# ANIMAL PRODUCTION SCIENCE

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#### Preface

With this Conference the Australasian Pig Science Association (APSA) celebrates 30 years of providing ground breaking and innovative science which has aided both the Australian and international pig industries. From very humble beginnings in Albury, the APSA Conference has become a significant pig science conference attracting papers and participants from around Australia and the globe. The Conference is unique in that it covers many disciplines, in fact any discipline associated with pork production, and while there are keynote speakers, each session is truly a plenary session with none of the sessions held concurrently.

In the past 30 years, Australian pork production has also experienced significant changes to the technologies and management used on-farms. Many changes were made possible because of the outcomes of significant research and development, quite a few of which were shared and communicated at past APSA Conferences. Some issues have been solved but others continue to plague both the Australian and global pig industry. Seasonal Infertility was one of the first major production syndromes that had research outcomes presented at APSA. The summer can be one of the most challenging times of the year for maintaining reproductive performance as the combination of heat and increased day-length create environmental conditions that can be difficult to cope with and manage. Over time, the international pig industry has spent considerable resources in addressing the issues that affect pig production during the summer. Seasonal Infertility is a perfect example of how even with the best intentions and substantial robust research, some issues continue to cause production losses. The A.C. Dunkin Memorial lecture will provide collated information from international and Australian projects including those commissioned over time by Pig Research and Development Corporation (PRDC), Australian Pork Limited (APL) and the Cooperative Research Centre for High Integrity Australian Pork Limited (Pork CRC), especially those that were presented at APSA, to assist industry to alleviate the problem of seasonal infertility. In addition, potential knowledge gaps and recommendations for future research will be identified. I can think of no better candidate for this presentation than Dr Ray King. Dr King is not only an internationally respected Australian pig industry researcher and consultant, he also was the first APSA President and one of the very few that have taken on that role twice; it is very fitting that he will provide the A.C. Dunkin Memorial lecture on this significant milestone in APSA's history.

While the Australian industry is small by world standards, it is well served by a robust and well-structured research program, provided for by the peak industry body, APL as well as the Pork CRC. This current Pork CRC is due to wind-up mid-2019, and its work will continue through a successor organisation, already formed, in the Australasian Pork Research Institute Limited (APRIL). APRIL is a cooperative research entity which will build on the Australian pork industry's current collaborative approach to research and development. The formation of APRIL, heralds an exciting new change for our industry, with its focus of delivering research outcomes as well as commercial technology, which will generate financial returns to APRIL and support ongoing R,D&E. All this will be carried out in conjunction with APL and other R&D programs which support industry activity. The Australian pork industry R&D programs will not compete with each other, rather they will complement each other, providing research outcomes that will undoubtedly be presented at future APSA Conferences, while also proving outcomes that will aid the sustainability of the pork industry.

This last year has been a particularly difficult one for our Australian pork industry; unexpected and devastating events have unremittingly impacted on the well-being and profitability of the entire production chain, affecting the livelihood of many pork industry stakeholders and the well-being and stability of numerous regional communities. This severe downturn only emphasises that the production system is still looking for answers to problems and issues that may not have been considered previously, or are still not sorted out. Research outcomes can aid industry, through cost reduction, production improvements, risk reduction/management and provision of innovation.

From its humble beginnings, the APSA Conference has always provided a valuable source of knowledge to aid industry, but it also provided excellent networking and collaborative opportunities especially for our younger scientists. All segments of the pig industry chain are represented at the APSA Conference from pig industry research providers as well as every part of the pork value and supply chain. The APSA Conference provides a unique opportunity to discover new ideas and innovations, whilst also taking advantage of the extensive pig industry network that is the APSA community. I'd like to urge all delegates to take advantage of the opportunities at this Conference, and to borrow an Australian sporting term, 'Go Hard!!!!!'

Thanks to all for your valued contributions that have made APSA such an excellent pig science body, and I hope that you will continue your support of APSA and its biennial Conference.

Dr Pat Mitchell President

#### **APSA Awards**

#### The APSA Fellow Award

The APSA Fellow Award was first presented in 2007. This prestigious award is offered in recognition of past and present members who have made an outstanding contribution to APSA as well as their contribution and commitment to pig science. Nominations for the APSA Fellow Award are received from the current APSA Committee and the initial nominations are supported by a verbal statement addressing the selection criteria which are weighted to assist the committee in its deliberations. The selection criteria for the deliberation of APSA Fellow nominations cover contributions to:

- The APSA Committee (President, Vice-President, Secretary, Treasurer, ordinary committee member)
- Technical content contribution (editor, referee, scientific authors, etc.)
- Membership development (students, national and overseas)
- · Contribution and commitment to Pig Science (research, involvement in post-graduate training)

All past APSA fellows were significant contributors to APSA and pig science, and given both their professional and personal commitments at the time, their substantial contributions are more utterly astonishing and valued.

Previous recipients:

Dr Ray King (2007) Dr David Hennessy (2007) Dr Michael Taverner (2009) Dr Ian Williams (2011) Professor Frank Dunshea (2013) Dr Bruce Mullan (2013) Professor John Pluske (2015)

#### The Batterham Memorial Award

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

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

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

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

Robert van Barneveld (1995) John Pluske (1997) Kaye Coates (1999) Darryl D'Souza (2001) Patricia Mitchell (2003) Eva Ostrowska (2005) David Cadogan (2007) Rebecca Morrison (2009) Cherie Collins (2011) Robert Smits (2013) Heather Channon (2015)



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#### **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

From the modest beginnings of the inaugural conference in Albury, the APSA Biennial Conference has become one of the most important international pig science conferences. But such conferences don't just happen; the growth and success of APSA has only occurred because of the hard work and dedication of many people since the very first conference was held in 1987. The Oxford living dictionaries define the term 'Acknowledgement' as '...an expression of appreciation; a thing done or given in appreciation or gratitude'. This term falls far short of what is required to thank the various people whose contributions, hard work and dedication over the past 30 years have ensured the success of the biennial APSA Conferences. The proceedings have grown from just over 60 papers (including reviews and symposia) in the 1987 Conference, to over 110 papers (including reviews and symposia) for the current Conference.

The 2017 Conference would not have been possible without the contributions of a great many people. Foremost are the APSA members as without your continued support, including the submission of papers, these proceedings would be nothing more than a well bound and presented blank note book. I would also like to thank all those who have attended the 2017 Conference, especially those that have travelled considerable distances to become part of the extended APSA Community; your contributions to the interactions and discussions during the Conference are very much valued.

The APSA Committee typically invites a number of international speakers to each Conference, whose expertise and knowledge adds greatly to the information presented. This year the international speakers invited by the Committee were John O'Doherty, Dorian Garrick and Matt Culbertson, and their contributions are greatly appreciated. Furthermore, I acknowledge the following Australian researchers who also contributed to the success of the symposia and reviews: Darren Trott, Heather Channon, Darryl D'Souza, Evan Bittner, Heather Brae, Kim Bunter and Alan Tilbrook. The A. C. Dunkin Memorial Lecture is an important part of any APSA conference, and the Committee thanks Ray King for accepting the honour of presenting the 2017 Lecture. The APSA Committee also thanks this year's chairpersons and judges.

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 Editors, Lucy Waldron and Shay Hill are acknowledged. Also, the Editors' job would be much harder without a band of very dedicated and obliging referees; many of whom were provided with very short time limits to complete their reviews, but as always, rose to the occasion with little fuss, much skill and no complaints. The contributions of these referees (named elsewhere in the proceedings) are gratefully acknowledged.

Since 2015, the proceedings have been published in full as a Special Issue of *Animal Production Science*. This move to publication in a journal provides greater exposure for APSA and ensures our authors are more widely recognised for their outstanding scientific contributions. The APSA committee would like to thank Claire Gibson, Alice Hall and Helen Pavlatos from CSIRO Publishing, for their patience, support and all their hard work during this process.

The 16th Biennial Conference would not have been possible without the generous support of our many sponsors. Once again, Australian Pork Limited (APL) and the Cooperative Research Centre for High Integrity Australian Pork Limited (Pork CRC) are the joint Principal sponsors for the APSA Conference. Many of our sponsors have increased their sponsorship for this Conference, to ensure that increases in registration fees for delegates were minimised, while also ensuring that the quality of networking events are not diminished. All of the Sponsors are listed on the Sponsors page, and their contribution to the success of the 2017 APSA conference is gratefully acknowledged.

I believe that one of the most important functions of an association such as APSA is to foster and support the development of young scientists. The APSA Conference provides a mechanism for students to be able to meet, gather information and network not only within the student group but also the pig industry research community in general. Each student meeting held at an APSA Conference has always been a resounding success, an outcome due largely due to the support of APL and the Pork CRC, as well as the hard work of a number of people. I would like to thank APL and the HIAP CRC for making the APSA student meeting a premier event for the pig student group and I would also like to thank Lechelle van Breda and Ashley Norval from APL for their considerable efforts in making the student meeting at the 2017 APSA Conference such a great event.

The organising committee works hard for two years to organise each conference, and the current Committee has been no exception; stepping up to the plate whenever demanded, whatever needed doing, all to ensure the success of the APSA Conference. Accordingly thanks go to Cherie Collins (Past President), Stuart Wilkinson (Vice President), Frank Dunshea (Treasurer), Cameron Ralph (Secretary), Robert Hewitt, John Pluske, Charlie Rikard Bell and Robyn Terry. Thanks are also due to Jo Healy from The University of Melbourne for her assistance in APSA financial matters. I would also like to provide

a heartfelt thanks on behalf of the entire Committee to Amy Lealiifano (APSA Committee Secretary for the 2013 and 2015 Conferences), who continued to assist the current Committee in so many ways, and was always there to help when we needed her. She streamlined many processes, and saved the Committee considerable time and effort for which we are truly grateful and forever in your debt.

The 2017 Conference was organised once again in conjunction with YRD who acted as the secretariat for this Conference. YRD proved themselves equal to any challenge that was given them; working with Kate Murphy and her team has truly been a wonderful experience.

It has been a pleasure to be the President of APSA for the past two years and to have been part of the organisation of such a premier pig science conference that provides a great networking opportunity for all those involved in pork production. Thank you for your support of the conference and please continue to support the new Committee so that the future success of APSA can be assured.

Dr Pat Mitchell President

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**G** From a very young age I have called the Murray River region of Corowa, home. Earlier this year I was delighted to learn that I had been given the opportunity to join the Agriculture graduate program. I was excited for the challenges and opportunities that come with joining a large and diverse Agri-food company which proudly supports its local community. The graduate program is designed to provide a hands on experience whilst also developing innovative thinking for the future. So far, I have had the opportunity to work alongside a range of people and professionals in multiple areas of the company, learning and discovering my areas of interest. Rivalea's Agriculture Graduate Program is a very exciting, supportive way to begin a career in the Pork industry.

**G** Relocating from Central West NSW to Corowa has definitely been a rewarding journey. I chose the Agricultural Graduate program to kick start my working career as it is such a versatile company and has numerous pathways to venture into. The program has allowed me to start at the base level of the business in production, learning the everyday basics of pig farming and continues to push me in many ways to lead on to future roles. It has provided many opportunities to work on production projects and contribute to discussions on current and future ideas for pig farming operations. Being a company with so many locations and business sectors, it proves to be an encouraging environment that will suit any person looking to find their mark within Rivalea.

#### Georgia Cluff, 2017 Graduate

**G** The Agricultural Graduate Program at Rivalea has been a great introduction into the industry. I completed my degree in Animal Science in South Australia, and made the move to Corowa to accept the position. Rivalea is a highly integrated company, giving you access to a wide variety of areas within the business. The Graduate program allows you to experience a range of roles that are of interest to you, and enables you to get a sound understanding of how such a large company operates. I have met a lot of great contacts throughout the program, and benefited greatly from having access to mentors along the way. I have thoroughly enjoyed a wide variety of work across my 18 months in the program and am confident that it has given me a great start to my career.

Megan Quinn, 2016 Graduate

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### Seasonal infertility in pigs: what have we achieved and where are we up to?

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**Abstract.** The most common manifestations of seasonal infertility are delayed puberty, prolonged weaning to oestrus intervals and a reduced farrowing rate brought about by increased returns to oestrus, including a proportionally higher incidence of irregular returns to oestrus. Over the past 40 years, there has been considerable investment in Australian pig research that has generated extensive knowledge about the physiological mechanisms behind seasonal infertility. While some of the physiological mechanisms allowing the expression of seasonal infertility still remain unclear, a number of possible intervention strategies have been developed and investigated to ameliorate the effects of seasonal infertility in commercial production. For commercial pork producers, there is considerable information available that is based on both research and practical experience, which the farmers can use to identify strategies to minimise the impact of seasonal infertility, although, in the future, it may be more targeted to identifying interventions to ameliorate the impact of seasonal infertility in affected herds, rather than undertaking intensive studies into the possible mechanisms and reasons behind this very complex syndrome.

Additional keywords: farrowing rate, pregnancy loss, sows, weaning-to-service interval.

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#### Introduction

The first reports defining seasonal infertility in Australian pig herds appeared in the late 1970s. Stone (1977), Paterson *et al.* (1978) and Love (1978) independently reported on the incidence and characteristics of a seasonally occurring drop in the farrowing rate in commercial piggeries in South Australia, Western Australia and New South Wales respectively. About this time, seasonal infertility was reported in many other countries, including Denmark (Tomes and Nielsen 1979), France (Aumaitre *et al.* 1976), the United Kingdom (Stork 1979) and the USA (BeVier and Backstrom 1980; Hurtgen and Leman 1980).

In Australia, seasonal infertility was manifested primarily as an increase in the number of sows failing to hold to service because of an increase in the number of sows that exhibited extended and irregular returns to service (Love 1978; Paterson *et al.* 1978). The reduced farrowing rate, which typifies seasonal infertility, was also due to an increase in the number of mated sows that did not return to service at all but were found to be nonpregnant at term. The other main reproductive parameter that was affected by season was weaning to oestrus interval, with considerable delays during late summer and early autumn (Love 1978). There was little evidence from these field studies in Australia that litter size was affected by season (Love 1978; Paterson *et al.* 1978).

While the incidence of seasonal infertility was observed in many herds in Australia in the 1970s, the incidence of seasonal infertility across Australian pig herds has not been fully evaluated. Certainly, from anecdotal evidence, the magnitude of seasonal infertility varied widely among herds, with time of the year, parity, housing systems and other management and environmental factors, as well as within herds from year to year. However, the main feature of seasonal infertility that was observed in commercial herds was a lower farrowing rate that was most common in late summer and early autumn. The causes of seasonal infertility were not due to any one factor, whether it was temperature, photoperiod or other stressors, and it was concluded that seasonal infertility was, indeed, a very complex problem among Australian pig herds. Seasonal infertility continues to generate discussion and debate within the scientific community and affects the profitability of individual farms to a varying degree in Australia. A report compiled by Black et al. (2002) presented an analysis of the reproductive performance of 31 Australian piggeries ranging from 40 to 3000 sows. A substantial seasonal variation was observed for several important determinants of reproduction, including farrowing percentage, returns to service and weaning to service interval. These variables were all poorer during the summer period, with the effects being observed 1-2 months earlier in the northern latitudes. Black et al. (2002) also observed that the variation throughout the year was substantially lower for the better-performing farms.

## The golden years of seasonal infertility research and development (1980s to mid-1990s)

The reports in the late 1970s initiated several field and more controlled laboratory experiments in industry-funded research

projects and post-graduate studies into the underlying causes of seasonal infertility and interventions to alleviate seasonal infertility in Australian herds. Much of the research work in seasonal infertility by the Australian pork industry in the early 1980s was presented at a symposium at the inaugural Australasian Pig Science Association (APSA) conference held in Albury in New South Wales, in November 1987. The symposium, 'Seasonal infertility in the pig' (Hennessy 1987), examined the results from this work and implications for the reduction of seasonal infertility. The symposium papers examined the roles of temperature and photoperiod on fertility in sows and boars, and the role of a generalised stress response in causing seasonal infertility. The main conclusions concerning seasonal infertility from this extensive symposium at APSA in 1987 were as follows:

- The main symptom of seasonal infertility is a seasonal decrease in the farrowing rate of sows and gilts. Sows either show a delayed return to oestrus, or a failure to return to oestrus after mating.
- High temperatures on the sow may cause endocrine disturbances, particularly around ovulation and high temperatures for boars can directly affect semen quality.
- Modern sows may still retain some tendency for seasonal reproduction.
- Other stressors that relate to management, husbandry and housing of sows also contribute to seasonal infertility.
- There is individual variation among animals in their ability to respond to the stressors.
- The association between the adrenal responsiveness to stress and the severity of seasonal infertility suggests a way to reduce the incidence of seasonal infertility by selecting pigs with a low stress responsiveness.
- The secret to reducing the incidence of seasonal infertility in a herd is to reduce the magnitude and frequency of relevant stressors in the period between just before mating and ~3 weeks after mating.

Research work into seasonal infertility continued to be well supported by industry and many projects were initiated, along with support for several PhD studies during the period from the 1980s to the mid-1990s.

Using the APSA proceedings as a default mechanism to examine research into seasonal infertility in sows in Australia, funding support for this type of work seemed to dry up towards the mid-1990s. Towards the end of this sustained period of seasonal-infertility research and development, one of the last papers published in the APSA proceedings was a paper by Love et al. (1993) who reported that a high-level feeding of grouped sows during early gestation in summer-autumn offered a simple strategy for mitigating the reduction of fertility often encountered at this time. Pregnancy rates were increased from 56.5% to 79.8% when daily energy intake for the first 4 weeks of gestation was increased from 24 MJ digestible energy (DE) to 44 MJ DE during February-March (Love et al. 1993). The results of subsequent trials conducted by Love et al. (1995) in two Australian piggeries confirmed that low levels of feeding in the summer-autumn period were associated with increased numbers of delayed returns to oestrus and a low farrowing rate (<75%) typical of the infertility that occurs at this time of the year. Furthermore, Love *et al.* (1995) found that accommodation in individual stalls, rather than in groups, removed the seasonal effect on fertility, an observation also made earlier in the USA (Hurtgen and Leman 1980). With the move towards group housing in Australia, it seems that higher feeding levels in early gestation should be recommended to ensure adequate intake for all sows, not only in the summer–autumn period, but throughout the year (Sawyer *et al.* 2013).

The work conducted in Australia during the 'golden' years of seasonal-infertility research and development (the period between the mid-1970s and mid-1990s) was well supported by the industry funding bodies. While the work did not completely overcome the impact of seasonal infertility in commercial production, the results did provide industry with better knowledge about the factors contributing to seasonal infertility, and different strategies were developed to help alleviate seasonal infertility in herds, through improvements in housing, stocking density, oestrus detection procedures, pregnancy diagnosis and ensuring adequate feed intake.

#### Research and development after mid-1990s

Despite the large investment in research through the 1980s and up to the mid-1990s, seasonal infertility remained a major constraint to productivity in many Australian herds. However, support for seasonal-infertility research and development waned somewhat for the next decade or so, as other research priorities were identified. After this hiatus of reduced investment in seasonal-infertility research, there appeared to be a resurgence with the introduction of the Pork Co-operative Research Centre (Pork CRC) in 2005. The increased investment in pig research and development through the Pork CRC initiated the examination of possible mechanisms leading to seasonal infertility, as well as management interventions to help alleviate seasonal infertility.

Rather than conducting an extensive literature review of seasonal infertility, I will rely on using papers published in APSA over the recent years, together with Pork CRCand Australian Pork Limited (APL)-supported research and development, to assess what we now know about seasonal infertility and possible interventions to alleviate seasonal infertility.

The first seasonal-infertility paper that has appeared in the APSA proceedings since 1993 is a 2007 paper on the use of injectable progesterone on Day 34 post-mating that failed to reduce pregnancy loss during a time of seasonal infertility (Johnston *et al.* 2007). From 2009, the Pork CRC seasonal-infertility program, primarily based at the University of Adelaide and the University of Sydney, began to produce several papers addressing both the understanding of the mechanisms underlying seasonal infertility, as well as investigating practical interventions that may alleviate seasonal infertility.

#### Mechanisms underlying seasonal infertility

Results obtained by Michael Bertoldo, from the University of Sydney, showed the following two clear effects of the season on the follicular characteristics in oocytes collected from sows 4 days after weaning:

 The concentration of progesterone in follicular fluid from both small and large follicles was ~50% lower in summer than in winter (Bertoldo *et al.* 2009b). - The quality of oocytes recovered from large follicles, measured by their capacity to develop to the blastocyst stage of embryonic development, was severely reduced in summer (21%) compared with winter (55%) (Bertoldo 2010).

These results were the first to demonstrate that oocyte quality in pigs may be compromised during the summer months. Furthermore, these findings supported the hypothesis that suppressed endocrinological control mechanisms during lactation in summer adversely affect oocyte quality at the postweaning oestrus.

The University of Adelaide had several researchers, including William van Wettere, David Kennaway and Sean O'Leary, leading Pork CRC projects that investigated aspects of seasonal infertility. Puberty is often delayed during late summer and autumn, with daylength often being implicated as a contributing factor. In the Pork CRC seasonal-infertility program, Kennaway *et al.* (2015) investigated whether melatonin implants altered the oestradiol negative-feedback mechanism in gilts. However, they failed to demonstrate any effects of season or melatonin on oestradiol feedback or effects of melatonin on puberty (Kennaway *et al.* 2015).

Oocyte competency during the summer months was also investigated by Swinbourne *et al.* (2014) who reported that there were fewer larger follicles in ovaries collected 19 days after the pubertal oestrus in summer months than there were in winter months. In addition, intrafollicular concentrations of luteinising hormone were higher, whereas oestradiol concentrations were lower. These data confirm that season can affect oocyte competency and reproductive hormone concentrations in follicular fluid.

Season may also affect the concentration of circulating hormones in early pregnancy. van Wettere *et al.* (2011) found that there were lower circulating progesterone concentrations on Day 19 and Day 23 after mating in sows mated during summer, than there were in those mated in winter. These lower progesterone concentrations may affect embryo development and contribute to the decreased sow fertility in summer. Furthermore, the earlier rise in progesterone in summer-mated sows observed by van Wettere *et al.* (2011) may imply that either luteinisation occurs more rapidly after ovulation, or that ovulation occurs sooner after the onset of behavioural oestrus in summer than in winter.

In later studies, van Wettere (2013) confirmed that circulating progesterone concentrations rose more rapidly after oestrus and were higher on Days 3-7 after mating, for sows weaned in summer than for those weaned in winter. Furthermore, he found that timing of ovulation (as monitored by regular trans-rectal ultrasound) relative to when oestrus was first detected was significantly affected by season, with sows ovulating an average of  $\sim 10$  h earlier in summer than in winter (van Wettere 2013). It could, therefore, be suggested that this alteration in the timing of ovulation, and hence inseminations occurring outside the optimal period relative to ovulation, could be partly responsible for the reduced fertility of sows mated during summer. It may be worthwhile to examine the mating protocol to ensure that summer-mated sows are inseminated as soon as they are detected to be in oestrus, so as to better synchronise insemination with the earlier ovulation that appears to occur in summer. This may be an area of further investigation in commercial herds that experience a high incidence of seasonal infertility.

#### Interventions to alleviate seasonal infertility

While much of the Pork CRC seasonal-infertility program investigated the physiological mechanisms that underlie seasonal infertility, several experiments also investigated different management strategies to help alleviate seasonal infertility.

Seasonal infertility is often manifested by late pregnancy loss (commonly referred to as 'not in pig', or NIP) contributing to the observed reduced farrowing rate. Bertoldo *et al.* (2009*a*) investigated some of the factors that lead to late pregnancy loss in retrospective analyses of the reproductive performance of 13 213 sows and gilts from three herds known to be affected by farrowing-rate decline in the summer and autumn periods. The project analysed the risk factors associated with late pregnancy loss during the seasonal-infertility period. Bertoldo *et al.* (2009*a*) found that the probability of late pregnancy loss increased with

- an increasing parity,
- an increasing weaning-to-oestrus interval,
- a decreasing lactation length, and
- a decreasing litter size weaned.

To avoid reductions in sow reproductive performance during the seasonal-infertility period, pork producers may manipulate their management during the seasonal-infertility period by practising longer lactations, ensuring that the first oestrus after weaning is identified in a higher proportion of animals, and culling higher parity sows earlier.

The success of a mating program is dependent on the accurate detection of oestrus in gilts and post-weaned sows and the optimal time for insemination is usually based on the onset of behavioural oestrus. However, if behavioural oestrus is harder to identify in summer and the timing between oestrus and ovulation in summer is different (van Wettere et al. 2011), the use of a fixed-time artificial insemination may be an effective strategy to alleviate seasonal infertility. O'Leary (2013) developed a fixed-time artificial-insemination protocol and examined this in a series of experiments using research and commercial herds. The preferred protocol was an injection of 1000 IU of equine chorionic gonadotrophin (Folligon<sup>®</sup>, Intervet, Bendigo, Vic., Australia) 24 h after weaning, followed by 50 µg of gonadotrophin-releasing hormone analogue (Gonavet<sup>®</sup>, Gonadorelin, Veyx-Pharma GmbH, Schwarzenborn, Germany) 96 h after weaning. Insemination with one dose of  $3 \times 10^9$ spermatozoa followed 24 h after the Gonavet injection.

This fixed-time artificial-insemination protocol with one insemination reduced weaning-to-service intervals, maintained subsequent litter size and increased pregnancy and farrowing rates, without requiring boar stimulation and synchronisation of heat (O'Leary 2013). Because of these positive preliminary results, this type of insemination protocol should be evaluated in further field studies, particularly under conditions where fertility may be compromised, as in seasonal-infertility situations.

van Wettere *et al.* (2012) found that adding betaine to sow gestation diets to provide a daily intake of  $\sim 9$  g betaine per

sow during gestation increased litter size by 1.5 piglets in olderparity sows that were mated in January and February. The results of a later experiment involving a total of 1079 sows that were mated between March and May confirmed the positive response of litter size to 3 g/kg supplemental betaine in older-parity sows (van Wettere *et al.* 2013). Furthermore, an extra 20 mg/kg folic acid and 150  $\mu$ g/kg vitamin B12 added to the gestation diet decreased the incidence of early pregnancy failure in all sows in this large-production type study (van Wettere *et al.* 2013).

#### Where are we up to?

Despite a significant effort having been spent on understanding and ameliorating the effects of seasonal infertility, we have yet to fully understand the complicated interaction among management, environment, temperature, sows and boars. The effects of seasonal infertility can vary markedly from farm to farm, even within the same area and with similar genotypes.

The commitment of the Australian pork industry to the voluntary phasing out of gestation sow stalls by 2017 has led to the wide adoption of group housing during gestation over the past few years. There has been evidence from industry that the fertility of sows was adversely affected immediately after the change from stalls to group housing, particularly during the summer–autumn period. The earlier results by Love *et al.* (1993) were confirmed recently by Sawyer *et al.* (2013), namely that providing higher feeding levels in early gestation to ensure adequate intake for all individual sows in group-housing systems will help alleviate seasonal infertility.

The impact of seasonal infertility on the farrowing rate in two herds over successive years is shown in Figs 1 and 2. The herd in Fig. 1 regularly experiences a significant dip in the farrowing rate, beginning with matings occurring from late January, whereas the average farrowing rate in the other herd (Fig. 2) does not change during the year. The litter size in both herds, although different between herds, was not affected by the month of farrowing.

Both these herds were group-housed during gestation and were operated by the same company. Efforts have been made in more recent years to reduce the incidence of seasonal infertility, particularly in the high-risk herd, through increasing space allowance, moving to smaller group sizes, reducing stress associated with mixing, reducing the ambient temperature in the sheds, as well as introducing several nutritional interventions. However, the high-risk herd often still experiences seasonal infertility in most years. It is worth noting that the obvious dip in the farrowing rate is usually only for a short period of up to 2-3 months, but this depression in fertility for those 12 weeks or so can have a marked effect on throughput of pigs unless pork producers increase the number of matings during the seasonal infertility period.

For a 1000-sow herd that has 45 matings/week, a reduction in the farrowing rate of 25 percentage units, from 85% to 60%, will reduce the number of pigs weaned each week from 383 pigs to 273 pigs. Thus, over a 12-week seasonal-infertility period, 1320 fewer pigs will be weaned, which would affect the revenue and annual profitability. Overall, on this 1000-sow piggery, if there were no seasonal infertility (low-risk farm in Fig. 2), there would be 19 890 (10 piglets weaned  $\times$  45 matings/week  $\times$ 52 weeks  $\times$  85% farrowing rate) pigs weaned each year, or 19.9 pigs weaned/sow.year. If the herd experienced seasonal infertility for 12 weeks (high-risk farm in Fig. 1), only 18 570 (19890 - 1320) pigs would be weaned, which is equivalent to 18.6 pigs weaned/sow.year. Producers who experience predictable seasonal infertility try to account for the lower fertility by mating more sows during the seasonal-infertility period, thereby maintaining throughput and reducing the impact of seasonal infertility on the revenue and profitability.

The Pork CRC and Australian Pork Limited have attempted to collate much of the information generated over the past 40 years or so and have recently prepared two guides that pork producers may use to identify strategies on their farms that may minimise the effects of seasonal infertility (Hughes and van Wettere 2010; King and Mitchell 2013).

With the emergence of the new Pork CRC (Co-operative Research Centre for High Integrity Pork) in 2011 and its lower emphasis on productivity research and development, Australian Pork Limited began developing priorities in productivity research and development that did include scope for some investment in seasonal-infertility research and development. Subsequent Business Plans for Specialist Group 2 (Genetics, Reproduction and Welfare) in 2014–2015 (APL 2014) and



Fig. 1. Impact of seasonal infertility on farrowing rate at a 'high-risk' farm.



Fig. 2. Impact of seasonal infertility on farrowing rate at a 'low-risk' farm.

Student	PhD thesis
Andy Paterson	Paterson AM (1979) The reproductive performance of sows and gilts under intensive conditions. University of Western Australia, Perth, Australia.
Bronte Stone	Stone BA (1985) Biochemical aspects of early pregnancy in the pig. University of Adelaide, Adelaide, Australia.
Tony Peacock	Peacock AJ (1991) Environmental and social factors affecting seasonal infertility in pigs. University of Sydney, Sydney, Australia.
Mark Lorschy	Lorschy ML (1994) The physiological regulation of heat exchange in the lactating sow exposed to high ambient temperatures. University of Sydney, Sydney, Australia.
Corinna Klupiec	Klupiec C (1995) Endocrinological and nutritional aspects of seasonal infertility of domestic pigs. University of Sydney, Sydney, Australia.
Michael Bertoldo	Bertoldo MJ (2010) Seasonal effects on pregnancy loss and oocyte quality in sows. University of Sydney, Sydney, Australia.

Table 1.	Post-graduate students that completed their PhD into aspects of seasonal infertility in pigs and were supported
	by funding from the Australian pork industry

2015–2016 (APL 2015) included the following objectives respectively:

- Management interventions that reduce the impact of seasonal infertility on herds that regularly suffer from productivity losses each summer through the application of combined basic and applied-type science or demonstration studies.
- Investigation of different mating strategies that reduce the impact of seasonal infertility on herds that regularly suffer from productivity losses each summer.

While seasonal infertility remains a problem in some herds, some of the time, the emphasis in the future will be to identify different cost-effective interventions that may help reduce or ameliorate the impact of seasonal infertility in these cases. In view of the difficulty in inducing seasonal infertility in the controlled research setting where it can be confidently replicated, there seems to be little justification in significant investment in projects to help further understand the physiological mechanisms of seasonal infertility. It may be better to use the extensive existing knowledge to identify possible interventions and to test these under commercial situations. There are likely to be other higher priorities and challenges facing the Australian pig industry that would benefit more from the limited research funding available in Australia. For example, an ex ante cost-benefit analyses of the various priorities indentified by the APL Specialist Group 2 in 2015 showed that the estimated present value of net benefits to the Australian pork industry over the following 10 years from APL investment in seasonal infertility was only AU\$11 million, compared with that from investment in optimising weaning weight, which was AU\$54 million (APL 2015).

## Intangible benefits of investment in seasonal-infertility research

There has been considerable investment in seasonal-infertility research and development in Australia since the 1970s and it may be questionable whether this investment has produced significant benefits relative to costs against other research and development investments. However, it should not be forgotten that the seasonal-infertility research investment has also supported several post-graduate students, some of whom have made valuable contributions to the Australian pork industry and pig science in Australia, and to the livestock industries generally. Table 1 identifies several students that received industry support for their post-graduate studies from the 1970s through to more recent years.

#### Conclusions

The most common manifestations of seasonal infertility are delayed puberty, prolonged weaning-to-oestrus intervals and a reduced farrowing rate brought about by increased returns to oestrus, including a proportionally higher incidence of irregular returns to oestrus.

Over the past 40 years, there has been considerable investment in Australian pig research that has generated extensive knowledge about the physiological mechanisms behind seasonal infertility. While some of the physiological mechanisms allowing the expression of seasonal infertility still remain unclear, several possible intervention strategies have been developed and investigated, to ameliorate the effects of seasonal infertility in commercial production.

For commercial pork producers, a considerable amount of information is available that is based on both research and practical experience and that they can use to identify strategies to minimise the impact of seasonal infertility on their farm. Many of these strategies have been identified by King and Mitchell (2013) and include the following:

- Increase boar contact and pay particular attention to oestrusdetection procedures during the seasonal-infertility period
- Mate gilts and sows at the first sign of standing oestrus, rather than delay insemination
- Increase the emphasis on checking for returns to oestrus after insemination. Check sows for oestrus with boars 18–23 days after insemination and conduct more regular pregnancy checks with ultrasound after Day 30 of gestation
- Maximise nutrient intake of lactating sows
- Increase feed intake during the first 3–4 weeks of gestation to ensure that all sows receive adequate nutrient intake during this critical time
- Supplement the gestation diet with betaine
- Consider a fixed-time insemination protocol
- Provide cooling for sows and boars

• Mate more sows to cover the expected shortfall in expected farrowings.

The industry still provides some support to research and development efforts to address seasonal infertility, although, in the future, the efforts may be more targeted to identifying interventions to ameliorate the impact of seasonal infertility in affected herds, rather than undertaking intensive studies into the possible mechanisms and reasons behind this very complex syndrome.

#### **Conflicts of interest**

The author declares no conflicts of interest.

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#### Consumers want pork with 'adjectives'

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**Abstract.** Pork is the most consumed meat globally, but its consumption varies widely across the major pork-consuming nations. Consumers consider a wide variety of intrinsic and extrinsic cues, and credence attributes, when making purchasing and consumption decisions for food products. Brand recognition has been an important extrinsic cue for consumers, especially in the case of pork-product quality. However, the branding of fresh pork products in Australia has not been very prominent, due to the dominance of retailer 'home-brand' labels. However, increasingly these retailer labels are using information and branding relating to adjectives (credence attributes), for example, animal welfare, production systems, environment. The role of these credence attributes in Australia are now very much regarded by consumers as surrogate indicators of pork quality. The present paper will look at consumer preferences and attitudes to pork and the role credence attributes play when consumers purchase pork. In addition, the paper looks at the role of retailers in delivering pork with adjectives.

Additional keywords: credence values, intrinsic and extrinsic factors, willingness to pay.

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#### Introduction

Pork is the most consumed meat globally, but its consumption varies widely across the major pork-consuming nations. Within the EU, pork consumption in many countries is over 60 kg per capita per year, and is considered an everyday, inexpensive meat, not suitable for special occasions (Bryhni et al. 2002; Ngapo et al. 2004). In Asia, this is certainly a different story. China (including Hong Kong), for example, will increase its per capita pork consumption from ~30 kg to ~40 kg (OECD-FAO 2016) over the next 10 years, with pork being very much a staple meat, yet strongly linked with its traditional customs and beliefs. In Australia, one of the highest meat-consuming countries globally, with per capita meat consumption more than 111 kg (ABARES 2015), per capita pork consumption (25.6 kg) has grown steadily over the past 10 years and is on par with beef consumption (25.7 kg). So, across these varied levels of consumption, what do consumers want when purchasing pork?

Dr David Hughes (Emeritus Professor of Food Marketing, Imperial College, London) frequently stated that 'consumers around the world increasingly want adjectives with their meat'. A trip to the supermarket or the butcher shows that most pork products offered will have a range of adjectives, perhaps better defined as credence attributes, on the product labels. Furthermore, it appears that these values attributed to the product are linked or implied to be linked to the overall quality of the product (Ratcliff 2009). The need to link product with credence attributes has driven the meat industries to shift focus from a traditional supply chain mentality to one that focuses on what the consumer wants. This appears to be very much the case for pork, with the pork industry responding to consumers increasingly basing their decisions to purchase pork on values, as well as value.

#### **Consumer preferences**

#### Intrinsic and extrinsic cues

The drive to align quality of meat products with consumer demands, expectations and desires arises from the consumer's subjective evaluation of quality (Bryhni et al. 2002). In the case of meat products, including pork, product quality may be defined in terms of either a holistic or an excellence approach (Grunert 2005). The holistic approach includes within the concept of meat product quality 'all the desirable characteristics a product is perceived to have', and tends to focus on the inherent properties of meat or intrinsic cue. By contrast, the excellence approach sees product quality as referring to characteristics that pertain to a higher, and more exclusive (or 'superior'), specification of the meat or pork product or extrinsic cues. The holistic approach provides a wider scope for interpretation in which quality can mean conforming to production or faming standards (including standards pertaining to the environment, local specialties, organic production, ethics, and even taste and smell) and often is based on subjectively perceived quality attributes (Dries et al. 2006).

The challenge for the Australian pork industry over the past 10–15 years has been to consistently deliver a high-quality pork product that meets the consumer needs. Consumer perception of pork quality has traditionally been based largely on intrinsic cues

Ngapo *et al.* (2007) conducted one of the few global consumerpreference studies (Fig. 1), and identified and compared the most important characteristics of fresh pork that determined



**Fig. 1.** Preferences for four pork characteristics from surveys conducted in 23 countries (Ngapo *et al.* 2007). ic, inconsistent colour; if, inconsistent fat; im, inconsistent marbling; id, inconsistent drip.

consumer choice (12 590 consumers) in 23 countries from all five continents. Ngapo et al. (2007) reported that across all countries, colour was the most consistently chosen characteristic, followed by fat cover, marbling and drip-loss characteristics. The Australian consumers were by far the most consistent, with 84% giving consistent choices, in contrast to only half of the Yugoslavian consumers giving consistent choices. Over all countries, similar numbers chose the dark red and the light-red pork. Australian (73%), Irish (67%) and Polish (63%) consumers showed the strongest preference for the light-red pork, whereas the Taiwanese consumers (66%) showed a strong preference for the dark-red pork. Although the largest differences were found between countries, there was little evidence that ethnic origins were strong, except for the similarities of the Asian (Taiwan, Japan and Korea) countries, which were different from the other countries. However, these countries were also very different from one another. Irish and Polish consumers clearly had quite different preferences and both had preferences different from those of the other European countries in this survey.

So, what role do these intrinsic cues play? Do they provide the consumer with specific cues on pork quality?

Grunert *et al.* (2002) conducted a study (Fig. 2) with German consumers in a focus group discussing parameters that influence pork quality. The consumers were confident that they could judge the sensory quality of pork themselves. The consumers were then presented with 22 cues and asked to (1) indicate whether the consumers understood what the cue was all about, and (2) rank the cues by perceived importance for pork quality. However, the results indicated that of the top five cues as measured by both knowledge and importance, none was related to sensory quality, but was, instead, related to technological, nutritional and hygienic quality of pork.



Fig. 2. Understanding and importance of 22 extrinsic pork cues (Grunert et al. 2002).

Pork with adjectives

Meat has always been an 'experience-good' product (Grunert 2005), which means that its quality is unknown before the purchase, and must be inferred from quality cues. In Australia, the sourcing of these quality cues has changed significantly. The primary sourcing of meat-quality cues was from the butcher, i.e. familiarity with the seller (D. Hughes, pers. comm.) and ability to inspect, including touch and feel, were important factors. With a shift in meat purchases towards supermarkets, most of the quality cues are no longer available. This has seen a shift in quality cues to the packaging and, more specifically, the information related to price, quality grade, brand, label, shop, country of origin on the meat packaging.

Brand recognition has been an important extrinsic cue, especially in the case of meat quality. However, the branding of fresh-pork products in Australia has not been very prominent. The main reason for this has been the dominance of retailer 'home-brand' labels (commonly referred to as retailer private labels). More recently, this has been gradually changing with some dual-branding of meat products in Australia, but these mainly appear to be in the 'value-add' fresh-pork category. The retailer private-label category provides a choice of products based either on price (value) or quality (premium), thus catering for a wide range of consumers within the same store. However, increasingly, retailer labels also include information and branding relating to individual or in some cases multiple credence attributes such as, for example, animal welfare, production systems, environment, animal nutrition (Dunshea et al. 2016). The role of these credence attributes in Australia is now very much regarded by consumers as surrogate indicators of pork quality.

#### 'Adjectives' or credence attributes

#### Animal welfare

The animal industries, especially intensive industries, have received considerable scrutiny and criticism from various segments of the animal welfare lobby (María 2006). This has been especially evident in Europe, Australia and North America (Garcés et al. 2008). Consumer concerns about animal welfare are increasingly influencing the 'ethical positioning' of food companies around issues relating to indoor confinement, stocking densities (de Barcellos et al. 2011), handling, longdistance transport, livestock truck accidents, slaughter (Mirandade la Lama et al. 2010) and religious slaughter, i.e. halal or kosher (Fuseini et al. 2017). Similarly, in Australia, animal welfare has been front and centre for pork industry. As a small agricultural industry (GVP of <AU\$1.5 billion; ABS 2016), the industry has invested significantly in research and development (R & D) to address several welfare issues, including confinement, space allowance and husbandry practices, such as tooth clipping and tail docking.

In 2009, in response to a damning and, at times, factually incorrect report 'an expose on the pig industry' on 60 Minutes Australia, a television program, the Australian pork industry voluntarily committed to cease the use of sow gestation stalls beyond 5 days after the last mating. Prior to the decision by industry (i.e. 2010), a major retailer launched a welfare standard, sow gestation stall use only permitted for 24 h, and committed to source all its fresh pork from sow stall-free production systems in

Australia. This was the first major animal welfare marketing initiative undertaken by a retailer in Australia to differentiate its 'stall-free pork' in the market place. Interestingly, in the case of 'sow stall-free' pork in Australia, this position was adopted across all its fresh-pork category, thereby limiting consumer choice. In addition, 'sow stall-free' pork was priced without any significant premium. Since 'sow stall-free' pork, animal welfare continues to be the most significant credence attribute influencing consumer pork-purchasing behaviour in Australia.

Credence attributes around supposed 'welfare-friendly' production systems have also significantly changed the pig production landscape in Australia, with almost a doubling of pigs (from 8% to 14%; APL 2015) produced from outdoor productions systems over the past 5 years. The dual branding of free-range pork with compliance certification by animal welfare groups such as the RSPCA in the market place has played a significant role in this increased growth of fresh-pork category over the past 3–5 years. Similar trends have also been observed in the UK for well over 10 years. Unlike 'sow stall-free' pork, free-range pork does attract a significant premium.

#### Product safety

Food industries globally appear to be beset with food-safety issues ranging from residues in food (Pei *et al.* 2011) and outbreaks of food-borne disease (Moffatt *et al.* 2016). However, for the most part, in developed countries, while meat safety concerns have been influential in shaping consumer attitudes towards meat (Verbeke and Vackier 2004; McCarthy and Henson 2005), the perceived food-safety risk needs to be put into perspective. In Australia, consumers take product safety as a given and, in many instances, tend not to mention food safety, especially when factors such as animal welfare or farming systems are in the mix.

In addition, factors such as genetically modified foods or food integrity (especially in the context of product substitution) seem to be increasingly capturing the consumer attention within this food-safety space, suggesting that perhaps consumer decisions to eat meat are gradually becoming more influenced by perceived nutrition and health considerations rather than safety concerns per se (Verbeke et al. 2007). One such example is the use of technologies such as metabolic modifiers to improve animal growth performance. These technologies, although benefiting producers (improved production efficiencies), processors (improved processing efficiencies due to higher lean-meat yield) and the consumer (leaner meat and less expensive), have been subjected to external influences such as market differentiation and trade barriers as well as consumer resistance that challenge the use of technologies (Dunshea et al. 2016). Consequently, these retailer strategies have limited, and, in some instances, banned, the use of some very viable technologies, with little or no supporting evidence. In the case of Australia, these retailer strategies seem to have focused on the mantra of 'do not mess with my food', with the supposed aim of improving food safety and animal welfare, the two issues that seem to resonate very well with modern consumers.

In the case of the EU, consumers are interested in how food is produced, and this appears to be a consequence of consumers being sensitised to disease outbreaks that have affected foodsafety credibility in the past (Aloha 2008). It has also been shown that consumers increasingly view high animal-welfare standards as an indicator that the resulting food is safe, healthy and of high quality (Fallon and Earley 2008; Weddle-Schott 2009). This view is becoming increasingly prevalent in Australia, especially among food-opinion leaders. The popularity of television cooking shows has seen consumers becoming more accepting of the opinions of leading chefs (e.g. Jamie Oliver and Curtis Stone, to name a few) linking animal-welfare aspects of production systems with the end-product quality, i.e. tasting better and being better for the consumer, with the resulting impact being shifts in consumer purchasing behaviour, i.e. increased consumption of free-range pork.

#### Health

In Australia, most health claims for pork are based on its lowfat content (pork tenderloin equivalent to skinless chicken breast) and natural levels of minerals (potassium and zinc) and vitamins (B6, thiamine and niacin) and, in many ways, has stood the test of time. In 1979, the Australian Pork Corporation and the Australian pork producers developed a new campaign designed to increase consumption of the new leaner pork. Leaner pork in Australia was marketed as 'newfashioned pork' (Batterham et al. 1982). In 1991, a new promotional campaign 'new-fashioned pork - the other white meat' was launched to increase consumption by infrequent pork consumers in Australia (Perren 2006). This campaign was adapted around the 'other white meat' concept undertaken by the pork industry in the USA in 1987 and was aimed at taking advantage of favourable consumer attitudes towards white meats such as chicken and fish. The 'new-fashioned pork the other white meat' campaign led to a more favourable change in the image of pork to suit the health-conscious consumer reinforced by the National Heart Foundation Food Approval Program, giving the tick to 14 of the 22 cuts of 'new-fashioned pork' (Saxelby 1990). The current industry marketing campaign around 'low-fat' still appears to resonate most with consumers. However, the extremely high levels of obesity in Australia, and recent reports linking processed meats with increased incidence of cancer, has seen increased commentary around the need for consumers to reduce their meat intake. In Australia, this messaging, for the time being, has not affected either per-capita pork or total meat consumption (red-meat consumption has decreased substantially; ABARES 2015).

#### Environment

Livestock production occupies  $\sim$ 75% of agricultural land (Foley *et al.* 2011), consumes 35% of the world's grain, and produces 14.5% of anthropogenic greenhouse-gas emissions (Gerber *et al.* 2013). With demand for meat and dairy products forecast to increase 60% by 2050, it is imperative that pork-production systems cater for this demand sustainably. The level of apprehension and concern relating to environmental issues continues to increase, especially regarding impacts of carbon dioxide emissions on climate (e.g. IPCC 2013), and impacts of human intervention in the global hydrological cycle (Rost *et al.* 2008). Food security, nutrition and poverty alleviation,

particularly in poor nations, are threatened by climate change (FAO *et al.* 2015).

Organic livestock farming has set itself the goal of establishing environmentally friendly production, sustaining animals in good health, realising high animal-welfare standards, and producing products of high quality (Sundrum 2001). By striving for these goals, organic livestock farming, on paper at least, meets the demands of an increasing number of consumers who are critical of conventional production methods. However, there does not appear to be much in the way of supporting data to substantiate these claims. In Australia, organic pork production does not even register on the pork production-system categories and remains an extremely small niche category. The growth of organic production farms in Europe has been higher, with countries such as Denmark, Germany, France and Switzerland recording significant growth; however, the proportion of the organic pig production to national pig production is still extremely small (<0.4%; Früh et al. 2014).

#### Usage and attitudes of pork consumers in Australia

Consumers consider a wide variety of intrinsic and extrinsic cues, and credence attributes when making purchasing and consumption decisions for food products. A key focus for the Australian pork industry, like for other livestock producers, has been to better understand the Australian consumer preferences for pork-product attributes. A usage and attitude study was conducted (APL 2010) to investigate the various consumer segments consuming pork and their preferences for a range of pork-product credence attributes. Australian pork consumers were segmented into the following five groups: (1) foodies who love cooking; (2) consumers with routine tried and tested pork recipes; (3) large households looking for value for money but still healthy and nutritious pork meals; (4) older consumers that are traditionalists in that they eat some pork but mainly consume beef and chicken; and (5) new-age older consumers that eat some meat but are increasingly moving to a vegetarian diet. The first three groups account for ~90% of pork consumed in Australia. In terms of attributes influencing pork-purchase decision, the top five attributes (Fig. 3) in order of importance were taste, price, health, Australian produce and easy to cook.

Similarly to the Australian usage and attitude study, Lusk and Briggeman (2009) studied 11 different food values, namely, naturalness, taste, price, safety, convenience, nutrition, tradition, origin, fairness, appearance and environmental impact. The top three food values identified as most important to consumers were safety, price and taste. In contrast, Cummin *et al.* (2016) investigated US consumer perceptions of the relative importance of seven different pork-production and -product attributes. Of the attributes studied, animal welfare was ranked third-most important, following food safety and taste. The order and share of preference for the seven pork attributes studied by Cummin *et al.* (2016) were food safety (40.6%), taste (21.5%), animal welfare (15.6%), price (9.7%), environmental impact (5.1%), locally raised/farmed pigs (3.9%) and locally processed pork (3.6%).

In looking at the consumer preferences (Lusk and Briggeman 2009; APL 2010; Cummin *et al.* 2016), it is somewhat surprising that the credence attributes such as animal welfare and the



Fig. 3. Australian pork consumer usage and attitude study (APL 2010).

environment were rated outside the top five attributes. This is particularly surprising for Australia, considering the significant scrutiny and focus on pork-production practices, such as the use of sow gestation stalls. In this context, it is important to understand the distinction between the roles of individuals as consumers and citizens (Grunert 2006). In the case of community attitudes, people may have specific views about various forms of pork production, but these may not be reflected in their behaviour as consumers. This is further emphasised, even though products with supposed 'higher' animal-welfare standards may be available. Verbeke *et al.* (2010) looked to further quantify the relationship between consumer behaviour and citizen attitudes, and further demonstrated that this relationship is not very strong.

#### Willingness to pay

Willingness to pay (WTP) is a measure of value of goods or services to an individual (Hanley *et al.* 2001), and is defined as the price premium or maximum price an individual is willing to sacrifice to obtain a certain benefit or to avoid undesirable characteristics (Hanley *et al.* 2001; Breidert *et al.* 2006). So, are consumers willing to pay a premium for pork with adjectives?

In the case of animal welfare, it would appear that only a small proportion of consumers is willing to pay a premium, even though most would rate this as being very important. The clearest example of this has been the significant growth in both volume and value share of free-range pork in Australia. The Australian Usage and Attitude Study (APL 2010) seemed to indicate that only 15% of consumers rated animal welfare as 'high' importance and were willing to pay premium for this 'high-welfare' pork. Typically, WTP studies have tried to quantify concerns in relation to the value placed on animal lives, their welfare conditions (Lagerkvist and Hess 2011) and the higher expected benefits associated with them, including product quality that consumers tend to associate with improved animal welfare (European Commission 2007; Verbeke 2009). As in Australia, there has been strong interest in identifying the market potential of speciality products focusing on animal welfare that exceed minimal requirements imposed by law (Christensen *et al.* 2012; Heerwagen *et al.* 2015). Markets for such speciality products with welfare traits exceeding those in standard products would provide consumers with an opportunity to align their meat consumption with their concerns.

In the case of health, the Australian Usage and Attitude Study (APL 2010) indicated that a significant proportion of consumers rated this as 'high' importance. This is unsurprising, given the issues around incidence of obesity in Australia. The number of products with implied health benefits available on the supermarket shelf continues to grow and this trend is unlikely to change soon. Bellhouse et al. (2010) investigated whether there would be an increase in consumer WTP and purchase if reduced-cholesterol pork was introduced to the Australian market. The results from a stated choice-survey analysis indicated that, at present, most consumers are unconcerned about the cholesterol content of fresh pork and that there is a minimal effect of such concerns on fresh-pork purchases. However, a niche market group of consumers were identified, who currently reduce purchases of fresh pork due to cholesterol-content concerns, that had a significantly higher willingness to pay than other respondents to the survey. Slattery et al. (2010), examined the potential economic impact from the introduction of a hypothetical low-cholesterol pork product into the Australian market reported by Bellhouse et al. (2010). Six different scenarios were examined that are combinations of a 10%, 20% or 30% increase in consumer demand, with and without a 10% increase in the costs of producing the more valuable pork. In choosing one of the more 'realistic' scenarios from the above, Slattery et al. (2010)

reported that if aggregate WTP increased 10% and cost of production increased 10%, and if adoption were only 15% of pork supply, then total annual industry benefits resulting from the development of low-cholesterol pork would be ~AU\$13 million.

Several studies have pointed to the existence of the following three distinct consumer segments where preferences for food products with improved animal welfare are concerned: one segment is very interested in animal-welfare issues, a second shows no particular interest in animal welfare, while the third segment, lying somewhere between these two, encompasses consumers who care about animal welfare to an extent but are also focussed on other attributes, including product quality, impacts on their own health and price (Meuwissen and Van der Lans 2005; Mørkbak et al. 2010; Vanhonacker and Verbeke 2014; Heerwagen et al. 2015). Denver et al. (2017) investigated the market potential of pork labelled to indicate medium and high levels of animal welfare, to determine the risk that Danish consumers would abandon high-level welfare pork if less expensive products with a medium level of animal welfare became available. The results indicated that the Danish market could accommodate more than one pork product with a welfare label but the price differential separating medium- and high-level animal-welfare pork would have to be quite narrow. The results reported by Denver et al. (2017) were further supported by Clark et al. (2017), who conducted a citizen and consumer metaanalyses of WTP for farm-animal welfare. Clark et al. (2017) reported that the meta-analyses indicated a small, positive WTP (0.63 standard deviations), i.e. WTP a small premium for farmanimal welfare.

So outside of free-range pork, it would appear that for the most, consumers, while wanting pork with adjectives, are not willing to pay a premium. But the market continues to deliver pork with adjectives even in the absence of a premium, with retailers being the main drivers in this instance.

The retailer 'sow stall-free' pork initiative in Australia did not need to rely on the consumer WTP for 'improved' animal-welfare production systems. The two key elements of this retailer initiatives were (1) all fresh pork was sourced from sow stallfree production systems, thereby removing the need to force consumers to choose between lower- or higher-welfare pork, and (2) 'sow stall-free' pork was priced without any significant premium. In this instance, the 'sow stall-free' pork, along with the 'hormone-free' beef and lamb campaign seems to have assisted the retailer in attracting consumers to shop at its outlets, as evident with the retailer growing its market share in Australia. I state this with some caution, given this animal-welfare initiative is probably just one among a range of strategies implemented by the retailer that may be responsible for the increased market share. This is an important example of the role the retailer plays in potentially increasing the relevance of key credence attributes of pork and other commodities. In addition, it would be fair to say, certainly in Australia, that the retailer appears to be the main driver behind consumers wanting more credence attributes linked to their pork.

#### The future of pork with adjectives

Consumers wanting pork with adjectives is here to stay for a while yet. Current attributes such as animal-welfare especially related to animal-production systems and animal health are also likely to remain at front and centre. However, if we look to the beef and lamb industry in Australia, a real gap exists in defining good sensory-based eating quality for the pork consumer (Channon and Warner 2011). Meat Standards Australia (MSA) eating quality-assured and branding of beef and lamb continues to grow, as does retailer and MSA co-branding of beef and lamb. The co-branding by retailers as seen in the case of MSA and the retailer 'home brand' appears to be working quite well, as seen by the increase in beef-product range and sales. Given that 'taste' was rated as the most important attribute influencing pork-purchase decision in Australia, and the fact that meat has always been an 'experience-good' product, an eating-quality cue, once understood by the consumer, suggests that there is a real opportunity for pork to develop an eating-quality cue for consumers in Australia. Interestingly, in the case of MSA beef, consumers were willing to pay a premium for MSA beef (Lyford et al. 2010). While the retail price of pork has increased marginally in recent times, it remains to be seen whether an eating quality-assured cue such as MSA will result in a willingness to pay a premium. Nevertheless, the recent retailer entrants into the Australian market appear to be more willing to co-brand, and this provides an opportunity to implement pork an eating quality 'brand' cue for its customers.

Like taste, being Australian produce was rated as an important attribute influencing pork-purchase decision (APL 2010). In a market where locally grown product increasingly has to compete with cheaper imported products, the cues around country of origin and provenance are becoming more prominent on the supermarket shelf and the same applies for pork. Apart from seafood, pork is one of the few meats in Australia that has had to compete with imports. Currently, all fresh pork sold in Australia must be sourced from Australian pig farms; however, more than 70% of ham and bacon sold in Australia is sourced from imported pork. Very clear country of origin labels indicating 'Australian pork' is increasing and, if anything, will continue to increase as value-add and heat and eat pork products become more prominent in Australia. This is likely to be the case for the retailer 'home brand' and branded pork products.

#### Conclusions

Pork consumption in Australia continues to increase and now ranks alongside beef. Consumer usage and attitudes indicate that, in the case of pork, a good eating experience is key to repeat purchase behaviour, while factors such as animal welfare and the environment are well down this list. In the case of animal welfare, community or citizen attitudes reflect expectations for higher welfare standards, and majority of consumers are unwilling to pay a significant premium. The retailers, who continue to promote their 'home-brand' labels (retailer private labels) over branded pork have seen the use of credence attributes such as 'sow stall free' to successfully differentiate their pork from that of their competitors. Looking forward, within the fresh-pork space, it is highly likely that consumers will continue to want more adjectives with their pork, but, on the basis of current trends, will for the most part be unwilling to pay significant premiums. Given the success of campaigns such as 'sow stall-free' pork and

'hormone-free' beef, retailers will continue to use credence attributes to promote their pork private labels.

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# Innovation in an expanding market: Australian pork is not a commodity

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**Abstract.** The growing Asian middle class, the proliferation of export markets and a more discerning domestic consumer base are creating new opportunities and challenges for the Australian pork industry. To fully capitalise on these opportunities and face these new challenges, the right questions need to be asked by the Australian pork industry. We need to know not only what our consumers want, but who our consumers are. The present paper aims to demonstrate that novel approaches to investigate consumer attitudes will be required, and it cannot be assumed that current productions systems, products and marketing strategies are optimal for the changing environment and the creation of new premium market opportunities. With new markets and new products come new consumers; identifying who those consumers are, the networks they operate within as food consumers, and what influences their purchasing decisions are the key to their adopting Australian pork as premium produce in a new global market.

Additional keywords: consumer-aided design, export, Kano model.

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#### Introduction

The Australian agricultural sector is predicted to become a AU\$60 billion industry in 2017, with rural produce expected to exceed AU\$100 billion by 2025 as the Asian food boom affects our producers (Anonymous 2017). Continuing population growth worldwide, coupled with economic growth in an emerging middle class, is significantly enhancing the purchasing power of consumers in Asia (Peel 2014). Several newly established and recently agreed-on free-trade agreements between Australia and countries within the Asia-Pacific region further enhance this great opportunity for agricultural expansion in Australia. The increased buying power of Asian consumers and pending increases in market access are obviously of major interest to Australian pork. Domestically, the Australian pork industry is enjoying a successful period, with consumption per capita continuing to grow ahead of projected figures and pork currently holding a price advantage over beef and lamb (APL 2016). China alone raises and consumes over 50% of global pork (Schneider and Sharma 2014), while Australia accounts for a mere 0.4% of world production (APL 2010). The immense size of the Asian pork market is what attracts us to it, but we will never be able to cater for it in bulk. Furthermore, the cheap labour and feed costs in this region mean we should never be looking to compete at the commodity end of the market where we become price takers not price setters. Australia has the opportunity to become a premium producer, that can demand higher prices for its highly desirable products, but to achieve this we must diversify what we sell, how we sell it and who we sell it to. Our reputation internationally, particularly as a 'clean and green' producer of food is essential in the establishment of an Asian export market, where food safety and a lack of trust in local food are major concerns. However, this reputation alone is not enough, as this tag also applies to agricultural products from the USA, South America, New Zealand and the EU. Domestically and internationally, consumer interests are becoming ever more individualised and fragmented. New methods to investigate who our target consumers are and what they want are required. At times, it may not be the entire market we need to understand, but specific individuals, their networks within those markets and where they operate as food consumers.

Understanding individual consumers can be as powerful as understanding an entire market. We have an understanding of the Chinese pork market, but we do not currently have an export protocol allowing us to sell pork to China, delaying many of the benefits of this knowledge. However, there are ~46 million Chinese people living outside of China, Hong Kong, Taiwan and Macau (Anonymous 2014). Thirty million of these migrants live within the Association of South-east Asian Nations (ASEAN), constituting ~10% of the population of South-east Asia, and close to one million within Australia (Anonymous 2014). A detailed understanding of these individual consumers can open opportunities within far more than a single market, including one million consumers in our domestic market, to whom pork is a staple. It is time that we re-think traditional market segmentation via, for example, sex, age, parental status and marriage status, and embrace the cultural diversity of Australian consumers. Australian pork must be careful not to approach marketing and product design as if catering for a monoculture, when we arguably live in the most diverse country on earth.

Two main avenues hold the brightest opportunities for Australian pork, namely, (1) developing export markets, and (2) shifting pork into the premium food category domestically. Research and development (R&D) along the food value chain clearly highlight benefits to both producers and processors for export demand-driven R&D that can deliver competitive premiums (CIE 2012). The aim of the present paper is to challenge the thinking of the Australian pork industry in how it approaches these opportunities, and provide an example of how they can be approached differently. Both export and domestic pork markets are complex, with ever-evolving and diversifying groups of consumers. The first question we should be asking is not whether we know what our consumers want, but rather whether we know who our consumer are? The answer is a moving target, depending on what you are trying to achieve, and will be different for individual businesses. Both the product design and marketing strategy need to consider this targeted consumer group. An urban 'foodie' who frequents farmers' markets and is willing to pay premium prices for locally sourced food is a far different target to a working mother who just needs something quick and easy to prepare for a whole family at a reasonable price. However, if we agree that the best avenue for increased revenue is creating premium pork products for export and domestic sale, then the primary target becomes wealthy consumers who purchase high-end products.

#### It's not as easy as 'let's be the next Uber...'

The call to find the next 'Uber' has become a popular sentiment, usually followed by a series of facts and charts of the profit of the company and its quick rise to global dominance. However, applying disruptive technologies and business practices into the agriculture sector, and even more specifically to the pork industry, is a complex proposition. For all the excitement around these new companies such as Uber, Facebook and Twitter, we really do not think about their application seriously enough. According to Forbes, 90% of start-ups fail (Patel 2015), and even within established businesses, only four in seven new product and/or service projects enter development, one and a half are launched, and only one succeeds (van Wulfen 2015). Even a successful, innovative idea that is adopted by a consumer base is not a guarantee of profit. While the benefits of such ideas for consumers are obvious, the benefits for workers and businesses are more nuanced and complex (Thompson 2015). Twitter has ~390 million daily active users (Anonymous 2016), yet, for all this appearance of a successful disruptive technology, the company has not been able to monetise their customer base, having serious problems generating enough income to keep its share prices afloat (Dans 2016). Thinking of the idea is in fact only half the battle, the more difficult part is identifying the idea when you come across it, and using it to directly benefit you or your industry. We need to stop acting as if there is a simple recipe for business, like for a cake or casserole, and start thinking in terms of

2014). Google became the world's most valuable company, worth nearly US\$520 billion (Hooker 2016) through many examples of innovation and disruption. In 2004, Google's email platform, Gmail, offered a full gigabyte of data for email storage for free (Covert 2014). At the time this was almost unthinkable, as data storage was extremely expensive and seen as a major issue for the technology sector. When forced to react, their biggest rival Yahoo, offered only a mere 100 megabytes in response (Covert 2014). Data are now a hugely valuable commodity, and, through such actions, Google has somewhat of a global monopoly on it. Yahoo failed to recognise the innovation and today Gmail dominates the email market.

how the factors that influence businesses are connected (Satell

While these ideas have changed global industries, they can rarely be replicated or directly compared among industries. There is a plethora of unseen connections, and networks of consumers that drive our industries and brands that determine how things get done, or do not get done, in our enterprises (Satell 2014). There are also internal networks within your company or industry that require similar understanding so that you can communicate your ideas and apply them, understanding both is critical to innovation. The structure of those unseen connections and how they relate to our objectives are increasingly becoming the difference between success and failure (Satell 2014). A great idea can change an industry, but great ideas cannot be guaranteed or expected. However, we can move forward by applying research into understanding these networks and connections, specific to what we are trying to achieve. Even a general awareness of how your consumers, competitors and colleagues operate within their networks can make you more likely to be Google and less likely to be Yahoo in the above example.

#### Premium pork and the Kano model

To create a successful 'premium' product, you require two things, namely, a differentiated product that has attributes of a higher desire to the target consumer, and a marketing strategy that clearly translates these benefits at the point of sale. If we are creating new premium products, our target consumers are going to be wealthy individuals, who are willing to pay for quality and prestige. This group has an entirely different buying psyche from that of the general population, who generally shop for price per kilo over all else. Add to this the fact that we are looking to create these new products for several different cultures and countries, and the task becomes even more complex.

Cultural factors are perhaps the most powerful determinants of which foods we consume (Prescott and Bell 1995). A primary issue for the expansion of our industries into Asian export markets will be understanding the differences between Australian and Asian cultures in their preferences for the sensory characteristics of foods, and how the perception of food qualities such as taste, flavour or texture differs between

dissatisfied

the cultures (Prescott and Bell 1995). Consumer, market and sensory research will be required to fully appreciate how Australian pork can be competitive in Asia. We also require a better understanding of domestic consumers who are willing to purchase premium products on Australian shelves, rather than assuming they all have similar influences. Investigating the attributes of Australian pork products, and what among them influence these consumers, is the first step.

The Kano model (Kano *et al.* 1984) differentiates among three types of product requirements, which influence customer satisfaction in different ways when they are present or absent in a product. Importantly, it gives insight into which product features have a more proportional influence on satisfaction, and which attributes are an absolute must in the eye of the consumer (Sauerwein *et al.* 1996). Customer satisfaction is the major concern and a prerequisite for competitiveness in today's global market (Chen and Chuang 2008). The three types of requirements are shown in Table 1.

Figure 1 shows how these different requirements interact in terms of customer satisfaction. The advantages of classifying customer requirements by means of the Kano model are very clear (Sauerwein *et al.* 1996), particularly when you know the interests of your target consumers. For a premium product, we need to shift our focus away from one-dimensional requirements that the consumers can articulate and demand (e.g. free-range and welfare friendly), and find delighter attributes that consumers cannot articulate, but result in an immediate shift of the product into the premium space. That is not to say that the product cannot have one-dimensional attributes, just that they should not be the focus of the new product.

As the delighter or attractive requirements cannot be articulated, we need novel ways of investigating what they might be with our target consumers. If consumers are asked directly about their desires and purchasing motives in the exploratory phase, the results are usually disappointing and the answers already known (Sauerwein *et al.* 1996). Finding these

Table 1. The three types of requirements as explained by the Kano model (adapted from Sauerwein et al. 1996)

Must-be requirements	One-dimensional requirements	Attractive or delighter requirements
<ul> <li>The basic requirements of a product.</li> <li>If not fulfilled as expected, the customer will be extremely dissatisfied.</li> <li>These requirements are taken for granted and do not add to satisfaction.</li> <li>They are taken as a pre-requisite and not explicitly demanded.</li> <li>i.e. four wheels on a car, it is expected, you would not demand it. A consumer would also be unwilling to pay for more than what is expected. If one or more wheels are missing the comparement would have a tremely and the comparement of the comparement of the comparement.</li> </ul>	<ul> <li>These are requirements where satisfaction is directly proportional to the level of fulfilment.</li> <li>The more the better.</li> <li>The consumers are aware of them and will demand them.</li> <li>i.e. the fuel mileage of a car, the further you get on each tank is proportional to your satisfaction with the car.</li> </ul>	<ul> <li>These requirements have the greatest influence on customer satisfaction.</li> <li>They are neither requested nor expected from the consumer.</li> <li>Their absence will not lead to dissatisfaction.</li> <li>These are new or innovative ideas that the consumer did not realise they wanted, but when exposed to them lead to a vast increase in satisfaction.</li> <li>i.e. satellite navigation or bluetooth connectivity when first introduced.</li> </ul>



Fig. 1. Kano's model of satisfaction (Berger et al. 1993).

attributes involves a step-by-step process engaging target consumers, mapping what we know, finding gaps and testing with consumers across different stages and processes.

#### Consumer-aided design (CAD)

To identify these attributes that our target consumers are not able to express, we need novel approaches. CAD is a marketbased innovation concept focusing on consumers' current and future needs to design improved food products with added value (Costa *et al.* 2004). It can 'translate' subjective consumer preferences (e.g. healthy, welfare friendly) into objective product specifications to substantiate the fulfilment of these requirements through the creation of a core product (Costa *et al.* 2004). This approach allows us to search for delighter attributes within any product or concept that we wish to test.

Essentially, the aesthetics of the product are being tested to see what influences the consumer. Scientifically and efficiently enhancing product design can be achieved by gauging consumer responses to product aesthetics and correlating these perceptions to form elements of product design (Chen and Chuang 2008). This also goes beyond packaging, meat quality and presentation. Using current research being conducted with pork, with this technique, we can test the aesthetics of different production systems, farrowing accommodations and welfare approaches, among other things. Thus, rather than ask a consumer 'would you like a welfare friendly product?', we present the consumer with visual images to demonstrate different welfare conditions in real production systems, and gain feedback on what they find appealing or unappealing in this context, drawing out their subconscious beliefs. Too often when prompted with such a question directly, particularly in a group setting, a consumer will tell you what they think they should say rather than their true belief as would actually influence their buying behaviour. Applying CAD overcomes this issue as it does not rely on direct questioning of the consumers.

These detailed data provide a deeper understanding of why an attribute is important to a consumer, and allow you to apply this to your product. In the welfare example above, the direct question lets you know you need to have a welfare friendly product. Using CAD, you can look at your production system, know what attributes are appealing or unappealing within that system, and how they influence your consumers. Therefore, you can market your product to directly target specific types of consumers. There are six different processes involved in CAD, as displayed in Fig. 2. Once you know who your target consumers are and understand the networks that you are working within, it becomes a powerful feedback and development tool for the enhancement of current products, and the creation of new ones.

Over the past year at the University of Melbourne, we have been using CAD to test consumer attitudes towards Australian pork across different cultures. By testing different Australian production systems (free-range, indoor and barn-raised) among groups of Australian and Asian consumers from several different countries, interesting and often unexpected Kano attributes were identified. To give an example of how CAD works, the following includes some brief unpublished results from the Australian consumers.

#### Step 1: knowledge mapping (literature review)

The knowledge-mapping process, within a scientific setting, was akin to a literature review. All known beliefs on the psychology and buying habits of both Australian and Asian consumers were identified, both in how they purchased pork specifically, and more broadly in how they purchased premium products of any kind. From this extensive and in-depth research, we identified what we thought would be most appealing and unappealing to both consumer groups, as it related to current Australian pork production. So as to stretch the parameters of the test and capture all possible attitudes of the consumers, extremes were added from the harshest view of an animal rights activist to a utopian fantasy of pig farming. The knowledge mapping process suggested that for pork to be sold as premium to an Australian consumer it had to be free range.



Fig. 2. The six steps of consumer-aided design (CAD). Knowledge mapping, qualitative multivariate analysis (QMA), hypothesis, research strategy, consumer testing and Kano attributes.

#### Step 2: qualitative-multivariate analysis (QMA)

With the psychology of premium buyers in mind, we tested this range of stimuli with both consumer groups using qualitativemultivariate analysis (QMA). This is a method for sorting groups of items (in this case, Australian pork-production systems) in reference to each other (e.g. similarities and differences), on the basis of their qualities (Lopetcharat and Beckley 2012). This allowed us to capture insights from the consumers and discover linkages between different and important values as they related to each system. This methodology can reflect empathy, appropriately unlocking consumer behaviour and needs, and, when coupled with asking relevant questions, can completely capture the consumer views and beliefs on a product (Lopetcharat and Beckley 2012). With an emotive issue, such as pig-housing systems and animal welfare, this is a far more robust method than is conventional marketing research. The results of the QMA gave us our first initial list of possible Kano attributes as they might relate to current and future products. Through the QMA process, it became evident that a free-range system was not a positive attribute for an Australian consumer looking to buy a premium product, as was expressed in initial questioning. All farming methods were associated negatively as soon as we moved past superficial cues, but other aspects of pig production in Australia did arise as beneficial Kano attributes that could be used in the creation of a premium product.

#### Research strategy and consumer testing

At this point, we took the results to our industry partner, and developed a research strategy for the next round of consumer testing. We designed and developed new products that we then presented to our consumer groups. They identified specific Kano attributes, including delighter attributes, which can be added to the product design of the company. This process could not only lead to improvement of on-shelf products but also allows for the development of new premium products that are not currently available. This is where the real power of CAD is seen, as these new products and attributes were not able to be expressed by the consumer but have now been captured and identified.

#### Kano attributes

When consumers are surveyed using traditional methods, they tend to rate basic requirements with high importance (Tontini 2007). In this example, it was clear that when asked, consumers requested a free-range animal. However, through rigorous testing using CAD, it was clear that when specifically looking for a highquality, premium Australian pork product, free-range was not a delighter attribute, but, rather conversely, was a negatively associated attribute. This is not to say that a premium pork product cannot be free-range. Premium product buyers are discerning, and want detailed information on the product that they are paying for, hearing detailed information about farming practices does not lead to increased satisfaction nor does it influence buying. Therefore, an optimal premium pork product on an Australian shelf should focus on attributes other than free-range farming methods.

Consumer satisfaction is related to the fulfilment of consumer needs, and this fulfilment depends on the existence and performance of certain requirements in the product or service (Tontini 2007). This is fluid, and the impact changes over time as consumers become used to attributes or they are replaced by other products and changing lifestyles. It is, therefore, important in this competitive environment to not only identify which attributes are important and influence buying behaviour, but also to follow changes in the market and constantly evaluate this process (Tontini 2007). CAD and the Kano model allow you to achieve this rather simply. It requires a clear understanding of who your target consumers are, and an awareness of their networks. If performed properly, it can create a feedback loop with your target consumer base, which can be applied cross-culturally to properly capitalise on the opportunities that the Australian agriculture has presented to it in the coming years.

#### Conclusions

Compared with other agricultural sectors in Australia and overseas, the Australian pork industry has a strong reputation for innovation and adoption of research outcomes to gain maximum industry benefit (van Barneveld 2013). As the Australian pork industry increases exports into Asia, we will be required to live up to this reputation to survive. The challenges associated with the globalisation of food are equal to the opportunities, and both need to be addressed in a serious matter. We need to think big, be disruptive and innovate. However, we need to do so intelligently and strategically. Our consumers have become far more sophisticated over the past decade, with evermore knowledge at their fingertips. Our industry requires new techniques to help us as researchers get beyond linking metrics. CAD allows for forward thinking and can assist in generational planning for the Australian pork industry, creating a superior layout for the future compared with traditional market research. It allows us to keep up with our modern consumer base, and investigate where we fit with international consumers. As we globalise, Australian pork must get away from a commodity mindset, we must sell pork products, not pigs. We will never be competitive in price, or market share in Asia. We have inbuilt advantages to make us competitive as premium producers in overseas markets, but we need to ask what those advantages are, and gain an understanding of how they differentiate us, and not assume that we already know.

#### **Conflicts of interest**

The authors declare no conflicts of interest.

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# What are they thinking? Consumer attitudes to meat production in Australia

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**Abstract.** Meat production has come under increasing scrutiny from consumers and citizens who feel that certain practices are unethical and negatively affect farm-animal welfare. Animal welfare can be viewed as both a scientific and social concept, and purchasing products with animal welfare claims can be considered an act of 'ethical consumption'. The present paper reviews research that examines consumer attitudes to animal welfare and highlights tensions between consumer and citizen attitudes and behaviours, and assumptions that are made within these studies. We present our own research into motivations to purchase free-range eggs as an example of research that attempts to unpack these assumptions, in particular, that such purchases are made out of concern for animal welfare. We present a further example of our own research that attempts to identify how attitudes to meat production are socially constructed. We conclude with recommended strategies to engage the broader community in discussions about animal production, so as to improve industry–community communication about farm-animal welfare in meat-production industries.

Additional keywords: animal production, animal welfare, ethical consumption.

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#### Introduction

The practice of raising animals for meat has come under increasing public scrutiny in recent decades, particularly in western, developed societies where food is mostly plentiful. Most of these concerns relate to what is broadly termed 'animal welfare'; however, it is becoming clear that different actors within the food system think very differently about the meanings associated with this term (Dockès and Kling-Eveillar 2006; Vanhonacker et al. 2008; Hansson and Lagerkvist 2012; Coleman *et al.* 2016), and this difference in opinion has resulted in animal welfare becoming a point of tension and debate. More recently, concerns about the impact of animal production on the environment, and the sustainability of meat production, also have been raised (Verbeke et al. 2010); however, animal welfare continues to be the main ethical issue for consumers and the community, at least with respect to the pork industry in Australia, and thus is the focus of the present paper.

The diversity of opinions about farm-animal welfare among food-system actors, changing opinions among these actors over time, increasing scrutiny of food-production methods within the media (Phillipov 2016*a*), combined with ongoing and increasing demand for affordable animal-protein products presents challenges for livestock production. The purpose of the present paper is first to outline research into both community and consumer attitudes to livestock production from a range of disciplines and across locales including Australia, with particular

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focus on the assumptions about consumers that underpin this research given the methodologies employed. Second, we present findings from our own research (Bray *et al.* 2016; Bray and Ankeny 2017), which show how Australian consumers think about animal welfare. Third, we suggest strategies for engaging the community in discussions about farm-animal production on the basis of our findings and literature within the scholarly field of public understanding of science.

#### Background

#### Defining animal welfare

Although humans have drawn general parallels between themselves and non-human animals for thousands of years, the understanding that animals suffer, and beliefs that humans should not cause undue suffering even in the context of meat production, has been a much more recent phenomenon. Often framed as a response to food shortages after the Second World War, intensive livestock production has been enabled through scientific and technological innovations together with policies that aimed to increase food production. In the UK, the 1964 publication of *Animal Machines* by Ruth Harrison (Harrison 1964) mobilised public interest and led to the inclusion of the positive concept of 'welfare', rather than focus merely on cruelty, in legislation referring to the treatment of production animals (Woods 2012).

Research efforts into farm-animal welfare initially aimed to maximise productivity while addressing the welfare needs of animals in production systems, and focused on the connection between animal biology and an animal's 'welfare state' (Fox 1980). Improved understandings of motivation, cognition and the intricacy of social behaviour has led to a rapid development of animal welfare science in the past 30 years (Broom 2011). Considerations about animals focus on the following three sets of issues: physical attributes (such as growth and health), mental feelings (pleasure or suffering), and naturalness (environmental or behavioural), or all three combined (Fraser et al. 1997; Veissier and Miele 2014). These approaches are characterised in what are termed the 'five freedoms', namely freedom from injury and disease, hunger and thirst, discomfort, fear and distress, and freedom to perform normal behaviour (Farm Animal Welfare Council 1997, as cited by Appleby 2005), which form the basis of some theories of animal welfare.

More recently, definitions of animal welfare have broadened to include other concepts that people value, such as the dignity and integrity of animals (Appleby 2005), positive welfare states (Mellor and Beausoleil 2015) and 'quality of life' (Mellor 2016; see also Cornish et al. 2016 for a more detailed review). However, much of the research on farm-animal welfare has had a strong emphasis on scientific concepts of welfare and the impact of associated practices on the profitability and the supply chain, rather than on how members of the broader public conceptualise animal welfare. While there is scientific evidence to assist in justifying how some farm animals are raised, some contend that these justifications align more closely with the profitability of the system, rather than with the moral obligations towards animals that many in Western societies believe that we should have. To put it even more bluntly, it could be argued (as it is by activist groups when arguing against industry domination of research efforts) that the aim of much research on farm-animal welfare has been to identify production environments that have the least negative impacts on the animal, rather than developing optimal environments.

As Fraser (2008) stated, 'our understanding of animal welfare is both values-based and science-based. In this respect, animal welfare is like many other topics of 'mandated' science. . .where the tools of science are used within a framework of values', with acceptance of removal of the animal from its 'natural' environment being one of those values. However, the extent to which an animal should be able to have a natural life within an artificial environment is one of the key areas of tension between scientists and the broader community. Broom (2011), Rollin (1990, 1995), Fraser et al. (1997) and Fraser (2008) all agreed that animals should be able to live reasonably natural lives. However, in defining what counts as 'natural', there is considerable emphasis on the biological functioning of the animal and its interactions with its environment. Broom (2011) also argued that the environment provided to an animal should fulfil the needs of the animal but does not have to be the same as it would be in the wild. However, as shown in international studies, members of the broader community place much more emphasis on how the animal may feel in its environment, often connecting animals' happiness to their abilities to express their natural behaviours (Vanhonacker et al. 2008).

#### Consumers, citizens and ethical consumerism

Individuals can have roles as consumers, who purchase and eat animal products, and as citizens, who voice opinions or participate in activities related to policy or regulation (Coleman et al. 2015), and it has been noted that these roles may not be well coordinated with respect to meat production (Verbeke et al. 2010). Not all members of society agree that it is appropriate to consume animals or products made from animals, and those who avoid meat and other animal products may not be considered 'consumers': however, their views and behaviour as citizens are still important to the livestockproduction sector. Those who do eat animal products can act as both consumers and citizens in different contexts. Ethical consumerism aims to reconcile these behaviours to some extent and typically refers to voluntary food choices made out of concerns for a 'moral other' (such as a food animal) because of a consumer's values and beliefs, and may involve choosing certain foods over others because of perceived ethical superiority, or avoiding foods that can be morally problematic (Ankeny 2012). For example, someone who purchases sow stall-free pork because he or she believes that it is morally wrong to confine pregnant sows and gilts in pens is participating in an act of ethical consumerism. Ethical consumerism also can be thought of as a political or economic act, aimed at changing or eliminating certain types of practices by consumers 'voting with their dollar' (Shaw et al. 2006; Willis and Schor 2012) or 'voting with their forks' (Parker 2013); an example would be purchasing sow stall-free pork (rather than that produced using other methods), with the aim of using market forces to eliminate the use of sow stalls.

Public interest in 'ethical' food production and consumption also has been raised in recent years by TV shows featuring celebrity chefs such as Jamie Oliver, popular books including Michael Pollan's The Omnivore's Dilemma (Pollan 2006), and films such as Food, Inc. (Kenner *et al.* 2008), all of which have drawn attention to avoidance of food produced from intensively farmed animals. The awareness of ethical claims on food products also has been brought more into the mainstream in recent years by retailers who have 'reconceptualise[d] values by promoting particular standards or principles of judgement to apply to food decision-making' (Dixon 2003, p. 37). Major sponsorship of popular television cooking shows by retailers strengthens their location at the centre of popular discourse about food production and consumption (Phillipov 2016*b*).

However, there is an inherent tension between people acting as citizens and consumers, which has been noted by some food studies scholars; for instance, Johnston (2008) and Guthman and Brown (2016) found that in circumstances where people are encouraged to act as citizens and, hence, make decisions on the basis of the 'greater good', such as shopping at a Whole Foods Market (Johnston 2008) or posting comments online opposing the use of an agricultural chemicals (Guthman and Brown 2016), consumerism still becomes dominant (see also Ankeny 2016 for more on the contrast between food citizens and consumers). Other scholars using a critical animal studies approach (Jenkins and Twine 2014) have contended that consumers are not as 'free' as we might think when making food choices, given dominant sociocultural norms, particularly about animal consumption. They have also stressed that food choices, for instance, whether to be vegan or to consume animal products, are moral rather than lifestyle decisions, and, hence, should not be viewed via the consumer model. As we discuss further in the present paper, we agree that there are limitations to focusing solely on consumer behaviours, for instance, by utilising only market mechanisms such as willingness to pay to assess public opinions; other behaviours such as citizen behaviours (including voting and advocacy in relation to relevant issues) are important to examine, so as to understand community attitudes to animal production. However, studies that unpack assumptions about why consumers make their choices still provide insights into how consumers think about animal production, as we discuss in the next section.

## Consumer attitudes to animal welfare and purchasing behaviour

Various European, American and Canadian studies have demonstrated that consumers generally focus on the animal's resources, notably the access that animals have to unenclosed areas, believing that such settings will lead to happy and healthy animals (Lassen et al. 2006; Miele et al. 2011; Spooner et al. 2014). Consumers also have a strong preference for animals to be reared in natural environments (Boogaard et al. 2008; Spooner et al. 2014), support humane handling practices (Boogaard et al. 2008, 2011; Vanhonacker et al. 2008), and express concerns related to humane transport and slaughter (Spooner et al. 2014). Consumers often object to animal suffering or pain associated with modern production methods (Vanhonacker et al. 2008; Tuyttens et al. 2010; Spooner et al. 2014). Economic studies have been used to examine how consumers value products that have animal welfare claims. In economic terms, animal welfare is a credence attribute, that is, it cannot be directly discerned from the product itself by consumers at the time of purchase or after consumption, in contrast with experience attributes such as flavour. The motivations for purchasing products with increased animal welfare attributes are associated with consumer sociodemographic characteristics, knowledge of animal welfare issues and trust in information about rearing systems (Toma et al. 2012; Gerini et al. 2016); for instance, choice experiments in the USA demonstrated a higher willingness to pay for animal welfare attributes verified by a trusted authority such as the USDA (Olynk et al. 2010). Providing information about animal welfare may not increase willingness to pay for some products (Elbakidze and Nayga 2012); however, European studies have indicated that consumers are willing to increase their meat expenditure by about a third in response to a welfarelabelling regime (Kehlbacher et al. 2012). Despite sector growth, average consumer willingness to pay for cage-free and organic eggs was much less than the estimated price premiums (hence, their smaller market share) in a US study by Chang et al. (2010). This research also found that price premiums were higher than the increased costs of production, highlighting the importance of retailer pricing strategies in this market.

Although animal welfare concerns are not a strong driver of purchasing behaviour, at least compared with other attributes such as taste or health attributes, recent studies have shown that consumers consider animal welfare to be connected to both of these attributes, and so 'animal welfare' (as understood by the consumer as opposed to other food system actors) may be an increasingly important driver of purchasing as it is a proxy for taste and health, as we discuss in more detail below. International studies have shown that consumers view high animal welfare standards during production as an indicator that the resulting meat is safe, healthy, better tasting and of high quality (Verbeke *et al.* 2010). A link between food safety and farm-animal welfare in terms of antibiotic and growth hormone use in livestock production has been documented (Spooner *et al.* 2014), as well as concerns about genetically modified products (Lagerkvist and Hess 2011).

Animal welfare labels also can alter the perceived quality of a product, with high animal welfare standards leading to higher quality expectations (Carlucci et al. 2009), or attribution of other characteristics such as nutritional value (Anderson and Barrett 2016). Food labels can be thought of as boundary objects (Star and Griesemer 1989), which are objects that form an interface between one group and another. Boundary objects such as food labels are interpreted differently across groups and, hence, are flexible in various ways, but also maintain their integrity, remain recognisable, and serve as interpreters among communities based on some underlying content that remains stable or static (see Bray and Ankeny 2015 for a more in-depth discussion about ethical food labels). Labels clearly are not free-floating bundles of information but arise in a context that is strongly shaped by a variety of factors that may explain the broader associations that consumers may have towards animal products with ethical claims, in particular the attribution of superior characteristics (Lee et al. 2013; Anderson and Barrett 2016). However, confusion about the claims made on labels is not just about the public's failure to receive and act on information provided by 'experts', as might be claimed under a deficit model of public understanding. People's eating habits and food choices do not occur in a cultural, social or historical vacuum, but within broader sociocultural, moral and historical contexts that oftentimes go unrecognised in conventional approaches to these issues. Consumers may wish to make 'informed choices', but struggle to do so within the context of real shopping, which is limited by time as well as economic and other resources. To focus merely on the need for more education about the 'facts' about various types of food categories is to overlook the context within which food choices occur, and the diverse values that people bring to these choices.

#### Australian attitudes to meat production

There has been comparatively less research in Australia than in Europe or North America aimed at understanding community and consumer attitudes to farm animal welfare; however, it is generally understood that Australia lies midway between Europe and the USA in terms of both attitudes and policy responses. Although animal agriculture is important economically, historically and culturally, Australia is highly urbanised, with 80% of people living in the major cities (Australian Government Department of Infrastructure and Regional Development 2015). There is evidence that our food habits and systems differ in important ways from those in other countries; we have lower rates of vegetarianism than in other locales and define this category

differently (Beardsworth and Keil 1992), have higher average rates of intake of meat, and deep cultural identification with being meat eaters (Ankeny 2008; Chen 2016). In addition, Australia's quarantine restrictions on imported animal products for human consumption result in a heavy reliance on domestic production, and the duopoly in our retail sector means retailers play major roles, perhaps greater than producers and consumers, in how food products come to be valued (Dixon 2003). Last, because of the relatively short period of time over which European food and fibre production activities have taken place in Australia, and because the species of plants and animals used in agriculture have all been introduced, agricultural activities are not seen as 'part of nature' (Saltzman *et al.* 2011) and, hence, attitudes towards what is 'natural' for animals in production systems may differ from those in other countries.

Surveys have shown that Australians believe that farmers do a 'good job' of looking after their animals (Cockfield and Botterill 2012; Worsley et al. 2015) and that farmers have the highest level of trust among food-system actors (Henderson et al. 2011). However, one critique of these studies is that we do not know what understanding of the term 'farmer' was employed by the participants in these studies; for instance, whether a caged-egg producer is thought of as a 'farmer' in the same way as a beef cattle producer, and whether there are differential levels of trust depending on the type of production system. We do know via popular media and commercial intelligence that Australian consumers are increasingly concerned about animal welfare in Australia's livestock industries. Recent media reports have focused on practices that some consumers believe are unethical, such as sow stalls, caged hens, bobby calves and live export of beef cattle and sheep. Heightened attention to these issues may be due in part to recent activist activity focused on these practices, especially in the case of live export (Tiplady et al. 2013). Other prominent local campaigns include Animals Australia's 'no way to treat a lady' (http://www.animalsaustralia.org/no-way-to-treat-a-lady, accessed 24 September 2017) and 'make it possible' (http:// www.makeitpossible.com/, accessed 24 September 2017) campaigns, featuring local celebrities and television and billboard advertising aimed at caged-hens and intensive housing in the pig industry respectively.

A lack of knowledge about animal production practices within the community is often linked with increasing community concern about farm-animal welfare, and studies have shown that Australians do have generally poor knowledge of agriculture (Worsley *et al.* 2015). Australians self-report a wide variability of knowledge of farming practices, but often do not perform better than chance when asked factual questions about farming practices (Coleman 2010; Coleman *et al.* 2015). While these previous studies have provided insight on general attitudes and knowledge regarding animal welfare, they do not give us an understanding of the impact of attitudes and knowledge on actual purchasing behaviours or on community behaviours that may exert regulatory pressure on animal production practices.

Few studies have explored willingness to pay for products with welfare claims in Australia; Taylor and Signal (2009) is one exception, but this research used self-reporting within a survey rather than behavioural-economics methods, and thus faces the usual limitations presented by reliance on selfreporting, including a tendency to promote positive bias towards issues presented as of concern. This research showed that only 6% of participants were not concerned about farm-animal welfare, and 37% described themselves as 'concerned'; 34% would pay 5–10% more for products made in ways that ensure the five freedoms (Taylor and Signal 2009). Interestingly, selfrated knowledge did not increase willingness to pay among rural participants, but did among those from metropolitan areas, suggesting that these groups of consumers are working with different types of knowledge, or that the knowledge that they have has led to different perspectives and, hence, diverse conclusions.

## Why are consumers motivated to purchase products with animal welfare claims?

Although the research discussed so far in the present paper has shown important findings for our understanding of attitudes to farm-animal welfare and willingness to pay for products with welfare claims, almost all of it has assumed that there is shared understanding between the researchers and the research participants about what animal welfare is, i.e. that it is related to animal wellbeing, similar to how it is defined in the five freedoms. The findings of Taylor and Signal (2009), Coleman et al. (2015) and others have highlighted that consumers have different understanding of animal production and animal welfare, yet the motivations and reasonings behind why consumers may be concerned about animal welfare have not been critiqued and have been broadly interpreted as concern for animal wellbeing in production systems. Similarly, a willingness to pay for products with welfare claims is assumed to be motivated by desires on the part of consumers to improve animal wellbeing. Thus, industry efforts to address wellbeing may be insufficient, unless there are further efforts to understand how consumers think about animal welfare in relation to meat production.

As part of a much larger study examining ethical consumption, we recently explored why consumers purchased free-range eggs (for a full description of this work see Bray and Ankeny 2017). For this research, we conducted interviews and focus groups with over 70 Australians from diverse backgrounds in a qualitative investigation of their purchasing behaviours, and, in particular, whether they made any purchases that they viewed as 'ethical'. We asked participants explicitly whether they purchased food with animal welfare claim; free-range or cage-free eggs were the most commonly mentioned products. However, often those who had preferences for free-range eggs did not prefer meat with animal welfare claims. Our participants suggested reasons for this apparent inconsistency, namely that the labelling on egg products was larger, and that they were easier to find in the supermarket, but perhaps most importantly that the price difference as compared with the conventional product was manageable within their budgets, whereas meat was already an expensive item and, therefore, the premium for welfare claims made it 'too expensive'.

When participants talked about free-range meats, it was more common for them to mention chicken than pork, and there was little discussion of beef and sheep meat. One of the main issues
that people raised in connection with meat production was confinement, revealing their perceptions that it is common practice for pigs and meat-birds to be confined, which they do not think is the case with other meat animals. Although efforts on behalf of retailers to credential their products may be having one of their desired effects, namely to reassure their customers that they are concerned about animal welfare, participants in our research were confused about some of the claims, for example, confusing sow stalls with farrowing crates.

Confinement was not an issue for our participants for the reasons that most animal scientists and even possibly producers would expect. Confinement was seen as preventing animals from exhibiting natural behaviours (i.e. moving around), which, in turn, was thought to be important because it enabled animals to access their 'natural' diets. In contrast, participants described the diets of housed animals as 'unknown'. It may be the case that some of our participants thought that access to a 'natural' (in their words) diet is a welfare issue, in other words that certain foodstuffs may reduce an animal's wellbeing or even make animals ill. However, we suggest that it is more likely that our participants felt that an 'unknown' animal diet increased the risk associated with the resulting food products. Specific examples provided by participants that were connected with these fears include grain that may have been sprayed with pesticides, been genetically modified, or contain 'unknown' chemical additives (presumably referring to antibiotics or 'hormones' that many think are used in animal food production), all of which were thought to be negative and to decrease the safety of the resulting product. In addition, several participants described positive effects of a 'natural' diet, which, in turn, improves the quality of the product; animals that have natural diets somehow naturally express that diet in the resulting product, which is, in turn, of higher quality.

Although further work is needed to understand what the community thinks of as a 'natural' diet for pigs, there are three important implications from these findings. First, although a preference for products with welfare claims may appear to be an act of 'ethical consumption', it appears instead that welfare claims are being used by consumers as proxies for quality in terms of both nutrition and safety. This finding is critical as it changes the category of behaviour from one that is 'ethical' and oriented towards the moral other (e.g. the animal whose higher welfare is desired or even the environment which might be affected by production practices), to one that is motivated by the needs and desires of oneself and one's family. In short, it may well be the case that preferences for animal welfare products are not based on what we typically consider to be 'ethical' considerations.

Second, these findings force us to revisit research that has identified preferences for welfare claims, especially willingnessto-pay (WTP) studies, where it is concluded that people will pay more for products from production systems with better animal welfare, and where animal welfare is understood by the researchers to relate to a 'scientific definition' (and may not be analysed in additional detail with the participants). If welfare is a proxy for quality, then the WTP for animal welfare actually may be a WTP for a better-quality product. If consumer perceptions of superior sensory characteristics of products with welfare claims are correct, then animal welfare should not continue to be considered to be a credence value. In other words, consumers believe that it *can* be directly discerned from the product itself on the basis of appearance at the time of purchase or sensory characteristics detected during consumption.

Last, to be precise, our work has not shown that people do not consider the welfare of animals when they make their purchases or engage in citizen behaviour related to animal welfare, but instead that consumers think about animal welfare in much broader and holistic terms than simply defining it as animal wellbeing, and, in particular, that they often associate animal wellbeing closely with access to a 'natural' diet. They also feel very strongly that better welfare is connected to improved product quality and safety, a finding which echoes those in international studies mentioned previously.

### How do Australians talk about meat production with their children

So far, in the present paper, we have emphasised that attitudes to and understanding of animal welfare differ among different members of the community, and that these attitudes typically do not relate specifically to 'factual knowledge' of animal production systems. So as to understand how attitudes towards meat production are socially and culturally constructed, we explored how Australian families talk about meat production with their children (for a full description of this work see Bray et al. 2016). Talking about animal death is generally considered to be a sensitive topic in countries such as Australia, especially in front of children, and until very recently, there were few educational programs aimed at children that deal expressly with meat production. We hypothesised, on the basis of tracking discussions on social media, that this might also be a difficult subject for parents in meat-consuming families to discuss because of fears that their children might become emotional, or that it may seem to contradict messages about caring for animals. Parents, particularly those in urban areas, also may feel that they lack knowledge of animal production required to discuss the issue. We also could find no information about what Australian parents thought was an appropriate age for children to learn about the animal origins of meat, or whether certain activities such as attending agricultural shows were important for teaching children about meat production. To address these questions, we surveyed 225 primary carers of children from Australian households where meat was consumed. Most of respondents (93%) had talked with their children about meat production and 60% felt that these conversations were appropriate when the children were five or younger. Most conversations occurred when preparing (67%) or eating (65%)meals. Parents stressed that it was important from an early age for children to know where their food comes from. They also noted that if children were older when they were told where meat comes from, they were more likely to become upset. There were some differences in the ways that women and men thought about meat eating; for instance, women were more likely to agree that children should make conscious decisions about eating meat. In addition, women were more likely than men to be understanding if their children stopped eating meat and more likely to feel conflicted themselves about eating meat. Men were more likely to believe that meat should be eaten as part of a healthy diet, and that children should eat what is put in front of them without question. As the links with meat and masculinity have been well documented, the gendered aspects of our findings are perhaps not surprising. More generally, women had greater general concerns about animal welfare and were more likely to avoid meat than men.

We also found that those who lived in cities found conversations about food animals and meat more difficult than those who lived in rural areas. Families in rural areas did not perceive these types of conversations to be difficult or to be avoided and believed that children should be shown aspects of animal-food production practices. People who lived in urban areas were more likely to feel that they lacked some of the necessary knowledge to talk about meat production and had preferences for avoiding these conversations.

Most of the participants provided details about how their children learned about the origins of meat. Some (particularly those who lived in urban areas) described cases where children became upset and chose not to eat meat for a period of time. In contrast, parents of rural children noted that knowing about the origins of meat was part of their day-to-day lives, and some were directly involved in raising farm animals for food. For some rural participants, their roles in animal production may be linked to their attitudes, but may also be connected to other rural values. Most participants, be they rural or urban parents, thought that it was critical to communicate a sense of respect to their children, namely that animals should be treated well on farms and killed humanely, and that the effort that goes into producing meat should be recognised.

Our research also found that the home environment is typically where children first learn about food production, including meat. In addition, parents talk to children about meat in ways that reflect their own values about meat production. We contend that one of the most important findings was the value of respect stressed by most study respondents, which we believe is an encouraging starting point for a broader conversation about the future of ethical, sustainable, and affordable food based on shared values.

### Moving forward: why education and information are insufficient

Knowledge and trust are clearly both important factors for consumers when they choose their food. As we have shown, 'farmers' enjoy high levels of trust in Australia, and that this trust is not associated with a high level of technical knowledge about food production. In the past, communication efforts to encourage the community to accept controversial food production methods, such as, for example, the production of genetically modified crops, have concentrated on increasing the community's knowledge about the science behind such methods. This approach to science communication is termed 'the deficit model' and has largely been rejected by scholars in the fields of science communication and public understanding of science, as it is both based on flawed assumptions and is highly ineffective, although it persists as a dominant mode of communication (Simis et al. 2016). Hence, while it is tempting to treat worries about animal welfare practices as based on a deficit of knowledge about current management practices that maximise welfare (at least in the opinions of scientists and arguably producers), it is unclear whether increasing awareness and knowledge of these practices would create more community acceptance or change consumer behaviours.

We argue that trust is more important than knowledge or information. While it is difficult to gauge community sentiments towards pig production for the reasons we have outlined above, on the basis of the available literature in related domains, it is likely that concerns for animal welfare do not regularly influence the food choices made by the majority of consumers. Instead they rely on what is termed 'habitual trust' (Bildtgård 2008), that is, the assumption that events occurring in the world will continue in the same way as they have before; as long as this assumption is not betrayed, trust will be more or less habitual and automatic. Habitual trust is very different from 'reflexive trust', where a person 'consciously weighs different values and corresponding forms of knowledge against each other, while trying to determine which systems and actors to trust' (Bildtgård 2008, p. 118). Knowledge becomes important when and if people become aware that practices do not reflect what they thought occurred in practice; if the reality is more negative than perceptions, they can feel that their trust has been betrayed. This betrayal of trust is increasingly being described as a loss of a 'social licence to operate' by a particular industry or sector (Martin and Shepheard 2011).

Maintaining or building trust is key to community and consumer support for animal production. We know that shared values are more important for the formation of opinions, well ahead of technical knowledge (Sapp *et al.* 2009), and, so, we recommend that industry communication efforts must be based on shared values. However, it is dangerous to assume that just by 'talking' about shared values, an industry will be able to convince the community that what they are doing is 'right'. Engagement does not work if it occurs only in one direction; dialogue and a preparedness to change has to exist on both sides. A clear picture of the values and attitudes of both parties needs to be at the core so as to foster any effective dialogue.

Consumer and citizen behaviours are both complex. Understanding the physiological basis of animal welfare has been an area of considerable international and interdisciplinary research effort for decades, and at least a similar effort will be required to determine what society members feel are appropriate ways to raise animals for meat. Researchers from various fields such as psychology, economics, media studies, sociology and science communication can help reveal some parts of the picture using their own particular lenses, but it will take sustained and coordinated investment across disciplines to ensure alignment in attitudes to and understanding of animal welfare between meat producers and the broader public.

#### **Conflicts of interest**

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# What does the 'closed herd' really mean for Australian breeding companies and their customers?

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Abstract. The perception that the genetic background of the Australian pig population is limiting for genetic improvement of commercial pigs in Australia is considered in the context of well established theory combined with practical evidence. The diversity of pig breeds used in modern commercial pig-breeding programs is diminished worldwide relative to all the pig breeds available. Australia is no different in this respect. The use of predominantly three main breeds (Large White, Landrace, Duroc) and synthetic lines, with contributions from other minor breeds to form the basis of a cross-breeding system for commercial pig production is well established internationally. The Australian concern of relatively small founder populations is potentially of relevance, from a theoretical perspective, for (1) the prevalence of defects or the presence of desirable alleles, and (2) the loss of genetic variation or increase in inbreeding depression resulting from increased inbreeding in closed nucleus lines, potentially reducing response to selection. However, rates of response achieved in Australian herds are generally commensurate with the performance recording and selection emphasis applied, and do not appear to be unduly restricted. Moreover, favourable alleles present in unrepresented breeds are frequently present in the three major breeds elsewhere, and therefore would be expected to be present within the Australian populations. Wider testing would provide confirmation of this. Comparison of estimates of effective population size of Australian populations with experimental selection lines overseas (e.g. INRA) or other intensely selected species (e.g. Holstein cattle) suggest adequate genetic diversity to achieve ongoing genetic improvement in the Australian pig industry. However, fitness traits should be included in breeding goals. What remains to be seen is whether novel phenotypes or genotypes are required to meet future challenges, which might be imposed by changes in the environment (e.g. climate change, disease) or market needs. Given probable overlap in genetic merit across Australian and foreign populations for unselected attributes, we suggest that sufficient genetic resources are already present in Australian herds to continue commercial progress within existing Australian populations that have adapted to Australian conditions.

Additional keywords: genetic improvement, genetic variation, inbreeding.

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#### Introduction

Selection to achieve genetic changes in breeding populations is a numbers game, and this game is played by every breeding company on behalf of their clients every day of the year. Essential to the game is sufficient genetic variability that can be explored to achieve genetic improvement of economically important traits. Australian breeders no longer import new pig genotypes and several questions have been raised. What is important for breeding companies to achieve the genetic changes that their clients require? Can genetic improvement be achieved with the breeds and populations that are available within Australia? Does the history of the founder populations limit genetic gains that we can expect in Australia? Or, is the perception of the level of genetic merit in Australia altered by factors other than genetics? What about the possibilities for adapting to future needs, brought about by climate change, new disease challenges or changes to consumer requirements?.

In the present paper, we discuss some of the issues important for genetic gain within Australian breeding herds, without too much detail on the extensive theory that underpins knowledge in this area.

## The development of modern commercial breeding programs

Breeding programs in all livestock species have changed dramatically over time. Prior to the 19th century, the use of performance recording and selective breeding to generate permanent change in livestock was relatively poorly understood. In the early 20th century, significant developments were made in understanding Mendelian genetics and selection theory. Among many works, a mathematical framework for quantifying the relationships among individuals and the calculation of inbreeding (Wright 1921, 1922), the use of selection index to combine multiple selection criteria (Hazel 1943), and the implications of selection for prediction of response and inbreeding in over-lapping generations typical of animal-breeding populations (Hill 1977) was established. Throughout the 1960s, commercial breeding companies made

their mark for easily measured traits, such as weight or fat depth, largely through direct comparison of contemporaries available for selection. The development of best linear unbiased prediction (BLUP) in the 1970s (Henderson 1975) was a game changer that improved the accuracy of comparisons among individuals, across environments and generations, particularly for lowly heritable traits. This enabled a much greater flexibility in the structure of breeding programs and also potentially larger populations evaluated for selection (Webb and Bampton 1988, cited in Webb 1991). In the late 1980s to 1990s, these sophisticated genetic evaluation systems were more routinely implemented. driving gains in more difficult-to-measure traits that require family information (e.g. sex-limited, fitness and carcass traits). In this century, technological developments have enabled genomic information to become accessible routinely, but commercial implementation is patchy. Use of genomic technology requires significant investment, interest in appropriate trait groups that maximise returns on investment and economies of scale to take full advantage of the improvements in accuracy that can be obtained from using this information source. The use of genomic technology is also driven by phenotypic data, often recorded in resource populations, discussed further in this symposium by (Garrick 2017) and Culbertson et al. (2017).

Essentially, in modern breeding programs, the selection of breeding stock has largely become based on performance data, backed up by sophisticated software and powerful computers for predicting genetic merit of individuals, and this has been reflected by increasing rates of genetic improvement in an increasingly wider range of traits. Commercial application of new technologies (e.g. genomics, sequencing) will generate even larger genomic databases that will contribute to further improvements in the accuracy of genetic evaluation and selection of breeding stock. However, with the exception of some commercially available genetic tests (e.g. see Rothschild *et al.* 2007), the application of genomic information in pig breeding is currently predominantly a feature of consolidated breeding companies and national breeding schemes.

Modern breeding companies demonstrate the effectiveness of their breeding programs by obtaining a sustained response to selection in the right direction(s) for the traits that are considered economically important to their customers, and therefore included in their breeding goals. They also require the ability to add new selection criteria to their repertoire, should the need arise. Within-breed selection for dual-purpose goals has evolved, such that breeding companies now typically retain more than one selection line, largely to accommodate differences in the selection emphasis required for terminal versus maternal lines, but also to create marketing flexibility. The commercial sow and slaughter progeny are then derived from the crossing of independent 'nucleus' lines or products. Therefore, providing the nucleus lines are maintained as essentially distinct populations, most of commercial sows and slaughter progeny in the Australian Industry are not hindered by issues relating to inbreeding, such as inbreeding depression, because they are outbred by the process of line crossing. The performance of multiplication and maternal tiers then represents the average genetic merit of the parents, as well as any heterosis that is expressed by the crossbred dams and progeny.

There are some fundamental requirements, well known in the field of quantitative genetics and outlined in detail (Hammond 1992; Harris and Newman 1994), which must be met to obtain effective genetic change within nucleus lines and to disseminate this improvement to commercial herds. These include the following:

- (1) A defined breeding goal, which is usually short-medium term in nature (e.g. 5–10 years) and driven by client's needs. Breeding goals identify which traits need changing and their relative importance. Breeding goals are typically balanced across lines, so that selection effort is not wasted for attributes which are not required within each specific line. Breeding goals can also change over time in response to new requirements.
- (2) Genetic variation in measureable and heritable traits (i.e. phenotypes) that have been identified as important in an economic context. Some phenotypes have no intrinsic economic value in themselves (e.g. IGF-I or haemoglobin), but provide indirect genetic information for other economically important traits, such as feed conversion ratio (Bunter *et al.* 2005) or pork quality (Hermesch and Jones 2012). Some attributes that are important for performance cannot be changed by genetic selection because they are not heritable. For example, it is difficult to obtain improved meat quality by selecting for an altered gender balance (i.e. all female offspring). Many traits are heritable, but not all are easy to measure or are of economic importance.
- (3) Accurate performance-recording systems combined with the best mathematical methodology for genetic evaluation (e.g. BLUP). This enables accurate selection decisions based on the phenotypes available. This is particularly important for hard to measure traits, such as sex-limited, late in life traits (e.g. sow longevity or reproduction); lowly heritable traits, or traits which can only be recorded on relatives and not the selection candidates themselves (e.g. survival, disease or meat quality traits). The potential contribution of genomic information to increasing the accuracy of selection fits into here, but it is important to note that genomic information does not eliminate the need for phenotypes somewhere on those traits.
- (4) A population of large enough size to enable high selection intensity (i.e. selecting the best animals), minimise random drift and keep inbreeding rates to an acceptable level. The reality is that the reproductive physiology of a species determines how easy it is to achieve this. Moreover, the size of the population managed for animal-breeding programs generally depends on associated costs relative to the predicted response to selection.
- (5) Control and monitoring strategies. Control of generation intervals. Control of selection and mating strategies. Use of tools such as mate selection (Bunter 1996; Kinghorn 2011) and optimum contribution selection (Henryon *et al.* 2014) to balance response to selection with inbreeding, or other restrictions.

These fundamentals for effective breeding programs are generally well adhered to by breeding companies, who moreor-less can demonstrate improvements in traits for which they record data. Evidence for progress in Australian populations for common traits is shown in Table 1, and the improvements seen are generally comparable to genetic progress published elsewhere. Examples from Canada and Denmark are shown for comparison. There are also a range of other traits that independent breeding companies might consider, such as, for example, mortality and meat-quality traits, or traits relating to welfare. What is not so clear is how the raw material they have started with might influence their ability to achieve future change, compared with, say, a difference in breeding goals among companies, which has a clear impact on the genetic changes achieved. In addition, implementation of current (e.g. panels of singlenucleotide polymorphism (SNP) panels) and future (e.g. gene editing) genomic technologies offer new opportunities that need to be explored further in Australian populations.

#### Population size and founder-population effects

While data from large breeding populations can be processed in modern BLUP-based genetic evaluation systems, implying potentially more genetic diversity and higher selection intensities due to a larger number of breeding animals (Hammond 1982), simulation studies have also demonstrated that selection using BLUP can increase rates of inbreeding over other forms of breeding schemes (Belonsky and Kennedy 1988), particularly under single-trait selection for lowly heritable traits, such as litter size. This is of some concern for breeding companies because inbreeding contributes to loss of genetic variance and inbreeding depression. Loss of genetic variation then depletes longer-term opportunities to obtain a response to selection, while inbreeding depression reduces fitness traits, which are typically reproduction or survival traits.

Elevated inbreeding under BLUP-based selection is less problematical with diverse breeding objectives leading to multiple traits being included in the breeding goal, including fitness traits, such as litter size and survival. Moreover, selection and mating strategies exist to limit the accumulation of inbreeding and loss of diversity, while still achieving high rates of genetic gain. Minimising loss of genetic variation is a major goal for the long-term conservation management of small populations. In contrast, maximising the short- to mediumterm response is the major goal for commercial breeding operations and, by definition, inbreeding within a closed herd or population can only increase. Therefore, some balance among these potentially antagonistic goals is required. There is little commercial sense in maintaining large populations for the sake of diversity if a large proportion of animals can contribute no beneficial genes (i.e. they are not competitive selection candidates) to the required breeding program. For this reason, modern pig breeding has become focused on using few breeds with superior performance in economically desired characteristics (e.g. litter size, growth rates, and carcass-quality attributes).

#### Breeds that contribute to commercial pig breeding

In 2007, the FAO published a massive 524-page treatise on the state of the world's genetic resources for all domesticated livestock species. In this compilation, there were 566 pig breeds identified and 541 (96%) were considered local breeds (occurring within a limited geographical range), mostly originating in Asia and Europe. About 141 breeds were already listed as extinct, 59 breeds existed with varying population sizes in more than one country, and only a handful of the breeds actually contributed to commercial pig-breeding herds worldwide. These breeds are Large White (or Yorkshire), Landrace, Duroc, Hampshire and Pietrain pigs. Only Holstein-Friesian cattle were more widely distributed globally than were these five pig breeds. Large White and Landrace breeds form the basis of commercial maternal and terminal lines in nearly all countries, while the remaining breeds typically contribute predominantly to terminal lines. Of these five breeds, only the purebred Pietrain is absent from Australia. Clearly, nearly all commercial breeding operations worldwide have lost genetic diversity if one considers how many breeds were originally available. However, similar to the dominance of the Holstein breed for milk production, the reduction in pig breeds has been based on the clear superiority of these breeds for economically important traits. Breed choice and distribution is generally driven by the desire of breeders and producers to obtain genotypes that perform optimally in a given production environment (Peters and Meyn 2005), such as the modern intensive production environment for pigs.

Dispersion of these breeds around the world was then facilitated by commercial breeding companies, globalisation and new reproductive technologies (e.g. artificial insemination commencing in the 1960s, and embryo transfers in the 1980s) that enabled transfer of genes without requiring the transfer of live animals. Relative to the other main livestock species, pigs

### Table 1. Average annual genetic gains for 28 Australian populations (AUS28), along with trends reported for Yorkshire (Y), Landrace (LR) and Duroc (DU) populations in Canada (CA) and Denmark (DK)

AUS28 from Hermesch (2006); CA from CSIS (2015); DK from DanBred International (2015)

Trait	AUS28			Yorkshire		Landrace		Duroc	
	Mean	Top 25% index	Top 25% trait	CA	DK	CA	DK	CA	DK
Growth rate									
0–90 kg (g/day)	+5.0	+7.52	+9.59	+3.99		+2.84		+5.31	
0–30 kg (g/day)					+1.1		+0.1		+4.0
30–100 kg (g/day)					+12.1		+10.5		+20.6
Backfat (mm)	-0.15	-0.26	-0.28	-0.01		-0.01		-0.07	
Muscle depth (mm)	+0.05	+0.014	+0.20	+0.15		+0.07		+0.30	
Feed conversion ratio (kg/kg)	-0.01	-0.027	-0.028	-0.011	-0.027	-0.010	-0.031	-0.014	-0.048
Litter size (piglets/litter)	+0.07	+0.12	+0.18	+0.14	+0.16	+0.13	+0.21	+0.02	n.a.

are somewhat hindered in this respect because both semen and embryos of pigs do not freeze well, so introductions to Australia were largely confined to live animals at high import expense. In addition, Australia obviously lacked any local pig populations well adapted to the environment to draw from.

The first pigs, of unreported breed composition, arrived in Australia with European settlement and were dispersed by settlers in the 19th century. These pigs, along with escapees from later introductions, have contributed to the very large population (>20 million) of feral pigs that free-range throughout Australia (Department of Sustainability, Water, Population and Communities 2011). Later introductions of Large White (McPhee 1965), Landrace (Treacy 1976) and Duroc (Taylor et al. 2005a, 2005b, 2005c) from a range of populations occurred sporadically up until 1990. Since the early 1900s, the Australian Pig Breeders Association (APBA) supported purebred registrations for nine breeds in Australia, namely Large White, Landrace, Duroc and Hampshire, as well the Tamworth, Large Black, Berkshire, Wessex Saddleback and Welsh breeds (https://lbcentre.com.au/Australian Pig Breeders Society\_Australia.php, verified 27 September 2017), but several of these are now listed as rare breeds on the basis of registrations. None of the major Australian breeding companies registers purebred pigs with the APBA, so their lines do not have direct contemporaries within the herds registering with the APBA. The last known imports to Australian breeding companies were Norwegian Landrace and Finnish Large White, but with no current agreed import protocols, there have been no further introductions since 1990 (Brian Luxford, Rivalea Australia Pty Ltd, pers. comm.).

Could any of the above populations be considered as a resource for Australian commercial companies? This might be true if these populations carried at least some genes of merit not present in commercial populations, such as, for example, genes for disease resistance. However, when significant genetic change has already been made in modern herds, it becomes increasingly difficult to introduce new genetic material into a commercial population without incurring substantial production losses, and there is also no guarantee of success. An example of the average production losses associated with introducing unselected boars into Australian nucleus herds was demonstrated by Jones and Hermesch (2009). For other traits, in which there has been less change in the commercial populations, greater overlap could potentially be expected across populations, as was illustrated for acrossand within-breed sire comparisons for meat- and eating-quality traits in Australian populations (Bunter et al. 2008). Generally, independent breeding companies that have tried introductions from outside their herds have been disappointed, and this practice has subsequently become uncommon.

Some examples of introducing genes from new breeds into existing lines include the introgression of desired alleles from Meishan into maternal lines in Europe. The aim of introgression is to retain only the desired variant(s) of interest from an outside population within the recipient genome. Introgression of desired alleles, therefore, requires matings to fix the desired alleles, followed by multiple generations of backcrossing to retrieve the majority of the recipient line content, which concurrently creates genetic lag, and it is inevitable for some of the donor genome to be retained. While Meishan has many other undesirable characteristics (slow growth, fat), the large breed advantage of Meishan for litter size, combined with the use of genetic markers for desirable genes, and a receptive customer market, was enabling for this strategy (Wall et al. 2005). Nevertheless, some of the causative loci responsible for the superior performance of Meishan with respect to litter size are already present in Large White commercial lines. The French achieved higher litter size simply by screening their national Large White population for hyperprolific sows. In contrast, the attempt to introgress genes from breeds or related species resistant to African swine fever has been largely unsuccessful (FAO 2007). The difficulty of achieving introgression without losing too many other desirable attributes, and the sparsity of known single genes with a large effect, generally motivates the pursuit of selection strategies within existing commercial lines for desired characteristics such as disease resistance. Some examples include selection against the Halothane gene and for resistance to strains of E. coli, both of which require some individuals carrying the favourable alleles within the existing population. Direct selection for survivors within a disease-affected population is generally not an accepted practice in the major livestock species, although this strategy has been used successfully in other species, such as oysters and poultry.

#### Implications of closed populations

As noted above, the sample of breeds contributing to Australian pig breeding is consistent with the dominant breeds used worldwide by commercial pig-breeding companies, with the general exception of Pietrain. In fact, breeding in pigs and poultry has become dominated by a few large companies that have been in business since the 1960s, and these companies generally do not exchange genetic material unless there is a takeover or merger. So, worldwide, there are a series of closed populations, each subjected to the selection pressures determined within the company, and each derived potentially from a small founder population. As a result of this, some line 'breed' characteristics are now quite inconsistent across (and even within) companies and countries. For example, the historically 'maternal' Large White breed heavily selected for production and carcass characteristics might end up as a terminal line, less 'maternal' in attributes than a Landrace line arising from a carcass-orientated background, but selected for maternal performance. Company breeding goals can have a large impact on what are thought of as traditional breed characteristics, and over long-enough time frames, can create larger differences between within-breed sublines than among breeds, as has been previously shown in some US comparison trials (Johnson and Goodwin 1995). Thus, overall variability within breed might not be declining, because selection within independent subpopulations from a common base also increases diversity. Dispersal of breeds into subpopulations is generally accompanied by differential selection and adaptation (FAO 2007).

### How do we assess genetic diversity (and how little is too little)?

The role of population size and selection intensities for long-term response (i.e. maintaining variability for selection) and fitness in

closed populations have been extensively studied both in theory and through selection experiments. The general observation has been that variability in the traits under selection is generally maintained over very long time frames by segregation and, to a lesser extent, mutation, but that fitness attributes may decline concurrently as a correlated response to selection unless they are also included in the breeding goal (Hill and Zhang 2009). The founder population can be important in this respect, but effective population size plays the major role with respect to maintaining variation both in production and fitness traits (Hill and Zhang 2009). A decline in population size or a loss of diversity within an otherwise large population (e.g. Holstein–Friesian) can make a numerically large population a very small effective population.

The original number of pigs imported to Australia, representing the founders for each breed, is hard to establish accurately. A large sample of unrelated founder animals maximises genetic diversity, but this is generally not what happens in reality. The implication of relatedness among founders for diversity is exacerbated if the subsequent contributions from individual founding parents vary widely. A small founder-population size or extensive use of influential individuals in closed herds can be problematical, particularly when deleterious variants of genes are present in that population. This is because accumulating inbreeding increases homozygosity and, therefore, the opportunity for expression of deleterious recessives. The potential founderpopulation effect is generally difficult to quantify for livestock species because of long generation intervals and low reproductive rates. In fact, closed selection lines with a very long history of selection are often demonstrated to retain variability and have sustained response to selection, even when selection is for a fairly narrow breeding goal. For example, poultry selection lines showed no evidence for a plateau in the rate of response to selection for bodyweight gain, despite a four-fold change in weight over 50 generations of selection (Hill and Zhang 2009).

#### The concepts of variability and effective population size

Phenotypic and genetic diversity are both important for selection. Phenotypic diversity enables discrimination among the performances of individuals who are potential selection candidates. A lack of phenotypic diversity is probably more illustrative of a poor choice of phenotype, or methodology for obtaining phenotypes, than of a lack of genetic diversity per se. For example, measuring fatness in whole centimetre instead of millimetre increments would immediately remove phenotypic diversity for fatness, even though the genetic control of fatness is moderate regardless of the poor choice of measurement. Therefore, phenotypic diversity does not necessarily reflect genetic diversity, and vice versa. Similarly, phenotypic levels of performance are, particularly for lowly heritable traits, typically more indicative of environmental than of genetic factors. This is why performance levels should be compared only among populations in the same environment, or with genetic linkage between populations. Phenotypic and genetic variability are evident for a wide range of economically important traits in Australian populations. Genetic variation exists in Australian populations for sow characteristics such as reproductive performance, including piglet survival, sow longevity and sow mature weight, as well as for traits describing feed efficiency and lean meat growth, carcass and meat-quality traits of growing pigs, and traits describing behaviour, adaptation and robustness (Bunter *et al.* 2015; Hermesch 2004; Hermesch and Jones 2012; Lewis and Bunter 2011*a*, 2011*b*; Li and Hermesch 2016; Tholen *et al.* 1996, respectively). Parameter estimates from these studies have been generally similar to comparable estimates from populations in other countries.

The maintenance of genetic diversity, however, is typically described in terms of effective population size (Ne), and it is related to the number of breeding animals, selection and mating strategies, and the resulting accumulation of inbreeding. Ne represents the number of breeding animals in an idealised population (for example, no selection, random mating, equal contributions from parents, no mutation) that would lose heterozygosity at the same rate as the observed population. Effective population size can be difficult to calculate for farm livestock populations because of the dynamics of breeding populations (Welsh et al. 2010). These dynamics can include over-lapping generations, rescaling sow herd size, variable boar use, irregular introductions of new animals, and bottlenecks caused by restrictive re-population due to disease, or restrictions on selection candidates due to genotype. Since calculation of Ne is typically pedigree based, loss of pedigree information (e.g. due to grading up) can also be erroneously interpreted as increased diversity, because unpedigreed introductions are considered to represent new, unrelated animals.

Until the provision of POPREP software (Groeneveld *et al.* 2009), there were no published estimates of parameters that describe genetic diversity for commercial populations. This software enables anyone to monitor their populations using available pedigree. The INRA experimental selection line for reduced residual feed intake (RFI), which has shown a sustained response to single-trait selection for RFI over nine generations so far, have an estimated *Ne* of 35, using the method of Gutiérrez *et al.* (2009). This *Ne* is typical of an experimental line, with a comparable rate of inbreeding of <1.5% per generation. In addition to a sustained response for RFI, fecundity in this line (a fitness trait, which was not selected for) has increased (Gilbert *et al.* 2017), demonstrating that small closed lines do not always lose fitness under selection. Estimates from other populations for the major pig breeds are shown in Table 2.

### Table 2. Effective population size (Ne) calculated from pedigree for common pig breeds

For USA,  $Ne=1/(2\Delta F)$  used by Welsh *et al.* (2010). For Croatia, Ne is average across 2008–2012 used by Krupa *et al.* (2015). For Australia, values are based on method of (Gutiérrez *et al.* 2009) used by D'Augustin *et al.* (2017), time span 2010–2015. NPIP, National Pig Improvement Program

Country Data Source	USA Breed associations	Croatia Pig breeders associations	Australia NPIP
Berkshire	77	n.a.	n.a.
Duroc	113	34	42-61
Pietrain	n.a.	82	n.a.
Hampshire	109	n.a.	n.a.
Landrace	74-80	60	52-108
Large White/Yorkshire	113	138 (dam line) 118 (sire line)	64–98

While estimates of Ne (D'Augustin et al. 2017) for genetically connected but independent herds in the Australian National Pig Improvement Program have generally been smaller than those reported for the same breed from the USA (Welsh et al. 2010), the lowest Ne of 42 are consistent with rates of inbreeding of <1.2% per generation, and the highest Ne are equivalent to <0.5% per generation. These rates of inbreeding are generally considered acceptable for selected populations. Estimates of Ne for Croatian populations (Krupa et al. 2015) have been variable and inflated by missing pedigree for most breeds, but have showed sustained low Ne only for Duroc. The paper of Krupa et al. (2015) also very nicely demonstrated how the impact of founder parents to diversity diminished over time. Estimates from a privately owned Landrace-based nucleus line within Australia exceed 100; Landrace is the Australian breed considered to have the most limited founder population. This is a larger Ne than is estimated from pedigree or molecular data for the worldwide Holstein-Friesian breed (Rodriguez-Ramilo et al. 2015), suggesting that the effective population size should not be limiting for selection in commercial Australian pig populations.

#### Genetic diversity assessed using genomic information

Genomic information can also be used to assess genetic diversity and inbreeding. For example, genomic estimates of co-ancestry can be obtained from identity by descent segments, while inbreeding is illustrated by runs of homozygosity (Rodriguez-Ramilo et al. 2015). Similar estimates of diversity should be obtained from full-pedigree and genomic information. Using the Porcine 60K SNP panel, Zhang and Plastow (2011) concluded that the long history of domestication of pigs contributed generally to higher inbreeding (lower heterozygosity) than with humans and cattle, implying a lower Ne. However, there was considerable genetic distance between European and Asian breeds, with more heterozygosity within (the less selected) Asian breeds. In their data, the joint effects of selection and genetic drift, due to different numbers of ancestral stocks, produced different patterns of structured genomic diversity (e.g. heterozygosity and associations between SNPs).

Genomic data from a recent study using an Australian population demonstrated that average heterozygosity of SNPs for the Australian Large White were similar to those of the European Large White (35.5 vs 33.9%; Gore *et al.* 2017). Moreover, breed proportions estimated from constrained genomic regression (Boerner 2017) suggest that the Australian Large White sample genotyped contained breed contributions not only from European Large White (36%), but also non-trivial contributions from Landrace (5.8%), Welsh (12.6%), Pietrain (12.5%) and Middle White breeds (15.9%). This would suggest that characteristics of the Pietrain breed are not entirely absent from Australian populations, and contribute towards the existing genetic variation in that breed. Similar studies could be performed with other samples of breed within Australia.

#### Conclusions

While small founder size of Australian pig populations might be considered limiting in theory, there is no substantial evidence that this is a major limiting factor for response to selection in commercial Australian populations. There is no known evidence for excessive expression of deleterious alleles. Estimates of effective population sizes from pedigree or limited genomic data also do not indicate that there is cause for concern in current populations where data are available. The authors suggest that genetic resources are already present in Australian herds to continue commercial progress within existing Australian populations. Cost-effective routes to implement new technologies such as genomics, which can enhance rates of genetic gain, should be investigated within the Australian industry.

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### The role of genomics in pig improvement

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Abstract. Genomic prediction uses marker genotypes distributed throughout the genome to track the inheritance of chromosome fragments and quantify their contribution to the superiority or inferiority of breeding merit. It does this by using a so-called training population of historical animals with both genotype and phenotypic measures. Genotyping adds additional costs to an improvement program, so these costs must be offset elsewhere for there to be net benefit from adopting genomics in pig improvement. Genomic information is used implicitly or explicitly to predict the merit of young selection candidates more reliably than is the case when using only pedigree and phenotypic performance information. More accurate genomic prediction of index merit in young selection candidates results in faster genetic progress. Further, the technology allows good use to be made of phenotypic measures from non-traditional sources, including descendants of nucleus animals whose performance is measured in the commercial sector. This facilitates nucleus selection to include more reliable predictions for disease-resistance, and carcass and meat-quality traits, other traits with low heritability or those measured late in life, and to directly target selection for crossbred rather than purebred performance. Collectively, these features allow genomic prediction to provide a more balanced response to selection with respect to the entire portfolio of traits that influence income and costs in pig-production systems. Achieving the full cost-benefit potential from using genomics will not occur from simply genotyping nucleus animals and using this information in prediction, it requires innovation, ongoing phenotyping and genotyping, and re-examination of all the systems and processes involved in pig improvement.

Additional keywords: crossbred performance, evaluation, selection.

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#### Introduction

Global pig production is mostly represented by pigs in the so-called 'commercial' sector, for which the rate of genetic improvement is dictated by that achieved from selection in a much smaller 'nucleus' sector. This industry structure is typically coupled with a 'multiplication' sector that sources improved pigs from the nucleus sector, which it multiplies to supply parents that improve productivity and profitability in the commercial sector (Bichard 1971). Such a structure means noteworthy per-animal investment in genetic improvement in the nucleus is recouped by a much larger number of small per-animal returns in the commercial sector. Competition among different breeding companies representing the nucleus sector rewards market share to those that can achieve faster rates of genetic gain, while selling breeding animals at competitive rates. Companies, therefore, seek ongoing innovations to achieve enhanced annual rates of cost-effective genetic gain. One such innovation is genomic prediction and it offers the potential to achieve faster progress in increasing productivity and profitability of commercially managed descendants.

#### **Pig improvement**

Over recent decades, many innovations have been introduced by breeding companies that have enhanced the genetic merit of pigs for lifetime productivity and profitability. These include the following: exploitation of breed complementarity and heterosis; the routine prediction of breeding values through best linear unbiased prediction (BLUP), based on pedigree and performance information for key performance traits; the development of breeding objectives that extended the portfolio of economically relevant traits under selection; the use of correlated or indicator traits to improve the accuracy of prediction in hard-to-measure economically relevant traits, such as those that are one or more of sex-limited, low heritability, measured at the end of life, impractical or expensive to measure; and mate selection (optimising response to selection while managing the rate of inbreeding).

Despite all these advances, the annual realised rates of genetic gain are still well below those that could be achieved in the same-sized nucleus if the selection candidates could be more reliably evaluated at an early age (Smith 1984). Increasing the reliability of genetic evaluations directly increases the effectiveness of selection, and, when available at a much younger age, allows both increased selection intensities and reduced generation intervals. Increasing the reliability of evaluations is in itself straightforward. Progeny testing can be used to achieve near-perfect accuracy, but it is expensive in financial terms and the delay in using selection candidates widely while progeny information is obtained will increase generation intervals. Measuring additional indicator traits can improve the accuracy of evaluations, but the contribution of indicator traits is limited by their heritability and genetic correlation with the economically relevant traits, and only a small number of useful indicator traits has been found.

Selection typically involves choosing replacements with the most favourable index values that balance superiority and inferiority over the evaluations of all the economically relevant traits (Hazel 1943). Selection response can be viewed as arising from a among-family component that comprises selecting the better families and a within-family component that comprises selecting the best offspring from within the better families. Among-family and within-family selection can also be considered in terms of predicting, for each selection candidate, the parent average merit and its deviation from parent average, which is technically referred to as being due to Mendelian sampling. Conventional pedigree and performance recording cannot predict Mendelian sampling for breeding merit without phenotypic measurement on the selection candidate, or its offspring; all other relatives contribute only to the accuracy of the parent average term (VanRaden and Wiggans 1991). Theoretically, the most appealing way to improve the accuracy of evaluation at a young age is to better predict Mendelian sampling directly, by characterising the values of the parental chromosome fragments in one or more genomic regions and identifying which of these fragments are inherited by each particular offspring. This approach, applied to no more than a few regions in the genome, is referred to as marker-assisted selection or gene-assisted selection, the latter in the case where the actual causal variant(s) is used (Dekkers 2004). When applied to all the regions that collectively comprise the genome, this method is known as genomic prediction (Meuwissen et al. 2001). In its computer implementation, alternative algorithms might explicitly predict a breeding value for each fragment (Meuwissen et al. 2001), or implicitly use the inheritance of every fragment to infer the degree of relationship among genotyped individuals through the construction of a genomic relationship matrix (Nejati-Javaremi et al. 1997). Genomic prediction using one or other approach has now been widely adopted in several species. Methods that predict a breeding value for each fragment have advantages when there is interest in identifying those regions that make the largest contribution to genetic variation, or when some fragments are assumed to have no effect on genetic variation. In contrast, methods that use a genomic relationship matrix have an advantage when fewer animals have been genotyped than the number of loci genotyped on each animal, and when every fragment is assumed to contribute equally in some sense to genetic variation.

#### Genomic prediction

So as to be successful, genomic prediction requires many thousands of animals both to be genotyped and to have relevant phenotypes measured on them or their offspring. The genotypes need to comprise a panel of informative markers that can track the inheritance of chromosome fragments regardless of their location, and several such panels have now been developed for use in various pig breeds using markers representing single nucleotide polymorphisms (SNPs). The first of such panels used in most livestock species comprised ~50 000 SNPs (50K)

and were introduced progressively by Illumina, with the pig version made available in 2009 with ~64 000 SNPs derived from sequencing Duroc, Landrace, Large White and Pietrain breeds and a wild boar population (Ramos et al. 2009). The cost of genotyping such a panel of markers depends on both sample volumes and the number of markers, and this led to the development of cheaper lower-density panels (e.g. 10 K) that were more widely used, but which can still be 'imputed' to the higher density (Kong et al. 2008; Hickey et al. 2011; Sargolzaei et al. 2014). Imputation requires that a large number of individuals have initially been genotyped at the higher density. Imputation became routine in some livestock applications, and its cost savings led to rapid increases in the number of genotyped animals, but higher-density genotyping is now much cheaper than it was, due to economies of scale, reducing the cost advantage from using lower densities and then imputing to a higher density.

The phenotypes need to be measured on the genotyped animals, or on other relatives including offspring. The simplest approach to develop genomic predictions involves the genotyping of a training or reference population of 2000, or preferably many more, individuals, each with reliable pedigree and performance-based estimated breeding values (EBVs), so as to predict the breeding values of the chromosome fragments present in related selection candidates (Meuwissen et al. 2001). Obtaining reliable EBVs for animals in the training population requires that the traits of interest have high heritability, or that the animals have many offspring measured for the phenotypes of interest. An obvious choice of animals to use in the so-called training analysis would be widely used parents. However, in many cases, there may not be that many parents available, and genotyping will need to be undertaken on animals with a less reliable EBV, whereby a much larger number of genotyped individuals (ideally 10 000 or more) will be required to achieve the same accuracy of prediction (Goddard and Hayes 2009). Predictions of selection candidates can be reliable only if the chromosome fragments they have inherited have been characterised previously in the training population. For this reason, genomic predictions will not usually have any value unless ancestors and other close relatives of the selection candidates were in the training population (Habier et al. 2007). Genomic predictions must, therefore, be separately developed for each breed, line or cross from each breeding company. For the selection candidates in successive years to all be close relatives of their training populations, which is a prerequisite for reliable predictions in complex traits, the process of phenotyping and genotyping in each breed, line or cross must be recognised as requiring ongoing effort. The training or reference populations will not therefore be static, but need to be annually enhanced through new phenotypic and genotyping efforts. This might not be the case if causal mutations were genotyped, rather than the intergenic or intronic SNP markers that are predominately used on current SNP panels, but few such causal mutations have been discovered for complex traits such as growth, survival or reproduction.

Early implementation of genomic prediction typically estimated the effects of chromosome fragments in a training analysis, and then used that knowledge in a second step to genomically predict the merit of the selection candidates (Meuwissen et al. 2001). Commonly, the selection candidates would also have pedigree and performance information, providing conventional EBVs. The genomic predictions and the conventional EBVs would then need to be blended in a separate analysis (VanRaden et al. 2009; Harris and Johnson 2010). Today, the preferred approach is a single-step analysis, that does not involve a prior training analysis or post-analysis blending, but still requires an appropriate reference population to be incorporated into the evaluation dataset. Such an analytical method is referred to as a single step, and combines all pedigree, performance and genomic information in a single analysis (e.g. Legarra et al. 2014). Nevertheless, it can still be implemented either in a manner that directly predicts EBV for all animals in the pedigree after accounting for genomic relationships, a method known as ssGBLUP (Aguilar et al. 2010), or in a manner that computes the values of chromosome fragments and uses these explicitly to predict EBVs for all animals (Fernando et al. 2014, 2016), a method known as a single step-marker effects model, ssMEM. In Australia, multi-trait ssGBLUP has been implemented for sheep (Swan et al. 2014) and is being implemented in beef cattle (Johnston et al. 2017).

#### New opportunities arising from genomic prediction

The cost of genotyping an individual in the training population has nothing to do with the amount of trait information that is available on an animal, but the benefits will be greater if the animal has been characterised for a wide range of phenotypes. In some circumstances, that issue has stimulated deliberate efforts to extend the nature and scope of phenotypes collected on animals for the training population so as to implement genomic selection. In this respect, the use of genomics in pig improvement could be viewed as a disruptive technology. Collecting pedigree and the same kinds of performance information as was used in the past and adding genomic information would not represent much innovation and would fail to capture the real benefits from genomics. The benefits from genomic prediction are greatest for hard-to-measure traits and these were often ignored in conventional pedigree-based breeding programs.

Conventional pedigree and performance recording focused phenotypic measurement on the selection candidates, for example, for growth rate, fatness and feed conversion, or where this was not possible, on close relatives such as measurement of the reproductive performance of the dam. Further, there were practical difficulties in collecting pedigree information on commercial descendants, which limited the use of phenotypes collected from descendants outside the nucleus. Genomic prediction offers the opportunity to separate the individuals for whom the phenotypes are measured from those of the immediate selection candidates, without as severely compromising prediction accuracy as would have occurred in conventional pedigree-based analyses. This separation of the training population from the selection candidates offers several appealing advantages in a pig-improvement context, particularly for disease traits, carcass and meat-quality traits, other traits with low heritability or those measured late in life, and to directly target improved crossbred rather than purebred performance.

#### Disease resistance

Nucleus herds are normally managed under circumstances that avoid disease to a greater extent than would be the case for commercial pig herds. This is the case for several reasons. First, catastrophic losses due to disease would compromise the ability of the breeding program to meet demand for stock, resulting in loss of market share. Second, pigs from the nucleus herds are moved to other herds and this activity would not want to be associated with the introduction of a disease not already present in a multiplier or commercial herd. Third, the loss of young animals to disease reduces the number of available replacements and the loss of adult pigs increases the replacement rate, both of which will reduce selection intensity. Fourth, the accuracy of phenotypic comparisons for growth, body composition or reproduction may be compromised when animals have variable exposure to disease, and that disease reduces their phenotypic performance. Nucleus herds are, therefore, maintained free from specific pathogens. This means, in the context of conventional pedigree and performance recording, that there cannot be any natural or artificial selection for resistance to disease unless there is a known indicator trait that is correlated with disease resistance when measured in animals free of the disease. Such indicator traits are rare. However, disease phenotypes could be measured in dedicated experiments (e.g. Boddicker et al. 2012) or in genotyped commercial descendants of the nucleus animals, and used to form the training population for genomic prediction. Nucleus animals could, therefore, have genomic predictions based on natural- or artificially induced disease challenges and be selected for resistance to the disease. The role of genomics in selection to improve animal health was recently reviewed by Plastow (2016), and includes pig examples.

#### Carcass and meat quality

Animals in nucleus herds are seldom used for direct collection of carcass and meat-quality phenotypes. The best selection candidates are used for breeding, and measuring only the cull animals for carcass and meat-quality traits would provide a biased sample, and, in any event, would not improve the accuracy of predicting the selection candidates before their selection as parents. Genomic prediction allows for relatives, such as descendants included in the training population, to be genotyped and measured for carcass and meat quality and informs the ranking of selection candidates. Ultrasound scanning in purebreds could also be used to measure indicator traits for genomic prediction of carcass and meat quality, in the same manner as it was used before the introduction of genomic prediction. Strategies for implementing genomic selection to improve pork quality are reviewed by Miar *et al.* (2015).

#### Difficult traits

Low heritability or sex-limited traits and those measured late in life result in low accuracy of predictions for selection candidates, yet high accuracies can be achieved if large numbers of offspring can be performance recorded in a well managed progeny test. Progeny testing is expensive to undertake properly, relative to simply measuring and genotyping large numbers of nucleus descendants from the commercial sector that lack individual information as to their ancestry. Samorè and Fontanesi (2016) reviewed many studies related to genomic prediction and selection in pigs, including those related to genomic prediction of low-heritability maternal traits in pigs.

#### Crossbred performance

Cost-effective genetic improvement necessitates that the size of the nucleus of a particular breed or line be as small as possible, within the context of being sufficiently large to achieve genetic gain, but the small size combined with intense selection will increase the rate of accumulation of inbreeding in that breed or line. Inbreeding is seldom an issue in the commercial pig sector, as sows are typically crossbred and mated to an unrelated breed or line of terminal sire boars. First-cross sows will not exhibit the inbreeding of their nucleus or multiplier parents and grandparents, nor will terminal sire offspring exhibit the inbreeding of their ancestors. This industry structure can readily exploit specific lines selected for maternal or terminal performance, and take advantage of breed complementarity and heterosis. However, this structure does cause a problem in that the goal of the selection should be to improve crossbred performance, but the phenotypes measured in the nucleus to improve the accuracy of prediction are typically based on purebred performance. It has often been assumed, for convenience, that selection for purebred performance will perfectly correlate with improved crossbred performance, but that is not exactly the case in practice. It would be better to predict index merit of nucleus selection candidates in terms of the crossbred performance of their descendants (Dekkers 2007; Ibánez-Escriche et al. 2009; Esfandyari et al. 2015). It is possible to collect pedigree and performance information through the multiplier and commercial tiers of the industry, but this increases costs in a similar manner as would occur if the size of the nucleus was expanded, and is not as straightforward as simply genotyping and phenotyping commercial pigs using SNP marker panels to form a crossbred training population to use in nucleus prediction targeted at crossbred performance. Genomics allows young boars to have simultaneous predictions for both purebred and crossbred performance.

The accuracy of genomic predictions in distant relatives is predicted to be poor (Habier *et al.* 2007) unless the training population is large, comprising tens or hundreds of thousands of genotyped and phenotyped individuals (Goddard and Hayes 2009). Such a size of training population required for accurate prediction in distant relatives is often greater than the total numbers of nucleus and multiplier animals in some breeding programs. However, accuracy of genomic prediction is much higher from close relatives than from distant relatives, so genotyping all the parents in small populations is a practical option that can provide genomic prediction in small populations with a better value proposition than would be the case for a large outbreeding breed in other livestock species.

#### Gene discovery, gene introgression and gene editing

Genomic prediction also provides for the discovery of genes responsible for simply inherited genetic defects from small numbers of affected individuals. Such genes are seldom problematic in the commercial sector because of the crossbred nature of commercial sows and their terminally sired offspring, but such defects can nevertheless markedly reduce selection intensities in the nucleus sector. Discovery of genes or genomic regions responsible for defects allows these defects to be readily managed, by avoiding matings between carrier animals, if not by eliminating all carrier animals from being used as parents.

The discovery of favourable alleles in other breeds or lines that do not exist in a particular nucleus line has traditionally been exploited by introgression, involving crossing the disparate breeds or lines to introduce the novel allele, then successively backcrossing the offspring to the nucleus line while selecting for the favourable allele so that a new line is produced with background genome as close to the original line as possible, except for the immigration of the favourable allele, ultimately in homozygous state. This can be done more efficiently in complex traits using marker- or gene-assisted introgression (Dekkers 2004), rather than using phenotype, but the process is expensive in terms of reductions in selection intensity that result from limiting selection to only those candidates that inherited the favourable allele, and because the process requires several generations to minimise the contribution of the outside line genome to the background genome of the nucleus line. Gene editing is a newly developed molecular technique that overcomes these limitations associated with introgression, as well as offering new opportunities for genetic improvement and avoidance of inbreeding.

In addition to introducing known alleles that might not currently exist in a particular breed or line, gene editing offers enormous potential for generating new and improved lines of livestock, by reducing the frequency, if not eliminating, unfavourable alleles, and increasing the frequency of favourable alleles. New alleles could even be constructed, producing quantum improvement, as was the case, for example, in producing pigs with complete resistance to porcine reproductive and respiratory syndrome virus (PRRSV; Burkard et al. 2017). Currently, there are several challenges to the adoption of gene editing, including legal aspects and issues of consumer acceptance. However, a technical problem has been the lack of knowledge of targets for gene editing. This is now beginning to change with the development of large training populations for genomic prediction and the routine genome-wide association studies that use those phenotypes and real or imputed genotypes, not just for selection of parents but also to discover major gene effects for both complex and more simply inherited traits.

#### Conclusions

Genomic prediction offers the first practical approach for selection at young ages to fully exploit within-family selection for any complex trait. That is, it allows distinction at a young age between those full-sib selection candidates that inherited betterthan-average and those that inherited poorer-than-average genomic fragments, and it can do this without necessarily phenotyping the selection candidates themselves. Phenotyping of close relatives to maintain a training population will, nevertheless, remain critically important, perhaps even more so than was the case in the pre-genomics era of pedigree and performance recording when only the most important traits warranted routine measurement.

Increased understanding of genomics, and its role in improvement, along with reduced costs for collecting genotypes using marker panels and next-generation sequencing will guarantee that the role of genomics in pig improvement will only increase relative to its position today. Those companies that appropriately invest in relevant genomics research and development, and are innovative in its application, will increase market share relative to their competitors that adopted the technology too early, too late, or inappropriately. Although some findings related to the role of genomics are readily available in the public domain in terms of oral presentations and other forms of published scientific findings, many of the precise details have not been publicly shared, yet attention to some of these finer details can seriously affect both the costs and the benefits from the use of genomics in the pig industry. This means that the application of genomic technology needs to be done innovatively, within a sufficiently well resourced internal or collaborative research and development program.

#### **Conflicts of interest**

The author acknowledges joint ownership of ThetaSolutionsLLC, a company that develops and licenses BOLT software used internationally in genetic evaluation, including single-step genomic prediction, but declares there is no other conflicts of interest.

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# Genetic improvement and dissemination for the global commercial swine industry

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**Abstract.** Commercial swine production has become an increasingly globalised industry, with global meat trade demanding that all regions compete on cost and differentiation of pork products. The utilisation of continually improving genetic populations can be one input that helps maintain, or increases, the competitiveness of an individual producer or regional industry. So as to deliver these improving genetic populations, genetic providers of today must focus on developing and implementing best science that delivers improvement on traits affecting commercial profitability. Providers must also efficiently multiply and disseminate the improved merit to the commercial hog production level. The swine-genetics industry has made considerable progress in driving a faster genetic gain over the past 30 years by systematically combining ever-changing computing power, accurate data capture and emerging genomics information. The combination of these technologies today has resulted in hundreds of thousands of animals being genotyped for tens of thousands of markers, and this information is being combined with extensive phenotypic data to deliver rates of genetic gain nearly double what we were able to achieve 20 years ago. As importantly, this scientific advancement can then be combined with the ability to continue to understand and evaluate emerging traits related to animal robustness, well-being and consumer demand, resulting in the most comprehensive definition of selection targets in the history of modern animal improvement. Finally, managing the dissemination of these genes through boar stud and multiplication systems helps ensure that the commercial level minimises lag and utilises the highest-merit genetics available.

Additional keywords: genetic lag, performance testing, selection objectives.

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#### Introduction

The creation and utilisation of genetic improvement within the global livestock industries has been continually evolving. However, even with that evolution of process and practice, the core principles have always been built on the concept demonstrated by Fisher (1919) that the observed resemblance among relatives is due, at least in part, to genetic factors that are inherited. As a swine-genetics industry, we continue to build on this platform today, by striving to do a more accurate job of describing the kinship among animals and linking it to accurate and meaningful traits important to commercial pig production.

The definition of desirable characteristics has evolved from a very subjective classification that is difficult to quantify (and repeat) to an increasingly comprehensive and objective definition linked to economic viability of commercial operations. Likewise, the utilisation of modern testing and selection capabilities has allowed for increasingly accurate, large-scale capture of phenotypic information on individual animals within breeding populations. This information allows for a detailed understanding of animal performance through the various stages of the life-cycle and for traits expressed in separate environments or those that are gender specific. Scientific advancements in computing, genomic science and theoretical genetics have increasingly converged, allowing dramatic changes in the way that we combine these measures of relatedness with phenotypic information, to make accurate genetic predictions of individual-animal merit. The initial outline of the modern genetic evaluation system was originally proposed by Dr Henderson in the early–mid 1970s and is now commonly referred to as best linear unbiased prediction, or BLUP (Henderson 1974). These statistical genetic techniques, which began to allow for utilisation of data and comparison of animals across farms and discrete windows of time, would prove pivotal for the modern improvement program utilising large and diverse populations. Unfortunately, it was really not until the late 1980s that availability of computing power allowed for the initial widespread implementation of these tools.

Today, many genetic providers have continued to build on these original proven principles by focusing more resources on detailed and robust data capturing the performance potential of elite animals. In addition, genomic science has become increasingly cost efficient and incorporated into genetic improvement programs via methods ranging from single nucleotide polymorphism tests to incorporation of marker effects to comprehensive inclusion into the genetic relationship matrix (Legarra *et al.* 2009).

Ultimately, the creation and realisation of genetic improvement can be visualised and deconstructed into four primary factors, as shown in the following equation:

Genetic improvement per year = <u>Accuracy × Selection intensity × Genetic variation</u> <u>Generation interval</u>

#### Accuracy of selection

Accuracy of selection could be considered the primary driver affecting annual genetic improvement as it encompasses both the accuracy of the genetic prediction process as well as the specific traits going into the evaluation that ultimately form the selection objective.

#### Identification and inclusion of traits for genetic evaluation

Selection theory in animal breeding and genetics have long proposed selecting animals for a combination of traits weighted to reflect their impact on the overall value of the animals (Smith 1936; Hazel 1943). Today, we still utilise the same base theory to combine an ever-increasing number of traits that are included due to their impact on describing the future economic value of an animal as a parent of the next generation. The inclusion of traits is evaluated by ultimately combining the economic incentive presented by consideration coupled with the practical reality of capturing accurate and meaningful volumes of data. Within our program, the most recent considerations of traits have primarily been focused on exploring additional metrics related to phenotyping across expanded environments, as well as including new phenotypes related to emerging areas of animal robustness, well-being and final product value.

As a global provider of elite genetics, we must continually challenge ourselves to select animals and develop products that work across a range of production systems and environments. Today, that means coordinating data capture that incorporates animal performance across sow-housing systems, growing pig environments, various definitions of well-being-based programs (e.g. intact males, intact tails) and a range of disease challenges. To achieve this, we continuously capture data from 700 global farms representing nearly every design, geographic region and health status common to the global swine industry. In addition, we supplement this with a focused capture of commercial growing pig performance via our GNXbred system. The PIC GNXbred system utilises the initial semen production from elite nucleus boars to produce individually identified commercial pigs that are then tested and monitored from birth through harvest and processing. This program allows elite genetics in high-health environments to be evaluated for growth, robustness, efficiency and carcass value, via progeny test, across a range of more challenged environments. Without this type of data source, selection improvement for livability and robustness would be greatly reduced.

Simultaneously, we are constantly evaluating new and emerging traits that help more completely define the profit contribution an individual animal might make to commercial production. Over the past 10 years, this has expanded to include our initial traits related to post-weaning survivability and robustness. In addition, more recently, we have expanded the opportunity to increase piglet robustness by including individual piglet birthweight into the genetic-evaluation process and have realised a substantial impact on response to selection for both birthweight and pre-weaning survivability (Fig. 1). Current focus areas include additional exploration of increasing robustness as well as further refining lean value selection to include genetic gain in yield of specific carcass primals as well as components affecting consumer eating satisfaction and buying decisions. The generation of large volumes of detailed carcass data from commercial slaughter facilities has enhanced our definition of saleable yield to utilise the specific value of competing primals and the initial inclusion of tenderness and eating satisfaction values are now also increasingly available.

#### Genetic prediction of merit

Until recently, we utilised panels of a few DNA markers because of the cost of individual animal genotyping and available



**Fig. 1.** Genetic trends for total born (...) and pre-weaning survivability (—) in the PIC Genetic Nucleus maternal populations.

bench technologies. In 2009, a new genotyping chip became commercially available that allowed for a quick description of roughly 60 000 genotypes on an individual animal spread across the porcine genome. This platform was the first application in the market that allowed for a sufficiently expansive description of an animal's genetic makeup with a robust chemistry platform (e.g. few genotyping errors, high call rates, automated platform).

During this same time period, Legarra *et al.* (2009) developed an algorithm to utilise such dense marker information from the new chip to more accurately describe the genomic segments animals had in common from any density platform. Their models were and are scalable as the technology cheapens and changes over time. Their approach utilised current best linear unbiased prediction models, but augmented the pedigree relationships with genomic relationships. Practically, it individually estimates the gene segments any two animals have in common on the basis of their genotype results, instead of the theoretical expectation of any parent donating a random half of its genes to an offspring. This method is commonly referred to as single-step genomic evaluation or relationship-based genomic selection (RBG).

PIC published its first RBG genetic evaluation in January 2012. Initially, we introduced the technology evaluating reproductive traits within our maternal lines by primarily genotyping boars already in stud that had or were destined to produce daughter litter records. We have since expanded its use to all lines and all traits and, today, we genotype over 100 000 animals each year to increase the accuracy of selection and to fuel future genetic improvement.

Combining a powerful genotyping platform with large numbers also enables us to more cost effectively and strategically genotype the animals that can provide the greatest genetic progress for our customers. In other words, we are able to genotype most animals well in front of their selection event to be sure that we capture those animals that possess unique genetic combinations of economically important traits. We, in turn, quickly process those genotypes through our global genetic-improvement system combined with a vast database of pedigrees and numerous economically important traits, allowing genomics to have its impact on our indexes. This genetic-evaluation platform is now efficient and robust enough to be utilised not only within the large global PIC populations, but also in some of the elite regional populations that operate independent of the primary larger global populations.

#### Selection intensity and genetic variation

Selection intensity and genetic variation also have the potential to drive genetic progress. Utilising the highest-merit animals as parents of the next generation will create greater selection differential and, ultimately, when combined with accurate selection, should maximise gain in the next generation. Overall, the decision on balancing selection intensity and retaining genetic variation for future generations is dependent on the population size and structure.

#### Generation interval and genetic lag

Generation interval is typically defined as the average age of the parents when their offspring are born. Ultimately, it is difficult and expensive to change the underlying biology of the animal in a way to decrease generation interval. Today, common management practices such as artificial insemination, structured gilt development and managed replacement rate deliver a generation interval that is operating at a near optimum level.

Genetic lag can also be described as the amount of time it takes to get the most elite genetic merit from the nucleus level to the commercial level. Although not directly related to creating annual genetic gain, genetic lag and the controllable decisions related to it can have a dramatic impact on the realisation of the genetic gain at the commercial level.

The full inclusion of breeding populations, including boar studs and genetic-production sow farms, into a modern genetic



Fig. 2. PIC genetic trend for commercial pig equivalent.

management system allows for individual animal tracking and, subsequently, information-based management decisions. The current PIC genetic management system utilises various decision support tools to identify boars for replacement, the most elite sows to mate pure and various metrics related to success of implementing on-farm processes.

#### Conclusions

Continued advancement in computational power, statistical genetic theory and genomics are being combined to allow faster genetic progress than was previously possible (Fig. 2). The next generation of these developments will be driven by current research initiatives into further refining the currently available technologies and harnessing the capabilities of next-generation initiatives such as genome sequencing and gene editing.

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### Neurophysiological assessment of animal welfare

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**Abstract.** Livestock industries such as the pork industry are striving to continuously improve the welfare of animals. Inherent to the success of this is the ability to rigorously assess the welfare of animals in the field. While much progress has been made towards the development of methodology to assess the welfare of animals, there have been major challenges to establishing practical and definitive procedures to assess the welfare of animals. These include, but are not limited to, establishing a universally accepted definition of animal welfare and the choice of measures that are taken from the animal to assess its welfare. Measures of biological functioning and affective (emotional) state of the animal have been common, but there have been many limitations in terms of practical application. Some of the reasons for this include the choice of physiological measures, which are often restrictive in providing information about welfare, affective measures being restricted to specific behavioural measures and the biological-functioning and affective-states approaches being undertaken in isolation. Biological and affective functioning are integrated and controlled by the brain. Many of the regions of the brain involved in the regulation of biological and emotional functioning have been identified. Furthermore, there is considerable knowledge about the roles and interactions among the neurophysiological systems in these brain regions. We propose a strategy to use this knowledge to develop procedures to assess animal welfare. The initial phase is to identify the neural pathways that regulate the physiological and emotional processes that allow animals to adapt and cope. The next phase is to determine the activity of these pathways in conscious animals in the field. This requires the identification of biomarkers of specific neuronal activity that can be measured in the conscious animal in the field. Emerging technologies are offering promise in the identification of such biomarkers and some of these are already applicable to the pig. There is now the opportunity to apply this strategy within the pork industry to assess the welfare of pigs throughout the value chain.

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#### Introduction

Most livestock industries are now committed to the continuous improvement in the welfare of their animals. This is certainly the case for the pork industry. To achieve this, it is first paramount to have a reliable and accurate means to assess the welfare of animals throughout the value chain, which includes the public, consumers, processors, retailors, producers and accreditors. Despite a vast research effort to achieve this, the reality is that there are not methods readily available for practicable application to assess the welfare of animals, such that the assessments can be ultimately used in the development of means to improve welfare. This critique will highlight some of the key challenges to the assessment of animal welfare and will propose a strategy to provide robust methods to assess the welfare of animals. The intention is that this strategy will be able to be applied throughout the value chain.

#### Challenges to the assessment of animal welfare

One critical challenge to the development of methods to assess the welfare of animals is to establish a universally accepted

definition of animal welfare. We and many others (Hemsworth et al. 2015; Ralph and Tilbrook 2016; Tilbrook and Ralph 2017) have dissected the various approaches that have been presented to define the welfare of animals. These include, although are not limited to, the 'five domains' model (Mellor and Reid 1994; Fraser et al. 2013; Beausoleil and Mellor 2015b, 2015a; Mellor 2016), the 'two question' approach to defining good welfare (Dawkins 2008, 2017), the Welfare Quality Project<sup>®</sup> (European Union; http://www.animalwelfareplatform.eu/Welfare-Qualityproject.php, accessed 4 September 2017), the biologicalfunctioning, affective-states and natural-living frameworks of animal welfare (see below) and the concept that animals have 'lives worth living' (Green and Mellor 2011; Mellor 2016). Of the definitions developed, we are of the view that the most useful in terms of understanding, and thereby assessing, animal welfare are the biological-functioning and affectivestates frameworks (Hemsworth et al. 2015; Tilbrook and Ralph 2017). We accept that both frameworks have limitations, especially if considered in isolation of each other and, in this regard, have suggested that the biological and affective-states frameworks should be integrated (Hemsworth et al. 2015).

Indeed, we have discussed the value of integrating these frameworks in understanding the welfare of group-housed sows (Hemsworth *et al.* 2015).

Another impediment to progress in developing methods of assessment of animal welfare is the choice of measures to take from the animal. With respect to biological functioning, the most common measures are physiological, although behavioural measures are also collected. These are usually feasible to collect but often do not directly provide specific information about the state of the welfare of an animal. The most common measures to assess affective states are behavioural and, while useful, behavioural measures on their own do not always provide a complete assessment of the welfare of the animal. Here, we discuss principal issues in establishing measures of both physiological and emotional functioning that will be useful in the assessment of the welfare of animals.

#### The spectrum of animal welfare

Clearly, development of methods to assess the welfare of animals cannot afford to deal with the biological functioning and affective states in isolation but, instead, methods must establish how these frameworks are related and where they intersect. The development of practical measures of both physiological and emotional functioning are essential. Importantly, there is a spectrum of levels of welfare of animals that ranges from negative to positive (Mellor 2012, 2015b, 2016; Fraser et al. 2013; Hemsworth et al. 2015; Tilbrook and Ralph 2017), so methods of assessment of animal welfare must be able to provide critical information about the welfare of animals throughout this spectrum of welfare. It is now recognised that the continuous improvement of animal welfare requires the ability to move animals from the negative to the positive regions of the welfare spectrum. In other words, the ultimate goal is to provide animals with positive states of welfare.

#### **Biological functioning and affective states**

The biological-functioning and affective-states frameworks were initially seen as competing but it is now evident that they are integrated and knowledge of the interactions between these frameworks is paramount to understanding animal welfare (Boissy et al. 2007; Barnett and Hemsworth 2009; Green and Mellor 2011). For instance, biological functioning includes affective experiences (Boissy et al. 2007; Barnett and Hemsworth 2009; Green and Mellor 2011). This may not seem evident when one considers that this state appears to be primarily concerned with the extent of biological activity that is associated with an animal attempting to cope and involves a range of physiological systems in the body such as those classically associated with stress as well as immunological responses, repair systems and various behavioural responses. Nevertheless, the reality is that emotions are not independent of other biological processes (see below). Indeed, affective experiences are recognised as products of biological functioning (Spruijt et al. 2001; Boissy et al. 2007; Hemsworth et al. 2015). Affective experiences are generated by sensory inputs that reflect the internal physiological state of the animal and contribute to the awareness of the animal of its external circumstances (Mellor 2012, 2015a; Hemsworth et al. 2015).

A common approach to assessing biological functioning in animals has been to take measures of the physiological systems that are activated to allow an animal to cope and adapt. These include the stress systems, immunological measures, measures of pain and measures of body repair, among others. We have recently critically assessed a range of these measures in terms of their value in assessing animal welfare and there are frequently serious limitations (Ralph and Tilbrook 2016; Tilbrook and Ralph 2017). In addition to the measurements often being limiting in terms of assessing the welfare of animal, they have often been taken in isolation of measures that will assist in the integration of biological and emotional functioning.

In comparison to biological functioning, it was long considered that assessing emotions was virtually impossible, but this belief is now uncommon and a variety of approaches have been undertaken to quantify affective states. Most of these have been behavioural approaches, with one of the most common being research that investigates the choices that animals make for a chosen environmental option or motivation to perform a type of behaviour (for review, see Hemsworth et al. 2015). The premise underlying this approach is that animals will make choices that are in their best interest (Fraser and Nicol 2011). Other approaches to assessing affective states include measures of behaviour and cognitive bias (Boissy et al. 2007; Mendl et al. 2009) and qualitative behavioural assessment, which uses the intuitive perception of human observers (Wemelsfelder and Mullan 2014). Many of these measures are limited by not taking account of biological functioning. Furthermore, most approaches to measure emotions have focused on assessing negative affective states and there is a need to be able to measure positive affective states, consistent with the desire to not simply mitigate against poor animal welfare, but to strive for positive animal welfare.

### Neurophysiological approaches to assessing animal welfare

Irrespective of the behavioural and physiological functions being considered in the assessment of animal welfare, these functions are controlled by the brain. Indeed, given that animal welfare is a state within the animal, the animal must be sentient (Hemsworth *et al.* 2015). In other words, the brain of the animal must have the capacity and functional sophistication to transduce impulses in sensory and other nerves into experienced sensations (Hemsworth *et al.* 2015). It is generally agreed that the welfare of animals relates to experienced sensations, which means that an animal must also be conscious (Mellor *et al.* 2009). A corollary of this is that an understanding of how the brain controls both physiological and behavioural functions is essential to assess these functions in terms of the welfare of an animal.

In a peculiar twist, the key advancements in this area of animal science may come from a vast research effort in biomedical science. Animal models have been developed to understand human psychology, including emotion. The field of affective neuroscience is dedicated to understanding the processes within the brain that control and regulate emotional experiences. Biomedical research has defined the brain regions, cell types and physiological processes that control cognition, emotion, behaviour, anxiety, fear and reward (Davis 1997; Berridge and Kringelbach 2013). It has been shown, primarily with rodents, sheep and primates, that there are measurable changes in defined regions of the brain that indicate the affective state of an animal (Bergholm et al. 1984). Moreover, the structure and function of the brains of humans and many animals are similar (Panksepp 2005; Boissy et al. 2007; Mellor 2015a). Much of this biomedical research has relevance to understanding the emotional states of animals, especially as the animals have often provided the original basis for the research. It is entirely plausible that many of the approaches used in affective neuroscience can be applied to the understanding and, in turn, the assessment of animal welfare. On the basis of the existing literature in this section of biomedical science, the approaches may be most useful when assessing reward, fear and pain.

### Development of practical neurophysiological measures of animal welfare

Recently, we reviewed the regions in the brain, and the key neural pathways and neurotransmitters involved in regulating positive and negative affective states (Ralph and Tilbrook 2016). There is extensive knowledge of the neuroanatomy and neurophysiology of reward, fear and pain that can serve as the basis to understand the central regulation of these emotions in animals and it should be possible to integrate this understanding with the biological functioning framework of animal welfare. We argue that substantial progress can be made in developing procedures to assess animal welfare by identifying the neural pathways that generate key emotions in animals, such as reward, fear and pain, and then by developing the means to determine the activity of these pathways. Fortunately, much is already known about the central generation of emotions. The challenge lies in the development of the means to quantify this generation. Given that the objective is to assess the welfare of an animal along the welfare spectrum, the assessment must consider both negative and positive affective states, and consider the central generation of both.

The brain is organised into discrete regions and neuronal systems that regulate complex emotional states. Various regions of the brain and neuronal systems have been shown to be associated with reward, or positive affect. These include the mesolimbic dopaminergic system (the nucleus accumbens and the ventral tegmental area), the medial prefrontal cortex and the basolateral amygdala (Boissy et al. 2007; Taber et al. 2012; Berridge and Kringelbach 2013). The neurotransmitters involved in the communication and regulation of reward signals within these regions of the brain are dopamine, glutamate and gamma-aminobutyric acid. Research has also shown that the opioidergic systems (endorphins, enkephalins and dynorphins) interact with the dopaminergic system to regulate reward (Boissy et al. 2007; Benarroch 2012). The serotoninergic system, originating in the raphe nucleus of the brain stem has also been shown to have a strong effect on reward (Beaudoin-Gobert and Sgambato-Faure 2014). Each of these neural pathways is a candidate worth investigating in terms of understanding the regulation of positive emotions in animals. Nonetheless, it is apparent from the biomedical literature that the affective neuroscience that underpins reward and positive affective states is complex and rarely are feelings of reward mediated solely by one region, one neuronal system or one neurotransmitter. Thus, future research should avoid investigating these neuronal systems in isolation, and, instead, will need to establish how the interactions among and within these regions of the brain regulate positive affective states.

Biomedical research in affective neuroscience has also provided a sound understanding of the regions of the brain that regulate negative affect, such as fear. These have been shown to include the medial prefrontal cortex, the amygdala and the periaqueductal grey (Chan et al. 2011; Tovote et al. 2015). It is believed that the medial prefrontal cortex regulates fear behaviour by modulating the amygdala and the periaqueductal grey (Chan et al. 2011). Two subregions of the medial prefrontal cortex are believed to play opposing roles in fear behaviour (Chan et al. 2011). The pre-limbic subdivision promotes the expression of fear by increasing neural output from the amygdala, while the infra-limbic subdivision inhibits the expression of fear behaviour by decreasing the neural activity within the amygdala. The medial prefrontal cortex has direct projections to the periaqueductal grey and regulates defensive behaviour (Davis 1997; Courtin et al. 2013). The amygdala is essential for proper expression of fear behaviours and, in particular, conditioned fear (Kolber et al. 2008). The amygdala has direct neural projections to the lateral hypothalamus that appear to be involved in activation of the sympathetic autonomic nervous system during fear (Davis 1997). In addition, there are direct projections from the central nucleus of the amygdala to the paraventricular nucleus and indirect projections to the paraventricular nucleus via the stria terminalis and preoptic area (Davis 1997; Hartley and Phelps 2010). These projections are likely to mediate the increase in glucocorticoids seen during fear behaviours, while electrical stimulation of the amygdala has been shown to increase corticosterone release in rats (Mason 1959; Dunn and Whitener 1986). In addition, corticotrophin-releasing hormone is increased in the central amygdala after conditioned fear and when corticosterone is administered to the central amygdala (Shepard et al. 2000; Roozendaal et al. 2002). This is clearly a complex integrative neuronal system but the foundation exists to unravel the roles of these regions in the regulation of negative affect in animals.

Identifying regions of the brain and key neuronal pathways that regulate positive and negative affective states in animals will provide cutting-edge knowledge that is essential to the development of novel methods to assess animal welfare. Nevertheless, the knowledge will be insufficient in and of itself because it will not provide a practical means of quantifying affect in the field. The practical value of this knowledge will rest in the identification of the means to identify the activity of these neuronal systems in the conscious animal. For the most part, there has not been a straightforward means to measure the central regulation of emotion in animals. Functional magnetic resonance imaging is possible in a live animal (Ferris et al. 2006, 2008), but is not suitable in a production environment and rarely can be conducted on a conscious animal. Electroencephalogram can provide valuable information on brain activity (Perentos et al. 2017), but, again, this method is not suitable in a production environment. Hence, while experimental approaches exist to evaluate the function of the brain in animals, the scarcity of peripheral measures, or biomarkers, of central function that can be used in the field is a barrier to the practical application of the assessment of animal welfare. Therefore, there is need to develop biomarkers for positive welfare states such as reward and contentment and novel biomarkers for negative welfare states such as pain, anxiety and distress.

Clearly, the research pursuit of the assessment of animal welfare must include both identification of the neurophysiological systems that regulate affective states, and the development of biomarkers of the activity of these systems. Candidates for biomarkers of central neuronal activity, among others, may be specific micro RNAs (miRNAs) that are expressed in response to the activation of various neuronal systems. These are small non-protein-coding RNA molecules transcribed from a range of DNA sequences, including introns (Codocedo and Inestrosa 2016). The miRNAs target and bind mRNA of genes and prevent gene expression by either targeting mRNA for degradation or by blocking translation of a mRNA into protein. After their release into circulation, miRNAs can be indicative of cellular processes or states. There is evidence from studies in rats and humans that specific miRNAs can be measured in the blood and provide specific information about cellular processes in the brain relevant to affective states (Baudry et al. 2010; Issler and Chen 2015; Kocerha et al. 2015). For example, miRNA-34c was upregulated after chronic social defeat and led to anxiety and depression-like behaviour, miRNA-135 was indicative of stress resilience, miRNA-9 was regulated by chronic unpredictable stress and maternal deprivation, miRNA-21 was upregulated by multiple types of peripheral pain and miRNA-124 was upregulated in key reward centres during rewarding experiences (Balakathiresan et al. 2014; Issler and Chen 2015; Basak et al. 2016; Codocedo and Inestrosa 2016; Issler et al. 2014). Furthermore, miRNA-29C, miRNA-135 and miRNA-124, miRNA-9 and miRNA-34c have been shown to be expressed in the brain of pigs (Li et al. 2010; Podolska et al. 2011) and miRNA-34a, miRNA-107, miRNA-103, miRNA-25, miRNA-93, miRNA-92b, miRNA-128, miRNA-16 and miRNA-106 have all been measured in the blood of pigs (Wang et al. 2015). These studies suggest a potential to assess the function of the brain and, thereby, welfare states from the measurement of miRNAs in the blood.

It is generally agreed that assessments of animal welfare should consider quantification of pain. The perception and regulation of pain is extremely complex, involving both the central and peripheral nervous systems (Hazel *et al.* 2015); however, a recent systematic review of novel approaches to measure pain in animals identified a variety of possible biomarkers of pain that may be measured peripherally and, therefore, may have practical applications (Hazel *et al.* 2015). These include immune-related, inflammation-related and pain-related biomarkers.

While the foregoing discussion has primarily focused on the regulation of emotional states, this does not detract from the importance of understanding physiological responses in the context of animal welfare. As indicated earlier, the assessment of animal welfare requires an understanding of both the biological functioning and affective state of an animal. Notably, the principles that we are proposing here to develop the means to assess affective state, identification of critical neural pathways and identification of biomarkers of the activity of these pathways, apply equally to the understanding of biological functioning. The central regulation of many of the physiological systems involved in the coping and adaption of animals are well known (Ralph and Tilbrook 2016) and many of these are also involved in the regulation of emotion (see above). This further underscores the integration between the biological-functioning and affective-states frameworks. Consequently, while some peripheral biomarkers of the central regulation of physiological and emotional systems may vary, there will likely be many in common.

# Application of neurophysiological approaches to assess pig welfare

There is vast potential to exploit the principles that we have proposed here to advance the assessment of the welfare of pigs, but, hitherto, this approach has been lacking. We recently identified that most research on the welfare of sows housed in groups has used the biological-functioning framework (Hemsworth et al. 2015). The basis for this has been that suboptimal biological functioning accompanies negative affective states (Green and Mellor 2011; Mellor 2015a, 2015b). These studies have used various behavioural measures, such as aggression, and physiological measures such as circulating concentrations of cortisol and measures of immune function, reproductive function and fitness (Hemsworth et al. 2015). While there have been fewer studies on affective states than biological functioning, there has been general agreement in the findings. Nonetheless, serious constraints with the research remain, in terms of application to the industry due to the limitations of many of these physiological measures regarding assessing welfare (Ralph and Tilbrook 2016; Tilbrook and Ralph 2017). There has also been a lack of integration between the frameworks and a tendency to focus on measures of negative welfare states instead of assessing welfare across the full spectrum. Furthermore, the research has not been extended to pigs in other stages of development and across the value chain.

The pork industry is well poised to make use of the advances in biomedical science, especially affective neuroscience, so as to develop practical measures of the central regulation of physiological and emotional functions. Many of the key neurophysiological systems have already been identified. The miRNAs offer potential as biomarkers of positive and negative welfare states because relevant miRNAs are expressed in the brain of the pig and can be measured in the blood (see above). Furthermore, there are also promising biomarkers of pain in pigs (see above) that have potential for practical use.

#### Conclusions

Substantial progress can be made in the development of procedures to assess the welfare of animals by identifying the neural pathways that regulate the physiological and emotional processes that allow animals to adapt and cope, and then determining the activity of these pathways in conscious animals in the field. This is a challenging and complex objective, but it is possible. There is now extensive knowledge of the neurophysiological regulation of physiological and emotional processes that are fundamental to influencing the welfare state of an animal. There are also numerous experimental approaches available to study the function of brains in animals but these are generally not able to be applied practically in the field. This means that there is the need to identify biomarkers of the activity of important neurophysiological systems that can be measured in conscious animals in the field. Fortunately, there are emerging technologies that provide the potential to identify such biomarkers that can be used in the field to assess the welfare of animals. This should be a foremost objective of research in animal welfare science.

The pork industry is well poised to undertake the strategy that we have proposed here. There is a high likelihood that the neurophysiological regulation of fundamental processes that influence the welfare state of pigs is like for other mammalian species, and some key biomarkers of neural activity and of pain have been identified in the pig. Research should extend this knowledge to the development of practical measures of biological functioning and affective state in pigs at all stages of development and throughout the value chain.

#### **Conflicts of interest**

The authors declare no conflicts of interest.

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Abstract. Post-weaning complications in piglets are characterised by a reduction in feed intake and growth, atrophy of small-intestine architecture, upregulation of intestinal inflammatory cytokines, alterations in gastrointestinal microflora, diarrhoea and heightened susceptibility to infection. Traditional measures to reduce weaning-associated intestinal dysfunction have centred on dietary inclusion of antibiotic growth promoters in weaning pig diets, or high concentrations of dietary minerals in the form of zinc oxide. However, these strategies are under scrutiny because of their role in promoting multi-drug resistant bacteria and the accumulation of minerals in the environment. Up to recently, the main focus on finding alternatives to in-feed antibiotic growth promoters has been on dietary manipulations post-weaning, through the use of feed additives in the post-weaning diet. However, there are also other strategies that could enhance the growth and health of the newly weaned pig. One of these strategies is the use of maternal nutrition to improve growth and health in her offspring. The development of the immune system begins in utero and is further developed after the colonisation of the gastrointestinal tract with microbiota during birth and post-natal life. The early establishment of this relationship is fundamental to the development and long-term maintenance of gut homeostasis. There are significant efforts being made to identify natural alternatives to support the development of the piglet gastrointestinal tract, in particular during the weaning period. Chemodiversity in nature, including microorganisms, terrestrial plants, seaweeds and marine organisms, offers a valuable source of novel bioactives. This review will discuss the development of the intestinal tract in the pig during gestation, lactation and post-weaning periods and the factors that influence intestinal health post-weaning. It will also discuss how feeding marine bioactives in both the maternal diet and the piglet diet can be used to alleviate the negative effects associated with weaning.

Additional keywords: chitosan, intestinal health, pigs, seaweed extracts.

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#### Introduction

In the modern swine industry, weaning is a major cause of stress to piglets due to multi-factorial stressors such as nutritional, environmental and physiological factors, resulting in an immediate drop in voluntary feed intake post-weaning (Pluske *et al.* 1997; Lallès *et al.* 2007*a*). The decreased feed intake affects the intestinal health balance, inducing undesired morphological, physiological and microbial changes in the gastrointestinal tract (GIT). Subsequently, these changes give pathogenic microbes the opportunity to colonise the intestinal tract and reduce the barrier function of the intestine, and cause intestinal inflammation and post-weaning diarrhoea (PWD; Pluske *et al.* 1997). The occurrence of PWD and subsequent growth check cause large economic losses for the pig industry worldwide.

Traditional measures to reduce weaning-associated intestinal dysfunction have centred on dietary inclusion of antibiotic growth promoters (AGP) in weaning-pig diets (Williams *et al.* 2001), or high concentrations of dietary minerals in the form

of zinc oxide at doses well above nutritional requirements. The direct purpose of these additives is to suppress the growth of pathogenic bacteria such as Escherichia coli and Salmonella. The over use of antibiotics is closely related to the growing number of antimicrobial-resistant agents and this raises important concerns about animal and also human health. As a result of this, the European Union implemented a full ban on AGP usage in livestock diets in January 2006 (European Communities (EC) Regulation no. 1831/2003). There is also pressure in other pig-producing regions worldwide to minimise or completely eliminate the inclusion of in-feed antibiotics in livestock diets (Reijnders 2006) and there are concerns regarding the feeding of pharmacological doses of zinc oxide to pigs. This is due to the relationship between zinc oxide usage and an increase in antibiotic resistance (Bednorz et al. 2013) and zinc accumulation in the environment (Miller et al. 2009). Consequently, a diverse range of feed additives has been researched to replace in-feed AGP (de Lange et al. 2010). Up to recently, the main focus of finding alternatives for prophylactic AGP use has been on direct supplementation of piglets post-weaning, through the use of feed additives in the post-weaning diet. However, there are also other strategies that could enhance the growth and health of the newly weaned pig. One of these strategies is the use of maternal nutrition to improve growth and health in her offspring. Recent research has shown that maternal supplementation with  $\beta$ -glucans has a positive effect on piglet growth performance and health during lactation and post-weaning (Leonard et al. 2011; Shen et al. 2011; Heim et al. 2015). The present review will discuss the development of the intestinal tract in the pig during gestation. lactation and post-weaning periods and the factors that influence intestinal health post-weaning. It will also discuss the effects of diet composition and the supplementation of novel bioactives and additives to both the maternal and post-weaning diets, so as to alleviate the negative effects associated with weaning.

## Influence of maternal nutrition on intestinal health of the piglet post-weaning

The development of both the GIT and the immune system of the pig is influenced by maternal factors throughout gestation. The GIT develops early in embryonic life, during the process of gastrulation that occurs on about Day 8 of gestation. The immune system has begun developing by Day 16 of gestation, with the development of the lymphoid-cell population. The developing thymus and spleen are evident by Day 22 of gestation (Šinkora and Butler 2009). The first B-cells can be detected in the liver at about Day 30 of gestation, and surface IgM cells are found in the spleen at about Day 50 (Šinkora and Butler 2009). The porcine fetus develops in the absence of maternal immunoglobulins, due to the specialised epitheliochorial placenta. Hence, B-cells and the humoral component of the immune system are naive at birth, having developed without anti-idiotypic influences from the mother during fetal life. The number of circulating lymphocytes and their functional activity declines after birth and for a period of 14-21 days (Hammerberg et al. 1989). This is important as this decline in the circulating lymphocytes coincides with weaning under commercial husbandry conditions and could potentially contribute to weaning dysregulation.

The concept of microbial succession in mammals is an ecological principle that describes the succession of microbes over time culminating in a 'climax' community, which is stable in a homeostatic GIT environment. Many factors contribute to microbial succession in the pig. At birth, the GIT of pigs is sterile due to the sterile environment in utero (Le Dividich et al. 2005). Immediately after birth, the GIT of the neonatal piglet is colonised by bacteria derived from the sow's vaginal tract and faeces and from other maternal and environmental sources (Mackie et al. 1999). From this moment onward, the microflora has to develop from a simple, unstable community into a complex and stable community, a process that can be influenced by diet, environmental factors and the genotype of the piglet itself (Konstantinov et al. 2006). The establishment of the pig intestinal microbiota is a complex process that involves an initial colonising phase, during which the intestine of the newborn is rapidly invaded by bacteria, followed by different successional steps where diverse groups become predominant (Zoetendal et al. 2004). This process continues as the pig matures, resulting in a characteristic and dynamic bacterial population for each individual (Zoetendal *et al.* 2004). The microbiota establishment after birth plays an important role in the development of the neonatal intestinal and immune systems (Mukherjee and Hooper 2015). Initially, there is a high degree of similarity in the microflora of the dam and piglet. However, the microbial patterns change in piglets within a few days, resulting in patterns that are rather different from those of the dam, and are characteristic for each individual (Zoetendal *et al.* 2004).

There are a few ways to manipulate the colonisation process in the new-born piglet's GIT, since there is evidence that when neonatal pigs are less exposed to potentially pathogenic bacteria, they have a lower chance of developing PWD (Bauer et al. 2006a, 2006b; Heim et al. 2014a). The first way is to reduce the number of potentially pathogenic bacteria or promote the number of beneficial bacteria in the GIT and faeces of the sow. This will benefit the colonisation of the piglet's GIT. Piglets are known to explore the farrowing crate and can ingest large quantities of faeces from the sow (Demecková et al. 2002). The maternal administration of probiotics containing beneficial bacteria can result in a higher colonisation of these bacteria in the piglet GIT, thereby reducing colonisation of pathogenic bacteria (Baker et al. 2013). Both Leonard et al. (2012) and Heim et al. (2014a) showed that supplementing pregnant-sow diets with seaweed extracts containing laminarin and fucoidan during late gestation reduced the Enterobacteriaceae population in the sow's faeces, while also reducing colonic Escherichia coli numbers in the piglets at weaning. This indicates that manipulation of the microflora of the sow has the potential to reduce the abundance of pathogenic bacteria in the intestinal tract of her offspring.

Second, the composition of the colostrum and milk from the sow that is ingested by the piglet has the potential to deliver antimicrobial effects (Bauer et al. 2006a; Perez et al. 2007) and immune-enhancing properties (Leonard et al. 2010). Both colostrum and milk contain a lot of different antimicrobial substances such as glycoproteins, glycolipids, mucins and oligosaccharides (Bauer et al. 2006b). They also contain lactoferrin, which has bactericidal properties and prevents the induction of cytokines that can cause inflammation (Bauer et al. 2006b). Some of these components in milk can support the establishment of a beneficial commensal microbiota (Bauer et al. 2006a). The immunoglobulin IgA, which is the main immunoglobulin in milk, is important in the defence against intestinal pathogens, and also serves an essential role in preventing bacterial translocation beyond the GIT (Green-Johnson 2012). Higher levels of immunoglobulins in the colostrum and milk can improve the acquired passive immunity in new-born piglets. From studies that evaluated the effect of sow nutrition on colostrum and milk composition, it can be concluded that dietary manipulation affects the nutrient composition and immunoglobulin content of both colostrum and milk (Farmer and Quesnel 2009). The use of short-chain fructo-oligosaccharides (Le Bourgot *et al.* 2014) and  $\beta$ -glucans from different strains of yeast (Jang et al. 2013; Zanello et al. 2013) result in improved immunoglobulin concentrations in colostrum. Other feed additives associated with increased immunoglobulin concentrations in colostrum and milk are seaweed extracts (SWE) containing the bioactive compounds laminarin and fucoidan (Leonard *et al.* 2012; Heim *et al.* 2014*a*). Maternal SWE supplementation increases piglet serum IgG concentration on Day 14 of lactation (Leonard *et al.* 2012), while piglets suckling SWE-supplemented sows have improved leukocyte phagocytosis capacity (Leonard *et al.* 2010).

While maternal supplementation with SWE appears to have enhanced the immunoglobulin status of the piglets, it also appears to have enhanced the ability of the piglets to fight pathogenic bacterial challenges. Piglets suckling sows supplemented with SWE containing laminarin and fucoidan have improved resistance to enterotoxigenic E. coli (ETEC) infections and reduced shedding of this pathogen post-weaning following an ETEC challenge (Heim et al. 2014a). More recently, Bouwhuis et al. (2017a) showed that piglets suckling sows supplemented with laminarin have improved resistance to an experimental Salmonella Typhimurium challenge and reduced shedding of this pathogen post-weaning. The IL-22 expression in the colon is reduced in pigs weaned from laminarinsupplemented sows. The presence of S. Typhimurium is linked to the expression of *IL-22* in the intestinal tract, since IL-22 is involved in maintenance of the mucosal barrier and tissue generation, especially during microbial challenges (Behnsen et al. 2014). Indeed, IL-22 is thought to play a unique role in S. Typhimurium infections (Behnsen et al. 2014).

In a longer-term study, sows were supplemented with laminarin and fucoidan from Day 87 of gestation and the offspring were monitored until time of slaughter (~90 kg). Pigs from laminarin- and fucoidan-supplemented sows had greater nutrient digestibility and increased numbers of faecal *lactobacilli* spp., greater villous architecture at weaning and had higher daily gain than did control pigs for the duration of the studies (Heim *et al.* 2015; Draper *et al.* 2016). These studies indicated that the ingredients used in the maternal diet can have a substantial influence on growth and gastrointestinal health of the offspring in post-natal life.

#### Effect of weaning on the intestinal health of the pig

Weaning is a critical period in pig husbandry. Weaning of pigs in commercial systems is abrupt and is undertaken at an early age, normally 14-28 days after birth. Weaning induces transient and acute changes in the morphology and physiology of the GIT, which are most likely to be related to the post-weaning reduction in feed intake. This is followed by a period of intestinal maturation, corresponding to resumption of feed intake (Lallès et al. 2007a). The villi and crypts that line the epithelium of the small intestine are essential for the digestive and absorptive processes, and their structure and function after weaning are affected by many factors (Pluske et al. 1997). However, Vente-Spreeuwenberg et al. (2003) concluded that diet composition had only marginal effects on the smallintestinal morphology of the weaned pigs, and the level of feed intake was the most important determinant of mucosal function and integrity. Food deprivation in piglets leads to a lack of luminal stimulation and induces a reduction in villous height and an increase in crypt depth (Pluske et al. 1997). Villous height is minimised after 2-5 days post-weaning and is associated with cell loss and reduced absorption of nutrients (Hedemann et al. 2003). Intestinal villous height starts to recover in feed-deprived piglets at ~4 days after feeding is restarted, but can take more than 10 days to fully recover (Boudry *et al.* 2004). Crypt hyperplasia is an indication of increased cell production in the crypt (Hedemann *et al.* 2003). The epithelial cells originate from the crypts and move from the crypts to the top of the villi. The production of these cells is increased in the immediate post-weaning period (Heo *et al.* 2013).

As well as the intestinal morphology being affected by weaning, the activity of many brush-border and pancreatic digestive enzymes such as sucrase, lactase, the peptidases aminopeptidase A and N and lipase are also reduced (Lallès et al. 2004). A continuous supply of nutrients is essential for maintenance of gastrointestinal-tract integrity, as the absence of nutrients in the small-intestine lumen will have marked effects on the rate of cell differentiation and turnover (Pluske et al. 2003; Heo et al. 2013). However, due to the reduced feed intake post-weaning, this supply of nutrients is reduced. Part of this reduced supply can also be attributed to the change in nutrient source. The sow milk is highly digestible, contains bioactive substances and the main energy sources are fat and lactose. The post-weaning feed is typically solid, has a much higher dry-matter content, is less digestible and the main energy source is starch (Kim et al. 2012a). This reduced digestibility is partly due to the insufficient enzyme production in the piglet. The endogenous enzymes are adapted to the digestion of nutrients from sow milk (Heo et al. 2013). Therefore, sufficient amounts of lipase, amylase and other necessary enzymes needed to digest the post-weaning diet are not produced until ~3-4 weeks postweaning (Torrallardona 2010). The provision of creep feed supplemented with the necessary enzymes may be a useful tool to prepare piglets during lactation to the consumption of solid feed and to avoid the post-weaning anorexia. The activity of the brush-border enzymes is also decreased post-weaning, which is thought to be due to villous atrophy and crypt hyperplasia. This has been related to the loss of mature enterocytes and rapid renewal of the immature enterocytes as the villi shortens. The fast renewal of the immature enterocytes does not allow them to fully express maximal brush-border enzyme activity (Hedemann et al. 2003). Another reason for the reduced enzyme production could be the immature GIT itself, which is not capable of producing sufficient enzymes to efficiently digest the unfamiliar nutrients provided by the post-weaning diet (Bauer et al. 2011). The reduced production of enzymes in the brush border and subsequent reduced nutrient digestibility also reduce the secretion and composition of pancreatic juice, which is rich in digestive enzymes, into the proximal small intestine (Lallès et al. 2004).

It has been reported in numerous studies that weaning stress results in an impaired intestinal barrier function and an increased intestinal permeability as a result of a reduced energy uptake and increased inflammatory response in the GIT (Boudry *et al.* 2004; Bauer *et al.* 2011; Wijtten *et al.* 2011). Maintenance of intestinal barrier function is dependent on the appropriate biological functioning of both the mucosal layer and the tight junctions. The mucosal layer, which is the primary barrier between the internal environment and external environment, protects the epithelial layer against the microbes and antigens present in the intestinal lumen (Farhadi *et al.* 2003; Kim *et al.* 2012*a*).

The tight junctions form protein bonds between the individual epithelial cells, forming a physical barrier (Hu *et al.* 2013).

Tight junctions consist mainly of transmembrane protein complexes such as claudins and occludins and the cytosolic proteins zonula occludens (ZO-1, ZO-2 and ZO-3), which join the transmembrane proteins to the cytoskeletal actins (Smith et al. 2010; Hu et al. 2013). There is a cascade of events affecting intestinal barrier function. The mucosal layer in the intestine prevents pathogenic adhesion (Kaper et al. 2004; Smith et al. 2010). The most important component of the mucosal layer in the GIT is the intestinal epithelial layer (Burkey et al. 2009). The intestinal epithelial cells are coated with a layer of viscous mucus to protect the epithelial cells against pathogens (Linden et al. 2008). The intestinal epithelial cells excrete, together with the underlying leukocytes, many defensive compounds into the mucosal layer, including mucins, defensins, antibodies and many more (Linden et al. 2008). However, once pathogenic bacteria are able to break through the mucous layer, they are able to penetrate the tight junctions with, for example, fimbria (enterotoxigenic E. coli), subsequently releasing toxins that trigger inflammation and PWD (Pluske et al. 2002). An increase in the production of pro-inflammatory cytokines such as TNF- $\alpha$ , IFN- $\gamma$  and IL-6 is associated with an increase in intestinal permeability, while anti-inflammatory cytokines such as IL-10 are associated with a decrease in intestinal permeability (Hu et al. 2013). The increased cell turn-over in the intestine results in lower numbers of mature enterocytes, where tight junctions are not fully developed, leading to an increase in the intestinal permeability (Wijtten et al. 2011). Therefore, it is very important to try and improve the small-intestinal morphology by improving feed intake in the immediate post-weaning period, so as to reduce the intestinal permeability and, thus, pathogenic susceptibility and intestinal inflammation.

### Microbiological changes in the gastrointestinal tract post-weaning

In the immediate post-weaning period, the balance between a healthy and unhealthy microflora can be easily changed towards a pathogenic profile (Zoetendal et al. 2004). The weaning process also results in a greater variation in microbial profiles among animals. Castillo et al. (2007) indicated that weaning resulted in more heterogeneous microbial profiles among pigs than during the suckling period. According to Bauer et al. (2011), the numbers of Lactobacillus spp. and other beneficial bacteria decrease in times of stress, as do their beneficial effects. When lactic acid bacteria, which are harmless bacteria, are attached to the mucosal layer of the intestinal tract, they can prevent pathogens adhering to the enterocytes, thus preventing the multiplication of these pathogens and production of their toxins (Janczyk et al. 2007). This reduces the opportunities for pathogenic bacteria to increase their abundance and reduces the risk of the onset of PWD (Kaper et al. 2004).

The gastric pH is an important first barrier against pathogens entering the intestine and influencing the intestinal microbiota. The conversion of lactose to lactic acid by microbes during the suckling period helps maintain this low gastric pH. However, the weaning-associated anorexia can result in an increased gastric pH, and reduces the acidic protection against microbial pathogens (Torrallardona 2010). The introduction of a solid diet requires an increase in anaerobic bacteria in both number and diversity, but also favours the risk of microbial instability (Konstantinov et al. 2006). Furthermore, reduced feed intake immediately post-weaning leads to rapid changes in the microbiota as substrates available for microbial fermentation are depleted. As a consequence, the microbiota becomes unstable during the first week post-weaning, with a marked decrease in biodiversity, which will be restored after a reestablishment period of 2-3 weeks (Inoue et al. 2005). The decline in microbial diversity coupled with increased proliferation of pathogenic bacteria, such as β-haemolytic E. coli, can result in PWD (Konstantinov et al. 2006; Castillo et al. 2007). Diarrhoea generally occurs in pigs 3-10 days post-weaning and is often associated with an over-population of ETEC bacteria carrying specific heat-labile and/or heat-stabile enterotoxins (Carstensen et al. 2005; Lallès et al. 2007b).

While all microbial-diversity studies agree that Firmicutes and Bacteroidetes are the most abundant phyla in the faecal microbiota of piglets, accounting for more than 90% of the faecal bacterial community at both pre-weaning and postweaning periods, there is some confusion in the literature with regard to the specific proportions of gut microbial communities and how they change at the weaning time period (Kim *et al.* 2011, 2012*b*; Kim and Isaacson 2015). These varying results may reflect advances in microbial characterisation and quantification methodologies, but may also indicate that there are marked differences among different breeds, husbandry conditions and diets.

### Diet composition and gut health and development in post-weaned pigs

Extensive research programs have explored the impact of a wide range of feed ingredients and nutrients on various aspects of gut health and development in pigs during the post-weaning period (Lallès *et al.* 2007*a*; de Lange *et al.* 2010; Pluske 2013; Thacker 2013). However, it is beyond the present review to summarise this information. The focus of this section of the review is to highlight key underlying concepts that allow a better understanding of the value of selected nutrients and feed ingredients in stimulating gut health and development.

In recent years, the recommended levels of amino acids have increased (Hermes et al. 2009; NRC 2012) in diets for weaned pigs and the crude protein content is ~200-230 g/kg according to the NRC (2012) guidelines. This level of protein can be quite problematic to weaned pigs. Not all dietary protein is available for the newly weaned pig, because the pig's ability to digest and absorb high protein diets is generally compromised postweaning. This results in protein entering the large intestine to be fermented, which encourages the growth of N-utilising bacteria such as E. coli, producing potentially toxic substances such as ammonia, amines, indoles, phenols and branched-chain fatty acids, which have been implicated in the pathogenesis of PWD (Pluske et al. 2002). The studies by Bikker et al. (2006) and Heo et al. (2009, 2010) all proved that a reduced crude protein content reduces protein fermentation in the intestine, which results in reduced ammonia concentrations, an improved faecal

consistency and faecal dry-matter content but does not negatively affect growth performance, even after an experimental challenge with enterotoxigenic *E. coli*.

Dairy products are known to have beneficial effects on feed intake, growth performance, feed efficiency and health in newly weaned piglets. This is due to the high palatability and digestibility of protein and energy in dairy products (Lallès et al. 2007a). The inclusion of dairy products and other highly digestible and palatable diets such as sugar, rolled oats, animal by-products and cooked rice is a common practice in piglet nutrition and enhances feed intake in the immediate postweaning period (Dunshea et al. 2002; Molist et al. 2014). Dietary inclusion of lactose results in rapid fermentation into lactic acid due to the presence of lactic acid bacteria (among others, Lactobacilli spp.; Pierce et al. 2007). It is thought that the increased production of lactic acid and subsequent lowering of the pH in the GIT may delay the multiplication of pathogenic bacteria, therefore improving gastrointestinal health (Pierce et al. 2007). The inclusion of other fermentable carbohydrates such as sugar-beet pulp, inulin and resistant wheat starch also promotes colonic microbial stability and diversity and stimulates gastrointestinal health (Lallès et al. 2007a). The inclusion of these fermentable carbohydrates results in an increased transient time of the digesta in the stomach and small intestine, giving the endogenous enzymes a better chance at hydrolysing the nutrients (Lallès et al. 2007a). The inclusion of the right proportion of insoluble and soluble fibre reduces the abundance of Enterobacteriaceae (Molist et al. 2009).

Dietary components originating from leguminous plant proteins (e.g. soya bean meal and peas) are known to have negative effects on growth and health post-weaning. The inclusion of soya bean meal leads to morphological changes due to delayed hypersensitivity reactions and the antigenic effects associated with the feed (Dréau *et al.* 1994). The inclusion of these legumes can result in a localised immune response, villous atrophy, crypt hyperplasia and decreased growth (Heo *et al.* 2013). However, Pierce *et al.* (2004) showed that high dietary concentrations of lactose allow for increased soya bean meal inclusion (>200 g/kg) in weanling pig diets, without affecting performance or health.

#### Feed additives in the post-weaning diet

A diverse range of feed additives has been researched, so as to replace antimicrobial growth promotors (de Lange et al. 2010). Various (natural) materials have been investigated as efficient alternatives to AGPs, such as zinc oxide (Heo et al. 2010; O'Shea et al. 2014; Cho et al. 2015), copper sulfate (Edmonds et al. 1985), prebiotics such as galacto-oligosaccharides (Tzortzis et al. 2005; Searle et al. 2010), yeast β-glucans (Kogan and Kocher 2007; Zanello et al. 2013), mannan-oligosaccharides (Castillo et al. 2008), organic acids (Roselli et al. 2005; Kuang et al. 2015; Stensland et al. 2015), probiotics (Valeriano et al. 2017), spraydried plasma proteins (Torrallardona 2010), exogenous feed enzymes (Torres-Pitarch et al. 2017) and essential oils (Zeng et al. 2015). These feed additives can beneficially affect the microbiota composition, and health and growth performance of pigs. However, according to de Lange et al. (2010), only a limited number of feed additives are effective in stimulating

gut development and health of pigs that are managed under wide-ranging conditions of housing, management, feeding and health status; these authors concluded that no alternative additive has been found that results in an improvement in growth performance and reduces the occurrence of diarrhoea, similarly to the inclusion of in-feed AGP. However, it is important to remember that we cannot consider these feed additives as alternatives to in-feed antibiotics any longer, because they are now required on their own to improve digestive health in the absence of in-feed antibiotics. There is fundamental requirement to explore the underlying mechanisms of activity when evaluating the functional properties of feed ingredients and feed additives, so as to better understand under what conditions it is possible to achieve the optimal response to dietary interventions (Pluske 2013). Key aspects of gut functionality that should be considered include digestive capacity (activity of pancreatic and brush-border enzymes), absorptive capacity (villi architecture and nutrient-transporter expression), chemical and physical barriers, microbiota load and diversity and immune function (de Lange et al. 2010).

#### Novel bioactives in post-weaning diets

Chemodiversity in nature, including microorganisms, terrestrial plants, marine macroalgae and marine organisms, offers a valuable source of novel bioactives (Vondruskova et al. 2010; Redondo et al. 2014). Diverse organisms have evolved diverse chemical and molecular mechanisms for a variety of homeostatic activities, including cell-to-cell signalling, receptor sensitivity, inflammasome activity and gene activation (Sweeney and O'Doherty 2016) and, hence, offer great potential as preventatives and prophylactics in mammals. Marine macroalgal extracts are showing a wide range of biological activities (i.e. antioxidant, anticancer, anticoagulant and anti-inflammatory activities, among others), with potential use in the food and nutraceutical markets. They are a rich source of structurally diverse bioactive compounds with valuable pharmaceutical and biomedical potential. Brown marine algae contain large amounts (~40% of the dry matter) of polysaccharides, particularly laminarin and fucoidan, which are resistant to hydrolysis by human endogenous enzymes and are, therefore, valuable dietary fibres for bacterial fermentation in the large intestine (Hoebler et al. 2000).

#### Laminarin

Laminarin is a specific type of  $\beta$ -glucan extracted from seaweed species. The biochemical characteristics of laminarin vary with the seaweed species of origin. In general, it has a low molecular weight of ~5 kDa and is soluble in water (Lynch *et al.* 2010). It is composed of  $\beta$ -(1, 3)-D-glucan with  $\beta$ -(1, 6) branch chains that vary with species in distribution and length. It has been suggested that the immunoprotective effects of orally administrated  $\beta$ -glucans are mediated through receptor-mediated interactions between  $\beta$ -glucans and epithelial microfold cells in the GIT. The specialised microfold cells are primarily responsible for the transport of macromolecules within the Peyer's patches (Tsukada *et al.* 2003). The  $\beta$ -glucan is taken up by the Peyers patches and is presented to underlying dendritic cells to influence cytokine production. Dietary supplementation of 50-day-old pigs with  $\beta$ -glucan from *Laminaria digitata*, *L. hyperborea* and *Saccharomyces cerevisiae* downregulated the expression of a panel of inflammatory cytokines in the colon and liver (Ryan *et al.* 2012; Sweeney *et al.* 2012) and mucin gene expression in the ileum and colon (Ryan *et al.* 2010; Smith *et al.* 2011). Similar observations were subsequently reported in the newly weaned piglet (Walsh *et al.* 2013*a*). Interestingly, supplementation of laminarin derived from *Laminaria digitata* increased the expression of *IL-6* and *IL-8* in lipopolysaccharide challenged colonic tissue (Bahar *et al.* 2012; Smith *et al.* 2011). These data suggest that laminarin decreases the expression of cytokines in the quiescent GIT, but increases the capacity of cytokines to respond to a pathogenic challenge (Smith *et al.* 2011).

Beta-glucans can agglutinate certain bacterial species, inhibiting subsequent attachment and colonisation of epithelial mucosal surfaces (Kogan and Kocher 2007). Experiments investigating the effects of  $\beta$ -glucan supplementation in protecting pigs against ETEC infection after weaning showed reductions in the faecal excretion of F4 E. coli and a reduced F4specific serum antibody response, thus decreasing susceptibility to ETEC infection (Stuyven et al. 2009). Supplementation of a Laminaria spp.-derived seaweed extract containing laminarin to pigs suppressed enteric Enterobacteriaceae populations (Reilly et al. 2008; Lynch et al. 2010) and decreased faecal E. coli numbers (McDonnell et al. 2010; O'Doherty et al. 2010). The species of seaweed that the laminarin is extracted from is important as laminarin extracted from Laminaria hyperborea is more effective in reducing coliform counts than is laminarin extracted from Laminaria digitata (Murphy et al. 2013; Gahan et al. 2009).

Most importantly, weanling piglets supplemented with laminarin at a rate of 300 mg/kg from the Laminaria spp. have improved growth and feed-efficiency in the absence of in-feed antibiotics (Walsh et al. 2013b; Heim et al. 2014b) and have a growth performance similar to those supplemented with zinc oxide. Purified laminarin extracts improve nutrient digestibility, improve villous structure and increase the gene expression of the nutrient transporters GLUT1, GLUT2 and SGLT (Heim et al. 2014b). The laminarin produced by different methods may vary in its structure, chemical composition, or both, and this may influence its activity and the amount that should be added to get a growth response. Feeding excess laminarin can have detrimental effects on pig performance. It is possible that diets supplemented with excess laminarin promote the secretion of pro-inflammatory cytokines instead of decreasing secretion (Smith et al. 2011). Similar responses have been reported with yeast  $\beta$ -glucans.

#### Fucoidan

Fucoidan represents a class of fucose-enriched sulfated polysaccharides extracted from the extracellular matrix of brown algae, with L-fucose 4-sulfate building blocks as the major component (Fitton 2011). The molecular weights of fucoidans vary from ~100 kDa to 1600 kDa and Usov *et al.* (2001) suggested that the biological activity of fucoidan is dependent on the molecular weight and sulfation content.

Research has indicated that fucoidan possesses antimicrobial, immunomodulatory, antioxidant and antiviral properties (Walsh et al. 2013a; Rajauria et al. 2016; Sweeney and O'Doherty 2016). Fucoidan is thought to affect leukocyte recruitment following an infection and reduces tissue break down during inflammation (Fitton 2011). However, the main activity of fucoidan and sulfated polysaccharides seems to be their prebiotic potential. Fucoidan supplementation results in an increased Lactobacillus spp. abundance in colonic digesta in pigs (Lynch et al. 2010; Sweeney and O'Doherty 2016). Recently, Bouwhuis et al. (2017b) showed that supplementation of a Laminaria spp.-derived seaweed extract containing predominantly fucoidan reduces the shedding of S. Typhimurium following a S. Typhimurium challenge in finisher pigs. Dietary supplementation with this SWE also resulted in a suppression of a panel of selected cytokines that are associated with the NF- $\kappa\beta$  pathway, demonstrating the anti-inflammatory effects of the SWE. In a dextran sodium sulfate challenge, supplementation of fucoidan or a combination of fucoidan and laminarin improved bodyweight loss, diarrhoeal scores and clinical variables associated with colitis, in tandem with a reduction in colonic IL-6 mRNA abundance (O'Shea et al. 2016). Other effects were also evident as inclusion of seaweed extracts containing predominantly fucoidan in the diet of pigs decreased lipid oxidation in meat products and increased total antioxidant capacity in the serum and muscle of the supplemented pigs (Rajauria et al. 2016).

#### Chitin and its derivatives

There is substantial variation in the literature on the biological properties of chitosan and chitooligosacharide (COS; Liu et al. 2006). This variation is most probably due to the widely different molecular weights (MW) of compounds used across studies. Indeed, Walsh et al. (2013c) found that the biological activities of COS are very much influenced by the MW. The supplementation of COS at a particular MW (from 5-10 kDa COS upward) range may be an effective substitute for in-feed antibiotics during the post-weaning period. The inclusion range of 10-50-kDa COS seems to be the optimum MW range to increase growth performance (Walsh et al. 2012) by enhancing small intestine structure, improving nutrient digestibility, modifying E. coli populations and reducing diarrhoea in pigs. A possible reason for the higher-MW COS (5–10 kDa upward) exhibiting an antibacterial activity is that COS may interact with the membrane of the cell to alter cell permeability (Chung and Chen 2008). The second mechanism is that COS penetrates the nuclei of the bacteria and interferes with RNA and protein synthesis (Liu et al. 2004).

#### Conclusions

Traditional practices have focussed on supplementing the piglet diet in the post-weaning period with AGP and zinc oxide to alleviate post-weaning complications. Several nutritional and management strategies have been suggested as alternative means to enhancing post-weaning growth performance and controlling PWD in piglets. The ban on in-feed AGP also reinforces the importance of maternal sow nutrition, to protect the offspring from intestinal disorders via a healthy microflora and an increase in immune cells in colostrum and milk. Several studies have shown the positive effects of sow-diet supplementation on the neonatal piglet by enhancing the immune response and reducing the shedding of pathogenic bacteria. Recent research has shown that the inclusion of marine-derived bioactives laminarin, fucoidan and chitosan could affect the pig intestinal health and growth performance in the post-weaning phase. These supplements could, therefore, be suitable substitutes for in-feed antibiotics and will have a positive effect on the intestinal physiology, morphology, microbiology and immune response of the post-weaned piglet, leading to an improved growth performance. A combination of these management and nutritional strategies will be required to avoid occurrence of PWD in pigs, particularly in the absence of in-feed antibiotics and zinc oxide in the post-weaned pig diet.

#### **Conflicts of interest**

The authors declare no conflicts of interest.

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# Guaranteeing consistently high quality Australian pork: are we any closer?

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Abstract. Considerable investment has been made by the Australian pork industry over several decades, to address key factors that affect pork quality, so as to improve consumer acceptability of pork and pork products. These outcomes have been utilised to inform on-farm quality assurance programs, develop effective solutions to negate boar taint issues associated with the production of entire males, drive continuous improvement in animal management and encourage new technologies to be implemented in both the production and processing sectors of the Australian pork supply chain. Australian Pork Limited's Strategic Plan 2015–20 is focused on maintaining profitable and sustainable growth in existing markets and developing strong foundations to support new opportunities, both in Australia and internationally. Guaranteeing that pork available for purchase is always consistently high in eating quality will support ongoing consumer demand for pork through increased consumption frequency. However, achieving this on an everyday basis presents industry with significant challenges due to the many complex interactions among the production, processing and post-slaughter factors experienced by pigs, carcasses and pork that can influence final product quality, either singularly or in combination. The present paper describes recent quantitative studies to determine the size and effect of pathway parameters on eating quality attributes of fresh pork and knowledge gaps identified. Outcomes of consumer sensory studies to inform the development of a non-prescriptive cutsbased eating quality system for pork and commercially validate particular pathway interventions are detailed. Through the implementation of validated pathway interventions to optimise pork eating quality, the overall industry objective is to reduce eating quality fail rates of different pork cuts to less than 10%.

Additional keywords: cuts, eating quality, interventions, pigs, system.

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#### Introduction

The ability of the Australian pork industry to consistently supply high quality fresh pork is integral to supporting growth in fresh pork consumption by Australian consumers, which has increased from 9.79 kg/capita.year in June 2015 to 10.06 kg/capita.year as at February 2017. This growth, resulting from current higher beef prices, improved knowledge of how to cook fresh pork at home and changing consumer demographics of the Australian population, has also contributed to pork consumption overtaking beef in Australia for the first time (ABARES 2017). Consumers expect to be able to reliably purchase, and consume, meat of premium quality and value that meets their requirements for flavour, tenderness and juiciness (Pethick et al. 2006). However, eating quality of meat is difficult to evaluate before purchase as it is highly variable and not visible (Verbeke et al. 2010). This presents significant challenges for the meat industry, given the heterogeneity of the product, with variability occurring both among muscles within an animal due to differences in anatomical function and among animals in response to genetic and environmental factors. For the red meat sector, variability in eating quality has largely been addressed through the development and adoption of the Meat Standards Australia eating quality program for both beef (Polkinghorne *et al.* 2008; Watson *et al.* 2008) and sheep meat (Thompson *et al.* 2005). These programs were initiated to address consumption frequency issues associated with eating quality inconsistencies and recognition that grading systems in place did not differentiate carcasses on an eating quality basis. This is also the case for pork. The grades and categories used on the slaughter floor to describe carcass quality, such as gender, carcass weight, fatness level, lean meat yield content and evidence of secondary sexual characteristics, are inadequate descriptors of eating quality.

Taste is the most important attribute to consumers when eating pork, followed by convenience (Verbeke *et al.* 2005). As part of Australian Pork Limited marketing program, online main grocery buyer representative surveys are undertaken biannually to establish fail rates of pork, lamb, beef and chicken on the basis of the last three dinner meals consumed by the consumer. Estimated eating quality fail rates, determined from stated perceptions of consumers, in the May 2016 survey were 10% for pork loin steaks compared with 7% for chicken, 8% for lamb and 9% for beef. In addition, fail rates for smell and taste were higher for pork (3%) than the 2% for the other three meat proteins evaluated. These data, together with fail rate data obtained from centrally located sensory studies, where consumers are asked to rate each piece of pork on a 1 (unsatisfactory) to 5 (excellent) scale (e.g. D'Souza *et al.* 2012; Channon *et al.* 2014*b*), indicate that an ongoing industry effort to develop and implement an eating quality system for pork is required.

The Australian pork industry is focused on differentiating Australian pork from other Australian meats as well as from imported sources, with this objective supported by improved product quality and consistency, as described in Australian Pork Limited Strategic Plan 2015–2020. For ongoing sustainability, profitability and competitiveness of the Australian pork industry, market diversification is also being sought to increase sources of demand, as 90% of Australian pork is currently consumed domestically. An effective system for the industry to guarantee its product quality is a central enabling pillar to underpin export opportunities in the global pork market, where Australian pork exports currently only comprise ~0.5% of international trade. Such eating quality systems for pork have not yet been developed or implemented anywhere in the world, other than blueprints (Warkup 1993) or pork quality targets (Meisenger 2001), implying that this is no simple task. The conversion of muscle to meat alone is very complex, involving many biochemical processes and structural changes, let alone accounting for the myriad of production, processing, post-slaughter and cooking factors that can affect eating quality in combination. This said, the implementation of a cuts-based eating quality system for Australian pork, supported by solid supply chain commitment, would strongly position Australia as a reliable and consistent supplier of high quality pork.

#### Industry investment to improve pork quality

#### Causes and incidence of PSE

Over the past 25 years, the Australian pork industry has heavily invested in improving the quality and consistency of pork. Initially, the National Pork Quality Improvement Program demonstrated that the occurrence of pale, soft and exudative (PSE) pork could be reduced by ~40% through improved handling and management of pigs and carcasses at all critical points in the production and processing chain (Eldridge et al. 1995; Warner 1997a; Trezona et al. 2001). An extensive pork quality research program focused principally on reducing the incidence of PSE, with issues investigated including mixing of entire males (D'Souza et al. 1999a), muscle type (O'Halloran et al. 1995), electric prodder use (D'Souza and Hofmeyr 2001), pre-slaughter handling and rate of processing (D'Souza et al. 1998a), on-farm and pre-slaughter handling (D'Souza et al. 1995), stockperson behaviours (Hemsworth et al. 2002), interactions between halothane gene and stunning procedures (Channon et al. 2000) and magnesium supplementation of pigs for several days before slaughter (D'Souza et al. 1998b, 1999b). These outcomes also informed quality assurance standards in the Australian Pork Industry Quality Assurance program. However, this substantial body of work did not assess the eating quality attributes of pork, namely tenderness, juiciness,

flavour and overall acceptability or liking, which can influence quality grade and consumer re-purchase intention.

#### Boar taint

In addition to pork quality issues related to PSE, technologies to reduce the incidence of boar taint arising from the production of entire male pigs by Australian producers, in preference to castrated males to better meet consumer requirements for lean pork, were also being explored (Hennessy and Wan 1993; Hennessy et al. 1997). The preference of Australian consumers for pork from low taint male carcasses and female carcasses compared with high taint male carcasses has been demonstrated (Laing et al. 1995; Laing 1996). Subsequently, Hennessy et al. (1997) showed that boar taint concentrations (defined as androstenone and skatole concentrations above the international thresholds of  $1.0 \,\mu g/g$  and  $0.2 \mu g/g$  respectively) were high in entire males slaughtered at 85-110 kg liveweight. Developed in Australia, the immunisation of entire males against gonadotropin-releasing factor suppresses the production and accumulation of androstenone and skatole in adipose tissue of entire male pigs (following the second immunisation administered 2-6 weeks before slaughter). This inhibits testis function and reduces androstenone and skatole concentrations in adipose tissue of entire males to below the limit of detection (Hennessy et al. 1997; Dunshea et al. 2001; Moore et al. 2017) and sensory detection thresholds (Font i Furnols et al. 2008; Font i Furnols et al. 2009). Intramuscular fat content can be increased and shear force reduced in pork from immunocastrated male pigs compared with entire males (Batorek et al. 2012), and the pattern of this increase is related to the progressive increase in feed intake. Pork from immunocastrated male pigs has been shown (as summarised by Allison et al. (2009)) to be superior in quality to entire males and of equivalent quality to females and surgically castrated males. Its use by Australian pig producers continues to increase, reflecting its effectiveness in minimising flavour issues associated with boar taint and, in particular, improving animal welfare on-farm by reducing sexual and aggression activities of immunocastrated male pigs (Cronin et al. 2003).

#### Pork eating quality research and development

Bennett (1997), in the opening paragraph of the final report of her Pig Research and Development Corporation-funded study examining the feasibility and desirability of an eating quality program, stated the following:

> The future success of the Australian pig industry will be critically dependent on its ability to understand and satisfy the changing needs and expectations of consumers in a highly competitive food market. In recent years, the industry has progressively implemented quality assurance programs which aim to meet the requirements of domestic and export markets in relation to carcass quality, antibiotic residues and meat hygiene. The proposed next step in this process is an Eating Quality Improvement Program which would focus on pork tenderness, juiciness and flavour, with the objective of increasing market value and market share through improved consumer satisfaction.

This led to an extensive eating quality research program being initiated by Pig Research and Development Corporation to address pork eating quality issues for the Australian pork industry and develop management strategies, both on farm (Campbell 1998; D'Souza and Mullan 2002, 2003; D'Souza *et al.* 2008) and during processing (Channon *et al.* 2003*a*, 2003*b*, 2004, 2014*b*) Importantly, these studies involved consumer evaluation of pork eating quality, with cooking protocols also developed to meet preferred consumer requirements for a medium to well done degree of doneness (Channon *et al.* 2001), but largely only evaluated eating quality attributes of the *M. longissimus dorsi* (loin).

The key outcomes and recommendations arising from this program were presented at the APSA Conference in 2001 (Channon 2001; D'Souza and Mullan 2001) and risk ratings for factors that may influence pork eating quality through increasing intramuscular fat concentrations, reducing drip loss and improving tenderness, juiciness and flavour were proposed (Taverner 2001). These are presented in Table 1. The strong emphasis of pathway factors that may reduce PSE incidence is noteworthy. Taverner (2001) considered that cooking has the greatest impact on pork eating quality, suggesting that even if high quality pork was available for purchase, the cooking methods used by consumers at that time (Bejerholm and Aaslyng 2004), together with their confidence, knowledge and cooking skills, would influence the final eating quality outcome. Moisture infusion, ageing period, hanging method, chilling and gender (in a declining order) were the next five factors rated as having a significant effect on pork eating quality. A pathway approach was implemented in Western Australia, involving the production of immunised entire males, magnesium supplementation for 5 days before slaughter and moisture infusion, resulting in improvements in eating quality and reduced fail rates from 30% to 3% when these interventions were implemented (D'Souza *et al.* 2012). While an industry-wide eating quality system for the Australian pork industry did not culminate from this program, the outcomes and data generated from this work are underpinning substantial industry efforts to implement a cuts-based eating quality system for Australian pork.

#### Quantitative analysis of pathway factors

Extensive qualitative reviews on production, processing, postslaughter and cooking factors on pork eating quality attributes have been published (including D'Souza and Mullan 2001; Rosenvold and Andersen 2003; Ngapo and Gariepy 2008; Wood et al. 2008; Bonneau and Lebret 2010; Channon and Warner 2011). Flavour, tenderness, and juiciness are the key pork eating quality attributes that influence the overall liking of pork (Wood et al. 1986; Cameron et al. 1990; Channon et al. 2004, 2014b) and are highly correlated with each other (Channon et al. 2014b). These attributes may be influenced by boar taint, intramuscular fat content, the rate and extent of muscle pH decline post-slaughter (and its influence on water holding capacity, meat colour, flavour, proteolytic enzyme activity and the breakdown of myofibrillar structure) and connective tissue content and solubility. Channon and Warner (2011) outlined a systems approach that may be applied to produce consistent high quality pork and briefly described the proposed approach to establish an eating quality system for Australian pork, with support from both Australian Pork Limited and the High Integrity Australian Pork CRC.

 Table 1. Collation and risk rating of the factors that might have an impact on pork eating quality across the value chain (adapted from Taverner 2001)

Critical management point	Rating <sup>A</sup>	Issue
Breed	**	Duroc known to boost marbling
		• Hal gene known to be linked with pale, soft and exudative (PSE)
		Unknown status of RN gene
Gender	***	Castration reduces risk of boar taint
		Castration increases marbling
Age and weight at slaughter	*	Little effect of weight within current commercial range
Nutrition	**	• Taint from certain ingredients (fish meal/oil)
		Benefits of Mg to reduce PSE and vitamin E on pork quality
		Risks to eating quality of metabolic modifiers
Housing	*	Risk of skatole taint
On farm handling	*	Possible link to stress, incidence of PSE
Transport	**	<ul> <li>Stresses of mixing and handling, incidence of PSE</li> </ul>
Lairage and pre-slaughter handling	**	<ul> <li>Stresses of mixing and handling, incidence of PSE</li> </ul>
Time off feed	**	• 6–24 h before slaughter
Stunning	*	• Low risk if done correctly with either electrical or CO <sub>2</sub> stunning
Stimulation	**	<ul> <li>Low voltage electrical stimulation 5–10 min post-slaughter</li> </ul>
Carcass processing		
Chilling	****	• Effect on PSE
Hanging	****	<ul> <li>Benefits of aitchbone hanging on tenderness</li> </ul>
Product preparation		
Ageing	****	• Benefits of ageing: >2 and up to 7 days on tenderness
Pumping	****	<ul> <li>Benefits on juiciness and tenderness</li> </ul>
Consumer preparation	*****	$\bullet$ Cook pork cuts to end-point temperature between 65°C and 71°C

<sup>A</sup>Impact on eating quality: \*, low to \*\*\*\*\*, high.

The pathway approach and consumer evaluation methodologies adopted by Meat Standards Australia (Channon and Warner 2011) and statistical modelling work undertaken as part of a large European Union-funded project, Q-Pork Chains (that aimed to develop innovative, integrated and sustainable food production chains of high quality pork products to meet consumer demands; Verbeke et al. 2010), informed quantitative approaches to commence the development of predictive eating quality models for Australian pork (Channon et al. 2016b). Meta-analysis approaches, using published data, have been used to predict the effect of various production parameters on pork quality, including metabolic modifiers, such as porcine somatotropin and ractopamine (Dunshea et al. 2005), fasting, transport and lairage (Salmi et al. 2012), vitamin E supplementation (Trefan et al. 2011), halothane gene (Salmi et al. 2010), gender (Pauly et al. 2012), housing (Demori et al. 2012) and gender, breed and carcass weight (Trefan et al. 2013). However, for those studies investigating eating quality traits (Dunshea et al. 2005; Pauly et al. 2012; Trefan et al. 2013), only loin data were used.

Trefan et al. (2013) conducted a random-effects meta-analysis using 43 published studies to quantify the effect of different pig genders on pork quality traits across a range of carcass weights and different breeds. The additive model for each trait contained linear and quadratic terms of carcass weight as covariates, gender and muscle as a fixed effect and breed as a random effect. A large range in the effects of breed on pork quality traits was identified and may partly be explained by breed effects on intramuscular fat content (Cameron et al. 1999). No relationship between carcass weight (ranging between 65 and 85 kg) and juiciness or tenderness scores for the loin was found. The lack of a relationship between carcass weight and eating quality traits of female pigs was also reported by both Channon et al. (2014b), for carcasses averaging 60 or 80 kg, and Channon et al. (2013b), who found that overall liking scores for loin steaks and silverside roasts were not influenced by age at slaughter (20, 21 or 24 weeks), corresponding to average hot carcass weights of 68, 70 and 88 kg respectively. Trefan et al. (2013) also showed that intramuscular fat content, drip loss and muscle lightness were not related to carcass weight, between the range of 45.9 and 135.9 kg. As slaughter age of female pigs increased from 16 to 25 weeks, higher intramuscular fat content was found in the silverside muscle, but not the loin or M. supraspinatus, despite increases in P2 fat depth and carcass weight (D'Souza et al. 2004).

Estimated tenderness and juiciness scores were not significant among immunocastrated males, surgical castrates, entire males and females (Trefan *et al.* 2013). Similar meta-analysis outcomes for juiciness and tenderness between genders were also reported by Pauly *et al.* (2012), who concluded, on the basis of these outcomes, that the production of immunocastrated males or entire males as an alternative to the surgical castration of males in Europe, in response to voluntary banning of castration of pigs by 1 January 2018 (Bee *et al.* 2015), would not result in pork quality concerns. Gispert *et al.* (2010) also considered that immunocastrated males may be an alternative to surgical castration as no meat quality differences due to gender, other than higher intramuscular fat concentrations in pork from immunocastrated males, were identified. Martinez-Macipe *et al.*  (2016) also found no difference in eating quality among Iberian surgical castrates, females and immunocastrated males when vaccinations against gonadotropin-releasing factor were conducted at 11, 12 and 14 months of age. However, higher scores for rancidity (aroma and taste/flavour) were found for pork sourced from immunocastrated males. Although it was not assessed, it is suggested that this may have been due to a higher concentration of polyunsaturated fatty acids in intramuscular fat in loins from immunocastrated males than from females or surgical castrates as a result of vaccination timing. Both Trefan et al. (2013) and Pauly et al. (2012) noted that limited studies were available for immunocastrated males for their analyses and this, together with differences among studies in how eating quality was assessed (i.e. use of consumer or trained sensory panels), may explain the lack of gender effect on eating quality. While higher intramuscular fat concentrations and marbling scores of castrates (when data for immunocastrated males and surgical castrates were pooled) were found compared with pork from entire male and female pigs (Trefan et al. 2013), it may not necessarily improve eating quality performance. The magnitude of the correlation between intramuscular fat and tenderness, while significant, is low (Huff-Lonergan et al. 2002; Channon et al. 2004).

While these studies quantified the effects of individual pathway factors on pork eating quality, these analyses were not conducted with a view to informing the development of an eating quality model for pork. It was, therefore, apparent that a more comprehensive approach that encompassed a larger number of pathway factors to quantitatively determine the size and effect of these factors, as well as interactions among them, on pork eating quality was needed. This requirement was also identified by Warkup (1993, p. 66) who stated that 'due to unforeseen and unidentified interactions, the specification advantage in one link of the chain is negated by an interaction with another element of the specification'. So as to do this, peerreviewed journal articles, identified from systematic searches of digital databases, and several unpublished Australian studies (Campbell 1998; Saunders et al. 1999, 2000; Channon et al. 2001; Wilkinson 2002; D'Souza and Moore 2005) reporting eating quality data for effects of production, pre-slaughter, post-slaughter and cooking parameters on pork eating quality of various cuts assessed by inexperienced consumers and trained or experienced panellists, and objective measurements, were obtained. A database was compiled, with all details of each study, including experimental treatments and statistical analyses, were recorded and a Monte Carlo simulation approach was used to estimate effects in eating quality attributes due to supply chain parameters imposed (Channon et al. 2016b).

The use of this methodology aimed to explore whether probability distributions of correction factors, established by determining relative changes in sensory tenderness, juiciness and flavour scores of pork in response to pathway parameters imposed on pigs, carcasses and/or pork cuts, could be used as quantifiable indicators of pork eating quality (Channon *et al.* 2016*b*). Table 2 details the pathway factors that were investigated together with variables that were arbitrarily set for a 'standard pig' and comparative variables within each pathway factor. The proportional change in each attribute in comparison

Pathway parameter	Variable (x)	Comparative variable $(y_0)$
Gender	Female	Entire male, physical castrate, immunocastrated male
Genotype	White	≥50% Duroc, Pietrain, Hampshire, Berkshire
Halothane gene	Normal (NN)	Heterozygote (Nn), homozygous recessive (nn)
Housing	Indoor/conventional	Outdoor, straw bedding
Plane of nutrition	ad libitum	Restricted
Metabolic modifiers	None	pST, ractopamine
Mixing	Not mixed	Mixed
Stunning method	$CO_2$	Electrical
Electrical stimulation	No	Yes
Ageing period	1–2 days	3-7 days, >7 days
Hanging method	Achilles	Aitchbone (tenderstretch)
Moisture infusion	None	Moisture infusion
Final internal temperature	70–74°C	<70°C, >74°C
Intramuscular fat content	<1.6%	>1.6%
Ultimate pH	5.5–5.7	<5.5, >5.7

Table 2.A	Arbitrary variables of the Australian	'standard' pi	ig set for each key	pathway parameter	(from Channon et al. 2016b	, with permission)
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to the variable set for the 'standard' pig for that attribute was determined by dividing the mean for the comparative variable by the mean data for the 'standard' pig variable. Correction factors for each of the 'standard' pig specifications were set at 1 and the uncertainty for the correction factor for each of the pathway parameters was characterised using a Normal distribution. Improvements, of greater than 10%, in the means of probability distributions of correction factors for sensory attributes compared with the 'standard' pig were obtained for gender (immunocastrated males vs females: tenderness), Hampshire gene (tenderness and juiciness), moisture infusion (tenderness and juiciness), hanging from the aitchbone vs Achilles (tenderness and juiciness), ageing period (3-7 days vs 1-2 days (tenderness); >7 days vs 1-2 days (tenderness and juiciness)) and intramuscular fat (>1.6% vs <1.6% (juiciness)). Means of probability distributions of correction factors for moisture infusion were increased by 33% and 27% for tenderness and juiciness respectively, compared with non-infused pork, with the increases being larger than the shifts in correction factor distributions found for both hanging method and ageing period. Negative shifts of greater than 10% in the means of the probability distributions of correction factors were found for pork cooked to an end-point temperature of >74°C (compared with  $70-74^{\circ}$ C) and from homozygous recessive pigs for the halothane gene (compared with homozygous dominant pigs). Low ultimate pH of <5.5 reduced the mean of the probability distribution of the correction factor for juiciness by ~10%, compared with a pH of 5.5-5.7, indicating the influence that ultimate pH can have on water holding capacity.

Overall, these outcomes generally support the risk ratings proposed by Taverner (2001), at least in relation to tenderness and juiciness. Flavour scores were not influenced to the same extent as observed for tenderness and juiciness by any of the pathway parameters investigated in the study. As previously noted for gender comparisons (Pauly *et al.* 2012; Trefan *et al.* 2013), the analysis comparing eating quality traits of pork from immunocastrated males and those from females was limited by lack of studies, with only three studies used. Importantly, this was also the case for many other comparisons in addition to gender. While the Monte Carlo simulation approach used by Channon *et al.* (2016*b*) may be a useful approach to account for the effects of pathway factors on eating quality, only pairwise comparisons of explanatory variables could be made due to lack of data. It was also not possible to generate significant differences between means. For an eating quality system to be successful, it will be necessary that the size and effect of multiple pathway parameters, and their interactions, on eating quality traits are quantified.

The application of random-effects meta-regression statistical methods, using restricted maximum-likelihood models, were investigated to obtain effect estimates and determine whether effect sizes among different explanatory variables within a pathway parameter were significant (Channon et al. 2017). The database developed for the Monte Carlo simulation study (Channon et al. 2016b) was used, with selection criteria modified such that only studies reporting eating quality effects on the loin muscle only and measures of variation were included in the final data file, comprising a total of 294 studies. The pathway parameters investigated were largely similar to those outlined in Table 2, subject to sufficient studies meeting the selection criteria, with the difference being that all of the explanatory variables within a pathway parameter were assessed within the one analysis. Briefly, for production factors, no effect of gender (entire male, immunocastrated male, surgical castrate and female), plane of nutrition, housing and ractopamine supplementation (up to 10 mg/kg for 28 days before slaughter) on eating quality traits were found (Table 3). Genotype differences on eating quality for tenderness and juiciness were identified, potentially reflecting breed differences in intramuscular fat content. Pathway interventions implemented after slaughter, including tenderstretching (or hanging from the aitchbone), ageing pork for more than 2 days post-slaughter and moisture infusion, each improved eating quality traits, while estimated means for tenderness, juiciness and flavour scores were reduced as end-point temperature increased from 70–74°C to >80°C. As earlier Australian studies were conducted using an end-point temperature of 75°C, this suggests that eating quality benefits from cooking Australian pork to an end-point temperature of 70°C need to be further quantified. Some outcomes, including those for effects of gender, are in contrast to those of Channon

## Table 3. Normal probability distributions (mean ± s.d.) of correction factors determined for sensory tenderness, flavour and juiciness scores of each comparative variable of pathway parameters investigated (from Channon et al. 2016b, with permission) n, number of studies; c, total number of comparisons

Description		Tenderness			Flavour			Juiciness	
1	<i>n</i> ( <i>c</i> )	Mean $\pm$ s.d.	95% CI	n (c)	Mean $\pm$ s.d.	95% CI	<i>n</i> ( <i>c</i> )	Mean $\pm$ s.d.	95% CI
					Sex				
Entire male	16 (45)	$1.022\pm0.077$	0.871-1.173	14 (38)	$0.974\pm0.063$	0.855 - 1.102	13 (38)	$1.028\pm0.059$	0.912-1.144
Physical castrate	29 (55)	$1.027\pm0.169$	0.696-1.358	22 (33)	$0.998 \pm 0.104$	0.794-1.202	27 (47)	$1.001\pm0.094$	0.817-1.185
Immunocastrate	3 (8)	$1.131\pm0.215$	0.710-1.552	5 (10)	$0.990\pm0.121$	0.752-1.227	4 (9)	$1.004\pm0.112$	0.784-1.223
				Ge	notype				
>50% Duroc	24 (50)	$1.071 \pm 0.169$	0.740 - 1.402	22 (39)	$1.033\pm0.087$	0.862 - 1.204	24 (54)	$1.062\pm0.145$	0.778-1.346
Pietrain	4 (6)	$1.038\pm0.143$	0.758-1.318	4 (6)	$1.008\pm0.056$	0.898-1.118			
Berkshire	3 (5)	$1.087\pm0.111$	0.869-1.305				4 (7)	$1.104\pm0.135$	0.839-1.369
Hampshire	7 (19)	$1.184\pm0.209$	0.774–1.594	10 (22)	$1.046\pm0.053$	0.942-1.150	7 (19)	$1.127\pm0.098$	0.935-1.319
				Haloth	hane gene				
Nn (carrier)	10 (13)	$0.979\pm0.122$	0.740-1.218	6 (8)	$1.023\pm0.034$	0.956-1.090	11 (16)	$0.937\pm0.086$	0.768-1.106
nn (reactor)	7 (9)	$0.917\pm0.185$	0.554 - 1.280	5 (6)	$0.956\pm0.078$	0.803-1.109	7 (9)	$0.845\pm0.123$	0.603-1.086
				Feed	ing level				
Restricted	7 (9)	$0.969\pm0.047$	0.877 - 1.061	6 (8)	$0.982\pm0.026$	0.931-1.033	7 (9)	$1.005\pm0.051$	0.905-1.105
				Metabol	ic modifiers				
pST	12 (46)	$0.927\pm0.105$	0.721-1.133	10 (31)	$0.983\pm0.064$	0.858 - 1.108	11 (43)	$0.946\pm0.103$	0.744-1.148
Ractopamine	6 (13)	$0.952\pm0.066$	0.823-1.081	5 (12)	$0.987\pm0.044$	0.901-1.073	6 (13)	$0.982\pm0.066$	0.853-1.111
				Electrica	l stimulation				
All systems	5 (14)	$1.083\pm0.139$	0.810-1.355	4 (7)	$0.999\pm0.058$	0.885-1.113	5 (11)	$0.969\pm0.111$	0.751-1.187
				Hangir	ng method				
Aitchbone	3 (26)	$1.206\pm0.203$	0.808-1.604	2 (25)	$1.079\pm0.100$	0.883-1.275	3 (27)	$1.183\pm0.287$	0.620-1.746
				Ageing p	eriod (days)				
3–7 days	9 (49)	$1.123\pm0.174$	0.782 - 1.464	7 (43)	$1.066\pm0.096$	0.877 - 1.254	8 (47)	$1.055 \pm 0.223$	0.618-1.492
>7 days	4 (12)	$1.138\pm0.090$	0.962-1.314				4 (12)	$1.127\pm0.138$	0.857-1.397
				Moistu	re infusion				
Yes	7 (32)	$1.333\pm0.216$	0.908-1.755	8 (49)	$1.054\pm0.119$	0.821 - 1.287	7 (47)	$1.268\pm0.201$	0.874-1.662
				Internal ter	nperature (°C)				
<70°C (60–68°C)	6 (14)	$1.065\pm0.074$	0.919-1.211	10 (36)	$0.977\pm0.103$	0.777 - 1.178	14 (41)	$1.075\pm0.101$	0.877-1.272
>74°C	8 (18)	$0.919\pm0.066$	0.790 - 1.048	13 (97)	$0.918\pm0.128$	0.667-1.169	18 (103)	$0.836\pm0.117$	0.606-1.066
				Ultir	nate pH				
<5.5	6 (8)	$0.975\pm0.106$	0.767 - 1.182				6 (8)	$0.908 \pm 0.094$	0.723-1.092
>5.7	5 (14)	$1.081\pm0.054$	0.975-1.187	3 (7)	$1.046\pm0.041$	0.966-1.126	5 (14)	$1.024\pm0.165$	0.701-1.347
			Intra	muscular fa	t concentration (%	6)			
>1.6%	3 (9)	$0.984\pm0.192$	0.607-1.360	2 (8)	$1.089\pm0.059$	0.973-1.205	2 (6)	$1.153\pm0.240$	0.683-1.623

*et al.* (2016*b*), reflecting the different comparisons made due to the statistical methods used, as well as different studies included in the analysis.

#### Limitations from quantitative analyses

Several limitations and knowledge gaps were identified from these two quantitative analyses that affected the development of a non-prescriptive, cuts-based predictive model for eating quality of pork:

 Many multifactorial studies did not report predicted mean data for non-significant interactions on pork eating quality, limiting meta-regression analyses to investigate the effects of one pathway parameter at a time (in contrast to inclusion of additive or multiple fixed terms to determine interactions) due to lack of studies.

- Few multifactorial studies have investigated effects of similar pathway parameters on eating quality of pork.
- Weightings for different correction factors, and their combinations, could not be determined to account for effects of additional pathway interventions on eating quality.
- Estimated means of sensory traits for many pathway parameters were determined based on very small number of studies.
- Additional eating quality data are needed for many pathway parameters, most notably for (but not limited to) immunocastrated male pigs, due to limited studies available and meeting eligibility criteria.

- Minimal eating quality data for porcine muscles, other than the loin and when prepared and cooked as different cuts.
- Interactions among cut type, cooking method and end-point temperature could not be determined, even for the loin.

#### Optimising pork eating quality

The Co-operative Research Centre for High Integrity Australian Pork aims to reduce fail rates of pork to less than 10% through the implementation of validated pathway interventions to optimise pork eating quality (Channon and Warner 2011). Several multifactorial eating quality studies (Channon et al. 2013a, 2015a, 2015b, 2015c; Jose et al. 2013; Akit et al. 2014a, 2014b; Peng 2015; Jin et al. 2016; Moore et al. 2017) were conducted, in a staged approach, to address these knowledge and data gaps as well as to validate key pathway parameters identified to confirm effects of selected pathway interventions on pork quality in the Australian pig population. All data generated are now being used to inform the development of predictive models for pork eating quality; work is currently underway to address this. Initially, Channon et al. (2016a) investigated the effects of gender (entire male, surgical castrate, female), ageing period (2 days, 7 days), end-point cooking temperature (70°C, 75°C), muscle (leg, M. biceps femoris; shoulder M. triceps brachii; M. supraspinatus; loin) and cut type and cooking method (roast, stir-fry, grill, not all cuts used for all cook methods) on consistency of pork eating quality. Consumers were used to evaluate eating quality traits and as part of this, quality grade was assessed on a 1-5 scale (1 = unsatisfactory to 5 = excellent). Fail rate was determined as the percentage of consumer evaluations that scored either 1 or 2 for quality grade, with a fail rate of <10% targeted.

Significant, but small, effects on eating quality due to gender were identified (Channon *et al.* 2016*a*), with lower scores for juiciness and flavour and higher fail rates found for pork from entire males than for pork from surgical castrates, with females being intermediate (Table 4). Furthermore, boar taint incidence of entire males, determined by international threshold levels described by Dunshea *et al.* (2001), was 15%. Fail rates of loin steaks from entire males were found to be 9.1 percentage units higher than those from immunocastrated males, even though there were no differences due to gender in tenderness, juiciness and flavour scores (Moore *et al.* 2017). Jose *et al.* (2013) found that the fail rate for quality grade of pork from

entire males was 4.7 percentage units higher than that from females. Conversely, Channon et al. (2013a) did not identify any differences in eating quality or fail rates between pork from entire male and that from immunocastrated male pigs in a study designed similarly to that of Channon et al. (2016a). Lower androstenone and skatole concentrations in belly fat of entire males (together with a lower boar-taint incidence of 10%) may, in part, explain the lack of gender difference. Furthermore, Akit et al. (2014b) reported a trend for a higher failure rate of pork from entire males than pooled values for females and immunocastrated males. The inclusion of pork from entire males into an eating quality system, therefore, presents inherent risks in being able to consistently guarantee its freedom from boar taint, in the absence of any rapid, online assessment tools for determining androstenone and skatole concentrations on the slaughter floor. Commercial validation studies have also shown that fail rates of pork cuts from immunocastrated males were comparable or lower (Channon et al. 2015a, 2015b, 2015c) than those from females, supporting the inclusion of immunocastrated males, together with females, in an eating quality system.

In contrast to the quantitative analyses for ageing period (Channon et al. 2016b, 2017), ageing for 7 days postslaughter, rather than 2 days, did not improve any eating quality traits (Channon et al. 2016a). In contrast, Channon et al. (2013a) showed that ageing pork for 7 days postslaughter, compared with 2 days, improved only overall liking scores. Fail rates were not influenced by ageing period in either study. Commercial validation studies conducted, in conjunction with several supply chains, to validate these findings identified that ageing vacuum-packaged pork cuts resulted in no improvement in eating quality after either 7 (Channon et al. 2015a) or 14 days ageing (Channon et al. 2015b) or only very small increases in overall liking scores (Channon et al. 2015c). Channon et al. (2013b) also found that ageing pork loin steaks and silverside roasts for 7 and 28 days post-slaughter did not improve overall liking scores. Given that extended post-slaughter ageing is a key intervention in both the beef- and sheep-meat Meat Standards Australia systems to optimise eating quality of various cuts, this outcome for pork is perplexing. It suggests that improvements in eating quality of Australian pork due to ageing may plateau by 2 days post-slaughter. This presents new challenges to understand the biochemical mechanisms that may be impacting on post-

 Table 4.
 Effect of gender (entire male, immunocastrated male, surgical castrate, female) on quality-grade fail rates (%) of pork

Reference		Gender	ſ		P-value
	Entire male	Immunocastrated male	Surgical castrated male	Female	
Channon et al. (2016a)	23.0		17.1	19.1	0.005
Channon et al. (2013a)	17.8	15.7			0.190
Jose et al. (2013)	23.8			19.4	0.190
Channon et al. (2015c)		15.8		19.6	0.004
Channon et al. (2015a)		18.7		21.9	0.032
Channon et al. (2015b)		19.1		19.1	1.000
Moore et al. (2017)	29.8	20.7			0.007

slaughter tenderisation. Unfortunately, our studies did not extend to the investigation of mechanism to understand what may be contributing to this failure to age. Peng (2015), in a subsequent, multi-factorial study, using pork supplied through an alternate supply chain, compared the effects of ageing period (1 or 7 days) and packaging method (vacuum or modified atmosphere packaging (80% O2:20% CO2) and showed that sensory tenderness, juiciness and overall liking of vacuum-packaged loin steaks aged for 7 days was improved compared with pork aged for 1 day post-slaughter or modified atmosphere-packaged pork aged for 7 days. While ageing in vacuum packaging for 7 days was effective, these outcomes also confirmed those of Lund et al. (2007), who identified that packaging loin steaks in 70% O<sub>2</sub>: 30% CO<sub>2</sub> for up to 14 days results in protein oxidation, negatively affecting tenderness. This is another issue that needs to be better understood to ensure that pathway interventions employed by producers and processors to improve eating quality before packaging are not negated. Further work (Ha et al. 2017) is currently investigating the mechanisms affecting tenderisation and water-holding capacity of pork packaged in a high oxygen atmosphere system and how this may be influenced by ageing period and packaging method used.

It may be that the low ultimate pH observed in both the loin and silverside (*M. biceps femoris*) results in a rapid attainment of tenderness or impedes further ageing resulting from the inactivation of proteolytic enzymes in muscle, as observed in beef and sheep meat (Kim *et al.* 2014; Warner *et al.* 2014). In support of this, tenderness, juiciness and flavour scores of roast and stir-fry from shoulder muscles, with a higher ultimate pH, consistently outperformed comparable cuts from silverside and loin muscles with a lower ultimate pH, with fail rates of <10% found only for shoulder cuts (Table 5; Channon *et al.* 2013*a*, 2016*a*). Continued focus on identifying effective interventions is needed for the loin and silverside, so as to implement feasible strategies to improve eating quality performance. To address this, Jin *et al.* (2016) evaluated the effect of cooking method (grill, roast and sous vide (waterbath for 12 h) to an end-point

temperature of 70°C) and ageing period (2 or 7 days) on tenderness of loin and silverside muscles, objectively assessed by shear force. Loin muscles were more tender than the silverside, reflecting the higher connective tissue content of the silverside. Ageing for 7 days (compared with 2 days) and sous vide cooking (compared with roasting and grilling) were also shown to be additive factors, each independently reducing the shear force of pork. Further work to explore the effects of dietary lecithin on pork cuts with a high collagen content, such as the silverside, is needed to ascertain whether this may be a useful intervention to improve eating quality performance (Akit et al. 2014b). The importance of providing appropriate, clear and simple cooking instructions to consumers to best prepare various pork cuts so as to optimise their eating quality was also demonstrated. End-point temperature alone was not shown to influence eating quality, with end-point temperature effects observed at the cut type  $\times$  cooking method level (Channon et al. 2013a, 2016a). Cooking loin steaks to a 75°C end-point temperature reduced both juiciness and flavour scores, as well as increased fail rates, compared with cooking to 70°C (Channon et al. 2016a), and eating quality of stir-fry cuts was negatively influenced when cooked to the lower end-point temperature of 70°C, compared with roasts. Increasing internal temperature can increase water loss during cooking in response to collagen shrinkage, collagen solubilisation, shortening of sarcomeres and myofibrillar protein denaturation (Christensen et al. 2000).

Jose *et al.* (2013) found that consumers favoured loin steaks with a higher ultimate pH (up to pH 5.7), rather than pork with an ultimate pH of 5.35 to 5.5. Glycogen concentrations at slaughter were also found to be strongly correlated with ultimate pH, suggesting that this may need to be more closely managed to optimise the rate and extent of pH decline, so as to deliver consistently high-quality pork. This was illustrated as part of the commercial validation study (Channon *et al.* 2014) in which three supply chains were involved, and interventions including electrical stimulation or tenderstretching/aitchbone hanging, ageing for 7 or 14 days and moisture infusion were evaluated

Table 5. Means and standard error of difference (s.e.d.) of overall liking scores (0, dislike extremely; 100, like extremely) due to ageing period (A), end-point temperature (T), cut (C), cooking method (roast or stir-fry; CM), between loin steaks and all other cuts (S) and fail rates (%) for quality grade for cut × cooking method (from Channon *et al.* 2013*a*, 2016*a*, with permission) \*, *P* < 0.05; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001

Ageing	Temp	Sho	oulder		Loin		Silv	rerside	s.e.d.	Significance
(days)	(°C)	Roast	Stir-fry	Roast	Stir-fry	Steak	Roast	Stir-fry		-
				(	Channon et al. (	2016a)				
2	70	60.5	69.2	56.3	56.8	52.4	46.5	56.4	1.30	CM, C, S***
2	75	61.5	71.4	53.3	60.1	49.3	43.2	53.8		$CM \times C^*$
7	70	66.3	70.7	58.3	58.8	44.1	47.3	53.9		$T \times S^{**}$
7	75	63.8	72.0	58.7	60.1	48.6	49.4	54.9		
Fail ra	te (%)	10.0	5.4	19.2	15.2	30.2	36.0	21.5		***
				C	Channon et al. (	2013a)				
2	70	69.6	72.3	58.1	62.0	58.9	55.0	54.7	1.66	A*
2	75	69.8	75.0	57.2	57.1	51.2	55.2	52.0		CM, C, S***
7	70	67.8	75.8	62.5	68.4	56.9	52.7	59.0		$CM \times C^*$
7	75	71.7	82.4	60.3	59.2	52.8	54.4	56.6		$T \times C^{**}$
										$A \times CM^{***}$
Fail ra	te (%)	5.6	5.3	19.1	13.4	25.0	26.9	21.9		*

on loin and silverside cuts (roast, stir-fry, steak (loin only)) from immunocastrated and female pigs. An accelerated rate of glycolysis very early post-slaughter (with explanations for this remaining unclear) was observed in loin muscles from one supply chain, which may have negated the response to electrical stimulation. However, average ultimate pH of loin and silverside muscles (assessed at 2 days) were within the normal range (5.5-5.7) and small improvements in eating quality due to ageing for 7 days were found. In contrast, the average ultimate pH of muscles from the other two supply chains were low (<5.5) and ageing for either 7 or 14 days was not effective. Tenderstretching was shown to be an effective physical intervention; however, processor reluctance hampers its adoption (Channon et al. 2015a). Moisture infusion improved eating quality scores and reduced fail rates compared with the other processing interventions imposed across all supply chains; however, it was unable to result in a fail rate of <10% for all cut × cooking treatments assessed.

Overall, none of the interventions imposed, or their combinations, enabled a fail rate of <10% to be consistently met across all pork cuts. These inconsistencies in the effectiveness of pathway interventions to reduce the variability in eating quality among supply chains present additional challenges in developing robust predictive models and emphasise that the non-prescriptive approach is needed to allow supply chains to choose which particular interventions are implemented to optimise pork eating quality. Further work to identify alternate interventions is needed to provide supply chains with additional options, given these observed variabilities in response.

Channon and Warner (2011) outlined work undertaken, particularly for beef, to identify consumer willingness to pay for high quality meat. As part of the Pork CRC program, limited information on consumer willingness to pay for pork on the basis of scoring of individual cuts for re-purchase intention from which fail rates were determined has been obtained. Typically, fail rates for re-purchase intention are higher than those for quality grade (Channon *et al.* 2016*a*). Where willingness-to-pay studies have been conducted in relation to eating quality of beef (Lyford *et al.* 2010; Bonny *et al.* 2017), premiums of between 150% and 200% more for premium beef were identified (Bonny *et al.* 2017). The preparedness of pork consumers to pay for consistently high quality pork needs to be determined to support the implementation of an eating quality system for Australian pork.

#### Conclusions

Warner (1997b) concluded that 'In the year 2010, the pig industry will look back in amazement to 1997 and realise how its perceptions and definition of quality have changed. The industry will be measuring quality and the variation in quality will be minimal. There will be a precise definition of the critical control points between conception and consumption which influence quality using a 'palatability analysis of critical control points' approach.'. While it may be considered that the industry may be in a position of 'so near, yet so far', the Australian pork industry now has extensive and comprehensive datasets and new information to assist with generating eating quality-predictive models. Significant knowledge gaps have

been addressed to generate much-needed data that have quantified both the size and effect that each of the different pathway interventions, and their combinations, have on pork eating quality, to enable a cuts-based system to be established. These significant datasets are now being analysed to develop predictive models, with outcomes expected by December 2017. While it would be ideal that the outcomes are undisputed and clear cut, further challenges as well as inconsistencies have been identified and will require further investigation. It is exciting to see that the Australian pork industry is getting closer to being in a position to implement a robust cuts-based system for pork to ensure that high-quality Australian pork can be consistently delivered by the pork supply chain to our customers, both domestically and internationally. Taverner (2001, p. 116) concluded that 'to manage pork eating quality, there must be a coordinated, integrated effort that extends beyond the producer and the processor. Manufacturers, distributors, retailers, foodservice and export agencies need to be involved ... '; this will be paramount and relationships with all supply-chain stakeholders will be critical to successfully implement of an eating quality system for high integrity Australian pork.

#### **Conflicts of interest**

The authors declare no conflicts of interest.

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### Current and future antimicrobial resistance issues for the Australian pig industry

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Abstract. Antimicrobial use and antimicrobial resistance (AMR) in intensive pig production and its potential impacts to human and animal health are very much under the spotlight, both internationally, and within Australia. While the majority of AMR of medical importance is associated with the exclusive use of antimicrobials in humans, resistance in zoonotic foodborne pathogens such as Salmonella and Campylobacter, and livestock commensal bacteria such as Escherichia coli and *Enterococcus* spp., is under increased scrutiny. This is primarily due to the current reliance on many of the same drug classes as used in human medicine for treatment and control of bacterial diseases of livestock. Furthermore, the development of multidrug resistance in pathogens such as enterotoxigenic E. coli may drive off-label use of critically important drug classes such as 3rd-generation cephalosporins. This could lead to the emergence and amplification of resistance genes of potential public health significance in both pathogens and commensal bacteria. Livestock-associated and communityassociated methicillin-resistant Staphylococcus aureus has also recently been detected in Australian pigs as a result of human-to-animal transmission and are a potential public health issue for in-contact piggery workers. Australia is in a unique position compared with many of its international trading partners due to its isolation, ban on importation of livestock and conservative approach to antimicrobial registration, including reservation of the fluoroquinolone class for use in humans and companion animals only. Cross-sectional AMR surveys of pathogens and commensals in healthy pigs have identified only low frequency of resistance to critically important drug classes. Nevertheless, resistance to critically important antimicrobials has emerged and careful antimicrobial stewardship is required to ensure that these low levels do not increase. In this report, we review AMR of significance to the Australian pig industry and identify potential prevention and control measures.

Additional keywords: antimicrobial stewardship, pig production, prudent use.

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#### Introduction

Antimicrobial resistance (AMR) is described as one of the greatest long-term threats to human health, on the same scale as climate change and terrorism (Viens and Littmann 2015). For only the fourth time in its history for a health issue, the United Nations General Assembly met in September 2016 and committed to 'a broad, coordinated approach to address the root causes of AMR across multiple sectors, especially human health, animal health and agriculture' (WHO 2016). If left unchecked, AMR among pathogenic microorganisms is predicted to cause 10 million deaths in the Year 2050, with a cumulative economic impact exceeding 100 trillion US dollars (O'Neill 2016).

The term 'antimicrobial' (often used as both an adjective and a noun as the short form of 'antimicrobial agent') is used collectively to describe agents that can be administered parenterally, orally or topically to humans, animals and plants, that either kill microbes (bacteria, protozoa, fungi) or inhibit

their growth by mechanisms that exhibit selective toxicity. The term 'antibiotic' is in more general use, but in its strictest definition, it applies only to inhibitory compounds directly produced by microorganisms themselves (such as penicillin), which are the building blocks for many of the antimicrobials in current clinical use today. AMR mechanisms have coevolved with antimicrobials in the struggle for life in complex microbial ecosystems such as soil, water or the vertebrate gut (Laehnemann et al. 2014). In developing antimicrobials, humans have increased the rate of evolution among a number of bacteria that cause clinical infections, to the point where they have developed multiple mechanisms of resistance (Quinn et al. 2011). Scientists had very little idea when they first developed new antimicrobial classes that, within one human generation, they would be struggling to find new antimicrobials for certain infections, particularly those caused by the group of organisms collectively termed the ESKAPE pathogens (including *Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas* and *E. coli/Enterobacter*; Boucher *et al.* 2009). While it is generally accepted that the majority of AMR encountered in human pathogens directly results from the use of antimicrobials in humans (both hospitals and the community), animals, and in particular the livestock industries, have come under increasing scrutiny (Marshall and Levy 2011; Collignon 2012). This is related to both the historic use of some antimicrobials at subtherapeutic levels for growth promotion, the prophylactic use of antimicrobials in feed or water to prevent infections in large groups of animals, and the use of antimicrobials in livestock that are deemed as critical to human health (the 'critically important', 'last resort' or 'top shelf' antimicrobials (ASTAG 2015)).

#### The issue of AMR in animals

Globally, there is much debate concerning the significance of antimicrobial usage in animals, both in livestock produced for food, and pets sharing close contact with humans, and its proportional impact on public health (Marshall and Levy 2011; Collignon 2012; Page 2012; Trott 2013; Abraham et al. 2014c; Shaban et al. 2014). This is largely because the development of AMR is complex, driven by both clonal expansion of multidrugresistant (MDR) strains and the evolution and horizontal transmission of mobile genetic elements carrying AMR genes (ARGs). These include plasmids, transposons, integrons and integrative conjugative elements that constantly evolve under antimicrobial selection pressure (Marshall and Levy 2011; Toleman and Walsh 2011; Mukerji et al. 2017). Some understanding of these terms is necessary to appreciate how bacteria develop resistance, and, in particular, how a susceptible strain of a bacterium can move to a MDR strain in one genetic step.

#### Plasmids

Plasmids are extra-chromosomal autonomous DNA that are separate from bacterial chromosomes. Conjugative plasmids that can be readily transferred to closely related bacterial species may serve as a carrier for several bacterial genes including AMR genes (Bennett 2008).

#### Transposons

Transposons are mobile genetic elements also referred to as jumping genes. Transposons have the ability to move within, attach to or detach from bacterial chromosomes or plasmids. They play a vital role in disseminating AMR genes, by the ability to move among bacterial cells. Transposons generally do not have specific preference or affinity for target binding sites on the plasmid or chromosome. As a result, they are highly efficient and effective at mobilising AMR genes (Lorian 2005).

#### Integrons

Integrons are genetic elements that can effectively capture and express exogenous genes. Integrons are known for their ability to acquire gene cassettes that carry AMR genes (Recchia and Hall 1995). The integrons that carry AMR genes are usually mobile. There are multiple (>100) different gene cassettes that carry AMR genes described and gene cassettes carried by mobile integrons

can render resistance to most classes of antimicrobial used in human and veterinary medicine (Gillings 2014).

#### Integrative conjugative elements

Integrative conjugative elements are self-transmissible, mobile genetic elements that integrate into and replicate as part of the host chromosome. However, integrative conjugative elements also contain conjugative machinery genes that enable their transmission to other bacteria in a similar way that plasmids are transferable between donor and recipient bacteria (Wozniak and Waldor 2010).

## *Critically important antimicrobials (CIAs) and resistance in Gram-negative bacteria*

The CIAs, which are the last-line therapies in humans, include third-generation cephalosporins (3GCs), fourth-generation cephalosporins (4GCs), fluoroquinolones (FQs), carbapenems and colistin (WHO 2017). Antimicrobials are rated as critically important when they are the only or one of few treatment options available for treating severe, life-threatening infections in humans. While CIA-resistance determinants readily circulate among human isolates, driven by the selection pressure of antimicrobial use in humans, the examples below show that they can also be acquired through the food chain.

As stated previously, the World Health Organisation has recently highlighted the major public health risks posed by resistance to CIAs such as 3GCs, 4GCs, FQs, and carbapenems among Enterobacteriaceae (WHO 2014). This is due to the risk of transmission of these resistant bacteria to humans, primarily through health-care settings and person-toperson contact, but also potentially through the food chain and/or the environment (Laxminarayan et al. 2013; Woodford et al. 2014). Internationally, emergence and spread of resistance to CIAs has been reported in both food-producing and companion animals (Pulss et al. 2017). Plasmid-mediated 3GC resistance (mediated by bla<sub>CMY-2</sub>) was first detected in E. coli from US livestock in 1996, and in Salmonella Newport shortly thereafter, with both being linked to the use of 3GCs in livestock (Bradford et al. 1999; Allen and Poppe 2002). Similarly, in Asia and Europe, 3GC resistance in E. coli isolated from livestock has been attributed to emergence and spread of plasmid-mediated resistance genes (Yang et al. 2004; Aarestrup et al. 2006; Wang et al. 2010; Jiang et al. 2012). In addition, several countries in these regions have reported extensive use of FQs in some food-animal species. This has been linked to the emergence of FQ-resistant E. coli and Salmonella in livestock (Yang et al. 2004; Wang et al. 2010; Marshall and Levy 2011; Jiang et al. 2012). More recently, resistance to carbapenems has been reported in Enterobacteriaceae isolated from livestock systems in both Asia and Europe (Woodford et al. 2014) and recently from pigs in North America (Johnson 2017). However, the most significant development in recent years is the emergence and spread of transferable colistin resistance among humans and animals due to plasmid-mediated mcr genes (Liu et al. 2016; Litrup et al. 2017). Colistin, an antimicrobial commonly administered to pigs in several countries in Asia and Europe, was once considered too toxic for human use, but is now prescribed for infections caused by bacteria that are pan-resistant to all antimicrobial classes, including the other CIAs (Gilad and Carmeli 2008). The rapid emergence and spread of colistin and other CIA resistance in both humans and animals has resulted in calls for reductions in the veterinary use of antimicrobials, especially those also used in humans (i.e. 'shared-class drugs'), with a particular focus on the CIAs (Abraham *et al.* 2014*c*, 2016).

## Rapid transfer, dissemination and maintenance of CIA resistance

Development of CIA resistance in pig production systems can occur via the following three possible mechanisms: (1) development of resistance under selection pressure induced by use of CIAs in pigs; (2) direct transmission of resistant bacteria such as *E. coli*, *Salmonella* sp. or methicillin-resistant *Staphylococcus aureus* (MRSA) between humans and pigs; and (3) transfer of mobile genetic elements containing resistance genes from commensal human microbiota into pig commensal microbiota such as *E. coli* or *Salmonella* sp. or, potentially, through an intermediate source of transmission, such as migratory or scavenging wild birds (Mukerji *et al.* 2017). Once introduced into a piggery environment, CIA resistance may circulate and be maintained in the production system for an indefinite period for several reasons, which are discussed below.

Since most of the CIA resistance is encoded on mobile genetic elements such as plasmids and integrative conjugative elements, the transfer of these genes can readily occur among bacterial species and between humans and animals. In addition, critically important AMR can be linked to lower-importance AMR by a process known as co-selection. For example, a carbapenem resistance plasmid may also possess genes encoding resistance to routinely used first-line antimicrobials such as  $\beta$ -lactams, tetracyclines, macrolides and trimethoprim-sulphonamide combinations. As a result, these MDR, promiscuous plasmids can be selected, transferred and maintained in a production system, through the use of these first-line drugs, or indeed other CIAs registered for use in livestock such as 3GCs. Coselection may also involve elements other than AMR genes. For example, many plasmids also carry genes conferring some form of resistance to metal ions, such that in the presence of metal contaminants or some feed additives, bacteria harbouring these plasmids have a fitness advantage (Tetaz and Luke 1983). AMR transfer and maintenance is complicated, and special consideration is required to limit or prevent the entry of the CIA-resistant bacteria into pig production systems.

#### AMR in Australian livestock

Cross-sectional studies have demonstrated that Australia has low levels of CIA-resistant Gram-negative bacteria in foodproducing animals, possibly due to a reliance on extensive livestock production systems (mainly cattle and sheep) and conservative management of the registration of antimicrobials (Abraham *et al.* 2014*a*, 2015). Australia is the only country never to have permitted use of FQs in livestock produced for food (Cheng *et al.* 2012). 3GCs are not used in poultry, 4GCs are not registered for use in any animal species (Cheng *et al.* 2012; Trott 2013), and colistin has not been registered for veterinary use for more than 25 years, with potentially very low historic use prior to this date (S. Page, pers. comm.).

A survey of antimicrobial resistance in Australian enterotoxigenic E. coli (ETEC) isolates undertaken in the mid-2000s found no resistance to ceftiofur or fluoroquinolones, despite the fact that ceftiofur use was reported on 25% of Australian piggeries (Jordan et al. 2009). Furthermore, no plasmid-mediated ceftiofur resistance genes were identified in a study of commensal E. coli isolated from finisher pigs originating from 72 Australian farms (Smith et al. 2016). Further studies confirmed that Australian porcine ETEC are distinct from isolates obtained in other parts of the world with respect to their genetic profile and the absence of resistance to CIAs (Abraham et al. 2014b). Other studies have also suggested that the biology of AMR among Gram-negative bacteria (Enterobacteriaceae) isolated from Australian food-producing animals differs from that in other parts of the world (Abraham et al. 2014a, 2014b). This is predominantly attributed to Australia's geographic isolation, restrictions on the importation of livestock and some fresh meat and strong regulation on the use of CIAs such as 3GCs, 4GCs, FQs and colistin. At present, resistance to carbapenems has yet to be reported among Enterobacteriaceae from Australian livestock. However, a recent study has demonstrated the first detection of resistance to 3GCs and FQs among clinical E. coli isolates from Australian food-producing animals, attributable largely to globally disseminated FQ- and 3GC-resistant E. coli lineages such as ST10 (Abraham et al. 2015). In the study by Abraham et al. (2015), only three FQ- and/or 3GC-resistant E. coli isolates were detected among a total of 114 isolates obtained from pigs. However, only one of the isolates was a pig pathogen (porcine ETEC, ST100) and two of the three strains have been previously reported in humans overseas (ST744 and ST10). One strain (ST744) was resistant to both 3GCs and FQs and this strain has been identified previously as representing an extendedspectrum  $\beta$ -lactamase-producing E. coli lineage associated with wild birds in Bangladesh and with human extra-intestinal infection in Laos (Hasan et al. 2012). To our knowledge, these potentially zooanthroponotic E. coli strains have not been identified previously in Australia, either from humans or livestock. Their low frequency among clinical E. coli isolates from Australian livestock suggests that they have potentially been introduced via human carriers or, possibly, migratory birds (Manges and Johnson 2012; Hasan et al. 2012; Poirel et al. 2012). Since FQ use in Australian food-producing animals is illegal, it is unlikely that the FQ-resistant E. coli ST744 strain evolved from a livestock-associated progenitor strain under local FQ selection pressure. Further to this report, a recent Australian regional survey of faecal samples from 22 pig farms found that 6.1% (20/325) of E. coli isolates were ceftiofur-resistant with a range of extended-spectrum cephalosporinresistance genes identified, including those encoding CTX-M and AmpC β-lactamases (van Breda *et al.* 2016). An epidemiological investigation of piglet scours at the same farms identified recent disease events and use of bedding or not maintaining fresh bedding as the most significant risk factors for post-weaning diarrhoea (van Breda et al. 2017). Further work using Baysian Network analysis confirmed that managing smaller production units could also reduce the incidence of piglet scours and

potentially, the number of antimicrobial treatments (McCormick *et al.* 2017).

In Australia, until recently, carbapenemase-producing Enterobacteriaceae have been reported only in hospital settings (clinical and environmental sources) where the  $bla_{IMP-4}$  gene is considered endemic and is often carried on a highly transmissible  $bla_{IMP-4}$ -qacG-aacA4-catB3 cassette array (Espedido et al. 2008; Partridge et al. 2012; Sidjabat et al. 2014, 2015b, 2015a). This cassette array that confers resistance to carbapenems and other antimicrobials is generally carried on IncA/C or IncL/M plasmids in New South Wales and Victoria (Espedido et al. 2008; Partridge et al. 2012), and IncHI2 or IncL/M plasmids in Queensland (Sidjabat et al. 2015a, 2015b).

Australian livestock have until recently remained free of multidrug-resistant Salmonella DT104 (Crerar et al. 1999) and extended-spectrum cephalosporin-resistant Salmonella Newport (Abraham et al. 2014a, 2014b). Ceftiofur-resistant Salmonella harbouring an extended-spectrum  $\beta$ -lactamase gene has recently emerged in dairy cattle in Victoria, with closely related strains also isolated from sporadic cases of salmonellosis in humans (Sparham et al. 2017). Recently, isolation and genomic characterisation of a carbapenemase-producing Salmonella enterica Typhimurium from cats in Australia (Abraham et al. 2016) demonstrated the acquisition of an IncHI2 plasmid that carried the same *bla*<sub>IMP-4</sub>-qacG-aacA4-catB3-sul1 cassette array as was previously identified in human isolates by a broad host-range S. enterica Typhimurium sequence type (ST19) (Abraham et al. 2016). In addition, another recent study has identified carbapenem-resistant E. coli and other Gram-negative bacteria that carry the same bla<sub>IMP-4</sub> cassette array from a single offshore seagull colony in Wollongong, New South Wales, Australia (Dolejska et al. 2016). While such isolates have not yet been identified in food-producing animals in Australia, these reports illustrate the ubiquitous nature of such plasmids in the environment and the possibility that agents other than antimicrobials (e.g. heavy metals) may be exerting co-selection pressure for their maintenance and transmission among different host species (Yu et al. 2017).

The potential for multidrug-resistant zooanthroponotic pathogens to enter Australian production systems, presumably by human-to-animal or wild bird transmission has also been highlighted by the emergence of MRSA ST398 in Australian piggeries (Groves *et al.* 2014, 2016). Whole genome sequencing of the strains isolated has suggested multiple incursions of European-origin clonal lineages, most likely from human carriers visiting European piggeries (Sahibzada *et al.* 2017). In contrast to this, the recent detection of MRSA ST93 in Australian pigs (a common, more virulent human disease-associated clone in Australian communities) represents a 'home grown' biosecurity issue and highlights the timeliness of the Australian Pork Limited-sponsored MRSA survey in Australian piggeries (Sahibzada *et al.* 2017).

#### Prevention and control of AMR in animals, with a focus on the Australian pig industry

Australia's livestock and veterinary industries, particularly those involved with intensive farming of animals, such as pigs, will require innovative action to rapidly adapt to the changing international climate in the post-antibiotic era. The following six caveats provide a road map for industry to control AMR at the animal-human interface.

## • Development of robust One Health antimicrobial prudent use guidelines

The current WHO dogma has antimicrobials classified into first choice (empirical treatment), second choice (following first choice failure or the results of culture and susceptibility indicating resistance to first choice antimicrobials) and third choice (i.e. CIAs). This simple system will be adopted in all countries and extended to cover both human and animal health. A relevant example is the use of FQs. Fluoroquinolones resistance varies greatly among countries. In those countries where they have often been used empirically as first choice antimicrobials, rates of resistance in pathogens are as high as 60% (AURA 2016). By contrast, in Australia where the use has been more tightly regulated, FQ resistance in human and companion animals has, until the most recent Antimicrobial Use and Resistance in Australia Report, consistently remained below 10% (Cheng et al. 2012; AURA 2016). FQ-resistant E. coli isolates have been detected in animal production systems in Australia, including in pigs, but they are largely restricted to globally distributed, zooanthroponotic clonal lineages of limited virulence that move freely between humans and animals (Abraham et al. 2015). Nevertheless, they emphasise that AMR is a biosecurity issue for Australian piggeries, requiring consideration of both animal and human movement.

#### • Antimicrobial stewardship principles

Adopting a successful antimicrobial stewardship program is underpinned by the 5Rs (Weese *et al.* 2013; Page *et al.* 2014), as follows:

- (1) Responsibility. The appropriate use of antimicrobials is a shared responsibility between the prescribing veterinarian, who accepts responsibility for the decision to use an antimicrobial agent, and the livestock producer, who is responsible for following all directions for use and implementing associated management changes. This approach safeguards the health and welfare of the animals, while minimising the likelihood of any immediate or longerterm adverse impacts on the individual animal, other livestock, or on public health.
- (2) *Reduction.* Wherever possible, means of reducing the use of antimicrobials should be implemented. Infection control and prevention measures underpin animal health and welfare and are supported by meticulous hygiene, precision nutrition, biosecurity, vaccination, and expert animal husbandry. When these measures are effectively combined, they ensure that infectious disease incidence (and need for antimicrobial treatment or prophylaxis) is minimised.
- (3) *Refinement.* Refined use means the right diagnosis, the right drug, at the right time, at the right dose, the right route, and for the right length of time. Information about each use of an antimicrobial agent should be recorded, so that total use can be evaluated and future use fine-tuned.
- (4) *Replacement.* The use of antimicrobials should be replaced whenever available evidence supports the efficacy and safety of an alternative.

- (5) Review. Antimicrobial stewardship initiatives should be reviewed regularly and a process of continuous improvement adopted to evaluate compliance with initiatives and ensure that antimicrobial use practices reflect contemporary best practice. Effective antimicrobial stewardship operates through a process of continuous improvement, through the following approach:
- (a) Undertake a stocktake (review) of current usage practices (what is being used, how much, why and when?), sources of information and continuing professional development.
- (b) Develop antimicrobial stewardship objectives by identifying ways to Reduce, Refine and Replace current antimicrobial use.
- (c) Implement the antimicrobial stewardship plan.
- (d) Measure progress, review achievements, identify barriers and enablers, critically appraise, and develop new objectives.
- (e) Repeat! For continuous improvement.

## • New antimicrobials and alternatives to traditional antimicrobials

Any newly developed classes of antimicrobial will be for human use only; the animal health industry will need to adopt prudent use guidelines and antimicrobial stewardship principles to preserve the lifespan of 'shared class' antimicrobials and explore new or repurpose existing 'animal only' antimicrobial classes.

There will be an increasingly profitable market for animal products produced, with demonstrably minimal antimicrobial interventions, and industries will be increasingly reliant on good management, improved diagnosis of infectious disease and a range of novel non-antimicrobial control measures to treat and prevent infections. For example, by maintaining gut microbiota integrity through the use of credible prebiotic and probiotic combinations during the critical pre-weaning phase, the need for antimicrobial interventions during the growing period can be reduced. Despite this, antimicrobial usage in livestock is predicted to increase by 67% between 2010 and 2030 (Van Boeckel *et al.* 2015).

While vaccines will not be able to provide protection from the full spectrum of microbial infections in Australian pigs, reliance on efficacious, cost-effective vaccines will likely increase, with loss of effective antimicrobials due to resistance development in the future leading to an increased requirement for novel strategies to vaccine development and registration in Australia. Current legislation on the development and use of autogenous vaccines requires urgent review, as these often represent a highly cost-effective and efficacious alternative to antimicrobials for the control of several endemic bacterial diseases of pigs in Australia, such as greasy pig caused by *Staphylococcus hyicus* (P. Mitchell, pers. comm.).

#### • Infection control and biosecurity

Infection-control and biosecurity principles have been the mainstay of effective prevention of many endemic diseases on Australian piggeries for many years (Fig. 1).

(1) Primary prevention. External biosecurity (bioexclusion). Minimising the introduction of animals, minimising the number of sources of introduced animals. Cleaning and disinfection of transport vehicles and containers, isolation of sick animals before introduction, provision of clean water, feed, air and housing, which must exclude pests, control human access, and filter exhaust to reduce pathogen load.



Fig. 1. Schematic of biosecurity principles applied to pigs. 1, primary prevention: external biosecurity (bioexclusion). 2, secondary prevention: internal biosecurity (biocontainment). 3, tertiary prevention: individual animal resilience.

- (2) Secondary prevention. Internal biosecurity (biocontainment). All-in–all-out production systems, hygiene, infection control protocols, housing design (ventilation, drainage, litter/bedding materials, early diagnosis of disease, once pathogen present, introduce measures to eliminate or reduce transmission) guided by on-farm microbiological risk assessment, reduced stocking density, segregation, sick pens.
- (3) Tertiary prevention. Individual animal resilience (adaptive capacity to changing environment). Genetic selection, vaccination, management (handling, low stress, enrichment), nutrition, housing (ventilation, temperature, stocking rate, hygiene).

However, few of the above biosecurity principles have been adapted and applied specifically to AMR and the role of humanto-animal transmission in AMR-resistant pathogens gaining entry into piggeries. Multidrug-resistant zooanthroponotic (i.e. moving frequently among humans and animals) clonal lineages could potentially colonise the skin, mucous membranes or gastrointestinal tract of piggery workers, and we hypothesise that this risk would be compounded if the same workers frequently travelled overseas to countries with poor regulation of antimicrobial use in both human and animal health sectors (Abraham *et al.* 2015).

#### • Use of effective diagnostics

Traditional diagnostics have been based on varying forms of culture and antimicrobial susceptibility testing methods for detection of disease causing organisms and determining their level of resistance to tested antimicrobial classes. While these systems are highly validated and useful, it is becoming apparent that they must be used in conjunction with molecular methods for a number of reasons, including the following:

- (a) Epidemiological validity. While a small number of swabs from clinically affected individuals, followed by interrogation of a single colony growth, may provide some information on the animal's carriage status, it is only a snapshot of a larger system. Following swabbing an epidemiologically relevant on-farm sample set, high throughput robotics now enables processing of hundreds of bacterial colonies from a single swab, giving a much broader representation of the gut microbiota (Shaban *et al.* 2014).
- (b) Rapid testing. Polymerase-chain reaction (PCR) testing is available for many antimicrobial resistance gene classes. Using these methods, a laboratory can screen swab samples for the presence of genotypic resistance in a matter of hours, with less than one day's turnaround from the samples leaving the farm to the submitter having the results. Such tests will become very important in future quality assurance programs (Pérez-Pérez and Hanson 2002).
- (c) Next-generation sequencing. As mobile genetic elements carry many resistance genes that readily transfer among bacteria, it may not just be an outbreak of disease caused by a particular bacteria undergoing diagnostic investigation, but also an 'outbreak' of promiscuous transfer of the mobile genetic element within unrelated bacteria. Next-generation sequencing is a rapid, high-throughput method allowing detailed interrogation of the bacterial genome to the level of identifying mobile genetic elements. This allows tracing of mobile elements between bacteria of the same species or

across species. It also allows rapid typing of bacterial clones, potentially allowing tracing back from humans, imported stock or wildlife (Groves *et al.* 2016; Worthing *et al.* 2016; Abraham *et al.* 2017).

#### • Surveillance

The defining feature of surveillance is continuous repeated measures (rather than one-off surveys) so that the effects of the above five intervention strategies on AMR can be effectively and credibly reported. Sustainability over time, and the ability to provide data needed to establish AMR trends that support public health-related decision-making are important characteristics to consider.

AMR surveillance generally tends to focus on commensal isolates such as E. coli and Enterococcus and pathogens important to public health such as Campylobacter and Salmonella spp. (Shaban et al. 2014). However, AMR surveillance should also focus on the main livestock pathogens driving antimicrobial use, which, in pigs, constitute the major respiratory pathogens (Actinobacillus pleuropneumoniae, Pasteurella multocida and Haemophilus parasuis) as well as pathogenic E. coli, clinical Salmonella and Brachyspira hyodysenteriae isolates. In the recently concluded Zoetis national survey of AMR in animal pathogens, over 100 porcine ETEC isolates were obtained and widespread resistance to drugs registered for use in pigs, including aminoglycosides, amoxicillin, sulphonamidetrimethoprim and tetracyclines was observed. However, rates of resistance to off-label drugs (florfenicol and ceftiofur) were still at comparatively low levels by international standards (Abraham et al. 2015). AMR surveillance in animal pathogens requires the continued cooperation of veterinarians and veterinary diagnostic laboratories, the adoption of common testing methodologies and the continued funding of reference laboratories to archive the isolates.

#### · Education and stakeholder engagement

The best designed programs to reduce the public health impact of antimicrobial use in pig production are doomed to fail without adequate uptake across all areas of the supply chain. Specialist veterinarians, managers and piggery workers must be committed to making the necessary changes to reduce antimicrobial use and maintain retention of susceptibility among pig pathogens. This has a positive beneficial effect not only to the consumer but may result in decreased production costs due to overall reductions in antimicrobial use. If retailers demand greater accountability in antimicrobial stewardship, even if the link between antimicrobial use in pig production in Australia and MDR in the human pathogens that cause the most severe, life-threatening infections is tenuous, the industry must be ready to respond. Low use of antimicrobials in production and confirmation of freedom of CIA resistance genes could also open up further export opportunities. In many neighbouring countries, unregulated antimicrobial use in both human and animal health has created a significant public health risk (Smith et al. 2014; Doyle 2015).

## Conclusions: what does the future hold for antimicrobial use and AMR in pigs in Australia?

It has been recognised since the original Swann report (Swann 1969) that antimicrobials should not be used in preference to

improved management in intensive animal production systems. This has become even more crucial since the emergence of resistance to CIAs in many livestock systems throughout the world, and the first detection of such resistance in Australia. On the basis of reported use of ceftiofur on 25% of Australian pig farms in the mid-2000s and the recent emergence of resistance among both ETEC pathogens and commensal E. coli isolated from pigs (Abraham et al. 2015; van Breda et al. 2016), we hypothesise that 3GCs may still be in use in some piggeries in Australia. Denmark introduced a voluntary ban on 3GC use in pigs following the detection of 3GC resistance in pig commensal E. coli isolates (Agersø and Aarestrup 2013). In both humans and animals, 3GC use results in gut microbiota changes that can predispose the gut to clostridial enteritis (Slimings and Riley 2014). 3GC administration to suckers, in particular, significantly alters the intestinal microbiota, which may possibly affect whole life performance (S. Mukerji, R. Kirkwood, M. Barton and S. Abraham, unpubl. data). As ceftiofur is the only shared-class CIA used in pigs in Australia, it is now certainly the time to review the cost-benefit of ceftiofur use by the industry.

Without the availability of cheap and effective vaccines, judicious selection of antimicrobials combined with good management may be used to protect the welfare and health of the herd. If the production systems adopted increase the environmental stressors through increased stocking rates and continuous flow to generate maximum meat production from the available area, the situation may be exacerbated. The economic theory behind higher stocking rates is that space is a fixed cost so the economic optimum is to produce as many pigs as possible through the system (Fablet et al. 2012). However, Australian research over 20 years ago recommended that pig stocking densities and shed designs needed to provide at least 3  $m^3$  air volume and 0.6  $m^2$  floor space per pig to improve respiratory health status and, at the time, many sheds often did not provide a satisfactory environment for optimum health (Buddle et al. 1997). In the current post-antibiotic age, such systems may be neither profitable nor sustainable, as animal production moves en masse to low antimicrobial use systems, both from the point of view of public health and consumer preference as well as to further reduce the costs of production.

Introduction of true all-in-all-out production systems can mean fewer pigs weaned per week than in continuous flow systems, but superior production and less porcine respiratory disease (and potentially other systemic and enteric disease) and, concomitantly, a reduced requirement for mass antimicrobial medication, more than compensate for these losses. Four-week batch farrowing, i.e. 13 groups per calendar year, and strict compliance to all-in-all-out post-weaning breaks the most common production-limiting respiratory disease cycles by creating homogeneous subpopulations in terms of herd immunity, as well as decreasing environmental infectious pressure between batches, thereby reducing total antimicrobial usage (Díaz et al. 2017). This significantly reduces incidence of clinical cases of porcine respiratory disease, prevalence of lung lesions at market weight, improves production performances such as average daily gain and feed conversion ratio and days to market (Scheidt et al. 1995). This technique has proven very effective in both North America and Southeast Asia and, if combined with herd closure, allows successful eradication of several bacterial pathogens and reductions in throughput of poorquality pigs (Cargill *et al.* 2017). A cost–benefit analysis of Australian production systems, directly comparing continuous flow to all-in–all-out systems with particular focus on disease, antimicrobial use and AMR, would generate useful data for future planning in the post-antimicrobial era.

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## Mannitol and galactose as markers of gastrointestinal tract morphology in pigs after gradual or conventional weaning

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Intermittent suckling sows and litters suckle, piglets suck (IS), where a sow and her piglets are separated for a period of time each day before weaning, can attenuate weaning-associated villous atrophy in progeny from multiparous sows (Berkeveld *et al.* 2009). The effect of such a management strategy on progeny from primiparous sows might be different given differences in gastrointestinal (GIT) function at weaning (Cottrell *et al.* 2017). Sugar absorption tests (SAT) using mannitol (MAN) and galactose (GAL) were used to assess GIT morphology with results validated using standard histological methods. Mannitol and GAL are usually absorbed *in vivo* across the epithelium via transcellular passive or active pathways, respectively. It was hypothesised that (1) MAN and GAL SAT would detect GIT changes at weaning, and (2) changes would be less profound in IS pigs from primiparous sows due to habituation with creep feed and maternal separation in lactation.

Gilt litters (n = 15), Large White x Landrace, were allocated to one of three weaning regimes: (1) conventional weaning (CW), where piglets had continuous access to the sow until weaning at 26.4 ± 1.34 days (mean ± s.d.), (2) IS, where piglets were separated from the sow for 16 h overnight (0700 to 1500 h) for three nights before weaning (IS16), and (3) IS for 8 h per day (0700 to 1500 h) for 6 days before weaning (IS8). Creep feed was offered *ad libitum* from 10 days of age. At weaning, litters were mixed within treatment and housed in pens of 9.8 ± 0.41. Two hours (d 0) and 4 days after weaning one piglet per pen was selected, fasted for 3 h and given an oral dose of 20% MAN (2.5 mL/kg bodyweight (BW)) and 20% GAL (2.5 mL/kg BW). A blood sample was taken 20 min later. Pigs were then killed and the jejunum was removed for histological examination. Plasma MAN, GAL and jejunum villous height were compared between treatments using the GLM procedures of SPSS (v22.0, IBM, Armonk, NY, USA). No differences in SAT between treatments were found (P > 0.05), hence data were combined to compare SAT data with GIT histology. Quadratic regressions ( $y = a +bx + cx^2$ ) were calculated for all relationships.

Plasma MAN and GAL were highly correlated with the jejunum villous height (r=0.76, P<0.001 and r=0.73, P<0.001 respectively), with all measures decreasing 4 days after weaning compared with the day of weaning (P<0.05; Fig. 1). These results suggest that MAN and GAL (as single marker probes) are effective measures of changes in small intestine surface area, which supports the first hypothesis. However, given IS8 pigs had the lowest villous height (P<0.01; Fig. 1C) compared with the other treatment groups 4 days after weaning, there may be some limitations to MAN and GAL SAT when differences between treatments are more subtle. Furthermore, IS did not improve GIT morphological adaptation to weaning in progeny from primiparous dams, with IS8 pigs performing worse than CW pigs with respect to villous height in the immediate post-weaning period. Therefore, our second hypothesis was not supported.



**Fig. 1.** (*a*) Plasma MAN concentration, (*b*) plasma GAL concentration, and (*c*) jejunum villous height in pigs killed either at weaning or 4 days later. CW = black (n = 5), IS16 = black stripe (n = 5) and IS8 = grey (n = 5).

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### Dietary fibre improved ileal morphology without reducing ileal digestibility in weaned pigs housed in an inferior environmental condition

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A recent study demonstrated that insoluble non-starch polysaccharide (iNSP) could attenuate gastrointestinal disturbance by suppressing proliferation of unfavourable microbial population and by maintaining intestinal integrity (i.e. increasing villous height; Pluske *et al.* 2001). In addition, the notion existed that dietary NSP could support a healthy gut to resistant enzymatic digestion and could be beneficial to microbes in the large intestine (Choct 1997). The hypothesis tested in the present study was that supplementing iNSP would reduce the incidence of post-weaning diarrhoea (PWD) through improving intestinal morphology without impairing apparent ileal digestibility (AID) in weaned pigs.

A total of 108 male pigs (Duroc × (Yorkshire × Landrace); initial birthweight (BW)  $6.2 \pm 0.4$  kg (mean  $\pm$  s.e.m.)) were randomly allocated to one of three dietary treatments and two environmental conditions (sanitary *v*. unsanitary) (six replicate pens per treatment with three pigs per pen). Diets were formulated to contain similar digestible energy content with increasing amounts of cellulose as top dressing (0, 1 and 2%). Chromium oxide was added as an indigestible marker to measure the AID of dry matter, crude protein and energy. One pig per pen (n = 6) was killed to harvest ileal digesta, tissue at the terminal ileum on d 0, 7 and 14 as described by Heo *et al.* (2010). The effects of cellulose supplementation and sanitary conditions were analysed using the general linear model (GLM) procedure of ANOVA of SPSS software (v22.0, IBM, Armonk, NY, USA).

There were interactions between sanitary conditions and dietary treatments in crypt depth and villous-crypt ratio (V : C) on d7(P < 0.01) and 14(P < 0.001) (Table 1). Pigs that were housed in poor sanitary conditions had lower (P < 0.05) AID of dry matter than pigs that were housed in sanitary conditions on d 14. A diet supplemented with 2% cellulose decreased (P < 0.05) AID of crude protein compared to pigs fed a diet with 0 or 1% cellulose. Our results indicated that a diet with 1% added cellulose increased V : C ratio, but feeding a diet containing cellulose impaired the AID of crude protein and energy on d 14 in both sanitary and poor sanitary conditions.

Item	Environmental conditions		s.e.m. <sup>A</sup>	Γ	s.e.m.	P-value <sup>B</sup>				
	Sanitary	Unsanitary		Cellulose 0%	Cellulose 1%	Cellulose 2%		Е	D	$\mathbf{E} \times \mathbf{D}$
			Пе	eal morphology						
Villous height (µm)	806.23	703.58	9.127	767.70	758.79	738.22	9.513	***	NS	NS
Crypt depth (µm)	648.67	561.88	8.342	652.12	560.56	603.16	8.434	***	***	***
V:C	1.30	1.32	0.021	1.26	1.41	1.27	0.020	NS	**	**
			Apparent il	eal digestibility (AL	D; %)					
Dry matter (%)	74.00	71.13	0.59	73.86	72.73	71.11	0.65	*	NS	NS
Crude protein (%)	74.82	72.11	1.24	77.22	74.42	68.75	1.02	NS	*	NS
Energy (%)	77.68	74.14	1.11	76.81	75.22	73.47	1.15	NS	NS	NS

Table 1.	Effects of environmental	conditions and	dietary treatment	s of cellulose	on ileal	morphology	and apparen	t ileal	digestibility	(AID;	%)
			in weaned	pigs over 14 d	lays						

<sup>A</sup>Pooled standard error of the mean. <sup>B</sup>Significance level: NS, not significant;  $\dagger$ , P < 0.1; \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001.

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## Increasing dietary tryptophan and decreasing other large neutral amino acids increases weight gain and feed intake in weaner pigs infected with *Escherichia coli*

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Manipulating endogenous production of neurotransmitters in the peri-weaning period by increasing the ratio of tryptophan (Trp) to other large neutral amino acids (LNAA) in the diet increases serotonin production and dopamine metabolism in the brain (Fernstorm 2013). Tryptophan is a precursor for the synthesis of serotonin, a neuromediator associated with appetite regulation and down-regulation of the hypothalamic–pituitary–adrenal axis (Le Floc'h and Seve 2007). Other LNAA compete with Trp to cross the blood–brain barrier, therefore regulating LNAA in plasma, which can influence Trp availability and thus serotonin biosynthesis (Shen *et al.* 2012). In this study it was hypothesised that increased supplementation of Trp and/or reduction in LNAA, to increase the Trp:LNAA ratio, in diets for weaned pigs experimentally infected with enterotoxigenic *E. coli* (ETEC) would improve growth performance and reduce cortisol levels.

A total of 96 male weaned pigs (Large White x Landrace) with the Mucin 4+ allele (affecting resistance to *E. coli* infection) were individually housed and allocated into treatments based on weaning weight, sow parity and location in the building (eight treatments  $\times$  12 pigs = 96 pigs). The study was designed as a 2 × 4 factorial arrangement with respective factors being without/with ETEC infection and four dietary Trp:LNAA (LNAA: tyrosine, valine, phenylalanine, isoleucine and leucine) ratios (Table 1). Pigs in the infection group were inoculated with 0.8 mL of ETEC (serotype O149; K88) solution in two gelatinised capsules, on d 7 and 8 after weaning. Faecal consistency score, diarrhoea index, faecal β-haemolytic *E. coli* shedding and number of therapeutic antibiotic treatments were recorded. Blood samples were collected on d 6, 9 and 14 from eight pigs per treatment, plasma cortisol was assessed using ELISA (Enzo Life Sciences, NY, USA). Data were analysed by two-way ANOVA using SPSS (v21, IBM, Armonk, NY, USA).

Diet 4, with the highest Trp:LNAA, had higher ADG (P < 0.05) during d 8 to 14 and 15 to 21 periods (Table 2), and from d 0 to 21 when compared to Diet 3 and Diet 1. During d 8 to 14, pigs in the infection group grew more slowly (P = 0.04) than their non-infected counterparts, and had increased incidence of diarrhoea (60.4% v. 39.6\% respectively; P = 0.017). Between d 15 and 21, ADFI was higher in pigs fed Diet 4 compared to Diets 1 and 3 (527 g v. 429 g and 438 g, respectively; P = 0.021). Plasma cortisol at d 9 was higher in ETEC pigs (11.9 v. 16.3 ng/mL respectively; P = 0.05), but there were no dietary differences. Data suggested that increased dietary Trp and reduction in LNAA (Diet 4) for weaned pigs improved ADG and ADFI irrespective of infection with ETEC or not, but did not modulate the stress response, as assessed by cortisol levels.

I uble II	Diet description	and analysed stan	auf albea neur algest	note ripili and e	sinposition

Diet description and analysed standardised ileal digestible Trp-I NAA composition

Diet	Description	SID Trp	SID LNAA	SID Trp:LNAA
1	Low Trp, High LNAA	0.222	5.006	0.044
2	Low Trp, Low LNAA	0.216	4.176	0.052
3	High Trp, High LNAA	0.317	4.966	0.064
4	High Trp, Low LNAA	0.315	4.231	0.074

Table 2.	Effects of dietary treatments, ETEC infection or sham-infection on average daily gain (ADG), average daily feed intake (ADFI) and feed
	conversion ratio (FCR) from d 0 to 21 after-weaning

Parameter	Dietary (D)			Treatn	nent (T)	s.e.m.	<i>P</i> -value			
	1	2	3	4	Control	Infected		D	Т	$D \times T$
ADG (g)	162 <sup>b</sup>	184 <sup>a,b</sup>	163 <sup>b</sup>	219 <sup>a</sup>	179	185	13.6	0.010	0.681	0.729
ADFI (g)	259 <sup>b</sup>	284 <sup>a,b</sup>	261 <sup>a,b</sup>	315 <sup>a</sup>	279	281	16.2	0.050	0.936	0.753
FCR	1.51	1.41	1.71	1.21	1.56	1.36	0.152	0.126	0.158	0.657

<sup>a,b</sup>Mean values within a row that have different superscript are significantly different (P < 0.05).

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Table 1

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## Effects of feeding conjugated linoleic acid (CLA) and medium chain fatty acids (MCFA) to gilts and sows on survival of their progeny

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Feeding lipid sources such as conjugated linoleic acid (CLA) and medium chain triglycerides or their acids (medium chain fatty acids; MCFA) to the sow in late gestation and lactation has been shown to improve the survival of piglets, in particular those of low birthweight, through increased energy and immunoglobulins available in colostrum and milk (Azain 1993; Bontempo *et al.* 2004). Gilt progeny (GP) have higher rates of mortality and medication compared to sow progeny (SP; Smits 2011). It was hypothesised that feeding CLA and (or) MCFA would improve survival of both progeny groups, with improvements more pronounced in the lighter, more immunocompromised GP.

A total of 129 primiparous (Parity 0; GILT) and 123 multiparous (Parities 2 and 3; SOW) sows and their piglets (PrimeGro<sup>TM</sup> Genetics, Corowa, NSW; 1367 GP and 1546 SP) were involved in the experiment. Diets consisted of different sources of dietary lipid: (1) 6% tallow (CON); (2) 2.5% tallow replaced with a commercial CLA product (Lutrell<sup>®</sup> Pure; BASF; 50% c-9,t-11 and 50% t-10,c-12 CLA isomers); (3) 0.1% tallow replaced with a commercial MCFA product (Aromabiotic<sup>®</sup> Pig, Nuscience, Drongen, Belgium); and (4) equal parts of the CLA and MCFA diets (by weight, i.e. 1.25% CLA, 0.05% MCFA; BOTH). Experimental diets were fed from an average of d 107 of gestation until weaning at d 27 of lactation. Cross-fostering between litters was carried out as per standard production protocols to equalise litter numbers. A serum sample was collected from a subsample of piglets (n = 144) 3 days after birth. A sample of colostrum was collected at birth, and a milk sample was collected on d 21 of lactation from a subsample of sows (n = 68). Serum samples were assayed for immunoglobulin G (IgG) and  $\beta$ -hydroxybutyrate ( $\beta$ HBA) concentrations using commercial kits. Colostrum (IgG<sub>d0</sub>) and milk samples (IgG<sub>d21</sub>) were assayed for IgG concentration. All piglet mortalities were recorded. Continuous variables were analysed as a linear mixed model using the MIXED procedure of SPSS (v24.0, IBM, Chicago, IL, USA). Mortality was analysed using  $\chi^2$ . The diet\*parity interaction was not significant for any trait ( $P \ge 0.10$ ).

Lower IgG in GP compared to SP (P < 0.05; Table 1) despite similar levels of IgG<sub>d0</sub> and IgG<sub>d21</sub> ( $P \ge 0.10$ ) suggests that GP may absorb less IgG through colostrum and milk than SP. Contrary to the current hypothesis, feeding 2.5% CLA or 0.1% MCFA (or a combination of both) in the late gestation and lactation diet did not significantly improve immune status, energy levels or pre-weaning mortality rates in gilt or sow progeny.

	mortality in gilt and sow progeny	
Traatmant	Least square mean $\pm s$ a	D voluo <sup>A</sup>

Table 1. Effects of feeding different lipid sources in late gestation and lactation on colostrum, milk and serum metabolites, and pre-weaning

Treatment		Least square mean $\pm$ s.e.										
Trait <sup>B</sup>		Die	t (D)		Parit							
	CON	CLA	MCFA	BOTH	GILT	SOW	D	Р				
IgG <sub>d0</sub>	82.8 ± 12.7	$81.0 \pm 10.4$	$85.5 \pm 10.7$	$78.8 \pm 14.2$	93.1 ± 9.5	$70.9\pm7.5$	NS	*				
IgG <sub>d21</sub>	$0.34\pm0.06$	$0.30\pm0.05$	$0.37\pm0.05$	$0.33\pm0.06$	$0.36\pm0.04$	$0.31\pm0.04$	NS	NS				
IgG <sub>S</sub>	$16.3 \pm 1.7^{ab}$	$17.4 \pm 1.5^{\mathrm{a}}$	$12.3 \pm 1.4^{b}$	$14.0\pm2.0^{ab}$	$13.1 \pm 1.3$	$16.9 \pm 1.1$	*	**				
βΗΒΑ	$0.85\pm0.07$	$0.84\pm0.06$	$0.82\pm0.06$	$0.84\pm0.08$	$0.90\pm0.05$	$0.78\pm0.05$	NS	*				
Mortality	14.4 <sup>ab</sup>	11.2 <sup>a</sup>	16.0 <sup>b</sup>	15.0 <sup>b</sup>	14.0	14.3	*	NS				

<sup>A</sup>NS, not significant,  $P \ge 0.10$ ; \*P < 0.10; \*P < 0.05. <sup>B</sup>IgG concentration expressed as mg/mL,  $\beta$ HBA expressed as mM, mortality expressed as %. <sup>a,b</sup>Different superscripts within rows denote significant pairwise differences between diets (P < 0.05).

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### Dietary essential oil volatiles are transferred to milk and amniotic fluid in sows

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Maternal food cues during pregnancy and lactation have been demonstrated in several mammalian species, including pigs, to impact offspring food preferences later in life (Hepper *et al.* 2012). However, the evidence that dietary volatile compounds are transferred, and to what extent, into maternal fluids in pigs remains elusive. We hypothesise that the efficiency of transfer of dietary compounds into maternal fluids will be specific to each compound and related to their chemical nature. This study aimed to trace and quantify dietary essential oil (EO) compounds in milk and amniotic fluid in sows.

A total of 38 multiparous Large White sows were selected at 104 days gestation. The experiment was divided in two trials: Trial 1 (T1) aimed to assess the kinetics of the potential transfer of two EO compounds (geraniol and anethole) as a proof-of-concept; and Trial 2 (T2) studied the transfer of eight different EO (oregano, thyme, clove, cinnamon, lemon myrtle, lemon ironbark, peppermint gum and nerolina). In T1, six sows per treatment were fed a normal gestation or lactation diet supplemented with one morning dose consisting of 450 ppm of each EO (EO1.1), or the same total amount of each EO but administered in two meals with 225 ppm of each compound (EO1.2). Control group (C1) received non-supplemented feed. Amniotic fluid samples were collected by squeezing the placental tissue. Milk/colostrum was collected on 1 d and 5 d lactation hourly for 6 h immediately following the morning meal. In T2, eight EO were added in equal amounts to feed to a final dose 1 kg/ton (EO2) and compared to a non-supplemented control group (C2). Sows were induced to farrow in order to be able to collect fresh amniotic fluid samples. Colostrum was collected on d 1 lactation, 1 h after the morning meal. All samples were stored at  $-20^{\circ}$ C and analysed by GC-MS. The statistical analysis included *t*-test and ANOVA (Minitab 16, Minitab Inc., State College, PA, USA).

Results for the T1 showed a significant increase of geraniol and anethole in colostrum (P=0.02 and P=0.036 respectively). However, no significant differences (P > 0.5) could be measured in amniotic fluid. Results for T2 are shown in Table 1. All dietary EO were significantly transferred to amniotic fluid and colostrum, except for lemon myrtle. The results also showed significant differences (P=0.001) in the rate of transfer of the different EO, thyme being the most efficiently transferred to colostrum and clove the most efficient in amniotic fluid. Overall, there was a higher (P < 0.05) transference to amniotic fluid than colostrum for all the EO except peppermint gum, cinnamon and oregano.

In conclusion, our data proves that all dietary EO tested, except lemon myrtle, were transferred to maternal fluids in sows, but in a different rate and quantity. The results confirmed that dietary volatile compounds might be present in colostrum and amniotic fluid of sows and therefore foetuses and newborn piglets could potentially experience perinatal conditioning, hence improving weaning, welfare and performance.

		Amnio	otic fluid		Colostrum					
Essential oils	C (ug/L)	EO (ug/L)	P-value	Transfer (%)	C (ug/L)	EO (ug/L)	P-value	Transfer (%)		
Oregano	1.147	393	0.000	0.437	0.281	0.820	0.008	0.000912		
Thyme	3.210	258	0.000	0.422	0.184	5.080	0.000	0.0083		
Clove	3.540	1539	0.000	1.559	0.097	4.600	0.000	0.00466		
Cinnamon	0.335	1.232	0.001	0.00136	2.158	2.886	0.040	0.00238		
Lemon myrtle	0.661	1.454	0.066	0.00245	0.168	0.135	0.790	0.000227		
Lemon ironbark	0.257	1.426	0.013	0.00452	0.098	0.240	0.013	0.000761		
Peppermint gum	0.108	1.242	0.040	0.00193	0.142	2.270	0.018	0.00353		
Nerolina	7.020	135.060	0.005	0.233	0.425	1.710	0.035	0.00295		

Table 1. Trial 2 results on transfer of dietary EO to amniotic fluid and colostrum in sows comparing the control (C) and the EO treated (EO) groups

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# Effects of different amounts of wheat bran and oat hulls on production of short chain fatty acids in the hindgut of pigs

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Fermentation of soluble fibre in the large intestine of pigs favours beneficial microbiota, but can also reduce feed intake (FI) by stimulating the 'intestinal brake' (Black *et al.* 2009). Numerous studies (Black *et al.* 2009) have associated short chain fatty acids (SCFA) to ileal and colonic brakes in the gut, and increased transit-time and reduced FI. Measurement of end products of fermentation such as SCFA in faeces is a valid method to assess large intestinal fermentation activity (Bauer *et al.* 2004). The hypothesis tested was that fibre source alters the extent of hindgut fermentation.

Different amounts of an insoluble fibre, oat hulls, OH: 0, 2.5, 5, 10, 15 and 20% or a partially soluble fibre, wheat bran, WB: 0, 5, 10, 15, 25 and 35% were added to a highly digestible base diet containing maize starch and dextrose (67%) as the main energy source. Pigs were assigned to diets in a randomised block design with a minimum of five pigs on each diet. Pelleted diets were fed *ad libitum* to pigs housed individually with free access to water over 21 days and FI (Ratanpaul *et al.* 2017) was found to be higher with OH than with WB. At the end of d 7, 14 and 21 faeces from each pig were collected, stored in an air-tight container, and transferred to a freezer  $(-18^{\circ}C)$  within 1 h. Short chain fatty acids from faeces were extracted with water and quantitatively determined by gas chromatography. The data were analysed using a linear mixed modelling approach using ASReml version 3 (VSN International, Hemel Hempstead, UK).

On d 7, 14 and 21 (Fig. 1), pigs fed OH diets showed no difference in amounts of SCFA produced, whereas WB on d 7 at 25% (216.91 mmol/L) was found to have produced over 22% more (P = 0.031) SCFA than any other diet. Wheat bran at 35% (165.81 mmol/L) produced 23% less SCFA than WB at 25%. Since the intake of fibre diets at 25% and 35% WB was similar (Ratanpaul *et al.* 2017), the most likely reason for the lower amount of SCFA produced at 35% WB was reduced transit-time in the large intestine at such a high proportion of WB (Wilfart *et al.* 2007). Overall, with the exception of WB at 10%, the amount of SCFA produced increased with increasing amount of WB up to 25% and then declined at WB 35% for d 7, 4 and 21. On d 21, WB diets were collectively found to have produced over 20% more (P = 0.009) SCFA than OH based or control (0% WB) diets.

It has been reported elsewhere using *in vitro* fermentation studies that WB is more fermentable than a variety of carbohydrate substrates including OH (Bauer *et al.* 2004). WB is likely to favour growth of healthy microbiota in the hindgut with possible activation of the 'intestinal brake' and a decrease in FI. OH may not trigger the 'intestinal brake' because of its low fermentability, when fed with a highly digestible base diet. Oat hulls have low or no fermentability when compared to WB diet. Thus, this study confirms that fibre source alters the extent of hindgut fermentation.



Fig. 1. Total SCFA (mmol/L of faecal water) in faeces from (a) OH diets and (b) WB diets.

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# Effects of L-citrulline supplementation on lactation performance of sows in summer

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Facilitating improved heat dissipation in sows may improve lactation performance in summer by reducing the negative effects associated with heat stress. Vasodilation is one adaptive strategy pigs can use to dissipate heat when exposed to high temperatures. Nitric oxide is required for thermal-induced vasodilation in skin (Charkoudian 2003) and a previous study showed supplementing a nitric oxide donor, L-citrulline, reduced respiration rates in heat-stressed pigs (Kvidera *et al.* 2016), suggesting it has the potential to reduce heat stress. Therefore, we hypothesised that supplementing 1% L-citrulline in lactation diets may reduce heat stress and improve lactation performance of sows in summer.

A total of 221 mixed parity sows (Large White × Landrace, PrimeGro<sup>™</sup> Genetics, Corowa, NSW) were allocated to two dietary treatments with a similar parity distribution ( $2.4 \pm 1.76$  days, mean  $\pm$  s.d.). The wheat-based control lactation diet contained 14.9 MJ/kg digestible energy (DE) and 15% crude protein (CP). The L-citrulline diet was similar to the control diet but 1% wheat was replaced with 1% L-citrulline. The diet contained similar DE and 16% CP. The sows were fed either a control diet (n = 111) or L-citrulline diet (n = 110) at entry to the farrowing house ( $5.8 \pm 1.78$  days before farrowing) until weaning ( $26.4 \pm 1.53$  days lactation). Sows were restrict fed 2.5 to 4.0 kg from entry until d 3 after farrowing and then fed *ad libitum*. The experiment was conducted over summer at Corowa, NSW from 20 January to 6 March 2017. The average daily minimum temperature of  $15.6 \pm 4.56$ °C (mean  $\pm$  s.d.) and maximum temperature of  $32.6 \pm 5.49^{\circ}$ C were beyond the thermo-neutral zone for sows (12 to  $22^{\circ}$ C) (Black *et al.* 1993). A total of 64 sows were monitored for signs of heat stress by measuring respiration rate and rectal temperature every 3 h from 0800 h until 1700 h on d 19 post-farrowing (maximum shed temperature was over 30°C). Bodyweight and P2 backfat thickness of sows was measured at entry and weaning. Feed intake of sows was recorded daily. Litter size and weight were recorded after cross-fostering and at d 21 post-farrowing. Lactation performance and physiological data were analysed by univariate and repeated-measures procedure of General Linear Model (SPSS v24.0, IBM, Armonk, NY, USA) respectively for the effects of parity (gilt or sow), diet and their interaction. The main effects of dietary treatment are presented. Rectal temperature was similar between the treatments on the days when maximum shed temperature was over  $30^{\circ}$ C; however, respiration rates tended to be lower in those sows supplemented with L-citrulline compared to controls (P = 0.095) (Table 1). L-citrulline did not affect sow daily feed intake, bodyweight loss or backfat loss over lactation. Number of piglets born alive and post-foster was similar between the treatments. L-citrulline supplementation tended to increase (P = 0.094) litter size from 9.5 to 10.1 at 21 days post-farrowing but did not affect piglet average daily gain (ADG).

In conclusion, supplementation of 1% L-citrulline tended to reduce the sign of heat stress and improve lactation performance of sows in summer.

	Sow RT <sup>A</sup> (°C)	Sow RR <sup>B</sup> (breaths/min)	Sow feed intake (kg/d)	Sow weight change (kg)	Sow backfat change (mm)	Piglets born alive	Litter size, post-foster	Litter size, 21 d	Piglet ADG (g/d)
Control	39.2	50.7	6.4	-19.20	-0.40	12.0	11.9	9.5	208
L-citrulline	39.2	44.8	6.3	-18.10	-0.80	11.7	12.1	10.1	207
Standard error	0.06	2.55	0.10	1.75	0.57	0.52	0.17	0.24	6.8
P-values	0.93	0.095	0.32	0.65	0.73	0.47	0.51	0.094	0.92

Table 1. Physiology and lactation performance of sows fed a Control or L-citrulline diet in summer

<sup>A</sup>RT, rectal temperature on d 19 of lactation. <sup>B</sup>RR, respiration rate on d 19 of lactation.

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### The effect of heat stress on respiratory alkalosis, blood acid base balance and insulin sensitivity in cinnamon supplemented pigs

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With increases in the frequency, intensity and duration of heat waves forecast, heat stress (HS) is both a current and emerging problem for pig producers. As insulin improves peripheral blood flow and radiant heat loss (Allwood et al. 1959; Cottrell et al. 2015), we hypothesised that cinnamon (Cinnamonium zevlancium) would improve insulin sensitivity and ameliorate the effects of HS in pigs. To test this, 36 female Large White × Landrace (ca. 41.4 kg) pigs were allocated to either Cinnamon (0 v. 1.5% in a standard grower diet) and HS (thermoneutral (TN) v. HS) conditions in a  $2 \times 2$  factorial design (n = 9 treatments per group). Pigs were acclimatised to experimental diets for 14 days before being challenged with cyclic HS (35°C 9 am to 5 pm/28°C) or TN (20°C) for 7 days. Respiration rate (RR), rectal (RcT) and skin temperature (ST) were measured five times daily. On d 7 an intravenous glucose tolerance test (IVGTT) was performed and blood acid-base balance quantified. Data were analysed via an REML using GENSTAT v18 (VSN International, Hemel Hempstead, UK) with blocking on the experimental replicate.

Cinnamon did not ameliorate RR, RcT and ST or blood acid-base balance. Fasted glucose concentrations were lower in cinnamon supplemented pigs (6.55 v. 5.65 for Control v. Cinnamon, P = 0.050), but no interaction with HS was observed and no other influence of cinnamon on glucose and insulin kinetics were observed. Therefore the hypothesis that cinnamon would improve insulin sensitivity in HS pigs was not supported. The effects of HS on pig thermoregulation, blood biochemistry and insulin sensitivity were marked. Heat stress increased RR ~8-fold, from 22 to 172 breaths/min. The increase in respiration resulted in reduced blood CO<sub>2</sub> concentrations (pCO<sub>2</sub> 53.2 v. 47.3 mmHg for TN v. HS P < 0.001). As blood pH regulates the rate of biochemical reactions on a global basis it is very tightly regulated; however, HS pigs tended to have more alkaline blood than TN pigs (7.419 v. 7.432, P = 0.087). Blood HCO<sub>3</sub><sup>-</sup> concentrations were reduced in HS pigs (34.4 v. 31.6 mmol/L, P = 0.002), indicating increased buffering by excretion of excess HCO<sub>3</sub><sup>-</sup>. Compared to TN, urinary pH was lower in HS pigs (6.38 v. 5.41, P < 0.001), indicating that urinary bicarbonate excretion occurred before 7 days during HS. Heat stress reduced haematocrit (31.3 v. 26.1%, P < 0.001), indicating an expansion in plasma volume possibly due to reduced  $H_2CO_3$  formation from  $CO_2$  and  $H_2O$ . Heat stress reduced the maximal glucose (9.62 v. 8.18 mmol, P=0.014) and insulin concentrations (1.06 v. 0.67 ng/mL) following the intravenous (IV) glucose bolus. Glucose area under the curve (AUC)<sub>0-60</sub> was not influenced by HS; however, the insulin AUC<sub>0-60</sub> was significantly lower in HS pigs (0.242 v. 0.169 ng/mL.h, P = 0.005). Collectively these results show that HS pigs had a lower insulin response to an IVGTT. This may be in part due to reduced glucose concentrations after the IV bolus due to an expansion in blood volume or an increase in insulin sensitivity. In summary HS alters blood acid-base balance, leading to a loss of minerals such as bicarbonate and an apparent expansion in blood volume. It is expected that the energetic cost of panting, buffering and loss of nutrients such as bicarbonate contribute to compromised production efficiency in HS pigs.

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### Dietary phytate, calcium and phytase levels affect mineral utilisation in weaned pigs

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Dietary phytases degrade phytate and so prevent possible phosphorus (P) deficiency, reduce P excretion and maintain animal well-being (Guggenbuhl *et al.* 2016). The aim of this experiment was to evaluate the effects on P and calcium (Ca) utilisation in weaned pigs fed diets with different levels of phytate (phy), Ca and phytase (R) (*Citrobacter braakii*; Ronozyme HiPhos, DSM). The hypothesis tested was that R inclusion levels would modulate P and Ca utilisation in the presence of high levels of dietary phy and Ca. The experiment was conducted with 128 28-day-old castrated male weaned pigs (Large-White x Redon) having an initial bodyweight of 7.2  $\pm$  1.2 kg (mean  $\pm$  s.e.). Pigs were randomly allotted into eight treatment groups of 16 animals each (four pens of four piglets). They were fed *al libitum* for 42 days with mash diets based on corn, soybean meal and rapeseed meal. Eight diets were formulated to meet the animal requirements for weaned pigs according to NRC (2012) (crude protein (CP), 198 g/kg; metabolisable energy (ME), 13.0 MJ/kg; total P, 0.47%; total lysine, 1.40%). The experiment was conducted in a 2 × 2 × 2 factorial design with two dietary phy (0.18 and 0.31%), Ca (0.45 and 0.80%) and R (1000 and 2500 FTU/kg) concentrations. The coefficient of total tract apparent digestibility (CTTAD) of P and Ca, excretion of P and Ca, and femoral bone characteristics were evaluated at the end of the experiment. Data were analysed as a 2 × 2 × 2 factorial ANOVA and differences between groups were determined by the Student–Newman–Keuls multiple-range test (significant at *P* < 0.05) (StatGraphics Centurion XVII, Manugistics, Rockville, MD, USA).

The CTTAD of P was improved (P < 0.05) and P excretion reduced (P < 0.05) with the higher level of R (Table 1). For both P and Ca, the CTTAD was higher (P < 0.05) and the excretion lower (P < 0.05) with low dietary inclusions of phy or Ca. Bone strength was improved (P < 0.05) with increasing amount of R, whereas high dietary Ca reduced (P < 0.05) the bone breaking force. High dietary Ca significantly reduced the overall impact of R on P and Ca digestibility, increased their excretion, and reduced bone strength. High dietary phy induced the same effects except in bones where the breaking force was improved. The high Ca level was in excess and was largely excreted. Ca is an essential nutrient having a high ability to chelate phy and most nutrients released by R (Selle *et al.* 2009). The binding of Ca to phy reduced the R accessibility to phy, increased the response time and may partially inhibit the R activity.

Data from the present study showed that high dietary R could not compensate for the poor P and Ca utilisation observed in pigs fed a diet with high levels of Ca, irrespective of the dietary level of phy.

Phy (%)	Ca (%)	R (FTU/kg)	CTTAD P (%)	P excretion (g/kg DM)	CTTAD Ca (%)	Ca excretion (g/kg DM)	Bone ash (%)	Breaking force (N <sup>A</sup> )
0.18	0.45	1000	61.13 <sup>de</sup>	2.02 <sup>b</sup>	76.14 <sup>c</sup>	2.07 <sup>a</sup>	63.28	451.33 <sup>ab</sup>
		2500	66.68 <sup>e</sup>	1.71 <sup>a</sup>	76.13 <sup>c</sup>	$2.09^{\rm a}$	66.88	509.46 <sup>ab</sup>
	0.80	1000	48.45 <sup>ab</sup>	2.63 <sup>cd</sup>	61.15 <sup>ab</sup>	5.13 <sup>b</sup>	63.08	345.65 <sup>a</sup>
		2500	53.69 <sup>bcd</sup>	2.35 <sup>bc</sup>	62.99 <sup>ab</sup>	4.65 <sup>b</sup>	63.67	433.39 <sup>ab</sup>
0.31	0.45	1000	55.85 <sup>bcd</sup>	2.27 <sup>bc</sup>	73.55 <sup>c</sup>	2.39 <sup>a</sup>	61.68	486.03 <sup>ab</sup>
		2500	57.97 <sup>cd</sup>	2.16 <sup>b</sup>	67.33 <sup>b</sup>	2.84 <sup>a</sup>	63.60	615.42 <sup>b</sup>
	0.80	1000	$43.70^{a}$	2.91 <sup>d</sup>	$58.87^{a}$	5.18 <sup>b</sup>	62.55	392.01 <sup>a</sup>
		2500	50.73 <sup>bc</sup>	2.63 <sup>cd</sup>	60.82 <sup>ab</sup>	5.14 <sup>b</sup>	63.31	457.22 <sup>ab</sup>
s.e.m.			0.99	0.05	0.99	0.16	0.51	19.2
Treatment effect P-value			< 0.001	< 0.001	< 0.001	< 0.001	NS	0.021
Main effects P-value								
		Phy	< 0.001	< 0.001	0.008	0.009	NS	NS
		Ca	< 0.001	< 0.001	< 0.001	< 0.001	NS	0.003
		R	< 0.001	0.001	$NS^B$	NS	NS	0.020

Table 1. P and Ca CTTAD and faecal excretion, bone ash and bone breaking force in weaned pigs fed different levels of Ca, phy and R

<sup>A</sup>N, Newton. <sup>B</sup>NS, not significant. <sup>a-c</sup>Means in a column not having the same superscript are significantly different.

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# Potential of blood biomarkers to estimate optimum amino acid requirements for pig growth

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Current requirements of the branched chain amino acids (BCAA) isoleucine (Ile), leucine (Leu), and valine (Val) have been estimated empirically in dose–response experiments using pig growth as the response criteria. The discriminating metabolites to the optimum dietary BCAA levels may be an alternative to animal growth as the response criteria, and could use fewer animals in short-term studies. Previous dose–response studies in our laboratory demonstrated that  $0.52 \pm 0.1$  standardised ileal digestible (SID) Ile:lysine (Lys),  $0.70 \pm 0.07$  SID Val:Lys, and  $0.93 \pm 0.1$  SID Leu:Lys are the minimum BCAA requirements to support the best growth performance of weaned piglets (Soumeh *et al.* 2014, 2015*a*, 2015*b*). The objectives of the current study were to first identify biomarkers of BCAA intake status that are linked to animal growth and second to develop a method to study BCAA requirements in pigs based on blood metabolites in a short-term trial.

Three dose–response experiments were conducted to study growth performance of pigs (10 to 20k g, n = 96 per study) that were fed with increasing levels of SID IIe:Lys (0.42, 0.46, 0.50, 0.54, 0.58, and 0.62); SID Val:Lys (0.58, 0.62, 0.66, 0.70, 0.74, and 0.78); and SID Leu:Lys (0.70, 0.80, 0.90, 1.00, 1.10, and 1.20). At d 8 and 15 of each experiment, after an overnight fast, pigs were supplied with 25 g/kg BW<sup>0.75</sup> of feed and blood samples were collected 3 h later from eight pigs per treatment. Blood samples were analysed by a HPLC–MS in a non-targeted metabolomics approach to determine the metabolic profile of pigs fed increasing dietary levels of BCAA:Lys. Principle component analyses (PCA) and partial least-squares regression (PLS) were used to identify discriminating metabolites. The identified biomarkers were used as response criteria in the next trial using the diets of the previous studies (stored at  $-20^{\circ}$ C) in a 6×6 Latin square design (six BCAA levels×six pigs per BCAA level). The experimental diets were fed for 2 days and then the next diet was fed for a total of 12 days. Blood samples were taken after 2 days and analysed for identified biomarkers. Performance data was analysed using the MIXED procedure of SAS (v9.3, SAS Institute Inc., Cary, NC, USA) and metabolic profiling and biomarker identification analysed using multivariate analysis (LatentiX v2.12, LatentiX Aps, Frederiksberg, Denmark). Of the several identified discriminating metabolites in each study, few showed a significant response to increasing dietary levels of IIe, Leu, and Val in the 2-day trials. Fitting different statistical models to these metabolites (Table 1), however, allowed estimation of a minimum requirement for each BCAA that were close to the values determined using traditional growth performance criteria (Nørgaard *et al.* 2017).

The results indicate that blood biomarkers have potential as response criteria in short-term dose-response studies to estimate BCAA requirements in pigs.

		Fitting model			Previous dose-response	studies
	$BL \pm s.e.$	$CLP \pm s.e.$	$Q, R^2$	Mean/n metab. <sup>A</sup>	Reference	Value
Ile:Lys	$0.53 \pm 0.09$	$0.54\pm0.18$	0.54, 0.59	0.53	Soumeh et al. (2014)	0.52
Leu:Lys	$0.96 \pm 0.14$	$1.06 \pm 0.24$	1.10, 0.89	1.04	Soumeh et al. (2015a)	0.93
Val:Lys	$0.66\pm0.14$	$0.68\pm0.22$	0.69	0.68	Soumeh et al. (2015b)	0.70

Table 1. Optimum SID IIe:Lys, Leu:Lys and Val:Lys values for pig growth estimated by broken-line (BL), curvilinear-plateau (CLP) and quadratic (Q) models fitted to blood metabolites

<sup>A</sup>Mean of the three models/number of unique amino acids and other metabolites used for modelling plus/minus standard error or regression coefficient. Mean number of metabolites were 6, 16, and 2 for Ile, Leu, and Val studies, respectively.

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### Factors influencing the measure of creatinine in nonreproductive pigs

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Creatinine (Crea) is generated as a metabolic waste product of muscle metabolism and movement, it is released into plasma and transported to the kidneys where it is filtered and passes into urine. There is little published on the possibility of using Crea as a marker of muscle catabolism in pigs, initial studies (Muller *et al.* 2015) aimed to determine whether a handheld, portable meter (Nova Biomedical<sup>TM</sup> StatSensor<sup>TM</sup> Creatinine and GFR Meter, RHCG, Rosebery, NSW, Australia) could provide an instant measure of Crea that may reflect sow catabolism, rather found Crea to correlate with feed offered across gestation and lactation at two breeder sites. As with all metabolites, daily fluctuations can be caused by many external variates, in the case of Crea, feeding has been suggested as one possible cause of variation. The aim of this study was to investigate the relationship between Crea and feed intake in non-reproductive pigs, which may be useful in group housing systems to identify low intake pigs.

Creatinine levels (whole blood) were assessed in 18-week-old finisher pigs and its correlation to feeding. A total of 64 male finisher pigs were randomly allocated and housed in four pens of 16 pigs with each pen fitted with electronic Feed Intake Recording Equipment (FIRE) which recorded information on individual feed events including individual feed intake and entry and exit time. Individual Crea measurements, a 30 s test using a drop of blood collected from the ear vein and placed directly onto the testing strip of the handheld meter, were taken for three consecutive days at 1300 h, on two separate occasions, 1 week apart. Ambient temperature, at the time of testing, was also recorded. Data were analysed using the Univariate GLM and correlation procedures (GENSTAT 18, VSN International, Hemel Hempstead, UK).

There were a high number of correlations between the measured variables; however, most significant correlations (P < 0.001) show a weak relationship (Table 1). Crea levels measured were moderately positively correlated with temperature (r = 0.49), which suggests levels of measured Crea may be influenced by the pigs ambient temperature. These results support the findings from prior studies and illustrate the need to consider external stimuli when measuring daily concentrations of Crea using the Nova StatSensor Creatinine Meter.

	Crea	Crea Test Time	Prior Intake	Exit Time	Time Between	Total Exits	Total Intake	Temp
Creatinine		< 0.001	0.006	< 0.001	0.168	0.750	< 0.001	< 0.001
Test Time	0.22		0.402	< 0.001	0.518	< 0.001	< 0.001	0.585
Prior Intake	0.16	0.05		0.541	0.081	< 0.001	< 0.001	0.006
Exit Time	0.19	0.62	-0.04		< 0.001	< 0.001	< 0.001	0.039
Time Between	-0.08	-0.04	0.10	-0.77		< 0.001	< 0.001	0.003
Total Exits	0.02	0.29	-0.21	0.48	-0.39		< 0.001	0.011
Total Intake	0.21	0.37	0.43	0.50	-0.35	0.32		0.001
Temp	0.49	-0.03	0.16	0.12	-0.18	-0.14	0.19	

#### Table 1. Relationships and correlations between creatinine and feed intake in pigs

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# Effects of dietary Ca and digestible P concentrations and addition of phytase on growth performance of nursery pigs

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Appropriate dietary calcium (Ca) and phosphorous (P) concentrations are essential for nursery pig performance. The total Ca and digestible P requirements estimated by NRC (2012) are 0.83 and 0.45%, respectively, for 6 kg pigs. Research has shown that feeding excess dietary Ca impairs P digestibility, therefore reducing growth performance and bone development in nursery pigs (González-Vega *et al.* 2016). The objective of this study was to evaluate growth performance and bone ash concentration of nursery pigs in response to combinations of dietary Ca and P levels provided by inorganic sources or phytase (1000 FTU of Ronozyme HiPhos 2500; DSM Nutritional Products Inc., Parsippany, NJ, USA).

A total of 720 pigs (PIC  $1050 \times 280$ , initially  $6.1 \pm 0.23$  kg) were used in a 42-day growth study. Pens of pigs (10 pigs/pen, 12 pens/treatment) were blocked by initial pen weight, and within blocks, pens were allotted randomly to one of six treatments. Dietary treatments were arranged in a 2 × 3 factorial with two levels of Ca (0.58 v. 1.03%) and three standardised total tract digestible (STTD) P treatments (0.33 and 0.45% without phytase, and 0.45% with 0.12% of the P being released by phytase). Diets were provided in three phases with pigs fed experimental diets in Phase 1 (d 0 to 14) and Phase 2 (d 14 to 28), followed by a common Phase 3 diet from d 28 to 42. Average daily gain (ADG), feed intake (ADFI), and feed efficiency (G : F) were determined every 7 days. Data were analysed using the Proc GLIMMIX of SAS (v9.4, SAS Institute Inc., Cary, NC, USA).

For the majority of the feeding periods, Ca × P interactions were observed for growth responses (P < 0.05). From d 0 to 28 (Table 1), when diets contained a low Ca concentration, pigs fed 0.45% STTD P with phytase had greater (P < 0.01) ADG and ADFI compared with those fed 0.45% STTD P without phytase, and pigs fed 0.33% STTD P. When high Ca was fed, ADG and ADFI were similar among pigs fed 0.45% STTD P with or without phytase, but were greater than those fed 0.33% STTD P. Feed efficiency was reduced (P < 0.01) when low STTD P and high Ca were added to diet, compared with other dietary treatments. During Phase 3, pigs previously fed 0.33% STTD P had similar ADG, but decreased (P < 0.05) ADFI and improved G : F compared with pigs previously fed 0.45% STTD P with or without phytase. However, pigs fed 0.33% STTD P with high Ca were not able to fully compensate the negative effects of P deficiency resulting in decreased (P < 0.05) overall ADG and ADFI compared with pigs fed 0.45% STTD P diet with or without phytase. On d 21, one median-weight gilt from each pen was killed and fibulas were collected for analysis of bone ash content. Pigs fed 0.33% STTD P had decreased (P < 0.05) bone ash concentration compared with those fed 0.45% STTD P with or without phytase when high Ca was added to diets, but this P effect was not observed when diets contained low Ca (Ca × P interaction, P = 0.007).

In conclusion, excess Ca in diets decreased nursery pig performance and bone ash content only when diets were deficient in STTD P. Adding phytase to achieve 0.45% STTD P improved ADG and ADFI of pigs compared with diets containing 0.45% STTD P without phytase, indicating a potential underestimation of the P release from phytase or an increased availability of other nutrients liberated by phytase.

			Treat	ment				Pr	obability, P	<
Ca (%)	0.58	0.58	0.58	1.03	1.03	1.03	s.e.m. <sup>A</sup>	$Ca \times P$	Ca	Р
STTD P, no Phytase (%)	0.33	0.45	0.33	0.33	0.45	0.33				
STTD P, with Phytase (%)	-	-	0.45	-	-	0.45				
ADG (g)	365°	365°	411 <sup>a</sup>	312 <sup>d</sup>	379 <sup>bc</sup>	398 <sup>ab</sup>	0.7	< 0.001	0.002	0.001
ADFI (g)	493°	501 <sup>bc</sup>	554 <sup>a</sup>	485 <sup>c</sup>	528 <sup>ab</sup>	556 <sup>a</sup>	0.7	0.042	0.217	0.001
G/F (g/kg)	740 <sup>a</sup>	729 <sup>ab</sup>	742 <sup>a</sup>	642 <sup>c</sup>	718 <sup>ab</sup>	715 <sup>b</sup>	0.6	< 0.001	0.001	0.001
Bone ash (%)	44.1 <sup>bc</sup>	45.6 <sup>ab</sup>	45.8 <sup>ab</sup>	42.6 <sup>c</sup>	$48.0^{\mathrm{a}}$	45.5 <sup>ab</sup>	0.61	0.007	0.692	0.001

Table 1.	Effects of Ca and P	concentrations on	growth performance	e of nursery	pigs from	d 0 to 28
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<sup>A</sup>s.e.m., standard error of the mean. <sup>a-d</sup>Means with different superscripts within a row differ (P < 0.05).

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# The expression of bitter taste receptors (T2Rs) in the porcine gastrointestinal tract epithelium and smooth muscle

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The expression of bitter taste receptors (T2Rs) was originally discovered in taste sensory cells of the tongue's taste buds. However, in recent years, it has been found that T2Rs are expressed beyond the oral cavity in mammals including pigs where they seem to play a fundamental role orchestrating the hunger-satiety cycle (Roura *et al.* 2016). However, little is known about their specific expression profile across tissues. This project aimed to investigate the expression of a subset of porcine T2Rs throughout the epithelium of the gastrointestinal tract (GIT), to quantify the abundance and localisation of the genes of interest expressed, in particular in the stomach, duodenum and oesophagus.

Six Large-White male piglets with  $10.2 \pm 0.53$  kg bodyweight were used to perform the intestinal gene expression analysis. Five porcine bitter taste receptors T2R1, T2R4, T2R7, T2R10, T2R20 and T2R39 were selected based on their high gene sequence homology with the human orthologues and their affinity to several compounds (i.e. caffeine, quinine, amarogentin and saccharin) known to be bitter to humans and pigs. Epithelium layers and smooth muscle layers in the stomach, duodenum and oesophagus were separated and analysed for the expression of the bitter taste receptor genes (Tas2rs). Total RNA was extracted from the tissue samples using the TRIZOL-chloroform method. RNA purification, cDNA synthesis and real-time qPCR assays using SYBR green were performed. The relative gene expression levels were estimated using the Pfaffl method (Pfaffl 2001) taking into account the cycle threshold values of both the candidate genes and of the two reference genes ACTB<sub>3</sub> and GAPDH. GraphPad Prism 7 software (GraphPad Software Inc., La Jolla, CA, USA) was used to analyse the gene expression data using a one-way ANOVA and Fischer's least significant difference (l.s.d.) analysis for multiple comparisons.

The porcine Tas2r20 gene had a higher expression in the oesophagus than in the duodenum and stomach (P < 0.01; Fig. 1a). Tas2r39 resulted in higher expression rates in the stomach compared to the oesophagus and duodenum (P < 0.01; Fig. 1a). Porcine Tas2r7 and Tas2r39 genes both had a greater relative expression in the epithelium than the smooth muscle layer (P < 0.01; Fig. 1b). Porcine Tas2r10 and Tas2r20 genes both had a greater relative expression in the epithelium than the smooth muscle layer (P < 0.05; Fig. 1b).

The results confirmed the expression of porcine Tas2r genes beyond the oral cavity into the gut with the level of expression being gene and tissue specific. In addition, our data shows that the expression of the genes of interest occurs preferentially in the epithelial cell layer of the GIT. These results are relevant in the context of understanding the functionality, including tissue specificity, of food-borne compounds relevant to appetite enhancing or inhibition.



**Fig. 1.** Expression of porcine Tas2r1, 4, 7, 10, 20 and 39 in the gastrointestinal tract of pigs (mean  $\pm$  s.e.m., n = 6) (*a*) Normalised expression of bitter genes of interest in the pig duodenum, oesophagus and stomach. (*b*) Normalised expression of the bitter genes of interest in the epithelium and smooth muscle layers across the three aforementioned tissues. \*, P < 0.05; \*\*, P < 0.01.

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## Importance of connectivity grains for AusScan NIR prediction accuracy

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The AusScan near-infrared (NIR) calibrations for predicting available energy content of cereal grains for pigs and broiler chickens, are based on results from many experiments that commenced in the mid-1990s (Black et al. 2014). Robust NIR calibrations require information from hundreds of measurements. Limitations in infrastructure capacity, concurrent availability of grains varying widely in characteristics that affect energy availability, and research funds meant that many small experiments were conducted and the results aggregated to develop the NIR calibrations. Results from the first three years of the Premium Grains for Livestock Program could not be used for development of NIR calibrations because only one grain ( $\sim$ 3% of grains in an experiment) was constant across experiments and this was insufficient to satisfactorily adjust for differences between experiments. Consequently, ~30% of grains (known as connectivity grains) used in each experiment have been included in previous experiments to account for variations in environmental conditions across experiments. Inclusion of connectivity grains reduces the number of new grains included in each experiment and increases cost. The impact of including connectivity grains on variance of available energy values and therefore accuracy of NIR calibrations was assessed. The value of connectivity grains was assessed for pig faecal digestible energy (DE), when grains were fed without enzymes, and for broiler apparent metabolisable energy (AME) for combined gender, males and females, when grains were fed with and without enzymes. For each assessment, the unadjusted (raw) measured values with standard errors (SE) were compared with statistically adjusted values using connectivity grains (Table 1). Inclusion of connectivity grains reduced SE of the estimated energy content of grains across all comparisons by 48%, with the decrease in SE ranging from 0.079 MJ/kg as fed (25%) for male broilers with enzymes to 0.231 MJ/kg as fed (82%) for combined gender broilers without enzymes.

The impact of reducing SE of measurement on the accuracy of NIR calibrations can be estimated because the accuracy is approximately twice the mean SE of the values used to develop the calibration. Thus, including connectivity grains improved the accuracy of NIR predictions from  $\pm$  0.16 (0.079\*2) MJ/kg as fed for male birds fed diets with enzymes to  $\pm$  0.46 MJ/kg as fed for combined gender broilers fed diets without enzymes. The corresponding value for pig faecal DE was  $\pm$  0.22 MJ/kg as fed. An increase in error of prediction from the NIR calibrations of these magnitudes, if connectivity grains were not used, would substantially reduce the practical value of NIR calibrations for use by the pig and broiler industries. These analyses indicate that inclusion of connectivity grains should be continued for future experiments.

Variable	Statisticall	y corrected	Raw m	easured	Ratio SE	SE difference
	Mean	SE	Mean	SE	Raw/Corrected	Raw-Corrected
Pig DE	13.64	0.151	13.53	0.259	1.72	0.108
Broiler AME – without enzymes						
Combined	12.91	0.282	12.76	0.513	1.82	0.231
Male	12.75	0.3	12.62	0.478	1.59	0.178
Female	13.2	0.296	13.02	0.396	1.34	0.100
Broiler AME – with enzymes						
Combined	13.58	0.289	13.41	0.432	1.49	0.143
Male	13.43	0.313	13.29	0.392	1.25	0.079
Female	13.73	0.299	13.54	0.388	1.30	0.089
Total Means	13.32	0.276	13.17	0.408	1.48	0.133

Table 1.	Effect of statistically correcting across experiments for connectivity grains on mean grain energy content (MJ/kg as fed) and SE for all
	grains for which NIR calibrations are available

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## A double-choice model to quantify negative preference to bitterness in pigs

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Bitter compounds have shown potential to decrease feed intake and fat deposition in pigs (Fu *et al.* 2015). However, identifying and quantifying bitterness in pigs is complex, since the standard double-choice (DC) models are based on motivation to consume. It works well when measuring positive preferences, but does not reliably identify negative preferences. This project aimed at developing a new DC model to measure negative preferences in pigs. Our hypothesis was that bitterness would counteract the positive preference for sweetness. Post-weaning piglets (Large White, 6.5 to 7.5 kg, half male and half female) were given a 2 min choice between a sweet solution (50 mM sugar) or and the same sweet solution added with one of 13 compounds (five pure chemicals and eight plant extracts) known to be bitter to humans at two doses (1 mM or 0.1 mM). The piglets were grouped in same sex pairs with similar bodyweight and randomly assigned to 24 pens with unrestricted access to a standard commercial feed and water. The DC tests ran twice daily (Sessions 1 and 2) per pen for 14 days (three blocks of 4 days and two additional days used to repeat missed results). This resulted in 18 replicates per treatment following an incomplete randomised block design where every treatment had six replicates per block (three in Session 1 and three in Session 2). Spare pens in every session were used to test a control treatment (sugar *v*. sugar). The amounts of each solution consumed were recorded and used to calculate preference rates. Data were analysed by using Duncan's statistical analysis with ANOVA (SPSS v22.0, IBM, Armonk, NY, USA) in the General Linear Model.

The preference results are shown in Fig. 1. At the high dose, the addition of all compounds tested resulted in significant (P < 0.05 or P < 0.01) rejection except for orange peel, quinine, caffeine and amarogentin. In contrast, only quinine was significantly rejected in the lower dose. The results relevant to the extracts need to be interpreted with caution since non-bitter sensory factors may apply. We concluded that our model was successful in identifying negative preferences for bitter compounds and may become the foundation of future research on the functionality of bitter tastants in pigs.



**Fig. 1.** Mean ( $\pm$  s.e.) preference rate (as % of the total double choice solution intake) between a 50 mM sugar solution and the same solution added with one of thirteen bitter compounds or plant extracts. <sup>A</sup>Treatments with the superindex are plant extracts. \* or \*\* indicates that the preference values are significantly different from the neutral value of 50% at P < 0.05 (\*) or P < 0.01 (\*\*). <sup>a-c</sup>Bars with all the letters different indicate significantly different values (P < 0.05).

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## Preference thresholds for four limiting essential amino acids in piglets

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Promoting feed intake at weaning is important to prevent growth check. Thus, highly palatable and easily digestible ingredients including appetite enhancers are commonly used in piglet diets. For example, glutamate is known to stimulate taste and appetite in pigs. In addition, the long-term appetite for limiting essential amino acids has been well documented, particularly when piglets are fed deficient diets. However, little is known about the potential sensing of these amino acids Lysine (Lys), Methionine (Met), Tryptophan (Trp) and Threonine (Thr) by the peripheral system (i.e. taste and smell) and if this was related to preferences. It was hypothesised that pigs have a high preference for essential limiting amino acids unrelated to dietary deficiencies or other potential metabolic imbalances. The current experiment examined preference thresholds (defined as the lowest dose with a preference significantly higher than 50%) for Lys, Met, Trp and Thr solutions in pigs using a 2 min double-choice (DC) model consisting of two stainless steel bowls containing either water or the amino acid solution under evaluation following the method described previously by Roura *et al.* (2011).

Ninety-six piglets were selected and housed in 48 pairs of males or females in two environmentally controlled rooms. Pigs were trained on a DC procedure. The test solutions were either sugar (at 200 mM), a positive control, or: Lys (at 1.0, 2.5, 5.0, 7.5 and 10 mM), Met (at 0.25, 0.5 0.75, and 1 mM), Trp (at 1, 10, 15, 20, 25, 30 mM) and Thr (at 1, 10, 15, 20, 25, 30 mM). The dose range selected was based on unpublished preliminary data. Test solution preference, measured as a percentage ratio of test solution consumed over total consumed (test + water), was compared to the neutral no-preference value of 50%. Preference trends were analysed using a linear mixed model incorporating cubic smoothing splines. The package ASReml v3 for the statistical computing software R (VSN International, Hemel Hempstead, UK) was used to fit the models.

Preference results are shown in Fig. 1. Pigs had a significant preference of 74% for the sugar solution and an average intake of 230 g and is represented as a dotted line in the graphs. The highest significant preference values for Lys, Met and Trp were 56%, 60% and 62% at 10, 1 and 20 mM, respectively. Preference thresholds (P < 0.05) were set at 5, 0.25 and 10 nm, respectively. No significant preference was observed for Thr. In conclusion, the results showed that piglets have an accurate oral perception of Lys, Met and Trp but not Thr as shown by preference values compared to water. Also, the preference threshold differed among the amino acids tested, with Met having the lowest value.



Fig. 1. Dose-dependent preference values for Lysine, Methionine, Tryptophan and Threonine solutions in pigs. Preference is shown as a fraction of 1 (equivalent to 100%). Shaded regions in the figures represent approximate 95% confidence bands (P < 0.05). The dashed lines depict the preference value for the positive control (200 mM sugar solution).

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## Effects of standardised total tract digestible phosphorus on performance, carcass characteristics, and economics of 24 to 130 kg pigs

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The 2012 National Research Council (NRC) adopted the concept of standardised total tract digestibility (STTD), which was based on a factorial approach to report the phosphorus (P) requirements of pigs. There is a need for more data to validate the model-derived digestible P requirement since there was only one empirical estimate for pigs greater than 65 kg bodyweight (BW) included in these recommendations. The objective of this study was to determine the effects of STTD P on growth performance, bone mineralisation, carcass characteristics, and economics of 24 to 130 kg pigs housed under commercial conditions. A total of 1130 barrows and gilts (PIC;  $359 \times$  Camborough, initially 24.1  $\pm$  0.73 kg BW) were used in a 111 day growth trial. Pens of pigs were randomly assigned to one of six dietary treatments in a randomised complete block design. Treatments were formulated to contain 80, 90, 100, 115, 130, and 150% of the NRC (2012) STTD P requirement for growing-finishing pigs within each phase. There were seven replicate pens per treatment and 26 to 27 pigs per pen (at least 13 barrows and gilts per pen). The experimental diets were corn-soybean-meal-based and fed in four phases. Treatments were achieved by increasing the inclusion of limestone and monocalcium phosphate at the expense of corn. A similar 1.14:1 to 1.17: 1 total Ca: Pratio was maintained, with no phytase added to the diets. Data were analysed using generalised linear and non-linear mixed models, and polynomial contrasts were implemented with pen as the experimental unit. Competing models, including a linear model, quadratic polynomial (QP), broken-line linear, and broken-line quadratic were fit using GLIMMIX and NLMIXED procedure of SAS (v9.4, SAS Institute Inc., Cary, NC, USA) according to Gonçalves et al. (2016). For the overall period, increasing STTD P increased average daily gain (ADG), feed efficiency (G:F), final BW, and hot carcass weight (quadratic, P < 0.05). Average daily feed intake, grams of STTD P intake per day, ashed bone weight and bone percentage ash increased linearly as the inclusion of STTD P increased in the diets (P < 0.05). Carcass yield decreased with increasing STTD P (linear, P < 0.05), while there was a decrease in backfat and increase in fat-free lean (P < 0.10). No evidence for differences were observed for loin depth measurements (P > 0.10). Feed cost per pig increased linearly (P < 0.05) with increasing STTD P levels while gain value per pig increased quadratically (P < 0.05). Similarly, income over feed cost increased in a quadratic manner (P < 0.05). For ADG and G : F, the OP model demonstrated best fit (Fig. 1). For ADG, the maximum response was estimated with STTD P at 122% of current NRC estimates, with 99% of maximum ADG achieved at 102% STTD P. For G: F, the maximum response was estimated with STTD P at 116% of current NRC estimates, with 99% of maximum ADG achieved at 82% STTD P.

In conclusion, the estimated STTD P requirement for pigs from 24–130 kg to maximise growth performance ranged from 116% to 122% of the NRC (2012) recommendations for each phase, depending on the response criteria and statistical model.



Fig. 1. Fitted QP regression model for ADG and G: F as a function of increasing STTD P in 24–130 kg pigs.

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## Saccharomyces cerevisiae boulardii improves performance of pigs fed low and high energy diets in summer

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Reduced feed intake over summer, and the consequential slow growth of finisher pigs is an important issue for the Australian pork industry. Diets with higher starch and lower fibre (e.g. high wheat and low barley or millrun, to increase energy density) have been shown to reduce the digestive heat increment experienced by pigs (Noblet *et al.* 1985). However, raw material costs can make this much more expensive than feeding diets containing high levels of barley or millrun. Levucell SB (LSB) (*Saccharomyces cerevisiae boulardii*, CNCM I-1079) is a live yeast used to maintain healthy and balanced gut micro-flora in pigs (Collier *et al.* 2011). Levucell SB has been found to increase growth and reduce heat stress in cattle and pigs. It is hypothesised that LSB will increase growth performance of finisher pigs over summer in low digestible energy (DE) diets more so than high DE diets.

Eight hundred and forty Improvac vaccinated male pigs (PrimeGro<sup>TM</sup> Genetics, 57.8 kg  $\pm$  0.66 kg) were randomly allocated to four dietary treatments in a 2×2 factorial design (high and low DE level and LSB addition at 0 or 10<sup>9</sup> CFU/kg), with Pen as the experimental unit. Pigs were housed in commercial pens (14 pigs/pen and 15 pens/treatment) with feed and water available *ad libitum*. The experiment commenced in February 2016 and over the 42 day experimental period there were 25 days with maximum pen temperatures exceeding 30°C and 12 days over 35°C. Diets contained 14.0 or 12.8 MJ DE/kg and 0.62 g standardised ileal digestible lysine/MJ DE. Pen weights and feed use was measured at 0, 21 and 42 d. Carcass fat depth (P2) was measured after slaughter. Statistical analysis was conducted using ANOVA (SPSS v21.0, IBM, Armonk, NY, USA).

As expected, pigs fed diets with higher DE grew faster and more efficiently and had fatter carcasses than those fed a low DE diet (Table 1). The addition of LSB at 10<sup>9</sup> CFU/kg increased feed intake of pigs fed low-energy, but not high-energy, diets. Pigs fed LSB also had improved feed efficiency, particularly in higher DE diets. There was an interaction between DE and LSB for carcass fat at the P2 site. Pigs fed high DE diets with LSB were leaner than those without LSB, whereas in low DE diets LSB addition produced a fatter carcass. The results support the hypothesis that in hot weather, LSB increased feed intake in pigs fed a low energy diet, most likely due to reduced heat increment during digestion. However, in high DE (high starch) diets feed efficiency is improved. The use of the live yeast Levucell SB is a useful tool for maintaining pig growth performance over hot weather.

	ADG <sup>A</sup> (kg/d)	FCR <sup>B</sup>	ADI <sup>C</sup> (kg/d)	Carcass P2 <sup>D</sup> (mm)
14.0 MJ DE/kg	1.039	2.44	2.538 <sup>ab</sup>	12.2 <sup>d</sup>
14.0  MJ/kg + LSB	1.049	2.37	$2.484^{a}$	11.9 <sup>c</sup>
12.8 MJ DE/kg	0.982	2.59	2.539 <sup>b</sup>	10.3 <sup>a</sup>
12.8 MJ DE + LSB	1.016	2.57	2.609 <sup>c</sup>	11.0 <sup>b</sup>
SEM	0.01	0.02	0.02	0.14
Significance DE $(P =)$	0.001	0.001	0.060	0.001
Significance LSB $(P =)$	0.086	0.022	0.001	NS
Significance DE x LSB $(P =)$	NS	NS	0.033	0.017

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<sup>A</sup>ADG, average daily gain. <sup>B</sup>FCR, feed conversion ratio. <sup>C</sup>ADI, average daily intake. <sup>D</sup>P2, fat depth. NS, not significant. <sup>a-d</sup>Means in a row not having the same superscript are significantly different (*P* < 0.05).

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### Cooling innovations for loose farrowing pens in summer

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The summer environment is a high risk time in loose farrowing systems as sows and piglets share cooler areas of the pen, resulting in piglets being at risk of being overlain (Morrison and Baxter 2014). The aim of this experiment was to assess piglet and sow lying behaviour and growth and survival of piglets in a SWAP (Sow Welfare and Piglet protection) loose farrowing system that included cooling innovations. The hypothesis was that the piglets would spend more time in the creep area in the cooled treatments resulting in improved piglet survival and growth.

The experiment was conducted over four time replicates utilising 190 mixed parity sows (Large White × Landrace PrimeGro<sup>TM</sup> Genetics, Corowa, NSW, Australia) in open-sided, naturally ventilated sheds. A 2 × 2 factorial design was used with the main factors being: (1) cooling (standard pen v. cooled tiles in creep and fan in nest; a 30cm fan was attached to the creep and airflow directed over the nest area and three 'cool' tiles covering the whole creep area (MIK International, Germany), and (2) floor type (solid v. slatted nest). The treatments were: (1) standard pen/solid nest; (2) standard pen/slatted nest; (3) cooled pen/solid nest; and (4) cooled pen/slatted nest. All floors were plastic. The cooled treatments were activated 4 days post-birth when the ambient temperature was greater than  $25^{\circ}$ C and remained on throughout lactation. The surface temperature of the cooled tiles was ~2°C cooler than the surrounding area. The total number of piglets born, number of piglets born alive and number of piglet deaths were recorded for each litter. Piglet mortality was calculated for each litter and live weights of litters were recorded at birth and weaning (~24 days of age). The location of the sow and piglets was recorded by direct observation scan sampling on a daily basis (1300 h) over lactation. The internal shed temperature was recorded from *in situ* temperature loggers located on the wall of the shed immediately before the behaviour observations. The scan data were converted into percentage of the sows and litter in each location. Sows were assigned to either the nest or dunging passage area. Univariate GLM analysis (SPSS v21.0, IBM, Armonk, NY, USA) was undertaken using each sow/litter as the experimental unit with the sow as the blocking factor. Differences in piglet location at different temperature ranges were analysed (range from less than or equal to 23.8°C to greater than 36°C) and the results are shown in Table 1.

There were no significant (P > 0.05) interactions between treatments or differences in location preference of the sows. There was no significant difference (P > 0.05) in the location preference of piglets in the control and cooled treatment up to 36°C. Above this temperature, there was a greater proportion of piglets in the cooled creep area; however, this did not improve piglet survival and growth as there was a trend (P < 0.1) for higher piglet mortality and reduced weaning weight in the cooled treatment. There was no significant (P > 0.05) effect of floor type on sow or piglet location preference. Therefore, based on these results, our hypotheses was not proven and further research is warranted to assess alternative cooling strategies in loose systems in open-sided, naturally ventilated sheds.

	Cooling		Floo	r type	s.e.m.	P-value	P-value
	Control	Cooled	Solid	Slatted		Cooling	Floor type
Live born mortality (%) <sup>A</sup>	20.6	25.8	23.6	22.8	1.31	0.094	0.776
Av. number piglets weaned <sup>A</sup>	9.5	9.2	9.1	9.6	0.14	0.330	0.135
Av. piglet rate of gain (g/day) <sup>B</sup>	233.2	223.7	226.8	230.0	2.91	0.152	0.624
Av. piglet weaning weight (kg) <sup>B</sup>	7.9	7.5	7.6	7.7	0.09	0.081	0.678

Table 1.	Piglet survival,	number of piglets	weaned and growth	performance

<sup>A</sup>Number of piglets born alive used as covariate in analysis. Liveborn mortality figures are calculated for each litter (taking into account fostering adjustment). <sup>B</sup>Piglet birthweight used as covariate in analysis.

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## Dietary cellulose could reduce cytokine responses without compromising growth performance in weaner pigs under a farm-like circumstance

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Insoluble non-starch polysaccharide (iNSP) could reduce growth performance of host animals due to decreased access of endogenous enzymes, and a subsequent increased flow rate of dietary chime; however, indigestible particles increase health-promoting bacteria along with suppressed protein fermentation in the large intestine of weaner pigs (Heo *et al.* 2013). The present study tested the hypothesis that dietary iNSP would reduce cytokine responses without compromising growth performance in weaner pigs.

A total of 108 male pigs (Duroc × (Yorkshire × Landrace)) weaned at 21 days of age with initial bodyweight (BW) of  $6.2 \pm 0.4$  kg (mean  $\pm$  s.e.m.) were randomly allocated to one of three dietary treatments (0, 1, 2% cellulose; Accent Microcell Pvt. Ltd, India). The BW was measured individually on d 0, 7, and 14. Feed consumption was recorded weekly on a pen basis. The concentrations of interleukin 1 $\beta$  (IL-1 $\beta$ ; R&D Systems, Minneapolis, MN, USA), tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ; R&D Systems), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>; MyBioSource, San Diego, CA, USA), leukotriene B<sub>4</sub> (LTB<sub>4</sub>; MyBioSource), and cyclooxygenase-2 (COX-2; MyBioSource) in plasma were quantified using commercially available ELISA kits according to the manufacturers' instructions described by Piñeiro *et al.* (2009) on d 0, 7 and 14. Data were analysed as completely randomised block design, using general linear model procedure of ANOVA (SPSS v22.0, IBM, Armonk, NY, USA).

Pigs reared under sanitary environmental conditions had higher ADG (P < 0.05) and improved feed efficiency for 14 days after weaning compared to their counterparts (Table 1). Pigs housed in poor sanitary conditions reduced the cytokines TNF- $\alpha$ , COX-2, and PGE<sub>2</sub> (P < 0.05) for 14 days after weaning compared to those in sanitary environmental conditions. Feeding a diet with dietary cellulose (i.e. up to 2%) lowered COX-2 concentration (P < 0.05) without compromising growth performance for 14 days after weaning independent of environmental conditions. Our results indicated that pigs fed a diet supplemented with cellulose (i.e. up to 2%) did not impair growth performance of weaned pigs, and could maintain and/or reduce plasma cytokine concentrations (i.e. COX-2) for 14 days after weaning regardless of environmental conditions.

Item	Environmental conditions		s.e.m.	D	Dietary treatments			<i>P</i> -value	
	Sanitary	Poor sanitary		Cellulose 0%	Cellulose 1%	Cellulose 2%		Environmental conditions	Dietary treatments
ADG (g)	339.7	167.6	18.8	280.1	254.4	226.5	27.51	0.003	0.617
ADFI (g)	392.8	317.8	24.44	382.1	372.0	312.0	25.7	0.229	0.566
FCR (g/g)	1.2	2.1	0.17	1.5	1.9	1.6	0.2	0.036	0.666
Diarrhoea index (%)	12.8	24.0	2.58	24.8	17.9	12.5	2.66	0.041	0.196
IL-1 $\beta$ (pg/mL)	0.0	13.3	4.80	0.0	19.9	0.0	4.84	0.179	0.144
TNF- $\alpha$ (pg/mL)	5.1	17.6	1.90	16.9	10.8	6.4	2.21	0.002	0.063
COX-2 (pg/mL)	102.8	123.7	2.67	125.3	112.4	102.1	3.29	< 0.001	0.023
PGE2 (pg/mL)	17.5	60.3	4.10	38.5	40.8	37.4	5.57	0.002	0.947
LTB4 (pg/mL)	258.0	413.7	49.45	323.6	436.3	247.6	53.57	0.168	0.297

#### Table 1. Effects of environmental conditions or dietary treatments of cellulose on growth performance, diarrhoea index and circulating proinflammatory cytokines in weaned pigs

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## Dietary phytate, calcium and phytase levels affect growth performance in weaned pigs

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Phytase addition to swine diets improves mineral utilisation and bone strength with less consistent effects on performance (Selle and Ravindran 2008). The aim of this study was to evaluate the effects on performance in weaned pigs fed diets with different levels of phytate (phy), calcium (Ca) and phytase (*C. braakii*; Ronozyme HiPhos, DSM). The hypothesis tested was that the phytase (R) concentration would modulate weaned pig performance in the presence of different dietary levels of phy and Ca.

An experiment was conducted with 128 28-day-old castrated male weaned pigs (Large White × Redon) having an initial bodyweight of 7.2  $\pm$  1.2 kg (mean  $\pm$  s.e.). Pigs were randomly allotted into eight treatment groups of 16 animals each (four pens of four piglets). They were fed *ad libitum* for 42 days with mash diets based on corn, soybean meal and rapeseed meal. Eight diets were formulated to meet the animal requirements for weaned pigs according to NRC (2012) (crude protein (CP), 198 g/kg; metabolisable energy (ME), 13.0 MJ/kg; total P, 0.47%; total lysine, 1.40%). The experiment was conducted in a 2 × 2 × 2 factorial design with two dietary phy (0.18 and 0.31%), Ca (0.45 and 0.80%) and R (1000 and 2500 FTU/kg) concentrations. Growth performance parameters were recorded throughout the study and average daily gain (ADWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. Data were analysed as a 2 × 2 × 2 factorial ANOVA and differences between groups were determined by the Student–Newman–Keuls multiple-range test (significant at  $P \leq 0.05$ ) (StatGraphics Centurion XVII, Manugistics, Rockville, MD, USA).

High dietary phy had a positive effect (P < 0.05) on ADWG and FCR for the first period (d 0 to 14) and overall period (d 0 to 42), but increasing (P < 0.05) the ADFI for these periods (Table 1). By contrast, high dietary Ca had a negative impact (P < 0.05) in all periods on ADWG and FCR (Table 1). High dietary Ca inclusions are well documented to significantly reduce the overall impact of phytase (Selle *et al.* 2009). Indeed, the ability of Ca to bound to phy reduced the potency of phytase to produce digestible P by degrading phy and by that impacted the performance of the weaned piglets (Kim *et al.* 2017). Furthermore, the high Ca concentrations affected less ADFI, indicating that the reduced ADWG may be due to less available P coming from phy.

Data from the present study showed that high dietary phytase could not modulate the performance of weaned pigs fed a diet containing a high amount of Ca.

Phy (%)	Ca (%)	R (FTU/kg)		ADWG (kg	()	ADFI	(kg)		F	CR (kg/kg	)
			0–14	15-42	0-42	0–14	15-42	0-42	0–14	14–42	0–42
0.18	0.45	1000	0.21 <sup>ab</sup>	0.56 <sup>c</sup>	0.39 <sup>abc</sup>	0.29 <sup>ab</sup>	1.24	0.59 <sup>abc</sup>	1.42 <sup>ab</sup>	1.99 <sup>a</sup>	1.48 <sup>b</sup>
		2500	0.27 <sup>b</sup>	$0.50^{\rm abc}$	0.41 <sup>bc</sup>	0.32 <sup>ab</sup>	1.28	$0.57^{ab}$	1.21 <sup>a</sup>	2.29 <sup>ab</sup>	1.29 <sup>a</sup>
	0.80	1000	0.16 <sup>a</sup>	0.48 <sup>abc</sup>	0.33 <sup>a</sup>	0.26 <sup>a</sup>	1.36	0.52 <sup>a</sup>	1.61 <sup>ab</sup>	2.51 <sup>ab</sup>	1.46 <sup>b</sup>
		2500	0.19 <sup>ab</sup>	0.49 <sup>abc</sup>	0.34 <sup>ab</sup>	$0.28^{ab}$	1.22	$0.53^{\mathrm{a}}$	1.49 <sup>ab</sup>	2.23 <sup>ab</sup>	1.43 <sup>b</sup>
0.31	0.45	1000	0.26 <sup>b</sup>	0.54 <sup>bc</sup>	0.44 <sup>c</sup>	$0.40^{b}$	1.31	0.68 <sup>bc</sup>	1.58 <sup>ab</sup>	2.17 <sup>ab</sup>	1.42 <sup>b</sup>
		2500	$0.24^{ab}$	0.51 <sup>abc</sup>	0.43 <sup>c</sup>	$0.37^{ab}$	1.35	$0.70^{bc}$	1.55 <sup>ab</sup>	2.38 <sup>ab</sup>	1.50 <sup>b</sup>
	0.80	1000	0.22 <sup>ab</sup>	0.42 <sup>a</sup>	0.39 <sup>abc</sup>	0.36 <sup>ab</sup>	1.20	0.68 <sup>bc</sup>	1.68 <sup>b</sup>	$2.70^{b}$	1.59 <sup>b</sup>
		2500	0.25 <sup>b</sup>	$0.45^{ab}$	0.43 <sup>c</sup>	$0.40^{b}$	1.206	$0.70^{c}$	1.61 <sup>ab</sup>	2.43 <sup>ab</sup>	1.51 <sup>b</sup>
s.e.m.			0.01	0.01	0.01	0.01	0.02	0.02	0.04	0.05	0.02
Treatment effect P-value			0.010	0.001	< 0.001	0.008	NS	0.009	0.039	0.016	0.002
Main effects P-value											
		Phy	0.023	NS	< 0.001	< 0.001	NS	< 0.001	0.014	NS	0.003
		Ca	0.013	< 0.001	0.002	NS	NS	NS	0.025	0.006	0.011
		R	$NS^A$	NS	NS	NS	NS	NS	NS	NS	NS

Table 1.	Growth performance in wear	ned pigs fed different levels of Ca, Phy and R
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<sup>A</sup>NS, not significant. <sup>a-c</sup>Means in a column not having the same superscript are significantly different.

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## Dietary phytate, calcium and phytase levels affect the small intestine and plasma *myo*-inositol concentrations in weaned pigs

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Dietary phytase, has the potential to degrade phytate to the *myo*-inositol (INO) ring increasing plasma INO concentration (Cowieson *et al.* 2015; Guggenbuhl *et al.* 2016). The aim of this study was to evaluate the INO concentrations in the small intestine content and plasma in weaned pigs fed diets with different levels of phytate (phy), calcium (Ca) and phytase (*C. braakii*; Ronozyme HiPhos, DSM). The hypothesis tested was that the phytase (R) inclusion levels would modulate the INO production in the presence of high dietary levels of phy and Ca.

An experiment was conducted with 128 28-day-old castrated male weaned pigs (Large White × Redon) having an initial bodyweight of  $7.2 \pm 1.2$  kg (mean  $\pm$  s.e.). Pigs were randomly allotted into eight treatment groups of 16 animals each (four pens of four piglets). They were fed *ad libitum* for 42 days with mash diets based on corn, soybean meal and rapeseed meal. Eight diets were formulated to meet the animal requirements for weaned pigs according to NRC (2012) (crude protein (CP), 198 g/kg; metabolisable energy (ME), 13.0 MJ/kg; total P, 0.47%; total lysine, 1.40%). The experiment was conducted in a 2 × 2 × 2 factorial design with two dietary phy (0.18 and 0.31%), Ca (0.45 and 0.80%) and R (1000 and 2500 FTU/kg) concentrations. *Myo*-inositol concentration of digesta from the small intestine segments and plasma were determined at the end of the trial (Leung *et al.* 2011). Data were analysed as a 2 × 2 × 2 factorial ANOVA and differences between groups were determined by the Student–Newman–Keuls multiple-range test (significant at  $P \le 0.05$ ) (StatGraphics Centurion XVII, Manugistics, Rockville, MD, USA).

In all four compartments, the 0.31% phy level gave a higher (P < 0.05) INO concentration than the 0.18% phy level (Table 1). By contrast, the INO concentration was lower (P < 0.05) with the 0.80% Ca level than with the 0.45% Ca level. A higher INO concentration was measured in the duodenum, jejunum, and plasma with the highest dietary R inclusion of 2500 FTU/kg.

*Myo*-inositol is the end product of phytate degradation and requires joint action of dietary phytase and endogenous phosphatases. In the duodenum, jejunum and plasma, INO concentration was increased by the higher levels of phy and R, indicating more phytate degradation. In the ileum, the lower INO levels and the lack of the R effects could be explained by an early absorption in the jejunum. Calcium significantly reduced the overall impact of R on INO production. This was particularly verified in plasma and duodenum where INO levels were low when Ca inclusion was high and phy inclusion low. Data from the present study showed that R could modulate INO concentration in the duodenum, jejunum and plasma in weaned pigs fed diets containing low Ca and high phy concentrations.

Phy (%)	Ca (%)	R (FTU/kg)	Duodenum (mg/g DM)	Jejunum (mg/g DM)	Ileum (mg/g DM)	Plasma (µg/mL)
0.18	0.45	1000	0.77 <sup>a</sup>	0.87 <sup>ab</sup>	0.32 <sup>a</sup>	10.1 <sup>ab</sup>
		2500	1.20 <sup>ab</sup>	$1.07^{ab}$	$0.37^{a}$	10.5 <sup>ab</sup>
	0.80	1000	0.61 <sup>a</sup>	$0.56^{a}$	0.45 <sup>a</sup>	8.1 <sup>a</sup>
		2500	0.99 <sup>b</sup>	$0.91^{ab}$	$0.69^{a}$	$8.2^{\mathrm{a}}$
0.31	0.45	1000	1.17 <sup>ab</sup>	1.02 <sup>ab</sup>	$0.48^{a}$	11.4 <sup>b</sup>
		2500	1.73 <sup>b</sup>	$2.06^{\circ}$	$0.68^{a}$	15.7 <sup>c</sup>
	0.80	1000	$0.97^{a}$	1.41 <sup>b</sup>	1.53 <sup>b</sup>	12.2 <sup>b</sup>
		2500	$0.86^{a}$	$1.07^{ab}$	$0.97^{ab}$	12.7 <sup>b</sup>
s.e.m.			0.07	0.08	0.08	0.33
Treatment ef	fect P-value		0.005	< 0.001	0.001	< 0.001
Main effects	<i>P</i> -value					
		Phy	0.036	< 0.001	0.002	< 0.001
		Ca	0.009	0.035	0.003	0.002
		R	0.020	0.015	NS <sup>A</sup>	0.011

Table 1.	Myo-inosito	concentrations	in the	small	intestine	segments	and	plasma ir	ı weaned	pigs
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<sup>A</sup>NS, not significant. <sup>a-c</sup>Means in a column not having the same superscript are significantly different.

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## Enhanced *E. coli* phytase at 2500 FTU improved piglet performance in both animal and plant protein-based diets

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The presence of phytate (inositol-6-phosphate, IP<sub>6</sub>) and its lower degree phosphorylation (IP<sub>X</sub>) esters have been reported to significantly reduce digestion and absorption of minerals and proteins in monogastric animals, even at low concentrations (Wilcock and Walk 2016). *In vivo* reduction of IP<sub>6</sub> and IP<sub>x</sub> ester content through superdosing of enhanced *Escherichia coli* phytase consistently improved nutrient utilisation efficiency and growth performance of monogastric animals (Wilcock and Walk 2016). Moreover, as the pharmaceutical dose of zinc oxide in piglet diets interacted with phytate and other divalent minerals, supplementation of 2500 FTU/kg phytase improved performance in weaned pigs (Walk *et al.* 2013). However, there still is a perception in the pork industry that piglets will not respond to phytase at 2500 FTU/kg will improve performance of piglets offered both animal protein (AP) and plant protein (PP)-based diets, containing low and moderate amounts of phytate, respectively.

A total of 1147 weaned piglets weighing  $6.25 \pm 0.16$  kg (mean  $\pm$  s.e., PrimeGro<sup>TM</sup> Genetics) were randomly stratified to a 2 × 2 factorial arrangement with the respective factors being diet type (animal or plant protein-based diet) and enhanced *E. coli* phytase (0 or 2500 FTU/kg; Quantum Blue<sup>®</sup>, AB Vista). Each treatment consisted of 20 pen replicates (10 male, 10 female) with 14 or 15 pigs per pen. The experimental duration was 29 days after weaning with a three phase feeding system (Starter: 0 to 7 days, Weaner 1: 8 to 21 days, Weaner 2: 22 to 29 days). Phytase was supplemented over the top for the Starter and Weaner 1 diets, while a mineral matrix release from phytase (0.15% available phosphorous (P) and 0.16% calcium) was applied for the Weaner 2 diets. Starter, Weaner 1, and Weaner 2 diets were formulated to contain 14.9, 14.8 and 14.4 MJ digestible energy (DE)/kg, respectively and 0.9 g standardised ileal digestible lysine/MJ DE for all three-phase diets. Animal protein diets included meat and bone meal, fish meal and blood meal which were partly or entirely replaced by soybean meal and lupins in PP diets. For Starter, Weaner 1, and Weaner 2 diets, the calculated phytate-P contents in AP diets were 0.16, 0.20, and 0.23%, while phytate-P contents in PP diets were 0.20, 0.25, and 0.28%, respectively. Piglets had *ad libitum* access to the respective pelleted diets and fresh water throughout the experiment. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were measured (Table 1). Data were subjected to two-way analysis of variance using JMP statistical software (SAS JMP pro v13.1, SAS Institute Inc., Cary, NC, USA).

The hypothesis tested in this study was supported. Inferior performance of AP diets compared to PP diets may be due to amino acid shortfall in AP used. Piglets fed PP diets and 2500 FTU phytase significantly improved ADG (P < 0.001) due to increased ADFI (P < 0.05) with a better FCR (P < 0.001). There was an interaction between diet type and phytase for FCR such that the improvement was greater in piglets fed AP-based diets (P < 0.05). However, lack of interaction between diet type and phytase for ADG and ADFI indicates that 2500 FTU phytase can reduce anti-nutritional effects of phytate even at low dietary phytate levels, especially in diets formulated based on AP.

Diet type	Animal protein		Plant	protein	Pooled SE	Significance		
Phytase (FTU/kg)	0	2500	0	2500		Diet	Phytase	Interaction
Start weight (kg)	6.3	6.2	6.3	6.3	0.16	NS	NS	NS
Finish weight (kg)	13.7	14.8	14.9	15.7	0.28	0.058	0.066	NS
ADG (g)	256	301	298	326	5.20	0.001	0.001	NS
ADFI (g)	396	426	424	454	5.83	0.020	0.020	NS
FCR (g/g)	1.55	1.42	1.43	1.39	0.013	0.001	0.001	0.012

#### Table 1. Effect of diet type and 2500 FTU/kg phytase on performance of weaned pigs

NS, not significant; SE, standard error.

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## Alternative milk derivatives do not reduce weaner pig performance

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The inclusion of dried milk derivatives (DMD) in diets of newly weaned pigs has been shown to have beneficial effects (Cromwell *et al.* 2008), providing a highly digestible source of nutrients. Improvements in performance are associated with both their high quality protein fractions (Tokach *et al.* 1989) but more specifically their lactose content (Mahan 1992). Whey, both liquid and dried, has been widely used in pig production; however, expansion in the use of DMD for human nutrition has seen an increased offering for pig nutrition. The aim of this study was to evaluate a range of DMD available for inclusion in weaner diets, with the null hypothesis that there would be no difference in growth performance between DMD treatments.

Five hundred and sixty male pigs (20 days of age,  $5.92 \pm 0.16$  kg) entered the experiment over a 4-week period, were sorted by size and assigned to pens (n = 14). Pigs within each pen were weighed and allocated to one of four treatments using a randomised block design, resulting in 10 replicates per treatment, with pen as the replicate. Treatments consisted of isoenergetic and isonitrogenous first stage weaner diets (15.0 MJ DE/kg, 0.9 g standardised ileal digestible lysine (SID L)/MJ DE) including either 13% whey protein concentrate (WPC), 13% skim milk powder (SMP), 13% whey powder (WP) or a mix of 6.5% SMP and 6.5% WP fed for the first 14 days post-weaning. All treatments received the same second stage weaner diet (14.8 MJ DE/kg, 0.85 g SID L/MJ DE) from d 15 to d 28 post-weaning. Data were analysed by ANOVA with treatment as a fixed factor, entry week as blocking factor and entry weight as a covariate (GENSTAT 18, VSN International, Hemel Hempstead, UK), with pairwise differences between treatments determined by 1.s.d. (P < 0.05).

Inclusion of different DMD did not influence the weight gain of pigs in the period immediately post-weaning, nor the growth performance in the second stage (Table 1). The inclusion of WPC did significantly decrease feed efficiency in the first stage (P < 0.001), but there was no difference in the second stage.

Results suggest these products are all suitable for use in weaner diets; however, their use may be largely based on economics.

		Tre	atment				P-value	
	WPC	SMP	WP	SMP/WP	SED	Treat	Week	ΤxW
Weight (kg)								
Entry (d 0)	6.0	6.0	5.9	5.9	0.6	0.997	0.725	0.999
d 14	8.4	8.7	8.5	8.7	0.2	0.074	0.003	0.411
d 28	14.3	14.6	14.1	14.7	0.3	0.179	0.258	0.436
Stage 1 (d 0 to c	114)							
ADG (kg/d)	0.176	0.195	0.181	0.200	0.010	0.074	0.070	0.411
ADFI (kg/d)	0.27	0.25	0.23	0.25	0.01	0.085	0.206	0.408
FCR (kg/kg)	1.53 <sup>a</sup>	1.30 <sup>b</sup>	1.27 <sup>b</sup>	1.26 <sup>b</sup>	0.04	< 0.001	0.137	0.129
Stage 2 (d 15 to	d 28)							
ADG (kg/d)	0.421	0.421	0.402	0.425	0.012	0.220	< 0.001	0.285
ADFI (kg/d)	0.61	0.62	0.58	0.62	0.02	0.117	0.109	0.295
FCR (kg/kg)	1.46	1.49	1.45	1.47	0.03	0.731	< 0.001	0.842

 Table 1. Performance of weaner pigs fed diets containing whey protein concentrate (WPC), skim milk powder (SMP), whey powder (WP) or a combination of skim milk powder and whey powder (SMP/WP) from d 0 to d 14 immediately post-weaning (Stage 1) and a control second stage weaner diet (Stage 2)

<sup>a,b</sup>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 of means; Treat, treatment effects; Week, entry week effects; T × W, interaction effects.

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## Levan-type fructan improved growth performance and nutrient digestibility of weaner pigs

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As possible alternatives to antibiotic growth promoters, prebiotics have shown positive effects on growth performance and gut health of weaner pigs (LeMieux *et al.* 2003). Levan-type fructan is a homopolymer of fructose linked by the  $\beta$ -2, 6 fructofuranosidic bonds. It is considered to be a prebiotic with health- and growth- promoting effects in pigs (Zhao *et al.* 2013; Zhang and Kim 2014). The information on supplementation with different doses of levan-type fructan in weaner pigs is still scarce. The objective of this study was to evaluate the effects of different dose levels of levan-type fructan on growth performance, digestibility, and blood characteristics in weaner pigs.

A total of 144 weaner pigs ((Yorkshire × Landrace) × Duroc) with an average bodyweight (BW) of  $7.92 \pm 0.86$  kg were randomly allocated to four experimental diets with six replicate pens per treatment based on initial BW and sex (three barrows and three gilts per pen) for a 6-week experiment. Dietary treatments were basal diets supplemented with 0%, 0.01%, 0.05%, and 0.10% levan-type fructan (RealBioTech Co., Daejeon, South Korea). Individual pig BW and feed consumption on a pen basis were measured at the beginning and end of the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G : F). Chromic dioxide marker (0.2%) was added to feed from d 36 to d 42 to estimate the apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP), and gross energy (GE). At the end of this experiment, blood samples were randomly collected via jugular venipuncture from two pigs (one gilt and one barrow) from each pen. Serum was harvested from centrifugation at 3000g for 15 min at 4°C and concentrations of calcium, blood urea nitrogen, and creatinine were determined using an automatic blood analyser (Technicon RA-1000; Bayer, Tarrytown, NY, USA). Serum iron concentration was determined using an automatic blood analyser (Hitachi 747, Hitachi, Tokyo, Japan). All experimental data were analysed using linear and quadratic contrasts (SAS v9, SAS Institute Inc., Cary, NC, USA). The results are shown in Table 1.

Average daily gain and ADFI as well as ATTD of DM, CP, and GE linearly increased when pigs were fed increased levels of levan-type fructan (P < 0.05). Fructan linearly increased the concentrations of calcium and iron in serum (P < 0.05).

The results indicated that levan-type fructan could be a prebiotic to enhance growth performance, nutrient digestibility and improve the absorption of calcium and iron in weater pigs.

Parameter		Levan-type fructan (%)				P-	P-value	
	0	0.01	0.05	0.10		Linear	Quadratic	
Average daily gain (g)	449	457	470	485	7	0.001	0.604	
Average daily feed intake (g)	675	691	690	720	10	0.009	0.510	
Gain to feed	0.664	0.658	0.684	0.672	0.013	0.422	0.804	
ATTD dry matter (%)	78.8	80.1	80.4	82.1	0.42	< 0.001	0.792	
ATTD nitrogen (%)	76.0	76.2	77.2	80.3	0.82	< 0.001	0.134	
ATTD gross energy (%)	78.8	80.0	81.5	82.3	0.43	< 0.001	0.323	
Serum calcium (mg/dL)	9.4	10.3	10.5	10.7	0.21	< 0.001	0.051	
Serum iron (µg/dL)	52.0	74.8	83.0	135.0	12.62	0.001	0.276	

Table 1. Effects of levan-type fructan on growth performance, digestibility, and blood characteristics

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## Using hydroxyl selenomethionine as an antioxidant for weaned piglets

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After weaning, piglets face various challenges, such as relocation, new housing, mixing with unfamiliar piglets, dietary switch from milk to solid feeds, resulting in stresses to their metabolic system that often lead to gut inflammation accompanied with increased level of oxidised products, which further increases the severity of any intestinal disorder. The selenium (Se) based enzyme, glutathione peroxidase (GSH-px) is widely present in the intestinal villi, and protects the intestines against oxidative stress. The efficiency of Se uptake from diet into the body tissues depends on the source supplied, and Se-yeast has been found more nutritionally effective than selenite (Mahan *et al.* 1999). The main shortfall of the yeast source is its Se occurs as many different molecules, which are inconsistent and make it difficult to define the level of selenomethionine (SeMet).

A new commercial compound, hydroxyl selenomethionine (OH-SeMet, Selisseo, MinAscent Leuna Production, GmbH, Germany), produced from chemical synthesis, has been reported to be 40–60% more efficiently incorporated into the liver and muscle than Se-yeast (Jlali *et al.* 2014). The following trial was conducted to ascertain the efficacy of this source of Se and how it can promote antioxidant status in piglets. This study used 252 hybrid piglets, weaned at d 21, randomly allocated to seven treatments (36 pigs/treatment, six replicates × six pigs/pen). The basal diet contained 0.13 ppm Se, and was supplemented with either selenite (0.3 ppm) or OH-SeMet at five levels. Measurements included feed intake, growth, feed conversion and number of pigs and days on diarrhoea *v*. total number of pigs and days. The trial lasted 28 days. At the end of the trial, one pig per replicate was randomly selected and sacrificed in order to analyse tissue to determine Se status. Data were analysed via ANOVA using Software SPSS v20.0 (IBM, Armonk, NY, USA).

The results (Table 1) showed that Se source and level did not alter performance although addition from either selenite or OH-SeMet increased Se content (P < 0.05) in the liver, and increased sequentially with higher doses of the OH-SeMet. However, when GSH-px was measured, responses to dietary treatments were inconsistent. Addition of OH-SeMet at 0.2 ppm and above significantly reduced diarrhoea (P < 0.05) compared to the unsupplemented control group.

In conclusion, OH-SeMet appeared to be effective in enhancing liver Se content, which may have been involved in promoting antioxidant status in weaned piglets, leading to reduced diarrhoea occurrence.

	Basal	Selenite		Hydı	oxyl selenomethi	onine	
Se inclusion (ppm)	_	0.3	0.1	0.2	0.3	0.4	0.5
Se analysed (ppm)	0.13	0.38	0.20	0.33	0.42	0.53	0.62
Start weight (kg)	7.70	7.75	7.72	7.68	7.71	7.52	7.66
Average daily gain (g)	344	358	348	348	364	346	357
Feed conversion ratio	1.76	1.76	1.75	1.72	1.73	1.74	1.76
Se in liver (mg/kg)	$1.07^{a}$	1.45 <sup>b</sup>	1.53 <sup>b</sup>	1.89 <sup>c</sup>	2.27 <sup>d</sup>	2.51 <sup>e</sup>	3.09 <sup>f</sup>
GSH-px in liver (U/mg)	43.4 <sup>a</sup>	52.2 <sup>b</sup>	47.8 <sup>ab</sup>	53.8 <sup>b</sup>	51.2 <sup>ab</sup>	51.8 <sup>b</sup>	49.2 <sup>ab</sup>
Diarrhoea (% of herd affected)	13.1 <sup>a</sup>	10.8 <sup>ab</sup>	10.9 <sup>ab</sup>	7.6 <sup>b</sup>	6.4 <sup>b</sup>	7.7 <sup>b</sup>	6.4 <sup>b</sup>

Table 1. The effect of supplementing Se sources and levels on antioxidant status and performance

Means not bearing the same superscript within a row differ significantly (P < 0.05).

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### In vivo digestion of encapsulated essential oils in weaned pigs

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The antibacterial properties of essential oils (EO) are well known (Hammer *et al.* 1999). However, most EO are readily absorbed in the gastrointestinal tract (GIT), which potentially limits their *in vivo* efficacy. Previous studies have demonstrated that effective encapsulation of EO using Ca-alginate does not compromise antibacterial activity (Wang *et al.* 2009). This study tested the hypothesis that the encapsulation of a mix of three EO with proven antimicrobial activity against enterotoxigenic *E. coli* could help release the active principle onto the small intestine of recently weaned pigs.

A 200 g mixture including thyme, nerolina and peppermint gum EO were added to a solution of 20 g/L of alginate and 0.5 g of Tween 80 (Croda International, Snaith, DN14 9AA, UK) and blended for 1 min using an Ultraturrax homogeniser (IKA Works, Staufen im Breisgau, Germany). A low flow peristaltic pump with a 21 G needle dropped the emulsion into a 20 g/L CaCl solution. The droplets were left to harden for 30 min. Microcapsules were washed, drained, and stored in a sealed container at 4°C. A catheter was surgically implanted in the external jugular vein of 12 5-week-old male pigs. Treatments consisted of a single oral dose of free EO (FEO) mixed with the morning meal (0.45 mg/kg BW of the principal compounds of each EO (PCEO)), encapsulated EO (EEO, 76 mg/kg BW of microencapsulation) and control (C, feed without EO). Blood samples were collected at 5, 10, 15, 30, 45, 60, 120, 180, 240 and 300 min. Animals were euthanised ~10 h after receiving the treatment and gastrointestinal content samples were collected. Samples were analysed by gas-chromatography/mass spectrometry. Data were analysed by ANOVA using Minitab16 (Minitab Inc., State College, PA, USA).

Compared to the FEO group the serum data indicated that pigs consuming EEO had a significantly (P < 0.05) higher concentration of PCEO at 30 min post-ingestion and thereafter (Fig. 1). A peak for FEO was seen as early as 5 min after intake followed by a slow but constant decrease in the PCEO concentration up to the 5 h timeline. The EEO group exhibited a fast increase followed by a plateau reaching a high level in serum after 5 min and a peak 60 min after intake. In addition, the gastrointestinal data showed a significant (P < 0.05) higher concentration of PCEO from the EEO compared with FEO in gastric contents. The average concentrations for the encapsulated treatment in duodenal and jejunal contents (Fig. 2) were also numerically higher but no statistical significance could be found due to a high variance. The results indicated that the encapsulation with Ca-alginate liberates only part of the EO (probably the exterior bonded EO) in the stomach while releasing the rest of it in the small intestine.

In conclusion, the results suggested that the encapsulation of EO with Ca-alginate improved the efficiency of absorption of the EO both short and long-term together with a protective effect of the EO from GIT conditions, particularly in the stomach.

100



 (b)
 80

 (b)
 60

 (c)
 40

 (c)
 20

 (c)
 (c)

 (c)
 (c)

FFO

FEO

Control

**Fig. 1.** Concentration of principal components of EO (PCEO) found in serum of pigs after oral treatment with free (FEO) or encapsulated (EEO) essential oils. Asterisks show significant differences between treatments at P < 0.05.

Fig. 2. Concentration of principal components of EO (PCEO) found in the GIT of pigs around 10 h after oral treatment of free (FEO) or encapsulated (EEO) essential oils. Superscripts show significant differences between treatments at P < 0.05.

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## Elucidation of the complex carbohydrate structures of canola meal fibre by commercial feed enzymes

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Inclusion levels of canola meal (CM) in pig diets may be restricted by the concentration of glucosinolates (Schone et al. 2001) and by relatively high concentration of fibre (plant cell walls) in the meal. However, new varieties of canola containing more protein and less fibre than conventional CM have been identified. Due to the likely higher total glucosinolate levels in the high protein CM (CM-HP) variety (15 µmol/g) compared to the conventional CM (CM-CV) variety (8.69 µmol/g), it was observed that the standardised ileal digestibility of amino acids in pigs fed with CM-HP from black-seeded canola was not greater than in CM-CV (Berrocoso et al. 2015). The high fibre components in CM include mainly non-cellulosic polysaccharides (13-16%). The fibre in CM is antinutritional and is responsible for the less than optimum digestibility values for constituents such as protein (70-75%) (Sauer et al. 1980). Since non-ruminants do not possess fibre degrading enzymes, use of exogenous enzymes seems to be essential. In vitro microscopy work elucidating the cell wall structure of soybean and the effect of enzymes on the same has been published earlier (Ravn et al. 2015). In the current in vitro work, using techniques devised by Ravn et al. (2015), the complex fibre structure of canola/CM and the impact of enzymes was examined. Using a dye staining acidic polysaccharides orange (Coriphosphine O, TCI America, Portland, OR, USA) and antibodies targeting plant cell wall structures, identification of the cell wall of canola was possible, showing that the structure of cell wall of canola is different from that of soybean (Ravn et al. 2015). Using a cell wall specific antibody recognising xyloglucan epitopes, a pure commercial xyloglucanase from Megazyme International Ltd (Co. Wicklow, Ireland) and a commercial feed xyloglucanase (Ronozyme MultiGrain, DSM, Wagga Wagga, NSW, Australia), the outmost cell wall layer of canola was identified as xyloglucan (Fig. 1Aii). Removal of the xyloglucan layer after incubation at room temperature for 3 h by either enzyme, washing the sample and re-staining with either Coriphosphine O (Fig. 1*Aiii*) or use of antibodies revealed a pectin layer below the xyloglucan layer. (Ronozyme VP, DSM, Wagga Wagga, NSW, Australia) (containing pectinase) removal of the pectin layer revealed protein underneath, indicating protein accessibility on removal of cell wall fibre (Fig. 1Aiv). Each experiment was repeated three times.

This research on elucidation of cell wall morphology of differing protein sources and the use of enzymes to degrade the same can be used to highlight the importance of using the right combination of enzymes. In turn this may assist in increasing nutritional worth of canola and reduce feed costs while maintaining performance.



**Fig. 1.** (*a*) Canola meal (*i*) is stained with Coriphosphine O, which stains cell walls orange, and proteins show blue autofluorescence (*ii*). On incubation at RT for 3 h with Ronozyme<sup>®</sup> MultiGrain a xyloglucanase containing product the outer intact cell walls disappear, revealing a more diffused cell wall structure: white arrow (*iii*). On further incubation at RT for 3 h with Ronozyme<sup>®</sup> VP a pectinase containing feed enzyme product, pectin solubilisation of occurs, exposing blue fluorescing proteins (*iv*). (*b*) Schematic drawing of CM fibre (cell wall) composition as visualised in A (*i* to *iv*).

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## Effects of different amounts of wheat bran and oat hulls in a starch-based diet on voluntary feed intake in grower pigs

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Feed intake in pigs fed *ad libitum* increases with decreasing feed energy density until a threshold is reached (Black *et al.* 2009). Indigestible fibre stimulates digesta passage rate and feed intake until the gut-capacity threshold is achieved. Undigested fibre in the distal ileum and colon stimulates the 'intestinal brake', which reduces digesta passage rate and feed intake (Black *et al.* 2009). The relative effects of passage rate increasing feed intake and the 'intestinal brake' reducing feed intake cannot be determined with conventional grain diets, as these contribute to both effects. The hypothesis tested in this experiment was that fibre source and level alters intake of a highly digestible starch-based diet diluted with various amounts of an indigestible fibre (oat hulls, OH) or a partially soluble fibre (wheat bran, WB).

The base diet contained maize starch (52.1%), fish meal (20%), dextrose (15%), soy protein (5%), Opticell lignocellulose (4%), amino acids, minerals and vitamins (2.4%) and sunflower oil (1.5%). Different amounts of OH (0, 2.5, 5, 10, 15 and 20%) or WB (0, 5, 10, 15, 25 and 35%) were added to the base diet and pelleted. A minimum of five pigs (male, Large White, initial bodyweight (BW)  $19.7 \pm 0.88$ kg (mean  $\pm$  s.d.), 49 to 56 days old) were assigned, in a randomised block design, to each diet, which was fed *ad libitum* to pigs housed individually with free access to water over 21 days. Average daily feed intake (ADFI) was measured on dry matter basis at d 7, 14 and 21. Data were analysed using a linear mixed modelling approach, with initial BW as a covariate. Hydration capacity (HC; mL/g) was measured as the amount absorbed after soaking in excess water for 24 h and subsequent centrifugation. HC data were analysed by one-way ANOVA and Tukey test using ASReml, version 3 run on R platform (VSN International, Hemel Hempstead, UK). ADFI of OH diets tended to be higher (P = 0.053) than WB diets during 0 to 7, 7 to 21 and 0 to 21 days. ADFI of OH diets were over 4% higher (P = 0.021) than WB based diets for the 7 to 14 days period.

During 7 to 21 days (Fig. 1), basal diet intake increased ~7% as OH increased to 5%, but then decreased by ~8% with 20% OH. This suggested OH increased rate of passage and total intake, with intake maximised at 5% OH. Lower intake of the 10% OH diet could be due to higher HC (P < 0.001) compared to other OH diets (1.43 v. 1.23 to 1.31 mL/g), as this increases bulk and decreases intake (Kyriazakis and Emmans 1995). Feeding 10% WB reduced intake by ~4%. A steeper decline in intake to 30% below control for 35% WB diet was consistent with HC increasing from 1.30 (10% WB) to 1.63 mL/g (35% WB). Three factors may be interacting in WB diets: (1) initial increased rate of passage due to bulk; (2) reduced intake due to HC and bulk; (3) soluble fibre stimulating the intestinal brake. In conclusion, fibre source affects intake of basal diet.



**Fig. 1.** ADFI (kg) from 7 to 21 days for whole diets containing OH (a) or WB (b). Intake of the base diet was calculated by subtracting the proportion of WB or OH from whole diet.

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# Effects of dietary supplementation of *Curcuma aromatica* and inositol monophosphate on performance and IgG of blood in lactating sows and piglets

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Several authors have observed that the herb *Curcuma aromatica* (CA) and inositol monophosphate (IP) perform several biological activities such as anti-inflammatory, antioxidant and intracellular calcium ( $Ca^{2+}$ ) concentration control (Shen *et al.* 2002; Steger *et al.* 2002; Sikha *et al.* 2015). Due to these activities, it was hypothesised that adding these compounds into feed may influence sow and piglet performance. This study was conducted to evaluate the effects of dietary supplementation of CA and IP on the performance and immunoglobulin G (IgG) concentrations in blood from lactating sows and piglets.

Eighteen (Landrace × Yorkshire) third parity sows and their litters were used in a 28 days feeding trial (21 nursing days). Sows with an average initial bodyweights (BW) of  $249.9 \pm 3.2$  kg were allotted into three treatments with six replicate pens per treatment on d 108 of gestation, based a randomised block design. Dietary treatments (formulated to meet NRC 2012) included: (1) basal diet (CON); (2) basal diet + CA 0.5% in feed (CA); and (3) basal diet + IP 0.1% in feed (IP). Sow BW and backfat were recorded before farrowing, within a few hours after farrowing and after weaning. Piglet BW was recorded on d 0, 1, 7, 14, and 21 (weaning) and the number of piglets for each sow was recorded on farrowing day and weaning to evaluate piglet survival rate. Blood, colostrum and milk samples were taken before farrowing and weaning day. All data were analysed using the GLM procedure of SAS (v9, SAS Institute Inc., Cary, NC, USA). The individual sow or litter of piglets were used as the experimental unit. Differences among the treatment means were determined by using the Tukey's test with P < 0.05 indicating significance. The results are shown in Table 1.

Piglet weaning weight and average daily gain (ADG) in the IP treatment group were higher than those in CA treatment (P < 0.05) but both IP and CA supplementation did not improve litter performance over those fed CON. The CA diet numerically improved sow bodyweight loss, back fat loss, blood IgG in sows and piglets compared with CON but was not significant (P > 0.10).

In conclusion, in this study, supplementation of 0.5% CA and 0.1% IP in feed failed to affect growth performance or IgG in sow and piglets compared with CON.

Parameter	CON	CA	IP	$SE^2$
Sow backfat thickness loss (mm)	4.2	4.0	4.2	0.6
Sow bodyweight loss (kg)	35.8	30.6	34.8	3.9
Sow lactating daily feed intake (kg)	5.85	5.60	5.04	0.26
No. of piglets	10.2	10.8	10.3	0.5
Piglet initial weight (kg)	1.47	1.33	1.43	0.08
Piglet weaning weight (kg)	6.98 <sup>ab</sup>	6.21 <sup>b</sup>	7.36 <sup>a</sup>	0.28
Piglet ADG (g)	230 <sup>ab</sup>	203 <sup>b</sup>	251 <sup>a</sup>	10
Farrowing Sow IgG (mg/dL)	706	755	691	54
Weanling Sow IgG (mg/dL)	896	922	905	59
Weanling Piglet IgG (mg/dL)	271	315	270	18

Fable 1.	Effect of Curcuma	aromatica (O	CA) and	inositol	monophosphate	(IP)	supplementation	in	lactating
			Parity 3	sows an	d piglets				

CON, basal diet; CA, basal diet + CA 0.5%; IP, basal diet +IP 0.10%; SE, standard error of the mean. <sup>a,b</sup>Means in the same row with different superscripts differ significantly (P < 0.05).

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### NSP-ase and phytase improve growth performance and upholds carcass traits

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It has been largely established that non-starch polysaccharides degrading enzymes (NSP-ase) are able to improve digestibility of nutrients in growing and finishing pigs fed on common ingredients (Emiola *et al.* 2009; Cozannet *et al.* 2012), but research on the effect of dietary enzymes on carcass quality in pigs remains scarce. The aim of this study was to evaluate the effect of a combination of NSP-enzymes and phytase on the performance and carcass traits in growing and finishing pigs fed reformulated wheat-, wheat bran- and soybean meal-based diet.

The study was conducted in Schothorst Feed Research facility, The Netherlands. In total, 360 crossbred gilts (Talent x (Great Yorkshire × Finnish Landrace)) with an average liveweight of 23.8 kg were used in the trial, which consisted of three treatments with 12 replicates of 10 pigs per replicate. The three treatments included: (1) positive control (PC), formulated as a typical commercial Dutch diet; (2) negative control (NC), reduced NE: -100 kcal/kg, calcium (Ca): -0.8 g/kg and digestible phosphorous (P): -1.0 g/kg, in accordance with the manufacturer's guidance; (3) negative control + NSP-enzymes (Rovabio, Adisseo, Singapore) 200 mL/mt, containing mainly the activities of xylanase and  $\beta$ -glucanase + phytase 500 FTU/kg. Feed (pellets) and water were provided *ad libitum*. The pigs received a grower diet during the first 5 weeks followed by a finisher diet until the end of the experiment. At the end of the experiment, 12 pigs per treatment were killed to determine bone ash content (fat-free basis) in the metacarpus 2. Carcass weight, back fat thickness, muscle thickness and lean meat percentage were assessed per pig after slaughtering. Results were screened for outliers using the Doornbos test and subsequently statistically analysed as a randomised block design by ANOVA, using GENSTAT 14 (VSN International, Hemel Hempstead, UK). If a significant treatment effect was found, the least significant difference test (l.s.d.) was used for comparing treatment means. Differences were considered to be significant when P < 0.05.

Growth performance of the pigs is presented in Table 1. Compared with the PC, the reduction of nutrients (NC) significantly (P < 0.001) decreased weight gain, and numerically decreased feed intake and feed efficiency. The enzyme supplementation significantly improved weight gain (P < 0.001), and partially improved feed intake and feed efficiency. In terms of carcass traits, the reformulation significantly reduced bone ash content (-4.13%, P < 0.001), but the addition of the enzymes restored this parameter to the same level of the PC group. Lowering the specifications in NE, Ca and P led to a significant (P < 0.001) decrease of carcass weight without affecting meat percentage, back fat and muscle thickness. Adding the enzymes to the reformulated diet significantly (P < 0.001) improved carcass weight percentage. The results indicate that down-specification on energy, Ca and P has detrimental effect on performance and carcass traits, and the addition of NSP-ase, and phytase is capable of degrading cell wall constituents and phytic acids, hence can improve the digestibility of key nutrients, and restore performance and some carcass characteristics. In conclusion, the results clearly demonstrate the benefits of supplementing both NSP-ase and phytase to diets of grower and finisher pigs. Moreover, exogenous enzymes can restore bone ash and carcass weight in pigs fed on a nutritionally marginal wheat diet.

Table 1.	Effect of the NSP-	enzyme + phytase o	on growth perfo	ormance and carcass traits
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	Positive control	Negative control	NC + (NSPase + Phytase)	P-value
Initial weight (kg)	23.9	23.9	23.6	0.020
Final weight (kg)	98.9 <sup>a</sup>	94.3°	96.9 <sup>b</sup>	< 0.001
Weight gain $(g/d)$	823 <sup>a</sup>	773°	805 <sup>b</sup>	< 0.001
Feed intake (kg/d)	1.91	1.87	1.86	0.35
FCR <sup>A</sup>	2.33	2.41	2.32	0.054
Ash in bone (g/kg)	556 <sup>a</sup>	533 <sup>b</sup>	558 <sup>a</sup>	< 0.001
Carcass weight (%)	89.0 <sup>a</sup>	86.0 <sup>b</sup>	$88.0^{\mathrm{a}}$	< 0.001
Lean meat (%)	58.3	58.5	58.4	0.35
Backfat (mm)	14.2	13.6	13.8	0.096
Muscle thickness (mm)	60.2	59.1	59.2	0.29

<sup>A</sup>FCR, feed conversion ratio. <sup>a-c</sup>Means with different superscripts within a row differ (P < 0.05).

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### The reproductive value of enrichment to sows at farrowing

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Pre-parturient sows in traditional farrowing environments are confined at a time when they are highly motivated to perform nest building behaviours (Westin *et al.* 2015). Providing them with hay may help alleviate the frustration associated with confinement, and lead to welfare improvements for the sow and her piglets. In this study, sows were provided with lucerne hay, which acted as a food-based enrichment and nest building material, and the impact on reproductive performance was studied. It was hypothesised that the provision of lucerne would reduce parturition time and decrease the number of stillborn piglets.

Sixty-nine Large White x Landrace sows (Parity 0 to 2) over six farrowing batches were allocated to either the Control (n = 33) or Lucerne enrichment (n = 36) treatments. Prior to farrowing ( $6.5 \pm 0.3$  days), sows receiving enrichment were given ~1 kg of lucerne hay/d into their feeding trough after their morning ration. Weaning occurred at  $16.4 \pm 0.3$  days. Current farrowing duration and piglet numbers and outcomes were measured from video observations. Subsequent mating performance was taken from farm records. All data were analysed to assess the effects of enrichment. In SPSS (v24.0, IBM, Armonk, NY, USA), general linear models with parity, treatment and their interaction as fixed effects, and batch as a random term, were run for the following variables: farrow duration ( $\log_{10}$ ), piglet interval ( $\log_{10}$ ), total piglets born, piglets born alive, and piglet interval only. The same model was used for number of piglets born dead and post-natal piglet deaths but a generalised linear model with Poisson distribution was applied, and a binomial distribution was applied to the number of sows that were mated the next batch. The number of piglets born dead was reduced by 0.3 piglets in the lucerne treatment (Table 1). There was no difference in farrowing duration, piglet birth interval, total number of piglets born, or piglets born alive. A significant parity by treatment interaction existed for percentage of sows mated within the batching requirements. Sows displaying oestrus within 2 weeks of weaning were bred, whilst the remainder were not bred that batch. More gilts from the Lucerne treatment were mated immediately following weaning (81%) than Controls (60%), but this relationship was reversed in multiparous sows (Lucerne 67% v. Control 90%; P < 0.05).

The difference in number of piglets born dead in the absence of any change in farrowing duration is intriguing. One possible explanation is that allowing the sow to perform nest-building activities had positive effects on uterine blood flow and so risk of piglet hypoxia was reduced. This notion needs confirming. Behaviour at parturition from this experiment is being analysed to assess if this contributed to the difference in the number of stillborn piglets. The finding that gilts may show improvements in re-breeding success is interesting, but viewed with caution given the short lactation length and consequent poor subsequent performance. This experiment is being replicated on a larger scale in a commercial piggery, and the nutritional impacts of lucerne are being quantified to evaluate these results further.

Tuble 1. The encets of the provision of facerne before and at partainable of som reproductive performan	Table 1.	The effects of the	provision of lucerne	before and at	parturition on so	w reproductive	performance
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	Control		Lucern	Lucerne		
	Mean	s.e.m. <sup>A</sup>	Mean	s.e.m.		
$Log_{10}$ farrowing duration (min) <sup>B</sup>	2.22 (166.0)	0.05	2.31 (204.2)	0.04	0.174	
$Log_{10}$ piglet interval (min) <sup>B</sup>	1.18 (15.1)	0.05	1.24 (17.4)	0.05	0.364	
Total piglets born	10.9	0.5	11.0	0.5	0.834	
Piglets born alive	10.4	0.5	10.9	0.5	0.450	
Piglets born dead	0.4	0.1	0.1	0.1	0.027	
Piglet deaths	0.8	0.2	0.6	0.1	0.328	
Piglets weaned	10.5	0.3	10.6	0.3	0.865	

<sup>A</sup>s.e.m., standard error of the mean. <sup>B</sup>Back-transformed means are presented in brackets.

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### A preliminary examination of sham chewing behaviour in group-housed, nulliparous sows

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Stereotypies are relatively invariant motor acts, repeated frequently, that have no apparent function (Mason and Rushen 2008). Whilst the origins and mechanisms of stereotypic behaviour remain unclear, it has been suggested that sows may develop stereotypies, such as sham chewing, as a means to cope with their environment (Mason and Rushen 2008). Despite the move from stall to group-housing during gestation, stereotypies are still anecdotally observed in Australian sow herds. Sham chewing is the most common and frequently observed stereotypy in group-housed sows (Vieuille-Thomas *et al.* 1995). The characteristics and welfare implications of stereotypic behaviour in group-housed sows have received little examination and their causation remains unknown. This preliminary study is part of a larger project investigating the relationships between sham chewing and the welfare and productivity of group-housed gestating sows.

This study aimed to characterise sham chewing behaviour in group-housed gestating sows with regard to average bout duration, bout frequency and the persistence of sham chewing, and develop a valid method of sampling this stereotypy. Archive video footage of 20 group-housed, nulliparous sows (two groups of 10 sows) in their first gestation was utilised. Gilts were twice artificially inseminated and within 7 days of insemination randomly mixed into groups of 10 (1.8 m²/gilt). A standard commercial gestation pelleted diet (13.1 MJ/kg DM and 12.8% protein; 31.3 kg per feeder per drop and 2.5 kg/sow/d) was delivered onto the floor in four feeding bouts drops (~0730, 0930, 1100 and 1500 h). Water was supplied *ad libitum*. One video camera with built-in infrared lights was positioned above each pen during gestation and video recordings were conducted from 0700 to 1700 h on d 3 (D3) and 8 (D8) post-mixing. An ethogram was developed; a sow was deemed visible if the observer could clearly view the snout and jaw, and sham chewing was defined as jaw movement without contact with any substrate. The performance of sham chewing was assessed using continuous sampling, instantaneous point sampling at 2 min intervals (2 min IPS), and instantaneous point-sampling at 5 min intervals (5 min IPS). Wilcoxon signed-rank tests were used to compare continuous sampling with the two instantaneous point sampling methods.

The average duration of sham chewing bouts was 54 s on D3 (s.d. 47) and 78 s on D8 (s.d. 173), and the difference in average bout duration between the 2 days was not significant (Z = -1.11, P = 0.27). The average frequency of sham chewing bouts was 8 bouts/sow/d on D3 and 5 bouts/sow/d on D8, and the difference in average frequency of bouts between the 2 days was significant (Z = -2.16, P = 0.03). 85% of sows were identified as performing sham chewing behaviour on D3 and 70% of sows on D8; however, the difference between the days was not significant (Z = -1.34, P = 0.18). Sham chewing was observed across the day, both pre- and post-feed drops. The proportion of sows identified as performing sham chewing behaviour with continuous sampling was significantly different to 5 min IPS on both D3 (Z = -2.45, P = 0.01) and D8 (Z = -2.65, P = 0.01), and 2 min IPS on D3 (Z = -2.00, P = 0.05) but not D8 (Z = -1.73, P = 0.08). When comparing the two instantaneous sampling methods, the proportion of sows identified as performing sham chewing behaviour on D3 (Z = -0.82, P = 0.41). Using 5 min IPS, 65% of sows were identified as performing sham chewing behaviour on D3 and 35% of sows on D8. While for 2 min IPS, 55% of sows were identified as performing sham chewing behaviour on D3 and 55% of sows on D8. Thus, while 2 min IPS was more accurate in identifying sows sham chewing than 5 min IPS, both instantaneous point sampling methods failed to identify some sows that continuous sampling identified.

These findings suggest that a point sampling frequency of 30 s is likely to be an effective method of sampling sham chewing. This is currently being investigated, with the subsequent aim of examining sham chewing behaviour in 200 group-housed gestating sows and its implications for their welfare. Whilst this preliminary study involves a small number of animals, it provides valuable data on the characteristics of sham chewing behaviour in group-housed sows and the development of an appropriate sampling method for use in larger scale studies.

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### Influence of access to maize silage on sham chewing and stomach ulcer of gestating sows

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Diets of gestation sows are typically restricted to ~60% of their *ad libitum* intake. In addition, these diets are often offered daily with a concentrated feed, and as a result, the sows are likely to have an unfulfilled feeding motivation. Both hunger and lack of ability to perform foraging behaviours contribute to stereotypic behaviours, such as sham chewing (D'Eath *et al.* 2009). O'Connell (2007) and Stewart *et al.* (2010) found that the combination of a high fibre diet with access to straw reduced sham chewing compared to a control diet without straw. Giving finishers permanent access to wrap hay from racks, in addition to standard Danish pelleted diet, (Poulsen and Thoning 2015) could reduce the incidence of gastric ulcer. The aim of this study was to assess the effect of providing supplementary maize silage on stomach ulcers in culled sows and sham chewing of gestating sows in groups. It was hypothesised that maize silage would reduce stomach ulcers and sham chewing.

A total of 2988 gestation sows in two different Danish herds with group housing were included in the study. Both herds had ~1200 sows and the sows were inserted into the gestation pens 4 weeks after weaning. In each herd, the sows were either given 3 kg of maize silage on the floor at feeding once daily, or no roughage (control). All sows were offered straw as bedding (legislation). Data was collected on farm by a technician from the Pig Research Centre. Sows chewing with white foam and no feed/straw in the mouth were defined as sham chewing. In both herds, the sows were examined 5 h after feeding. After weaning, stomachs from culled sows were examined for stomach ulcers and given a score from 1 to 10 according to severity (Jensen *et al.* 2017).

Stomach ulcers were analysed by a Chi-test and data on the percentage of sows per pen sham chewing were analysed by a *t*-test (SAS v7.1, SAS Institute Inc., Cary, NC, USA). Each herd was analysed separately and the results are shown in Table 1.

Maize silage given as a supplement during the gestation period had no effect on stomach ulcer in culled sows at weaning. Maize silage reduced the proportion of sows sham chewing significantly in both herds compared with sows only offered straw as bedding. In conclusion, 3 kg of maize silage per sow per day in the gestation period had a positive influence on the percentage of sows sham chewing, but no influence on stomach ulcer at weaning.

	He Free acc	rd 1 cess stalls	He Floor	rd 2 feeding
Group size	35		14	
Area (m <sup>2/</sup> sow)	3	.5	3.0	
	Straw	Maize	Straw	Maize
Number of sows included in the study	554	1,068	608	758
Number of pens	16	16	47	25
Sham chewing, % sows 5 h after feeding	62 <sup>a</sup>	48 <sup>b</sup>	41 <sup>a</sup>	16 <sup>b</sup>
Number of sows examined	62	90	47	77
Stomach ulcer, % of sows with score over 6	48	45	32	46

Table 1.	Effect of maize silage on stomach ulcers	at weaning and sham ch	ewing five hours after feeding
	8		

<sup>a,b</sup>Significantly different (P < 0.05).

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## Agonistic interactions in stable group housed swine using a Gestal<sup>®</sup> free access stall over two gestation periods

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In the United States, producers are transitioning sow farms from individual housing to group housing utilising electronic sow feeders (ESF) for delivering feed in gestation. These feeding systems represent a significant investment and require that equipment is designed to ensure individualised feeding regimens. Group housing sows inevitably results in unwanted aggression during either the initial 48 h period post-mixing (Anil *et al.* 2006), as a result of re-mixing in dynamic group systems or competition for resources (Jensen *et al.* 2000). The Gestal<sup>®</sup> ESF (Jyga Technologies, Saint-Lambert-de-Lauzon, QC, Canada) is a gated unidirectional feeder that targets loose housing sow systems, and protects sows from unwanted aggression during feeding. The objective of this study was to quantify agonistic interactions in sows housed in a stable group gestation housing system, utilising one Gestal<sup>®</sup> free-access stall with radio-frequency identification (RFID) technology during the first 48 h post-mixing over two gestation periods (first gestation = unfamiliar sows; second gestation = familiar sows).

Thirty-eight unfamiliar gilts were randomly mixed into two stable groups (n = 20 and n = 18) and housed in gestations pens ( $6.8 \times 5.5$  m) with a single gated Gestal<sup>®</sup> unit and two water nipples located on the side of the pen. The number of initiated agonistic bouts (biting, chasing, and displacing) and corresponding pen location (concrete flooring (C), drinking area (D), and feeding area (F)) were recorded for each sow continuously over 48 h postmixing for two gestation periods (first and second gestation). Individuals were grouped into three ranked categories based on a calculated agonistic interaction index (high, intermediate and low; adapted from Galindo and Broom (2000)). Due to low chasing and displacement counts, agonistic data was pooled. Data were transformed into normality using a  $\log_{10}$  transformation and analysed using a PROC Mixed ANOVA in SAS (v9.4, SAS Institute Inc., Cary, NC, USA). Overall, agonistic interactions decreased between the first and second gestation period ( $13.5 \pm 1.1 v. 9.8 \pm 1.1$ , bouts  $\pm$  s.e., P = 0.005) for both pens. High and intermediate ranked sows performed a greater number of agonistic initiations compared to low ranking sows ( $20.1 \pm 1.2$ ,  $16.0 \pm 1.1$  and  $4.8 \pm 1.1$ , bouts  $\pm$  s.e., respectively, P < 0.0001). A greater number of agonistic initiations took place in the F area compared to C and D area across gestation periods (Fig. 1, P < 0.0001).

Agonistic initiations varied among sows housed in small group sizes and fed utilising a Gestal<sup>®</sup> system. This is likely a demonstration of the dominance hierarchy established within the group. Agonistic interactions decreased between gestation periods highlighting the value of stable groups compared to mixed groups. The greatest number of agonistic initiations was in the F area indicating that resource guarding is likely a major factor in inter-sow aggression, regardless of the ability to individually feed sows.



**Fig. 1.** Mean ( $\pm$  s.e.) agonistic initiations performed (bouts/48 h, n = 38) in group housed sows across two gestation periods (first gestation **s**; second gestation **s**) and area within in pen (F, feeding area; D, drinker area; and C, concrete flooring). Data are presented as back-transformed values. Superscripts indicate a difference between pen locations across gestation period (<sup>a-c</sup>P < 0.0001).

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## Group lactation compromises piglet performance and shifts injuries from weaning to lactation

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Group lactation systems provide sows with increased movement and the ability to interact with piglets, compared to conventional farrowing crates. The effects of grouping sows during lactation on the welfare and performance of piglets have been studied to a much lesser extent (Li *et al.* 2012). The nutritional, environmental and social changes that occur during weaning, makes it a stressful period for piglets. This has implications for subsequent post-weaning growth. It was hypothesised that piglets from a group lactation system are better adjusted to weaning and hence would have improved welfare as indicated by growth performance and post-weaning injuries.

Primiparous sows (n = 196) were randomly allocated to one of two treatments; Control sows (n = 49) were housed in traditional farrowing crates for the duration of lactation, and Grouped sows (n = 147) were housed in traditional farrowing crates until 14 days before weaning, at which point they were mixed into groups of three with litters until weaning. On d –14, –12, –1, +1, +7, +14 and +30 relative to weaning, piglets were weighed and assessed for injury score (with the exception of d +14) using a modified injury score (Widowski *et al.* 2003), which consisted of a four-point scale for scratches around the head and ears of each piglet. Statistical analyses were conducted using a generalised linear mixed model (Proc MIXED) in SAS 9.1 (SAS Institute Inc., Cary, NC, USA) with grouped lactation sows treated as a group. Data are expressed as least-squares means  $\pm$  s.e.m.

Grouped piglets were lighter following the grouping event. On the day before weaning, Control piglets weighed 0.56 kg more that Grouped piglets (7.2 kg v. 6.6 kg; P < 0.05; Fig. 1) and d 30 post-weaning Grouped piglets were still lighter (by 0.77 kg) but not significantly. Variation in grouped piglet weight increased with age to d +14, but decreased in Control piglets during the same period. Post-mixing on d -12 relative to weaning the number of injuries sustained by grouped piglets was double that of control piglets (Fig. 2). By d +7 post weaning, injuries were highest in control piglets.

Whether piglets are mixed in lactation or after weaning, they sustain injuries most likely attributed to fighting behaviour. This study could not determine whether these injuries were related to competition at the udder or dyadic agonistic interactions. Grouped lactation slows the growth performance of piglets which is not compensated for post weaning.



**Fig. 1.** Pre- and post-weaning piglet growth from control piglets and piglets grouped for the last 14 days of lactation. Day 0 represents weaning.



Fig. 2. Pre- and post-weaning injury scores from control and piglets grouped for the last 14 days of lactation. Day 0 represents weaning.

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## Confinement of sows at parturition increases the incidence of behaviours thought to indicate pain

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As gestation progresses, a sow's tolerance to pain is increased to the point that, immediately before and during farrowing, they are almost non-responsive to adverse stimuli (Jarvis *et al.* 1997). What is less clear, is the impact of the farrowing environment on this parturition-induced hypoalgesia. The aim of this study was to determine the impact of confinement during parturition on behavioural and physiological indicators of pain. We hypothesised that reduced confinement of the sow leading up to and during parturition would result in reduced pain-related behaviour.

Sows (Parities 1 to 3) were housed in '360 farrower' pens (Midlands Pig Producers Ltd, UK), a design that fits the standard footprint of a traditional crate but has adjustable bars, which gives the animal space to move around but also enables containment. Two treatments were applied; OPEN: pen was open from sow entry (5 days before farrowing) until the sow stood for the first time following parturition, at which point they were closed, and CLOSED: pen was closed from sow entry. Blood was collected hourly via an ear vein catheter 24 h before the birth of the first piglet through until the birth of the last piglet and analysed for plasma cortisol concentration using radioimmunoassay (n = 18 CLOSED, n = 15 OPEN). Video cameras remotely collected footage during parturition (from the birth of the first until last piglet) for a subset of sows (n = 12 CLOSED, n = 14 OPEN). Behaviours indicative of pain (Ison *et al.* 2016) and stereotypies were analyszd using continuous sampling (Observer XT, Noldus, The Netherlands). A generalised linear model with poisson distribution was used for behavioural count data in SPSS v24.0 (IBM, Armonk, NY, USA). Cortisol data were analysed using a linear mixed model with sow id as the unit and time as the repeated-measure.

Animals that farrowed in the OPEN treatment demonstrated a reduced number of tail flicks, back leg forward and strains (P < 0.05; Table 1). Additionally, OPEN sows displayed reduced nosing of crate fixtures and increased number of postural changes (P < 0.001; Table 1). No differences in bar-biting/champing or plasma cortisol concentration at any time were observed (P > 0.05).

We have shown that allowing the sow greater movement leading up to and during parturition resulted in a reduced incidence of behaviours indicative of pain. These findings suggest that confinement may result in a reduction in the level of parturition – induced hypoalgesia, but further more objective measures of pain are required to support this notion.

Fable 1.	The number of pain related behaviours observed during farrowing for animals housed in OPEN
	or CLOSED pens

	CLOSED	OPEN	<i>P</i> -value
Number of events			
Nosing crate fixtures	$7.3 \pm 0.8$	$4.1 \pm 0.4$	< 0.001
Bar biting and champing	$2.8 \pm 0.5$	$2.8 \pm 0.5$	0.999
Sitting	$3.7 \pm 0.6$	$4.4 \pm 0.6$	0.360
Tail flicking	$27.6 \pm 1.5$	$\textbf{8.4} \pm \textbf{0.7}$	< 0.001
Back leg forward	$162.4 \pm 3.7$	$122.0 \pm 3.0$	< 0.001
Straining	$182.9 \pm 3.9$	$146.1 \pm 3.2$	< 0.001

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### Nurse sows display altered reproduction in the next gestation

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An increase in the total number of piglets born alive has resulted in excess piglets in relation to available functional teats (Baxter *et al.* 2013). Nurse sows are now commonly employed to accommodate excess piglets born and those that are not thriving on their birth sow. To do this, sows receive foreign piglets after weaning their biological litter. In hyper prolific sows, this practice increases number of pigs born in the next litter, most likely due to the extended lactation length, and stockperson bias in sow selection (Bruun *et al.* 2016). We hypothesised that the subsequent reproductive output of a nurse sow would differ to that of a non-nurse sow.

Data was extracted from the herd management software for the years 2015 and 2016 for a large commercial breeder unit located in South Australia. Nurse sows were identified in the dataset as being those with a second lactation subsequent to a single gestation (n = 849), and were randomly paired with sows recording a single lactation (n = 723). All data were analysed in SPSS v24.0 (IBM, Armonk, NY, USA) with year and shed as random terms, parity group (1, 2–4 and 5+), season (summer, autumn, winter and spring), and treatment (control and nurse) as fixed effects. Weaning to service interval, number of piglets born, and piglets born alive were analysed using a general linear model. Percent bred by <10 days, pregnancy rate and farrowing rate were analysed using a generalised linear model with binary distribution. Number of piglets born dead was analysed using a generalized linear mixed model with Poisson distribution.

Nurse sows were on average younger  $(3.1 \pm 0.1)$  than controls  $(3.6 \pm 0.1; P < 0.001)$ . There was no difference in the total number of piglets born before treatment  $(12.3 \pm 0.2; P > 0.05)$ . First lactation length was reduced in nurse sows  $(25.1 \pm 0.2 \text{ and } 25.8 \pm 0.2 \text{ for controls}, P < 0.001)$ , and nurse sows averaged  $13.8 \pm 0.3$  days in the second lactation. Weaning to service interval was increased in nurse sows, and percent bred <10 days, pregnancy rate and farrowing rate were reduced (Table 1). Total number of piglets born and piglets born alive were increased in nurse sows, but piglets born dead were similar to controls.

Our hypothesis was supported. Using the predicted means for farrowing rate and total piglets born alive (Table 1), the number of piglets per 100 sows bred would be 899 for control and 842 for nurse sows. Given the usage of nurse sows is probably low in the Australian herd, this result would have little impact on the total productivity and output of breeder units. Future research will explore population dynamics of nurse sows to exploit reproduction advantages. This will be of high importance as the use of nurse sows is increased.

	Control	Nurse	Significance
Wean to service interval (d)	$5.4 \pm 0.0$	$7.3 \pm 0.0$	< 0.001
Bred $<10$ days (%) <sup>A</sup>	94 (91–96) <sup>A</sup>	84 (78–89) <sup>A</sup>	< 0.001
Pregnancy rate $(\%)^{A}$	95 (92–96) <sup>A</sup>	88 (84–91) <sup>A</sup>	0.001
Farrowing rate (%) <sup>A</sup>	81 (77–84) <sup>A</sup>	72 (67–76) <sup>A</sup>	< 0.01
Total piglets born	$12.3 \pm 0.2$	$13.1 \pm 0.2$	< 0.01
Piglets born alive	$11.1 \pm 0.2$	$11.7 \pm 0.2$	< 0.05
Piglets born dead	$1.0 \pm 0.1$	$1.1 \pm 0.1$	NS

#### Table 1. Mean ± s.e.m. reproductive output for Control and Nurse sow in the subsequent gestation

<sup>A</sup>95% confidence intervals rather than s.e.m. are presented for binary data. NS, not significant.

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## Farrowing performance of sows with increased magnesium concentrations in a transition diet

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Around birth, sows are subject to factors that result in stress, such as confinement in a crate, the parturition process, a change in state from gestation to lactation, and constipation. These can all potentially reduce piglet survival and hence pigs weaned per sow. Magnesium reduces stress in finisher pigs (D'Souza *et al.* 1998), is a fetal neuroprotectant (Marret *et al.* 2007), and an effective laxative. We hypothesised that increased magnesium in a transition diet of the sow would improve farrowing performance and number of pigs weaned.

Sows (n = 811, parity  $3.2 \pm 0.1$ ) were randomly allocated to treatment; CON fed lactation mash, MgSO<sub>4</sub> fed lactation sow mash with added magnesium sulphate (2.85 kg/tonne), SUPP fed lactation mash mixed with an algae supplement high in magnesium and calcium (5.5% and 30% respectively; 5 kg/tonne). Sows were fed 2.5 kg of the treatment diets from 5 days prior, and *ad libitum* to 3 days after farrowing. From 4 days to weaning, all sows were fed the CON diet *ad libitum*. Sows that farrowed between 0700 and 1600 h were checked every 40 min and if no progression was noted, were manually assisted. Sows were fostered to  $11.52 \pm 0.03$  piglets 12 to 24 h after farrowing, and where possible this was conducted within treatment. All data analyses were carried out in SPSS v24.0 (IBM, Armonk, NY, USA) with the number of times a sow was assisted, the number of piglets pulled manually and all piglet mortality using a generalised linear model with Poisson distribution, percentage of sows requiring assistance using a generalised linear model with binary distribution and total pigs born, pigs born alive and number of piglets weaned using a general linear model.

The percentage of sows requiring assistance was unchanged by treatment, but the number of sow assists, and number of piglets pulled manually were reduced in the SUPP treatment compared with CON and MgSO<sub>4</sub> (P < 0.05; Table 1). Total piglets born, and piglets born alive did not differ, but number of piglets born dead increased in MgSO<sub>4</sub> sows compared to CON (P = 0.01). More piglets died from d 1 to 3 in the SUPP treatment than CON (P = 0.05), but number of piglets weaned was similar for all treatments.

Although some small treatment differences were observed, the addition of two magnesium sources fed to sows during the transition phase from gestation to lactation did little to impact number of pigs weaned.

Table 1.	Mean ± s.e.m performance traits for sows fed s standard lactation diet (CON) and those supplemented with magnesium
su	lphate (MgSO <sub>4</sub> ) or an algae supplement high in magnesium (SUPP) from 5 days before 3 days after farrowing

	CON	MgSQ4	SUPP	Significance
	0011	118504	5011	Significantee
Sows requiring assistance (%) <sup>A</sup>	53 (38–68)	47 (34–61)	45 (31–59)	NS
Number of assists per sow	$0.9\pm0.1^{\mathrm{a}}$	$0.9\pm0.1^{\mathrm{a}}$	$0.6 \pm 0.1^{\mathrm{b}}$	0.05
Number of piglets pulled manually	$1.4\pm0.2^{\mathrm{a}}$	$1.4\pm0.2^{\mathrm{a}}$	$1.0 \pm 0.1^{b}$	< 0.05
Total number of piglets born	$12.7 \pm 0.2$	$12.5 \pm 0.2$	$12.8 \pm 0.2$	NS
Piglets born alive	$11.5 \pm 0.2$	$11.1 \pm 0.2$	$11.4 \pm 0.2$	NS
Piglets born dead	$0.7\pm0.1^{\mathrm{a}}$	$1.0 \pm 0.1^{b}$	$0.8\pm0.1^{\mathrm{ab}}$	0.01
Piglet death before fostering	$0.4 \pm 0.1$	$0.3 \pm 0.1$	$0.3 \pm 0.1$	NS
Piglet death from d 1 to 3	$0.5\pm0.1^{\mathrm{a}}$	$0.5\pm0.1^{\mathrm{a}}$	$0.7 \pm 0.1^{\rm b}$	0.05
Piglet death from d 4 to 21	$0.4 \pm 0.1$	$0.3 \pm 0.1$	$0.4 \pm 0.1$	NS
Number of piglets weaned	$9.5 \pm 0.2$	$9.6 \pm 0.2$	$9.1 \pm 0.2$	NS

<sup>a,b</sup>Means in a row with different superscripts differ significantly (P < 0.05). <sup>A</sup>95% confidence intervals are presented for binary traits rather than s.e.m. NS, not significant.

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## Play behaviour in piglets is infrequent and not altered by enrichment with lucerne when measured by scan sampling

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Play behaviour in early life may have an important role in the cognitive and social development of piglets. Research also supports play as a potential positive welfare indicator (Brown *et al.* 2015). Environmental enrichment can provide opportunities to express exploratory behaviours that could lead to increased play behaviour. Our aim was to determine the effects of access to lucerne on piglet play. The hypothesis was that the provision of lucerne throughout lactation would increase play behaviour in piglets. Approximately 1 kg of lucerne hay was added to the farrowing crate daily for the first week of lactation, then every second day until weaning. Piglets from nine control litters (n = 98) and 13 enriched litters (n = 141), born across several days, were recorded for 24 h on a set day, 2 weeks after the farrowing period begun. Behavioural analysis was carried out for 2 h of video footage (11:00 to 13:00) for all litters by instantaneously sampling every 15 s. Behaviours expressed were grouped into; play, active, rest or nursing, and were mutually exclusive to one another. Piglets were not individually identified, but each piglet age, and their interaction, fitted as factors and sow as a random factor were used to analyse incidents of play, nursing and active behaviours (P > 0.05). Age influenced nursing (P = 0.016) and play (P = 0.04), but the relationships were not linear. Behaviour varied widely between litters (Fig. 1). The occurrences of play were low and occurred sporadically, but, observationally, they appeared more common after a nursing event.

Contrary to the hypothesis and other published literature on environmental enrichment provided during lactation (Martin *et al.* 2015), the provision of lucerne did not increase play behaviour in piglets. The short intervals of scan sampling did identify play behaviour, but it was highly infrequent. Continuous observations or assessing behaviour at other time points during the day when pigs are active would provide a more comprehensive analysis and would give further insight on treatment effects.



**Fig. 1.** Behavioural profiles of two litters (Control (*a*) and Lucerne (*b*)), showing the proportion of nursing (thin black), active (broken), play (thick black) and resting (dots) over the 2 h observation period.

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### Strategies to reduce the pain of tail docking in piglets

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Tail docking is a common practice to prevent tail biting in piglets and strategies to provide pain relief for the short-term pain (Morrison *et al.* 2013) associated with this procedure are being investigated. The aim of this experiment was to assess the efficacy of the cauterisation technique with or without pain relief (meloxicam) in mitigating the acute stress response to tail docking. The hypothesis was that cauterisation and meloxicam would mitigate the stress response and reduce pain-related behaviours compared to the cauterisation or clipping the tail without meloxicam.

Seventy-two sows (Large White × Landrace; PrimeGro<sup>TM</sup> Genetics, Corowa, NSW) and their litters were used. Fostering was conducted in the first 24 h post-birth. Six entire male piglets were selected per litter at 2 days post-farrowing (432 piglets). Piglets were randomly allocated to the following treatments: (1) No Handling; (2) Sham: Handling; (3) Clipper: Tail docked using sanitised clippers; (4) Cauteriser: Tail docked using a cauterising iron (Stericut Tail Docker, Cotran Corp., Portsmouth, RI, USA); (5) Meloxicam + Clipper; and (6) Meloxicam + Cauteriser. Meloxicam treatments used Metacam (Boehringer Ingelheim, Sydney, NSW, Australia)<sup>®</sup> at 5 mg/mL injected intra muscularly 1 h before tail docking. Piglets in all treatments were handled in the same manner, for the same duration, by the same two technicians. The tail was cut ~2 cm from the base. Blood samples were collected via jugular venipuncture at 15 and 30 min and analysed for total plasma cortisol using an extracted radioimmunoassay (Immuchem<sup>TM</sup> Coated Tube Cortisol RIA kits; MP Biomedicals, Belgium). Pain-related behaviour was assessed by measuring the frequency of escape attempts (Marchant-Forde *et al.* 2009) and duration of vocalisations during treatment. After treatment, behaviour was recorded using mounted cameras (Signet Model QV-3063). The behaviour (postures, states and pain-related behaviours; Hay *et al.* 2003) were measured by continuously observing each piglet for 1 min every 5 min for 1 h post-treatment. Statistical analyses were performed using SPSS (v21.0, IBM, Armonk, NY, USA). Univariate GLM was used, with each pig as the experimental unit and sow as a random factor and post-hoc tests were conducted using least significant difference tests.

In comparison to the Sham treatment, cortisol concentrations at 15 min were higher (P < 0.05) in the Clipper and Cauterisation treatment whereas the Meloxicam + Clipper and Meloxicam + Cauteriser treatments were similar to the Sham. At 30 min post-docking, in comparison to the Sham treatment, cortisol concentrations were higher (P < 0.05) in the Clipper treatment (Table 1). The Cauterisation, Meloxicam+Clipper and Meloxicam + Cauteriser treatments were similar to the Sham treatment (Table 1). The Cauterisation, Meloxicam+Clipper and Meloxicam + Cauteriser treatments were similar to the Sham treatment. The duration of vocalisations and frequency of escape attempts during treatment were greater (P < 0.05) in all of the tail docking treatments compared to the Sham treatment. Piglets in the Clipper treatment spent more (P < 0.05) time with their head lowered compared to all other treatments and there were no significant differences (P > 0.05) between treatments in other postures and states observed. Cauterisation appears to be less aversive than the Clipper technique, based on the physiological stress response. The administration of Meloxicam did not mitigate the behavioural response during tail docking, however, it mitigated the physiological stress response. The commercial administration of meloxicam requires consideration before it is recommended for use compared to cauterisation alone.

Fable 1.	Mean total plasma cortisol	(ng/mL) concentrations at	t 15 and 30 min post-treatment
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	No handling	Sham	Clipper	Cauteriser	Meloxicam + Clipper	Meloxicam + Cauteriser	s.e.m.	P-value
Cortisol 15 min	88.6 <sup>a</sup>	138.4 <sup>b</sup>	186.7 <sup>c</sup>	169.5 <sup>cd</sup>	163.2 <sup>bcd</sup>	144.3 <sup>bd</sup>	3.97	< 0.001
Cortisol 30 min	212.6 <sup>a</sup>	276.2b <sup>c</sup>	317.7 <sup>b</sup>	267.5 <sup>c</sup>	261.8 <sup>c</sup>	238.7 <sup>ac</sup>	6.73	0.001

<sup>a-d</sup>Within rows values with different superscripts are significantly different (P < 0.05). s.e.m., standard error of mean.

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## Provision of enrichment blocks alters red blood cell parameters in sucker and weaner pigs

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Providing animals with enrichment can decrease antagonistic behaviours (Beattie et al. 1995; van Nieuwamerongen et al. 2015), enhance learning ability (Bolhuis et al. 2013), and decrease oxidative stress in hippocampal slice cultures though modification of immune cell secretions (Pusic et al. 2016). We investigated the effect of providing sucker and weater pigs with enrichment in the form of a specially formulated enrichment block for sucker and weaner pigs (Ridleys Corporation, Toowong, Qld, Australia). We hypothesised that the provision of enrichment blocks would attenuate the inflammatory response. Piglets (Large White × Landrace cross) were housed in conventional farrowing crates for 21 days during lactation and in group pens for 9 weeks post weaning. Prior to weaning pigs were housed in either a pen with enrichment blocks (enriched) or without enrichment blocks (barren). Enrichment blocks were provided from d 7 of life. At weaning, the pigs were weaned into pens with enrichment blocks (enriched) or without enrichment blocks (barren). This resulted in four treatments in a 2 × 2 factorial design: enriched in sucker and weater phases (EE; n = 10), enriched in the sucker phase and barren in the weater phase (EB; n = 12), barren in the sucker phase and enriched the weater phase (BE; n = 11) and barren in sucker and weater phases (BB; n = 12). Food and water were provided *ad libitum* during the weaner phase. Blocks were replaced weekly and increased in size to match piglet size. Blood samples (3 mL) were collected via jugular venepuncture at 1 day before weaning, 1 day post weaning, and 21 days post weaning for complete blood count on a haematology analyser (Abbott Cell Dyn 3700, Abbott Laboratories, Chicago, IL, USA). Data were analysed using a repeated-measures ANOVA and a general linear model in SPSS v23.0 (IBM, Armonk, NY, USA). Haemoglobin and haematocrit were less in the BB group than the BE, EB and EE groups (P < 0.05). Red blood cell (RBC) distribution width was greater in the BB group than in EE and BE groups (P < 0.05), but was not different than the EB group (P > 0.05). The number of platelets was greater in the BB group than the BE, EB or EE groups (Fig. 1; P < 0.05).

The difference in haemoglobin, haematocrit, variation in red blood cell width, and platelet concentration in the enriched groups are consistent with an attenuated inflammatory status. Our data therefore suggest pigs provided with enrichment blocks within the first 11 weeks of life have a lowered inflammatory status and the provision of enrichment blocks can influence inflammatory status and potentially immune function of sucker and weaner pigs. However, the implications of this for the long-term welfare and productivity of sucker and weaner pigs requires further investigation.



Fig. 1. The effect of enrichment on four red blood cell parameters. \*indicates significance (P < 0.05).

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## Group-lactation housing from 7 or 14 days post-partum: effects on piglet behaviour

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Group-lactation housing may improve sow and piglet welfare by increasing opportunities to move about and express social and maternal behaviours (van Nieuwamerongen *et al*. 2014). This study tested the hypothesis that piglets reared in multi-litter groups from 7 or 14 days of age would display more positive and less negative behaviours during lactation, and deliver less aggression when mixed post-weaning, compared to piglets reared in standard farrowing crates.

The litters (n = 1551 piglets) of 112 sows (Large White × Landrace, PrimeGro<sup>TM</sup> Genetics, Corowa, NSW, Australia) were allocated to one of three treatments over four time replicates: 1) Group lactation (GL) from 7 days post partum (GL<sub>7</sub>, n = 48 litters), 2) GL from 14 days post partum (GL<sub>14</sub>, n = 48 litters), or (3) Farrowing crate (FC; n = 16 litters). All dams farrowed in standard farrowing crates, where FC litters remained (with their dams) until weaning. GL<sub>7</sub> and GL<sub>14</sub> litters were transferred (with their dams) from farrowing crates to GL pens (one pen of five sows at 8.4 m<sup>2</sup>/sow and one pen of seven sows at 8.1 m<sup>2</sup>/sow, per treatment and replicate) at 7 and 14 days post partum, respectively. All treatments were weaned at 28 days post partum. Treatments were balanced for sow parity, weight and litter size, and there were no treatment differences in litter weight and sex ratio, or variation in these variables. Four focal piglets (one average-sized male and one average-sized female from a high and a low parity dam) per GL pen, and two focal piglets per FC (selected as per GL piglets), were video recorded from 0700 to1700 h on the day after mixing (D2) and 2 days before weaning (pre-weaning, PW). Of the four FC litters per replicate, two were video recorded on the same days as GL<sub>7</sub> (FC<sub>7</sub>) and the other two recorded on the same days as GL<sub>14</sub> (FC<sub>14</sub>). Piglet time-budgets were observed using point sampling with 5 min intervals. Behaviours were analysed with LMM and GLMM models (SPSS v23.0, IBM, Armonk, NY, USA), with the main and interactive effects of housing (GL v. FC), litter age at mixing (7 v. 14 days) and observation day (D2, PW), as repeated-measures and controlling for pen and replicate as random factors (Table 1). Effects of GL<sub>14</sub>, GL<sub>7</sub> and FC treatments on aggression following mixing with unfamiliar (ratio 50 : 50) pigs post-weaning was also analysed for one replicate (five pens/treatment, 12 pigs/pen).

Time spent suckling declined over lactation for all housing treatments. GL piglets suckled mostly from their dam at d 2 post-mixing, but mostly from other dams at d 26. The higher incidence of potentially harmful manipulations (manipulating ears, belly/tail) in FC compared to GL treatments at d 26 requires further investigation. No other piglet behaviours were affected by lactation housing. Post-weaning, FC pigs delivered 57 to 72% more single bites/knocks, and engaged in 43% more fights post weaning than GL pigs. The means for GL<sub>7</sub>, GL<sub>14</sub> and FC piglets were, respectively, 17.0, 11.2 and 39.8 bites/knocks per pig (P < 0.001) and 1.29, 1.48 and 2.45 fights per pig (P = 0.006). This suggests that group-lactation systems can reduce piglet's aggression post-weaning.

Table 1. Percent of observations piglets recorded performing behaviours, by housing (GL, FC), age of GL litter at mixing (7 or 14 days) and<br/>observation day (d 2 post-mixing, D2; 2 days pre-weaning, PW)

	G	GL <sub>2</sub> GL <sub>44</sub>		FC	FC <sub>7</sub> <sup>A</sup> FC		α B		P-value		
	D2	PW	D2	PW	D2	PW	D2	PW	Housing	Day	Age
Suckling (total) <sup>C</sup>	17.7	16.6	15.8	12.4	16.0	13.7	14.0	12.4	0.16	0.11	0.02
Suckling other dam <sup>C</sup>	3.8	10.6	3.9	9.1	N/A	N/A	N/A	N/A	N/A	< 0.01	0.66
Nosing sow <sup>C</sup>	1.4	1.5	0.9	1.4	0.6	1.0	1.1	0.9	0.18	0.05	0.37
Manipulating	0.1 <sup>a</sup>	0.2 <sup>a</sup>	$0.0^{\mathrm{a}}$	0.2 <sup>a</sup>	0.5 <sup>b</sup>	1.6 <sup>b</sup>	$0.8^{b}$	3.1 <sup>b</sup>	$0.06^{\mathrm{D}}$	< 0.01 <sup>D</sup>	0.48
Aggression <sup>E</sup>	0.7	0.7	0.7	0.2	0.8	0.8	1.2	0.7	0.13	0.14	0.08
Play	0.4	0.2	0.3	0.4	0.6	0.3	0.6	0.2	0.47	0.05	0.88

<sup>A,B</sup>Observed on the same days at GL<sub>7</sub> and GL<sub>14</sub>, respectively. <sup>C</sup>y = sin<sup>-1</sup>( $\sqrt{X}$ ) back-transformed means. <sup>D</sup>Housing × day interaction, P < 0.01. <sup>E</sup>Bites, knocks, fights. <sup>a-c</sup>When there was an interaction, superscripts indicate where means differ.

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## Effect of L-glutamine in late gestation sow diets on survivability and growth of piglets

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Increased sow prolificacy has led to an increase in pre-weaning mortality in Australia. Reducing pre-weaning mortality would not only have economic benefits for pig producers, but also social and animal welfare benefits. Previous research into the inclusion of L-glutamine in late gestation diets using gilts showed that supplementing gestation diets with 1% L-glutamine between d 90 and 114 of gestation increased average piglet birthweight and significantly reduced variation in piglet birthweight (Wu *et al.* 2011). The aim of the present study was to see if the inclusion of L-glutamine in late gestation diets fed to multiparous sows under Australian conditions would improve piglet birthweights, and decrease within-litter birthweight variability, therefore improving piglet pre-weaning survival and growth.

At d 80 of gestation, 460 multiparous sows (Parities 1 to 8; Large White x Landrace, PrimeGro<sup>TM</sup> Genetics, Corowa, NSW, Australia) were allocated to either a commercial gestation diet (n = 218) or the same diet containing L-glutamine (n = 242) at an inclusion rate of 1%. Sows were housed in groups of 40 or 80 and fed a 2.4 kg daily ration via an Electronic Sow Feeder. At 110 days of gestation, sows were transferred to their farrowing accommodation where they remained on their allocated treatment until they farrowed. Live piglets were individually weighed within 24 h of birth and at weaning. A blood sample was obtained from a subset of 427 piglets (Control, n = 216; glutamine, n = 211) within 24 h of birth for analysis of immunoglobulin G (IgG) as an indication of colostrum intake. Piglet mortalities and removals were also recorded. Data were analysed using GLM analysis or a Chi-squared ( $\chi^2$ ) test (for piglet survival) (SPSS v24.0, IBM, Armonk, NY, USA). The sow was the experimental unit and data means were analysed by least significant differences (P < 0.05). Sow parity was included in the analysis as a covariate. A coefficient of variation was calculated for each litter in order to measure within litter variation in birth and weaning weight.

There was no difference in the number of piglets born alive between treatments (Table 1). Average birthweight and variation in birthweight of piglets born alive did not differ between treatments, nor did the variation in individual weaning weights. There was a trend for piglets from sows fed the L-glutamine diet to have lower weaning weights than those from sows fed the Control diet (P < 0.10). Piglet survival within the first 24 h after birth was higher for those piglets from sows fed the L-glutamine supplemented diet (95 v. 94%;  $\chi^2 = 4.05$ , P = 0.044). However, overall pre-weaning survival was not different between treatments (83.3 v. 82.9%;  $\chi^2 = 0.13$ , P = 0.72), for the L-glutamine and Control diet fed sows, respectively. The average 24 h IgG concentration in piglet serum tended to be higher in the piglets from sows fed L-glutamine (21.42 ± 0.66 ng/mL) compared to Control sows (19.69 ± 0.65 ng/mL, P = 0.061).

The inclusion of L-glutamine in late gestation diets of multiparous sows did not lead to an improvement in piglet birthweights or overall pre-weaning survival or growth, despite higher 24 h IgG levels in piglets born to those sows fed the L-glutamine diet. Hewitt and van Barneveld (2012) suggest that glutamine levels in Australian sow diets may already be adequate due to the availability and inclusion of animal based protein meals. In conclusion, the addition of L-glutamine sow gestation diets fed to multiparous sows late in gestation in Australia to improve piglet birthweight and pre-weaning survival seems unwarranted.

Table 1.	Litter characteristics of sows fed either a standard Control diet or a diet supplemented with 1% Glutamine from
	d 80 of gestation until farrowing. Values are means $\pm$ s.e.m.

Control	Glutamine	P-value
$13.02 \pm 0.21$	$13.03 \pm 0.22$	0.975
$11.72 \pm 0.19$	$11.74\pm0.20$	0.945
$1.53 \pm 0.02$	$1.51 \pm 0.02$	0.318
$18.97\pm0.43$	$19.10\pm0.48$	0.843
$7.45\pm0.08$	$7.25\pm0.08$	0.070
$21.28\pm5.50$	$22.05\pm5.27$	0.316
	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c } \hline Control & Glutamine \\ \hline 13.02 \pm 0.21 & 13.03 \pm 0.22 \\ \hline 11.72 \pm 0.19 & 11.74 \pm 0.20 \\ \hline 1.53 \pm 0.02 & 1.51 \pm 0.02 \\ \hline 18.97 \pm 0.43 & 19.10 \pm 0.48 \\ \hline 7.45 \pm 0.08 & 7.25 \pm 0.08 \\ \hline 21.28 \pm 5.50 & 22.05 \pm 5.27 \\ \hline \end{tabular}$

<sup>A</sup>Coefficient of variation =  $(s.d./mean) \times 100\%$ . <sup>B</sup>Age at weaning included in the analysis as a covariate.

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## Pre-weaning growth of gilt and sow progeny is not improved by feeding conjugated linoleic acid and medium chain fatty acids during gestation

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Gilt progeny (GP) are generally recognised as having reduced growth performance compared to sow progeny (SP; Smits 2011). Feeding lipid sources such as conjugated linoleic acid (CLA) and medium chain triglycerides or their acids (medium chain fatty acids; MCFA) to the sow in late gestation and lactation improves the growth of piglets through increased energy available in colostrum and milk (Azain 1993; Bontempo *et al.* 2004). This study hypothesised that feeding CLA and (or) MCFA would improve growth rates of both gilt and sow progeny.

A total of 129 primiparous (Parity 0; GILT) and 123 multiparous (Parities 2 and 3; SOW) sows and their piglets (PrimeGro<sup>TM</sup> Genetics, Corowa, NSW, Australia; 1367 GP and 1546 SP) were involved in the experiment. The experimental design can be found in the companion paper by Craig *et al.* (2017). Sow bodyweight (SW) and P2 backfat were recorded at entry to the farrowing house and at weaning. Number of piglets born alive (NBA) was recorded and individual piglet bodyweights were recorded within 24 h of birth (PW<sub>d0</sub>) and at 21 days of age (PW<sub>d21</sub>). Variables were analysed as a linear mixed model using the MIXED procedure of SPSS (v24.0, IBM, Armonk, NY, USA).

Change in P2 ( $\Delta$ P2) was the only trait for which the diet\*parity interaction was significant (P = 0.004). Gilts on the BOTH diet treatment lost more backfat during lactation ( $-2.4 \pm 0.6$  mm) compared to CON gilts ( $-1.4 \pm 0.6$  mm), whereas all other diet\*parity combinations lost less backfat during lactation compared to CON (data not shown). Gilt progeny were lighter than SP at PW<sub>d0</sub> (1.39 v.  $1.56 \pm 0.02$  kg, respectively; P < 0.001) and PW<sub>d21</sub> (5.10 v.  $6.27 \pm 0.07$  kg, respectively; P < 0.001), with a lower ADG in this period (P < 0.05) than their SP counterparts (Table 1). There was no effect of diet on PW<sub>d0</sub> or PW<sub>d21</sub> ( $P \ge 0.10$ ), although feeding CLA resulted in reduced body fat loss in gilts and sows (P < 0.10) and a reduction in NBA (P < 0.05; Table 1).

The present study confirms numerous other investigations showing that GP are born lighter and grow slower than SP in the pre-weaning period. However, feeding CLA and (or) MCFA in late gestation and lactation at the current levels did not improve preweaning growth of sow or gilt progeny.

Table 1.	Effects of feeding different lipid sources in late gestation and lactation on gilt and sow reproductive performance and gilt and sow progeny
	growth parameters

Treatment	Least square mean $\pm$ s.e.							lueA
Trait		Die	et (D)		Parit	y (P)		
	CON	CLA	MCFA	BOTH	GILT	SOW	D	Р
$\Delta SW (kg)$	$-22.7 \pm 1.8$	$-18.0 \pm 1.8$	$-19.4 \pm 1.7$	$-17.9 \pm 1.8$	$-13.6 \pm 1.3$	$-25.4 \pm 1.3$	NS	*
$\Delta P2 (mm)$	$-3.6\pm0.4^{a}$	$-2.0\pm0.4^{\rm b}$	$-2.6\pm0.4^{ab}$	$-2.5\pm0.4^{ab}$	$-1.3 \pm 0.3$	$-4.0 \pm 0.3$	*	*
NBA	$12.6\pm0.4^{\rm a}$	$11.6 \pm 0.4^{\rm b}$	$12.3\pm0.4^{ab}$	$12.1 \pm 0.4^{ab}$	$11.2 \pm 0.3$	$13.1 \pm 0.3$	**	**
Piglet d 0 to 21 ADG (g/d)	$207\pm4$	$195\pm4$	$197\pm4$	$199\pm4$	$176\pm3$	$223\pm3$	NS	**

<sup>A</sup>NS, not significant, P > 0.10; \*P < 0.10; \*P < 0.05. <sup>a,b</sup>Different superscripts within rows denote significant pairwise differences between diets (P < 0.05).

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### Piglet birth weight is related to time to first walk after birth

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Pre-weaning mortality contributes significantly to the productivity of the breeder herd and hence herd feed conversion efficiency through the number of sows required to maintain progeny production volume. In farrowing houses, the major cause of pre-weaning mortality is sow overlay of neonatal piglets (50%), unviable piglets and runts (30%) and scours (12%) and over half of the total losses occur within the first 48 h after farrowing (Morrison *et al.* 2009). Morrison *et al.* (2009) identified that piglets at risk of dying in the pre-weaning period are those that have a low birthweight, do not suck within 40 min of farrowing, have a low rectal temperature 60 min post-birth, and have low serum immunoglobulin G concentrations 24 h post-birth. The aim of this experiment was to investigate the relationships between piglet birthweight and time to first breath, walk and suck. It was hypothesised that birthweight would be negatively related to time to first breath, walk and suck post-birth.

Eighty sows from Parity 1 to 9 (Large White × Landrace, PrimeGro<sup>™</sup> Genetics, Corowa, NSW) and their litters (1096 piglets) were monitored during the farrowing process. Individual live weight of piglets was measured immediately post-farrowing. Time points for each piglets' birth, first breath, first attempt to walk, and first suck were recorded. The data was linear and was analysed by Pearson's bivariate correlation, two-tailed analysis using SPSS (v21.0, IBM, Armonk, NY, USA).

There was a significant (P < 0.01) weak negative correlation between birthweight and time to first breath and suck (Table 1). There was a significant (P < 0.01) strong correlation between birthweight and time to first walk ( $R^2 = 0.76$ ), which means that birthweight accounts for 76% of the variability of time to first walk post-birth. Baxter *et al.* (2008) noted that piglets that have low birthweights often have poor thermoregulatory ability and depleted body energy reserves, causing them to receive small amounts of colostrum in the early stages of birth which reduces their viability and survivability. Strategies to increase birthweight of piglets may have a positive impact on the ability of these animals to walk (move away from sow and reduce risk of being overlain by the sow) and suck more quickly, which may improve piglet survival within the first 24 h post-birth.

## Table 1. Pearson correlation coefficients (r) between birthweight, time to first breath, walk and suckle

	Birthweight
Time to First Breath	-0.091**
Time to First Walk	-0.870**
Time to First Suck	-0.189**

\*\**P* < 0.01.

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## Oxytocin delivered intranasally to gilts immediately after the birth of the first piglet decreased colostrum intake and growth of piglets

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Oxytocin has a role in maternal care, maternal aggression and anxiety (Sabihi *et al.* 2014). When the effect of oxytocin was blocked in rodents, maternal care was impaired, conversely, increasing oxytocin improved maternal care and evoked maternal behaviour in virgin animals (Slattery and Neumann 2008). There are two pools of oxytocin; a central pool (within the brain) and a peripheral pool (outside of the brain). Delivery of oxytocin intranasally, via nasal spray has been shown to reach the brain, and actions of oxytocin through the brain are more likely to be directly involved with the control of behaviour (Neumann 2008). We hypothesised that a single intranasal dose of oxytocin delivered to gilts immediately after the birth of the first piglet would potentiate mother/offspring bonding, increase colostrum intake by piglets and increase the growth of piglets.

Gilts were randomly allocated to treatments and housed in conventional farrowing pens at Roseworthy piggery, Roseworthy, SA at ~110 days of gestation. The experiment was run over three replications in time. Treatments consisted of: a single 25  $\mu$ g dose of oxytocin diluted in 1 mL saline (Auspep pty ltd, Melbourne Australia. equivalent to 12 IU) (*n* = 18) or a 1 mL dose of saline (*n* = 13) up one nostril immediately after the birth of the first piglet. Gilts were continually monitored by technicians throughout parturition in order to collect the following measures: farrowing duration, inter-piglet birth interval, total piglets born, piglets born alive, piglets born dead, 3-day piglet weight, 18-day piglet weight and the number of piglets saved from the sow by the researchers. Colostrum intake in the first 24 h was calculated using the method described by DeVillers *et al.* (2007). Data were analysed using a general linear model (sow measures) or a mixed model (piglet measures) in SPSS v24.0 (IBM, Armonk, NY, USA).

The number of piglets that had to be saved by the researchers during parturition because of risks of savaging or crushing was greater for gilts that received oxytocin than for gilts that received saline (Fig. 1; P < 0.05). Colostrum intake, 3-day piglet weight and 18-day piglet weight were significantly lower in piglets from gilts that received oxytocin than those that received saline (P < 0.05) There was a trend (P = 0.065) towards a reduction in farrowing duration, saline 240.85 min  $\pm$  29.75 and oxytocin 168.16 min  $\pm$  22.04 (mean  $\pm$  s.e.m.), birth intervals and piglets born alive or dead did not differ between treatment (P > 0.05).

Our data do not support our hypothesis, as the administration of intranasal oxytocin to gilts during farrowing had a negative impact on their piglets' colostrum intake and growth. Gilts that were administered oxytocin spent significantly greater time ventral lying than lateral lying and this likely reduced the opportunity for piglets to suck. Future research is required to further explore the effect of intranasal oxytocin on gilt maternal behaviour, to determine if the dose of oxytocin was appropriate, and the most appropriate time to administer oxytocin intranasally.



Fig. 1. The effect of 25  $\mu$ g of oxytocin delivered intranasally to gilts, on colostrum intake, piglet weight at d 3 and 18 and the number of piglets saved. \*, P < 0.05.

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## Effect of maternal creatine supplementation prior to parturition on piglet growth and survival prior to weaning

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Over the past three decades, high incidences of piglet mortality before weaning have been a significant, and persistent problem for the Australian, and global pig industries. These piglet deaths, which are highest during the first 3 days following parturition, limit breeding herd efficiency and represent a significant welfare concern. Neonatal piglets die for several reasons; however, intra-partum oxygen deprivation is a primary cause as it results in poor vitality, impaired thermoregulation and delayed or insufficient colostrum intake (Herpin *et al.* 1996). Consequently, strategies that protect the piglet brain from the damaging effects of oxygen deprivation (i.e. neuroprotectants) may increase piglet vitality at birth and thus survival. The neuroprotective effects of maternal creatine monohydrate supplementation have been demonstrated in spiny mice (Dickinson *et al.* 2014). We have demonstrated previously that dietary creatine supplementation (2.5% of intake) for 5 days before farrowing reduced latency to stand and suckle in piglets (van Wettere *et al.* 2015). Consequently, the aim of the current study was to determine whether dietary creatine supplementation (2.5% of intake) of pregnant sows would increase piglet survival and growth to weaning.

Five days before farrowing due date, the diets of 64, multiparous, Large White / Landrace sows were supplemented with either 0% (Con) or 2.5% creatine monohydrate (CR) (n = 35 and 29 sows/treatment, respectively). Sows were housed in farrowing crates, and received 1 kg of the same diet three times per day (14.2 MJ/kg DE; 17.3% crude protein). The CR supplement was top-dressed onto the diet and divided equally across each feed allocation. Piglet cross-fostering was kept to a minimum, with cross-fostering only occurring when litter size suckled exceeded the number of functional teats possessed by the sow. Total litter size, number of piglet born alive and still born, piglet liveweight (LW) on d 1, 3 and 21 of life were recorded, as was piglet survival. Treatment effects were analysed using an unbalanced design analysis of variance, with suckled litter size included as a co-variate in the model when analysing piglet LW, growth and survival (GENSTAT 15, VSN International, Hemel Hempstead, UK). Data are presented as mean  $\pm$  standard error (SE).

There was no effect of treatment (Con v. CR; P > 0.05) on the total number of piglets born ( $12.2 \pm 0.5$  and  $11.2 \pm 0.6$ ), or the number of piglets born alive ( $10.8 \pm 0.5$  and  $10.2 \pm 0.5$ ) or dead ( $1.2 \pm 0.2$  and  $0.9 \pm 0.24$ ). Suckled litter size on d 1 post partum was similar (P > 0.05) for Con and CR sows ( $10.4 \pm 0.4$  and  $10.2 \pm 0.4$ , respectively). The proportion of piglets surviving from d 1 to 3 post partum was lower (P < 0.05) for Con compared with CR sows ( $0.92 \pm 0.01 v$ .  $0.96 \pm 0.01$ ), but was similar between d 1 and 21 post partum for Con and CR sows ( $0.84 \pm 0.02$  and  $0.88 \pm 0.02$ , respectively). Compared with Con, CR supplementation increased (P < 0.05) piglet weight gain (kg) between d 1 and 3 post partum ( $0.44 \pm 0.02$  versus  $0.28 \pm 0.02$ ) and between d 1 and 21 post partum ( $4.78 \pm 0.22 v$ .  $4.10 \pm 0.20$ ).

Overall, the current data provide evidence that supplementary creatine before parturition positively affects piglet growth from d 1 to weaning and survival during the first 3 days of life. Although, a positive effect of the numerically lower litter size of the CR sows on piglet growth and survival cannot be discounted, the current results are consistent with those of our previous study involving maternal creatine supplementation (van Wettere *et al.* 2015). Specifically, a reduced latency to stand and suckle (as previously observed in piglets born to creatine supplemented sows; van Wettere *et al.* 2015) is not only an indicator of increased neonatal vitality and vigour, but is also associated with increased colostrum intake and, thus survival and growth to weaning (Herpin *et al.* 1996). Although, further studies with larger number of animals are required, our current, and previous, data indicate that maternal creatine monohydrate supplementation can improve neonatal piglet vitality, early survival and growth before weaning.

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## The effects of provided enrichment in the sucker phase on piglet scratch score post-weaning

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At weaning, piglets are exposed to an array of changes; environmental, social and or nutritional. These changes can result in increased aggressive behaviour (e.g. fighting, mounting), as well as redirected behaviour (e.g. belly nosing, tail biting) (Cox and Cooper 2001; Dudink et al. 2006). Environmental enrichment can provide both a play stimulus and nutritional supplement. Few studies have demonstrated the effects of enrichment throughout the sucker phase. Therefore, this trial tested the hypothesis that the provision of enrichment during the sucker and weaner phases would result in a decreased scratch score and increased piglet growth. At 7 days of age, 96 piglets from 24 litters were randomly allocated to one of two treatments. Piglets were either provided with one cube shaped enrichment block (specifically formulated by Ridleys Corporation Ltd, Toowong, Qld, Australia) per four piglets (enriched), or no enrichment blocks were provided (barren). The blocks were adjusted in size to suit the age of the piglet during the sucker and weaner phases. Four focal piglets per litter were tagged for identification and weaned at 21 days of age, such that half of the piglets in each lactation treatment (enriched or barren) were weaned into either an enriched (with blocks) or barren (without blocks) environment. The final four treatments consisted of; enriched in sucker and weaner phases (EE), enriched in sucker phase and barren in weaner phase (EB), barren in sucker phase and enriched in weaner phase (BE) and barren in sucker and weaner phases (BB), n = 24 per treatment. The piglets were weighed and a scratch score of 0 to 3 (Widowski 2003) recorded weekly throughout the 11-week experiment. Six fresh blocks were introduced to the enriched pens each week. Data were analysed using a mixed model (ASReml v4, VSN International, Hemel Hempstead, UK), with treatment, phase and week as fixed effects. Results are presented in Fig. 1. There was no effect of enrichment on growth rate throughout the 11 weeks (P>0.05). There was a significant sucker  $\times$  weak interaction for scratch score (P < 0.05). Animals in EE treatment had significantly fewer scratches than those in BB, BE and EB treatment at d 7 post weaning (P < 0.05). Piglets in the EB treatment had significantly greater scratches than piglets in the other treatments at d 14 post weaning (P < 0.05).

The data partially supported the hypothesis as there were differences in scratch scores, but not in growth rate. Animals that were enriched in both the sucker and weaner phase had lower scratch scores at d 7 post-weaning, potentially indicating that enrichment reduced aggression at this time. Data suggested that providing piglets with enrichment in the sucker phase, but not in the weaner phase, could have negative impacts on behaviour; evidenced by the increase in scratch scores in EB piglets at 14 days post-weaning. Overall, the provision of enrichment in the sucker and weaner phase has the potential to reduce aggression and result in less scratches.



Fig. 1. The effects of enrichment on scratch score in piglets measured weekly around the weaning period. \*Indicates significant differences between treatments post weaning.

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## Amino acid complexed minerals in the diet increased mineral content in the hair and hoof of growing gilts

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Trace minerals play an important role in improving claw health and integrity thus reducing the incidence of lameness in pigs. Trace minerals that have been observed to have an important role in claw keratin formation include zinc (Zn), manganese (Mn) and copper (Cu). In particular, Zn and Mn are two trace minerals supplemented into livestock diets, each play a role in keratinisation of the hoof epidermis (Tomlinson *et al.* 2004). It has been stated that much of the inorganic Zn included in pig diets is excreted (Acda and Chae 2002) due to its low bioavailability; however, research has found bioavailability of trace minerals can be improved by binding them to organic ligands where one metal ion is bound to one amino acid ion. Muller *et al.* (2015) showed the ability to measure content through trace mineral analysis, using a chemical traceability system known as Physi-Trace<sup>®</sup>, of hair in mature sows. This experiment aimed to determine if trace mineral content was comparable within the hoof and hair, such that hair analysis could be a marker in the context of mineral bioavailability for hoof health.

Fifty female pigs were selected at weaning and randomly allocated (21 days, mean weight  $5.4 \pm 0.5$  kg (mean  $\pm$  s.d.)) into three dietary treatment groups (n = 25) and maintained through to slaughter (~21 weeks of age). Isoenergetic and isonitrogenous diets were fed to treatment groups throughout the experiment containing a base vitamin/mineral premix (120 ppm Zn for ZnSO<sub>4</sub>, 50 ppm Mn from MnO, 15 ppm Cu from CuSO<sub>4</sub>) with the inorganic treatment group receiving an additional 750 g/t of the premix. Amino acid complex (AAC) treatment received 750 g/t of Availa<sup>®</sup>Sow (Zinpro Corp., Eden Prairie, MN, USA) delivering an additional 50 ppm Zn, 20 ppm Mn and 10 ppm Cu from amino acid complexed minerals. Pigs were held in a group housing system with feed and water offered *ad libitum*. The left and right rump was shaved of all hair at the start of the experiment (Muller *et al.* 2015) and a pooled hair sample was taken before slaughter at 21 weeks. Hoof samples, from each of the rear and front limb, were also collected at 21 weeks, with both hair and hoof samples analysed for their trace mineral content. Data were analysed using the Univariate GLM and correlation procedures (GENSTAT 15, VSN International, Hemel Hempstead, UK).

Although not significant, and correlations between hoof and hair samples were not found (P < 0.05), levels of Zn and Mn appear to be higher in the hair of pigs fed the AAC diet, consistent with content seen in the hoof (Figs 1, 2). With these results, it is uncertain whether trace minerals were supplemented at an increased rate above that required physiologically and therefore, whether this may hold some validation for using hair analysis to predict levels of content in the hoof for future work.





**Fig. 1.** Concentration (ppm) of Mn, Cu, (primary-axis) and Zn (secondary-axis) in the hoof of growing gilts fed control diets ( $\blacksquare$ ) or diets supplemented with amino acid complexed minerals ( $\Box$ ; Mn, Cu, and Zn) from weaning to 21 weeks of age. <sup>a,b</sup>Means with different superscripts differ significantly (P < 0.05).

**Fig. 2.** Concentration (ppm) of Mn, Cu, (primary-axis) and Zn (secondary-axis) in the hair of growing gilts fed control diets ( $\blacksquare$ ) or diets supplemented with amino acid complexed minerals ( $\square$ ; Mn, Cu, and Zn) from weaning to 21 weeks of age.

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## A poured block reduces feeding associated aggression in sows during gestation

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The long-term hierarchical stability of group housed sows can be affected by the method of feeding (Arey and Edwards 1998). Aggression around feeding may lead to detrimental effects on reproductive parameters as a result of the physiological stress response of the sow. Limited opportunity for expression of key behaviours such as foraging and exploration in group feeding systems, heightens competition over access for food. It has been suggested that the behavioural effects of food restriction may be alleviated by providing sows with a substrate (Whittaker *et al.* 1999). It was hypothesised that the use of a poured block (Sow Enrichment Block, mostly comprised of molasses; Ridley Agriproducts, Pakenham, Vic., Australia) can reduce feeding associated aggression at mixing of sows during gestation.

A commercial gestation diet (14.5 MJ digestible energy (DE)/kg, 0.55 g standardised ileal digestible lysine/MJ DE) was floor-fed at a rate of 2.5 kg/sow/d to all treatments, in a randomised block design study. Groups of 15 multiparous sows were mixed immediately post-mating (d 0) and randomly allocated to one of three treatment groups. The control group was floor-fed daily with one 20 kg block placed in the pen (d 0); and the two block group was floor-fed daily with two 20 kg blocks placed within the pen (d 0). Each experimental replicate ran for 4 days and was replicated 10 times with a new group of sows. The measures taken during each 4-day observation period included aggressive behaviour observations (push, chase, attack, bite and threat), recorded for a period of 45 min after feed was presented. Individual sow scratch injuries were counted on a sub sample of sows per pen, on d 1 and 3, as an indicator of aggressive behaviour. The blocks were weighed daily until complete disappearance. Data were analysed using the Univariate GLM procedure (GENSTAT 15, VSN International, Hemel Hempstead, UK).

Although the presence of the supplement block did not reduce the incidence of fresh scratch injuries when aggressive behaviour is highest on d 1 of mixing sows, injuries were significantly reduced on d 3 (Table 1). This suggests there was a reduction in aggressive interactions as a result of the presence of the block; however, there was no change observed in aggressive behaviours recorded during the 45 min period after feed was presented. Perhaps suggesting aggressive interactions were occurring outside of the time chosen for recording. Block disappearance in groups of sows (P < 0.05) housed with one block was 83 g/sow/d and 75 g/sow/d in groups of sows housed with two blocks. This experiment shows that the provision of a supplement block or blocks reduces the prevalence of fresh scratch injuries by d 3 of mixing unfamiliar sows into group pens.

 Table 1. Mean number of fresh scratch injuries scored on d 1 (day after mixing) and d 3 (3 days post-mixing) of sows in the Control group, One block and Two block and mean time (mins) sows' spent engaged in aggressive behaviour over the 4-day observation period

Treatment	Control	One block	Two block	SED <sup>A</sup>	P-value
Batches, $n = 10$					
Fresh scratch injuries					
d 1	8.3	8.7	8.3	0.78	0.845
d 3	1.7 <sup>b</sup>	1.1 <sup>a</sup>	$1.0^{\mathrm{a}}$	0.30	0.038
Aggressive behaviour (min/d)	0.1	0.4	0.1	0.01	0.965

<sup>A</sup>SED, standard error of difference of the means. <sup>a,b</sup>Means in a row with different superscripts differ significantly (P < 0.05).

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## Sucker and weaner pigs prefer brick shaped enrichment blocks over cube or wedge shaped enrichment blocks

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Environmental enrichment is provided to captive zoo animals to improve welfare by increasing the frequency and diversity of (natural) behaviours (Newberry 1995). According to De Jong *et al.* (2000), pigs require manipulable objects or bedding for stimulation. Most often this is provided in the form of straw. Under commercial conditions in Australia, providing such enrichments can be problematic since the conventional indoor, pig-pen environment is not compatible with straw or other bedding, in particular during the sucker and weaner phases. An enrichment block originally developed for sows during gestation, was reformulated to reduce hardness, and reduced in size and weight, to suit sucker and weaner pigs (Ridleys Corporation Ltd, Toowong, Qld, Australia). We investigated the effect of enrichment block shape on oro-nasal contact by sucker and weaner pigs with the block, and whether pigs habituated to the blocks over time. We hypothesised that the brick-shaped block would induce more contact than the cube or wedge as it would stimulate facilitative (co-operative) group behaviour and that interest in the blocks would be maintained for at least 24 h.

The experiment was conducted at the May Farm pig unit, Camden, NSW, with 19 Large White × Landrace litters containing 197 piglets from 10 days to 9 weeks old. Litters remained together throughout the experiment, with weaning occurring at about d 26. Litters were allocated at random to one of three block-shape treatments: (1) Cube; (2) Brick; or (3) Wedge. Pigs and enrichment blocks (one block per pen, fixed on 10 mm thick steel rod) were weighed weekly, and blocks were replaced weekly with the same treatment shape. Within litters, the number of pigs observed to interact oro-nasally with the enrichment block was recorded from video (AHDI Mega Pixel Cameras and AHD 1080P Digital Recorder, CCTV Central, Mount Waverley, Vic., Australia) using a point sample technique. Four focal pigs per litter were also marked enabling quantification from the video of bout duration of interactions with the blocks. Interactions were recorded on each minute over the first 30 min after block replacement, on each minute over the first 5 min/h for the next 23 h after block replacement, and on each minute over the first 5 min/h on the fourth day after block replacement. Behaviour data were analysed using Generalised Linear Mixed Models while weight data were analysed with Linear Mixed Models in REML (GENSTAT 17, VSN International, Hemel Hempstead, UK).

Brick-shaped blocks attracted more oro-nasal contact (17.0% probability during observations) than cube and wedge shapes (13.2% and 12.7%, respectively; P = 0.002). While oro-nasal contact with the blocks was relatively infrequent before pigs were ~25 days old, thereafter there was a steady increase in interactions (P < 0.05). Further, the frequency of oro-nasal contact was greater (P < 0.001) if blocks were 'fresh' (i.e. during the first 24 h) compared to 4 days old, suggesting habituation to blocks occurred. From 25 to 60 days of age, the duration of oro-nasal bouts by focal pigs with the blocks was always longer (P = 0.014) during the first 30 min of exposure to a fresh block, than for the remainder of the first 24 h or on the fourth day after block replacement. The findings thus suggest habituation may have occurred as quickly as 24 h after the block introduction. The decrease in block weight within weeks was not affected by block shape (P > 0.05), nor was the decrease in block weight associated with pig weight change (P > 0.05).

Our data suggest sucker and weaner pigs preferred brick- to cube- or wedge-shaped blocks, and that habituation may have occurred after 24 h. Oro-nasal contact by sucker pigs with the blocks predominately commenced in the fourth week of lactation, suggesting that enrichment blocks may not be needed in the sucker stage until the fourth week of lactation. The brick-shape preference may be due to the wider surface available for oro-nasal contact, where multiple pigs could simultaneously interact with the block, stimulating facilitation of rooting/nosing behaviour. We speculate that simultaneous interaction with the brick-shaped block may be similar to a litter co-operatively massaging the sow's udder before suckling bouts.

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## The provision of lucerne to sows evoked greater arousal in response to an anticipatory behaviour test

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Environmental enrichment is thought to be beneficial for pigs. Exposure to enrichment develops resilience to future stressful events by exposing animals to mildly stressful experiences leading to adaptation (Crofton *et al.* 2015). Rodents housed in enriched environments had greater corticosterone concentrations (Benaroya-Milshtein *et al.* 2004) and grower pigs housed in enriched environments had greater salivary concentrations of cortisol (de Groot *et al.* 2000). We investigated the effect of providing lucerne to sows before parturition, on their anticipatory response to the introduction of a feed cart and of a feeding event. We hypothesised that sows provided with lucerne would produce greater concentrations of cortisol and would perform more postural changes than sows that were not provided with lucerne.

Large White x Landrace sows were loaded into conventional farrowing crates approximately 7 days before parturition. Sows in the lucerne treatment were provided with 1 kg of lucerne hay daily, and sows in the control treatment had no lucerne hay. Sows were fed manually twice daily from a feed cart at 7 a.m. and 3 p.m. After 3 days in the farrowing crate sows were subjected to an anticipatory behaviour test. On the day of the test blood was collected via an indwelling ear vein catheter every 15 min for 60 min before, and 60 min after, the introduction of the feed cart and feeding event. Behaviours were recorded via video for analysis. At 3 p.m. (normal feeding time) the feed cart was moved into the room and left for 3 min. After 3 min the sows were given their daily feed ration. For behavioural analysis there were n = 10 control sows and n = 11 lucerne sows and n = 9 control sows. Plasma was assayed for cortisol using a radioimmunoassay (MP Biomedicals LLC, Santa Ana, CA, USA). Cortisol data were analysed using a repeated-measures analysis of variance and behavioural data with a general linear model in SPSS v24.0 (IBM, Armonk, NY, USA). Data that were not normally distributed were  $\log_{10}$  transformed before analysis and all data are presented as back transformed means. The concentration of plasma cortisol was significantly greater for the sows receiving lucerne (Fig. 1) compared to sows that did not receive lucerne (P < 0.05). This effect was only seen after the introduction of the feed cart, therefore, the provision of lucerne altered the cortisol response of the animals to the feeding event. Sows that received lucerne displayed a greater number of behavioural transitions than the sows that did not receive lucerne (P < 0.05).

Our data suggest that sows provided with lucerne display greater levels of arousal in anticipation of the arrival of a feed cart and a feeding event, both in terms on hypothalamic-pituitary-adrenal axis and behavioural activity. This is in keeping with previous reports on the effects of enrichment. Therefore, our data indicate that lucerne may be an effective enrichment for sows before farrowing.



Fig. 1. The mean plasma concentration of cortisol (ng/ml) for sows for 60 min before, and 60 min after, the introduction of a feed cart. Arrow indicates the introduction of the feed cart.

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### Group-lactation housing from 7 or 14 days post partum: effects on sow behaviour

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Group-lactation housing may improve sow welfare by increasing the opportunity for the sow to move about and express social and maternal behaviours (van Nieuwamerongen et al. 2014). This study tested the hypothesis that group-housed lactating sows would (1) engage in less aggression and more positive social interactions when mixed at 7 rather than 14 days post partum; and (2) show better maternal behaviour (nursing behaviour and sow-piglet interactions) than sows housed in farrowing crates. One hundred and twelve sows (Large White × Landrace, PrimeGro<sup>™</sup> Genetics, Corowa, NSW; Parity 1 to 7) and their litters were allocated to one of three treatments over four time replicates: (1) group lactation (GL) from 7 days post partum (GL<sub>7</sub>, n = 48 sows); (2) GL from 14 days post partum (GL<sub>14</sub>, n = 48 sows); or (3) farrowing crate (FC; n = 16 sows). All sows farrowed in standard farrowing crates, where FC sows remained until weaning. However, GL<sub>7</sub> and GL<sub>14</sub> sows and their litters were transferred from farrowing crates to GL pens (one pen of five sows at 8.4 m<sup>2</sup>/sow and one pen of seven sows at 8.1 m<sup>2</sup>/sow, per treatment and replicate) at 7 and 14 days post partum, respectively. All treatments were weaned at 28 days post partum. Treatments were balanced for sow parity, weight and litter size, and there were no treatment differences in litter weight and sex ratio, or within pen/crate variation in these variables. For GL pens, two focal sows per pen (one high and one low parity) were video recorded from 0700 to 1700 on the day after mixing (D2) and 2 days before weaning (preweaning, PW). Of the four FC sows per replicate, two were video recorded on the same days as GL<sub>7</sub> (FC<sub>7</sub>) and two on the same days as GL<sub>14</sub> (FC<sub>14</sub>). Data gathered continuously from video records included sow aggressive and nursing behaviours while sow time-budgets were observed using point sampling at 5-min intervals. Behaviours were analysed with LMM and GLMM models (SPSS v23.0, IBM, Armonk, NY, USA), with the main effects of housing (GL v. FC), litter age at mixing (7 v. 14 days) and observation day (D2, PW), as repeated-measures and controlling for pen and replicate as random factors (Table 1). There were no significant interactions between main effects.

Aggression (bites/knocks) between GL sows increased by nearly 40% from d 2 to 26; however, there was no effect of age at mixing on aggression.  $GL_{14}$  sows engaged in more positive interactions with conspecifics (i.e. nosing sow) than  $GL_7$  sows. Sows spent less time lying in GL pens compared to FC and interacted with piglets more frequently. Whilst there was no difference in number of nursings between GL and FC treatments, GL housing disrupted nursing behaviour, as evidenced by reduced proportion of successful nursing bouts, a tendency for increased sow terminated bouts and a longer inter-nursing interval, compared to FC sows. Sow aggression and disrupted nursing behaviour in GL may result in compromised welfare and growth of sows and piglets, and is being investigated.

	G	L <sub>7</sub>	GL	-14	FC	7 A	FC	В 14		P-value	
	D2	PW	D2	PW	D2	PW	D2	PW	Housing	Day	Age
Bites/knocks <sup>C</sup>	8.38	13.8	9.50	15.1	N/A	N/A	N/A	N/A	N/A	0.03	0.62
Nosing sow <sup>D</sup>	0.002	0.003	0.006	0.01	N/A	N/A	N/A	N/A	N/A	0.18	0.01
Nosing piglet <sup>D</sup>	0.09	0.10	0.09	0.10	0.06	0.07	0.07	0.08	0.03	0.15	0.33
Lying <sup>D</sup>	0.69	0.62	0.63	0.58	0.73	0.69	0.63	0.58	0.02	0.05	< 0.01
Nursing <sup>C</sup>	15.1	12.1	11.6	11.0	15.1	11.9	13.8	10.4	0.81	< 0.01	< 0.01
Nursing success <sup>D</sup>	0.63	0.71	0.71	0.75	0.73	0.94	0.81	0.92	< 0.01	< 0.01	0.02
Sow terminated nursing <sup>D</sup>	0.52	0.74	0.62	0.90	0.30	0.58	0.46	0.64	0.06	< 0.01	0.06
Nursing interval <sup>E</sup>	52.6	62.5	71.4	76.9	52.6	55.6	58.8	66.7	< 0.01	0.04	0.06

Table 1.	Sow behaviour (per sow and pen/crate) in each housing treatment (Group lactation, GL; Farrowing crate, FC; Housing), relative to age of GL
	litter at mixing (7, 14; Age) and observation day (d 2 post-mixing, D2; 2 days pre-weaning, PW; Day)

<sup>A,B</sup>Observed on the same days at  $GL_7$  and  $GL_{14}$ , respectively. <sup>C</sup>Frequencies. <sup>D</sup>Proportion of observations or nursing bouts. <sup>E</sup>y = 1/x back-transformed means presented (min).

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## Efficiency to complete the maze test is decreased in young pigs enriched during the sucker phase

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Numerous studies have explored whether pigs housed in barren versus enriched environments differ in the ability to learn and hold shortterm memory (Kornum and Knudsen 2011). Most of the environmental enrichments provided in recent studies are not easily applied in a production setting, e.g. alternative penning systems and straw bedding (de Jong *et al.* 2000). In this study the authors used pig enrichment blocks that were specifically formulated for use with sucker and weaner pigs (Ridleys Corporation Ltd, Toowong, Qld, Australia). The enrichment blocks were malleable, edible, and degradable, at different stages of the sucker and weaner phase. It was hypothesised that providing enrichment in the sucker and weaner phases would improve pig cognitive ability, assessed via the pigs' ability to learn and navigate a maze.

At 1 week old, four focal piglets (Large White x Landrace) from 72 litters over three replicates (n = 288) were selected and allocated to either the enriched or barren treatment group. Enriched litters were given one enrichment block per four piglets in the litter, placed unfixed in the farrowing crate. Barren litters received no enrichment blocks. At weaning ( $18.7 \pm 0.1$  days), the litters and the four focal piglets from the litters were split into treatment groups, two into enriched and two into barren treatment pens. Thus there were four treatment groups per replicate consisting of: enriched during weaner and sucker phase, enriched during sucker phase only, enriched during weaner phases. During weaner phase, pigs were housed in groups of 24 and enriched pens were given one block per four pigs, unfixed, replaced weekly. Pigs were handled consistently across replicates and treatments. At 7 weeks of age, 18 pigs from each treatment group were exposed to a maze test. The maze arena measured 4.7 m × 2.0 m and consisted of an internal pathway constructed from metal mesh panels. This included two 'traps' ( $1.4 \text{ m} \times 0.75 \text{ m}$ ), designed to hinder pigs movement through the maze arena. Pigs could see two companion pigs through the maze, which incentivized their movement through the maze and a reward of whipped cream was provided at completion of the maze. Each pig was individually tested four times in the maze in 1 day. The time pigs took to emerge from the starting box, number of times traps were entered, the total time spent in the traps, and time to reach the end were recorded. Data were analysed using a general linear model in ASRemI (ASRemI v4, VSN International, Hemel Hempstead, UK), with treatment/test number as fixed effects and pig as the repeated-measure.

All pigs completed the maze except one. Pigs that were enriched as weaners, regardless of treatment grouping, emerged quicker from the starting box (P < 0.05). There were no effects of enrichment on the number of traps entered or total time spent in traps. However, there were trends for pigs that were enriched during the sucker phase taking longer to complete the maze than pigs housed in barren conditions during the sucker phase (Predicted Means  $41.7 \pm 1.1$  s and  $34.7 \pm 1.1$  s respectively, P = 0.053), and for females finishing quicker than males (Predicted Means  $34.4 \pm 1.1$  s and  $41.5 \pm 1.1$  s respectively, P = 0.056).

Enrichment had only minor effects on behavioural responses of the pigs in the maze test. However, an interesting trend was noted that pigs provided with enrichment in the sucker phase move slower through the maze. These pigs may have been less fearful when placed in the novel maze environment, and the decreased fear response may have allowed more explorative behaviour, hence the slower movement through the maze. Similarly, the gender difference in time taken to navigate the maze could indicate a superior learning ability of females or simply a greater motivation to reach the reward provided. The impact of enrichment on cognitive function and the use of the maze as a cognitive test to determine the effects of enrichment on pigs requires further study.

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## Seasonal effects can be separated from other challenges in the pig environment using time series analysis

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The pig environment can be quantified through the mean performance of a contemporary group (CG), adjusted for systematic and genetic effects (Li and Hermesch 2016). The objective of this study was to use time series analysis to decompose CG estimates into the seasonal, long-term trend and residual components generally observed in time series data. It was hypothesised that seasonal effects can be partitioned from the other environmental challenges that are simultaneously captured in CG estimates of average daily gain.

Production records from 1999 to 2013 were available from a commercial herd of Large White pigs located in Queensland, Australia  $(n = 31\ 230)$ . Bodyweight averaged 90.9  $\pm$  9.9 kg (mean  $\pm$  s.d.), measured at an average age of  $127.9 \pm 5.1$  days. Defined by birth month, there were 167 CG with an average size of 187 pigs. Using ASReml (Gilmour *et al.* 2009), CG estimates for average daily gain were derived using linear mixed models, fitting sex as a fixed effect, and additive genetic effect, CG and common litter environment as random effects. An additional model was evaluated to account for minimum monthly temperatures of test month (MinT; data from www.bom.gov.au) using splines (model described in Guy *et al.* (2017)). The CG estimates from each model were decomposed using the 'stl' function in R (v3.3.2, R Foundation, Vienna, Austria).

The CG estimates ranged from -67 to +55 g/d, and -52.0 to +37.9 g/d when adjusted for MinT. Figure 1 shows these estimates decomposed into seasonal, trend and residual components. The seasonal contribution accounted for between -26 to +21 g/d. Pigs born in April were born in the best growing environment, while October was the most challenging environment. Even though the CG estimates adjusted for MinT had a smaller range than unadjusted estimates, a seasonal component was still extracted, ranging from -11.9 to +10.5 g/d. This demonstrates that temperature, represented by MinT, accounts for some, but not all, seasonality for this Queensland herd. However, this is may vary depending on herd location.

The trend component ranged from -19 to +14 g/d, and described the gradual changes in environmental conditions over time. The residual component ranged from -33 to +33 g/d, and can be interpreted as irregular, short-term perturbations. Although there is possible confounding, the trend and residual components together can be seen as a measure of environmental challenges other than seasonal effects, including infection challenges. While decomposition may depend on parameter choice, different model parameters were explored and produced similar results. Therefore, decomposing environmental variability through time series analysis indicates that selection for improved robustness is partly for improved response to seasonal fluctuations, and partly for other environmental challenges, which may need to be considered



Fig. 1. Contemporary group (CG) estimates (— unadjusted and --- adjusted for minimum monthly temperatures), decomposed into seasonal, trend and residual components using time series analysis.

separately for genetic improvement of traits such as disease resilience. **References** 

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### Modifying procedures to assess immune competence in mature boars

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Survival of progeny through to slaughter age is a key driver directly impacting on profitability and animal welfare within the Australian Pork Industry. Vaccinations against diseases causing mortality, such as *Actinobacillus pleuropneumoniae* (APP), are not always effective, suggesting a proportion of animals are responding poorly to vaccination. Immune responsiveness, the body's ability to respond to foreign antigens and render it harmless, involves a complex network of factors (Mallard *et al.* 1992). Since it is not possible to identify all of the genes that contribute to enhanced immune competence, an alternative strategy is to consider immune competence as a quantitative trait with a measurable phenotype (Hine *et al.* 2012). Procedures using test antigens (Mallard *et al.* 1992; Wilkie and Mallard 1999) have been developed to assess immune competence phenotype in pigs, combining measures of an animal's ability to mount both an antibody mediated immune response (AMIR) and cell mediated immune response (CMIR). This study tested the hypothesis that similar procedures, using commercial vaccines rather than test antigens to induce measurable responses, could be used to assess immune competence in mature boars. Use of commercial vaccines removes the requirement for test antigens to be registered for use in food-producing animals.

To assess AMIR, nine mature boars were bled on d 0 to establish base line levels of anti-tetanus toxoid specific immunoglobulin G1 serum antibody before being vaccinated with Ultravac 5-in-1 (Zoetis, Rhodes, NSW, Australia), containing tetanus toxoid antigen. Boars were vaccinated again at d 21 and bled on d 30 to measure secondary antibody responses. An in-house ELISA was developed (Miller *et al.* 2008), to measure antibody levels, represented as a sample to positive (S/P) ratio calculated for each boar at each time point. Delayed type hypersensitivity (DTH) reactions to vaccine preparations were measured to assess CMIR in the same nine boars. The DTH reactions were measured as the increase in skin fold thickness at the injection site 48 h after the vaccine was injected intradermally. Two injection sites were investigated: (1) the base of left ear and (2) the perineal area. On d 30 after vaccination, each boar received intradermal injections of either Ultravac 5-in-1 or Equivac T (Zoetis<sup>®</sup>), both containing tetanus toxoid antigen. At each injection site the double skin fold thickness (mm), recorded in triplicate using a Harpenden Skinfold Caliper (Bowers Group, Burgess Hill, UK), was assessed both pre- (d 30) and 48 h post-injection (d 32). For CMIR, the phenotype analysed was the average value of the three replicates for skin thickness at each time point, fitting a mixed model where time (d 30 v. d 32), Site (Ear v. Perineum) and Antigen (Ultravac v. Equivac) were considered as fixed effect.

The antibody response was significant (P < 0.0001) between d 0 (S/P ratio:  $0.05 \pm 0.07$ ) and d 30 (S/P ratio:  $0.71 \pm 0.07$ ) demonstrating the vaccination induced a measurable antibody response. The DTH reaction was also significant (P < 0.0001), with average skin thickness increasing from d 30 ( $4.03 \pm 0.25$  mm) to d 32 ( $6.16 \pm 0.25$  mm). The DTH reaction measured at the base of ear ( $3.06 \pm 0.43$  mm) and perineal site ( $2.97 \pm 0.43$  mm) were not significantly different (P > 0.05), indicating that both sites are suitable for DTH testing. There was no significant difference in the magnitude of DTH reactions observed for Ultravac ( $5.31 \pm 0.21$  mm) and Equivac ( $5.63 \pm 0.21$  mm) antigens. In conclusion, a testing procedure based on the use of commercially available vaccines to induce measurable immune responses was developed to assess immune competence in mature boars.

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## Breeder dam parity does not affect lifetime reproductive performance

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Progeny born to primiparous sows contribute significant performance variation which impacts herd feed conversion efficiency. Compared to progeny from multiparous sows, the progeny of gilts are lighter at birth and weaning (Hendrix *et al.* 1978), have reduced lifetime growth rates (Rehfeldt and Kuhn 2006) and a greater susceptibility to disease (Miller *et al.* 2013). With gilts making up one quarter of the breeding herd (Koketsu 2007), selection of replacements from these gilt litters is likely, however little is known about the impact of dam parity on subsequent reproductive performance. The objective of this study was to compare the performance of breeders born to primiparous sows *v*. multiparous sows, with the null hypothesis that there is no difference in performance between breeders born from gilt or sow litters.

Performance records of 1034 breeders from first litter sows, and 4721 breeders from multiparous sows that farrowed at SunPork Farms, Tong Park Piggery, during 1 January 2006 to 31 December 2015, were included in this study. Data was mined from existing herd records (EliteHerd, Genetic Solutions Ltd, Palmerston North, NZ). Selection criteria of the 5755 breeders was broad, and was based on any gilt that entered the herd, successfully farrowed, and had a traceable pedigree. Analysis included; pedigree information, age at mating, reproductive performance from Parity 1 to 7, and removal information. Data was cleaned by tracking each sow within the herd recording software correcting obvious entry errors or discarding the sow if error was not obvious. Data was analysed using GENSTAT (GENSTAT 18.1, VSN International Ltd, Hemel Hempstead, UK). A general linear model ANOVA was used to analyse continuous variables (age at first mating, reproductive data, etc.) with dam parity (gilt *v*. sow) as the treatment factor. Chi-squared ( $\chi^2$ ) analysis was used to assess discrete variables (reasons for removal).

Breeders from primiparous sows were 1 day older at first mating than those from multiparous sows (Table 1). In the first parity there was no significant difference in total born per litter or number weaned, however, there was an increase (P < 0.05) in the number of stillborn piglets per litter, and wean-to-oestrus interval was extended compared to breeders of multiparous sows. In the second parity, breeders from gilts had an increased total born. Dam parity had no effect on the age and parity at which breeders were removed from the herd, nor was there any impact on the reasons for removal (not shown). Effects on stillborns and wean-to-oestrus interval may be a reflection of lower muscle fibre numbers in gilt progeny, reducing body size, uterine size and contractile ability, and decreased body reserves. The absence of differences in stage and reason for removals is unexpected, given known health effects, but may reflect poorer gilt progeny not being considered for selection.

This study suggests producers do not need to be concerned with selecting breeders from gilt litters as no difference was found in performance between gilt and sow litters.

 Table 1. Age at first mating (Age 1<sup>st</sup> Mate), reproductive performance, and parity (RemPar) and age (RemAge) at removal of breeders compared by dam parity, Gilt (Parity 1) or Sow (Parity 2+)

Dam parity	Age 1 <sup>st</sup> Mate	TB P1	SB P1	#W P1	WOI P1	TB P2	RemPar	RemAge
Gilt	228.8 <sup>a</sup>	11.25	1.67 <sup>a</sup>	9.91	8.76 <sup>a</sup>	11.69 <sup>a</sup>	4.31	890.4
Sow	227.5 <sup>b</sup>	11.15	1.56 <sup>b</sup>	9.88	7.61 <sup>b</sup>	11.45 <sup>b</sup>	4.36	893.8
SED	0.61	0.09	0.06	0.05	0.27	0.11	0.08	11.11
P-value	0.027	0.27	0.047	0.49	< 0.001	0.037	0.54	0.76

<sup>a,b</sup>Means in a column with different superscripts differ significantly (P < 0.05). SED, standard error of difference of the means; TB P1, total born/litter parity 1; SB P1, stillborn/litter parity 1; WOI P1, wean-to-oestrus interval parity 1; TB P2, total born/litter parity 2.

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## Early lifetime performance parameters affecting selection and reproductive success in gilts

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The sow replacement rate in Australia is 56.1%, with the average parity at which a sow is culled, currently sitting at 4.1 (Australian Pork Limited 2013). There are several key reasons for premature sow turnover, with failure to express pubertal oestrus and poor reproductive performance during the early parities considered a major cause for removal. Most on farm selection criteria for replacement gilts focus on gilt attributes at selection into the breeding herd. However, including criteria from early lifetime performance parameters such as birthweight, weaning weight, and pre-weaning growth may aid in selecting gilts with a higher probability of reproductive success (Knauer 2016). The hypothesis of this study was gilts that are born heavier and which do not have any growth deficits during the pre-weaning or post-weaning period will have a higher probability of (1) being selected into the breeding herd, and (2) displaying pubertal oestrus, resulting in at least one successful mating.

From January 2014 until March 2015, individual weights at birth and d 21 were recorded on 10 480 multiplier gilts (Large White × Landrace, PrimeGro<sup>TM</sup> Genetics, Corowa, NSW, Australia) born at the genetic supply unit of a large commercial pig producer located in southern New South Wales, Australia. As a matter of routine recording, the date of birth, parity and gestation length of the dam, as well the number of total piglets born in the litter, were known for each individual gilt. Post weaning weights were recorded 2 weeks after weaning on a subset of 3288 gilts. Of the 10480 gilts included in the project, 8852 (84.5%) gilts were selected to enter the breeding herd and were sent to five different sites on the one farm. A subset of selected gilts had data for weight (n = 7446) and P2 backfat (n = 3399) at selection. Of the gilts selected, 7612 (72.6%) were mated and 6870 (65.5%) farrowed at least once. The significance of early weights and development for the probabilities (0/1) of a gilt being selected (SEL), mated (MATE) and successfully farrowing at least one litter (FARR), was investigated using stepwise logistic regression (PROC LOGISTIC, SAS v9.4, SAS Institute Inc., Cary, NC, USA). Apart from year-quarter of birth (season) and dam parity group (four levels: Parities 1, 2, 3-5, 5+), early in life explanatory variables submitted to the procedure included deciles (allocated within birth year-quarter) and were all treated as class effects. Within each explanatory variable, gilts with missing records were allocated to a separate class. Site (five levels) was also submitted for MATE and FARR. Only factors significant at P < 0.05 were included in the final models for each trait. The significance of the difference between each factor level and the reference decile was assessed via the odds-ratio. The sixth decile was the reference level for each explanatory variable. The corresponding probability of gilts being selected, mated and farrowing for deciles significantly different to Decile 6 were back-calculated using the corresponding odds and the odds-ratios.

Season of birth was the most significant factor contributing to SEL (P < 0.0001). After season, gilts from the lowest decile for birthweight were 5% less likely to be selected than gilts in Decile 6 (P < 0.001). Gilts in the lowest 10% for 21 days weight and post-weaning gain had a 14% lower probability for SEL compared to Decile 6 (P < 0.0001). The probability of SEL was also reduced by 6% for gilts in the lowest 30% for weaning age (P < 0.05). Season and site were the most significant factors affecting both MATE and FARR (P < 0.0001). Relative to the reference level, gilts in the lowest 10% for pre-weaning gain, the lowest 20% for weight at selection, and the lowest 30% for P2 backfat at selection also had a reduced MATE, with corresponding reductions of 9%, 7% and 10%, respectively (P < 0.01). Gilts in the four lowest deciles for pre-weaning gain had a 10% reduction in the probability of FARR, whereas gilts in the highest 20% for post-weaning gain, or the top 30% for P2 backfat at selection, had an increased probability of FARR (+10% and +8%) relative to the reference level. The association between P2 backfat and FARR was generally linear. Being in the low deciles for birthweight, 21 days weight and post-wean gain were detrimental for surviving to selection or meeting the minimum weight requirement at selection age. Once selected, being in the lower deciles for pre-weaning gain, selection weight and P2 backfat at selection decreased the probability of being mated and successfully farrowing a litter whereas, being in the higher deciles for post-wean gain and P2 backfat at selection significantly increased a gilts' probability of success.

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### Caffeine increases gestation length on a commercial farm

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In recent years, pressure for sow prolificacy has been applied to increase the number of pigs sold/sow/year. However, selection pressure on sow prolificacy and the resultant increase in litter sizes has adverse consequences, most notably increased stillbirths, lower birthweights, lower viability at birth and hence poor survival (Quiniou *et al.* 2002). Small scale studies have demonstrated that maternal caffeine supplementation on the day before parturition decreased stillbirths and increased piglet temperature at birth (Superchi *et al.* 2013, 2016). Recent data (B. A. Dearlove and W. H. E. J. van Wettere, unpubl. obs.) indicated that 3 days of caffeine supplementation (6 g/d) before parturition extended gestation length and increased piglet temperature shortly after birth. The aim of the current study was to determine the impact of caffeine supplementation of sow diets before parturition on gestation length and the incidence of stillbirths under commercial conditions. We hypothesised that caffeine would extend gestation length and reduce stillbirths.

Large White, Landrace, and Duroc sows (n = 348, parity  $2.85 \pm 0.10$ ) were allocated to one of three treatment groups, Control (CTL, n = 122), 3 g/d caffeine (CAF3, n = 111) and 6 g/d caffeine (CAF6, n = 115). Treatment began at d 112 of gestation and continued until farrowing (CAF3: mean  $3.74 \pm 0.14$  days treatment; CAF6:  $3.91 \pm 0.14$  days treatment). Gestation length, total born, born alive and born dead, and piglet survival to processing (processing occurred within the first 24 h of life), d 4 and d 21 post partum were recorded. Statistical analysis was performed using a mixed general linear model (SPSS v24.0, IBM, Armonk, NY, USA) with treatment, breed, parity and whether the piglets were purebred or crossbred with total born as a covariate. Sow was treated as a random effect. Data from individual piglets were treated as repeated-measures on the sow in similar mixed linear models including the sow as a random factor. Data are presented as estimated means  $\pm$  s.e. from mixed models. Total born ( $12.04 \pm 0.39$ ), born alive ( $10.92 \pm 0.19$ ) and stillbirths ( $0.93 \pm 0.15$ ) were unaffected by treatment. Treatment also did not affect piglet survival to processing ( $96.76 \pm 0.86\%$ ), d 4 ( $91.50 \pm 1.34\%$ ) and d 21 post partum ( $89.54 \pm 1.88\%$ ). Gestation length was increased in the CAF6 group compared to the CTL group (CTL:  $115.22 \pm 0.21$ ; CAF3:  $115.76 \pm 0.22$ ; CAF6:  $116.11 \pm 0.23$ ; P < 0.01) and tended to be increased in the CAF3 compared to the CTL group (P < 0.08). There was also a breed difference in gestation length with Landrace and Duroc sows having an extended gestation on the CAF6 treatment (Table 1).

The failure of caffeine to reduce stillbirths in this study may have been due to the intense supervision received under experimental conditions compared to commercial conditions (Superchi *et al.* 2013, 2016), suggesting that further studies should be conducted to confirm if caffeine supplementation does represent a commercial strategy to improve piglet survival. However, consistent with our previous data, gestation length was increased in response to caffeine supplementation. This is an important finding, as sows which farrow prematurely (<115 days) are likely to produce more stillborn and low viability piglets, with fewer of their piglets surviving to weaning (Vanderhaeghe *et al.* 2011). Maternal caffeine supplementation may, therefore, be a useful strategy for herds in which premature farrowing is a problem.

Table 1. Gestation length (d) for Large White, Landrace and Duroc sows receiving either no (CTL), 3 (CAF3) or6 (CAF6) caffeine a day from d 112 of gestation until farrowing

	CTL	3 g	6 g
Large White $(n = 111)$ Landrace $(n = 203)$	$114.67 \pm 0.33$ $115.30 \pm 0.19^{a}$	$115.47 \pm 0.29$ $115.59 \pm 0.27^{ab}$	$114.77 \pm 0.34$ $116.35 \pm 0.27^{\circ}$
Duroc $(n = 34)$	$114.42 \pm 0.44^{a}$	$115.13 \pm 0.47^{\text{ ab}}$	$115.99 \pm 0.43^{b}$

Data are represented as means  $\pm$  s.e. Significant differences within sow breed are highlighted using superscripts ( $a^{-c}P < 0.05$ ).

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## Sow dimensions increase with increasing parity but not with increasing litter size

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In most of indoor farrowing accommodations, sows are housed in crates. During mating and gestation many sows are in stalls, or are being fed in feeding stalls or in Electronic Sow Feeders. Increasing consumer awareness of sow welfare makes it important to ensure that sows do not risk injuries associated with crates or stalls that are too small (Pedersen 2015). Therefore, it is important that crates, stalls and feeding stations meet the space requirements of sows. Dimensions of Danish crossbred-sows were measured several years ago (Moustsen et al. 2011). Based on those measurements, recommendations for dimensions of crates, stalls, and pens were decided on. Body dimensions of sows increase with increasing parity but whether or not increased litter size leads to increases in body dimensions, or whether larger sows give birth to larger litters, has not been investigated. The objective of the present study was to determine whether body dimensions of Danish crossbred sows had increased (Moustsen et al. 2011) and if larger sows gave birth to larger litters. The average parity of the measured sows was 3.2, ranging from Parity 1 to 10. Sows farrowed, on average, 8.8 days before measuring. Length, width, height and depth of ~40 hyper-prolific crossbred (Large White × Landrace) sows, in 10 production herds, were measured (Table 1). The sows were in a standing position on a level surface when measured. Length was measured with a carpenter's rule as a straight line from snout to behind hind legs. Three measurements were taken and the average used in analysis. Depth, and width at the shoulders was measured using a specially developed calliper. Depth was measured in the middle section of the sow between the front and hind legs, from the dorsal to the ventral surface, and can be used as an estimate of the width of the sow's body when lying. The height was measured using the carpenter's rule. Number of liveborn and stillborn piglets were recorded. Data were analysed using generalized mixed models (SAS EG 7.1, SAS Institute Inc., Cary, NC, USA) with length, width, height and depth in turn as response variables, and parity group (1, 2 to 3, 4+) and total born (< or > than the median of the parity group) as explanatory variables. Herd was included as a random effect. In the statistical analysis, means were compared by Type 3-test. Length, width, height and depth increased significantly with increasing parity (P < 0.001), however, within parity group there was no difference in body dimensions between sows having litter size less or higher than the median of the group.

It is important that housing facilities allow the sows to stand up and lie down unhindered and enables all piglets easy access to the udder. Therefore, knowledge of sow dimensions is important. The average litter size in Danish production herds has increased over the years. However, this study concluded that there was no significant correlation between the measured sow dimensions and litter size. In addition, sow dimensions were similar to a previous study (Moustsen *et al.* 2011). It is expected that recommendations based on Moustsen *et al.* (2011) will continue to ensure sows' ability to stand up and lie down unhindered.

	Parity 1 (n	n = 114)	Parity 2 to 3	( <i>n</i> = 130)	Parity 4+ (	<i>n</i> = 161)	P-value
Total born (n)	$16.2\pm0.3^{a}$	11–22	$18.3\pm0.4^{\text{b}}$	9–25	$19.1 \pm 0.3^{b}$	13–25	< 0.001
Length (cm)	$169\pm0.8^{\rm a}$	155–183	$181\pm0.6^{\rm b}$	170-190	$192^{\rm c} \pm 0.5^{\rm c}$	180-203	< 0.001
Height (cm)	$83\pm0.4^{\rm a}$	76–89	$87\pm0.3^{\mathrm{b}}$	81–92	$90 \pm 0.3^{c}$	84–96	< 0.001
Width (cm)	$38\pm0.2^{\mathrm{a}}$	34-42	$40\pm0.2^{\mathrm{b}}$	36-45	$42 \pm 0.3^{\circ}$	38–47	< 0.001
Depth (cm)	$57\pm0.3^{a}$	51-63	$61\pm0.3^{b}$	56-66	$65\pm0.1^{c}$	60–71	< 0.001

Table 1. Litter size and body dimensions of 405 Danish crossbred sows in 2017. Mean ± s.e. and 5th to 95th percentiles (*in italics*)

<sup>a-c</sup>Significant differences between parity groups within rows.

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## A single intravenous injection of Kisspeptin evokes an increase in luteinising hormone in 15- and 18-week-old gilts

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Kisspeptin is a neuropeptide essential to the regulation of the gonadotrophin releasing hormone (GnRH) neuroendocrine system (Lehman *et al.* 2010). Sheep, rodents and humans deficient in the gene that codes for kisspeptin, KISS1, never reach sexual maturity and kisspeptin is a key regulator of seasonality in sheep (Goodman *et al.* 2007). Expression of KISS1 mRNA is attenuated in the non-breeding season of sheep and injection of kisspeptin during the nonbreeding season can stimulate oestrus in ewes (Smith 2012). While the role of kisspeptin in the neuroendocrine control of reproduction in humans, sheep and rodents is well established, little is understood about its role in pigs. We hypothesised that a single intravenous injection of kisspeptin would evoke an increase in plasma concentrations of luteinising hormone (LH) in 15- and 18-week-old gilts and that this increase would be of similar magnitude in each age group.

We conducted the experiment over two replicates at the Roseworthy Piggery, Roseworthy, SA. Gilts were identified and tagged at birth. One week before commencement of the experiment, gilts were transferred to individual pens to acclimatise to the experimental environment. Gilts were then fitted with indwelling ear-vein catheters. In Replicate 1, 18-week-old gilts were allocated to three treatments (n = 6 per treatment). They were injected with either saline, 5 mg of Kisspeptin 10 (Pheonix Pharmaceuticals Inc., Burlingame, CA, USA) or 10 mg Kisspeptin 10. In Replicate 2, 15-week-old gilts were allocated to two treatments (n = 6 per treatment). They were injected with either saline or 10 mg of Kisspeptin 10. On the day of the experiment blood was collected every 15 min for 1 h before injection and then every 15 min for 6 h after injection. Data were analysed using a repeated-measures analysis of variance in SPSS v24.0 (IBM, Armonk, NY, USA).

The concentration of LH after kisspeptin injection was greater than before kisspeptin injection for 15- and 18-week-old gilts (P < 0.05) (Fig. 1). Panel A (Fig. 1) shows that the increase in LH in 18-week-old gilts after a 5 mg injection of kisspeptin was not significantly different from the increase in LH after a 10 mg injection of kisspeptin. There was no significant difference in the increase in LH between 15-week-old gilts given 10 mg of kisspeptin or 18-week-old gilts given 5 mg or 10 mg of kisspeptin.

Our data indicate that the neuroendocrine production of LH in pigs is stimulated by kisspeptin. This effect is evident in pigs that are 15 weeks old. Further research into the role of kisspeptin in the control of reproduction and seasonality in pigs in warranted.



**Fig. 1.** Mean luteinising hormone concentration in response to a single intravenous kisspeptin (Kiss) injection for 18- (*a*) and 15-week-old (*b*) gilts. A 5 mg or 10 mg dose was given to 18-week-old gilts and a 10 mg dose was given to 15-week-old gilts. Injections were given at time 0 as indicated by the arrow. Blood samples were collected every 15 min commencing 60 min before injection and concluding 6 h after injection.

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## Lifetime sow productivity is influenced by both body protein and body fat reserves after first-litter weaning

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Sow longevity and lifetime productivity is a key factor in determining herd productivity and lowering production costs. Sow replacement rates are high in Australia, with an average of 59% sow turnover (Benchmarking Report 2015, R. Campbell, pers. comm.) and the trends do not indicate any improvement. The main cause of sow turnover includes low litter size and reproductive failure, with many sows being culled prematurely. Hughes *et al.* (2010) suggested management practices that reduce sows entering the herd with excessive body reserves and are too heavy, would promote sow longevity. This study tested the hypothesis that sow longevity and lifetime performance is affected by body reserves in young sows.

In a study herd of 1637 weaned, Parity 1, first-cross sows (Large White × Landrace, PrimeGro Genetics<sup>TM</sup>, Corowa, NSW, Australia), body reserves of protein and fat at mating following first litter weaning (Parity 1) were determined based on predictive equations (Smits *et al.* 2017). Sows were ranked independently for body protein and fat at Parity 1 mating as the lowest 25%, (LOW, n = 409), median 50% (MED, n = 819), and the highest 25% (HIGH, n = 409) cohort. The effects (mean  $\pm$  s.e.) of body protein or fat cohort at mating on lifetime performance were determined by one-way GLM univariate ANOVA (SPSS v21.0, IBM, Armonk, NY, USA) using body fat or body protein mass, respectively, as a linear covariate. Data excludes any sow culled or removed before mating as a Parity 1 sow. Sows ranked with a HIGH body protein mass, adjusted to a constant body fatness, produced fewer litters and less piglets (P < 0.05) in their lifetime performance, with MED or HIGH fat reserves, adjusted to a constant body protein, producing more piglets and lasting longer in the herd (LOW fat *v*. HIGH fat; P = 0.053). These results differ to other publications (Clowes *et al.* 2003), and this could be due to different genetics and feeding regimens resulting in sows with different protein and fat masses between studies.

In conclusion, our data provides evidence that sows with body protein not exceeding 26 kg, and body fat mass no less than 41 kg, last longer and are more productive than large lean sows. Furthermore, we suggest that breeding sows need to be individually fed throughout life so that body reserves, particularly fat levels, can be maintained as suggested by Bunter *et al.* (2010).

	Sow	P value		
LO	W (avg 26.0 kg)	MED (avg 28.8 kg)	HIGH (avg 31.5 kg)	Main effect protein
Lifetime litters	$4.4\pm0.1^{\rm y}$	$4.2\pm0.1^{ m y}$	$3.6\pm0.1^{\rm x}$	< 0.001
Lifetime live born	$49.0 \pm 1.3^{ m y}$	$47.3\pm0.9^{\rm y}$	$40.1 \pm 1.3^{\mathrm{x}}$	< 0.001
Lifetime total born	$54.5\pm1.5^{\rm y}$	$52.8\pm1.0^{\rm y}$	$45.5 \pm 1.5^{\rm x}$	< 0.001
	Sc	ow body fat mass at Parity 1 matin	g <sup>B</sup>	
LO	W (avg 31.4 kg)	MED (avg 40.6 kg)	HIGH (avg 51.0 kg)	Main effect fat
Lifetime litters	$3.8 \pm 0.1^{x}$	$4.2\pm0.1^{ m y}$	$4.1\pm0.1^{\mathrm{x,y}}$	0.013
Lifetime live born	$42.5 \pm 1.3^{x}$	$47.0\pm0.9^{\mathrm{y}}$	$46.4 \pm 1.3^{ m y}$	0.016
Lifetime total born	$47.7 \pm 1.5^{\rm x}$	$52.9 \pm 1.0^{\mathrm{y}}$	$52.0 \pm 1.5^{\mathrm{y}}$	0.014

Tabla 1	The offect of core body	protoin and fat mass at B	Davity 1 mating a	n lifetime com	norformonoo
Table 1.	The effect of sow bouy	notem and lat mass at 1	arity r mating o	n meunie sow j	periormance

<sup>A</sup>Main effect body protein analysed with body fat (mean BF:  $41.1 \pm 0.2$  kg) at Parity 1 mating included as a linear covariate. <sup>B</sup>Main effect body fat analysed with body protein (mean BP:  $28.8 \pm 0.1$  kg) at Parity 1 mating included as a linear covariate. <sup>x,y</sup>Within rows, mean values differ *P* < 0.05.

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## Effect of parity and stage of gestation on maternal growth and feed efficiency of gestating sows

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Previous research in regards to gestating sow nutrient requirements (Noblet *et al.* 1990) has been used to develop models based on the sow's body condition, parity and stage of gestation. However, data are limited pertaining to the application of these models in current commercial sow herds to determine maternal growth and efficiency of feed usage of modern sows. Therefore, the objective of this study was to evaluate the effect of parity and stage of gestation on maternal weight gain and efficiency of feed use in gestating sows from a commercial sow farm. A total of 712 females were group-housed from d 5 to 112 of gestation and individually fed with electronic sow feeders (ESF). Feed intake and bodyweight (BW) were recorded daily throughout gestation. Gilts (Parity 1) and sows, received 27.2 and 30.5 MJ ME per d based on set feeding strategies. Gilts and sows received 2.0 and 2.3 kg per day throughout the course of gestation. Maternal weight gain, not including products of conceptus, and feed efficiency was predicted using a series of equations to model nutrient utilisation in gestation (Dourmad *et al.* 2008). Data were divided into three parity groups and gestation was divided into three periods. Averages for each period were reported for all predictions with the exception of G : F, where the median for each period was reported. Data were analysed using PROC MIXED procedure in SAS (v9.4, SAS Institute Inc., Cary, NC, USA).

Parity 2 sows had the greatest (P < 0.05) energy use for maternal protein and fat deposition in all stages of gestation while Parity 1 sows had a negative energy balance during the final stage of gestation (Table 1). At every stage of gestation, maternal gain decreased with parity (P < 0.05). Regardless of parity, maternal average daily gain (ADG) decreased (P < 0.05) from d 39 to 74 before increasing (P < 0.05) during the final stage of gestation. Parity 1 sows had the greatest (P < 0.05) maternal ADG in all gestation periods. Parity 1 sow maternal G : F decreased (P < 0.05) in each sequential period of gestation. Parity 1 sow G : F was greater (P < 0.05) than Parity 2 and 3+ sows in most gestation periods.

Overall, this study and subsequent prediction models show how stage of gestation and parity affect growth of different tissue pools, sow maternal BW, and feed usage throughout the course of gestation. Further research is needed to investigate these differences and if there is an impact on subsequent performance.

	5–39	Day of gestation 40–74	75–109	Probability, P
Energy available	for maternal protein and li	pid deposition, KJ		
Parity 1	$5035^{ax} \pm 90.7$	$2895^{bx} \pm 90.7$	$-145^{cx} \pm 90.7$	< 0.001
Parity 2	$7395^{ay} \pm 104.4$	$5775^{\rm by} \pm 104.4$	$3016^{cy} \pm 104.4$	< 0.001
Parity 3+	$5431^{az}\pm86.3$	$4251^{bz} \pm 86.3$	$1397^{cz} \pm 86.3$	< 0.001
ADG (kg)				
Parity 1	$0.47^{ax} \pm 0.011$	$0.27^{bx} \pm 0.011$	$0.41^{cx} \pm 0.011$	< 0.001
Parity 2	$0.32^{ay}\pm0.013$	$0.04^{\mathrm{by}}\pm0.013$	$0.15^{\rm cy} \pm 0.013$	< 0.001
Parity 3+	$0.23^{az} \pm 0.011$	$-0.04^{bz} \pm 0.011$	$0.34^{cz} \pm 0.011$	< 0.001
G:F <sup>A</sup>				
Parity 1	$1.29^{ax} \pm 0.110$	$0.67^{bx} \pm 0.110$	$-1.24^{cx} \pm 0.110$	< 0.001
Parity 2	$0.67^{ay} \pm 0.127$	$-0.04^{\mathrm{by}} \pm 0.127$	$1.13^{\rm cy} \pm 0.127$	< 0.001
Parity 3+	$0.88^{ay} \pm 0.105$	$-0.34^{by}\pm 0.105$	$0.17^{\text{cz}}\pm0.105$	< 0.001

Table 1.	Maternal growth and	feed efficiency of sows	as influenced by	parity and stage of gestation
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<sup>A</sup>Maternal feed efficiency is reported as G : F and was determined using the following equation: G : F = Maternal ADG (kg)/energy available for maternal deposition (kg).

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## Development of non-invasive methods to monitor the transfer of dietary volatile compounds in pigs

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The transfer of volatile compounds from the maternal diet to amniotic fluid and milk has been previously evaluated using gas chromatography–mass spectrometry (GC/MS) (Palou *et al.* 2015). Both of these maternal fluids have disadvantages associated with the sampling procedures (i.e. sow monitoring at farrowing and oxytocin injection). Blood sampling is another method used to evaluate the transfer of dietary volatile compounds; however, this procedure is invasive, which causes stress and discomfort in the animals. The aim of this study was to develop a non-invasive user friendly methodology to assess the transfer of essential oil (EO) volatile compounds from feed to saliva and carpal gland secretion in pigs. We hypothesised that the quantification of volatile dietary compounds to saliva and carpal gland secretions will be indicative of the amount consumed and the transfer rate in the pre-absorptive and post-absorptive stages, respectively.

This trial was conducted at the Herston Medical Research Centre. Twelve post-weaning piglets were individually penned and allocated into one of three treatments: (1) single dose of a mix of EO in feed (EOF, 0.45 mg/kg BW of the principal compound of each EO (EOPC)); (2) single dose of a mix of EO intravenously (IV) injected (EOIV, 32 µg/kg bodyweight (BW) of each EOPC); and (3) a control consisting of standard feed without EO. Treatments were provided with the morning meal. The EO comprised lemon ironbark, peppermint gum, nerolina, clove, thyme, cinnamon, oregano, geraniol and anethole. Saliva, carpal gland secretion and serum were collected at different time points: 5, 15, 30, 45, 60, 120 and 180 min after administration of the treatments. Saliva was collected by approaching the mouth of the pig with a sea sponge attached to tweezers. While piglets were distracted chewing on the sponge, carpal gland secretion was collected by a procedure consisting of a gauze sponge impregnated in distilled water to gently wipe the skin in two directions (top to bottom and left to right) repeated three times ('skin washing'). In order to collect multiple blood samples a catheter was surgically implanted in the external jugular vein of all piglets. Data was statistically analysed using ANOVA in Minitab 16 (Minitab Inc., State College, PA, USA), to evaluate the transfer of EOPC to saliva, skin washing and serum between the three treatments at each time point. Paired *t*-test was performed to evaluate differences in the concentration of EOPC between serum with saliva and skin washing in treated pigs. The EOPC were analysed by headspace–solid phase micro extraction-CG/MS.

Overall there were significantly higher (P < 0.05) concentrations of EOPC in saliva compared to serum in EOF piglets while significantly lower (P < 0.05) concentrations were found in saliva compared to serum in EOIV piglets. Additionally, the concentrations of volatile compounds in skin washings were significantly lower (P < 0.05) than in serum in both EOF and EOIV treatment groups. These results indicated that the high levels of dietary EOPC found in saliva of piglets in EOF treatment are explained by direct contact of saliva with the volatile compounds present in feed. The result suggests that the levels of dietary volatile compounds found in saliva after oral consumption of EO are indicative primarily of aerial transfer from feed contents in the gastrointestinal tract, which, in turn, may be related to recent intake. On the other hand, the EOPC levels found in skin washings were absorbed from the gastrointestinal tract first, transferred to blood (or lymph) and then secreted through the carpal glands. Thus, EOPC in carpal gland secretions would indicate postabsorptive transfer efficiency and stability in biological tissues. To the best of our knowledge, this is the first report that monitors the transfer of dietary volatile compounds to saliva and carpal gland secretions.

It was concluded that the data offered two new approaches of monitoring the transfer of dietary volatile compounds to maternal fluids: one related to pre-absorptive (saliva) and the other to post-absorptive (carpal gland secretion) events. These results are relevant to the understanding of maternal-offspring imprinting through maternal diets in mammalian species including humans. Further investigation is required to evaluate the correlation between levels of dietary volatile compounds found in saliva and skin washing with those found in milk and amniotic fluid in pigs.

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## Predicting oestrus and ovulation in sows using the vulva, cervical mucus and body temperature

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The most common method of oestrus detection in sows relies on observing behavioural changes indicative of sexual receptivity. This technique is time consuming, labour intensive and due to its subjective nature, often provides inconsistent results between observers (Soede *et al.* 2011). Consequently, it would be beneficial to identify alternative markers for oestrus, and more importantly for the onset of ovulation, by validating more quantifiable and objective measures. The hypothesis follows that fluctuations in vulva size, mucus composition and body temperature will occur within 24 h of ovulation and would be suitable measures to assist with the determination of the onset of oestrus.

In this pilot study, a range of biological markers were measured on 46 multiparous (mean parity  $\pm$  s.e.m.  $3.22 \pm 0.15$ , range = 2–5) Large White × Landrace sows over the anticipated period of oestrus, based on time post-weaning. Vulva size, vulva and ear temperature and cervical mucus pH, viscosity and crystallisation patterns, were recorded. Measurements were recorded at 12 h intervals from 3 days post-weaning to 2 days after the last observed oestrus behaviour. Vulva size was measured with a ruler and calculated as length × width. Cervical mucus was extracted using a Rocket cervical mucus syringe and split into three aliquots. One aliquot was air-dried on a microscope slide, observed at ×100 magnification and classified into six patterns based on the predominant shapes present (Abusineina 1962). The remaining aliquots were used to detect pH using test strips and viscosity length by measuring the stretch of mucus with a ruler (Rijnders *et al.* 2007). Internal vulva and ear canal temperatures were obtained using an infrared gun at a distance of 10 cm from the body surface and corrected for ambient temperature. The time of predicted ovulation was defined as 30 h before an increase in faecal progesterone (P4) as determined by ELISA, accounting for the 24 h hormone passage rate (Shaw and Foxcroft 1985).

The measurements were mapped to three time points; the onset of behavioural oestrus determined by the first instance of standing heat in response to back pressure, 24 h before predicted ovulation and the point of predicted ovulation (Table 1). Each marker was analysed using an ANOVA in GENSTAT 16 (VSN International, Hemel Hempstead, UK). Mucus pH (P < 0.001) decreased at the point of predicted ovulation to facilitate sperm survival during conception. The predominant mucus crystallisation pattern changed from large irregular fern shapes at the onset of behavioural oestrus to shortened, linear patterns 24 h before predicted ovulation (P = 0.013) potentially indicating a decrease in ionic compounds resulting from elevated oestrogen levels. There were no significant differences in vulva size, mucus viscosity, vulvar temperature or ear temperature detected between the time points.

These results indicated that cervical mucus properties have potential as an alternative oestrus detection tool in addition to existing monitoring programs. Further investigation is required to determine if using these markers can reduce the time for oestrus detection and to predict insemination timing results in satisfactory fertility.

Table 1.	Mean values for biological markers measured at the first instance of standing heat in response to back pressure and 24 h
	before and at the time of predicted ovulation

Onset of behavioural oestrus	24 h before predicted ovulation	Predicted ovulation (30 h before P4 peak)
$7.1 \pm 0.9$	$6.7 \pm 0.8$	$4.9\pm0.8$
$7.7\pm0.1^{\mathrm{a}}$	$7.7\pm0.1^{\mathrm{a}}$	$7.2\pm0.1^{\mathrm{b}}$
$0.5 \pm 0.1$	$0.4 \pm 0.1$	$0.6 \pm 0.1$
Large, irregular shapes	Short linear streaks	Short linear streaks
$30.9 \pm 0.5$	$30.7 \pm 0.4$	$30.5\pm0.5$
$32.6\pm0.5$	$31.6\pm0.5$	$30.5\pm0.4$
	Onset of behavioural oestrus $7.1 \pm 0.9$ $7.7 \pm 0.1^{a}$ $0.5 \pm 0.1$ Large, irregular shapes $30.9 \pm 0.5$ $32.6 \pm 0.5$	Onset of behavioural oestrus24 h before predicted ovulation $7.1 \pm 0.9$ $6.7 \pm 0.8$ $7.7 \pm 0.1^a$ $7.7 \pm 0.1^a$ $0.5 \pm 0.1$ $0.4 \pm 0.1$ Large, irregular shapes $30.9 \pm 0.5$ Short linear streaks $30.7 \pm 0.4$ $32.6 \pm 0.5$ $31.6 \pm 0.5$

Data presented as mean  $\pm$  s.e.m. <sup>a,b</sup>Within the same row, values with different superscripts differ significantly.

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## The pre-ovulatory luteinising hormone surge is affected by the sex ratio of a gilt's birth litter

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Female reproduction can be affected by exposure to excessive concentrations of androgens *in utero*, resulting in masculinisation (Veiga-Lopez *et al.* 2009). Gilts may be exposed to excessive concentrations of androgens *in utero* from developing male littermates, as occurs in rodents (vom Saal and Bronson 1978). Gilts from male-biased litters may be masculinised and present with decreased reproductive potential due to differences in the functioning of their hypothalamo-pituitary-gonadal axis (HPG) (Veiga-Lopez *et al.* 2009). We hypothesised that the preovulatory surge of luteinising hormone (LH) of gilts from male-biased litters would be delayed in onset and would have an attenuated amplitude compared to gilts from female-biased litters.

Large White x Landrace gilts were selected from male-biased (>60% males, n = 10) or female-biased (>60% females, n = 9) litters. From 19 weeks of age gilts were rehoused into groups of four and began boar exposure in a detection mating area for 1 h daily for detection of puberty. To synchronise second oestrus, gilts received 5 mL/d of an orally active progestogen, altrenogest, commencing 12 days after the detection of puberty. Once all gilts had expressed puberty the altrenogest was withdrawn. Four days after withdrawal of altrenogest blood samples were collected via indwelling jugular vein catheters every 4 h until the end of subsequent oestrus. Plasma was stored at  $-20^{\circ}$ C until required for LH assay using a double antibody radioimmunoassay. The assay sensitivity was 0.4 ng/mL and the intra and inter assay coefficients of variation were, 11.9% and 20.3%, respectively. A surge was defined as described by Barb *et al.* (1982). Data were analysed using a one-way ANOVA (SPSS v22.0, IBM, Armonk, NY, USA).

The onset of the LH surge was significantly delayed ( $56 \pm 3.3 v. 43 \pm 3.8 h, P < 0.05$ ) for gilts from male-biased litters compared to gilts from female-biased litters (Fig. 1). The duration of the LH and the time from the onset of the LH surge to peak amplitude was significantly less ( $30 \pm 2.2 v. 38 \pm 1.2 h$ , and  $6 \pm 0.9 v. 12 \pm 1.4$ , respectively, P < 0.05) for gilts from male-biased litters than for gilts from female-biased litters. There was no difference between groups in the amplitude of the LH peak or the time from beginning sampling to reach the LH peak.

These data partially support our hypothesis in that the LH surge was delayed in gilts from male-biased litters but there was no difference in amplitude of the LH surge. Nonetheless, we present evidence that gilts from male-biased litters display a delayed LH surge, an attenuated duration of the LH surge and a reduced time from the onset of LH surge to the peak. Combined, these data suggest that the response of the HPG axis during oestrus is different between gilts from male-biased litters and gilts from female-biased litters. These differences may affect reproductive performance and therefore, with further research, the sex ratio could be used as a selection tool to improve the breeding herd.





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### The effects of the *in utero* environment on gilt performance

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Traditionally, the pork industry has focused most of its attention on the management of replacement gilts in the post-selection (16–21 weeks) phase. However, in many species it is now understood that the prenatal environment of an animal plays an equally important role as the pre-pubertal environment. In dairy cows, the age of the dam at first calving, the dam's milk yield and body condition score during gestation accounted for a significant proportion of the total phenotypic variance of calving interval and non-return rate of the daughter cows (Banos *et al.* 2007). There is growing evidence to suggest more research is needed on the long-term effects of the maternal environment. The aim of this study was to determine the efficacy of easily identifiable, early predictors of a gilt's lifetime reproductive performance. We hypothesised that the proportion of female to male fetuses would significantly affect the *in utero* environment in which a gilt develops, thereby affecting ovarian development and overall performance.

This study used 166 Large White × Landrace gilts, which were identified and weighed at birth, weaning, week 18 and week 21. Reproductive tracts were collected post slaughter at 21 weeks of age and antral follicles on both ovaries were counted and classified as either small (1–3.99 mm) or large (>4 mm). An analysis of variance (ANOVA), unbalanced design (GENSTAT 15, VSN International, Hemel Hempstead, UK) was used to determine the effect of proportion of females in the gestated litter (low, <40%; normal, 41–59%; high, >60%) on surface antral follicle counts, birthweight and growth rates.

The ovaries of gilts from female-biased litters contained a higher total number of surface antral follicles (P < 0.05) (Table 1) than those of gilts from litters with low proportions of females. The total small follicle counts were higher (P < 0.05) in gilts from femalebiased litters. Large antral follicle counts did not differ between females from male-biased, normal or female-biased litters. Female birthweight was reduced in female-biased litters in comparison to litters with low proportions of females ( $1.16 \pm 0.02 \text{ kg v}$ .  $2.09 \pm$ 0.02 kg; P < 0.05). Female piglets born into litters with low proportions of females showed an increased average daily weight gain to weaning (P < 0.05) and remained significantly heavier until slaughter (P < 0.05).

The results of this study support the hypothesis that the *in utero* environment in which a gilt develops significantly affects the birthweight, and subsequent growth performance of an individual animal. Sexual maturity appears to be occurring independently of the *in utero* sex ratios, as there was no significant difference observed in large antral follicle numbers. However, the significantly higher number of small antral follicles observed in gilts from female-biased litters, indicates that these animals may have a higher ovarian reserve in comparison to gilts from normally distributed or male-biased litters.

	Low <40%	Proportion of females Normal 41–59%	High >60%
No. of gilts	39	81	46
No. of follicles			
1–3.99 mm	$116.7 \pm 11.3^{\mathrm{a}}$	$138.1 \pm 7.9^{\rm a}$	$165.6 \pm 10.6^{\rm b}$
>4 mm	$23.1 \pm 2$	$18.5 \pm 1.4$	$20.7 \pm 1.9$
Total follicle number	$136.4 \pm 11^{a}$	$155.5 \pm 7.8^{a}$	$184.7\pm10.4^{b}$
Piglet weight			
D 1 weight (kg)	$2.09\pm0.02^{\rm a}$	$1.62\pm0.01^{\rm b}$	$1.16 \pm 0.02^{\circ}$
Average daily gain (g)	$210.9\pm13.5^{\rm a}$	$165.9\pm9.5^{\rm b}$	$168.1\pm12.8^{b}$

Table 1.	Piglet d 1 weight, average daily gains from birth to weaning, number of small (1-3.99 mm) and large (>4 mm) antral follicles present on the
	ovaries of 21-week-old gilts from gestational litters with low (<40%), normal (41–59%) and high (60%) proportions of females

<sup>a–c</sup>Means in a row with different superscripts differ significantly (P < 0.05).

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# Can serum levels of anti-Müllerian hormone and oestradiol in juvenile gilts be used to predict future reproductive performance?

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The current process for selecting breeding gilts results in significant economic loss for the pig industry. In Australia, it is estimated that around 40% of selected gilts are culled before Parity 3 (Plush *et al.* 2016). Thus, there is a need for effective markers of future reproductive performance in young gilts.

We previously tested whether serum levels of anti-Mullerian hormone (AMH) and oestradiol (E2) in 80-day-old juvenile gilts would be useful markers of future ovarian and uterine properties and found that AMH was indicative of uterine capacity while E2 marked puberty attainment at 160 days of age (Steel *et al.* 2016). We also examined whether AMH and E2 in juvenile gilts aged 60, 80 and 100 days were predictive of mating and litter parameters over three parities. It was found that basal levels of AMH were not associated with mating and litter parameters and while E2 was associated with the proportion of piglets born alive and proportion stillborn at Parity 1 (Steel *et al.* 2017). The present study was conducted to determine the repeatability of our previous experimental results across different herds. It was hypothesised that, similar to our previous studies, E2 levels in juvenile gilts could be used to predict Parity 1 reproductive performance and, in contrast to other species, AMH could not.

Blood samples were obtained from 80-day-old Landrace x Large White gilts from two Australian commercial piggeries located in South-Eastern QLD (Farm A: PIC Australia<sup>TM</sup> Genetics, Grong Grong, NSW, Australia; n = 101) and in Southern NSW (Farm B: PrimeGro<sup>TM</sup> Genetics, Corowa, NSW, Australia; n = 187). Sera AMH and E2 were measured via competitive inhibition ELISA kits, CEA228Po and CEA461Ge, respectively (Cloud-Clone Corp., Katy, TX, USA). The mean mating age of gilts was 227.8  $\pm$  12.5 (s.d.) days of age at Farm A and 208.6  $\pm$  19.6 days of age at Farm B. Age at first heat (only at Farm A), first mating outcomes, gestation length, number of mummified, stillborn, and live piglets from the first litter and any culling information were recorded. Data was analysed using regression, restricted maximum likelihood and generalized linear model methods, with Farm as a factor, via the statistical package R (v3.3.3, R Foundation, Vienna, Austria).

Our two previously mentioned studies (Steel *et al.* 2016, 2017), were conducted only at Farm B. Similar to previous results, a single sample of AMH at 80 days of age was not predictive of Parity 1 mating, litter or culling parameters at both farms (P > 0.05). In contrast to our previous findings at Farm B, serum E2 levels were negatively associated with age at first heat at Farm A (b = -0.021, P = 0.011,  $R^2 = 0.10$ ), positively associated with proportion of piglets born alive at both farms (OR = 1.005, P < 0.001) and were negatively associated with the proportion stillborn at Farm A (OR = 0.991, P = 0.003), but not at Farm B (P > 0.05). The inconsistent results between farms could be due to genetic variation studies, but reasons for disparities between experiments on the same farm are currently unclear. However, it is evident that at a time in juvenile gilts when ovarian cells first become responsive to gonadotrophins, serum E2 levels, which likely influence the development of the female reproductive tract, are indicative of first parity outcomes. It should be noted that the sample size of the present study (n = 288) was much greater than that of the earlier studies (Steel *et al.* 2016: n = 48; Steel *et al.* 2017: n = 72).

The findings of the present study validate those obtained previously showing that serum AMH levels in 80-day-old gilts are not predictive of first parity outcomes. The relationship between serum E2 levels at 80 days of age and the proportion of stillborns and piglets born alive at Parity 1 warrants further investigation.

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## Commercial evaluation of a mating in lactation protocol

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Alternative management of the sow and her litter in conventional farrowing accommodation to uncouple mating from the weaning process has been investigated over recent years in Australia. The main rationale behind this research was to enhance the weaning process for the piglet without compromising sow productivity. Extending weaning age or gradual weaning through intermittent sucking can help piglets adapt to the weaning process, resulting in a decrease in the incidence and/or severity of the post-wean growth check (Kuller *et al.* 2004). However, increasing weaning age will decrease the number of litters per sow per year and gradual weaning will result in some sows cycling whilst they are still lactating, leading to an increase in sow non-productive days (Downing 2015). In order to improve the piglet weaning experience in combination with maintaining sow performance, mating sows whilst they are still lactating may be a viable option. The aim of this study was to compare subsequent reproductive outcomes between sows that were mated during lactation and those that were mated after weaning under commercial production conditions.

This study was conducted over a 12 month period between August 2015 and July 2016. The lactation oestrus (LO) induction protocol consisted of sow and piglet separation (placement of a solid board within the farrowing crate that separated the piglets from the sow) along with fence line boar exposure during the last week of lactation. All sows were monitored for signs of oestrus and were mated in the farrowing crate by artificial insemination if they displayed standing oestrus. Those sows that did not display oestrus during lactation were mated at their first standing oestrus after weaning. Subsequent reproductive outcome data was analysed using GLM analysis or Chi-squared ( $\chi^2$ ) (SPSS v24.0, IBM, Armonk, NY, USA). The percentage of sows (Parity 3.1 ± 0.10) that had a lactation oestrus was 40%. Sows mated during lactation (responders, n = 166) had a lactation length (LL) of 29.8 ± 0.37 days and a wean to re-mating interval (WRI) of  $-1.3 \pm 0.34$  days compared to a LL of 28.8 ± 0.28 days and WRI of  $6.5 \pm 0.27$  days for those sows mated after weaning (non-responders, n = 253) (P < 0.05). The farrowing rate for responders was 78% compared to 88% for non-responders ( $\chi^2 = 7.99$ ; P < 0.05) and piglets born alive was  $10.7 \pm 0.46$  for responders and  $11.2 \pm 0.41$  for non-responders (P > 0.05). The number of sows that displayed oestrus during lactation dropped during summer and those that were mated had a lower farrowing rate and piglets born alive compared to both responders mated during winter, spring and autumn and non-responders mated in summer and winter, spring and autumn (Fig. 1).

In conclusion, lactation length and season had a significant effect on the number of sows that responded to the LO induction protocol. Additionally, subsequent reproductive outcomes were negatively affected for sows mated during lactation in summer compared to those mated during lactation in other seasons. However, there was no difference in subsequent reproductive outcomes between sows mated in lactation outside of summer and those mated after weaning.



Fig. 1. Farrowing rate and born alive for non-responders (NR) and responders (R) according to season. S NR (Summer – Non-Responders); S R (Summer – Responders); WSA NR (Winter, Summer and Autumn – Non-Responders); WSA R (Winter, Summer and Autumn – Responders). a,bare significantly different from each other (P > 0.05).

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### Melatonin fed in early gestation increases fetal weight

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Uterine blood flow and placenta size are determinants of the amount of nutrients reaching the attached fetuses within the uterus. Inevitably, selection for prolificacy increases the number of fetuses *in utero* and, therefore, already limits nutrient exchange between the maternal and fetal circulations. Uterine capacity is considered to be limiting when the number of developing conceptuses exceeds 14 (Dziuk 1968). Consequently, the excessive intrauterine crowding occurring in a proportion of mature prolific sows between d 25 and 40 of gestation leads to limited placental development by d 30, and subsequently to intrauterine growth retardation (IUGR) and reduced birthweight of entire litters (Foxcroft 2012). Melatonin has been used in sheep models to induce umbilical vasodilation and increase the amount of blood flow and nutrients reaching the fetus (Thakor *et al.* 2010), providing a valuable treatment for pregnancies experiencing IUGR. The aim of this project was to determine whether supplementing diets with melatonin between d 25 and d 50 of pregnancy in gilts would increase fetal growth to d 50 of gestation.

Pre-pubertal gilts (n = 29) were induced to ovulate at 22 weeks of age using a combination of PG600 (Intervet America, Inc., Millsboro, DE, USA) and daily boar exposure. At their induced oestrus, gilts were inseminated twice (24 h apart) using commercial pooled semen doses (80 mL doses of  $3 \times 10^{-9}$  sperm per dose,  $\leq 4$  days old: SABOR Pty Ltd, Clare, SA, Australia). Days 25 to 50 of gestation, gilts were treated with either 5 mL canola oil (CTL), 18 mg melatonin in 5 mL canola oil (MEL18) or 36 mg melatonin in 5 mL canola oil (MEL36). Day 50 of pregnancy, gilts were slaughtered at a commercial abattoir. Reproductive tracts were dissected, and the number of corpora lutea (CL; ovulation rate), number and weight of fetuses, fetal crown–rump length, as well as placental weights were recorded. Statistical analysis was performed using a mixed general linear model (SPSS 24.0, IBM, Armonk, NY, USA) with treatment, replicate, fetal sex and gilt as fixed effects and litter size as a covariate. Data are presented as estimated means  $\pm$  s.e. from mixed models.

The number of fetuses recovered was not different among treatments ( $9.82 \pm 1.70$ ,  $8.34 \pm 1.30$  and  $8.93 \pm 1.60$ , for CTL, MEL18 and MEL36 respectively). Melatonin treatment did not affect CL, fetal and placenta weights combined, placental weights and crown–rump lengths (P > 0.05). Fetuses from the MEL36 group were heavier (P < 0.05) compared to the CTL group (CTL,  $65.77 \pm 4.78$  g; MEL18,  $73.42 \pm 5.08$ , MEL36,  $81.85 \pm 3.80$ ).

The current limited data from an experimental gilt model provides preliminary evidence that oral supplementation with melatonin from early to mid-gestation can increase fetal weight at d 50. Studies with larger numbers, and in situations of more extreme intrauterine crowding and limited placental development, are needed to confirm the benefits of using melatonin to increase litter weights when intrauterine crowding in early gestation is linked to subsequent litter-wide IUGR and low birthweight. Studies that follow the pregnancy to farrowing also need to be conducted to ensure the effects of melatonin on fetal weight are continuing through to birth after treatment ceases at d 50 of gestation.

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## Aerobic and algal treatment for piggery effluent and water reuse: design of an integrated wastewater treatment plant

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Anaerobically digested piggery effluent (ADPE) has high ammonia (NH<sub>3</sub>) and suspended solids concentration, which precludes using it as a substrate for the growth of most microalgae for effluent treatment, disinfection and sustainable biomass energy production. Concentrations of NH<sub>3</sub> of  $3630 \pm 1250$  mg NH<sub>3</sub>-N/L, chemical oxygen demand 8933 mg/L (Hu *et al.* 2013), and phosphate levels of 620 mg/L (Olguín *et al.* 2003) have been reported in ADPE. In the absence of oxidation of ammonium-N (NH<sub>4</sub><sup>+</sup>-N) to nitrate (NO<sub>3</sub><sup>-</sup>), some dilution will always be needed if used for algae culture. A recent review (Buchanan *et al.* 2013) recommended using an integrated wastewater treatment approach that incorporates a nitrifying aerobic treatment step to reduce the NH<sub>4</sub><sup>+</sup> concentration in the effluent without dilution thereby promoting algal growth for biomass energy production, to likely reduce greenhouse gas (GHG) emissions. The proposed integrated system also has the potential to increase pig productivity and profitability via improving the quality of effluent reused in piggery operations. The objective of this project is to construct an integrated on-farm aerobic-algal treatment system and evaluate the performance in terms of biomass energy production, reduced GHG emissions and higher quality effluent for reuse. This research has the potential to improve profitability via increased on-farm bioenergy production, availability of high quality reuse water, improved pig health and reduction in operating costs.

The Roseworthy Piggery in South Australia was chosen for the pilot study and preliminary effluent analyses were conducted to enable design of the integrated wastewater treatment plant (Fig. 1). Anaerobically digested piggery effluent from an existing lagoon is transferred to a reception pit and followed by aerobic reactor at programmed intervals. NH<sub>3</sub> is converted to NO<sub>3</sub><sup>-</sup> under aerobic conditions using naturally occurring populations of aerobic nitrifying bacteria. The resulting treated slurry with greatly reduced NH<sub>3</sub> load is then pumped into a sedimentation tank where the supernatant liquid phase is delivered to a makeup tank. Finally, effluent from the makeup tank is transferred into high rate algal pond (HRAP) for nutrient removal via growth of the algal biomass. The HRAP is mixed by an eight bladed paddlewheel and its hydraulic residence time is determined by the rate of slurry addition and/or change in HRAP depth (maximum operating depth 0.5 m). Treated HRAP effluent is returned to sump and then to anaerobic pond before land spreading. Through this direct demonstration, farmers can form an opinion about the feasibility of implementing an integrated waste system to their own operations. An integrated wastewater treatment system offers the potential to treat waste safely and provide additional on farm energy, while reducing GHG emissions and producing high quality water for reuse in piggery operations.



Fig. 1. Flinders University Roseworthy integrated aerobic-algal wastewater treatment research facility.

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## Assessing the suitability of microalgae biomass produced from piggery waste as a fertiliser

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The use of microalgae for the recapture of nutrients, water and bioenergy from the treatment of piggery effluent is a promising technology (Astals *et al.* 2015; Nwoba *et al.* 2016). The harvested microalgae biomass has the potential to be developed into a high quality, concentrated and balanced fertiliser due to its high nitrogen (N) and phosphorous (P) content that is easy to handle and transport (Mulbry *et al.* 2005). However, there is still uncertainty regarding the re-use of microalgae in terms of its impact on plant N uptake, dry matter production, N mineralisation, and soil microbial activity. The aims of this study were 2-fold: (1) to investigate the effectiveness of microalgae biomass to supply N to wheat in comparison with a synthetic N fertiliser, ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), and (2) to assess the release of inorganic N (potential N mineralisation rate) following the application of microalgae biomass to soil. Microalgae biomass grown on undiluted, untreated piggery anaerobic digestion effluent was obtained from the Algae R&D Centre, Murdoch University. First, a pot experiment having a 2 × 5 factorial arrangement of treatments was undertaken to test the feasibility of using microalgae as a fertiliser with factors being two N sources (microalgae biomass and NH<sub>4</sub>NO<sub>3</sub>) applied at five N equivalent levels (0, 10, 20, 40, 80 kg N/ha) for 8 weeks. The trial was planted with wheat (*Triticum aestivum L*.) and arranged in randomised block design with four replicates per treatment. Second, a laboratory incubation experiment was conducted to quantify the potential N mineralisation of the microalgae using the same experimental design for the pot trial but without the plant. Total dry biomass, root and shoot production were determined using standard methods. Soil mineral N was analysed colourimetrically using a modified hydrazine reduction method. The carbon dioxide (CO<sub>2</sub>) production (microbial activity) was measured using an infrared gas analyser.

Utilisation of the microalgae biomass as an alternative fertiliser significantly improved the overall wheat yield and shoot production (Fig. 1), particularly at higher application rates. However, shoot production was slightly lower in the plants receiving the microalgae when compared to the mineral fertiliser  $NH_4NO_3$ . The soil N mineralisation rate was positively correlated with the amount of microalgae biomass applied implying that the microalgae provides an available nitrogen source for plants but not to the same extent as  $NH_4NO_3$ . This is because a large proportion of the N in microalgae is in an organic form and needs to be mineralised by soil microorganisms before it is plant available. Consequently, an increase in microbial activities ( $CO_2$  production) was observed in the soil amended with the microalgae biomass. Further studies to determine the agronomic and economic value of microalgae as a fertiliser are needed.



**Fig. 1.** Effect of mineral fertiliser NH<sub>4</sub>NO<sub>3</sub> ( $\bigcirc$ ) and microalgae biomass ( $\bullet$ ) on shoot production (mean  $\pm$  s.e.m., where n = 3) for wheat when applied at different levels (equivalent to 0 to 80 kg N/ha).

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### Effect of feed wastage on piggery effluent characteristics

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Across the Australian pork industry, a 5% change in feed wastage is 82 000 tonnes feed/year, with a current value of approximately \$38m. While its importance is widely recognised, there are currently no practical robust methods to quantify feed wastage. Feed wastage influences feed efficiency, shed (manure), effluent management practices and effluent characteristics. Accordingly, the present study hypothesised that feed wastage could be estimated from effluent characteristics.

To relate feed wastage to effluent characteristics, the study used an innovative modelling and experimental approach to simulate different rates of feed wastage and assess its effects on piggery effluent. Quantities of pig feed (wheat and barley based grower diet), faeces, urine, flush water (clean bore water) and shed effluent (collected over 24 h in an agitated sump) were sampled from a commercial batch grower shed, housing 535 pigs (average 45 kg live weight at 13 weeks of age). Pre-determined proportions of these samples were mixed to simulate shed effluent having four different rates of feed wastage (Treatments A–D). Treatment A was composed of faeces, urine and flush water only, to simulate zero feed wastage. Treatment B was raw effluent discharged from the shed. Treatments C and D were composed of raw shed effluent with added feed, to simulate higher rates of feed wastage. The resulting samples were analysed to evaluate the total solids (TS), volatile solids (VS) and biochemical methane potential (BMP). Analyses of variance (ANOVA) followed by protected least significant difference (l.s.d.) testing were performed on the analysis results, at the 5% level, using GENSTAT 16.1 (VSN International, Hemel Hempstead, UK). The AUSPIG growth and production simulation model (Davies *et al.* 1998) was used to simulate the age, live weight, P2 back-fat and feed intake of the pigs in the trial shed over their entire growth cycle (wean-to-finish). The genotype settings and feed intake factors in the model were adjusted so that measured and predicted performance parameters (growth rate, P2 backfat and feed intake) were similar for the batch of pigs. For each of the four treatments, the extent of feed wastage was then estimated using the AUSPIG model (as reference for comparison) and separately by total solids mass balance.

Estimated feed wastage in the trial shed on the sampling day (Treatment B) was 4.2% from the mass balance calculations and 6.9% from the AUSPIG model. The difference between these two estimates would likely be indistinguishable with normal production data variability. This extent of wastage represents current industry best practice, supported by inspection of the shed indicating virtually no visible spilt feed. The analysis further confirmed that increasing levels of feed wastage resulted in an increased BMP due to the energy content of the waste feed, and also increased concentrations of TS and VS (Table 1). However, the increased feed costs associated with higher levels of feed wastage outweigh potential cost savings from increased methane recovery (higher BMP) for onfarm energy use because energy currently is a relatively minor contributor to total production costs (~4%) compared to feed (~60%).

Overall, the results support the stated hypothesis, showing that feed wastage consistently affects and can be estimated from effluent characteristics. A logical next step is to adapt industry-standard effluent characteristic models such as PigBal (Skerman *et al.* 2013) to also estimate feed wastage.

Parameter	Units	А	В	С	D	s.e.m.
Feed wastage (mass balance)	%	0.0	4.2	9.4	15.2	
Feed wastage (AUSPIG)	%	0.0	6.9	12.1	17.8	
TS	%	1.51 <sup>a</sup>	1.81 <sup>b</sup>	2.06 <sup>c</sup>	2.29 <sup>d</sup>	0.04
VS	%	1.24 <sup>a</sup>	1.46 <sup>b</sup>	$1.70^{\circ}$	1.93 <sup>d</sup>	0.03
BMP $(B_0)$	L CH <sub>4</sub> /kg VS	284.6 <sup>a</sup>	326.6 <sup>b</sup>	360.9 <sup>c</sup>	383.3 <sup>d</sup>	5.4

Table 1. Feed wastage and mean analysis results for treatments A, B, C and D

s.e.m., pooled standard error of mean. <sup>a-d</sup>Means in a row with different subscripts are significantly different (P < 0.05).

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# The effect of dam parity on growth, white blood cell count, haemoglobin and immunoglobulin levels of weaner pigs

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Dam parity affects the growth rate of finisher pigs and gilt progeny have lower growth rates than progeny from multiparous sows, although the magnitude of this effect varies between herds (Hermesch and Li 2013). Gilt progeny had lower levels of immunoglobulin (Ig) G and IgA at birth (Klobasa *et al.* 1986). However, differences in IgG and IgA were the reverse between piglets from gilts *v*. multiparous sows at 2 (IgA) and 3 (IgG) weeks of age, due to higher *de-novo* synthetisation of Ig in piglets from gilt litters (Klobasa *et al.* 1986). Similarly, Miller *et al.* (2013) found no dam-parity effect on multiple immune parameters measured in piglets. This study hypothesised that gilt progeny have reduced growth and similar haematological and immunoglobulin levels in weaner pigs in comparison to progeny from multiparous sows.

All traits were recorded from January 2013 until October 2014 in 799 Large White pigs that originated from 217 litters and 209 dams. Serum samples were taken at an age of  $37.7 \pm 3.4$  days and a weight of  $11.3 \pm 2.17$  kg after being weaned at  $27.1 \pm 2.4$  days with a mean weight of  $8.9 \pm 1.30$  kg. Capture ELISA were performed using purified Ig (Sigma Aldrich, Castle Hill, NSW, Australia) or known reference serum as standards and polyclonal antibody sets (Bethyl Laboratories, Montgomery, TX, USA). Quantitative analysis of the samples was executed using four parameter logistic fit (4PL) software. Dam parity and weekly batch were fitted as fixed effects for all traits using the SAS software (v9.4, SAS Institute Inc., Cary, NC, USA). Sex had significant effects on growth rate until weaning (GRW), white blood cell count (WBC) and haemoglobin (HGB). Age was fitted as a linear covariable for IgA. Dam parities above the sixth parity were defined as one level in the analyses.

The statistical power to detect differences between parities was large due to the large number of records and dam parity was a statistically significant effect for most traits in Table 1. Dam parity affected growth rate most, which was lowest for gilt progeny. For example, pre-weaning growth of gilt progeny was 20 g/d and 28 g/d lower than growth of progeny of second- and fourth-parity (P2, P4) sows. Similarly, growth from weaning to 5 weeks (GR5) was 25 g/d and 34 g/d lower in gilt progeny in comparison to progeny from P2 and P4 sows. These differences can be expressed relative to the mean (or standard deviation, s.d.) of each trait to make a comparison between traits possible. The growth gap of gilt *v*. P4 progeny was 9% of the mean (63% of the s.d.) for GRW and 16% of the mean (29% of s.d.) for GR5. Gilt progeny had superior WBC and HGB than progeny from multiparous sows. Further, IgA levels were not significantly different in gilt progeny compared to progeny from multiparous sows. The levels of IgG in progeny tended to increase with parity number and gilt progeny had significantly lower IgG levels than progeny from P3 or P6 sows; however, dam parity was not a significant effect overall.

These results illustrate the reduced capacity of younger sows to support the growth potential of their progeny. Haematological and immunoglobulin levels of weaner pigs were not inferior in gilt progeny.

Table 1.	Least square means (standard error) for dam parity (P1 to P6) of growth rate until weaning (GRW) and from weaning to 5 weeks (GR5)
	as well as white blood cell count (WBC), haemoglobin (HGB) and immunoglobulin (IgA, IgG) levels in weaner pigs

Trait	P1	P2	P3	P4	P5	P6	P-value
GRW	310 (3.75) <sup>a</sup>	330 (3.25) <sup>b</sup>	328 (3.65) <sup>b</sup>	337 (4.56) <sup>b</sup>	333 (5.67) <sup>b</sup>	330 (3.27) <sup>b</sup>	< 0.0001
GR5	$186(8.24)^{a}$	$211(7.14)^{b}$	205 (8.010) <sup>bc</sup>	$220(10.01)^{c}$	$206(12.45)^{bc}$	$224(7.17)^{c}$	< 0.023
WBC	15.5 (0.40) <sup>a</sup>	16.6 (0.35) <sup>bc</sup>	16.6 (0.39) <sup>ab</sup>	18.1 (0.49) <sup>c</sup>	$17.0 (0.60)^{bc}$	17.0 (0.61) <sup>b</sup>	0.0059
HGB	$117(0.78)^{a}$	$112(0.67)^{b}$	$114(0.75)^{bc}$	$113(0.94)^{b}$	116 (1.18) <sup>ac</sup>	$113(0.68)^{b}$	< 0.0001
IgA	$0.51 (0.025)^{a}$	$0.44 (0.021)^{b}$	$0.52(0.030)^{a}$	0.48 (0.037) <sup>ac</sup>	0.48 (0.037) <sup>abc</sup>	0.48 (0.021) <sup>abc</sup>	0.07
IgG	8.38 (0.46) <sup>a</sup>	8.77 (0.40) <sup>ab</sup>	9.66 (0.44) <sup>bc</sup>	9.73 (0.55) <sup>abc</sup>	9.48 (0.69) <sup>abc</sup>	9.91 (0.40) <sup>c</sup>	0.10

<sup>a-c</sup>Means in a row with a different superscripts differ significantly, P < 0.05.

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## The effect of parity on haemoglobin levels in sows prior to farrowing and in 1-day-old piglets

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A decreased haemoglobin level in piglets is associated with a decreased survival rate (Rootwelt *et al.* 2012). While haemoglobin levels have been studied in piglets, few studies have focused on sows. In sows, low iron levels were associated with reduced reproductive performance (Buffler *et al.* 2017). Haemoglobin levels in sows decrease with increasing parities (Gannon *et al.* 2011*a*). It has been suggested that the body haemoglobin reserves cannot be restored between parities (Auvigne *et al.* 2010). In this study it was hypothesised that with an increasing parity, both the sow haemoglobin and the piglet haemoglobin decrease.

In 2012 and 2013, haemoglobin samples were collected from 171 Landrace and 216 Large White sows with 581 litters on a breeding farm in the region of Adelaide, South Australia. The sow parity ranged from 1 to 5+, there were between 75 and 162 observations per parity. A droplet of blood was collected from sows  $5.0 \pm 2.7$  days before farrowing via the ear vein. The haemoglobin levels were estimated using the HemoCue Hb<sup>201+</sup> analyser (HemoVue AB, Angelholm, Sweden), which was validated by Gannon *et al.* (2011*b*). Some sows had observations in multiple parities, resulting in 568 observations on haemoglobin level. The haemoglobin levels of three piglets in each litter (selected visually as a light, medium and heavy piglet) were measured on 1-day-old piglets before iron supplementation based on the same procedure used in sows. The average haemoglobin level of the three piglets was used for analysis (n = 503). A linear model was fitted in R with haemoglobin measurement date (66 levels) and parity (five levels) added as fixed effects (R v3.3.2, R Foundation, Vienna, Austria). Breed was also tested as a fixed effect and was found not to be significant. The R function TukeyHSD was used to determine which means were significantly different from each other.

Sow haemoglobin ranged between 74 and 146 g/L, with a mean of  $111.6 \pm 12.8$  g/L. Average piglet haemoglobin ranged between 58 and 149.5 g/L, with a mean of  $103.3 \pm 15.1$  g/L. The linear model showed a decreasing trend of sow haemoglobin levels with increasing parity ( $R^2 = 0.27$ ), while the average piglet haemoglobin stayed relatively constant with increasing parity ( $R^2 = 0.12$ ) (Table 1).

This study showed that with increasing parity, sow haemoglobin level decreased, while piglet haemoglobin levels stayed constant. The haemoglobin levels found in this study were lower than found in the study of Gannon *et al.* (2011*a*). A haemoglobin level of 100 g/L has been suggested as the normal-range threshold for sows (Gannon *et al.* 2011*a*). In this study, the mean sow haemoglobin level was above this threshold for all parities. However, 6.8% of the sows in Parity 1 and 36% of the sows in Parity 5+ were below this threshold. Awareness of the increasing danger of anaemia in later parities could help identify problem cases earlier, thereby ensuring that sow health and welfare is not compromised.

	Parity 1	Parity 2	Parity 3	Parity 4	Parity 5+
Sow haemoglobin (g/L) Average piglet haemoglobin (g/L)	118.2 <sup>a</sup> (1.0) 101.5 <sup>a</sup> (1.4)	$111.1^{b} (1.1) 106. 8^{ab} (1.5)$	109.6 <sup>b</sup> (1.2) 103.3 <sup>a</sup> (1.6)	$107.1^{b} (1.3) 100.8^{ac} (1.7)$	$\frac{106.7^{b} (1.4)}{106.3^{a} (1.9)}$

Table 1. The least-squares mean (SE) level of sow haemoglobin and the average litter haemoglobin across parities

<sup>a-c</sup>Means in a row with a different superscripts differ significantly, P < 0.05.

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## The effects of selecting growing pigs for a high growth rate and low backfat on sow characteristics

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The effects of selecting for finisher traits on the development of breeding sows is not well understood (Lewis and Bunter 2013). It has been estimated by Hermesch *et al.* (2010) that for a 1 g increase in estimated breeding value (EBV) for average daily gain (ADG) of the growing pig, the sow weight (SWT) before farrowing increased on average by 0.32 kg. For a 1 mm decrease in backfat (BF) EBV of the growing pig, sow backfat (SBF) decreased on average by 1.56 mm. The aim of this study was to evaluate the effects of historical selection for a high ADG and low BF in growing pigs on the sows' weight, fatness and haemoglobin levels (Hb). It was hypothesised that sows with a higher genetic potential for lean meat growth as growing pigs are both heavier and leaner in each parity.

In 2012 and 2013 data were collected on 171 Landrace and 216 Large White sows with 581 litters. Sow weight, SBF, and Hb were measured ~5 days before farrowing, upon transfer to the farrowing facilities in Parity 1 to 5+. There were between 164 (Parity 1) and 75 (Parity 5+) observations per parity. PIGBLUP was used to obtain EBVs for ADG and BF on the growing pig, using own and relatives' records. Pedigree was available for 50 431 animals from 260 sires and 2055 dams born between 2004 and 2013. There were 563, 573 and 568 observations for SWT, SBF and Hb, with a mean (standard deviation; s.d.) of 266 (35.2) kg, 18.6 (3.6) mm, and 112 (12.8) g/L. The EBV for ADG ranged from -47.06 to 82.81 and the EBV for BF ranged from -2.63 to 0.19. The average SWT was 230, 253, 280, 297 and 308 kg from Parity 1 to Parity 5+. Observations that deviated more than 3 s.d. from the mean were excluded. Measurement date (65 levels), parity (five levels) and breed (only for SBF, two levels) were fitted as fixed effects in linear models for SWT, SBF and Hb using R (R v3.3.2, R Foundation, Vienna, Austria). In addition, litter weight and ADG EBV were fitted as linear covariables for SWT, ADG EBV was fitted as linear covariable for Hb, and BF EBV was fitted as linear covariable for SBF. Regressions within parity group were also estimated to evaluate the effect of EBV for ADG or BF on sow characteristics within parity.

Across parities, regression coefficients were only significant for BF EBV (Table 1). With a 1 mm decrease in BF EBV, sow fatness reduced by 0.6 mm. With a 1 g increase in ADG EBV in the growing pig, SWT increased by 0.32 kg and Hb decreased by 0.15 g/L in Parity 1. The effect of ADG EBV on SWT and Hb was not significantly different from zero in later parities. Lewis and Bunter (2013) found genetic correlations between weight at selection and weight across parities ranging from 0.54 in Parity 1 to 0.32 in Parity 5.

This study found that the EBV for ADG and BF affected SWT, SBF and Hb, although the magnitude of effects changed over parities. Until the first farrowing, gilts with higher genetic merit for growth had a higher SWT and lower Hb. Due to feed restrictions and other management strategies, sows might not be able to express their genetic potential for growth. The EBV for BF had a positive effect on SBF, but was lower than found by Hermesch *et al.* (2010). Feeding strategies might affect the observed relationship between growing animals and the mature sow herd. This relationship should be explored further for the development of selection strategies to improve growth of growing pigs while limiting mature size of sows.

Table 1.	The regression coefficients (SE) of EBV	for ADG or BF on sow	characteristics across	(additive model) an	d within (inte	eraction model)
		parity, significant effect	is in bold ( $P < 0.05$ )			

	Across	Parity 1	Parity 2	Parity 3	Parity 4	Parity 5+
ADG EBV on sow weight	0.02 (0.06)	<b>0.32</b> (0.12)	-0.07 (0.18)	0.07 (0.17)	0.00 (0.17)	-0.21 (0.17)
BF EBV on sow fatness	0.59 (0.26)	0.15 (0.52)	0.33 (0.80)	0.79 (0.80)	0.56 (0.82)	1.32 (0.82)
ADG EBV on sow Hb	-0.04 (0.03)	<b>-0.15</b> (0.06)	0.00 (0.09)	0.03 (0.08)	-0.04 (0.09)	0.01 (0.09)

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### Predicting body protein and body fat for breeding sows of a modern commercial genotype

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Modern sow genotypes have changed considerably since body composition studies were conducted over 25 years ago on Australian genotypes. Earlier predictive equations were developed on datasets of slaughtered gilts and first-litter sows (e.g. Mullan and Williams 1990) or used genotypes from the United Kingdom (Large White × Landrace crossed with Landrace × Meishan; Gill 2006). As part of a larger project to evaluate sow lifetime performance and longevity over several parities (Smits *et al.* 2017), we established predictive equations based on measured live animal data for body protein and fat of sows before mating for Australian maternal genotypes. The hypothesis was that predicted and actual tissues reserves were similar.

Over six replicates, six unmated gilts (40 weeks of age) and 52 mixed parity (1 to 9) sows at weaning were selected for the study (Large White × Landrace F1 cross, PrimeGro Genetics<sup>TM</sup>). Prior to slaughter, animals were measured for several traits including ultrasound (PieMedical, linear array probe 5 MHz) fat depth thickness measured at 65 mm from midline at last rib (P2) and 20 mm from midline at junction of the tail (LEG); loin muscle depth at P2; shoulder height; girth circumference at foreleg and last rib; live weight (LWT); and parity. After 24 h, carcasses were split into primals and measured for protein and fat content using dual X-ray absorptiometry (Suster *et al.* 2003). Viscera were collected from the abattoir and weighed full and empty, frozen and then ground, freeze-dried and analysed for protein and fat using chemical analysis methods. Live empty bodyweight was calculated from the empty cold carcass weight plus the estimated blood volume (7% of live weight). Regression was used to develop the predictive equations with the highest regression ( $r^2$ ) for protein and fat content in the empty live weight. The least significant effect was removed from the model containing all factors in a step-wise fashion to develop a parsimonius model.

The predictive equations described with the highest regression in the model were as follows:

Body Protein (kg) = 
$$6.13 + \alpha + 0.14 \pm 0.01 \times LWT - 0.18 \pm 0.05 \times P2 - 0.13 \pm 0.04 \times LEG (r^2 : 0.96, P < 0.05)$$
 (Eqn 1)

where  $\alpha = -2.16$  for parity 0; -0.83 for parities 1-2; 0.33 for parities 3-5; 0 for parities > 5

Body Fat (kg) = 
$$\alpha + 0.24 \pm 0.05 \times LWT + 1.07 \pm 0.24 \times P2 + 0.50 \pm 0.21 \times LEG - 37.3$$
 (r<sup>2</sup>: 0.79, P < 0.05) (Eqn 2)

where  $\alpha = 12.7$  for parity 0; 4.51 for parities 1–2; 1.69 for parities 3–5; 0 for parities > 5.

There was a high consistency between actual protein and fat contents in the empty bodyweight and predicted values (Table 1). Adding additional parameters such as loin muscle diameter, girth dimensions and shoulder height did not increase the accuracy of the equation for predicting body tissue mass when live weight was recorded.

These equations provide a valuable tool for predicting changes in body protein and fat reserves in this commercial genotype across a range of parities, weights and backfat measures.

## Table 1. Comparison of actual tissue values (mean ± s.e.) in the empty bodyweight of unmated gilts and sows with predictive body protein (BPROT) and fat (BFAT) tissue reserves

Parity	LWT (kg)	P2 (mm)	LEG (mm)	Actual BPROT (kg)	Actual BFAT (kg)	Predicted BPROT (kg) <sup>A</sup>	Predicted BFAT (kg) <sup>B</sup>
0 (6)	$193.0\pm5.8^{\rm C}$	$17.8 \pm 1.0^{\rm C}$	$21.0\pm1.0^{\rm C}$	$24.8 \pm 1.0^{\rm C}$	$48.4\pm3.1^{\rm C}$	25.9	50.8
1 (14)	$212.0\pm4.4$	$15.9\pm1.0$	$21.9\pm1.5$	$28.5\pm0.5$	$44.7 \pm 2.3$	30.3	45.4
2 (13)	$222.7\pm5.7$	$12.8\pm0.9$	$20.4 \pm 1.1$	$31.6\pm0.7$	$40.6 \pm 3.1$	32.6	43.8
4-5 (16)	$246.9\pm5.6$	$15.0\pm0.8$	$21.2 \pm 1.1$	$35.1 \pm 0.8$	$47.3 \pm 2.2$	36.8	49.5
7 (9)	$262.4\pm6.6$	$16.9\pm1.6$	$23.7\pm1.5$	$36.3\pm0.9$	$52.5\pm2.9$	38.0	54.7

<sup>A</sup>Body protein (kg) and <sup>B</sup>body fat (kg) in empty bodyweight, calculated from Eqns 1 and 2 above.

<sup>C</sup>Unmated at 40 weeks of age. (), Number of sows sampled in dataset.

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### The effect of pre-slaughter factors on meat quality varies between muscle cuts

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The Australian pork industry has focused on developing an eating quality pathway (Channon et al. 2016) to improve quality and consistency of pork; however, this has focused on conventional housed pigs with an average HCW of 74.9 kg. This study investigated the effect of housing type (H), carcass weight (W) and sex (S) on objective pork quality. The hypothesis tested was that housing type, carcass weight and sex, as individual and additive factors, does not affect objective quality of several pork cuts. A total of 384 Large White  $\times$  Landrace commercial pigs (PrimeGro Genetics<sup>TM</sup>, Corowa, NSW, Australia) were selected over eight replicates in a 2  $\times$  2  $\times$  3 factorial experiment with the main treatments being: (1) Housing: Conventional (CON) and deep-litter (DL); (2) Sex: Female or castrated pigs (IM); and (3) Carcass weight: Light 60-70 kg (L), Medium 70.1-80 kg (M) or Heavy 80.1-91 kg (HV). Methods are previously described by Lealiifano et al. (2017) with objective meat quality measurements (shearforce, pH, drip loss, L\*, a\* and b\*) conducted 24 h post-slaughter on Musculus longissimus thoracis et lumborum (loin), M. biceps femoris (silverside) and M. gluteus medius (rump) from the carcass's left side. Data was analysed using ANOVA (GENSTAT 16, VSN International, Hemel Hempstead, UK).

There was a significant main effect of H on the shear force values, such that overall meat cuts from DL were lower than meat cuts from CON pigs (Table 1). There was no effect of carcass weight on the objective meat quality measures of the loin. Shear force in the rump decreased as carcass weight increased (4.22 kg v. 4.02 kg v. 3.97 kg, SE 0.09 for L, M and HV, respectively, P = 0.012). The increase in carcass P2 with increasing weight could have affected the increased tenderness associated with heavier carcasses. The silverside drip loss increased with an increase in slaughter weight (3.19%, 3.41%, 3.64%, SE 0.002, P = 0.024 for L, M and HV carcass weights, respectively). The increased drip loss in the silverside could be due to increased muscle size and a slower chilling rate for the heavier carcasses. Loin objective quality was not significantly affected by S differences. IM had lower drip loss, higher pH in the rump and silverside (SS) compared to females (Rump Drip loss: 3.46% v, 3.96%, SE 0.01, P = 0.025; SS Drip loss: 3.12% v, 3.70%, SE 0.01, P = 0.005; Rump pH: 5.47 v. 5.44, SE 0.01, P = 0.005; SS pH: 5.53 v. 5.49, SE 0.01, P = 0.01 respectively). There were no significant interactions of shear force by cut type due to S, W, or interactions with H. Deep litter carcasses had an increased drip loss level in the silverside over the three carcass weights (L: 3.26% v. 3.12%, SE 0.27; M: 3.63% v. 3.18%, SE 0.27; HV: 4.10% v. 3.18%, SE 0.27 DL v. CON housed respectively P = 0.049). There were also significant H × W interactions (L: 44.99 v. 47.05, SE 0.42; M: 45.08 v. 45.83, SE 0.42; HV: 44.81 v. 46.32, SE 0.42 DL v. CON housed respectively P = 0.049) and S × H × W interactions (L IM: 45.62 v. 46.95, SE 0.59 respectively; L Female: 44.37 v. 47.15, SE 0.59 respectively; M IM: 45.30 v. 45.92, SE 0.59 respectively; M Female: 44.86 v. 45.74, SE 0.59 respectively; HV IM: 44.45 v. 46.84, SE 0.59 respectively; HV Female: 45.18 v. 45.80, SE 0.59 respectively P = 0.010) for L\* value in the silverside.

The hypothesis was rejected as these results show that housing had a significant effect on key objective meat quality measures, with carcass weight and to a lesser extent sex impacting meat quality. The effects however were varied between meat cuts.

Table 1.	Means and standard error of the difference (SED) for the effect of housing on pH, Minolta L*, drip loss (%) and
	shearforce (kg) in the loin, rump and silverside (SS)

Housing	Conventional				Deep Litter			
Objective measurement	Loin	Rump	SS	Loin	Rump	SS	SED	P-value
рН	5.45	5.49	5.53	5.44	5.42	5.49	0.01	< 0.001
L*	49.45	47.75	46.40	49.06	49.06	44.96	0.20	< 0.001
Drip loss (%)	5.97%	3.67%	3.16%	6.70%	3.74%	3.67%	0.15	0.010
Shear force (kg)	5.08	4.21	4.78	4.69	3.94	4.65	0.07	0.002

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## The incidence of pale soft exudative pork in entire male pigs from an Australian herd

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Pale, soft, exudative (PSE) pork is caused by protein denaturation resulting from high temperature and low pH conditions occurring in the early post-mortem period. This changes the light reflectance and water binding characteristics of meat. However, the description of PSE varies within the literature and the Australian Pork industry has no definition for PSE pork. Since the removal of the halothane gene from the Australian herd (Channon and Warner 2011), the incidence of PSE pork may have been underestimated, with no studies conducted to determine the prevalence of PSE pork as it is no longer seen as an issue. However, factors other than the halothane gene are known to lead to the temperature and pH conditions required to cause PSE (Scheffler and Gerrard 2007). The objective of this study was to present data for the incidence of PSE, based on published PSE classifications, in an audit of carcasses from an Australian pork farm.

Data from 198 randomly selected entire male, Large White × Landrace carcasses, sourced from one farm, were collected over 6 days in an Australian abattoir. The pH of the Musculus longissimis dorsi was measured at 0.75, 3 and 72 h post-mortem. A 50 g sample of the M. longissimus dorsi was collected at 72 h post-mortem to measure colour values for lightness (L\*) and was then subsequently used to measure drip loss over a 24 h period. Five published PSE definitions of varying parameters were selected to test the incidence of PSE pork in the samples collected (Table 1). The carcasses that fell outside these thresholds were considered PSE and are presented as a proportion of the consignment (%). The prevalence of PSE was highly variable depending on which thresholds were used. Only six carcasses in total (3%) had an L\* value of greater than 60, to be considered PSE in Category 1 (Warriss and Brown 1995). Although paleness is an attribute of PSE, the colour of fresh meat is not associated with eating quality and is a consumer preference, thus colour alone should not be used to determine PSE. Of the 198 carcasses sampled, 63.6% would be considered PSE based on Category 2 (Warner et al. 1997) for L\* and drip loss percentage. The fact that almost two-thirds of all carcasses had a drip loss over 5% highlights that water holding capacity can increase without L\* increasing to high levels. Determination of PSE using an ultimate pH threshold of pH 5.5 (Category 3; Gajana et al. 2013) resulted in 68% of carcasses being classified as PSE. However, ultimate pH is independent of the rate of pH decline, so can be low without causing protein denaturation (Scheffler and Gerrard 2007), therefore ultimate pH alone is a poor indicator of PSE. Categories 4 (Bee et al. 2007) and 5 (Offer 1991) take into account rate of pH decline hence are a more accurate presentation of circumstances in post-mortem muscle that cause PSE. Although the rates described are lower than Categories 2 and 3, high rates still existed with 28.3 and 39.4% respectively, highlighting a likely issue with PSE in Australian pork when 4 of the 5 published thresholds are applied.

The Australian pork industry must consider the development of an industry standard for describing PSE pork. The current data presents a high incidence of PSE across most definitions; thus, PSE could explain some eating quality variation in Australian pork.

Table 1.	The incidence	(%) of PSE i	n 198	carcasses ba	ased on	five	published	definitions	for	PSE
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Category	Source	Definition	Incidence of PSE (%)
1	Warriss and Brown (1995)	L* > 60	3.00%
2	Warner et al. (1997)	$L^* > 50$ ; Drip loss > 5%	63.60%
3	Gajana et al. (2013)	pHu < 5.5	68.20%
4	Bee et al. (2007)	pH(a) 3 h < 5.7	28.30%
5	Offer (1991)	Rate pH decline $\geq 0.02$ units/min <sup>A</sup> at 45 min	39.40%

<sup>A</sup>The rate of pH decline after 45 min post mortem, assuming starting pH was 7.

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## Prevalence survey of *Toxoplasma gondii* in hearts from Western Australian sows

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*Toxoplasma gondii* (*T. gondii*) is a protozoan parasite that infects warm-blooded animals, including humans, with an estimated 30% of the world's population seropositive for *T. gondii* (FAO/WHO 2014). Infection in humans can result from the consumption of uncooked or undercooked meat from infected animals. The World Health Organization has classified *T. gondii* as high priority in a report on the global burden of foodborne hazards based on the potential for serious ongoing pathological conditions (FAO/WHO 2014). *Toxoplasma gondii* from the meat of small ruminants, pork, beef and game ranked fourth in a global ranking tool of foodborne parasites by 'importance' and their primary food vehicle. Congenital toxoplasmosis may cause abortion, fetal death or central nervous system abnormalities, chorioretinitis and encephalitis in neonates.

The aim of this study was to estimate the prevalence of *T. gondii* in sow meat from Western Australia (WA). The sampling strategy was based on the numbers of sows required for a national baseline survey (a minimum of 300 samples would be required to give 95% confidence in a national prevalence estimate) using a randomised sampling framework and pig numbers proportional to annual production. Western Australia has 12% of the Australian sow population resulting in a total sample of 40 sow hearts from six free range and 14 intensive farms in WA. The sow hearts were collected at slaughter by abattoir employees, frozen to  $-20^{\circ}$ C and transported to the SARDI Food Safety and Innovation laboratory. Heart tissue underwent acid/pepsin digestion (Dubey 1998) followed by DNA extraction using the Wizard Genomic DNA Extraction Kit (Promega Corp., Madison, WI, USA). All DNA extracts were analysed using polymerase chain reaction (PCR) for a mammalian house-keeping gene (Frericks and Esser 2008) and the *T. gondii* 529 base pair fragment (Opsteegh *et al.* 2010).

*Toxoplasma gondii* DNA was detected in two samples from different intensive indoor production herds, resulting in an estimated prevalence of *T. gondii* in sow hearts from WA of 5% (s.d.  $\pm$  1.4%). An earlier pilot study using the same methodology (APL project 2014/506) estimated the prevalence in sow hearts (*n* = 92 from 62 herds) from south-eastern Australia at 9.8% (s.d.  $\pm$  3.1%). Combined, the prevalence of *T. gondii* in sow hearts is 8.3% (s.d.  $\pm$  3.2%) with no statistical significant difference between the prevalence estimates for WA and south-eastern Australia (*P* = 0.57). It should be noted that this data does not represent the national prevalence of *T. gondii* in Australian pork due to limited geographic range and numbers sampled. Despite this, the data does indicate that further investigation is warranted to determine the actual prevalence in the Australian herd so that strategies are appropriately implemented to minimise public health risks associated with the consumption of uncooked comminuted fermented meats.

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## Deep litter housed pigs have a faster pH decline compared to conventional housed pigs

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The Australian pork industry has focused on developing an eating quality pathway (Channon *et al.* 2016) to improve the quality and consistency of pork. However, studies have generally focused on conventional housed pigs with an average HCW of 74.9 kg. This study was conducted to identify the effect of deep litter compared to conventional housing systems, over three carcass weights and two sexes of pigs. The hypothesis tested was that housing (H), carcass weight (W) and sex (S), does not affect carcass objective quality.

A total of 384 Large White × Landrace commercial pigs (PrimeGro Genetics<sup>TM</sup>, Corowa, NSW, Australia) selected over eight replicates were used in a  $2 \times 2 \times 3$  factorial experiment with the main factors being: (1) housing: conventional partially-slatted birth to bacon housing system (CON) *v*. deep-litter grow-out system (DL); (2) sex: female (F) or castrated male pigs (IM) (Improvac<sup>®</sup>, Zoetis, Rhodes, NSW, Australia); (3) carcass weight specification (Trim 1): Light 60 to 70 kg, 8 to 16 mm P2 (L); Medium 70.1 to 80 kg, 8 to 16 mm P2 (M); Heavy 80.1 to 91 kg, 8 to 16 mm P2 (HV). Pigs of different sex were kept separately on the farms, during transport (~4 h) and in lairage. On arrival at the abattoir a sub-sample per housing type and sex were held in lairage with access to water before slaughter the next day. For each replicate, 48 pigs were selected within housing type, sex, and carcass weight specifications. Carcasses were chilled 24 h at 2°C. Carcass pH and temperature were measured (loin) at 45 min, 90 min, 3, 6 and 24 h post-slaughter using a pH meter (MPI, Topeka, KS, USA). Data were analysed using ANOVA (GENSTAT 16, VSN International, Hemel Hempstead, UK).

The effect of housing, carcass weight and sex on objective quality measures is reported separately (Lealiifano *et al.* 2017). DL carcasses were 1 mm fatter compared to CON (SE 0.26, P = 0.002), and were fatter at target carcass weights (L: 10.3 v. 9.8, SE 0.36; M: 11.8 v. 11.1, SE 0.36; HV: 13.5 v. 11.9, SE 0.36 DL v. CON respectively P = 0.023). This could be due to ambient temperature variability in the deep litter housed pigs. DL carcasses had a faster pH decline (Fig. 1*a*) perhaps indicative of increased glycolytic rate post-slaughter as a consequence of increased pre-slaughter stress or higher glycogen stores. Carcass temperature was unaffected by housing (P > 0.05). Rate of chilling was slower in HV carcasses compared to L; however, there was no difference for 24 h carcass temperature between the weight categories (Fig. 1*b*). Carcass P2 increased by 1 mm with every increase in 10 kg carcass weight (SE 0.22, P = 0.001). Carcass pH was unaffected by weight differences (P > 0.05). The combination of the increased muscle size and carcass P2 caused greater heat inertia and a slower chilling in the heavier carcass. There was an H × S interaction (P < 0.05) at 45 min post-slaughter only (IM: 6.35 v. 6.53 SE 0.04; F: 6.43 v. 6.47, SE 0.04, P = 0.031 for DL and CON, respectively). The hypothesis was rejected as these results show that H, W and S, significantly influence objective carcass quality but with a large variability between factors.





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## Development of an algorithm to correlate Physi-Trace pig liver data with pork meat data

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Physi-Trace was designed to facilitate the provenance of pork back to a point of origin. Throughout Physi-Trace, it has been shown that it is possible to validate whether a pork product is Australian or not and to establish the origin (farm/tattoo) of Australian pork. This has been possible through continued sampling of known origin pork meat and the subsequent trace elemental analysis and statistical interpretation of the resulting data. A system is now in place that will facilitate the traceability of fresh Australian pork. Associated with the traceability of fresh pork is the traceability of pork offal. Preliminary research by Kreitals (2013) indicated that it was possible to link the elemental profile of offal varieties to the elemental profiles of fresh pig meat using several algorithms thus allowing the origin determination of pig offal to be conducted using the Physi-Trace framework. The algorithms developed by Kreitals (2013) were limited to a single processor and required further research to verify their robustness. The objective of this research was to undertake a detailed study to develop a universal algorithm that could be used to trace analytical data for liver back to their equivalent pork meat data and establish region of origin.

The experiment required the collection of samples (livers and raw meat) from pigs at participating Physi-Trace export processors, and represented two large, two medium and one small grower from each processor. Seven samples were taken from each grower totaling 203 raw meat and 203 liver samples. The samples were digested in a mixed acid solution at 90°C overnight before dilution with high purity (18 M $\Omega$ ) water and were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) to determine trace element profiles. Due to the accumulation of some elements in the livers, a reduced set of analytes (Table 1) was developed for accuracy.

This investigation produced an algorithm that could be used to transform Physi-Trace elemental data for pork liver into 'equivalent' data that can be compared with pork meat data to determine property of origin. Fig. 1 is a linear discriminant analysis plot of the raw meat elemental data from all five growers from a specific processor with the addition of the transformed liver data from Grower 3.

From Fig. 1, all five growers can be easily discriminated and the transformed liver data for Grower 3 groups well with the raw meat data from Grower 3. Similar results were obtained for 86% of all the liver samples tested showing that the developed algorithm could be used to transform pork liver elemental data into data that could be used to compare with the elemental data of raw meat in the Physi-Trace database to assess the origin of the liver sample. To trace back to a processor of origin, the success rate of the algorithm was 97%.



 Table 1. Reduced set of analytes used for all 'conversion' of pork liver

 data to equivalent pork meat data

7 Li	9 Be	11 B	45 Sc	49 Ti
51 V	53 Cr	55 Mn	59 Co	66 Zn
69 Ga	74 Ge	75 As	77 Se	85 Rb
88 Sr	89 Y	98 Mo	111 Cd	115 In
121 Sb	126 Te	133 Cs	138 Ba	208 Pb
Na 589.592 nm	Mg 285.213 nm	Al 167.019 nm	P 178.222 nm	S 181.972 nm
K 766.491 nm	Ca 422.673 nm			

**Fig. 1.** Scatter plot showing discrimination between five groups from a single region. Modified liver data for Liver Group 3 agrees with corresponding Meat Group 3.

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# Reduced antimicrobial resistance in weaner pigs treated with Detach<sup>®</sup> following natural challenge with F4 *Escherichia coli*

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Aminoglycosides and zinc oxide (ZO) are used to prevent post-weaning diarrhoea (PWD) caused by Enterotoxigenic *Escherichia coli* (ETEC), but their use can lead to antibiotic resistance. Detach (Anatara Lifesciences Ltd, Brisbane, Qld, Australia) protects pigs from PWD but is not bactericidal. Detach<sup>®</sup> acts by (1) inactivating host receptors, preventing ETEC colonisation and (2) inhibiting intestinal fluid secretion caused by ETEC toxins (Mynott *et al.* 1997; Chandler and Mynott 1998). This study tested the hypothesis that Detach<sup>®</sup> could control PWD in weaner pigs without increasing the incidence of antimicrobial resistance (AMR) in *E. coli*.

Seventy-two weaner pigs (mixed gender) from nine gilt litters were randomly allocated into four treatment groups with two piglets from each litter per treatment. Each group, six pens of three pigs (n = 18 per group), were housed in separate rooms to avoid faecal contamination. Detach<sup>®</sup> (4 mL) was administered orally (DT) the day before weaning (d -1) and on d 7. All pigs were acclimatised to non-medicated feed between d 0 and 7. Zinc oxide (2500 ppm) or neomycin sulphate (8 mg/kg, NS) was administered in feed between d 7 and 20. Non-medicated feed was supplied to Control (CT) and DT pigs from d 0 to 40, and to Zn and NS pigs from d 20 to 40. Pen feed intake and individual weight gains were recorded weekly and faeces were collected from each pig on d 6, 19 and 39 and faecal consistency scores recorded daily. Pathogenic F4 ETEC were quantified in d 6 faeces to compare infection levels between groups. Four E. coli isolates from each faecal sample were tested for AMR to seven commonly used antimicrobials. Differences in proportions of resistant E. coli between groups were analysed with a Kruskal–Wallis One-Way ANOVA (GENSTAT 18, VSN International, Hemel Hempstead, UK). Two NS pigs died on d 19 and 39 due to a Streptococcus suis infection and chronic pericarditis, respectively. Low numbers of F4 ETEC were detected at d 6 in all pigs (median = 280 ETEC), with a significantly higher proportion in CT relative to NS pigs (P = 0.042). During the treatment period, diarrhoea was numerically higher in CT piglets between d 7 and 19 (44% to 11% in other groups), but mean faecal consistency scores did not differ between treatments, and CT pigs recovered after d 20. Immediately following removal of ZO and NS, 25% of NS and ZO pigs had diarrhoea requiring electrolytes. Weight gains over the trial period (d 7 to 39) were NS, 23.13<sup>a</sup> kg; ZO,  $22.02^{\circ}$  kg; DT,  $21.07^{\circ}$  kg and CT,  $22.77^{\circ}$  kg (different superscripts indicate P < 0.05). Feed intake was NS,  $25.77^{\circ}$  kg; ZO,  $24.45^{\circ}$  kg; DT, 23.66<sup>c</sup> kg and CT, 27.00<sup>d</sup> kg. At d 6, *E. coli* AMR was not significantly different between groups, but by d 19 *E. coli* from DT pigs had reduced AMR to neomycin (1.4% compared to 12.7%), tetracycline, sulphamethoxazole/trimethoprim and lincospectin relative to NS pigs (Fig. 1). At Day 39, ZO pigs had significantly increased resistance to tetracycline.

Detach<sup>®</sup> effectively controlled diarrhoea following natural challenge without inducing resistance to antimicrobials in *E. coli*, but did not lead to the growth advantages observed in NS treated pigs.



Fig. 1. Percentage of *E. coli* with resistance to tetracycline, sulphamethoxazole/trimethoprim (TMS) and lincospectin in — NS, — ZO, — DT and -  $\bigstar$  - CT pigs.

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## The emergence of community associated MRSA (ST93) in piggery workers and associated risk factors

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In Europe, livestock associated (LA) MRSA (ST398) carriage in pig enterprises has emerged as an occupational challenge where a high prevalence has been reported in pig farmers (Cuny *et al.* 2009) and pigs (Morcillo *et al.* 2015). Recently, a high prevalence of MRSA carriage has been found in pigs and people working in a pig farm in Australia, where 84% of total MRSA positive workers (n=31) were carrying community associated (CA) MRSA (ST93) and 16% LA-MRSA (ST398) (Sahibzada *et al.* 2017). The objective of this study was to determine the potential risk factors associated with different MRSA clone carriage with regard to occupational pig exposure on this farm.

Information was collected from the participants via questionnaires. Associations between MRSA carriage and the presence of potential risk factors were investigated using the statistical package R v3.3.3 (R foundation, Vienna, Austria), fitting univariable generalised linear models (GLM) with binomial distribution logit link function. Significance was set at 0.05. Clone-specific carriage trend was compared separately for each clone with the overall MRSA non-carrier by excluding the counter-clone from the baseline model.

A total of 52 piggery workers participated in the study, 77% male and 23% female. No significant association was found for MRSA carriage on this farm with age, gender, ethnicity, number of years working with pigs, chronic disease, or history of hospitalisation. Pig contact and contact intensity (number of hours working in direct pig contact), the role of pig workers, and level of education were noted to be significantly (P < 0.01) associated with MRSA and MRSA clone carriages. The prevalence of MRSA carriage was 85.2% for persons working in pig sheds, 60% for those in maintenance roles and 50% amongst feedmill workers. No MRSA carriage was found in those with administrative or pastoral roles. The odds ratio (OR) for MRSA carriage in workers who had high school or lower levels of qualifications was 3.96 (CI = 1.25–13.84, P = 0.02) compared with those with tertiary education. Univariable logistic regression analysis showed that the odds of ST93 carriage increased by 7% (OR 1.07, CI = 1.02–1.14) for each hour increase in pig contact in a week. A similar higher odds ratio was noted for ST398 (OR 1.08, CI = 1.02–1.21).

ST93 has been frequently isolated from communities in Australia. However, this strain has never been reported as an occupational risk, unlike ST398 which has been studied thoroughly and linked with pig contact and contact intensity.

We describe for the first time the CA-MRSA clone carriage of ST93 as an occupational risk for piggery workers which is strongly associated with intensity of contact between workers and pigs. Given that it has been reported on a single farm, it is important to investigate MRSA carriage in humans and pigs on other pig farms.

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# Disinfectant susceptibility in south east Australian pig herds

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*Escherichia coli* (ETEC) causes severe diarrhoeal diseases in piglets resulting in large production losses to the Australian pig industry. Antibiotics are often used to manage this problem, but resistance can develop over time. Disinfectants can be used as an alternative (or additional) control option for *E. coli*, by reducing *E. coli* environmental contamination. However, disinfectant effectiveness has been poorly investigated in Australia. To assess disinfectant resistance, a cross-sectional survey of 22 commercial pig herds located in south-east Australia was conducted between September 2013 and May 2014. Fifty faecal samples were collected from each herd, 10 from pre-and 40 from post-weaned piglets. Faecal samples were collected off the floor of piglet pens (approximately five samples per pen). Fifteen presumptive *E. coli* isolates were randomly selected from diarrhoeal and non-diarrhoeal samples (including  $\beta$ - and non- $\beta$ -haemolytic isolates) and confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) (Microflex LT MALDI BioTyper; Bruker Biosciences, Preston, VIC, Australia). Isolates (n = 325) were screened for susceptibility to six veterinary disinfectants using MIC broth microdilution and breakpoints according to CLSI guidelines (CLSI 2012). *Escherichia coli* isolated from pre- and post-weaned piglet pens showed high susceptibility ( $\geq 95\%$ ) to five of the six disinfectants screened (Table 1).

Disinfectant use in combination with strict cleaning and hygiene protocols could effectively manage *E. coli* disease and limit other enteric bacteria commonly found in the piglet pen environment. This study highlights the effectiveness of disinfectant use as a management tool for *E. coli* associated with diarrhoea in piglets. Further work could monitor the effectiveness of these disinfectants on-farm to reduce other species of endemic bacteria.

Disinfectant	Manufacturer	Concentration	E. coli resistance (%)
Virkon®	DuPont (Wilmington, DE, USA)	1:100	Nil
		1:200	Nil
Farm Fluid S®	Antec International Ltd (Sudbury, UK)	1:100	Nil
		1:200	Nil
Nu-quat <sup>®</sup>	Bunzl Distribution Midcentral Inc (St Louis, MO, USA)	1:50	1.8
-		1:100	4.0
Microtech 7000	Artech Technologies Pty Ltd (Breakwater, Vic., Australia)	1:500	Nil
		1:1000	3.4
$F10^{TM}$	Health and Hygiene Pty Ltd (Roodepoort, South Africa)	1:100	2.2
		1:200	4.6
Iodophore	Not commercially available	1:85	100.0
		1:170	100.0

Table 1.	Disinfectant phenotypic	resistance in 325 E	. <i>coli</i> isolates from	Australian domestic piglet pens
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# Investigation of a novel porcine bacterium by whole genome sequencing and mouse inoculation

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A bacterial isolate collected from a lung lesion of a pig at slaughter was identified as a member of the family *Pasteurellaceae* using standard biochemical testing. Antibiotic sensitivity was tested using the CDS method and the isolate was found to be susceptible to ampicillin, cephalexin and enrofloxcain and resistant to tetracycline. Further classification was inconclusive, and the isolate failed to group within any existing genus or species. The lung lesions were typical of those seen after infection with *Actinobacillus pleuropneumoniae*, but preliminary 16S rRNA gene sequencing indicated that the closest relative was *Haemophilus parasuis*. The work described here aimed to further investigate this novel bacterium by biochemical testing, full genome sequence analysis and assessment of its pathogenicity in a mouse model of infection.

Full genome analysis was completed using Illumina (Illumina Inc., San Diego, CA, USA) and Oxford (Oxford Nanopore Technologies, Oxford, UK) nanopore sequencing, with comparisons of conserved genes suggesting that the novel bacterium was most closely related to *H. parasuis*. Concatenation and phylogenetic assessment of the housekeeping genes, *atpD*, *infB* and *rpoB* showed that the organism lay in a distinct monophyletic position within the *Pasteurellaceae* phylogenic tree (Fig. 1). Assessment of the pathogenicity of the organism was performed by inoculation of 6 week old BALB/c mice with the novel bacterium at different concentrations via the intranasal and intraperitoneal routes. The lesions caused after infection were compared to those seen in a positive control group infected with *A. pleuropneumoniae*. The lungs, liver and spleen were assessed for the severity and type of lesions using histopathological examination. Intranasal inoculation resulted in interstitial pneumonia and bronchitis. The findings suggested that the lesions caused by this yet unclassified member of the *Pasteurellaceae* differed significantly from those described in previously published studies in which mice were inoculated with *G. parasuis* (De la Fuente *et al.* 2007).

The results suggested that further research is needed to assess the prevalence of this bacterium in pig populations, as well as to examine its pathogenicity for its natural host, as mouse models only provide an indication of the potential of the organism to cause acute disease. Its potential to cause chronic disease will need to be assessed to fully understand the type and severity of impact this bacterium may have on commercial pig farms.



**Fig. 1.** Phylogenetic tree based on concatenation of the *atpD*, *infB* and *rpoB* genes from selected members of the family *Pasteurellaceae*. The randomised axelerated maximum likelihood (RAxML) tree was built in Geneious *v*.7.1 using the rapid hill-climbing algorithm. Bootstrap values from 100 trees are indicated as percent confidence values for the branching.

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## Haptoglobin in oral fluid samples from pens of pigs can potentially be used to estimate herd inflammatory status

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Until recently, plasma haptoglobin (Hp) content has been used as a biomarker for immune system activation in pigs (Parra *et al.* 2006). The major limitation of this is the need to collect representative blood samples, which can be problematic and create errors in terms of collecting representative samples of a herd. Alternatively, measuring Hp in oral fluid samples to discriminate an unhealthy herd has been proposed (Soler *et al.* 2013). Therefore, the hypotheses of the present study were that (1) immune system activation of grower/finisher pigs can be discriminated by measuring Hp content in oral fluid (OF) samples, and (2) the Hp contents in the OF samples collected from pigs in a pen (the group) are significantly correlated with that in the OF samples collected from individual pigs in that group.

To test the hypotheses, seven commercial farms of varying health status (healthy *v*. unhealthy, as assessed by veterinarians) were selected. To test the first hypothesis, five pens per farm were randomly selected for OF and plasma sample collection from individual pigs. A total of 340 grower pigs were sampled for plasma and OF. To test the second hypothesis, two additional pens were randomly selected from each farm and an individual OF sample and a single-point OF sample were collected by hanging a cotton rope for 40 min in a pen. A total of 110 individual OF samples and 12 group pens OF samples were collected in this process. One group pen OF sample was removed from the dataset due to dehydration, as the water supply was interrupted on the sampling day. The samples were analysed for Hp content using a commercially available ELISA kit (Aviva Systems Biology, San Diego, CA, USA). Correlation and a simple linear regression analyses were conducted using GENSTAT 15 (VSN International, Hemel Hempstead, UK).

The mean ( $\pm$  s.e.) plasma and OF Hp contents determined from 340 pigs were 0.73 (0.03) mg/mL and 0.51 (0.04) µg/mL, respectively. The Hp contents in the OF samples were significantly correlated with that in the plasma samples (r = 0.44, P < 0.001). The cut-off point of the OF Hp content for discrimination between healthy and unhealthy pigs was 1.5 µg/mL (Fig. 1*a*). The single-point OF sample collected by hanging a cotton rope in a pen linearly correlated with the mean Hp content determined by collection of OF samples from individual pigs in the same group (r = 0.967, P < 0.001, Fig. 1*b*). This suggests that OF is a potentially useful sample for measuring the degree of immune system activation. However, and as the slope indicates in the regression equation, the Hp content in the group OF sample was higher by 2.56 µg/mL per unit of Hp than the average of individual OF samples. This would indicate an adjustment for the cut-off point may be required when single-point OF sample is used to evaluate inflammatory status of a herd.



Fig. 1. Oral fluid haptoglobin concentration in individual pigs (a), and the relationship between mean haptoglobin content in individual v. group oral fluid samples (b).

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## Novel feed additives controlling Salmonella typhimurium in pigs

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Non-typhoidal salmonellosis is estimated to cause 93.8 million cases of acute gastroenteritis and 155 000 deaths globally each year, ~85% of which are estimated to be foodborne. Pork products are among the top food-borne sources of salmonellosis globally (FAO/WHO 2016). Reduction of contamination in the feed-to-food chain is necessary to reduce the number of human cases. Since antimicrobial resistance is on the rise, there is an urgent need for new antibacterial strategies to reduce salmonellosis. This paper investigates antibacterial properties of a mixture of organic acids (formic and lactic acid) with two novel feed additives: mannanase-hydrolysed copra meal (CM) and rye overgrown with mycelium of *Agaricus subrufescens* (ROM) using a porcine *Salmonella typhimurium* infection model. Formic and lactic acid were included because of their antibacterial properties (van Immerseel *et al.* 2006), while the other components were supposed to reach the intestinal tract where they may competitively bind to *S. typhimurium*. Furthermore, mannanase-hydrolysed copra meal contains  $\beta$ 1–4 mannobiose, which may increase IgA-response and reduce *S. typhimurium* shedding after infection (Agunos *et al.* 2007).

*In vivo* activity of the feed additive blend was evaluated in a *S. typhimurium* challenge study with 24 piglets individually housed directly after weaning. Piglets were fed a control diet or the same diet supplemented with the combination of organic acids, CM and ROM. To provide the disease challenge, piglets received feed containing10<sup>9</sup> CFU *S. typhimurium* from 5 days after weaning for seven consecutive days. The pigs were monitored for *S. typhimurium* shedding and serum immunoglobulin A (IgA) (3 days post infection). Data were analysed using the GLM procedure (SAS v9.4, SAS Institute Inc., Cary, NC, USA).

In all treatment groups *S. typhimurium* infection resulted in a mild increase in body temperature (<0.5°C), mild diarrhoea. The combination of feed additives significantly decreased *S. typhimurium* peak shedding during the first week after infection ( $4.0 \pm 0.27 v$ .  $5.1 \pm 0.27 \log$  CFU/gram faeces; P < 0.01; Fig. 1). Immunoglobulin A serum levels, 3 days post infection, were negatively correlated with the level of shedding 3 days post infection in both the control group ( $r^2=0.66$ ; P=0.03) and the treatment group ( $r^2=0.62$ ; P=0,01); however, supplementation of the combined feed additives had no effect on IgA levels (Fig. 2).

In conclusion, the feed additive combination did not influence serum IgA levels in pigs. However, the combination of organic acids with CM and ROM showed inhibiting effects towards the disease and therefore may be useful to control *S. typhimurium* in pigs.



Fig. 1. Salmonella shedding after challenge.



Fig. 2. Correlation of Salmonella shedding with IgA levels.

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# *Lactobacillus acidophilus* fermentation product as an alternative to therapeutic zinc oxide in weaned pig diets on performance and response to *Escherichia coli* challenge

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Previous research in pigs with chronic K88+(F4) *Escherichia coli* has demonstrated that feeding *Lactobacillus acidophilus* fermentation product (LAFP; SynGenX<sup>®</sup>, Diamond V, Cedar Rapids, IA, USA) at 1 kg/MT, in combination with carbadox and zinc oxide (ZnO) improves health and reduces the frequency of injectable treatments and mortalities (Probst Miller *et al.* 2016). Additionally, the combination of 1 kg/MT LAFP and oxytetracyline, or 2 kg/MT LAFP alone, has been shown to reduce *E. coli* in weaned pigs (An *et al.* 2015). Zinc oxide, fed at therapeutic levels, has been used to alleviate diarrhoea associated with weaning stress. However, concerns about the environmental impact of high inclusion levels of zinc, as well as the potential of zinc to drive antibiotic resistance in bacteria, may limit its use in the future. Therefore, we hypothesise that LAFP can aid in reducing the impact of *E. coli* on pig health and performance, demonstrating similar benefits as therapeutic levels of ZnO.

In the first of two studies, 288 piglets were weaned at 28 days (7.41 kg bodyweight (BW)) and randomly assigned to one of four dietary treatments (12 pens per treatment; three boars:three gilts per pen): Negative Control devoid of antibiotics (NC); Positive Control – NC + 3000 ppm ZnO (PC); NC + 1 kg/MT LAFP in pre-starter and 0.5 kg/MT LAFP in starter (LA1); and NC + 2 kg/MT LAFP in pre-starter and 1 kg/MT LAFP in starter (LA2). Diets were provided for 35 days (Pre-starter from weaning to 12 kg BW; Starter from 12 kg BW to the end of the study). Pigs and feeders were weighed on d 0, 7, 14, and 35 to determine BW, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). Data was analysed using GENSTAT 17 (VSN International, Hemel Hempstead, UK). In the first week post-weaning, a trend was observed in ADG and FCR in which PC had higher ADG and lower FCR than NC, with both LA1 and LA2 being intermediate. Significant improvements in ADG (P = 0.024) and FCR (P = 0.013) were observed using contrast comparisons between NC and the combination of LA1 and LA2. In the starter period (d 14 to d 34) and in the overall nursery period PC had higher ADG and final BW (P < 0.01) than NC and LA1, with LA2 being intermediate. Feed-to-gain was higher in NC compared to LA1 and LA2 combined (1.51 v. 1.44 g/g; P = 0.03) in starter period and in the overall nursery period (1.44 v. 1.36 g/g; P = 0.022). There were no differences in performance between PC and LA2 throughout the study.

In the second study, 48 piglets, 24 boars:24 gilts (Topigs Tempo x (Great Yorkshire x Finnish Landrace)), weaned at 26 days of age (7.1 kg BW) were allocated to four treatments: Negative Control devoid of antibiotics (NC2); Positive Control – NC2 + 2500 ppm ZnO (PC2); NC2 + 1 kg/MT LAFP (SG1); and NC2 + 2 kg/MT LAFP (SG2). Diets were offered to the piglets for 22 days. On d 10, piglets were orally challenged with 5 mL of 8.9 Log of nalidixine resistant *E. coli*. During d 11 to 15, 18, 20 and 22, faecal scores were measured (Gerritsen *et al.* 2012) and samples were collected to quantify nalidixine resistant *E. coli* and data was analysed using GENSTAT (17th edition). On d 14 (P = 0.064), 15 (P = 0.064) and 18 (P = 0.041), faecal *E. coli* excretion was lowest in boars fed SG2, with no differences between gilts and boars fed other diets. On d 20, pigs fed SG2 showed lower faecal excretion of *E. coli* than pigs fed with NC2, PC2 and SG1 (3.4 v. 4.3, 4.3 and 4.4 Log CFU/g faeces, respectively; P = 0.031). Overall faecal score was lowest for SG2 (P < 0.05). Pigs provided PC2 tended to have higher ADG (P = 0.053) compared with NC2 and SG1 (466 v. 359 and 385 g/d, respectively), with SG2 intermediate (406 g/d).

In summary, inclusion of ZnO at therapeutic levels improved performance compared to negative control fed animals. Feeding LAFP at 2 kg/MT resulted in performance comparable to therapeutic ZnO and additionally reduced faecal score and shedding of *E. coli* following challenge in diets that did not contain antibiotics or other additives. In conclusion, LAFP at 2 kg/MT may be a potential alternative for therapeutic ZnO in piglet diets post-weaning.

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# Effect of the combination of three yeast strains on post weaning piglets after an experimental *E. coli* infection

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Weaning imposes stress on the piglets, which may affect their general health, therefore, it is necessary to keep a well balanced microflora to protect against colonisation of pathogenic microorganisms in the gastrointestinal tract (Jensen 1998). Yeast fractions have been shown to have a positive effect on gut health in weaning piglets. A blend of three inactivated yeasts strains (YANG<sup>®</sup>; Lallemand SAS, comprising two complementary species of *Saccharomyces cerevisiae* and one strain of *Cyberlindnera jadinii*) has shown positive antibacterial effects *in vitro* (Dunière *et al.* 2016). The objective of this trial was to evaluate the effect of the yeast blend on *E. coli* faecal counts and IgG blood concentration of weaned piglets with an *E. coli* challenge. It was hypothesised that both the *E. coli* excretion and the immunoglobulin G (IgG) concentration will be lower, as an effect of counteracting the effects of the challenge given by *E. coli* inoculation.

The trial was run at Schothorst Feed Research (SFR), and 36 male piglets (Topigs Tempo × (Great Yorkshire × Finnish Landrace)) aged  $25 \pm 0.91$  days and weighing  $6.83 \pm 0.51$  kg at weaning, were housed in pairs in 1 m<sup>2</sup> pens. Pens were allocated to one of the experimental treatments: negative control (NC), positive control (PC), and YANG (Y, 800 g/ton). The test product was offered in feed for the entire experiment. In PC treatment, piglets were daily treated with colistin (100 000 IU Coliplus, Bimeda, Llangefni, UK<sup>®</sup>) via drinking water at a dose of 2 000 000 IU/mL per kg of bodyweight. During the first 3 days, all piglets received colistin to reduce the presence of *E. coli* in the gut. On d 10, all the piglets were challenged orally with 5 mL of  $8.80 \pm 0.07$  log CFU *E. coli*/mL. The *E. coli* strain was isolated from an SFR herd and made resistant to nalidixin to facilitate its identification. Faecal samples were taken on d 8, 11, 15, and 22 and blood samples on d 8, 15 and 22. The experimental data were analysed using the mixed model (repeated-measurements) using GENSTAT 18 (VSN International, Hemel Hempstead, UK), with dietary treatment and day as fixed effects. Significance was set at P < 0.05, with pen being the experimental unit. Before challenge, faecal excretion of *E. coli* in all the piglets was lower than detectable levels. Piglets fed diet Y had lower faecal *E. coli* excretion than piglets fed NC (P < 0.001). Piglets treated with colistin showed the lowest *E. coli* excretion in the faeces. Piglets fed diet Y tended to have lower IgG blood concentration (P < 0.10) than piglets on the PC diet. The study design did not allow detection of growth performance between dietary treatments. Results are shown in Table 1.

It was concluded that the inclusion of YANG<sup>®</sup> at a dose of 800 g/ton decreased faecal *E. coli* excretion by ~0.5 log CFU compared with piglets in NC treatment. An effect on the humoral immune response was suggested, but needs further investigation.

	E	. coli excretio	on			IgG concentration					
Day	NC	PC	Y	d	$P^{\mathrm{A}}$	$d \times T$	NC	PC	Y	d	$P^{\mathrm{A}}$
8 <sup>A</sup>	1	1	1	_	_	_	3.31	3.10	2.96	_	0.15
11 <sup>A</sup>	7.63 <sup>b</sup>	3.26 <sup>a</sup>	6.44 <sup>b</sup>	_	< 0.01	_	_	_	_	-	_
15 <sup>A</sup>	6.67 <sup>b</sup>	1.44 <sup>a</sup>	6.12 <sup>b</sup>	_	< 0.01	_	2.82	2.84	2.73	_	0.93
22 <sup>A</sup>	4.09 <sup>b</sup>	1 <sup>a</sup>	4.30 <sup>b</sup>	_	< 0.01	_	2.93	3.19	2.73	-	0.16
Overall	6.26 <sup>c</sup>	1.48 <sup>a</sup>	5.66 <sup>b</sup>	< 0.01	< 0.01	< 0.01	3.02 <sup>xy</sup>	3.04 <sup>y</sup>	2.80 <sup>x</sup>	0.03	0.08

Table 1. E. coli excretion (log CFU/g faeces) in faeces and IgG concentration in blood (mg/mL) before (d 8) and after (d 11 to 22) E. coli challenge

<sup>A</sup>*P*-value from one way ANOVA analysis. d, day; T, treatment;  $d \times T$ , interaction day × treatment. <sup>a-c</sup>Means with different superscript are significantly different. <sup>x,y</sup>Different superscript indicates a trend for a significant difference (P < 0.10).

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# Pen hygiene and piglet mortality in farrowing pens with partly solid floor, changes through lactation

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Commercial viability of free farrowing pens relies on good performance and functionality of the pens. Design recommendations suggest a partly solid floor to provide a comfortable lying area and to accommodate sow nesting behaviour (Baxter *et al.* 2011). However, dunging behaviour can be difficult to control when sows are loose and maintaining good hygiene on solid areas can require more effort than fully slatted floors. Improving piglet survival can also be labour intensive, but experiences from practice show both performance and hygiene improves as experience with the system grow. The objective of this study was to investigate how hygiene and piglet mortality developed with increasing experience with the system.

The study was conducted over 3 years in two commercial Danish herds, each with 1200 sows, and SWAP (Sow Welfare And Piglet protection) farrowing pens. The pens had 60% and 40% solid concrete floor in Herd A and B, respectively, and cast iron slats. The solid floor was divided into different areas: 'creep', 'sow area' and 'area under sloping wall'. Areas were scored for cleanliness ('clean and dry', 'partly dirty' or 'soiled') once a week, and sow cleanliness was assessed ('clean and dry', 'partly dirty' or 'dirty'). Cause of death of piglets within herds was recorded ('weak', 'crushed', 'starvation/runt', 'leg problems', 'other') and Herd A also recorded weekly performance figures. Data on hygiene and performance was analysed for effects of herd, season, time and week in lactation using linear models (SAS v9.4, SAS Institute Inc., Cary, NC, USA). Results showed a decrease in total piglet mortality (stillborn and liveborn deaths of total born) from 26.8% in the first quarter to 22% in the last quarter (P < 0.01) 2 years later. In this period, there was an increase in the number of liveborn piglets/litter ( $16.5 \pm 0.15 v$ .  $17.9 \pm 0.15$ , P < 0.01) and a higher weaned piglets/ litter (13.3  $\pm$  0.24 v. 14.2  $\pm$  0.18, P < 0.05), indicating that performance improved over time. Crushing was the main cause of death (P < 0.001) with 58%, 64% and 59% of mortality attributed to crushing in lactation week 1, 2 and 3 + 4, respectively. Cleanliness of the sow area differed between herds (P < 0.001) and seasons (P < 0.001). The proportion of clean sow areas increased after farrowing (P < 0.001) whereas cleanliness of the area under the sloping wall decreased during lactation (P < 0.001) (Fig. 1). Only piglets can defecate under the sloped wall and the reduced cleanliness indicate that piglets developed unwanted dunging behaviour. Sows were cleaner in Herd B compared to Herd A (P < 0.001) but in both herds sow cleanliness decreased through lactation (P<0.001). In Herd A, 70% of sows were clean before farrowing, decreasing to 55% in lactation week 3+4. In Herd B, 96% of sows were clean before farrowing and 87% were clean in week 3 + 4. These results are consistent with the reduced cleanliness of pens and also indicate an effect of the different floor profiles on sow cleanliness.

This study indicates that experience with a system improves performance and that the effect of interventions might be improved by aiming specific interventions at specific time points.



Fig. 1. Cleanliness of pen areas through lactation in Herds A and B. Effect of lactation week: creep area, P < 0.001; area under sloping wall, P < 0.001; sow area, P < 0.001.

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# Topical zinc oxide improves shoulder lesion healing compared with other treatments

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A shoulder lesion is formed when the scapular spinal process is placed under prolonged pressure reducing blood supply and eventually causing tissue necrosis (Rolandsdotter *et al.* 2009). Shoulder lesions are developed primarily in the farrowing unit, and are more typical of sows with poor body condition, lameness, and scars from previous incidence (Kaiser *et al.* 2013). These lesions negatively impact sow welfare, and adversely affect consumer opinion of current conventional farrowing systems. The aim of this study was to assess the effectiveness of four treatments on the healing of shoulder lesions. We hypothesised that topical zinc oxide administration would improve the healing of lesions compared with cetrimide, benzalkonium and aluminium sprays.

A total of 399 lesions were graded (BPEX 2011) and measured for lesion diameter pre-treatment (~d 12 lactation), d 7 post-treatment and again at weaning (~d 25 lactation). Each shoulder lesion was randomly allocated using the Rand function in excel to one of the following treatments: 0.8% cetrimide solution (n = 103), 0.2% benzalkonium solution (n = 104), 4% aluminium powder ointment (n = 90) and, 15.25% zinc oxide (n = 102). All groups were topically treated daily until weaning. Lesion grade and diameter were analysed using a general linear model, shed was fitted as a random term, with farrowing month, parity, body condition score, and treatment as fixed effects (SPSS v24.0, IBM, Armonk, NY, USA). Percentage of sows with sores at weaning was analysed using binary logistic regression with the same model.

There was no significant difference in shoulder lesion grading or diameter before treatment. At both the post treatment measurements (d 7 post-treatment and at weaning), zinc oxide had significantly reduced the diameter of the lesion compared with cetrimide, with the other two treatments being intermediate (Table 1). A similar pattern was observed for lesion grade. Fewer sows from the zinc oxide treatment presented with lesions at weaning compared with cetrimide and benzalkonium, with aluminium being intermediate.

Using a 15.25% zinc oxide ointment to treat shoulder lesions improved healing compared with other available treatment options such as antiseptic sprays and aerosol band aids.

	Cetrimide		Benzal	konium	Alum	Aluminium		Zinc Oxide	
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	P-value
Lesion diameter (cm)									
Pre-treatment	3.5	0.6	3.5	0.6	3.4	0.6	3.6	0.6	NS
7 days post-treatment	3.6 <sup>a</sup>	0.2	3.3 <sup>ab</sup>	0.2	3.1 <sup>ab</sup>	0.2	$2.8^{b}$	0.2	0.03
Weaning	3.0 <sup>a</sup>	0.2	2.7 <sup>ab</sup>	0.2	2.5 <sup>ab</sup>	0.2	2.3 <sup>b</sup>	0.2	0.007
Lesion grade (score 0 to 5)									
Pre-treatment	1.7	0.2	1.7	0.2	1.7	0.2	1.7	0.2	NS
7 days post-treatment	2.0 <sup>a</sup>	0.1	1.9 <sup>a</sup>	0.1	1.8 <sup>ab</sup>	0.1	1.6 <sup>b</sup>	0.1	0.004
Weaning	1.2 <sup>a</sup>	0.3	1.2 <sup>a</sup>	0.3	$0.9^{\mathrm{b}}$	0.3	$0.7^{b}$	0.3	0.001
Weaning presence (%) <sup>A</sup>	94 <sup>a</sup> (8	36–97)	97 <sup>a</sup> (9	91–99)	87 <sup>ab</sup> (	76–93)	77 <sup>b</sup> (6	66-85)	0.001

# Table 1. Lesion diameter (cm) and grade (0 to 5) pre-treatment, d 7, and weaning and lesion presence at weaning for sows treated with cetrimide, benzalkonium, aluminium and zinc oxide daily

<sup>a,b</sup>Means in a row with different superscripts differ significantly (P < 0.05). <sup>A</sup>Confidence intervals are presented in brackets.

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# Microencapsulated feed additives allow improved production efficiency in weaner pigs

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Zinc oxide (ZnO) is widely used at high levels (3000 ppm) in order to control enteric disease in weaner pigs (Poulsen 1995), nevertheless, regulatory issues and the need to reduce environmental loads of zinc has resulted in lower allowable inclusion rate of zinc (Case and Carlson 2002). Industry must look at alternative methods that allow for lower inclusion levels without reducing efficacy. This study aimed to assess performance benefits offered by the inclusion in weaner diets of microencapsulated blends of ZnO, essential oils and organic acids as an alternative to high levels of ZnO, with the hypothesis that microencapsulated blends would show equivalent performance to the 'standard' ZnO treatment.

One hundred and forty pigs (C29 × 400, PIC, Grong Grong NSW) entered the experiment weekly (CHM PP 75/15) at weaning (21 days) and were on experiment for 28 days. Five weeks' worth of entries were utilised in this experiment with 10 pens of pigs (n = 14) entered per week, resulting in 10 replicates per treatment. A base starter diet (14.9 MJ digestible energy (DE)/kg, 0.9 g standardised ileal digestible lysine/MJ DE) was formulated, which included 3000 ppm of bentonite. Treatments differed by part or total substitution of the bentonite with the additive, such that diets remained isoenergetic and isonitrogenous, and were Control (Ctrl) with no additive, (ZnO) containing 3000 ppm ZnO, P(ZnO) containing 1000 ppm of protected ZnO (Zinco-Plus<sup>TM</sup>, Jefo Nutrition Inc., Canada), P(OA+EO) containing combination of 1000 ppm of protected organic acids and essential oils (Porcinat+<sup>TM</sup>, Jefo Nutrition Inc.) and P(ZnO)+P(OA+EO) containing combination of 1000 ppm of protected organic acids and essential oils and 1000 ppm of protected ZnO. Microencapsultaed additives were based on a fat matrix protection for controlled released of active ingredients in the gastrointestinal tract. Data were analysed by ANOVA using GENSTAT 18 (VSN International, Hemel Hempstead, UK) with treatment as main effect, entry week as blocking factor and entry weight as a covariate, differences between treatments were determined by l.s.d. (P < 0.05). There was a significant blocking effect related to entry week, however, there were no interactions, so only main effects are shown in Table 1.

The supplementation of a combination of P(ZnO)+P(OA+EO) had a significant positive impact on growth performance of weaner pigs by comparison to the control treatment, P(ZnO)+P(OA+EO) pigs had a higher exit weight (P < 0.008) and a lower feed conversion ratio (P < 0.032). The combination of P(ZnO)+P(OA+EO) did not differ in performance to the ZnO treatment and thus appears to be a viable alternative to high levels of free ZnO.

# Table 1. Weight and growth performance of Ctrl weaner pigs compared to weaner pigs receiving ZnO, P(ZnO), P(OA+EO) or a combination of both microencapsulated products for 4 weeks

	Ctrl	ZnO	P(ZnO)	P(OA+EO)	P(ZnO)+ P(OA+EO)	SED	P-value
d 0 weight (kg)	5.8	5.7	5.9	5.9	5.8	0.28	0.921
d 28 weight (kg)	13.2 <sup>a</sup>	14.3 <sup>c</sup>	13.6 <sup>ab</sup>	13.6 <sup>ab</sup>	13.9 <sup>bc</sup>	0.27	0.008
d 28 ADFI (kg/d)	0.38 <sup>ab</sup>	0.42 <sup>c</sup>	0.39 <sup>ab</sup>	$0.38^{a}$	$0.40^{\mathrm{bc}}$	0.012	0.005
d 28 ADG (kg/d)	$0.266^{a}$	$0.302^{\circ}$	0.277 <sup>ab</sup>	$0.279^{ab}$	0.291 <sup>bc</sup>	0.010	0.008
d 28 FCR (kg/kg)	1.44 <sup>c</sup>	1.40 <sup>abc</sup>	1.42 <sup>bc</sup>	1.35 <sup>a</sup>	1.39 <sup>ab</sup>	0.027	0.022

 $a^{-c}$ Means in a row with different superscripts differ significantly (P < 0.05). AGD, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SED, standard error of difference of the means.

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## Zinc oxide presentation can influence weaner performance

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Weaning is associated with a period of feed interruption and a transition to solid feed that can result in transient changes in gut morphology leading to impaired performance. Zinc oxide (ZnO) has been found to benefit gut health with reported effects such as increased expression of antimicrobial peptides, gut flora stabilisation and bactericidal function (Pluske *et al.* 2007). Zinc oxide is included in weaner diets at rates well above nutrient requirements. Environmental concerns due to low bioavailability and retention of Zn (Case and Carlson 2002) and implication in the development of antimicrobial resistance in enteric bacteria (Yazdankhah *et al.* 2014) have led calls to restrict the use of ZnO. This study investigated weaner performance when using modified organic versions of ZnO, with the null hypothesis that there would be no effect on performance between ZnO treatments.

Five hundred and sixty male pigs (20 days,  $5.86 \pm 0.17$  kg) entered the experiment over a 4-week period and were sorted by size and assigned to pens (n = 14). Pens were weighed and allocated to one of four treatments using a randomised block design, resulting in 10 replicates per treatment. Treatments consisted of isoenergetic and isonitrogenous weaner diets (14.85 MJ DE/kg, 0.87 g standardised ileal digestible lysine/MJ DE) including no ZnO (control), 3000 ppm ZnO, 1000 ppm of a protected ZnO (PZnO) or 300 ppm of an enhanced surface ZnO (EZnO). Diets and water were offered *ad libitum* throughout the 28 days of the experimental period. Pens of pigs and feed refusal were weighed weekly and water usage was also measured weekly. Data were analysed by ANOVA with treatment as a fixed factor, entry week as blocking factor and entry weight as a covariate. Removals were tested for significance via Chi-squared analysis. Significant differences between treatments were determined by 1.s.d. (P < 0.05, GENSTAT 18, VSN International, Hemel Hempstead, UK).

There was no significant difference between diets containing no ZnO (control), 3000 ppm of ZnO or 1000 ppm of the PZnO; however, the 300 ppm EZnO treatment pigs consumed less feed, grew slower and ended the experiment significantly lighter than all other treatments (P < 0.05; Table 1). There was no significant difference in morbidity and mortality between treatments ( $\chi^2(3) = 5.76$ , P = 0.124). The lower feed intake of the EZnO treatment suggests that this product may have affected the palatability of the diet, which is supported by a consistent, but not significant, increase in water usage compared to the other treatments.

The similar performance of the pigs fed PZnO compared to those fed ZnO confirms our null hypothesis and affords us a viable alternative if restrictions on the inclusion rate of ZnO are imposed in the future.

	Control	ntrol ZnO	PZnO	EZnO	SED	<i>P</i> -value		
						Treat	Week	$T \times W$
Entry weight (kg)	5.8	5.8	5.8	5.8	0.6	1.00	0.62	0.85
Exit weight (kg)	13.4 <sup>b</sup>	13.7 <sup>b</sup>	13.3 <sup>b</sup>	12.4 <sup>a</sup>	0.4	0.010	< 0.001	0.45
ADG (kg/d)	0.269 <sup>b</sup>	$0.279^{b}$	0.264 <sup>b</sup>	0.234 <sup>a</sup>	0.012	0.010	0.002	0.45
ADFI (kg/d)	0.39 <sup>b</sup>	$0.40^{b}$	0.38 <sup>ab</sup>	0.35 <sup>a</sup>	0.02	0.033	0.010	0.68
FCR (kg/kg)	1.45	1.44	1.42	1.50	0.03	0.07	0.029	0.06

# Table 1. Performance of weaner pigs fed diets containing no zinc oxide (-ve Control), 3000 ppm of zinc oxide (ZnO), 1000 ppm of a protected zinc oxide (Pro ZnO) or 300 ppm of an enhanced surface zinc oxide (ES ZnO)

<sup>a,b</sup>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 of means; Treat, treatment effects; Week, entry week effects; T × W, interaction effects.

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# A comparative study of the efficacy of Detach<sup>®</sup> versus zinc oxide to control post-weaning diarrhoea in pigs

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Diarrhoea is a major cause of ill-thrift and mortality in piglets post-weaning. Control strategies include improved hygiene, antibiotics and high concentrations of in-feed zinc oxide (ZnO). There are increasing concerns regarding the use of antibiotics and ZnO because of the potential for developing bacterial resistance, hence the need for non-antimicrobial alternatives to control diarrhoea. Detach<sup>®</sup> (Antara Lifesciences Ltd, Brisbane, Qld, Australia), comprised of bromelain, a proteolytic extract from pineapple stems, is one alternative as it has been shown to reduce diarrhoea in piglets through its anti-attachment and anti-secretory effects in the intestine (Chandler and Mynott 1998; Mynott *et al.* 1997). The objective of this study was to compare the efficacy of Detach<sup>®</sup> to ZnO in-feed treatment in a Spanish herd with a history of post-weaning diarrhoea due to *Escherichia coli*. Healthy, 25-day-old pigs were ear tagged and randomly assigned at weaning (d 0) to one of four groups (72 piglets per group): (1) Detach<sup>®</sup>; (2) Detach<sup>®</sup>+ZnO; (3) ZnO; or (4) nil treatment. Pigs in Groups 1 and 2 received a single 4 mL drench of Detach<sup>®</sup> on d 0, whilst 2500 ppm ZnO (Apsamix Zinc, Andres Pintaluba S.A., Reus, Spain) was included in the diet of pigs in Groups 2 and 3 from d 0 to 14. All pigs received a diet containing 2500 ppm ZnO from d 15 to 42, as per the standard practice on this farm. Pigs were housed in 48 pens (six piglets per pen) in two identical nursery rooms. Each pig was examined from d 2 to 16 and on d 19, 26, 33 and 42 and scored for faecal consistency (normal (0), pasty/semi-liquid (1) or liquid/watery (2) and clinical condition (0 = normal, 1 = depressed). The combined faecal and clinical scores were used to categorise pigs as morbid/sick (Score 2/3) or healthy (Score 0/1). The number of sick days per pen was analysed using a linear mixed model with room/gender as random effects and treatment as the fixed effect (GENSTAT for Windows, 2007). The number of pigs with diarrhoea per pen was analysed pa

Over the d 0 to 14 and 0 to 42 post-weaning period, Detach<sup>®</sup> and ZnO were equally effective at reducing diarrhoea compared to controls (P < 0.05) (Fig. 1). Detach<sup>®</sup> and ZnO-treatment groups had fewer sick days (P < 0.05) and less antibiotic treatments than controls (P < 0.05), with 15, 0, 2 and 33 treatments administered to the Detach<sup>®</sup>, Detach<sup>®</sup>+ZnO, ZnO and control groups, respectively. Weight gains from d 0 to 14 were Detach<sup>®</sup> 0.7 kg, Detach<sup>®</sup>+ZnO 1.14 kg, ZnO 1.03 kg and control 0.57 kg, with ZnO-treated pigs gaining more weight than the other groups (P < 0.05). Neither treatment had a significant effect on weight gain between d 0 to 42; Detach 12.4 kg, Detach<sup>®</sup>+ZnO 12.5 kg, ZnO 12.6 kg and control 12.2 kg.

Under the conditions of this study, a single dose of Detach<sup>®</sup> at weaning was as effective as in-feed ZnO in reducing the prevalence of diarrhoea and antibiotic treatments post-weaning compared to untreated pigs.



**Fig. 1.** Prevalence of diarrhoea in piglets treated with a single dose of Detach<sup>®</sup> at weaning, compared with ZnO added in feed for 2 weeks and nil treatment (control). Dashed line indicates d 14 when ZnO was included in the feed of all groups.

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# Effects of microencapsulated organic acids and essential oils supplementation on performance and rectal temperature in challenged weaning pigs

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Lipopolysaccharide (LPS) is a complex of lipids and sugars and is an essential component of the cell walls of gram negative bacteria (e.g. *Escherichia coli* and *Salmonella spp.*). Injection of LPS induces pathologic phenomena, which can lead to immune and inflammatory responses (Wu *et al.* 2015). Addition of organic acids in feed can improve the growth of piglets (Costa *et al.* 2013), and essential oils are considered anti-inflammatory (Yoon *et al.* 2010). The objective of the study is to determine the effect of microencapsulated blends of organic acids and essential oils (MOE) supplementation on rectal temperature and growth in weaning pigs subjected to a LPS challenge.

A total of 20 weaning (21 days) pigs ((Landrace × Yorkshire) × Duroc) with an initial bodyweight of  $4.43 \pm 0.6$  kg (mean  $\pm$  s.d.) were randomly allocated to four treatments with five replicates per treatment per pen for a 28 days experimental period. The average temperature of the pig accommodation area was  $30 \pm 1^{\circ}$ C. Treatments were: (1) basal diets + 0.2% MOE+ LPS injection; (2) basal diets + 0.2% MOE + saline injection; (3) basal diets + LPS injection; and (4) basal diets + saline injection. The basal diet contained 14.9 MJ/kg digestible energy and 1.65% lysine. On d 28, LPS (SIGMA, from *Escherichia coli* O111:B4, L2630) was injected at 100 µg/kg × bodyweight. Injections were injected intramuscularly into the thigh, and rectal temperature of the pigs was recorded after injection of LPS or saline 0, 4, 8, 12 and 16 h. All data were analysed by ANOVA using the General Linear Models (GLM) procedure of SAS (v9.2, SAS Institute Inc., Cary, NC, USA). Results are presented in Table 1.

Supplement with 2% MOE significantly improved the final bodyweight, average daily gain (ADG) and average daily feed intake (ADFI) (P < 0.05). However, feed efficiency (G:F) was not significantly different (P > 0.05). At 4, 8, 12, and 16 h after challenge, LPS (+MOE+LPS, -MOE+LPS) gave significantly higher rectal temperature than the saline treatment (P < 0.01). In conclusion, LPS injection can increase rectal temperature and MOE supplementation increased growth performance in weaning pigs, however, MOE could not inhibit the LSP-induced hyperthermia.

+MOE		OE	-MOE		s.e.m. <sup>A</sup>	<i>P</i> -v	value
Initial BW (kg)	4.	41	4.	4.45		0.	816
Final BW (kg)	7.	40	8.	47	0.31	0.	023
ADG (g)	0.	21	0.	29	0.02	0.	035
ADFI (g)	0.	27	0.	32	0.01	0.	0001
G: F	0.79		0.	0.89 –MOE		0.	358
	+M	+MOE				P-v	<i>P</i> -value
	+LPS	-LPS	+LPS	-LPS		MOE	LPS
0 h	39.8	39.4	39.5	40.1	0.144	0.891	0.497
4 h	40.5	39.4	40.3	39.2	0.177	0.300	0.0001
8 h	40.6	39.4	40.4	39.1	0.158	0.171	0.0001
12 h	40.3	39.2	40.3	39.3	0.134	0.647	0.0001
16 h	40.4	39.3	40.1	38.9	0.225	0.110	0.0001

 Table 1. Effects of microencapsulated blends of organic acids and essential oils on growth performance and rectal temperature in weaning pigs challenged by LPS

<sup>A</sup>s.e.m., standard error of the mean.

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# Effect of probiotic and xylanase on growth performance, nutrient digestibility, blood profiles, and faecal microflora in growing pigs

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There is evidence that probiotics, also referred to as direct-fed microbials (DFM), can improve gut microbial balance and intestinal health (Dersjant-Li *et al.* 2015). In addition, an interaction between multi-enzymes and DFM has been reported (Romero *et al.* 2013). The hypothesis of this study was that there might be a beneficial interaction when a probiotic and xylanase are fed to growing pigs.

A total of 120 growing pigs ((Yorkshire × Landrace) × Duroc) with an average bodyweight (BW) of  $25.22 \pm 1.88$  kg were randomly allotted to four experimental diets based on initial BW and sex (six replicate pens per treatment; two gilts and three barrows/pen). The experiment lasted for 6 weeks and dietary treatments included: CON, basal diet, CON + 0.002% *Enterococcus faecium* (EF1), and CON + 0.005% *E. faecium* (EF2) and CON + 0.002% *E. faecium* + 0.01% *Endo-1,4-β-xylanase* (9000 U/g) (EX). Feed intake and BW were recorded initially at week 3 and 6 of the experimental period to calculate average daily gain (ADG), feed intake (ADFI), and gain to feed ration (G : F). Chromium oxide was added to the diet as an indigestible marker at 0.20% of the diet for 7 days before faecal collection at d 42, when samples were collected by rectal massage from at least two pigs per treatment to determine the digestibility (ATTD) of dry matter (DM), nitrogen (N) and energy. At week 6, blood samples (3 mL) were collected (four pigs/treatment) into nonheparinised tubes to obtain serum to determine the total cholesterol, high-density lipoprotein (HDL), cholesterol, and low-density lipoprotein (LDL), creatinine concentrations, blood urea nitrogen (BUN), and glucose concentrations. Faecal samples were collected directly via massaging the rectum of two pigs (one gilt and one barrow) in each pen and then pooled and placed on ice for transportation to the laboratory where analysis was immediately performed to determine faecal *E. coli* and *Lactobacillus* counts. Data were analysed in accordance as a completely randomised design using the GLM procedure (SAS v9, SAS Institute Inc., Cary, NC, USA). Pen was used as the experimental unit. Differences among the treatment means were determined by using the Tukey's test with *P* < 0.05 indicating significance.

During the whole period, pigs fed the EX diet had increased ADG compared to pigs fed the CON and EF1 diets (718 g v. 643 and 681 g, respectively, P < 0.05). The ADG of pigs fed the EF2 diet increased compared to pigs fed the CON diet (703 g v. 643 g, respectively, P < 0.05). The G : F of pigs fed the EF2 and EX diets increased compared to pigs fed the CON and EF1 diets (0.431 and 0.436 v. 0.389 and 0.407, respectively, P < 0.05). At week 6, pigs fed the EF2 and EX diets had better G : F compared to pigs fed the CON diet (75.5 and 75.6% v. 73.2%, respectively, P < 0.05). Pigs fed the EF2 diet had decreased creatinine concentrations in the blood compared to pigs fed the CON diet (0.95 mg/dL v. 1.25 mg/dL, respectively, P < 0.05) at the sixth week. Pigs fed the EF2 and EX diets had higher faecal *Lactobacillus spp*. concentrations compared to the CON diet (7.55 and 7.61 v. 7.42 log<sub>10</sub> CFU/g, respectively, P < 0.05). In addition, pigs fed the EX diet had decreased *E. coli* counts compared to pigs fed the EF1 diet (7.61 v. 7.48 log<sub>10</sub> CFU/g, respectively, P < 0.05). Pigs fed the EX diet had decreased *E. coli* counts compared to pig fed the CON diet (6.23 v. 6.46 log<sub>10</sub> CFU/g, respectively, P < 0.05) at week 6. However, no significant difference was observed for ATTD of DM and N, Glucose, BUN, HDL, LDL of blood at week 6, or ADFI during the overall study among treatments (P < 0.05).

In conclusion, supplementation of growing pig diets with a combination of *E. faecium* and endo-1,4- $\beta$ -xylanase is capable of improving growth performance, nutrient digestibility, increasing faecal *lactobacillus*, reducing faecal *E. coli* counts and decreasing creatinine concentrations in the blood in growing pigs compared to pigs fed CON, and probiotic only supplementation. The data suggested that the combination of *E. faecium* and endo-1,4- $\beta$ -xylanase could offer more benefits than when used alone.

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# *In vitro* antimicrobial activity of essential oils against enterotoxigenic *Escherichia coli* found in a nation-wide commercial farm survey

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The antibacterial activity of some natural essential oils (EO) have the potential to reduce reliance on antibiotics to prevent post-weaning diarrhoea (PWD) caused by enterotoxigenic *Escherichia coli* (ETEC). However, a systematic screening of EO against the most common ETEC strains in Australia has never been established and is the main objective of the current research. We hypothesised that the sensitivity to the EO (alone or combined) by the selected ETEC strains prevalent in Australian piggeries was serotype-dependent or pathotype-dependent.

Ninety-one different *E. coli* isolates were collected from Victoria, New South Wales, Queensland, Western Australia and Southern Australia pig nurseries and characterised by the principal ETEC virulence genes (LT, Sta, Stb, stx<sub>1</sub>, stx<sub>2</sub>, *eaeA*, *ehxA*, F4, F5, F6 and F18) using multiplex PCR. An initial screening of 18 EO using a broth microdilution method against the two isolates with the most common ETEC serotypes (O:141ab and O:149) was done to find the minimum inhibitory concentrations (MIC). The EO with a MIC  $\leq 0.1\%$  for non-natives and  $\leq 0.65\%$  for native EO were selected and subjected to a chequerboard method to study potential synergies between EO combinations. A fractional inhibitory concentration index (FICI; defined as (MIC<sub>EO1</sub> in the combination/MIC<sub>EO1</sub>) + (MIC<sub>EO2</sub> in the combination/MIC<sub>EO2</sub>) of  $\leq 0.5$  was set as an indicator of synergistic activity in the EO combinations tested. Finally, the 91 isolates were tested against the most successful combinations of oils (Do *et al.* 2015).

Our results showed that the most common virulence gene was Stb present in 42 isolates followed by LT (33) and Sta (31). Twelve of the *E. coli* strains isolates had no ETEC virulence genes. Oregano, clove, thyme and cinnamon for non-natives and lemon myrtle, lemon ironbark, peppermint gum and nerolina for natives presented average MIC of 0.02%, 0.08%, 0.08%, 0.07% and 0.1%, 0.4%, 0.55%, 0.65%, respectively. The synergies between different EO combinations are shown in Table 1. Based on the MIC we observed resistance, low, medium, high, and very high sensitivities for 38.3%, 28.3%, 13.3%, 6.6% and 13.3% respectively. No relationship between sensitivity with serotypes and pathotypes was found.

In conclusion, our results confirmed that the virulence gene profile of ETEC found in Australian pig nurseries is similar to previous publications from elsewhere. Different ETEC serotypes showed different sensitivities to EO (Gutierrez *et al.* 2008). The non-native EO presented a lower MIC compared with native EO; however, the native EO had a better synergistic potential hypothetically due to a differential mode of action. Additional studies are warranted.

EO mix	Concentration (%)	Serotype	FIC
Oregano/clove	0.005/0.005	O:141	0.32
Thyme/nerolina	0.02/0.04	O:141	0.33
Clove/nerolina	0.02/0.04	O:141	0.33
Cinnamon/lemon ironbark	0.017/0.025	O:141	0.42
Lemon ironbark/nerolina	0.05/0.04	O:141	0.23
Peppermint gum/nerolina	0.068/0.081	O:141	0.25
Cinnamon/nerolina	0.0175/0.081	O:149	0.37
Peppermint gum/nerolina	0.137/0.081	O:149	0.37
Thyme/nerolina/ peppermint gum	0.0025/0.02/0.068	O:141	0.18
Clove/lemon ironbark/nerolina	0.005/0.025/0.08	O:141	0.27
Thyme/nerolina/peppermint gum	0.0025/0.04/0.068	O:149	0.21
Clove/lemon ironbark/nerolina	0.0025/0.05/0.08	O:149	0.27

Table 1.	Synergistic ar	itimicrobial a	ctivity of e	ssential oils i	n combinations	against E.	coli serotypes	0:141 a	nd O:	:149
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# Cereal and protein sources fed to pigs after weaning influence faecal shedding of β-haemolytic *Escherichia coli*

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Feeding different cereal and (or) protein sources after weaning influences production, the structure and function of the gastrointestinal tract, and the incidence of post-weaning colibacillosis (PWC; Heo *et al.* 2013). Several studies show that white rice can replace other cereals in nursery diets, and with animal protein sources, can reduce the incidence of PWC (Montagne *et al.* 2004). However, an imbalance between amounts and (or) types of carbohydrates and proteins entering the large intestine in pigs fed rice-based diets may induce PWC (Pluske *et al.* 2007). This experiment examined whether feeding a different cereal base (rice or wheat), in combination with sources of animal proteins or vegetable proteins, would influence the excretion of  $\beta$ -haemolytic *Escherichia coli* and antibiotic treatments given to pigs after weaning.

A total of 84 newly weaned pigs (Large White x Landrace), aged 24 days and weighing  $6.7 \pm 0.13$  kg (mean  $\pm$  s.e.m.), were used in a  $3 \times 2$  factorial arrangement of treatments with the respective factors being (1) three cereal types (extruded medium-grain rice, extruded long-grain rice, or hammer-milled wheat); and (2) two dietary protein sources (animal *v*. vegetable). Diets were formulated to contain adequate levels of energy and nutrients for pigs of this genotype and age, and had a crude protein content of 200 g/kg. Pigs were offered their respective diets in groups of four for the first 7 days after weaning, and for the final 2 weeks were housed individually ( $0.42 \text{ m}^2/\text{pig}$ ). Each pen was equipped with a nipple water drinker and a stainless steel feed trough. Pigs were swabbed for the presence or absence of  $\beta$ -haemolytic *E. coli* upon arrival and then on d 2, 5, 6 and 8 after weaning, and the number of pigs injected with antibiotics for clinical PWC (as assessed by the stockperson) was recorded. Treatment effects were assessed by two-way ANOVA for a factorial design using StatView 5.0 for Windows (AddSoft Pty Ltd, Australia). The results are shown in Table 1.

The number of antibiotic treatments given for clinical PWC after weaning was similar for all treatments (P > 0.05). Feeding vegetable proteins showed a strong tendency to reduce (P = 0.057) faecal shedding of *E. coli* after weaning compared to pigs fed animal protein sources. This difference was caused predominately by the greater swab score in pigs fed diets with long-grain rice plus animal protein diet (P = 0.069). This suggests feeding this form of rice, which has the most amylose and least amylopectin, in the presence of vegetable proteins reduced ETEC colonisation and subsequent shedding of  $\beta$ -haemolytic *E. coli*.

Cereal source	Protein source	Antibiotic treatments	Faecal swab score <sup>A</sup>
		(per pig)	
Medium-grain rice	Animal	1.4	0.5
	Vegetable	0.7	0.7
Long-grain rice	Animal	1.0	0.6
	Vegetable	0.6	0.3
Wheat	Animal	1.0	0.8
	Vegetable	1.0	0.8
Standard error of mean		0.07	0.03
		Probal	bility
Cereal source		0.792	0.461
Protein source		0.230	0.057
Cereal * Protein		0.638	0.069

Table 1.	Interaction means for antibiotic treatments and faecal swab score recorded in t	nigs after	weaning
I HOIC II	interaction means for antibiotic treatments and facture stud score recorded in	pigo aiter	

<sup>A</sup>Scored from 0 to 5: 0 = no growth of  $\beta$ -haemolytic *E. coli*; 5 = heavy colonisation of  $\beta$ -haemolytic *E. coli*.

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# A potential new species of porcine *Actinobacillus* defined by multi-locus sequence analysis

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In recent years the submission of *Actinobacillus*-like strains sourced from the respiratory tract of pigs to our laboratory has increased. Our usual diagnostic method for identification of non-routine organisms is 16S rDNA sequencing. However, this method does not have the discriminatory power to separate very closely related species in the *Actinobacillus* complex. This has prompted a project to improve the identification of *Actinobacillus*-like strains by using multi-locus sequencing analysis (MLSA). The *recN*, *rpoA* and *thdF* housekeeping genes were chosen for this analysis due to their proven ability to predict whole-genome DNA-DNA similarity.

For a total of 36 field isolates, identified by the 16S rDNA sequencing as unusual species – A. porcitonsillarum, A. minor, A. porcitonsillarum/minor complex, A. porcinus, A. indolicus and A. rossi – three genes, recN, rpoA and thdF, were sequenced and aligned to publicly available data of type strains within the genus Actinobacillus to make multiple alignments of DNA sequences constructed by Clustal in Geneious version 8.0.5. The genome similarity index was calculated according to Kuhnert and Korczak (2006). Two species specific PCR for Haemophilus parasuis were used to exclude this species from the study.

None of the strains were positive in the species specific *H. parasuis* PCR. A potential new species, consisting of 17 isolates, was identified with a genome similarity index of 0.56 to the closest related type strain – *A. indolicus* (similarity index > 0.4 and > 0.9 indicating the same genus and species, respectively; Table 1). The type strains of *H. parasuis* and *A. indolicus* formed a group with nine isolates. A further seven isolates did not fit into a group due to lack of congruence of the *thdF* gene phylogeny with *recN* and *rpoA* and their identity remains uncertain. The analysis also pointed to the inadequacy of the 16S rDNA identification as none of the strains identified as *A. porcitonsillarum/minor* were confirmed as such. It also highlighted that no single gene by itself can be used for identification.

Further work is in progress to look at the pathology associated with the 17 strains identified as a potential new species, to determine the significance of these organisms for the pork industry.

No. of isolates	16S identification	recN	%	rpoA	%	thdF	%	Monophyletic group with
8	A. indolicus/ H. parasuis/A. rossi	H. parasuis/ A. indolicus	>96.05	A. indolicus/ H. parasuis	>97.44	Uncertain	>92.20	H. parasuis/ A. indolicus
1	A. porcitonsillarum/ minor	Uncertain	84.96	A. indolicus/ H. parasuis	98.57/97.70	Uncertain	94.44	
3	A. minor	A. minor	100	A. minor	99.62	Uncertain	94.64	None
4	A. porcitonsillarum/ minor	No similarity	<84	no similarity/ minor	<84	A. minor/ uncertainty	<97.92	
2	A. porcinus	A. porcinus	<99.2	A. porcinus	99.87	A. porcinus	<99.71	A. porcinus
1	A. porcinus	Uncertain	84.96	A. porcinus	99.87	Uncertain	87.29	*
7	A. indolicus/H. parasuis	No similarity	<84	A. indolicus/H. parasuis	97.18/<97.50	Uncertain	85.22	New species
10	A. porcitonsillarum/ minor	No similarity	<84	A. indolicus/H. parasuis	97.18/97.44	Uncertain	>85.14	

 Table 1.
 Sequence of isolates and type strains were aligned with CLUSTAL. Values of greater than 96% belonged to the same species, values between 84 to 96% were uncertain and values below 84% had no similarity

#### Reference

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# Effects of a multi-strain probiotic on growth performance and faecal microflora in weaner pigs

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Weaning stress leads to pigs being susceptible to gastrointestinal disorders and digestive disturbances, thus resulting in a high incidence of diarrhoea and depressed growth rate (Lallès *et al.* 2007). It has been suggested that probiotics could promote intestinal health and alleviate weaning stress in young pigs (Lan *et al.* 2016). Therefore, the objective of the present study was to evaluate the effect of a multistrain probiotic (*Bacillus subtilis*  $1.0 \times 10^9$  cfu/g and *Bacillus lichenformis*  $1.0 \times 10^9$  cfu/g) on growth performance and faecal microflora in weaner pigs.

A total of 120 weaner pigs ((Landrace × Yorkshire) × Duroc) with an average bodyweight (BW) of  $7.84 \pm 1.75$  kg (28 days of age) were randomly allocated to four experimental diets: (1) CON, basal diet; (2) CON + 0.1% multi-strain probiotics; (3) CON + 0.2% multi-strain probiotics; and (4) CON + 0.3% multi-strain probiotics. All data were statistically analysed using the GLM procedure of the SAS v9 (SAS Institute Inc., Cary, NC, USA). Orthogonal comparisons were conducted using polynomial regression to determine linear, quadratic and cubic effects. Feed consumption was recorded on a pen basis during the experiment, and individual pig BW was recorded at initial, d 7, 14 and 42 of the experimental period to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain : feed (G : F). *Lactobacillus, E. coli* were determined by serial dilution  $(10^{-1} to 10^{-7})$  on selective media. The results are shown in Table 1.

In this study, during d 1 to 7, dietary supplementation of three concentrations of a multi-strain probiotic increased (linear effect, P < 0.05) the G : F. During d 14 to 42 and d 1 to 42, increasing the level of the dietary multi-strain probiotic improved (linear effect, P < 0.05) the ADG and G : F. At the end of experiment, dietary supplementation with the multi-strain probiotic decreased (linear effect, P < 0.05) the faecal *E. coli* counts.

In conclusion, dietary supplementation of a multi-strain probiotic (*B. subtilis* and *B. licheniformis*) exerted a positive influence on growth rate and faecal microflora in weaner pigs.

	CONA	TRT1 <sup>B</sup>	TRT2 <sup>C</sup>	TRT3 <sup>D</sup>	<i>P</i> -value			SEME
Items					Linear	Quadratic	Cubic	
d 1 to 7								
G:F	0.813 <sup>b</sup>	0.826 <sup>ab</sup>	0.852 <sup>ab</sup>	$0.860^{a}$	0.0118	0.8630	0.6096	0.013
d 14 to 42								
ADG (g)	497 <sup>b</sup>	515 <sup>ab</sup>	525 <sup>ab</sup>	544 <sup>a</sup>	0.0147	0.9895	0.7547	12
G:F	0.601 <sup>b</sup>	0.627 <sup>ab</sup>	0.639 <sup>a</sup>	$0.640^{a}$	0.0250	0.2989	0.9447	0.012
d 1 to 42								
ADG (g)	417 <sup>b</sup>	434 <sup>ab</sup>	441 <sup>ab</sup>	453 <sup>a</sup>	0.0141	0.7490	0.7147	9
G:F	0.653 <sup>b</sup>	0.672 <sup>ab</sup>	$0.688^{a}$	$0.689^{a}$	0.0028	0.2983	0.7395	0.008
<i>E. coli</i> $\log_{10}$ cfu/g	5.83 <sup>a</sup>	5.78 <sup>b</sup>	5.75 <sup>b</sup>	5.67 <sup>b</sup>	0.0135	0.5337	0.1790	0.02

Table 1.	Effect of probiotics on	growth and faecal	microflora in weane	r pigs
	p	8-0		- <b>F-B</b> -

<sup>A</sup>CON, basal diets. <sup>B</sup>TRT1, CON + 0.1% probiotics. <sup>C</sup>TRT2, CON + 0.2% probiotics. <sup>D</sup>TRT3, CON + 0.3% probiotics. <sup>E</sup>SEM, standard error of the mean. <sup>a,b</sup>Means with the same superscript are not statistically different.

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# Does dietary supplementation of gamma-aminobutyric acid improve performance in weaner pigs experimentally infected with *Escherichia coli* and given adrenocorticotropic hormone?

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Gamma-aminobutyric acid (GABA) is a non-protein amino acid and a major inhibitory neurotransmitter in the central nervous system. Previous research completed on rats and humans has shown that GABA stimulates voluntary feed intake and regulates stress hormone secretion in humans (Kruk and Pycock 1983). This neurotransmitter also plays important roles in motor function, emotions, appetite regulation and nutrient utilisation efficiency (Lelevich *et al.* 2009; Zhang *et al.* 2012). The aim of this study was to use both an enterotoxigenic *Escherichia coli* (ETEC) infection model and an adrenocorticotropic hormone (ACTH) injection to simulate a pathogenic and endocrine stressful weaning event. We hypothesised that an increased supplementation of GABA in diets of weaned pigs will improve growth performance and reduce cortisol production.

A total of 96 newly weaned male pigs (Large White × Landrace) with the Mucin 4+ allele were stratified into eight treatments based on weaning weight, sow parity and location in the building (eight treatments × 12 pigs = 96 pigs). The study was designed as a 2 × 4 factorial arrangement with respective factors being (1) without/with ETEC infection plus ACTH and (2) four dietary GABA (Sigma-Aldrich; MO, USA) levels (0, 60, 80, 100 mg/kg). All pigs were fed the same base diet (20% protein, 5% fat, 1.2% lysine, 5% crude fibre, 0.85% calcium and 0.4% salt). On d 8 and 9 after weaning, all piglets were orally inoculated with ETEC (0.8 mL via two gelatinised capsules; serotype O149;K88) as well as being given 5 IU ACTH (Synacther; Juno Pharmaceuticals, Vic., Australia) intramuscularly (IM), which occurred an hour beforehand. Pigs in the non-infected, non-ACTH group were given IM 0.2 mL of sterile saline and sham infected with two gelatinised capsules of phosphate-buffered saline. Faecal consistency score, diarrhoea index, and the number of therapeutic antibiotic treatments were recorded. Faecal β-haemolytic *E. coli* shedding was measured on d 0, 7, 10, 11 and 14 after weaning. Blood samples were collected on d 6, 9 (1 h after infection) and 14 from eight pigs per treatment. Plasma cortisol was assessed using ELISA from Enzo Life Sciences (Cat. No. ADI-900–071, NY, USA). Data were analysed by two-way analysis of variance using SPSS (v21.0, IBM, Armonk, NY, USA).

There were no differences (P > 0.05) between the four GABA diets for ADG, ADFI or FCR over the 21 days duration of the study (Table 1). Pigs in the non-infected, non-ACTH group gained 35 g/d more than pigs in the ETEC infection plus ACTH group during week 3 (P = 0.098; data not shown). In total, 73.2% of pigs in the infection plus ACTH group developed diarrhoea between d 8 to 14 compared to 26.8% in the non-infected, non-ACTH group of pigs (P = 0.001). These data indicate that eliciting both an ETEC infection challenge and an acute stress response after weaning caused no overall detrimental effects on production or diarrhoea; however, further investigation will establish blood measurements more indicative of the stress response during this period.

 Table 1. Effects of dietary treatments, ETEC+ACTH infection or sham-infection on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) from d 0 to 21 after weaning

Item	GABA (mg/kg) (D)			Treatment (T)		s.e.m.	<i>P</i> -value			
	0	60	80	100	Sham	Infection		D	Т	$D \times T$
ADG (g)	186	191	187	168	189	176	13.1	0.63	0.33	0.95
ADFI (g)	290	295	279	260	291	272	17.4	0.50	0.26	0.95
FCR $(g:g)$	1.62	1.58	1.53	1.60	1.57	1.59	0.02	0.76	0.72	0.67

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