight basis), with 25% of samples having an intramuscular fat content less than 2.0% (Murray and Johnson, 1998). The Swiss pork industry improved pork eating quality by including intramuscular fat content into the selection index with intramuscular fat content increasing from 0.8% to 1.5% in the Swiss Landrace and from 1.0% to 1.7% in the Large White between 1987 to 1995 (Schwörer *et al*., 1995).

Halothane gene - Pigs can be genetically susceptible to porcine stress syndrome, a homozygous recessive condition, associated with mutations in the ryanodine receptor isoform located on the sarcoplasmic reticulum in skeletal muscle. This gene was originally detected upon exposure of pigs to halothane, with susceptible animals exhibiting a stress syndrome. Pigs with the gene are characterised by extreme muscularity, anxious behaviour, muscle tremors and reddening of the skin and the muscle is hypersensitive to stimulation by various stressors. The presence of the halothane gene causes an increase in calcium release from the sarcoplasmic reticulum. These changes in calcium concentrations activate muscle contraction and glycolysis and result in increased drip loss in pigs carrying the halothane gene (Greaser *et al*., 1969). Pigs carrying the halothane gene (either homozygous recessive (nn) or heterozygous (Nn) are generally leaner and faster growing than homozygous dominant (NN) pigs, but are more likely to produce pale, soft and exudative (PSE) pork than normal pigs (Briskey, 1964) due to a faster rate of pH decline post slaughter resulting in a higher drip loss and a lighter surface colour. A significant halothane genotype by weight interaction has been observed which may indicate that the dominance of the halothane gene increases with increasing liveweight (Sather *et al*., 1991a). Any effect of the halothane gene in Australian commercial slaughter pigs on pork quality is being managed, and minimised, through cross-selection programs of sire lines by breeding companies.

A recent meta-analysis of the effect of the halothane gene on pork quality has confirmed the significant effect of the halothane gene for pH45, pHu, L*, b* and drip loss; Nn animals are intermediate between NN and nn animals for these quality traits (Salmi *et al*,. 2010). Pigs with the halothane gene have been shown to produce tougher pork than normal pigs (Bejerholm, 1984; Boles *et al*., 1991; Sather *et al*., 1991b). No differences in shear force between Nn and normal NN pigs at one day post slaughter were reported by Channon *et al*. (2000), but by five days post-slaughter pork from carrier pigs was less tender compared with pork from normal pigs. This increased toughness of pork from carrier pigs following five days of ageing suggests a reduction in ageing associated with PSE muscles (Fernandez and Tornberg, 1994; Warner, 1994). According to De Vries and Plastow (1998), the best breeding strategy for breeding organisations is to ensure that all dam lines of slaughter pigs are free of the halothane gene, while tailoring the halothane status of the sires to suit individual requirements of specific breeding programs.

Industry implementation - Whilst it may be possible to select for superior meat quality characteristics, under the present system of assessment and marketing, information about such traits is not readily available to either breeders or producers. There is a need for the industry to rectify this situation, so that pig farmers can choose to use semen from boars known to produce superior quality pork. Selection for meat quality characteristics would certainly need to be balanced with selection for other production traits, particularly as payments based on quality are not yet received by producers (Hermesch *et al*., 2000). However, if the industry wants to produce pork known for assured eating quality, the ability to select for meat quality will be vital.

Housing Systems

Consumer demand for free range and organic pork products have increased over the past decade, however, the effects on pork quality due to housing systems appears to be equivocal. Niche outdoor production of rare pig breeds (eg. Berkshire, Wessex Saddleback, Large Black, Gloucester Old Spot, Tamworth) is increasing in Australia, primarily

targeting high end retail and food service markets. In considering the effects of housing systems on pork quality, we can only speculate on the possible mechanisms involved. The studies comparing housing systems are often quite applied, without a systematic approach to try to understand any differences. In comparing pigs reared in conventional indoor systems to those in an outdoor or organic system, the variables that must be considered include different diets, physical mobility and use of the musculature, exposure to the elements and seasons, exposure to disease and bacteria, access to plants and soil etc.

Pork from conventionally raised pigs has been found to be more tender (Enfält *et al.*, 1997; Danielson *et al.*, 1999) than pork from organic or outdoor production systems. The difference was attributed to lower daily gains in pigs raised outdoors negatively influencing proteolytic potential in the muscle at time of slaughter (Therkildsen *et al.*, 2002). Outdoor born and raised pigs have been found to have higher growth rates and lower shear force values than conventionally born and raised pigs (Gentry *et al.*, 2002; Stern *et al.*, 2003), although sensory tenderness was not affected by birth or rearing environment. It was suggested that the period from birth to weaning may have influenced muscle development and of other tissues. In contrast, others have found that indoor finished pigs grow faster than outdoor finished pigs in both summer and winter (Enfält *et al.*, 1997; Sather *et al.*, 1997). In their review of the effects of production systems on eating quality attributes of pork, Bonneau and Lebret (2010) concluded that pork quality was not improved when pigs were reared in welfare-orientated conditions, including outdoor systems, provision of additional space in conventional indoor systems and floor type. It is more likely that any differences that were seen can be attributed to diet or nutritional differences, which may influence intramuscular fat levels, or proteolytic activity of the muscle.

Some studies have reported higher intramuscular fat content in *M. longissimus* muscles from organically (Sundrum *et al.*, 2000) and outdoor raised pigs (Lebret *et al.*, 2006) while others have found no differences in eating quality or intramuscular fat content between free range and intensively fed pigs (Gandemer *et al.*, 1990; Dworschák *et al.*, 1995). The fatty acid composition is generally more unsaturated in the subcutaneous fat of organic (Hansen *et al.*, 2002; Oksbjerg *et al.*, 2005; Hansen *et al.*, 2006) and free range pigs (Hoffman *et al.*, 2003) compared to conventionally reared pigs, most likely due to differences in the diet. This may result in potential problems with oxidation, rancidity and warmed over flavours during storage and further processing. Nutritional management of organic and outdoor raised pigs needs to be closely considered to minimise issues in fat quality, whilst optimising growth rate, carcase composition and eating and technological quality. Outdoor pigs are generally less aggressive, particularly during lairage, compared with pigs raised indoors, reducing the level of unacceptable skin damage (Terlouw *et al.*, 2005; Barton-Gade, 2008).

The housing environment used for pigs can influence the level of skatole in subcutaneous fat of pigs. Pigs kept in dirty pens have higher skatole levels compared with pigs housed in clean pens. Stocking density can also affect the amount of skatole absorbed from the faeces. For example, pigs lying in pens in a mixture of faeces and urine at high stocking rates (0.6 m^2/pig) for at least one week had higher levels of skatole and indole in subcutaneous fat compared with those at a low stocking rate (0.8 m^2/pig; Hansen *et al.*, 1994). Maw *et al.* (2001), in a study comparing husbandry and housing of pigs on 23 Scottish farms, found that concrete floors resulted in bacon with the strongest taint and slatted floors the lowest, indicating that the accumulation of faeces and urine is most probable on solid concrete floors and least likely on slats. Season is also shown to play a role in skatole levels, with higher levels incorporated into the fat of pigs over summer compared to winter (Hansen *et al.*, 1994; Potgieter *et al.*, 1996).

Age at Slaughter

It is difficult to compare the effects of age at slaughter on pork quality separate from liveweight, as pigs are generally slaughtered on the basis of liveweight. It is well understood that pigs fed on a high plane of nutrition grow faster and reach targeted slaughter weights at an earlier age. A 50 day difference in the age of pigs or a difference of 20 kg (60 vs 80 kg) in slaughter weight of female pigs produces pork of comparable meat (Ognjanovic *et al.*, 1973; Channon *et al.*, 2001) and eating quality (Channon *et al.*,. 2001). Rhim *et al.* (1987) also found no relationship between meat tenderness and age at slaughter, over the range from 120 days to 320 days of age. In contrast, Candek-Potokar *et al.* (1996) found that although heavier pigs slaughtered at 130 kg liveweight (ranging from 212 days for *ad libitum* fed pigs and 243 days for 0.7 *ad libitum* pigs) had higher intramuscular fat levels, pork produced was less tender compared with pigs slaughtered at 100 kg liveweight (ranging from 161 and 187 days for *ad libitum* and 0.7 *ad libitum* fed pigs, respectively). Feed restriction at 0.7 *ad libitum* reduced intramuscular fat levels and increase drip loss at both liveweights compared to *ad libitum* feeding. However, intramuscular fat levels of pigs were similar in 130 kg pigs fed a 0.7 *ad libitum* diet and 100 kg fed *ad libitum*. As no effects of increasing age and weight were found for muscle lightness, drip loss or ultimate pH, the observed difference in tenderness may have been due to lower proteolytic activity and/or lower collagen solubility in pigs slaughtered at 130 kg liveweight.

Nutritional Management

Plane of nutrition and effects on growth rate and quality - Studies investigating feeding *ad libitum* versus restricted diets to pigs have mainly investigated effects on growth performance and carcase characteristics, with few studies reporting effects on sensory attributes. Therkildsen *et al.* (2004) showed that long term feed restriction in pigs negatively impacted on muscle growth by decreasing the rate of protein synthesis as well as the rate of protein degradation, increasing shear force. This was associated with higher activity of μ- and m-calpain in pigs fed *ad libitum* prior to slaughter compared with restrictively fed pigs, with no effect of plane of nutrition found on calpastatin activity, which regulates the activity of calpains. Blanchard *et al.* (1999) found that pigs fed *ad libitum* from 30 kg to 90 kg liveweight produced more tender meat compared with pigs on a restricted diet. Restricted feeding of organic cereals plus *ad libitum* silage resulted in tougher pork compared to conventionally raised pigs and pigs fed organic cereals *ad libitum* (Hansen *et al.*, 2006). Feed restriction of outdoor reared pigs also resulted in tougher pork compared with pigs fed *ad libitum* and reared either indoors or outdoors (Oksbjerg *et al.*, 2005). Feeding pigs at 0.7 *ad libitum* for slaughter at 100 and 130 kg liveweight did not influence pork quality compared to pigs fed *ad libitum* and this was suggested to be due to pork used in sensory studies being aged for four days post slaughter, as differences in myofibrillar fragmentation observed between 0.7 *ad libitum* and *ad libitum* fed pigs at one day post slaughter were not observed at four days post-slaughter (Candek-Potokar *et al.*, 1998).

Compensatory growth can increase tenderness compared with restricted growth (Blanchard, 1994; Therkildsen *et al.*, 2002; Kristensen *et al.*, 2004) and Therkildsen *et al.* (2004) suggested that the rates of both protein synthesis and degradation increase during compensatory growth and exceed levels in pigs fed *ad libitum* at slaughter. Animals finished on high energy diets have elevated rates of protein synthesis and degradation and this may accelerate proteolysis post mortem due to increased calpain activity resulting in improved tenderness (Kristensen *et al.*, 2002; Kristensen *et al.*, 2004). Correlations between growth rate and pork quality parameters have been reported to be small and negative, but the magnitude may be genotype-dependent (Bidanel *et al.*, 1994; De Vries *et al.*, 1994; Bidanel and Ducos, 1996; Oksbjerg *et al.*, 2000), whilst others have found no effect (De Vries *et al.*, 1994). Nissen *et al.* (2009) found no effects on shear force, μ and m-calpain activity, ultimate pH, drip loss or meat colour (except a* value) of finishing castrate and female litter mates to a set hot carcase weight of 80±5 kg and slaughter over a three week period. This is not surprising as the difference in growth rate would have been quite small. Split-sex feeding and the provision of diets which are finely tuned to the potential of the pigs are promoted by some (Castell *et al.*, 1994) and such practices have been adopted by Australian pig producers to improve production efficiency. The positive effects of rapid growth on meat tenderness are generally attributed to either a higher content of newly synthesized collagen (McCormick 1994) or to enhanced proteolytic activity (Kristensen *et al.*, 2002; Kristensen *et al.*, 2004), both of which are conducive to tenderness.

Diets to change intramuscular fat - The most successful nutritional strategy in non-ruminants to dissociate fat accumulation in muscle from that in other parts of the body has been demonstrated with subtle protein-deficient diets, resulting in improved juiciness of the meat (Hocquette *et al.* 2010; Bereskin *et al.*, 1978). Alonso *et al.* (2010) conducted a study to determine the effect of protein content of the diet (either 17% or 14.9% crude protein) on meat quality attributes of pork from entire male pigs. These diets resulted in different percentages of intramuscular fat (1.76 vs. 2.63%, respectively) in loin muscles and pork from pigs fed the lower protein diet was more tender, less fibrous and juicier than pigs fed the high protein diet, which may reflect the higher intramuscular fat content. Teye *et al.* (2006) showed that feeding an 18% protein diet to pigs increased total lipid to 2.8% in the *M. longissimus* muscle compared with 1.7% in pigs fed a 20% protein diet and tenderness and juiciness scores were also improved.

Witte *et al.* (2000) investigated the effects of dietary lysine level (0.48% or 0.64%) on the intramuscular fat content in the *M. longissimus* muscle in pigs reared indoors at either 18°C or 32°C and found that although pigs fed a lysine deficient diet produced pork with a higher intramuscular fat contents, no diet effects were found for shear force. D'Souza *et al.* (2008) reported that reducing the lysine to energy ratio increased the intramuscular fat content and improved consumer sensory scores for pork whilst feeding a Vitamin A restricted diet or a protein deficient diet (-15% or -30% protein:digestible energy) also resulted in an increase in intramuscular fat content (D'Souza *et al.*, 2003b). In addition, Witte *et al.* (2000) showed that environmental temperature did not influence intramuscular fat content or objective tenderness, but feed intake and average daily gain were lower and feed:gain was higher in pigs kept at 32°C compared to pigs housed at 18°C .

Diets to reduce skatole - The addition of non-digestible oligosaccharides, fructo-oligosaccharides or inulin, in the diet of pigs has resulted in a decrease in skatole levels in backfat (Claus *et al.*, 1994a; Hansen *et al.*, 2006). The inclusion of non-digestible carbohydrates results in increased carbohydrate fermentation in the hind gut rather than protein, thus lowering production of off-odour compounds including skatole due to reduced tryptophan fermentation. However, this is not viewed as a commercially applicable method of influencing the deposition of skatole by entire male pigs in

Australia due to both the cost of inulin supplementation as well as having no impact on androstenone concentrations. Studies have also been conducted to determine the effect of supplementation of pigs with animal (tallow, meat, bone and blood meal) and plant sources (linseed and soybean oil) of fat on skatole levels and mutton flavours of New Zealand pork (Leong *et al*., 2010; Leong *et al*., 2011). Garlic essential oil was added to try to overcome undesirable 'mutton' flavours in pork, but resulted in skatole levels in subcutaneous fat above the 0.2μg/g threshold level reported for the EU or the 0.03μg/g level for Singaporean consumers. Higher skatole levels were found in fat from female pigs fed diets containing blood meal, tallow and meat and bone meal. It was postulated that feeding garlic essential oil may have reduced liver metabolism of skatole and indole compounds, decreasing the removal of skatole and indole from blood and resulting in its increased deposition in subcutaneous fat.

Soy lecithin - Intramuscular connective tissue is an important contributor to toughness of lean meat (Bailey and Light, 1989; Lepetit, 2008), particularly as the degree of cross-linking of collagen increases. The solubility of collagen is more important than total collagen in influencing meat tenderness (Bailey, 1988). Proline and hydroxyproline are two major amino acids present in collagen and are positively correlated with the thermal stability of collagen, due to the stabilising effect of the added hydroxyl groups on the collagen triple helix. A reduction in the proportion of hydroxyproline in intramuscular collagen can improve tenderness of meat as the triple helical structure of collagen cannot form without hydroxyproline and cross linking cannot occur. Soybean lecithin contains polyenylphosphatidylcholine, an antioxidant that can inhibit the activity of prolyl-4-hydroxylase that hydroxylates proline. Polyenylphosphatidylcholine can also reduce the transformation of lipocytes into collagen-producing transitional cells and stimulate collagenases that can destabilize collagen.

D'Souza *et al*. (2005) showed that dietary lecithin supplementation improved eating quality of pork by reducing the objective measurements of chewiness and hardness and postulated this was due to an influence on collagen structure. Akit *et al*. (2011b; 2011c) found no effect of dietary soybean lecithin (4, 20 or 80 mg/kg feed) on growth performance, P2 fat depth, muscle depth, ultimate pH, drip loss, cooking loss or cholesterol or intramuscular fat levels. However, lecithin supplementation was shown to reduce dressing percentage by about 2% and result in paler pork, with a higher a* value, compared with non-supplemented pigs. Although lecithin treatment did not influence shear force, collagen stability appeared to be affected; with lower hydroxyproline levels, chewiness and cohesiveness values found in pork from lecithin-fed pigs compared to control pigs. Akit *et al*. (2011a) showed that dietary lecithin decreased gene expression of procollagen, indicating that collagen content and synthesis could be manipulated by soy lecithin supplementation to improve tenderness of pork. As a dietary ingredient, the addition of soy lecithin could be readily taken up by industry to reduce the contribution of collagen to pork toughness. A reduction in the chewiness component of pork could therefore improve cooked pork texture and overall consumer acceptability of pork. Further sensory work is being conducted to confirm these results.

Metabolic Modifiers

Porcine somatotropin - Administration of porcine somatotropin (pST) improves carcase composition through a reduction in adipose tissue growth and increased bone and muscle growth (Campbell *et al*., 1989). Increased protein deposition in pST-treated pigs results from an increase in protein synthesis rather than a reduction in protein degradation.

Administration of pST has been shown to reduce intramuscular fat content and increase protein content, but the majority of studies found little to no effect on cooking loss or sensory characteristics of pork (Bechtel *et al*., 1988; Thornton and Shorthose, 1989; Beermann *et al*., 1990; McKeith, 1993). D'Souza and Mullan (2002) reported lower consumer scores for tenderness, juiciness and overall acceptability for pST-treated pigs, whilst Moore (2009) reported that pST treatment at 5 mg/day for four weeks prior to slaughter did not affect shear force or sensory attributes of pork from surgically castrated pigs. A meta-analysis of the effects of pST on quality reported found that pST decreased intramuscular fat by 12%, increased shear force by 9%, reduced drip loss by 6% and reduced sensory tenderness by 9% (Dunshea *et al*., 2005).

Ractopamine - The inclusion of ractopamine in the diets of pigs increases carcase muscling and reduces carcase fatness through decreased lipogenesis, increased lipolysis and reduced protease activity. The response of pigs to ractopamine is most pronounced in the first two weeks and then declines (Dunshea *et al*., 1993) due to down-regulation of β-receptors. Early studies with ractopamine involved its use at 20 mg/kg feed to maximise carcase responses, but this dose does not present the best return on investment for Australian producers who finish pigs at lighter liveweights than those in the United States (Rikard-Bell *et al*., 2009). In Australia, ractopamine is generally included in pig diets at 5–10 mg/kg for 28 days prior to slaughter and its use provides producers with a tool for consistent delivery of pigs that meet required carcase specifications.

In a meta-analysis of pork eating quality, Dunshea *et al.* (2005) found that ractopamine used at 5 mg/kg feed increased shear force by about 0.5kg, decreased tenderness by 6%, had little effect on flavour (1%) and no effect on juiciness. However, increasing the inclusion rate of ractopamine during the latter stages of treatment did maintain the animal's response to ractopamine. Reduced tenderness as a result of treatment with β-agonists, such as ractopamine, is most likely due to reduced post-mortem enzyme activity, as a result of higher calpastatin levels. β-agonists also decrease tenderness of lamb (Koohmaraie *et al.*, 1991) and cattle (Miller *et al.*, 1988) and the mechanism is considered to be due to increased activity of calpastatin. This over-expression of calpastatin leads to inhibited proteolysis, inhibiting the activity of μ-calpain and m-calpain and results in tougher meat, as post-mortem tenderisation occurs through the actions of these calcium activated proteases. Moore (2009) showed that growth performance was improved, backfat at the P2 site was reduced and the meat was tougher, with lower intramuscular fat levels, by the use of ractopamine and pST compared to control and ractopamine-only treated pigs.

Pre-Slaughter Conditions

Although a large number of studies have been conducted to determine the effect of transport time, transport distance, time off feed and resting in lairage on technological quality attributes, including rate of pH and temperature decline post-slaughter, ultimate pH, drip loss and meat colour, there is little published data on eating quality effects. It is known that even when carried out with care, the transport and livestock marketing process is inherently stressful, with pigs subjected to a number of stressors at this time, including unfamiliar noise and odours; deprivation of food and water; sudden vehicle acceleration and braking; extremes in temperature and fighting resulting from mixing of unfamiliar animals (Warriss, 1987). Pigs stressed in this way for long periods pre-slaughter, can quite commonly exhibit low muscle glycogen levels at slaughter and the development of DFD. PSE is more likely to develop in pigs stressed immediately before slaughter which results in an increased rate of post-slaughter breakdown of glycogen to lactic acid. Negative handling immediately prior to slaughter can have a major influence on final pork quality as pigs have no time to recover from any stress imposed on them. Higher line speeds and forceful handling of the pigs may lead to increased stress and the resultant poorer meat quality (D'Souza *et al.*, 1998).

Processing Factors

The greatest influence on pork eating quality occurs from off-farm factors (slaughter, dressing, chilling, post-slaughter handling, ageing period, muscle, cooking, final internal temperature etc).

Stunning, Shackling, Scalding and Electrical Stimulation

In Australia, carbon dioxide stunning and electrical stunning are the two main forms of stunning used prior to exsanguination. The majority of research on pig stunning has concentrated on animal welfare, worker safety and technological pork quality, with little research investigating overall eating quality. Electrical stunning has been reported to increase the rate of pH decline, relative to carbon dioxide stunning without influencing shear force (Channon *et al.*, 2002; Channon *et al.*, 2003b), with similar glycolytic rates following electrical stunning to that of electrical stimulation observed (Barton-Gade, 1993). Improvements in tenderness at 24 and 48 hours post slaughter and increases in drip loss were observed following electrical stunning (head to heart) relative to carbon dioxide stunning by Rees *et al.*(2003).

The duration for processing a pig carcase from stunning point to chiller is about 30-45 min in most commercial abattoirs, however, this time can vary significantly (Eldridge *et al.*, 1993). D'Souza *et al.*(1998) showed that delays in carcase processing ranging from 35 min to 70 min, resulted in paler pork in both the *M. longissimus* and *M. biceps femoris*, without influencing drip loss. Effects on shear force or eating quality were not determined.

Electrical stimulation results in an acceleration of rigor development resulting from extensive membrane depolarisation and muscular contractions during stimulation (Aalhus *et al.*, 1994). Electrically stimulated carcases have been reported to require shorter ageing time than unstimulated carcases in order to reach an acceptable level of tenderness (Savell, 1979). However, issues with drip loss and increased PSE incidence had been reported in pig carcases stimulated with constant voltage systems (Taylor and Martoccia, 1995; Taylor *et al.*, 1995b; Bowker *et al.*, 2000). Electrical stimulation using a constant current system with 150 mA for 30 sec at 2 min post-slaughter has not been adopted by Australian pork processors, despite Channon *et al.* (2003a) showing that consumer scores for eating quality were improved, with no detrimental effects on drip loss, colour and PSE incidence, following electrical stimulation. Anecdotal evidence has indicated issues with increased carcase shrinkage from stimulated pigs.

Carcase Suspension

Eating quality of pork can be influenced by the method used to suspend or hang carcases pre-rigor. Hanging carcases from the aitchbone has been shown to restrain or stretch the *M. longissimus*, *M. semimembranosus*, *M. biceps*

femoris and *M. gluteus medius* muscles that are otherwise free to shorten on sides of carcases hung from the Achilles tendon (Harris and Shorthose, 1988). Channon *et al.* (2001) showed that the improvement in sensory tenderness scores was greater in magnitude for the pork topside than the loin, when carcases were aitchbone-hung. However, the adoption of aitchbone hanging on a substantial scale would require an innovative engineering approach to minimise labour input, address occupational health and safety concerns, change in muscle shape and develop techniques to minimise risks of bone breakages due to inaccurate splitting. Australian processors remain resistant to introduce aitchbone hanging on a routine basis. In those Australian beef abattoirs where aitchbone hanging has replaced Achilles tendon hanging as normal hanging practice, it is common for aitchbone-hung carcases to be re-hung to the Achilles tendon at about 20 hours post-slaughter or at least until *rigor mortis* has occurred. Aitchbone hanging of beef carcases has been included in the Meat Standards Australia system for both beef and lamb carcases. In these systems, the recommended ageing time to reach acceptable quality is reduced with aitchbone hanging and a greater number of muscles and carcases can be allocated to a higher grade.

Chilling

The rate of chilling of muscles post slaughter is influenced by the size, shape and fatness of the carcase as well as the temperature, relative humidity, velocity and flow pattern of the chiller air. Chilling rate can influence pork quality as it influences the rate of muscle pH and temperature decline of muscles. In contrast to beef and lamb, pH decline is more rapid in pork and the muscles are therefore more likely to experience elevated temperatures during the onset phase of rigor. Due to the poor thermal conductivity of muscle, it is difficult to substantially change the temperature of muscles deep in the carcase. The optimal temperature conditions for rigor development in normal muscle (often defined as when the muscle pH is about 6) is about 15-20°C, but if the muscle attains a temperature below 10°C before the onset of rigor while the muscle pH is >6.2, cold shortening will occur (Honikel and Reagan, 1987). As pork has a higher proportion of white, type IIb muscle fibres present which have a higher glycogen content and are more likely to exhibit fast rates of pH fall, rigor onset may commence very early post-slaughter and be completed at about 6 h post-slaughter (Savell *et al.*, 2004). Cold shortening is therefore not as significant a problem in pork as in beef and lamb.

Although rapid chilling within several hours post slaughter diminishes the exudative and pale characteristics of susceptible muscles (Honikel, 1986), this is not a practice undertaken by Australian processors. Australian abattoirs conventionally chill carcases in batch chillers set at 0-3°C for 12 to 18 hours (Channon and Weston, 2006; Sweet *et al.*, 2009) with varying chiller conditions (chiller temperature and air speed). Rees *et al.* (2003) investigated the influence of chilling rate on pork eating quality and found that slower chilling rates (chilling at 14°C rather than 2°C) significantly reduced shear force and improved taste panel responses for tenderness, juiciness and overall acceptability, without impacting on L* value and drip loss. These improvements were considered to be attributed to an increase in sarcomere length and greater myofibrillar fragmentation.

Danish pork processors use chiller tunnels and equalisation chillers to chill pig carcases. The chiller tunnel is set at -20°C and the carcases are rapid chilled for 110 minutes to achieve 10 or 15°C in the muscles of pig carcases, inducing freezing of the outer surface of the carcase. Carcases are then placed into equalisation chillers for 6 hours at 10 to 15°C followed by rapid chilling to 4°C. Such a process has been anecdotally reported to reduce shrinkage losses to less that 1% of hot carcase weight due to reduced evaporative weight loss. Rosenvold *et al.* (2010) found that stepwise chilling treatments improved tenderness of the *M. longissimus* without compromising quality attributes such as pH, drip loss, or ham processing yield compared with conventionally chilled carcases. Tenderness of *semimembranosus* muscles (assessed as processed ham) and shear force of *M. biceps femoris* muscle was improved by stepwise chilling, with these improvements considered to result from higher temperatures favouring proteolytic enzyme activity.

Post-Slaughter Ageing

The improvements in tenderness of pork during the first 1-2 days post-slaughter are rapid and then continue at a slower pace to plateau at around six days post-slaughter (Dransfield *et al.*, 1980-81). Increasing demand for boxed, vacuum packaged pork primals by supermarkets and food service operators has improved the ability of the Australian pork industry to increase the ageing period of pork. Ageing of individually vacuum packaged pork loin, or of carcases, for 4-7 days post-slaughter, improves sensory tenderness and shear force by 13-16% and 20-25%, respectively, compared to ageing for two days (Channon *et al.*, 2001; Walker and Channon; 2003). Extending the conditioning or ageing time for pork from four to 10-12 days increases the taste panel scores for tenderness, flavour intensity and overall liking and reduces the scores for intensity of abnormal flavours, compared with pork aged for only one day (Wood *et al.*, 1996; Taylor *et al.*, 1995a). Wood *et al.* (1996) reported that ageing pork for 10 days post-slaughter had a greater effect than both genotype (Duroc vs. Large White) and feed level (high vs. 0.8 high) in improving tenderness.

Tenderness of pork may also be influenced by ultimate pH. Eikelenboom *et al*. (1996) reported that shear force and sensory tenderness at three and seven days post slaughter could be related to ultimate pH. As shear force at seven days post slaughter was more highly correlated with ultimate pH than at three days post slaughter, ultimate pH may influence ageing rate as well as initial tenderness. More tender meat may be associated with a high amount of intra-myofibrillar water and a low amount of extra-myofibrillar water, which occurs during ageing (Pearce *et al*., 2011).

Moisture Infusion

The adoption of moisture infusion techniques has provided the Australian pork industry with an alternative means of consistently producing high eating quality pork and provided opportunities for companies to uniquely brand their products to food service and retail markets. Brines used for moisture infused pork consist primarily of water, antimicrobial and antioxidant agents (eg. lactates, citrates), phosphates and sodium chloride and are generally added at 5-12% of initial weight of fresh pork. The flavour of moisture-infused pork can be influenced by these different brine ingredients and their levels of inclusion, even in neutrally flavoured brines. Technical issues with pork flavour, including excessive saltiness, metallic, soapiness and bitterness, can arise due to the form and levels of brine ingredients used and must be addressed to ensure consumer satisfaction is not affected. The injection of a 3 or 5% polyphosphate solution into pork increased tenderness and juiciness of pork from female and entire male pigs compared with 0% polyphosphate. Injection of 10% polyphosphate did not show any further improvement in eating quality (Sheard *et al*., 1999). The size of this effect on tenderness and juiciness is large, particularly when compared with other factors including breed and feeding level, chilling rate, aitchbone hanging (Channon *et al*., 2001), electrical stimulation (Taylor *et al*., 1995a; Channon *et al*., 2003a), ageing period (Wood *et al*., 1996; Channon *et al*., 2004; Bertram and Aaslyng, 2007) and cooking temperature (Wood *et al*., 1995; Bejerholm and Aaslyng, 2003; Moeller *et al*., 2010). Moore (2009) showed that the interaction between moisture infusion and ageing period was not significant for any of the sensory attributes assessed by consumers, except sensory tenderness. Moore *et al*. (2009) also reported no interaction between the use of ractopamine and/or pST and moisture infusion post-slaughter on sensory pork quality.

Flavour and juiciness of the final infused product can also be influenced by ultimate pH and water holding capacity of the raw pork used. As moisture infused pork products are generally branded, both in Australia and in the USA, companies have imposed quality standards and specifications for raw meat used in the manufacture of moisture infused pork to optimise product quality and reduce the incidence and costs associated with excessive purge. As brine ingredients are added to fresh pork, moisture infused pork is considered a manufactured meat product, according to the FSANZ Food Standards Code, and must be labelled appropriately to differentiate it from non-infused pork at the retail level.

Packaging

Centralised packaging of pork in high oxygen (70-80% O_2: 20-30% CO_2) modified atmosphere (MAP) packs is becoming commonplace in Australia for supply of case ready pork into large retail supermarkets. It is well-known that ageing of pork in a vacuum bag will result in increased tenderness and flavour. Conversely, data for pork and beef has shown that storing meat in MAP containing oxygen can inhibit the tenderisation process. Lund *et al*. (2007) compared 70% O_2:30% CO_2 MAP and skin packaging of pork *M. longissimus* steaks stored for 14 days at 4°C and found that tenderness, juiciness and drip loss were negatively impacted by MAP. Similar effects of high oxygen environments have also been reported with beef *M. longissimus* steaks. Lagerstedt *et al*. (2011a) found that MAP steaks were rated by semi-trained panellists as less tender, flavoursome and juicy than vacuum packaged steaks following ageing for a total of five and 15 days. MAP steaks also exhibited higher water losses than steaks that had only been aged for three days post-slaughter. This has been confirmed again in beef by Lagerstedt *et al*. (2011b) and Kim *et al*. (2010).

High oxygen levels in MA packs is considered to result in oxidation of calpains, resulting in a slowed or interrupted proteolysis and/or the formation of intermolecular cross-links in myosin due to the formation of disulphide bonds from oxidation by free radicals (Lund *et al*., 2007). The implication of this on eating quality consistency needs to be considered given that large retailers are increasingly requiring meat to be centrally packaged in MAP for retail sale.

Cooking

Cooking is a key factor that can influence eating quality attributes of pork. Unfortunately, this is one factor over which the Australian pork industry as a whole has very little control, except through marketing efforts. Pork tends to be overcooked by Australian consumers due to unfounded food safety concerns associated with cooking pork to a rare, medium rare or medium degree of doneness. It follows that Australian consumers prefer their pork to be cooked through rather than pink in the middle (Stollznow, 2008). The desire to obtain a perfect crackling on rind-on pork products can also result in overcooking of pork due to the high oven temperatures used to optimise crackle quality.

In May 2011, the USDA revised the minimum internal temperature for cooking of all whole raw pork, beef, lamb and veal (steaks, roasts, and chops) to 62°C, from 71.1°C, recommending the use of a food thermometer, followed by a resting period of at least three minutes to ensure food safety and quality before carving or consuming. For minced products, the recommendation of cooking to 71.1°C, with no resting required, remains. The Australian pork industry's Eating Quality Assurance Project, used a final internal temperature of 75°C to produce cooked pork loin steaks to a medium-well-done to well-done degree of doneness, which is preferred by Australian consumers (Channon *et al.*, 2001). Topside roasts were cooked to an internal temperature of 70°C in a fan-forced oven set at 160°C.

The final internal temperature, together with the cooking procedure, has a large impact on juiciness, tenderness and flavour of pork. At internal temperatures between 40 and 50°C, tenderness declines due to the denaturation of myofibrillar proteins which results in a loss of fluid and shrinkage of the muscle fibres within the endomysial sheath. Tenderness declines further at internal temperatures between 60-70°C due to longitudinal shortening and denaturation of the endomysium and perimysium, which also results in water loss from muscle (Bailey, 1988). Juiciness can be described as the feeling of moisture in the mouth during chewing and is a combination of the moisture and fat chewed out of the meat and saliva production mixed into the meat. Juiciness is affected most by increasing internal temperature due to water and fat loss (Wood *et al.* 1995) but is also dependent on the raw meat quality and cooking procedure (Aaslyng *et al.*, 2003). The end point temperature of pork has a significant influence on eating quality, with pork cooked to an internal temperature of 60°C judged as being juicier and more tender than when cooked to 80°C (Simmons *et al.*, 1985; Heymann *et al.*, 1990; Wood *et al.*, 1995; Prestat *et al.*, 2002; Fjelkner-Modig, 1985). McComber *et al.* (1990) found that consumers preferred pork loin cooked to an internal temperature of 71°C, compared with 85°C, with the lower temperature producing a more juicy and tender product.

Cooking procedure, including oven temperature, has an impact on final eating quality. Pork roasts cooked to a similar final internal temperature of 65-67°C are juicier when a low oven temperature of 90°C is used, compared to using an oven temperature of 140°C (Bejerholm and Aaslyng, 2003). Pork is also juicier, with a lower cooking loss, when cooked at 90°C rather than at 190°C (Aaslyng *et al.*, 2003).

Initial assessment of juiciness of meat can be influenced by the intramuscular fat content which may directly or indirectly stimulate salivation (Wood *et al.*, 1986). Saunders *et al.* (1999) found that intramuscular fat had a positive effect on initial juiciness (which refers to the moisture released in the mouth from meat as a result of initial chewing) of roasted fillets, but did not influence any other sensory attributes of a variety of roasted and grilled pork cuts. Pork with higher intramuscular fat levels also cooked faster (on a weight for weight basis) and the extra fat may improve thermal contact between the meat and the heating surface.

The flavour and aroma of pork is associated with a large number of compounds and it is difficult to identify all of the compounds that contribute to these sensations. Off flavours are potentially the largest problem associated with pork, in particular, boar taint and lipid oxidation during storage. Wood *et al.* (1995) hypothesised that the higher cooking temperatures used in the UK could explain the lack of sex effect on flavour when pork from entire males and females is compared, as volatile compounds may have a smaller effect on abnormal flavour at higher temperatures. The aroma of cooked meat is much more pronounced than that of raw meat, with cooked meat aroma being affected by the cooking method, the meat cut used and the treatment of the meat prior to cooking. Increasing final internal temperature results in improved pork flavour for different cuts that are roasted, grilled or pan-fried (Heymann *et al.*, 1990; Wood *et al.*, 1995; Aaslyng *et al.*, 2003; Bejerholm and Aaslyng, 2003; Bryhni *et al.*, 2003). The intensification of pork flavour with increasing temperature is caused by a greater activity of the Maillard reaction between amino acids and reducing sugars, the thermal degradation of lipid and increased N- and S-heterocyclic compounds (Mottram, 1985; Mottram, 1992).

Different methods used to cook meat, including roasting, grilling, frying and casseroling/stewing, result in differences in surface temperature of the meat, the temperature profile through the meat and the method of heat transfer (Bejerholm and Aaslyng, 2003). Wood *et al.* (1995) found that the effect of internal temperature on juiciness of six-day aged pork was lower in roast cuts than in grilled steaks, and this was considered most likely due to the larger surface area of steaks. In a study comparing different cooking methods on pork cuts, Saunders *et al.* (1999) found that loin chops were slower to cook by roasting compared with grilling and frying, but eating quality was similar. The effect of endpoint temperature on meat textural changes was also shown to vary between different cuts, with loins tending to become harder due to moisture loss as the degree of doneness increased, whilst larger cuts softened due to thermal degradation of the muscle structure. For stir frying, a cooking time of only two to four minutes was recommended to maximise juiciness and tenderness. It is noteworthy that very few studies have investigated the effect of stir frying on the eating quality attributes of pork, other than Saunders *et al.* (1999). Lower juiciness levels of fillets and forequarter chops were found in grilled samples than in roasted samples, reflecting higher cooking losses. Bejerholm and Aaslyng (2003)

considered that tenderness differences observed between muscles (silverside and loin) at different internal temperatures could be due to differences in connective tissue content and solubility and its resultant influence on collagen shrinkage, solubilisation and myofibrillar protein hardening. Saunders *et al.* (1999) concluded that low endpoint temperatures should be used to maximise juiciness and flavour of pork when oven cooking; chops should be cooked to 65-70°C at 160°C and large roasts cooked at 70-75°C at 180-190°C.

Development of the MSA Beef Grading System

Through examination of the eating quality systems developed for beef and lamb in Australia, it is envisaged that this will assist in the development of an assured eating quality system for Australian pork. Thus the development of the MSA beef is described below and the MSA lamb grading system is described in the next section.

Pathway Approach

Variable beef eating quality was a concern to the industry in the 1990's and was thought to contribute to the declining consumption of beef (Polkinghorne *et al.*, 2008a). The Meat Industry Strategic Plan, tabled for the beef industry by Meat Research Corporation in 1996, identified that improved product description and marketing systems were needed to deliver a better eating quality experience to the consumer (Thompson and Polkinghorne, 2008) as grading schemes did not differentiate carcases on eating quality. In 1997, a proposed structure for a new beef grading scheme was discussed and the approach and terminology involved in Hazard Analysis of Critical Control Points (HACCP) from the food safety area was borrowed and labelled as PACCP. PACCP stands for Palatability Assurance at Critical Control Points and was first described by Morgan (1992). The objective of PACCP was to identify and carefully control those production and processing factors that have the largest effect on palatability, so that it is possible to predict the quality of the final product. It was recognised that Australia produces beef from a diverse base of climatic extremes, breed and animal management practices and processing facilities. A consumer testing protocol was developed (Watson *et al.*, 2008a) and the MSA research program began to collect data across the range of production systems, breed, nutrition, animal management systems from farm to slaughter and post-slaughter management strategies such as period of ageing. Those factors that correlated with eating quality were initially combined as fixed 'pathway' parameters. The first MSA carcase pathway was released in November 1997 and additions and modifications to the pathways were made as new results emerged. In 1998, the carcase based grading scheme was extended to become a cuts-based grading scheme.

The MSA carcase pathways scheme used, and still uses, a total systems approach whereby compliance with the Critical Control Points from the production, processing and value adding sectors were used to assign a carcase grade (ie. unsatisfactory, three star, four star, five star; Thompson *et al.*, 1999). The specifications for a carcase to be eligible for MSA grading were two-fold. Firstly, a general set of specifications was aimed at minimising quality losses during the pre-slaughter period and optimising the post-slaughter environment. For example, general recommendations on cattle handling and management and time off water and feed were given and carcases were required to be within a post-slaughter pH-temperature window. The pH-temperature window was defined by MSA and describes the pH and temperature fall post-mortem in the loin muscle (Thompson, 2002). It defines ideal criteria for pH-temperature decline so that the extremes of the window, where cold-shortening and heat-toughening occur, are avoided. Both cold-shortening and heat-toughening result in inferior quality. The second set of specifications were specific to individual grades and included factors such as breed, growth path, hanging method, chiller assessment traits and ageing times. This enabled the carcases to be sorted so that failure rates could be reduced to achieve the grade standards established from consumer testing. In recent years, specifications for carcases have been added as research data has been generated. The recent research on eating quality has shown negative effects of the use of hormonal growth promotants and selling cattle through saleyards and positive effects of the milk fed vealer category, thus these have been incorporated into the MSA system as specifications.

Consumer Research Required to Obtain Sensory Data

Eating quality is generally measured using one, or a combination of objective and sensory measurements. Sensory evaluation is performed by either a trained, or consumer (ie. untrained) taste panel. A consumer panel is unbiased, by definition, but has a wide variance. This is in contrast to a trained panel, which will have a narrow variance but will be biased by the training received. Conversely, objective tests for quality, including the shear force test for tenderness, whilst being relatively cheap, are simple one-dimensional measures of a complex set of interactions which occur when cooked meat is chewed and masticated in the mouth (Polkinghorne and Thompson, 2010) thus not reflecting the full consumer experience. The variance of a consumer panel can be reduced by averaging, thus an untrained consumer taste panel was considered the most appropriate method to score beef palatability characteristics as it had the ability to integrate all sensory dimensions (Thompson *et al.*, 1999).

For the MSA consumer panel, consumers were (and still are) recruited across socio-economic groups and each consumer was only ever used once. Four 100 mm lines are shown on the score sheet, which are anchored by the words, 'very tough/tender' for tenderness, 'very juicy/dry' for juiciness and extremely like/dislike for flavour and overall acceptability. To allow the sensory scores to be allocated to grades, the consumer was also asked to tick one of four grades; 'unsatisfactory' (no grade), 'good everyday' (3 star), 'better than everyday' (4 star) or 'premium quality' (5 star). A standard grilling procedure was used and each consumer gets seven warm steaks. The first sample is linked across consumers and tasting sessions in order to allow standardisation.

Initially, 212 consumers were used to score the sensory attributes of three different muscles, from three different experiments and 143 beef carcases (Watson *et al.*, 2008a). This data was analysed and used to develop a composite meat quality score (MQ4) and the specification of pathways to predict eating quality of these cuts was developed. The later development of the MSA model, for prediction of individual cuts, using consumer data from a large number of cuts and cooling methods, is described below.

Analysis of Data

In the initial stages of MSA, when a pathways approach and carcase grading was being used, only three cuts were verified for assured eating quality; being striploin (*M. longissimus lumborum*), rump (*M. gluteus medius*) and tenderloin (*M. psoas major*). For the first consumer tests, a score sheet with 13 line scales was used and these line scales described overall liking, liking of cooked appearance, liking of texture, tenderness, ease of first bite, ease of chew, hardness, juiciness, dryness, fatty taste, flavour, typical beef flavour and liking of taste (Watson *et al.*, 2008a). Principal components analyses showed that these 13 traits could be classed into four main descriptors of tenderness, juiciness, flavour and overall liking. This analysis also showed that the highest weighting should be given to tenderness, with lower weightings for flavour and overall liking, and the lowest weighting for juiciness. Thus a consumer scoring sheet to include only four line scales (tenderness, juiciness, flavour and overall liking) and four category boxes (no grade, 3-star, 4-star, 5-star) was developed and used in subsequent consumer panels. The composite score MQ4 was developed as there was a need to combine the scores into a single score for describing eating quality at an industry level. A linear discriminant analysis, with star (see above) as the category, was used to determine the weighting on each variable. This analysis gave linear functions which could be used to determine the cut-offs between the star categories. There were a number of iterations to determine the weighting on each descriptor and the final result was:

MQ4 = 0.4 x Tenderness + 0.1 x Juiciness + 0.2 x Flavour and 0.3 x Overall Liking.

The cut-offs for each grade were determined to be 48 for fail/3-star, 64 for 3-star/4-star and 80 for 4-star/5-star. Initially, when compliance was verified against palatability scores in the data base, it showed that if a carcase met, or exceeded the specifications for 3-star, then the risk of failure, based on MQ4 score, was 11% (Thompson *et al.*, 1999). If the carcase did not meet the 3-star specification, then the risk of failure increased to 29%, although 71% of the carcases that failed to meet 3-star were deemed acceptable by the consumer. This was recognised as a problem with the pathways system, as some carcases classified as failing (no grade), would have actually been acceptable (3-star). But this conservative approach did ensure minimal risk of a poor eating experience to the consumer.

Using this four-variable discriminant analysis, 68% of carcases were correctly classified. Setting the lower cut-off at 48 better protected the consumer from unsatisfactory product, although it did result in rejection of a higher percentage of product which was actually of 3-star quality. Further analysis showed the importance of having a standard approach to aberrant data. Thus the two highest, and two lowest, consumer scores for each trait were removed, prior to calculating the mean. This combined trait was called CMQ4, with the 'C' standing for 'clipped'.

Model Development

The MSA model development allowed a change from a carcase-based grading system, to a cuts-based grading scheme. This improved the accuracy of the prediction of eating quality and also recognised the eating quality of a greater number of high value muscles than just the striploin, cuberoll, tenderloin and rump.

The model building project was a large meta-analysis of data from a series of small experiments (Watson *et al.*, 2008b). The model development process sought to identify and quantify variables which assisted in describing the consumer tested outcomes. The data set analysed contained consumer evaluation of 32,237 muscle samples from 38 muscles (or muscle groups) across five cooking methods. The five cooking methods were grill, roast, stir-fry, thin slice and slow cook (although not all cooking methods were tested on all muscles). High connective tissue cuts were not grilled and low connective tissue cuts were not slow cooked. Most of the data was collected on the striploin (*M. longissimus thoracis et lumborum*) comprising 68% of all grilled muscles and 34% of all muscles tested by all cooking methods (Watson *et al.*, 2008b).

The full data set was mostly collected from unrelated experiments and thus Watson *et al*. (2008b) described it as observational data. As these different experiments were conducted using different abattoirs, this obviously increased the variance but was also considered to be representative of the range of environments throughout Australia. The objective was to derive a plausible and smooth model that described the data well. The aim of the modelling procedure was to try to find an overall smooth pattern which aligned with known meat science principles. The model production process involved trying effects in the model, and retaining effects if they commonly appeared in a range of analyses at a similar level. Effects that did not fit the common pattern were investigated, to ascertain why, then either included or removed. In the final model, variables which consistently added to the predictive ability were retained in the model whereas those which demonstrated no relationship were dropped (Watson *et al*., 2008b). The criteria that producers and abattoirs have to follow as well as grading specifications in order for a beef carcase to be eligible for MSA grading are described in Table 2.

Model Validation

An indication of the accuracy of the prediction model was given by applying it to the MSA dataset. For each meat sample, the observed MQ value was compared with the predicted MQ value. In general, the difference was less than one MQ4 unit. Analysis showed that the standard error for most of the predicted MQ scores was less than one. However, the standard errors are based on the assumption that the model is correct and thus must be treated cautiously.

The analysis by Thompson (2002) calculated whether the consumer grade, in terms of eating quality, aligned with the grade assigned by the model. The tests required consumers to score samples based on tenderness, liking of flavour, juiciness and overall liking. Consumers also scored products into eating quality grades as either unsatisfactory, good everyday or better than everyday eating quality. This showed that the accuracy of the model was about 50-70%. Using an independent dataset, Thompson *et al*. (2008) showed that accuracy of grade allocation was in the order of 59% and 53%, for Korean and Australian consumers respectively.

How Successful is the MSA Beef Program?

The number of beef carcases that are MSA graded has increased from about 28,000 in 1999-2000 to about 1.3 million in 2009-10 (MSA 09-10 Annual Report, http://www.mla.com.au). In 2010, MLA reported that for the previous 12 months, the average wholesale prices and retail prices for MSA beef were higher by 14% and 7.5% respectively, relative to non-MSA beef (MSA 09-10 Annual Report, http://www.mla.com.au). Robert Plush, a beef producer from Victoria, reported that the average premiums he received for reaching MSA grade standards since 2007 were 15-20¢/kg compared to over-the-hook prices and it did not cost him anything to join MSA (http://www.mla.com.au/livestock-production/producer-case-studies/changing-production-to-pursue-premiums). Thus, it is evident that MSA beef has been highly successful in the number of carcases graded and increased returns to producers, processors and retailers. Furthermore, the MSA pathways team won the International Meat Secretariat Millenium Prize in 2000 and the Australian Museum 2011 Eureka Prize for an interdisciplinary team. These awards reflect peer recognition of the science integrity underpinning the MSA beef system.

Table 2. *Details of criteria that producers and processors must follow as well as grading specifications in order for a beef carcase to be eligible for MSA grading (derived from Smith* et al. *(2008)).*

	Criteria	Model Input, Effect on Score and Eligibility for Grading
Cattle for MSA Grading	Supplied from registered MSA producer and accompanied by statutory declaration including criteria below	
	Maximum % *Bos Indicus*	Negative
	Whether cattle are classified as milk-fed veal (calves weaned immediately before sale)	Positive
	Whether the cattle ever been implanted with a Hormonal Growth Promotant (HGP)	Negative
	Time of departure from the farm	
	Whether the consignment has been sold through an MSA-accredited saleyard[1]	Negative
Transport	Industry code of practice	
MSA Abattoir	Must be MSA licensed and meet minimum conditions below	
	• Slaughtering MSA cattle within 36 h of despatch from the supplying farm	Excluded from grading if not meeting criteria
	• Not mixing groups in lairage	Excluded from grading if not meeting criteria
	• Operating slaughter equipment (including electrical stimulation) according to procedures that have been monitored to control the relationship of carcase pH and temperature decline within a defined window where the loin temperature at pH 6 is below 35°C and above 12°C.	Excluded from grading if not meeting criteria
Slaughter-floor Inputs	Hanging method – Tenderstretch or achilles	Positive if tenderstretch
	Carcase weight (kg)	Used with ossification to calculate 'weight for age'
	Sex (female or steer, bulls excluded)	Positive if female
	Vascular infusion	
MSA Grading	Hump height	Used to verify vendor declaration
	Marbling (USDA; 100-1100)	Positive
	Ossification (USDA; 100-590) <300	Negative
	pHu< 5.7 (dark-cutters are excluded)	Pre-condition for inclusion for grading
	Rib fat >3 mm	Pre-condition for inclusion for grading
	Exclude dark coloured meat, AUSMEAT colour score >3	Pre-condition for inclusion for grading
	Days ageing - 5, 14 or 21 day minimum, depending on model inputs	Positive
	Cooking method	

[1]Producers must be registered to supply cattle through licensed saleyards. Agents must be registered to supply cattle through the pathway. Agents and saleyard operators must go through a training course. Saleyard responsibilities for selling MSA cattle include clear identification of MSA cattle at all times, vendor declaration to accompany cattle, no mixing of unfamiliar cattle, soft-standing yards, access to water at all times, adherence to *MSA standards manual for saleyard consignment*. Processor responsibilities for slaughtering MSA saleyard cattle include slaughter of cattle within 36 hrs of despatch from farm, deduction of five points for saleyard pathway (see http://www.mla.com.au/Marketing-red-meat/Guaranteeing-eating-quality/Meat-Standards-Australia).

How Does Sheepmeat MSA Compare to Beef?

In 2000, Meat and Livestock Australia (MLA), with the support of research partners and the industry, designed a Sheepmeat Eating Quality (SMEQ) research program to define best practice procedures. The sheepmeat industry took a similar approach to that used in the beef industry, through the identification of critical control points for eating quality. This research covered all aspects of the supply chain on behalf of producers, processors, retailers and foodservice operators. Again, similar to the approach taken for MSA beef, a total consumer focus was the foundation of SMEQ research and development. The target was to accurately establish and satisfy consumer set standards.

Consumers rated the samples for tenderness, juiciness, flavour and overall linking (Thompson *et al.*, 2005). Whereas MSA beef asked consumers to give one of four rankings (unsatisfactory, everyday, good every day, premium), the Sheepmeat Committee decided to use three rankings – inferior, good everyday and excellent (called CEQ – consumer eating quality). A total of 108 grill and 108 roast samples, from six different muscles and from carcases ranging in quality traits, were presented to 60 consumers (Thompson *et al.*, 2005) and resulting scores and rankings collated and analysed. A simple linear function of the sensory variables (tenderness, juiciness, flavour and overall liking) that best predicted the CEQ perceived (Young *et al.*, 2005) by the consumers was derived (Pleasants *et al.*, 2005). Since 2000, over 45,000 consumer taste tests of lamb and sheepmeat products have been completed.

In contrast to cattle, where grading is conducted on an individual carcase, the sheepmeat program recognised that it was unrealistic to classify individual carcases. Thus, the predicted CEQ for sheepmeat was on a group basis, rather than on an individual sheep/carcase basis.

The main determinants of quality were described as 1) the age of the animal, described as lamb, hogget and mutton in the industry, 2) the pH-temperature window for a carcase, with the aim being to avoid cold-shortening through the use of electrical stimulation, 3) cut x cook method, 4) hanging method (tenderstretch vs. Achilles hung), and 5) ageing of the meat post-slaughter, which was predicated on market destination (Young *et al.*, 2005). It was recognised that high muscling sires can produce inferior quality, particularly tenderness (Young *et al.*, 2005) but this was not incorporated into a pathway.

Similar to MSA beef, there were generic recommendations around the importance of preventing stress and low glycogen levels, which could result in dark-cutting carcases. Thus, the recommendation was that sheep should have adequate nutrition to achieve liveweight gains of 50g/day in the pre-slaughter period (Young *et al.*, 2005). Recommendations for avoiding stress pre-slaughter were also specified. Several pathways for providing an assurance of quality were developed and the resultant predicted qualities are shown in Tables 3, 4 and 5. It was not considered necessary to develop an MSA model for sheepmeat, as the pathways adequately described the range in quality. By understanding and controlling the factors throughout the chain, the identification of SMEQ critical control points and the translation of these control points into practical steps, it is considered that the sheepmeat industry has the potential to improve average eating quality and reduce variability.

Assurance of the eating quality of sheepmeat through MSA was not formally implemented, until 2007. The number of sheep carcases MSA graded has increased from 10,000 per month in the first year of implementation (2007-08) to over 80,000 per month in the latest year with data (2009-10). As MSA sheepmeat has only been running for several years, no data is available for premiums to producers, processors or retailers.

Development of an Eating Quality System for the Australian Pork Industry

What Key Lessons Can the Pork Industry Take Room MSA for Red Meat?

A pathway approach, similar to that developed for the beef and sheepmeat Meat Standards Australia programs, is required for pork. This would allow effects of eating quality arising from factors including production, processing, post-slaughter handling, muscle/cut type and cooking to be considered. However, unlike beef, the Australian pork industry cannot afford the sizeable research and development investment required to undertake extensive eating quality research to develop the empirical equations to support the beef MSA system for pork. Thus, published and selected non-published studies are being used in the development of a database. Importantly, the majority of the eating quality research conducted on pork has not been conducted with a pathway approach in mind, with few studies reporting interactions of factors from across the supply chain. The underlying challenge therefore is how the Australian pork industry implements an effective, science based-system with scant resources.

Q-Pork Chains

The Q-Pork Chains project commenced in 2007 and was completed in October, 2011. It was a five year project with a budget of €14.5 million, funded by the 6th EU Framework Program and was coordinated by the University of Copenhagen, Denmark. The aim of Q-Pork Chains was to improve the quality of pork and develop pork products that meet the future requirements of global consumers. As part of Q-Pork Chains, predictive models were to have been developed for pork quality, but the models do not cover the entire pathway from production to consumption, but rather focus on specific influences including animal welfare, genetic background, production characteristics and vitamin supplementation. These models are not yet available to those outside of the Q-Pork Chains network. Meta-analyses have been conducted to determine the effect of vitamin E supplementation on α-tocopherol concentration and lipid oxidation in pork (Trefan *et al.*, 2010) and halothane gene on pork quality attributes (Salmi *et al.*, 2010). The criterion used for selection of publications by Salmi *et al.* (2010) included castrated or female pigs, known breed, pH 45, pHu, objective colour measurements (L*, a*, b*) and drip loss of the *M. longissimus* muscle, but not objective or sensory assessments of pork eating quality.

Table 3. *Details of criteria that producers and abattoirs must follow in order for a sheep carcase to be eligible for MSA grading (http://www.mla.com.au/Marketing-red-meat/Guaranteeing-eating-quality/Meat-Standards-Australia).*

	Criteria
MSA Eligible Sheep Categories	LAMB – Female, castrate or entire male animal that has 0 permanent incisor teeth (~12 months)
	HOGGET – Female or castrate male that has 1 but no more than 2 permanent incisor teeth and, if male, has no evidence of secondary sexual characteristics (10-18 months)
	MUTTON - Female or castrate male that has at least permanent incisor tooth and, if male, has no evidence of secondary sexual characteristics (>10 months)
Sheep for MSA Grading	Sourced from a registered MSA producer and accompanied by statutory declaration, including criteria below, which must continue to saleyard/processor
	Minimum fat score 2
	Recommended growth rate of >150g/day at least one month prior to processing
	Sheep not to be shorn within 2 weeks prior to processing, >5mm wool length
	Separate pens for different age categories
	Separate pens for Merinos and Merino-cross breeds
	Access to water at all times other than time required for sale
	Must be processed within 48 hrs off feed
	A minimum of 2 weeks at consignment property before dispatch.
	•Maximum time in transit of 24 hours
Saleyards[1]	1st and 2nd Merino cross are accepted through saleyards.
	Greater than 50% Merinos or pure Merinos may be accepted through saleyards providing the processor can demonstrate animals through this pathway meet pH/temperature window and ultimate pH requirements as outlined in the MSA Sheepmeat Standards Manual
Direct Consignment	All categories eligible
Transport	Industry code of practice must be followed
MSA Abattoir	Must be MSA licensed and meet minimum conditions below
	Plants must have Quality Assurance systems in place to meet MSA standards
	Plants must be AUSMEAT accredited
	Lairage - The total time in lairage must be monitored to ensure total time off feed prior to slaughter, including transport, does not exceed 48 hours. Total time off water is to be less than 24 hours
	If livestock are held over in a holding paddock and fed at the processing plant, the processor must demonstrate that animals through this pathway meet pH/ temperature window requirements and pHu requirements as outlined in MSA Sheepmeat Standards Manual
	Lot and carcase identification - Verification between lot ID and individual carcase ID is required for traceback and verification purposes
	Operating slaughter equipment (including electrical stimulation) according to procedures that have been monitored to control the relationship of carcase pH and temperature decline within a defined window (see Table 4). The rate of carcase pH and temperature decline is measured, taking into account electrical inputs and chilling rate. The temperature at which the carcase enters rigor (pH6) is critical when determining and optimising eating quality
Ageing	The ageing rate of the carcase is dependent on chilling rates and storage temperatures. Factors including the pH window and hanging method also impact on how well the carcase will age in relation to eating quality

[1]Producers must be registered to supply sheep through licensed saleyards. Agents must be registered to supply sheep through the pathway. Agents and saleyard operators have to go through a training course. Saleyard responsibilities for selling MSA sheep include clear identification of MSA sheep at all times, vendor declaration to accompany sheep, no mixing of unfamiliar sheep, soft-standing yards, access to water at all times, adherence to *MSA standards manual for saleyard consignment*. Processor responsibilities for slaughtering MSA saleyard sheep include slaughter of sheep within 36 h of despatch from farm (see www.msagrading.com.au).

Table 4. *Processing and ageing conditions for sheepmeat for optimum eating quality in different markets. Source: Sheepmeat information kit at http://www.mla.com.au/Marketing-red-meat/Guaranteeing-eating-quality/Meat-Standards-Australia/MSA-lamb-and-sheepmeat.*

	Target market			
Conditions	Domestic chilled trade		Domestic or export chilled trade	Frozen
Hanging method	Tenderstretch	Achilles	Achilles	Achilles
Electrical stimulation needed	No	Yes	No	Yes
Enter rigor (pH 6) at:	8-35°C	18-25°C	8-18°C	18-25°C
Minimum ageing period	5 days*	5 days*	10 days	5 days before freezing
Storage temperature	1°C	1°C	-1°C	1°C then -18°C

*This is the optimum time to maximise sheepmeat eating quality. For most domestic/short markets, this is not feasible. Optimum quality will take five days. **Provisional results. Lower limit may be reached.

Table 5. *A summary of the sheepmeat cuts and cooking methods eligible for quality assurance for lamb (L), hogget (H) and mutton (M) in the MSA sheepmeat program, once all standards have been met. Note: a blank cell indicates that the cut/cooking method would be classed as not eligible for grading. Derived from the sheepmeat brochure at http://www.mla.com.au/Marketing-red-meat/Guaranteeing-eating-quality/Meat-Standards-Australia/MSA-lamb-and-sheepmeat.*

	Specification[1]	Grill	Roast	Stir-fry	Casserole
Leg - Chump on or off	4800, 4820, 4821		**L H**		
Shoulder	4980, 4994, 5050				
Forequarter – boneless	5047				
Neck fillet roast	5059				
Eye of shoulder	5151				
Chump (rump)	5074	**L HM**	**L HM**	**L H**	**L H**
Loin	4860		**L HM**		
Shortloin	4880				
Shortloin noisettes		**L HM**	**L HM**		
Topside	5073			**L H**	
Silverside	5071			**L HM**	
Outside	5075				
Chump chop	4790	**L HM**			
Shortloin chop					
Rack cap on/off - cutlet	4930				
Tenderloin	5080, 5081, 5082				
Backstrap	5109, 5153, 5150	**L HM**		**L HM**	
Eye of rack					
Eye of shortloin					
Foreshank	5030				**L**
Hindshank	5031				**L H**
Breast and flap	5010	No recommended cooking methods			
Neck	5020				
Spareribs	5015				

[1]Handbook of Australian Meat – specifications of cuts(http://www.ausmeat.com.au/custom-content/preview/ham/pdf/Sheepmeat.pdf)

Modelling of existing knowledge on animal welfare is also being conducted to predict technological and sensory pork quality based on genetic background, production characteristics, animal treatment and slaughter effects. Work is also being conducted to investigate whether pork from pigs disposed to pre-slaughter stress can be detected immediately post-slaughter and involves detection of stress related markers in blood collected at sticking.

Pork Eating Quality Assurance Program 1998-2001

Studies conducted as part of the Eating Quality Assurance Program for Australian pork identified that post-slaughter factors including correct cooking procedures (cooking to an end point temperature between 65-71°C), moisture infusion and ageing to improve tenderness, carcase handling (including tender stretching and low voltage constant current electrical stimulation) and sex of the pig had the greatest impact on reducing variability in pork eating quality (Taverner, 2001). Channon *et al*. (2001) showed that the incidence of unsatisfactory pork was reduced from 25% to 5% when a combination of the production and processing recommendations were implemented. Based on the parameters investigated by Channon *et al*. (2001), the effect of production factors were shown to be of lesser importance compared with processing effects on overall liking scores of consumers for pork loin steaks and topside roasts (Table 6). This was determined from mean overall liking scores for different factors, with larger differences between these means indicating a greater improvement in overall liking of pork.

Table 6. *Effect of production and processing factors on the size of the effect (Little, Moderate, Large) on consumer ratings of overall liking score of pork loin and topside steaks (Channon et al., 2001).*

Factor		Muscle	Tested variables	Size of effect on average overall liking scores		
				Little < 1.0	Moderate 1.1-5.0	Large 5.1-10.0
Production						
	Carcase weight	Loin	60 kg vs. 80 kg	✓		
		Topside	60 kg vs. 80 kg	✓		
Sex		Loin	Entire male vs. Female	✓		
content	Intramuscular fat	Loin	0% to <3.0%	✓		
Processing						
	Abattoir	Loin	Same vendor		✓	
		Loin	Multiple vendors			✓
	Muscle pH	Loin	pH < 5.4 vs. pH > 5.6	✓		
	Hanging method	Loin	Tenderstretch vs. Achilles		✓	
		Topside	Tenderstretch vs. Achilles		✓	
	Ageing period	Loin	7 d vs. 2 d post-slaughter		✓	
		Topside	7 d vs. 2 d post-slaughter			✓
	Hanging method	Loin	TS + 7d age vs. A + 2 d age			✓
	x Ageing period	Topside	TS + 7d age vs. A + 2 d age			✓

D'Souza *et al*. (2003a) reported outcomes of the *Select Pork* alliance that involved implementing eating quality interventions at the producer and processor level in two stages. The incidence of consumers rating pork as being below average, or the pork eating quality 'fail rate', was 30%, 15% and 3% for generic pork, *Select Pork* (Stage 1) and *Select Pork* (Stage 1 and 2), respectively. The Stage 1 pathway stipulated i) Halothane free pigs, ii) pigs with minimum of 50% Duroc sire lines, and iii) no entire males (pork from immunological castrates, surgical castrates and females only) and Stage 2 involved moisture infusion of fresh pork. *Select Pork* (Stage 1 and 2) was rated as having the best flavour, juiciness, tenderness, overall acceptability and quality grade by consumers (Table 7).

Table 7. *The effect of the* Select Pork *eating quality pathway on the consumer sensory quality of the* M. longissimus thoracis *muscle (D'Souza et al., 2003a).*

Brand	Generic Pork	*Select Pork* (Stage 1)	*Select Pork* (Stage 1&2)	LSD	Significance
Aroma[1]	55	63	57	6.54	0.002
Flavour[1]	54	66	76	6.11	<0.001
Juiciness[1]	43	58	75	6.85	<0.001
Tenderness[1]	41	59	75	7.40	<0.001
Overall acceptability[1]	48	64	76	6.67	<0.001
Quality grade[2]	2.9	3.5	4.0	0.279	<0.001

[1]Acceptability score (line scale); 0 = dislike extremely and 100 = like extremely. [2]Quality grade; 1 = unsatisfactory, 2 = below average, 3 = average, 4 = above average, 5 = premium. LSD, least significant difference.

Several studies have shown that consumers' overall liking of pork is predominantly influenced by flavour, followed by tenderness and juiciness (Wood *et al.*, 1986; Cameron *et al.*, 1990; Channon *et al.*, 2004). Channon *et al.* (2001) identified that overall liking of pork loin steaks from female pigs by consumers was influenced, in order of importance, by flavour, tenderness, odour and juiciness. The regression equation, that explained 87.7% of the variation in overall liking, was:

Overall liking = -5.41 + 0.533xFlavour + 0.337xTenderness + 0.120xOdour + 0.114xJuiciness.

For the pork topside, 95.3% of the variation in overall quality of pork topsides from 100 sides was influenced, in order of importance, by flavour, juiciness and tenderness. The regression equation was:

Overall quality = -0.91+ 0.602xFlavour + 0.215xJuiciness + 0.201xTenderness.

These analyses confirmed the importance of flavour on overall liking of pork, but the results for the topside were different to those for the loin, particularly the order of importance of juiciness and tenderness and the non-significance of odour. Possible reasons for these differences remain unclear but it is possible that differences in cooking method used, connective tissue and/or intramuscular fat content, for each cut may account for some of these differences. Channon *et al.* (2004) reported that the overall quality of pork loin steaks from female pigs of varying Duroc content (with standard errors for each term in brackets) could be described by:

Overall liking = -1.82(2.83) + 0.754 (0.064) x Flavour +0.295(0:040) x Tenderness (R^2 87:6%).

Juiciness was not a significant term in this prediction equation. Aaslyng *et al.* (2003) reported that consumers were influenced by flavour as well as tenderness and juiciness in their liking of pork. This underlines the importance of continued work within all areas of eating quality, rather than just focussing on one attribute.

Development of predictive equations

The variability in eating quality of Australian pork is still an issue of concern for the industry. In contrast to the red meat industry where the MSA grading system has been implemented to predict eating quality (Polkinghorne *et al.*, 2008b), the Australian pork industry has relatively underdeveloped systems for the systematic prediction of pork eating quality. Work to date has attempted to quantify the impact of key critical control points using a pathway approach that can influence eating quality attributes of fresh pork, with a view to implementing pathway interventions to improve the consistency of pork eating quality. An extensive database of previous research that reported effects on pork eating quality has been compiled (Channon *et al.*, 2011b). The majority of studies were obtained from peer-reviewed journals and several unpublished final reports from previous Australian pork quality research (Saunders *et al.*, 2000; Channon *et al.*, 2001).

The approach taken by Channon *et al.* (2011b) was to arbitrarily set parameters for a 'standard' Australian pig (Table 8). Linear and non-linear regression analysis (using a three parameter equation $y_0 = a/(1+\exp(-(x-x_0)/b))$; where x_0 describes the midpoint of the sigmoid curve is along the x axis; *a* describes the height of the curve; b describes the steepness of the transitional part of the sigmoid curve and y_0 is the y intercept) were undertaken for single-order relationships comparing different variables within each parameter with that for the 'standard' pig for sensory tenderness, flavour and juiciness. Linear regression models implied that gender, genotype class, halothane gene, nutritional management and the use of metabolic modifiers, ageing period, moisture infusion, internal temperature, cooking method and muscle influenced eating quality attributes when compared with the variables set for the 'standard' pig. The amount of variation explained by the linear and non-linear regression equations was similar for the effect of various production, processing, post-slaughter and cooking factors on eating quality attributes (namely tenderness, flavour and

juiciness), but work is still required to validate the sigmoidal relationships. Overall, the effects of production factors were shown to be of lesser importance compared with post-slaughter effects on sensory scores. Lack of sensory data for pre-slaughter factors (eg. mixing, handling, transport) did not allow analyses to be conducted. The relationship between CO^2 and electrical stunning for all sensory attributes was low (R^2 >0.05). The effect of gender on odour was not possible to evaluate given that few studies investigating boar taint with sensory results have included females. As Australian pork processors use batch chilling, there is no comparable data available for different chiller management practices on pork eating quality. Hanging method and electrical stimulation were not evaluated as potential interventions to improve eating quality in this current analysis, given that they are not favourably viewed by Australian processors.

Channon *et al*. (2011b) highlighted that more data are required to better quantify the relationship between immunocastrated males and females. Interactions between gender and nutritional management (including the use of metabolic modifiers) on eating quality do exist but the extent of these interactions were difficult to estimate, as few studies report effects on eating quality in addition to growth performance, carcase composition and fatty acid composition effects. Consideration of interactions with genotype, nutrition and other environmental effects are also required. Further work is also needed to understand the contribution of low ultimate pH to inconsistent pork eating quality given that there is neither an established pH/muscle temperature window nor ultimate pH cut-offs developed to optimise eating quality of pork. When compared with other pathway parameters, moisture infusion was found to have the greatest impact on sensory tenderness and juiciness, followed by ageing for 6-10 days post-slaughter.

The interaction between moisture infusion and ageing period was not significant for any of the sensory attributes assessed by consumers, except sensory tenderness (Moore, 2009), whilst the interaction term of hanging method x ageing period was not significant for sensory quality of the loin and topside muscle (Channon *et al*., 2001). Also, no interaction between Duroc content and ageing period of female pigs for sensory quality was found (Channon *et al*., 2004). This suggests that the inclusion of moisture infusion, hanging method and ageing period into a pathway model to improve pork sensory quality could each be additive factors. Individual equations generated (Channon *et al*., 2011a) cannot be used as multiplicative factors to account for situations where a number of quality interventions are imposed, given the statistical complexity in accounting for standard errors associated with each equation. Interactions between cooking method, final internal temperature, cut type and muscle on pork eating quality were not elucidated from the literature. Furthermore, many studies have only reported effects on the *M. longissimus* whilst variations in connective tissue content and solubility, intramuscular fat content and composition and ultimate pH can also influence sensory quality of cooked pork. To enable the system to be cuts-based rather than carcase-based, these relationships need to be better understood and quantified, as well as interactions between muscles aged for different periods from different genders, to fill knowledge gaps.

Table 8. *Arbitrary parameters set for the 'standard' Australian pig.*

Parameter	Variable (x)	Comparisons made (y_0)
Sex	Female	Entire male, Surgical castrate, immunocastrate
Genotype group	White	Duroc-sired, Hampshire sired
Halothane gene	Normal (NN)	Nn, nn
Housing	Indoor/conventional	Outdoor
Nutrition	*ad libitum*	Restricted
Metabolic modifiers	none	pST, ractopamine
Handling	minimal	negative
Mixing	not mixed	mixed
Stunning	CO^2	Electrical
Intramuscular fat	1.0-1.49%	0.5-0.99%, 1.5-1.99%, 2.0-2.49%, 2.5-2.99%, 3.0-3.49%, >3.5%
Ageing period	1-2 days	3-5 days, 6 to 10 days, > 11 days
Moisture infusion	None	moisture infusion
Internal temperature range	70-74°C	65-69°C, 75-79°C, ≥80°C
Muscle	*M. longissimus*	*M. semimembranosus, M. semitendinosus, M. biceps femoris, M. gluteus medius, M. psoas major*
Cooking method	Grill	Roast, Fry

Benefits to Industry

The benefits and costs associated with implementing an eating quality system into the Australian pork industry have not yet been determined. To evaluate the feasibility of an integrated program linking production management, processing factors and further handling of meat on the overall quality of the final product, industry requires information on the benefits and costs of such a system. If these costs are passed on to consumers, then information on their willingness to pay is needed to analyse the benefits and costs of such an integrated program. Estimating a consumer's willingness to pay is a common method used to provide information regarding the potential benefits and costs associated with particular activities.

Sanders *et al*. (2007) used contingent validation methods to determine consumer willingness to pay for US pork on credence and taste related attributes. The quality characteristics that consumers are prepared to pay for were leanness, juiciness and tenderness, with about 50% of consumers indicating that they were willing to pay premiums for juiciness, leanness and tenderness. Consumer willingness to pay (WTP) was influenced by prior purchasing behaviour, perceived importance of the quality related attributes and the level of price sensitivity. However, it was found that 62% of consumers were already purchasing pork that was branded or was a premium pork product. Overall, a premium of $0.20/lb was found for marbling, extending to $0.37/lb for tenderness and juiciness.

Lyford *et al*. (2010) reported willingness to pay outcomes for beef quality grades in Australia, Japan, Ireland and USA as part of the MSA beef program. At the completion of the MSA beef consumer evaluation studies, consumers were asked to consider their willingness to pay for the beef they had just graded as either unsatisfactory (2 star), good everyday (3 star), better than everyday (4 star) and premium (5 star) using discrete prices and/or a continuous line scale. It was found that all consumers rated 'unsatisfactory' or 2 star beef at about half of the value of 'good everyday' 3 star beef. Overall, Japanese consumers were found to be prepared to pay an increasing premium with increasing quality grade, with 4 and 5 star beef valued at 1.7 and 2.9 times the WTP price assigned to 3 star product. Australian consumers however were least likely to pay more for 4 and 5 star beef. MSA quality grade was the most significant variable that influenced consumer willingness to pay for beef. Griffith *et al*. (2009) stated that greater economic benefits would be attained if retailers utilised the full MSA system rather than using it as a tool to differentiate between unsatisfactory and good everyday (3 star) beef. Lyford *et al*. (2010) also reported an interaction between WTP estimates and age, with younger Australian consumers prepared to pay more for higher quality beef than older consumers. Griffith *et al*. (2009) estimated the cumulative economic benefit of the MSA beef system at the retail level from 2004/05 to 2007/08 to be $300 million, with total research and development and implementation costs of $74 million and a current annual benefit of $57 million. As there have been additional costs borne by industry in implementing beef MSA, the estimated industry benefit:cost ratio in 2007/08 was at least 2:1.

The challenge for WTP studies for pork is the design of the studies to ensure that data obtained is realistic and captures consumer preparedness to pay for pork of high, consistent eating quality. This would enable the industry to determine and implement marketing activities required to generate consumer demand and awareness of premium Australian pork. To assist with determining WTP for Australian pork, it is recommended that all consumer evaluation questionnaires include this component. For future data analyses purposes, these questionnaires should be not be changed to ensure that data obtained across studies can be pooled. If this is the approach taken for pork, it must be recognised that data obtained would be based on survey data rather an actual consumer purchasing behaviour. MSA beef sensory evaluations also recruited on the basis of consumer preference for medium degree of doneness and meat was consistently cooked to medium. It will be interesting to determine whether WTP studies for pork concur with findings of Lusk *et al*. (2001), who found that consumers who preferred beef steaks cooked to a higher degree of doneness were less likely to prefer or value tender beef steaks.

The ability of the Australian pork industry to implement a non-prescriptive eating quality system will potentially be eased due to the high degree of vertical integration of the major pork production, processing and wholesaling companies and the co-ordination and communication benefits that this imparts.

Conclusion

The development of a non-prescriptive eating quality assurance system for pork is underway and it has been proposed that the system would have two levels – standard and graded/eating quality assured. It is envisaged that the system will be flexible, allow industry to improve overall perception of pork as a quality meat and lead to a process of continuous improvement in pork eating quality as goal posts in the future continue to shift. Such an approach could also allow companies to individually determine which pathway interventions are imposed for targeted markets. It is acknowledged that work conducted to date has limitations, but has been very useful in identifying key critical control points, knowledge gaps and framing further work required in the development of an eating quality system for Australian pork. Further

sensory work, particularly including quality grades, re-purchase intention and willingness to pay, will provide opportunity to quantify the extent to which quality can be shifted by implementation of particular pathway interventions. It is also recognised that any system developed must take a conservative approach, such as that of Meat Standards Australia, to ensure that consumer expectations for eating quality assured pork can be consistently met.

Economic implications for all participants of the pork marketing chain, including consumers, will have to be monitored whilst success in improving pork consumption by providing pork of consistently high quality will require co-operation and quality management throughout the entire pork marketing chain. This implies that each sector needs to deliver product that is ideal for the next sector of the chain to work with. If one link fails to deliver products with uniform quality levels, it is expected that negative impacts on consumer acceptability will be experienced. The challenge for the Australian pig industry is to consistently deliver high quality fresh pork products that have a lower level of natural variability.

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