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Entire male pig production in Europe

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Abstract. In Europe the proportion of male pigs that are left 'entire' has been high for many years in the British Isles and Iberian Peninsula, and has recently increased in The Netherlands and to a lesser extent in Germany and France. Various European Union partners agreed in 2010 on a road map to abandon piglet castration by 1 January 2018. Despite significant commercial in-confidence research on instrumental methods for detecting boar-tainted carcasses at slaughter plants, nothing is currently being adopted at an industrial scale. A few abattoirs sort out the most heavily tainted carcasses, using human nose methods. However, there are major concerns with their accuracy, which is currently not documented in any publicly available technical report. The importance of androstenone and skatole for boar taint is still debated but a recent study (CAMPIG; G Backus, H Snoek, MA Oliver, M Font i Furnols, M Aluwé, F Tuyttens, M Bonneau, P Chevillon, MD Aaslyng, D Moerlein, L Meier-Dinkel, J Trautmann, J-E Haugen, unpubl. data) has established preliminary equations relating consumer dissatisfaction to androstenone and skatole levels. These equations still need further consolidation to integrate the impact of very high and very low androstenone levels on consumer acceptability. Reducing the incidence of boar taint at a production level and at the same time overcoming possible greater aggressive behaviour of entire male pigs are also critical for abandoning castration. Genetic selection is the most efficient way to reduce androstenone, but the selection of boar-taint-free genetic lines without any adverse consequence on the reproductive and growth performance will take time. Skatole levels can be efficiently reduced via feeding specific feedstuffs and good control of the animals' environment. Provided that the incidence of boar taint can be reduced to an acceptable level and the residual tainted carcasses can be sorted out at a reasonable price by mutually recognised methods, the abandonment of castration will result in high benefits, up to one-billion euros for both the pork industry, via a drastic reduction of production costs, and society at large, through improved animal welfare and reduced impact on the environment.

Additional keywords: animal welfare, boar taint, castration, consumer acceptance, detection, threshold.

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Introduction

Castration of piglets in the European Union (EU) is regulated by the Council Directive 2008/120/EC, of 18 December 2008, relating to the minimum standards for the protection of pigs:

Castration of male pigs

The castration of male pigs can be carried out by means that do not imply the tearing of tissues under the following conditions:

- In the case of those piglets below the age of 7 days, the castration will only be performed by a veterinarian or by a person that has been trained in animal welfare and that has experience in the carrying out of these techniques with adequate means and hygienic conditions.
- In the case of those piglets from their seventh day of age or older, the castration will only be performed, under anaesthesia and a prolonged analgesia, by a veterinarian.

Since the report written by the European Food Safety Authority (EFSA 2004) declaring that castration before the seventh day of age is painful, and recommending a checking of the regulations, a debate about banning castration of piglets in the EU has commenced. On the invitation of the European Commission and the Belgian Presidency, and following a workshop on alternatives for pig castration, representatives of European farmers, meat industry, retailers, scientists, veterinarians and animal welfare non-government organisations met at the end of 2010 in Brussels to discuss the issue of pig castration, its possible alternatives, and to evaluate the possibilities to end this practice. At that time, as a basis for discussion, results of a large project on alternatives to pig castration funded by the EU were available (Bonneau *et al*. 2000*a*, 2000*b*; Dijksterhuis *et al*. 2000; Matthews *et al*. 2000).

As previously mentioned the main driver for this initiative was the fact that enough scientific evidence exists, which indicates that surgical castration of pigs is a painful intervention (Prunier *et al*. 2005) even when performed on very young animals (Llamas Moya *et al*. 2008). Apart from being an animal welfare concern, piglet castration is a market-driven decision primarily implemented to avoid the incidence of boar taint, a very unpleasant odour and taste of pork from entire male pigs

(Tuyttens *et al*. 2012). In view of the necessity to improve pig production efficiency and concomitantly lower its environmental impact, omitting surgical castration could also contribute to reach this objective as entire male pigs are more efficient at converting feed to growth than barrows. Before the launching of this initiative, different alternatives to surgical castration such as rearing of entire male pigs or vaccination to reduce boar taint were already applied in and outside the EU (Fredriksen *et al*. 2009). In order to relieve pain, Norway in 2002 and Switzerland in 2010 implemented pig castration only with anaesthesia and anaesthesia and analgesia, respectively. At the same time, countries like The Netherlands, Belgium and Germany committed to the long-term phasing out of surgical castration of piglets. Furthermore, some large European meat retailers started to market pork from entire male pigs and pigs immunised against gonadotrophin-releasing factor (immunovaccination) or pigs which were surgically castrated with anaesthesia or analgesia. It was recognised that these different approaches within the EU could cause problems for trading within the EU as well as for exports to third countries (e.g. China, Hong Kong, Philippines and before the embargo, Russia). This was the starting point of the initiative aiming at a concerted European-wide approach to solve the issue of pig castration and obtain a mutual recognition of the alternatives, which ultimately will facilitate trading of pork. As an outcome of these meetings, the various partners agreed on a road map with the final goal to abandon piglet castration by 1 January 2018 (European Commission 2010). As a first step, from 1 January 2012, surgical castration of pigs, if carried out, should be performed with prolonged analgesia and (or) anaesthesia with methods mutually recognised. Furthermore, all parties agreed that implementation of the ban of surgical castration needed coordinated research and development on the following points:

(1) European recognised reference methods for the measurement of each of the compounds responsible for boar taint;
(2) Rapid detection methods for boar taint at slaughter plants;
(3) Reduction of boar taint compounds by pig breeding and/or management and feeding;
(4) The production systems and management of entire male pigs during rearing, transport and at slaughter, to minimise sexual and aggressive behaviours.

However, in the case of high quality pork specialties registered under 'traditional specialties guaranteed' or with 'Geographical Indications' (Protected Geographical Indication or Protected Designation of Origin), castration is currently unavoidable to meet the defined quality standards (e.g. fat cover of the ham, intramuscular fat content, size of the ham) as pigs are generally slaughtered at much greater bodyweight (>160 kg). To ensure a sustainable and competitive pig meat chain in the EU, a European partnership on pig castration, supported and funded by the European Commission, was established in order to:

(1) Ensure the acceptance of products from pigs not surgically castrated by the authorities and the consumers in the EU but also in third country markets.
(2) Develop information and training of farmers and other members of the whole pork chain.
(3) Launch a cost/benefit analysis on the consequences of the end of surgical castration, including an analysis of the change in production costs in various production systems, the costs/benefits affecting the different levels of the pork chain and the cost sharing plans between the economic actors of the chain.
(4) Develop a list of traditional productions (e.g. Protected Geographical Indication or Protected Designation of Origin) requiring heavier pigs.
(5) Publish the abovementioned annual report. The report will also include a part on the costs for implementing the end of surgical castration.

To achieve the aforementioned objectives, several projects funded by the European Commission were launched. In addition, in various European countries such as The Netherlands, Belgium, France, and Germany, nationally funded research programs were started with the main purpose to tackle the objectives but taking into account the national perspective. This review will give an overview on the current situation with respect to the share of entire male pigs already slaughtered in Europe, what kind of initiatives have been undertaken to reach the goal to ban piglet castration, what has been achieved so far, and what efforts need attention in the near future.

Current situation in the EU with respect to entire male production

Importance of entire male production

With respect to the importance of pig production, out of the 28 member states the eight largest producers, slaughtering ~81% of all pigs in Europe, are in decreasing order: Germany, Spain, France, Poland, Denmark, The Netherlands, Italy and Belgium, with 58.8, 41.4, 23.9, 20.5, 19.1, 13.8, 13.5 and 11.9 million slaughtered pigs per year, respectively. Four major average carcass weight categories exist: <70 kg (Greece and Portugal), 82–88 kg (Spain, Poland, Denmark, United Kingdom and Ireland), 90–95 kg (Germany, France, The Netherlands and Belgium) and 121 kg (Italy). By the end of 2014, the proportion of entire male pigs already produced in the member states of the EU differs markedly. Traditionally, >90% of the male pigs born in the United Kingdom and Ireland are raised as uncastrated males. Together this represents annually ~6.4 million pigs slaughtered as boars. Apart from these two countries, Spain, Portugal and Greece together with The Netherlands raise and slaughter >60% of their male pigs as entire male pigs, which represents ~24.15 million pigs, with Spain and The Netherlands having the greatest share with 20.35 million pigs. The proportion of male pigs slaughtered as boars in Germany and France is ~12% followed by 5% in Denmark. Because overall in Germany 2.5 times more pigs are slaughtered compared with France, the actual number in Germany is ~3.5 and in France 1.5 million boars. Up to now, from the aforementioned eight large European pig-producing countries, only Poland, Italy and Belgium are slaughtering very few entire male pigs. In Italy especially, one of the main reasons for avoiding raising entire male pigs is the fact that for ham production a large number of pigs are slaughtered at older ages and markedly heavier slaughter weights. Instead of raising entire male pigs, Belgian pig producers

perceive immunovaccination as a valuable alternative to pig castration. Of the 2 million IMPROVAC vaccines distributed by ZOETIS in Europe, the majority is used in Belgium.

How piglet castration currently is carried out

Surgical castration involves cutting and manipulating innervated tissues (Prunier *et al*. 2006) and if anaesthesia is not provided it will be painful (von Borell *et al*. 2009), as reflected by elevated blood cortisol concentrations (Carroll *et al*. 2006; Kluivers-Poodt *et al*. 2012), high-pitched squealing (Taylor and Weary 2000; Puppe *et al*. 2005) and pain-indicative behaviours, such as trembling and lying alone (Hay *et al*. 2003). Some behavioural indicators of pain may persist for up to 5 days (Puppe *et al*. 2005). In addition, surgical castration can entail some complications like haemorrhage, excessive swelling or oedema, infection, poor wound healing and, if the procedure is not performed well, failure to remove both testicles. As reviewed by Prunier *et al*. (2006), suppressed immunity leading to greater incidence of inflammation and pneumonia has also been linked to the castration procedure.

A survey of Flemish pig producers showed that farmers perceive surgical castration without anaesthesia as the best strategy with regard to profitability, performance and reduction of boar taint; however, they expected the lowest consumer acceptance for this strategy (Tuyttens *et al*. 2012). The farmers ranked surgical castration with anaesthesia as the most labour intensive and production of intact males as the least profitable and least effective at reducing boar taint. By contrast, several surveys showed that consumers are not generally aware that pigs are castrated nor are they aware of boar taint (Huber-Eicher and Spring 2008; Fredriksen *et al*. 2011). However, when informed, European consumers view surgical castration without anaesthesia as a serious animal welfare concern (Huber-Eicher and Spring 2008; Fredriksen *et al*. 2011; Tuyttens *et al*. 2011; Vanhonacker and Verbeke 2011). A survey of Swedish consumers and Belgian students showed that immunovaccination was viewed positively and preferred over surgical castration or no castration at all (Tuyttens *et al*. 2011; Vanhonacker and Verbeke 2011), whereas a similar survey of consumers in Norway showed them to be sceptical about immunovaccination (Fredriksen *et al*. 2011).

Based on this assessment, and from 1 January 2012, those who have voluntarily made use of the aforementioned declaration carried out surgical castration with anaesthesia and (or) prolonged analgesia. Currently producers of only one country, The Netherlands, which signed the declaration, perform piglet castration after anaesthesia. Norway and Switzerland, two countries which are not part of the EU and have a relatively small pork industry, were not part of this agreement but made the use of a local or general anaesthetic also mandatory. Thereby, different methods are used:

- In The Netherlands, male pigs which are still castrated are anaesthetised for up to 75 s with a gas mixture of 70% CO_2 and 30% O_2. After receiving an adequate training, the procedure is performed by the producer itself. The effectiveness of CO_2 as an inhalation agent has been proven (Svendsen 2006; Gerritzen *et al*. 2008). Nevertheless, it has also been shown that CO_2 causes distress in piglets (Kohler *et al*. 1998; Prunier and Bonneau 2006).
- Since 2002, local anaesthesia during castration using a combination of subcutaneous and intratesticular administration of lidocaine (2%) with adrenaline at an average age of 10 days is mandatory in Norway. The intervention is performed by veterinarians. As determined by Fredriksen and Nafstad (2006), two-thirds of the veterinarians but only one-third of the pig producers were satisfied or very satisfied with the implemented policy. However, after 2 years of experience the proportion of dissatisfied producers decreased to one-third. The main reason for the persisting negative attitude was that farmers raised doubts about the overall efficacy in improving welfare as performing local anaesthesia requires extra handling.
- On the basis of the study of Walker *et al*. (2004), and since 2010 the use of isoflurane, a highly effective anaesthetic (Kohler *et al*. 1998; Hodgson 2006, 2007), is mandatory when castrating piglets in Switzerland. In addition to the general anaesthesia, and 15 min before performing the procedure, piglets need to receive an intramuscular injection of an analgesic such as Metacam (Boehringer Ingelheim), Tolfedin (Vétoquinol GmbH) or Finadyne (MSD Animal Health). After appropriate training with the inhaler and information about safety issues regarding the handling and use of isoflurane, pig producers are allowed to perform the procedure themselves.

The Swiss solution is by far the most costly followed by the Norwegian and Dutch solutions. Nevertheless, despite its cost, producers from Germany and France manifested some interest in the Swiss procedure. However, in Germany the deadline for castrating piglets without anaesthesia was recently extended until the end of 2018 in order to give producers enough time to make adjustments (Buhl 2013). To our knowledge, none of the other member states wants to implement castration with local or general anaesthesia.

Roadmap for implementing (voluntary) ban of castration in Europe

Reference method for the determination of the main compounds responsible for boar taint

For the detection and quantification of androstenone, skatole and indole, the three most important compounds responsible for boar taint, a wide range of analytical methods, covering immunological and chromatographic approaches combined with different sample clean-up procedures, have been developed. The results of these analyses are used to establish androstenone, skatole and indole threshold levels with the aim to define sensory appreciation of meat from entire male pigs (Bonneau *et al*. 2000*b*; Pauly *et al*. 2010). However, results of a limited inter-laboratory comparison study of the various methods used showed a not-negligible variability demonstrating the urgent need for a harmonisation and standardisation of androstenone, skatole and indole analysis (Haugen *et al*. 2012). The authors also emphasised that the varying sensory threshold levels reported for the boar substances are not entirely due to differences in sensitivity among tested individuals, or in differences in the way the tested meat is prepared, but may also be partly explained by a systematic bias in the laboratory methods being used for measuring the concentration of the

three compounds. Furthermore, it is evident that for trading purposes a harmonised and validated reference method is a prerequisite, which helps to build trust among stakeholders within and between countries. Thus, the Joint Research Centre, which is the European Commission's in-house science service tasked to carry out research to provide independent scientific advice and support to EU policy, was mandated to develop the reference method. An analysis method based on a common sample preparation, starting from liquefied fat from neck fat tissue followed by a clean-up using size exclusion chromatography, was developed. The measurement of the target substances is performed by stable isotope dilution chromatography mass spectrometry (Thomas *et al*. 2014). The detection levels are below 0.01 mg/kg for skatole and indole and ~0.05 mg/kg for androstenone. The method has proven to be robust and free from matrix interferences. With respect to recovery rate, repeatability and measurement uncertainty, the method is reliable and sensitive enough to determine the off-flavour compounds at the sensory threshold values and its performance characteristics complies with the requirements for official food control methods in the area of food contaminants, and therefore is fit for its intended purpose.

Rapid detection methods for boar taint at slaughter plants

The objective of a survey carried out in the framework of the European-funded project BoarCheck (J-E Haugen, BE Nielsen, C van Wagenberg, N Panella-Riera, M Aluwé, unpubl. data; Table 1) aimed to assess the necessity of slaughter plants from seven European (Belgium, Denmark, France, The Netherlands, Germany, Spain, United Kingdom) and three non-European (Canada, New Zealand, Norway) countries to have at hand a reliable rapid boar-taint detection method. The majority (26 out of 31) of the European-based respondents regarded such a tool as important whereas it seemed less important for those from non-European countries (1 out of 4). Thus, apart from a reliable reference method, the development of on-/offline boar-taint detection method(s) that is (are) in compliance with industrial standards are considered crucial for the implementation of a sustainable European entire male production. In the BoarCheck project, the requirements of such methods were assessed and compared with existing and in-development instrumental or sensory-based methods for boar-taint detection developed for slaughter plants in the EU. From the point of view of the slaughter industry, suitable boar-taint detection methods need to be adaptable whenever changes in the parameters used to analyse boar taint are needed and comply with food and work safety regulations. Furthermore, a primary objective of the industry is to have a method at their hands that ensures complete consumer satisfaction without risking to downgrading too many untainted carcasses. Therefore, the following generic requirements were considered decisive to make boar-taint detection methods suitable candidates:

- The method has to be sufficiently sensitive, specific and reproducible and reflect with great accuracy the consumer perception for boar taint.
- The method should integrate the measurement of the relevant boar-taint marker substances in a single system. This system should also be incorporable in current industrial setting.

Table 1. Objectives and main outcomes of the BoarCheck project

BoarCheck (A study on rapid methods for boar taint used or being developed at slaughter plants in the European Union)	
Objectives	Main outcomes
(1) Describe the current situation of boar-taint detection in slaughter plants in the European Union	• Sensory-based/human nose methods are used at the slaughter line at major European slaughter companies in Denmark, Germany, The Netherlands, France and Belgium • One instrumental method, based on a colorimetric method (skatole equivalents) is in place in one slaughter line in Denmark
(2) Critically review the rapid boar-taint detection methods in the European Union, compare the feasibility, performance and cost of the methods and identify the most suitable and reliable ones	• Comparison of the performance of the sensory-based/human nose methods could not be performed • Currently[A] no instrumental method for the detection of boar taint that can be used at industry level is available • Relevant instrumental methods are at the research and development stage • Promising[B] instrumental methods, which fulfil the industrial requirements[C] for analysis time and and/or simplicity are ▪ based on mass spectrometry, which have the ability to detect androstenone and skatole levels. ▪ based on electrochemical sensors technology, which have the ability to detect androstenone and skatole and if required also indole levels

[A]At the end of the BoarCheck project, which was September 2014.

[B]The limits of quantification are low enough to accommodate the envisaged sensory thresholds for androstenone and skatole. The preliminary data obtained at laboratory conditions are compatible with the requirements for capacity and time. It remains to be established if this together with the sensitivity and specificity of the analysis holds under industrial conditions.

[C]The method should comply to measurement capacity of 100–800 entire male pig carcasses per h, which means 4–36 s per analysis with a cost of less than €2 per sample.

- The method should give results immediately or shortly after the measurements (preferably within minutes).
- The method should have high sample throughput capacity and be cost effective.

Instrumental measurements

Currently only one instrumental detection method for sorting out boar-taint carcasses is in use on an industrial scale. The colourimetric method, which measures the sum of both skatole and indole to yield 'skatole equivalents' (Mortensen and Sorensen 1984), was introduced in Danish slaughter plants in 1991 (Hagdrup 2009), and allows analysis of up to 360 samples per h. As shown by Hansen-Moller and Andersen (1994), the correlation between the skatole equivalents and the concentration determined by high-performance liquid chromatography of skatole alone or the sum of skatole and indole was 0.975 and 0.986, respectively. Together with the low analytical error of 0.04 ppm, determination of skatole equivalents proved to be a valid system. However, in addition to the relatively high maintenance costs and the required great expertise of the personnel working with the machine, the Danish colorimetric method allows determination of only a part of the known boar-taint compounds and therefore does not fully cover the defined requirements.

It has often been debated whether androstenone, skatole and indole explain the full chemical spectrum of boar taint. Although there is no clear answer to that question, it can be stated without risk that those compounds do not account for all the unpleasant odours that can be found in meat from entire male pigs. Aiming to develop a method, which does not rely on the androstenone, skatole and indole compounds, Ampuero and Bee (2006) evaluated the potential of the electronic nose (SMart Nose 151, LDZ, Neuchatel, Switzerland) with a mass spectrometer (quadripole) as a detector to classify boar-tainted carcasses. In the study, backfat samples of a small number of entire male pigs ($n = 35$) and barrows ($n = 3$) were analysed with the electronic nose and by a trained panel using an olfactory test. The obtained mass spectrometer spectra were subjected to Principle Component and Discriminant Factor Analysis, which revealed a 100% correct classification between samples of entire male pigs and barrows. Furthermore, the authors reported that samples could be discriminated between high androstenone, high skatole, low androstenone and low skatole levels. However, cross-validation with 23 unknown samples showed that eight were misclassified as false positive. Nevertheless, these results and a recent study of Kirsching *et al*. (2012) demonstrated the potential of the electronic nose to discriminate between high and low levels of boar taint without relying exclusively on the taint-related compounds. However, a lot of supplementary testing and validating the used chemometric models would be needed to allow these methods to comply with the required industrial standards.

In the BoarCheck project, additional instrumental methods were identified which complied with industrial requirements such as analysis time or simplicity with regards to both operation and complexity in technology. Instrumental methods based on mass spectrometry, which can specifically measure both androstenone and skatole simultaneously, seem to fulfil most of the aforementioned industrial requirements. In addition, the electrochemical sensors-based method and insect-based biosensing were recognised as approaches that also have the potential to fulfil the industrial requirements. To our knowledge, these methods are still at the research and development stage and documentation on performance and validity are still lacking. Apart from the challenges regarding analytical determination, tissue sampling, which is recognised to be a critical element for the industrial application, remains an unresolved issue.

Human nose method

As previously mentioned, except for the Danish colorimetric method, currently no objective instrumental measurement to detect boar taint is available. Thus, abattoirs in Germany, The Netherlands, France and Belgium who regularly slaughter entire male pigs introduced sensory-based sorting methods, also known under the term Human Nose Score (HNS). Regarding the accuracy with respect to sensitivity and specificity of the HNS system, so far only one study at industrial level has evaluated its validity (Mathur *et al*. 2012). The study was carried out with 6574 subcutaneous backfat samples from the neck regions collected from pure- and cross-bred entire male pigs originating from different sire and dam lines and from different farms. They were slaughtered at an abattoir in The Netherlands. Contrary to the practice of some commercial abattoirs, which evaluate boar taint at slaughter line speed (>500 samples/h), in the study of Mathur *et al*. (2012), sensory assessment was performed in a laboratory setting by nine panellists trained for detecting boar odour using the HNS (0 = no detectable boar taint; 1 = no boar taint but some off odour; 2 = more off odour but no boar taint; 3 = some boar taint odour; 4 = strong boar-taint odour). In general, the correlation coefficients between the HNS and androstenone and skatole concentrations showed great variability ranging from 0.25 to 0.55 and 0.32 to 0.89, respectively. Based on the somewhat closer relationship between HNS and skatole, Mathur *et al*. (2012) suggested that skatole is a better predictor of boar taint. However, this assumption can be questioned as the average reproducibility weighted for the number of samples between assessors was low at 23%, and was recently confirmed by Meier-Dinkel *et al*. (2015). Interestingly, out of the 6574 samples analysed, the proportion of samples with boar taint was only 8.7% (Mathur *et al*. 2012). When sorting samples based on commonly used thresholds for androstenone (1 ppm) and skatole (0.25 ppm) (Walstra *et al*. 1999; Pauly *et al*. 2010), Mathur *et al*. (2012) estimated that the proportion of samples with boar taint would markedly increase and reach values as high as 44%. In addition, it was observed that some carcasses with low levels of androstenone and skatole also have strong boar-taint odour, reaching HNS of 3–4. Thus, the authors of the study emphasised that relying on the androstenone and skatole levels alone for the detection of boar taint may result in a too great number of false positives whereas at the same time a certain percentage of false negatives can not be excluded (Mathur *et al*. 2012). Again this conclusion needs to be taken with great caution because it would be valid only if HNS itself would be a reproducible determination method.

Because the method is simple, easy to use, fast and rather cheap, the sensory-based sorting method has become the method of choice for those abattoirs slaughtering and testing carcasses of entire male pigs. However, different method protocols including training of assessors, sampling and criteria for assessing tainted carcasses are in place. Thus, there are serious concerns about the reliability of the HNS as it is currently applied by the industry (on-line assessment by one or two people at chain speed). Those concerns are based on general considerations about selection, training, varying olfactory acuity of the assessor, fatigue of the assessor over time, olfactory noise, lack of time to perform the test and possible carry-over effects from heavily tainted carcasses (Meier-Dinkel *et al*. 2013, 2015; Meinert *et al*. 2013; Mörlein *et al*. 2013). For assessors' selection purposes, Trautmann *et al*. (2014) presented tests for the psychophysical evaluation of olfactory acuity to key volatiles contributing to boar taint, which ultimately could help reducing the observed considerable variation of olfactory performance among assessors. Apart from the olfactory test itself, the various heating methods such as a soldering iron, microwave and pyropen affect the intensity score and thus the outcome of the results (Bekaert *et al*. 2013). For trading purposes and confidence-building measures within the pork production chain, the available field experience data together with the recent scientific results needs to be used for further optimisation and standardisation of the HNS protocol(s).

Consumers' acceptance of pork from entire males

Regardless of the selected instrumental or sensory-based sorting method, there is still an urgent need for more information on boar-taint sorting thresholds based on consumer testing. Results of a simulation study carried out by Bonneau *et al*. (2000*b*) revealed that when pork from entire male pigs instead of females reach the market, the degree of consumers' dissatisfaction due to deviation in odour (+6.5%) and flavour (3.0%) will undoubtedly increase. The greater degree of dislike for odour originated primarily from the underlying skatole and less from the androstenone concentration (Matthews *et al*. 2000; Font-i-Furnols 2012). By contrast, with respect to flavour, both boar-taint components were similarly important for the degree of dissatisfaction. Furthermore, the authors concluded that because some consumers are very sensitive to the odour of boar-taint compounds, it will be elusive that one threshold level for each of the three compounds will completely eliminate differences in consumer dissatisfaction between entire male and female pork. This would speak against the definition of universally recognised thresholds for an instrumental method, which relies only on androstenone, skatole and indole determination but favour the calculation of risks levels associated with each level of boar-taint compounds or each value provided by any instrumental method. The industry can then choose the risk level they are prepared to face and determine their sorting strategy accordingly. Recently, Blanch *et al*. (2012) studied consumers' acceptance of pork with different levels of boar taint according to their androstenone sensitivity in three different European countries (France, Spain and United Kingdom). The study confirmed the large variability in perception of androstenone and skatole and they reported that the percentage of consumers that may reject tainted meat was very variable (14.3–41.0%), being lower in countries where consumers are continuously (United Kingdom) or occasionally (Spain) exposed to pork from entire male pigs and being markedly greater where this exposure occurred only rarely at that time (France). For an updated and clearer picture, by including more member state countries on consumer perception towards pork from entire male pigs, a European project with the acronym CAMPIG (G Backus, H Snoek, MA Oliver, M Font i Furnols, M Aluwé, F Tuyttens, M Bonneau, P Chevillon, MD Aaslyng, D Moerlein, L Meier-Dinkel, J Trautmann, J-E Haugen, unpubl. data; Table 2) was launched in 2012. The following three objectives were formulated: (1) study the consumer acceptance of pork from entire male pigs in the EU and in third countries; (2) re-evaluate the relationship between levels of androstenone and skatole and the sensory assessment of meat from entire male pigs; and (3) establish the consumer attitudes towards factors related to the acceptance of alternatives of surgical castration of pigs. Primary results and derived conclusions were presented at the workshop on alternatives to pig castration organised by DG SANCO on 26 February 2015 in Brussels. In the sensory study, consumer tests were conducted with 1099 consumers in Germany, Denmark, France, Italy, Poland, Russia and China. For the sensory evaluation, standardised pork patties of either castrate or boar meat containing a fat content of 15–20% were prepared at one site. The androstenone and skatole content (expressed per g fat) of the adipose tissue used ranged from 0.5 to 2.0 and 0.1–0.4 ppm, respectively. In accordance with aforementioned observations (Bonneau *et al*. 2000*b*; Blanch *et al*. 2012), skatole levels affected more strongly consumer acceptance than androstenone. However, when only androstenone-sensitive consumers were included, preference for boar meat decreased rapidly when androstenone levels increased despite low skatole levels. The explorative study in Russia and China revealed a stronger disfavour of boar meat with medium to high levels of androstenone and skatole, which increases in consumers sensitive to skatole. Furthermore, over the whole range of skatole levels included in the study, patties prepared from boar meat were less appreciated than those from castrates and with increasing skatole levels the percentage of dislike increased even more. The role of androstenone is likely to have been underestimated in this study because androstenone levels higher than 2.0 ppm have not been explored, although they are quite commonly observed in entire male pigs (Baes *et al*. 2013; Strathe *et al*. 2013*a*). As expected and due to the fact that liking (in this case with patties) is not accelerating at defined androstenone and/or skatole threshold values, but is rather continuous instead, no single threshold values for the two boar-taint compounds could be determined.

Thus, the authors of the CAMPIG project suggest that companies have to determine their own thresholds, depending on the products and given their own risk assessment. In order to give the companies a guidance for the risk assessment, the authors of the CAMPIG project created a preference map depicting the importance of skatole in the liking of boar meat patties and also the relevance of androstenone when skatole levels are low: for instance from the preference map one can conclude that at a given androstenone level of 1.1 ppm, the percentage of dissatisfied consumers increases from 20% to

Table 2. Objectives and main outcomes of the CAMPIG project

CAMPIG (Consumer acceptance in the European Union[A] and third countries[B] of pig meat[C] obtained from male pigs not surgically castrated)	
Objectives	Main outcomes
(1) Study and assess possible differences in consumer acceptance of meat from entire male pigs within the European Union and in third countries	• Differences in consumer acceptance of pork with varying levels of skatole and androstenone between the European Union member states were not significant • Consumers in third countries displayed stronger disfavour of boar meat with medium to high levels of skatole and androstenone. The % dissatisfied consumers increased in those sensitive to skatole
(2) Establish the relationship between levels of androstenone and skatole and the sensory assessment of meat obtained from male pigs	• Over the whole studied range of skatole[D] and androstenone[E] the % dissatisfied consumers is higher for meat from entire male pigs compared with meat from castrated male pigs • Consumer acceptance is more strongly affected by skatole than by androstenone levels o Increasing skatole levels is related to decreasing preference for entire male pigs over meat of castrated pigs o In the low skatole range, androstenone-sensitive[F] people showed decreasing preference for meat from entire male pigs with increasing skatole levels
(3) Assess consumer attitudes[G] towards factors related to the acceptance of alternatives of surgical castration of male pigs	• The attitude of consumers related to the pork production are determined by four dimensions of motives, which are food safety and quality, animal and environmental friendliness, costs and regional identity o Animal and environmental friendliness is rated second in Belgium, China, Denmark, Germany, France, Greece, Italy, The Netherlands, Poland and Spain • Differences in consumer attitudes between European Union member states and Asian countries are smaller than the differences between the European Union countries • From 11 motives[H] for buying and eating pork animal welfare was together with no artificial ingredients and convenience the least often selected. This is not true for consumers from Denmark, Germany, Belgium, and The Netherlands, which considered this motive relatively more important (rank 4 or 5)

[A]Germany, Denmark, France, Italy, Poland.
[B]Russia, China.
[C]Meat = meat patties with 15–20% fat and a skatole content ranging from 0.1 to 0.4 ppm skatole and 0.5–2.0 ppm androstenone.
[D]Conclusion valid only for the range of skatole levels included in the study (0.1–0.4 ppm in the fat).
[E]Conclusion valid only for the range of androstenone levels included in the study (0.5–2.0 ppm in the fat).
[F]Sensitivity was assessed based on an odour liking test for androstenone (5 ppm) and skatole (1 ppm) using a 9-point hedonic scale. Subjects were classified as sensitive whenever they correctly discriminated on a triangle test correctly for androstenone or skatole.
[G]Consumer attitude was obtained with an online survey among 11 294 consumers from Belgium, Denmark, France, Germany, Greece, Italy, Latvia, The Netherlands, Poland, Spain, China, South Korea, Russia, USA.
[H]The 11 generic motives were quality, price, taste, healthy, safe, natural, appearance, no artificial ingredients, animal friendly, friendly to environment and convenience.

47% when the skatole level increases from 0.1 to 0.4 ppm; however, when skatole level is low (0.1 ppm), the percentage of dissatisfaction increases from 16% to 20% to 24% when androstenone level increases from 0.5 to 1.1 to 2.1 ppm. When considering only androstenone-sensitive consumers, which represent about half of the consumers, the percentage of dissatisfied consumers at a given androstenone level of 1.07 ppm increases from 31.0% to 57.0% when the skatole level increases from 0.1 to 0.4 ppm; however, when skatole level is low (0.1 ppm), the percentage of dissatisfaction increases from 17% to 31% to 46% when androstenone level increases from 0.5 to 1.1 to 2.1 ppm.

With an internet-based survey among 11 294 consumers conducted in 10 European member state countries (Belgium, Denmark, France, Germany, Greece, Italy, Latvia, The Netherlands, Poland and Spain) and four other countries (China, Russia, South Korea, United States of America), consumer attitudes towards alternatives of surgical castration were assessed. Of the respondents, 62%, 15%, 29%, and 29% knew the terms castration, immunocastration, immunovaccination and boar taint, respectively, and 29% were not familiar with either one of these terms. There is evidence that the participating consumers have no clear opinion on specific issues of pork production. In a choice experiment regarding the importance of production aspects consumers ranked, in decreasing importance order, the following attribute values: produced in a natural way > avoiding human health risks > ensuring the best taste > avoiding stress and pain as most important. Production costs and pharmaceutical interventions only in case of medical need scored lowest. These answers could be relevant when evaluating for instance acceptance of immunovaccination (not specifically studied in the CAMPIG project) as a tool to circumvent piglet castration. Lower acceptance levels can be expected if consumers relate immunovaccination with human

health risks or naturalness. To assess the general willingness to purchase and consume pork, consumers of the survey were also presented with 11 motives (quality, price, taste, healthy, safe, natural, appearance, no artificial ingredients, animal friendliness, friendly to environment, convenience) for such a decision. They were then asked to choose from the set of 11, the three most important ones. Quality, price and taste were on average most often selected. Animal friendly, friendly to the environment, and convenience were least often selected. The survey showed that large differences exited between countries in the attitudes for main drivers of banning piglet castration. For instance 15% of consumers ranked animal friendliness in the top 3, ranging from as high as 28.2% to as low as 2.1% from German to Latvian consumers, respectively. In conclusion, the results of the CAMPIG survey allows the companies to use the consumer attitudes to develop communication and selling strategies to place products from boar meat in specific market segments.

Reduction of boar-taint compounds by pig breeding and (or) management and feeding

Possible breeding strategies

All the sensory studies carried out with trained panellists or consumers clearly show that in order to diminish dislike for pork from entire male pigs, low skatole and androstenone levels needs to be targeted. It is well known that to circumvent the boar-taint problem other alternatives such as semen sexing, which would allow the production of only female pigs or immunovaccination, involving the immunisation of the pigs against gonadotropin-releasing factor (or hormone), exists. Whereas for other species such as cattle the use of sexed semen is common practice, the same is not likely to become available for swine in the near future (Vazquez *et al*. 2001, 2009). Although in many European countries immunovaccination is registered and has been tested under research and field conditions and proven to be effective to avoid boar taint, its use in a large-scale setting is, apart from one Belgian retailer, limited because of image concerns. Therefore, if entire male pig production is the final objective to avoid surgical castration then, despite the unclear concern of the possible antagonistic relationship to fertility (Strathe *et al*. 2013*b*; Frieden *et al*. 2014) and negative effects on average daily gain, drip loss and intramuscular fat content (Haberland *et al*. 2014), developing breeding strategies against the main compounds of boar taint seems a promising way forward. Such a strategy would be very effective due to high heritability (Frieden *et al*. 2012; Baes *et al*. 2013; Strathe *et al*. 2013*a*), encounter high consumer acceptance, and result in favourable effects on economically important performance traits like feed efficiency and carcass leanness (Pauly *et al*. 2009; Batorek *et al*. 2012). Integrating this approach into a practical breeding plan requires a reliable assessment of the traits of interest. As previously discussed, up to now, two methods for boar-taint detection are available: (1) determination of androstenone, skatole and indole concentration or (2) evaluating boar odour using the HNS. Although very cheap and fast, the HNS can be performed only at the abattoir on carcasses of siblings of selection candidates (Windig *et al*. 2012). Determination of androstenone, skatole and indole can be carried out either on backfat samples collected at slaughter from offspring of breeding boars or on biopsy fat samples from selection candidates as performed currently in Switzerland (Baes *et al*. 2013). Response to selection may be improved by including genomic information, which allows estimating breeding values based on genome-wide marker maps (Meuwissen and Goddard 2001). Aiming to assess the potential for selection against boar taint, Haberland *et al*. (2014) modelled a terminal sire line breeding program comparing information from the biopsy-based performance testing (Baes *et al*. 2013), from HNS assessment at the breeding station (Windig *et al*. 2012) or on genomic selection against either chemical compounds or HNS. The authors concluded that breeding against boar taint using the biopsy-based performance testing is an effective method for optimising both selection against the chemically determined boar-taint compounds and selection against HNS of boar taint, in terms of yearly obtained genetic gain and variable costs per selection candidate. Depending on the economic weights used per unit of androstenone, skatole and indole, in the specific Swiss population (PREMO; Baes *et al*. 2013) a reduction of 50% within 7, 6 and 8 years, respectively, can be obtained. In a simulation study, using the genetic background of boar-taint components estimated from 1010 Piétrain-sired crossbred boars of type Piétrain × crossbred sows, Frieden *et al*. (2012) calculated that for the sire line four generations are needed to reduce the number of boars with a androstenone levels >1 ppm from 50% to 5% when using the chemically determined level of the two main boar-taint compounds. For the simulation, heritabilities for androstenone and skatole of 0.6 and 0.4, respectively, were used. Assuming an antagonistic relationship between maternal fertility and androstenone level of 0.2, implementing boar taint into the breeding goal for dam lines is difficult because the time period needed to obtain a similar success in reduction is extended to nine generations. The same authors concluded that due to the lower heritability, the use of HNS would result in lower breeding gains.

In conclusion, there is clear evidence that breeding strategies will successfully help to reduce boar-taint compounds, however due to uncertain effects on fertility, sexual maturity, average daily gain and meat quality traits such as drip loss and intramuscular fat content, selection against boar taint must be implemented with caution (Willeke *et al*. 1987; Sellier and Bonneau 1988; Sellier *et al*. 2000; Grindflek *et al*. 2011; Strathe *et al*. 2013*a*, 2013*b*; Haberland *et al*. 2014). These uncertainties are reasons that most European breeding organisations are reluctant to include boar taint in the genetic selection programs. Furthermore, it remains unclear which trait(s) for boar taint should be used to breed against and currently it is not possible to estimate the economic value for boar taint to use in a breeding index. In addition, including additional traits in a breeding index will reduce the genetic progress for the original traits.

Impact of management strategies

Increased incidence of mounting and aggressive behaviour resulting in greater risk for leg and feet injuries as well as skin lesions is one of the major issues encountered when raising entire male pigs (von Borell *et al*. 2009; Ebschke *et al*. 2014). Whether

the greater aggression is related to greater androstenone levels is still open for debate. Giersing *et al*. (2000) found a relationship between aggressive behaviour of boars and their androstenone levels in the backfat whereas Zamaratskaia *et al*. (2005) did not find such a relationship. However, there is compelling scientific evidence that both characteristics of the farm and management of the pigs from the day of birth to the day of slaughter are potentially associated with boar taint. For instance, observations from earlier studies revealed that housing conditions such as pen surface per animal (Hansen *et al*. 1994), floor type (Kjeldsen 1993) and hygiene circumstances in the pens and the cleaning strategy (Hansen *et al*. 1995) could influence the prevalence of skatole in carcasses of entire male pigs. However, the latter has been recently questioned as no relationship between pen cleanness and incidence of boar-tainted carcasses was found (Aluwé *et al*. 2011*a*). Factors related to feeding such as type of feed, feeding strategy and feed ingredients (Andersson *et al*. 1997; Pauly *et al*. 2008, 2009; Zamaratskaia and Squires 2009), and the fasting period before slaughter (Kjeldsen 1993), have been shown to influence boar-taint levels in pigs. Stocking strategies such as grouping of piglets based on sex, together with enrichment of the environment, can reduce boar-taint development (Holinger *et al*. 2014, 2015). Several studies suggest that the genetic background is determinant for the development of boar taint (Aluwé *et al*. 2011*b*; Windig *et al*. 2012; Robic *et al*. 2014), which ultimately might determine the success of the breeding program. Recently, van Wagenberg *et al*. (2013) published results of a survey carried out among 152 Dutch pig producers used to raise entire male pigs. With a questionnaire, farm and management characteristics were identified that were potentially associated with farm-level boar-taint prevalence. Boar taint was assessed at the abattoir using the HNS (Mathur *et al*. 2012). In partial agreement with the aforementioned studies, the evaluation of the survey responses showed that lower farm-level boar-taint frequency was associated with smaller group size, smaller pen surface per boar, newer housing equipment, not practicing restricted feeding in the last period before delivery, a longer fasting period before slaughter, a higher stocking weight and a lower fraction of boars from purebred dam line sows or from Piétrain terminal boars. The authors concluded that this information could be used as a control tool to develop farm-level interventions strategies to reduce observed variability in boar-taint level (Aluwé *et al*. 2015). As shown by Rydhmer *et al*. (2013) and van Staaveren *et al*. (2015), mixing unfamiliar entire male pigs during transport to and during lairage at the abattoir will stimulate mounting behaviour resulting in increased risk of skin lesions with uncertain consequences on boar-taint development (Giersing *et al*. 2000; Zamaratskaia *et al*. 2005).

Impact of feeding strategies

In the production of non-surgically castrated male pigs, the composition of the diet can be used to target two objectives. On the one hand dietary nutrients have to cover the requirements for efficient protein deposition of non-surgically castrated male pigs and on the other hand, dietary ingredients can be used to minimise bacterial degradation of tryptophan in the large intestine and ultimately resulting in lower skatole and indole production.

Dunshea *et al*. (2013) concluded, based on results of a meta-analysis, that assuring maximal feed intake is primordial for exploiting the inherited protein deposition potential of immunovaccinated (at least up to the second vaccination) and entire male pigs. A feeding strategy aiming at *ad libitum* access to feed allows maximisation of energy intake and covers the lysine requirements. Nevertheless, due to the generally lower feed intake of entire male pigs compared with gilts and barrows, greater lysine requirements in the range of 5–8% for the bodyweight range of 25–50 kg and 50 kg to slaughter, respectively, may still be required (Quiniou *et al*. 2010; Moore *et al*. 2013; Rikard-Bell *et al*. 2013).

Increased energy input in the hindgut alters the intestinal microbiota in such a way that less skatole is produced and this results in reduced skatole levels in the fat (for review see Wesoly and Weiler 2012). To achieve this, feedstuffs providing non-digestible fermentable polysaccharides can be given to entire male pigs a few weeks before slaughter. Feedstuffs known to have a positive impact on skatole and indole levels are raw potato starch (Lösel *et al*. 2006; Pauly *et al*. 2008), chicory (Hansen *et al*. 2006; Jensen and Hansen 2006; Byrne *et al*. 2008; Zammerini *et al*. 2012; Aluwé *et al*. 2013), Jerusalem artichoke (Vhile *et al*. 2012) and lupin seeds (Øverland *et al*. 2011). Results of recent studies suggest that also secondary plant metabolites such as hydrolysable tanning have the potential to reduce skatole in entire male pigs (Wealleans *et al*. 2013; Bee and Ampuero Kragten 2015; Čandek-Potokar *et al*. 2015). However, in this respect further investigations are needed in order to establish the optimal dosage and the duration of feeding.

Economic analysis of the costs and benefits of ending surgical castration

To provide policymakers of the EU with a better understanding of the costs and benefits of ending surgical castration of male pigs by 1 January 2018, the Directorate General for Health and Consumers (DG SANCO) of the European Commission appointed the Food Chain Evaluation Consortium to identify, analyse and compare the costs and benefits of three options for producing pork in the EU:

- Option 1: surgical castration
  (a) surgical castration at less than 7 days of age without anaesthesia or analgesia;
  (b) surgical castration at any age performed with anaesthesia and/or analgesia.
- Option 2: immunovaccination.
- Option 3: entire male production.

The cost-benefit analysis study aimed to provide an estimation of the costs and benefits of ending surgical castration on different percentages of EU male pigs' population by 1 January 2018. The basic data for the calculation derived from in-depth desk research as well as from interviews with key stakeholders from member states and other countries, including national associations of pig breeders, pig producers, slaughterhouses, pig meat processors, retailers, animal welfare associations as well as other organisations, and competent authorities.

Animal welfare benefits for society

A key aspect, which has not to be underestimated because it was the main driver for launching the discussion of piglet castration, is the importance of valuating animal welfare improvements in economic terms. The benefits accruing from animal welfare improvements have a social dimension as society consider animal welfare to have an economical value. With respect to the study of the Food Chain Evaluation Consortium, animal welfare benefits for the society arise in avoiding the pain that castrated pigs are subjected to. Peoples' willingness to pay for improved animal welfare was used to determine the values they ascribe to these improvements. The benefits in terms of animal welfare associated with each of the aforementioned options was estimated using data from the Welfare Quality Assessment Protocol for pigs for scoring management procedures (http://www.welfarequality.net/network/45848/7/0/40, accessed 4 September 2015) and on willingness to pay regarding pig welfare collected by the ALCASDE project (http://www.alternativepig.eu/, accessed 4 September 2015. D.1.3.3. Preliminary test report on attitudes in Europe). It was estimated that compared with surgical castration without analgesia and (or) anaesthesia (option 1a), using analgesia and (or) anaesthetics for surgical castration (option 1b), immunovaccination (option 2) or renunciation of castration (option 3) increases animal welfare benefits by €2.13, €2.51 or €2.81 per carcass, respectively. Interestingly, surgical castration of male pigs without the use of analgesia or anaesthesia generates an animal welfare cost for society depending if performed by the farmer or the veterinarian of €0.19 or €0.43 per carcass, respectively.

Costs of surgical castration and its alternatives

Not unexpectedly, the costs of surgical castration with the use of a pain reduction method vary largely. The less costly method is estimated to be the use of an analgesic by the farmer (€–0.31 per carcass), or castration with anaesthesia via inhalation of the CO_2/O_2 mixture administered by the farmer (€–0.46 per carcass). By contrast, castration with isoflurane anaesthesia and analgesia administered by the veterinarian was almost 10 times more expensive (€–4.04 per carcass). Unfortunately, costs caused by this procedure when farmers would perform it, as it is currently done in Switzerland, were not calculated. For the less expensive methods, the costs of the interventions was more than compensated by the animal welfare benefits for society (€2.13 per carcass) resulting in a positive net benefit ranging from €1.66 to €1.82 per carcass. This was not the case for the most costly option (€–1.91). After taking into account the frequency of finding tainted carcasses (option 2 = 1%; option 3 = 4.2%), the cost-benefit analysis revealed that for producers the range of the net benefits per carcass resulting from the production of immunovaccinated male pigs (€1.56–6.14) was lower than that generated by raising entire male pigs (€5.20–10.77). Furthermore, from the results of the study it is notable that whether animal welfare benefits were included or not, raising entire male pigs is associated with the greatest range of benefits compared with any other option.

Cost-benefits estimation after 2018

Using these data, the costs and benefits for the European pig production chain were estimated. For that matter four different scenarios were assumed: the share of entire male pigs is 28% (as in 2013), 50%, 75% or 95%. If 28% of the total pig population stays non-castrated the production of pig meat from male pigs may create the lowest net benefit due to castration practices (€5.9 million). When 95% of all male pigs are raised as entire male pigs, the total net benefits range from €605.5 to €1306.2 million. Thus, with an increasing share of male pigs the net cost-benefit of pig production including animal welfare benefits can reach over one-billion euros.

Conclusion

Before castration of piglets can be completely abandoned, it is imperative that a universally accepted, objective and reproducible method to determine boar-tainted or malodorous carcasses is available. When such a method is available, one could imagine that all carcasses could be tested for malodorous compounds at the slaughter line. Of great help for all concerned key players were the results of the CAMPIG project, which clearly demonstrated that one unique threshold level for boar taint cannot be defined. This led to the elaboration of a preference map depicting the importance of skatole and androstenone in the disliking of tainted pork. Despite its limitations, with the reference map industry has now a tool at their disposal that helps to choose the risk level they are prepared to take and determine accordingly their carcass sorting strategy. With the BoarCheck and CAMPIG projects relevant knowledge has been obtained but there are still major open issues requiring further research efforts. Therefore, it will not be easy to attain the objective to completely ban castration in the EU by 1 January 2018, unless an unexpected breakthrough especially in the detection technology, which can be easily implemented in the slaughter line, occurs. Due to all the unanswered questions, the interest in producing entire male pigs, marketing and processing of their meat currently stagnates. Certainly, apart from raising entire male pigs, other viable alternatives such as immunovaccination and surgical castration with anaesthesia and analgesia are available. However, their implementation is hampered either in the case of immunovaccination because of concerns towards consumers' acceptance of the method or, in the case of surgical castration with anaesthesia and analgesia, by the costs for the apparatus, drugs and veterinarian as well as surplus labour time. Very promising as a method are the results obtained with breeding; and these efforts needs to be intensified. In the long term, low boar taint-pig breeds might not be only interesting for conventional slaughter pigs but also for heavy pigs, which are mainly used for pork specialties registered under 'traditional specialties guaranteed' or with 'Geographical Indications'.

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Chapter 2: Symposia – Functional carbohydrates

Feed non-starch polysaccharides for monogastric animals: classification and function

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Abstract. This review outlines the importance of understanding the true fibre content, which is the sum of non-starch polysaccharides and lignin, of feed in order for animal nutritionists to improve the precision of feed formulation in the future. The continuing use of crude fibre in feed formulation means that up to a quarter of the feed components, mainly non-starch polysaccharides and oligosaccharides that are lost during acid and alkali extractions, are ignored for ingredients such as soybean meal. Furthermore, the values for acid detergent fibre and neutral detergent fibre are not used for feed formulation. They also do not represent unique classes of chemically defined molecules. In some cases, neutral detergent fibre and acid detergent fibre values do not cover a large proportion of soluble fibre, for example, in leguminous crops that contain a high level of pectic polysaccharides. Non-starch polysaccharides and their associated lignin content represent the true fibre levels in ingredients and this is the basis from which structural and physicochemical elucidation of fibre can be attained. Only with such understanding will nutritional strategies be applied to target specific fractions/types of fibre in ingredients to produce desired nutritional and health outcomes in pigs and poultry. In this context, an example is given to illustrate how gut microbiota of animals can be manipulated to enhance production performance and immunity.

Additional keywords: fibre, monogastrics, NSP, nutrition.

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Introduction

The term ‘fibre’ has been in use since the mid-19th century when Weende Experimental Station in Germany published its methods for proximate analysis. In a feed context, it is called ‘crude fibre’ (CF), which refers to organic matter remaining after a series of acid and alkaline extractions. CF consists of phenolic compounds (mainly lignin), the majority of cellulose and a variable amount of other insoluble polysaccharides. Although CF is used in feed formulation to set the fibre level in feed, it is not at all a reliable, let alone accurate, indicator of the true fibre content of feed. For example, the CF content in soybean meal is ~5%, whereas its true fibre level is as high as 25% (Choct *et al*. 2010).

What is the true fibre content of feedstuffs? In the context of human nutrition, the term dietary fibre (DF) is used. The definition of DF provokes a great deal of controversy because there have been numerous, at times confusing, definitions over the years, including definitions based on the physiological effects of DF and those based on methods of determination. Of direct relevance to monogastric animal nutrition is the simple understanding that DF is the sum of non-starch polysaccharides (NSP) and lignin. There are two well established methods for measuring DF. One is the series of enzymatic-gravimetric methods provided by the Association of Official Analytical Chemists Total Dietary Analysis (Methods 985.20; 993.19; 991.42; 991.43; 992.16), which uses enzymatic removal of non-cell wall organic materials and then gravimetrically measures the residue corrected for ash. The other technique is known as the Uppsala Method. This method quantifies individual sugar residues by converting them into alditol acetates and measures them using gas chromatography (Theander *et al*. 1995). Lignin and uronic acids are determined separately in the Uppsala Method. There are several advantages in using the Uppsala Method, including the separation of the individual sugar composition of DF that gives an idea of the type of polysaccharides present in an ingredient, and the ability of fractionating NSP based on their solubility in water (the other method also offers this option). The essence of this is the inclusion all NSP together with lignin, which is a polyphenolic compound. This definition of fibre captures all of the organic components in plant ingredients indigestible by the endogenous enzymes of monogastric animals and hence is regarded as the true fibre content of feed ingredients for those animals.

Non-starch polysaccharides are a significant part of plant ingredients (10–75% depending on the ingredient) for monogastric animal feed. The soluble NSP proportion can exhibit anti-nutritive activities in pigs and poultry, leading to changes to gut physiology, gut microflora and gut health. Whereas considerable evidence in poultry suggests that the insoluble NSP proportion acts very differently to the soluble NSP, often imparting beneficial effects on gastrointestinal tract (GIT) development and endogenous enzyme secretion. It is therefore essential that the roles of NSP as the major constituent

of fibre be taken into account when formulating feed for pigs and poultry in the future.

Definitions and terms used to describe 'fibre' in feed and food

Crude fibre

The term 'crude fibre' was first used more than 150 years ago to describe the organic remnant of feedstuffs insoluble in hot, dilute sulfuric acid and sodium hydroxide (Henneberg and Stohmann 1859). Depending on the raw material, CF represents variable portions of the insoluble NSP and therefore setting it as a nutrient constraint in feed formulation has limited utility. Table 1 shows the nutrient contents of wheat, sorghum and soybean meal that are available in most databases used to formulate feed for pigs and poultry in Australia. These nutrients do not add up to 100% (92% for wheat, 93% for sorghum and 70% for soybean meal). The reason is simple; the CF values do not represent the true fibre levels in feed ingredients.

Acid detergent fibre and neutral detergent fibre

Clearly, not knowing or understanding up to a quarter of the chemical composition of the key ingredients, for instance soybean meal as shown in Table 1, used in feed formulation is not an ideal situation. The deficiency of the proximate analysis system, especially in relation to CF measurement, has been recognised since the early 1960s. Thus, a system for 'detergent fibres' was instigated (van Soest 1963). The system used a series of detergent extractions (originally of forage materials) to obtain one fraction called neutral detergent fibre (NDF) and another fraction called acid detergent fibre (ADF). This system relies on the differences in solubility of feed components and therefore it lacks precision with respect to chemical structures. Both NDF and ADF fail to account for most, if not all, soluble NSP present in feed. This system also adds to the confusion as to what the two fractions actually represent. This makes it questionable how relevant the various ADF and NDF values are for monogastric animal nutrition.

Graham and Åman (2014) compared the CF, ADF, NDF and NSP values in maize, wheat and soybean, together with the detailed carbohydrate composition. It can be seen from Table 2 that the values for NDF and NSP are similar for maize and wheat but are less than half for soybean meal. This is because the detergent extraction process used for NDF determination removes the pectic polysaccharides, which dominate the NSP present in soybean.

Table 1. The amounts of 'fibre' unaccounted for in wheat, sorghum and soybean meal

Nutrient (%)	Wheat	Sorghum	Soybean meal
Protein	13	9	47
Starch	60	65	1
Fat	2	3	1
Crude fibre	3	2	5
Water	12	12	10
Ash	2	2	6
Total	92	93	0
Missing	10	7	24

The NDF and ADF are not separate entities because both terms cover cellulose and lignin. Thus, the difference between the two gives the value for hemicellulose:

$$\text{NDF} - \text{ADF} = \text{hemicellulose}$$

The term hemicellulose is widely used but it does not refer to a single molecule as early research mistook plant cell wall components that were soluble in alkali as precursors to cellulose (Schulze 1891). Thus 'hemicellulose' covers arabinoxylans, mixed linked β-glucans, xyloglucans, mannans, galactomannans, galactans, arabinans and any other neutral polysaccharides other than cellulose. Unfortunately the term has a long history of use in both industry and scientific literature.

Classification of feed carbohydrates

Overview

Feed contains several different types of carbohydrates, including monosaccharides, disaccharides, oligosaccharides (3–12 sugars) and polysaccharides. Monogastric animals such as pigs and poultry can digest monosaccharides and disaccharides. Most oligosaccharides are also digested via fermentation. Polysaccharides make up the bulk of feed carbohydrates with starch and NSP being two key distinct classes.

There are numerous polysaccharides present in nature. These polymers differ in their physical and chemical properties due to several factors: (a) the monosaccharides that make up the polymers; (b) the ring forms (6-membered pyranose or 5-membered furanose) of the monosaccharides; (c) positions and configurations (α or β) of the glycosidic linkages; (d) the sequence in monosaccharide residues in the chain; and (e) whether or not non-carbohydrate substituents are present. In feed carbohydrates, the predominant six-carbon sugars (hexoses) include D-glucose, D-galactose and D-mannose,

Table 2. Composition of maize, wheat and soybean meal (%DM) (from Graham and Åman 2014)

Analytical component (%DM)	Maize	Wheat	Soybean meal
Ash	1.4	1.7	6.6
Crude protein	9.1	11.0	53.3
Crude fat	4.6	2.4	2.8
Sugars	2.6	3.5	3.5
Oligosaccharides	0.3	0.2	5.3
Fructans	0.6	1.8	0.9
Starch	69.0	66.5	0
Crude fibre	2.3	2.5	4.2
Acid detergent fibre	2.5	3.4	4.9
Neutral detergent fibre	9.2	10.0	8.4
Non-starch polysaccharides + lignin	10.0 + 0.8	11.0 + 1.0	20.8 + 1.0
Non-starch polysaccharides			
– rhamnose	Tr	Tr	0.7
– arabinose	2.3	2.7	2.3
– xylose	3.1	3.9	1.5
– mannose	Tr	Tr	1.4
– galactose	0.5	0.2	5.3
– glucose	3.4	3.7	6.0
– uronic acids	0.7	0.5	3.8

whereas the main five-carbon sugars (pentoses) are L-arabinose and D-xylose. In addition, there are acidic sugars, such as D-galacturonic acid, D-glucuronic acid and its 4-O-methyl ether, that come from pectic polysaccharides.

Starch is by far the most important polysaccharide present in pig and poultry feed. Starch is second only to cellulose in abundance in terms of polysaccharides synthesised by plants and represents the primary source of energy for many monogastric species, including humans, as they can digest starch using their endogenous amylolytic enzymes. However, the digestion and utilisation of starch are more complex than as outlined here as the botanical origin, grain harvest conditions, processing, formation of resistant starch, and the asynchrony of starch and protein digestion all affect the way starch is utilised. Starch is, however, outside the scope of the present review.

Apart from starch, a large number of polysaccharides are present in feed. These polysaccharides are collectively called NSP.

Non-starch polysaccharides

Classification

From a practical point of view, it is easier to classify NSP based on extraction techniques that can be implemented in quality control laboratories. For example, CF, ADF and NDF are routinely measured in many laboratories with a good degree of repeatability. However, from a scientific point of view, such a classification is inaccurate, confusing and does not make nutritional sense. This is because the three fibres are not distinct chemical entities and they overlap each other on some NSP, i.e. cellulose, and lignin, a polyphenolic compound. These systems also do not measure the same chemical entities consistently. Bailey (1973) attempted to classify NSP in three chemically definable groups, that is: cellulose, non-cellulosic polysaccharides and pectic polymers. Cellulose is self-explanatory but non-cellulosic polysaccharides encompass numerous polysaccharides, which were traditionally included under the term 'hemicellulose' because these polysaccharides could be extracted together (Neukom *et al*. 1967; Neukom 1976). Non-cellulosic polysaccharides include, but are not limited to, arabinoxylans (pentosans), mixed-linked β-glucans, mannans, galactans, xyloglucans and fructans. Likewise, the term 'pectic polymers' covers many polysaccharides that consist mainly of polygalacturonic acids substituted with arabinan, galactan and arabinogalactan.

NSP in common feed ingredients

Cereals

Between 10% and 30% of cereals are NSP. There is only a trace amount of pectic polymers are found in them, usually in the stems and leaves. The majority of the NSP are composed of arabinoxylans, cellulose and β-glucans.

Cereal grains can be classified into two groups, i.e. viscous and non-viscous cereals. Viscous cereals include rye, wheat, barley, triticale and oats, whereas the non-viscous cereals include corn, sorghum, rice and millet. This classification is based mainly on the amount of soluble NSP present in the grain. For instance, the arabinoxylans and β-glucans present in rye, wheat, barley, triticale and oats are partially soluble and can form highly viscous solutions and therefore these grains are known as viscous cereals. However, corn, sorghum and rice contain a low level of NSP, of which a very small fraction is soluble but it does not form viscous solutions. So, these grains are classified as non-viscous cereals. Table 3 presents the main chemical entities and the level of NSP for some cereals (Choct 1997).

The fibre fraction of cereals is concentrated in the by-products when starch and, to a lesser extent, protein are removed through the milling process. Thus, wheat bran has ~40% NSP, which consist mainly of arabinoxylans (Englyst 1989). The NSP content in rice bran is usually less than 25% and is composed of arabinoxylans and cellulose in almost equal amounts (Saunders 1985). Most of the NSP present in cereal by-products are insoluble and therefore do not raise digesta viscosity and will not form a highly viscous gel (Annison *et al*. 1996). Rice bran also contains some xyloglucans, which are not a common group of NSP found in other cereals (Shibuya and Iwasaki 1978). These xyloglucans have a β 1–4-linked glucan backbone, which is substituted at the O6 atoms with single units of α-xylose. Another cereal by-product that has become available in large quantities in various parts of the world is distillers dried grains with solubles. Choct and Petersen (2009) presented the chemical composition of six corn distillers dried grains with solubles samples obtained from the northern part of the USA. In terms of the carbohydrate composition, the samples contained, on an as-is basis, between 15.5% and 22.4% starch, 14.6% and 19.3% NSP and 1.4% and 8.3% free sugars (sugars extractable in 70% ethanol).

Vegetable protein sources

When vegetable protein sources are included in diet formulation, nutritionists usually think about the amino acids they provide, rather than the effects the NSP will have on the nutritive value of the diet. For instance, soybean meal, the most widely used vegetable protein source for monogastric animal feed, contains up to 35% carbohydrates, of which, ~14% are low-molecular-weight soluble sugars, and 21% are NSP. Of the NSP, between 5% and 7% are soluble (Choct *et al*. 2010). These carbohydrates are mainly pectic polymers, such as galacturonan, rhamnogalacturonans, arabinans and arabinogalactans (Aspinall *et al*. 1967), which are unaccounted for in the CF, NDF or ADF values. These pectic polymers are galacturonans or in the case of soybean, rhamnogalacturonans. Pectic polymers usually have (1–4)-α-D-galacturonan chains, which are inserted with (1–2)-α-L-rhamnose residues at various intervals. Side chains, such as D-galactose, L-arabinose, D-xylose, and less frequently L-fucose and D-glucuronic acid, can also be present and mostly are short. Some neutral pectic polymers like galactans and arabinans, xyloglucans and galactomannans have very complex side chains, whereas the carboxyl groups of the galacturonic acid residues of pectins can have a high degree of methyl esterification (Aspinall and Jiang 1974). For instance, highly methylated soluble pectic polysaccharides were isolated from mung beans (Goldberg *et al*. 1994). A high degree of esterification renders pectins insusceptible to endo-polygalacturonase, which requires at least two free carboxyl groups adjacent to each other (Jansen and MacDonnell 1945). In general, the molecular weights of

Table 3. The types and levels of non-starch polysaccharides present in some cereal grains and their by-products (%DM)

Cereal	Arabinoxylan	β-glucan	Cellulose	Mannose	Galactose	Uronic acid	Total
			Wheat[A]				
Soluble	1.8	0.4	–	t	0.2	t	2.4
Insoluble	6.3	0.4	2.0	t	0.1	0.2	9.0
			Barley[A]				
Soluble	0.8	3.6	–	t	0.1	t	4.5
Insoluble	7.1	0.7	3.9	0.2	0.1	0.2	12.2
			Rye[A]				
Soluble	3.4	0.9	–	0.1	0.1	0.1	4.6
Insoluble	5.5	1.1	1.5	0.2	0.2	0.1	8.6
			Oats[A]				
Soluble	0.8	2.8	–	t	0.1	0.1	3.8
Insoluble	14.7	–	10.1	0.2	0.1	t	24.5
			Triticale[B]				
Soluble	1.3	0.2	–	0.02	0.1	0.1	1.7
Insoluble	9.5	1.5	2.5	0.6	0.4	0.1	14.6
			Sorghum[B]				
Soluble	0.1	0.1	–	t	t	t	0.2
Insoluble	2.0	0.1	2.2	0.1	0.15	t	4.6
			Corn[B]				
Soluble	0.1	t	–	t	t	t	0.1
Insoluble	5.1	–	2.0	0.2	0.6	t	8.0
			Rice (pearled)[B]				
Soluble	t	0.1	–	t	0.1	0.1	0.3
Insoluble	0.2	–	0.3	t	t	t	0.5
			Wheat pollard[A]				
Soluble	1.1	0.4	–	t	0.1	0.1	1.7
Insoluble	20.8	–	10.7	0.4	0.7	1.0	33.6
			Wheat bran[B]				
Soluble	2.6	0.2	–	t	0.1	0.3	3.2
Insoluble	26.0	–	10.8	0.1	0.6	0.9	38.4
			Rice bran (defatted)[B]				
Soluble	0.2	t	–	t	0.2	t	0.5
Insoluble	8.3	–	11.2	0.4	1.0	0.4	21.3

[A]Englyst (1989).
[B]University of New England, Australia.

pectins range from 30 000 to 300 000 (Pilnik and Voragen 1970). The structure of a pectic polymer from lupins is shown in Fig. 1.

Non-conventional ingredients

Whether or not an ingredient is classified as 'non-conventional' depends very much on the country or region where this raw material is used. For instance, tapioca is regarded as an alternative ingredient in Australia but is very much a common raw material in South-east Asia. So in this section, ingredients that are not commonly used in commercial feed formulation in Australia will be briefly discussed.

Copra meal. Copra meal contains between 45% and 60% NSP (Sundu *et al*. 2009). The NSP present in copra meal are mainly mannans (galactomannans and mannans) although ~10% cellulose and trace amounts of other polymers, such as arabinogalactans, arabinomannogalactan and galactoglucomannans are also present (Saittagaroon *et al*. 1983; Zamora *et al*. 1989). It is reported that 30% of the copra NSP is soluble in water (Saittagaroon *et al*. 1983), but it is not known whether these NSP are viscous in nature.

Palm kernel cake. Palm kernel is rich in oil (50% oil) and the extraction process can leave 20–30% of the oil behind. There are two methods of extraction, i.e. expeller press and solvent extraction. The resulting by-product after expeller processing, palm kernel cake, and that after solvent extraction, palm kernel meal, differ in calcium, oil and fibre contents. For instance, oil and CF levels of 12.3% and 9.8% for the cake, and 16.6% and 5.2% for the meal, respectively, have been reported (Omar and Hamdan 1998). The same authors reported an NSP level of 74.3% in the palm kernel cake. Düsterhöft *et al*. (1992) elucidated the polysaccharide populations of palm kernel meal and showed ~80% of the NSP as insoluble linear mannans together with cellulose (12%) and a small amount

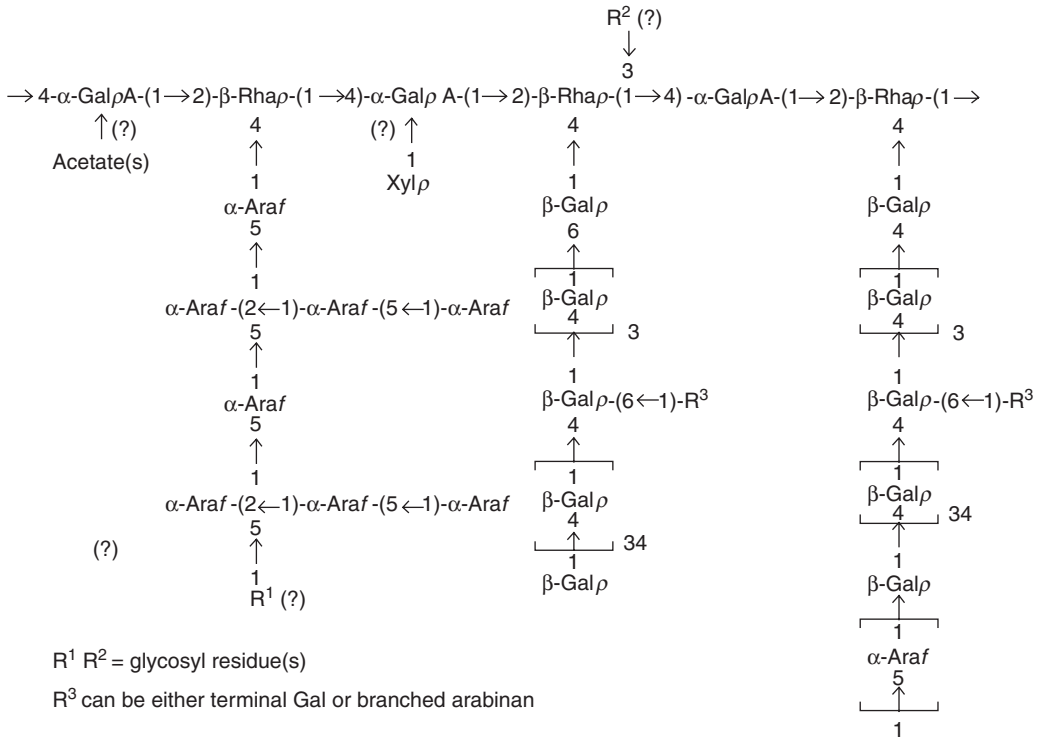

Fig. 1. Pectic polymer structure from lupins (from Cheetham *et al*. 1993).

of 4-O-methyl-glucuronoxylans (3%) and arabinoxylans (3%). Commercially available palm kernel cake or palm kernel meal has ~16% of crude protein and in excess of 60% of NSP.

Sunflower meal. Sunflower seeds are reported to contain 27.6% NSP, of which 4.5% was soluble (Irish and Balnave 1993). The soluble NSP were reported as uronic acids, which indicates the presence of pectic polysaccharides. It can be extrapolated that sunflower meal (assuming an oil extraction rate at 40%), may contain as high as 46% NSP. Düsterhöft *et al*. (1992) detailed the composition of the NSP found in sunflower meal as 42% cellulose, 24% pectins, 24% 4-O-methyl-glucuronoxylans, 5% (gluco)-mannans and 4.5% fucoxyloglucans.

Tapioca. In many parts of the world, potatoes, sweet potatoes, cassava, yams, taro (cocoyam) and other root plants are a major source of human food. Cassava is an important root plant in Asia. Cassava, also known as tapioca, contains 85–90% starch and 10–15% fibre. Cassava chips and pulp recently analysed at the University of New England (D. F. Tang, P. A. Iji, M. Choct, unpubl. data) contained 3.99% and 9.2% insoluble NSP and 0.78% and 0.26%, respectively. Arabinoxylans and glucans were the predominant NSP in the pulp whereas glucans were the main NSP in the chips. The nature of the glucans is not known but is likely to be mainly cellulose.

Nutritional properties

Soluble NSP. Solubility of NSP usually refers to water solubility and is an important measure of the physicochemical characteristics and nutritional properties of NSP for monogastric animals. Viscosity is not related to the linkage type or the sugar composition of a polysaccharide. Rather, it is determined by the physical properties of the polysaccharide, such as molecular weight, distribution and structure. In poultry diets, the anti-nutritive effects of soluble NSP are well characterised (Choct and Annison 1992; Choct 2006). In addition, there is a clear negative relationship between the amount of NSP and the nutritive value in poultry (Choct and Annison 1990; Annison 1991), in pets (dogs and cats) (Earle *et al*. 1998; Twomey *et al*. 2003) and in pigs (King and Taverner 1975). The effect of feeding viscous cereals on feed intake in pigs is large, which can be overcome by the use of appropriate xylanases (Cadogan *et al*. 2003). The results of this study indicated that the NSP were a major causative agent, but subsequent work (D. J. Cadogan, R. G. Campbell, M. Choct, unpubl. data) revealed little difference in digesta viscosity values between the two contrasting wheats. It is speculated that although the absolute value for digesta viscosity in pigs fed viscous grains is low compared with that in poultry (2–3 mP.s vs 8–12 mP.s), its impact on gut physiology and hence nutrient digestion/absorption in pigs is probably just as great as it is in poultry.

Insoluble NSP. Most NSP present in feed ingredients are insoluble in water. There is now clear evidence to suggest that coarse, structural materials consisting largely of insoluble fibre, enhances GIT development in poultry (Choct 2006; Hetland *et al*. 2007). Renteria-Flores *et al*. (2008) showed that there was interdependency between soluble and insoluble fibre digestibility in sow diets, where insoluble fibre digestibility was improved when soluble fibre intake increased. The authors stressed that knowledge of specific NSP components is necessary to accurately predict the effects of DF on digestibility.

Low-molecular-weight carbohydrates. Choct *et al*. (1996) used xylanase in a sorghum-based broiler diet with added

soluble NSP to examine whether the use of a well defined soluble NSP (wheat arabinoxylan in this case) would change the activity of the gut microflora. The study showed that the enzyme reduced fermentation in the ileum but increased it in the caeca. These results coincided with a reduced digesta viscosity and an increased digestibility of nutrients (starch, protein and fat). Traditionally, the small intestine of poultry was considered to have no fermentative activity. The significance of the study in question is not the detection of fermentation in the ileum or the amount of metabolisable energy arising from it, but the physiological and microbiota changes that small intestinal fermentation may bring about for the bird. However, changes to the GIT microbiota in response to enzyme supplementation is not really a surprise because when the substrates are modified, the organisms relying on them for their existence will have to adapt. Diet-dependent variation in GIT flora (Wagner and Thomas 1987) is another indication of the highly dynamic nature of more than 600 species of organisms harboured in the GIT of pigs and poultry. The complexity of the change is, however, difficult to understand. For instance, Bedford and Apajalahti (2002) showed a marked reduction in certain microbes in chickens by enzyme supplementation, leading to a significant reduction in the total number of microbes in the GIT. The hypothesis is that the removal of substrates from the GIT may leave the organisms to 'starve', leading to a reduction in numbers. Another thought is that the production of certain oligomers *in situ*, such as xylo-oligosaccharides (XOS), provides prebiotics to the GIT ecosystem and hence selectively stimulates the beneficial organisms while suppressing the growth of undesirable organisms. Courtin *et al*. (2008) reported that the benefit on feed conversion efficiency of broiler chickens induced by 0.5% of dietary XOS was similar to that obtained by xylanase supplementation, suggesting that these low-molecular-weight carbohydrates act as prebiotics. The mechanisms by which XOS exhibit their beneficial effects in humans and animals appear to be complex. Ebersbach *et al*. (2012) reported that XOS inhibit pathogen adhesion to enterocytes *in vitro*. In addition, Geraylou *et al*. (2013) demonstrated that XOS improved the non-specific immunity and changed gut colonisation in fish.

Specific XOS could be produced *in situ* through the use of appropriate xylanases in pig and poultry diets to tailor them for improved prebiotic action. Currently, there are several hurdles to overcome for this to happen. First, knowledge of the NSP substrates present in different diets remains poor as it is constrained by research capacities in the area of carbohydrate chemistry, and the lack of rapid methods to detect substrates in real time. Second, the understanding of GIT microbiota in pigs and poultry in terms of their functions, activity and substrate requirement is at a very early stage of development despite the advent of molecular technologies in microbiology. However, this is an area of immense potential as it holds the key to a sustainable animal production without the reliance on in-feed antibiotics.

Conclusion

Nutritionists in the future will need to move away from using CF in order to improve the precision of feed formulation for monogastric animals. This will require a comprehensive NSP database for ingredients used in pig and poultry feed. Understanding the chemical structure and physical attributes of fibre components of feed will also lead to a tailored use of different fibres to achieve desired outcomes in producing specific prebiotics *in situ* to enhance gut health or in releasing monosaccharides as an energy source.

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Dietary fibre and crude protein: impact on gastrointestinal microbial fermentation characteristics and host response

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Abstract. The role of the gastrointestinal tract microbiota in animal health and nutrition has become the subject of intensive research. Carbohydrates and crude protein are major components of swine diets and numerous studies have been performed looking at the effect of inclusion of dietary fibre with possible functional properties. In recent years, our understanding of the diversity and functionality of the gastrointestinal tract microbiota has increased further enabling the possibility for their targeted modulation. However, favouring potential beneficial bacteria, inhibiting possible pathogens or promotion of the formation of desired metabolites, is complex and underlies many factors and uncertainties. Approaches targeting this complex ecosystem (and discussed in this review) include the utilisation of fermentable carbohydrates such as resistant starch, cereal 1–3/1–4 β-glucans, arabinoxylans, inulin or other sources from legumes and industrial by-products. In addition, strategies regarding protein level and the protein : carbohydrate ratio are discussed briefly. Results are both promising and sometimes rather disillusioning considering the dietary concentrations needed to show biologically relevant effects. Deriving recommendations for an optimal inclusion rate of dietary fibre for weaning, growing pigs and sows and maximum levels for dietary crude protein may be one of the main challenges in the near future in the swine industry.

Additional keywords: animal health, gut ecology, nutrition, pigs.

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Introduction

Maintaining animal health and performance through prevention of gastrointestinal tract (GIT) disorders is one of the main challenges for the swine industry. The pig GIT harbours ~1000 different bacterial species, of which most belong to the phyla Firmicutes, Bacteroidetes and Proteobacteria. It is assumed that host phylogenetic background, the early environment and nutrition are the driving forces for the co-evolution of close microbe–host relationships. Whereas knowledge of bacterial diversity along the GIT of the pig has increased substantially in the past decade, further understanding interactions of the three-component system (diet-microbe-host) is pivotal to establish successful feeding and management strategies.

Regarding the beneficial effect of dietary fibre (DF) inclusion in pig diets, there are different assumptions regarding their modes of action promoting GIT health and animal welfare. These include:

(1) Promotion of certain bacteria (bifidobacteria, lactobacilli, butyrate-producers);
(2) Reduction of potentially harmful bacterial species;
(3) Maintenance of epithelial barrier function;
(4) Reduced detrimental effects from protein fermentation and reduction of nitrogen emission; and
(5) Impact on animal welfare, for example absence of abnormal or aggressive behaviour.

The GIT microbial ecology should be either manipulated in a way that zoonotic pathogens are limited in growth or that they are less harmful to the host through enhanced epithelial barrier function. This review will summarise some recent advances in the understanding of the influence of DF and protein on the targeted modulation of certain bacteria and their metabolites in young pigs.

Bacterial carbohydrate metabolism and host response

Microbial carbohydrate fermentation

Bacteria hydrolyse carbohydrate polymers to monosaccharides before uptake and intracellular metabolism. Whereas they utilise easily accessible compounds [glucose, amino acids (AA)] in the upper parts of the GIT, fermentation shifts towards the utilisation of more complex substrates in the large intestine. Thus sugars, starch, soluble oligosaccharides [e.g. oligofructose, inulin with a low degree of polymerisation (DP)], mixed-linked β-glucans and in part, soluble arabinoxylans (AX), are fermented in the upper GIT of the pig, whereas complex, low soluble substrates such as insoluble AX, cellulose in hulls, wheat bran or complex pectin structures are utilised in the lower GIT sections (Bach Knudsen *et al*. 2012).

Fermentation end products and intermediates depend on the bacterial community composition and their ability to utilise the monomers under the respective environmental conditions (e.g. redox potential, pH) in the different GIT sections and within a given time frame (i.e. digesta transit). Lactic acid predominates in the stomach and small intestine, and only low amounts of short-chain fatty acids (SCFA) can be detected illustrating the

metabolic priority towards lactate fermentation. Propionate can be produced from pyruvate through succinate decarboxylation, via the acrylate pathway from lactate as the intermediate metabolite, or via the propanediol pathway with the deoxy-sugars fucose and rhamnose as primary substrates (Reichardt *et al*. 2014). Acetate is the most abundant intestinal fermentation end product and contributes >90% of the SCFA in the proximal GIT of pigs, whereas propionate and butyrate can be found only in traces. The situation changes in the caecum and proximal colon of pigs, where lactate concentrations drop and SCFA concentrations increase up to 100 mmol/L with a typical ratio of 60% acetate, 25% propionate and 15% butyrate. Butyrate is formed via butyrate kinase or via butyryl CoA : acetate CoA transferase, and the latter seems to be the predominant and more efficient pathway in the GIT bacteria (Pryde *et al*. 2002; Louis *et al*. 2004). Typical butyrate-producing species are members of clostridial cluster IV (e.g. *Faecalibacterium prausnitzii*), cluster IX or clostridial cluster XIVa (e.g. *Roseburia* spp., *Eubacterium* spp., *Butyrivibrio* spp., *Ruminococcus* spp.), most of which can also be found in the pig GIT (Louis *et al*. 2010; Levine *et al*. 2013; Vital *et al*. 2013). In human faeces, the abundance of the *Roseburia/E. rectale* group showed a close relationship to total butyrate concentration (Scott *et al*. 2011). Butyrate production in the GIT may either occur directly via stimulation of bacteria that can produce butyrate from 'butyrigenic' substrates or via enhanced production of other intermediates.

Generally, SCFA produced by the GIT bacteria are rapidly absorbed from the GIT lumen and serve as energetic substrates. Acetate acts mainly as a precursor for fatty acids synthesis, whereas propionate in mainly used for gluconeogenesis in the liver. Butyrate, in turn, is mainly metabolised by epithelial cells and has been proposed as the main energy source for colonocytes (Hamer *et al*. 2008). Thus, there is some interest to enhance butyrate formation in the GIT as it may promote GIT health through enhanced epithelial integrity and barrier function. In the pig, utilisation of SCFA can contribute considerably to the daily energy requirement depending on the diet (Anguita *et al*. 2006). In the intestinal lumen, SCFA modulate the environmental conditions by reducing the pH, inhibit the growth of 'undesirable' bacteria and modulate bacterial metabolism. In addition, they can stimulate epithelial proliferation and barrier function, modulate immune response and satiety through receptor-mediated signalling (Hamer *et al*. 2008; Willing and Van Kessel 2010).

Promotion of beneficial bacteria and metabolites

Solubility of DF and thereby susceptibility to enzymatic hydrolysis largely depends on the primary structure and interconnection with other cell (wall) components, and influences digesta viscosity and water-holding capacity. A brief overview on the use of specific DF fractions to promote growth of beneficial bacteria is offered.

Resistant starch

Resistant starch (RS) may enhance butyrate production in the colon. The factors stimulating butyrate formation have not fully been elucidated. Initially, *Bacteroides* species were identified as degraders of RS in humans (Macfarlane and Englyst 1986). However, more recent studies in humans revealed that *Bacteroides*, *Bifidobacteria* and members of clostridial cluster XIVa, namely *Ruminococcus bromii*, efficiently utilise RS type 2 and 3 (Walker *et al*. 2011; Ze *et al*. 2012). Feeding a very high level of RS to pigs increased faecal bifidobacteria numbers and total SCFA (Regmi *et al*. 2011). Similarly, very high levels of RS from tapioca fed to pigs increased the abundance of *Ruminococcus bromii*, *Faecalibacterium prausnitzii*, propionibacteria or *Veilonella* spp., whereas the abundance of *Proteobacteria* (*E. coli*, *Pseudomonas* spp.) was reduced (Haenen *et al*. 2013). In the same study, increased SCFA levels were detected in the caecum and proximal colon. However, such studies may have more relevance for humans as inclusion of very high indigestible starch also reduced performance and increased ileal nutrient flow in pigs (Regmi *et al*. 2011). Interestingly, feeding RS increased expression of monocarboxylate transporter 1 (MCT1) in the caecum of pigs (Haenen *et al*. 2013). Improved expression of the transporter may improve energy supply to intestinal epithelial cells and promote barrier function. Utilisation of different starch sources (e.g. starch types) may also produce different levels of acetate, lactate, succinate and propionate (Giuberti *et al*. 2015). For example, studies with cereals differing in amylose/amolypectin content showed that amylose increased the abundance of *Clostridium butyricum*-like phylotypes, whereas amylopectin increased the abundance of *Clostridium ramosum*-like phylotypes and *Bacteroides*-like bacteria (Pieper *et al*. 2009; Bindelle *et al*. 2011). This did not enhance butyrate production but rather increased total SCFA and the propionate molar ratio. Other studies showed that barleys higher in amylose increased butyrate *in vitro* and *in vivo* (Bird *et al*. 2007; Jha *et al*. 2011).

Increased SCFA concentration due to RS feeding also seems to increase satiety in pigs although the definite mechanism is yet not established (Souza da Silva *et al*. 2014). A higher satiety due to high levels of SCFA in the large intestine is somewhat contradictory to the general aim of high productivity in growing pigs. RS favours a shift of microbial metabolism towards starch utilisation in the porcine large intestine and delays the fermentation of other DF fractions such as mixed-linked β-glucans and soluble AX (Jonathan *et al*. 2013). Finally, as pre-caecal digestibility of starch is between 90% and 95% in pigs, a considerable amount of undigested starch will enter the large intestine anyway. Thus, DF should be rather used to promote large intestinal fermentation with only minor effects on small intestinal starch utilisation.

Cereal mixed-linked β-glucans

Mixed-linked (1→3)/(1→4)-β-D-glucans (further referred to as β-glucan) can be found mostly in cereals such as barley and oats but with a considerably high variability (Brennan and Cleary 2005; Holtekjølen *et al*. 2006; Pieper *et al*. 2009). They have been shown to influence both the small and large intestinal microbial community composition and activity in pigs (Pieper *et al*. 2009, 2012*a*; Jha *et al*. 2010; Metzler-Zebeli *et al*. 2010). In contrast to low ileal digestibility and other negative effects in poultry, the apparent ileal digestibility of cereal β-glucan in pigs is quite high and varies according to cereal type and age of the animal (Bach Knudsen *et al*. 2012). In fact, Metzler-Zebeli and Zebeli (2013) reported that the ileal digestibility of β-glucan is between 15%

and 93% with a mean of 76%. Thus, cereal β-glucan is already partly fermented in the proximal GIT and β-glucan utilisation in pigs increases in the order weaning < growing < finishing pigs (Bach Knudsen *et al*. 2012). This is not surprising as the metabolic adaptation of small intestinal bacteria such as lactobacilli towards utilisation of complex plant-based carbohydrates increases with age (Pieper *et al*. 2008*a*). Very high dietary levels of β-glucan increased the abundance of bacteria with potential to degrade β-glucan in the colon of pigs (Pieper *et al*. 2008*b*). The adaptation can be delayed or shifted distally when the diet contains easily accessible substrates such as lactose from whey or starch (Bach Knudsen 2012; Jonathan *et al*. 2013). In addition, purified cereal β-glucans have different effects in the GIT of pigs than β-glucans within the grain matrix (Pieper *et al*. 2008*b*; Jha *et al*. 2010). Isolated β-glucans are easier fermented in the upper GIT whereas β-glucans embedded in the grain matrix shift fermentation towards distal parts.

In another study, 22 different barley and oat cultivars were used in an *in vitro* system simulating the digestion and fermentation process in the GIT of the pig (Pieper *et al*. 2009). The results confirmed that the concentration of β-glucans together with crude protein (CP) content were associated with high SCFA yield and butyrate molar ratio, and promoted members of clostridial cluster XIVa. The relation with CP content may be explained through the fact that most high β-glucan barley cultivars contain also high levels of CP. The butyrate-enhancing effects cannot be attributed to dietary β-glucan alone as CP also yields SCFA. *In vivo*, feeding high levels of β-glucan promoted the formation of lactic acid and propionate in the colon without an effect on butyrate (Jha *et al*. 2010; Pieper *et al*. 2012*a*). This seems contradictory to the above-mentioned results. However, lactate and propionate can serve as precursors for butyrate formation. In fact, feeding isolated β-glucans increased the abundance of the rate-limiting enzyme for butyrate formation, butyryl-CoA transferase, in the pig large intestine, which was in turn correlated with lactic acid-producing bacteria such as lactobacilli, bifidobacteria and enterococci (Metzler-Zebeli *et al*. 2010).

A promotion of lactic acid and total SCFA production may also have some GIT health implications with regard to inhibition of potentially pathogenic bacteria. Although co-inoculation studies with *Salmonella enterica* revealed that increased fermentative activity of high β-glucan cultivars can reduce Salmonella proliferation *in vitro* (Pieper *et al*. 2009), this was only in part confirmed *in vivo*. The colonisation with Salmonella was not affected but the transmission between penmates was reduced when pigs were fed high β-glucan barleys (Pieper *et al*. 2012*a*). Fermentable β-glucans also seem to reduce the small intestinal and caecal abundance of enterobacteria (Lynch *et al*. 2007; Pieper *et al*. 2008*b*) and bacterial pathogenic factors from *E. coli* (Metzler-Zebeli *et al*. 2010). The data suggest a total of three beneficial effects of cereal β-glucans: (i) promotion of beneficial bacteria such as lactobacilli and butyrate producers, (ii) promotion of large intestinal SCFA and butyrate production, and (iii) inhibition of putative pathogens.

The use of exogenous β-glucanases in diets can help to partially hydrolyse β-(1–3)(1–4)-D-glucans in the upper GIT, thereby making them more readily available for microbial fermentation. There are some indications that microbial communities are changed through exogenous enzymes (Bartelt *et al*. 2002; Vahjen *et al*. 2007). Increased access to nutrients and higher passage rate may facilitate the growth of small intestinal bacteria (e.g. lactobacilli) and change the flow of less fermentable substrates to the large intestine. *In vitro* analyses simulating pig large intestinal fermentation indicated a shift from propionate to acetate and butyrate and an increase in cellulolytic *Ruminococcus*- and xylanolytic *Clostridium*-like bacteria with the use of a multi-enzyme preparation with high β-glucan hulless barleys (Bindelle *et al*. 2011).

Little is known on the influence of cereal β-glucans on host response through altered microbial ecology. Oat β-glucans promoted propionate and butyrate concentrations and stimulated the expression of MCT1 in the caecum of pigs (Metzler-Zebeli *et al*. 2012). In addition, expression of the pro-inflammatory cytokine Il-1β was negatively correlated with propionate in the caecum. The expression of IL-6 was positively correlated with branched-chain fatty acids in the colon, indicating that concomitant fermentation of proteins with high β-glucan diets stimulate pro-inflammatory reactions (Metzler-Zebeli *et al*. 2012). Feeding barley-derived β-glucans changed the relative proportions of blood lymphocytes (i.e. CD4+ cells) but also increased the intestinal permeability and *E. coli* adhesion to enterocytes (Ewaschuk *et al*. 2012). Immunological reactions may be explained by the fact that some β-glucans induce receptor-mediated signals. For example, β-(1–3)(1–6)-glucans from yeast cell walls and algae (e.g. β-1–3-laminarin) are recognised by the host immune system and could indirectly modulate the intestinal environment through host-mediated mechanisms (Brown and Gordon 2003; Baert *et al*. 2015). Thus, very low concentrations of fungal β-glucans (0.02%) can already alter the immune response in pigs (Hahn *et al*. 2006). These observations might be important because: (i) yeasts are natural colonisers in the GIT of pigs and might be stimulated through dietary factors, (ii) yeasts can be found in higher numbers in fermented liquid feeds, and (iii) yeast cell walls might be present in higher concentration in by-products from the ethanol industry (e.g. distiller's dried grains with solubles), making them an interesting source of functional feedstuffs for growing pigs.

Cereal arabinoxylans

In contrast to studies on cereal mixed-linked β-glucans, less is yet known on the influence of AX from rye, wheat or triticale on bacterial ecology in the GIT. The AX represent a heterogeneous group of polysaccharides of high molecular weight and usually consist of a linear xylan backbone, substituted with various degrees of arabinose residues (Bach Knudsen 1997). Approximately 70–85% of AX disappears during the passage through the large intestine, whereas only a limited AX degradation was determined in the small intestine of pigs (Glitsø *et al*. 1999; Le Gall *et al*. 2009). Only a small fraction of rye AX can be fermented in the pig small intestine (Glitsø *et al*. 1998), but their fermentation in the caecum and colon of pigs can favour the formation of butyrate (Glitsø *et al*. 1998, 1999). However, these effects depend on the fractions of rye used. The AX present in the aleurone and endosperm showed higher fermentability (but comparable to that of whole rye) than in pericarp/testa (Glitsø *et al*. 1999). Interestingly, arabinose was

positively correlated with faecal butyrate concentration in pigs (Ivarsson *et al*. 2014). Studies on the targeted promotion of certain bacteria by AX from rye are limited. Given the complexity of xylans with numerous unique linkages compared with cereal β-glucans (two unique linkages), starch (two) or inulin (one), it is clear that the degradation and fermentation of AX relies on the ability of bacteria to cleave the AX side chains and the xylan backbone with a large number of different enzymes (Koropatkin *et al*. 2012). For example, *Bacteroides* spp. and *Prevotella* spp., which are also a dominant group in the pig large intestine, can produce several xylanases, mannanases and β-glucanases, and contribute to utilisation of soluble xylan (Dodd *et al*. 2011). As reviewed by Flint *et al*. (2012), the interaction with other bacteria including firmicutes or actinobacteria (i.e. bifidobacteria) seems to be important for the degradation of complex carbohydrates in the GIT. A recent study in pigs indicated that feeding diets very high in AX caused a higher number of *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, *Blautia coccoides–Eubacterium rectale*, *Bifidobacterium* spp. and *Lactobacillus* spp. in the faeces accompanied by higher butyrate in the large intestine (Nielsen *et al*. 2014).

There are some concerns on the inclusion of AX-rich feedstuffs at higher amounts into diets of young pigs because of their negative effects on nutrient digestibility (Bedford and Schulze 1998). Usually, exogenous enzymes are added to barley, wheat or rye-based diets for pigs. The degradation of complex AX to lower molecular compounds by exogenous enzymes (e.g. xylanases) decreases digesta viscosity but may also facilitate the access of small intestinal bacteria to fermentable substrates. For example, the addition of exogenous enzymes (xylanase, β-1–4 glucanase) to barley, rye or wheat bran-based diets of growing pigs increased the concentration of propionate in the small intestine indicating increased bacterial activity (Haberer *et al*. 1999). In addition, xylanase addition to wheat-based diets promoted lactobacilli such as *L. reuteri*, *L. acidophilus* and *L. mucosae* (Hirsch *et al*. 2006). However, a faster digesta transit and reduced water-holding capacity by AX reduced the microbial activity on the upper GIT of pigs, which coincided with reduced deconjugation of bile acids (Vahjen *et al*. 2007). This may in turn have implications on both the digestibility of fat and epithelial reactions due to changed levels of conjugated/deconjugated bile acids.

Finally, it should be noted that consumption of whole grain rye or their fractions increased satiety in humans (Isaksson *et al*. 2012), and this is also likely in pigs. This limits the use of rye fibres for targeted promotion of beneficial bacteria or metabolites in young pigs. Higher dietary inclusion rates could have beneficial effects in growing pigs but respective dose–response studies should be performed in order to define optimum inclusion rates without negative influence on pig performance.

Fructo-oligosaccharides and inulin

Inulin-type fructans have been studied quite intensively for their potential to promote specific groups of bacteria in the porcine GIT. Consequently, a broad variety of different findings are available and thus only a few aspects will be presented here. One of the basic ideas for using inulin-type fructans in pig diets is to support bifidobacteria and lactobacilli and thereby decrease possible pathogens (Verdonk *et al*. 2005). Most of the information about the effects of inulin on intestinal bacterial ecology comes from human or rodent studies (Bosscher *et al*. 2006), but the picture seems to be different in pigs. The main site of inulin fermentation has been postulated as being the caecum in young pigs (Yasuda *et al*. 2007). The site of fermentation depends on the DP of the product. For example, oligofructose with DP 2–7 was already partly fermented in the jejunum of pigs, whereas inulin with DP 20–60 was fermented in the caecum (Patterson *et al*. 2010). This is in agreement with other studies, where up to 90% of short-chain inulins (between DP 12–23) and oligofructose were fermented already in the upper GIT of pigs (Branner *et al*. 2004; Eberhard *et al*. 2007; Paßlack *et al*. 2012). Feeding inulin with DP 12 increased the number of pigs harbouring bifidobacteria in the colon (but cell counts were quite low) and increased the molar proportions of propionate and butyrate (Loh *et al*. 2006). In another study, long- and short-chain inulin enhanced the growth of lactobacilli and bifidobacteria in all segments of the GIT, whereas enterobacteria and clostridia were reduced (Patterson *et al*. 2010). Concomitantly, these inulin types decreased the expression of pro-inflammatory cytokines and increased genes related to iron metabolism, which may have positive effects for the host (Patterson *et al*. 2009). Feeding inulin with a DP57 increased the amount of fructans in the ileum, caecum and proximal colon of pigs and shifted the SCFA molar ratio from acetate to propionate in the proximal and distal colon, whereas lactate and butyrate were unaffected (Paßlack *et al*. 2012). Thus, the picture regarding the use of inulin in pigs is somehow heterogeneous.

It might be possible that other factors such as feed intake, diet composition (i.e. the presence of non-starch ploysaccharides in the basal diet), experimental conditions and/or the initial microbial community composition lead to different findings. For example, the influence of inulin on the GIT microbiota was reported in a commercial farm but not under high sanitary experimental conditions (Janczyk *et al*. 2010). Inulin supports the growth of bifidobacteria in humans, rodents and in pigs. However, the abundance of bifidobacteria in the GIT of pigs is lower compared with humans and their number declines further after weaning. Thus, a bifidogenic effect of inulin (although present) is probably less meaningful in pigs compared with other species. In addition, many other bacteria in the GIT are also capable of utilising inulin including enterobacteria (*Escherichia*, *Klebsiella*), *Staphylococcus* spp., *Enterococcus* spp., *Bacteroides* spp., *Lactobacillus* spp. and clostridia such as *C. butyricum* (Roberfroid and Delzenne 1998). Consequently, other bacterial groups and including some putative detrimental species may be favoured by inulin-type fibre, which could also lead to different outcome regarding SCFA and GIT health. With regard to infection with *Brachyspira hyodysenteriae*, only very high inclusion rates (8%, but not 2% and 4%) of inulin were reported to reduce the incidence of diarrhoea (Hansen *et al*. 2011).

Industrial by-products

By-products from industrial processing of feed and food are often considered as cost-effective ingredients for swine, which may have additional functional properties due to their content in fibre with different properties. Insoluble fibre sources are mainly

derived from by-products of cereal processing such as hulls from barley, oats or peas, cereal brans or purified cellulose. Other, more soluble fibre sources used in swine diets include sugar beet pulp (SBP) and by-products from various fruits (citrus pulp, apple pulp). Pectins are structurally and functionally the most complex polysaccharide in plant cell walls (Mohnen 2008). The SBP contains mainly soluble DF, with uronic acids and arabinose being the most predominant sugar monomers. Besides this pectin-rich fraction, SBP also contains considerable amounts of insoluble cellulose (Bach Knudsen 1997). Bacterial fermentation of pectins usually yields high amounts of acetate (Drochner *et al*. 2004; Anguita *et al*. 2007). Up to 37% of the pectin-rich fraction in SBP is already fermented in the small intestine, and another 50% disappears in the large intestine of pigs (Graham *et al*. 1986). Experiments from our laboratory showed that inclusion of SBP into the diets at 12% did not significantly change small intestinal metabolites but increased SCFA and promoted a significant shift from propionate towards acetate in the caecum and colon (Pieper *et al*. 2014). Konstantinov *et al*. (2004) determined a considerable microbial activity and increase in lactobacilli populations in the pig small intestine when diets containing SBP were fed. However, the initial microbial community composition and complex interactions between microbiota (e.g. bacteroidetes and firmicutes) during fermentation of highly complex pectins may favour either butyrate-producing bacteria or facilitate environmental conditions favouring the formation of butyrate. As an example, pectin favoured the growth of *Faecalibacterium prausnitzii*, an important butyrate producer in the human large intestine (Lopez-Siles *et al*. 2012). Interestingly, the inclusion of SBP or other by-products such as distiller's dried grains with solubles into growing pigs diets did not affect the shedding of Salmonella *in vivo* (Thomson *et al*. 2012).

There has been, and there is currently still, some debate whether soluble or insoluble fibre sources might have beneficial or detrimental effects in the GIT of young pigs affecting the susceptibility towards enterotoxigenic *E. coli* infections (Molist *et al*. 2014). Inclusion of soluble fibres has been reported as detrimental for weaned pigs whereas the inclusion of insoluble fibre could help to facilitate the proliferation of cellulolytic bacteria with possible beneficial effects later in life (Molist *et al*. 2014). For example, the inclusion of wheat bran may help to reduce the ileal *E. coli* counts and diarrhoea scores and increases the absolute and molar proportion of butyrate in the large intestine. However, inclusion of 5% of various sources of DF (straw, oat hulls, SBP, wheat middlings) into piglet diets increased the incidence of diarrhoea and reduced the apparent ileal nutrient digestibility (Berrocoso *et al*. 2015). The effects were more pronounced under optimal compared with poor hygienic conditions. The pig large intestinal microbiota could adapt to effectively utilise fibrous feedstuffs (Varel and Yen 1997). The microbial colonisation of the GIT is a gradual process and the microbial communities are better adapted to fibre-rich diets in older animals than in the young, weaned pig (Bach Knudsen *et al*. 2012).

Bacterial protein fermentation and host response

Microbial fermentation of undigested proteins

Dietary and endogenous proteins are utilised in the GIT through microbial fermentation. Bacterial AA metabolism occurs via oxidative and reductive reactions including deamination, decarboxylation, α- and β-elimination. A large variety of bacterial species such as *E. coli*, *Klebsiella* spp., *Campylobacter* spp., *Streptococcus* spp., *C. perfringens*, *C. difficile* and *Bacteroides fragilis* have been reported as dominant protein fermenters. Deamination of AA leads to formation of ammonia (NH_3) and the remaining carbon skeleton can be further metabolised to yield SCFA and branched-chain fatty acids from branched-chain AA. Bacterial AA utilisation also contributes to the formation of SCFA in the GIT. Protein fermentation products such as NH_3 have been primarily associated with toxigenic and damaging effects on the intestinal epithelium (Davila *et al*. 2013). Ammonia can interfere with the oxidative metabolism of SCFA in colonocytes, likely inducing energy deficiency in the cell (Blachier *et al*. 2007). Decarboxylation of AA yields several biogenic amines such as cadaverine, histamine tyramine, tryptamine, methylamine, ethylamine or agamatine. The role of polyamines such as spermidine, spermine and putrescine has been studied in detail due to their enhancing effect on cell proliferation (Seiler and Raul 2007). Histamine can induce chloride secretion and diarrhoea in the colon of pigs (Kröger *et al*. 2013). Little is yet known about the role of other biogenic amines but increased diarrhoea was associated with high intestinal concentrations of amines such as cadaverine and putrescine (Pietrzak *et al*. 2002). Several phenolic and indolic compounds may also be produced from the metabolism of aromatic AA including phenol, indole and p-cresol. A broad range of different bacterial species including *Clostridiales*, *Bacteroides*, *Prevotella*, *Enterobacteriaceae* and *Streptococcaceae* have been identified for their role in metabolism of sulfur containing substrates such as methionine, cysteine, taurine, sulfomucins and bile acids in the intestine. Sulfate-reducing bacteria were identified to play a major role in hydrogen sulfide formation in the pig, with the genus *Desulfovibrio* being most important. Hydrogen sulfide has been attributed to both beneficial and deleterious effects in the intestine (Blachier *et al*. 2010), but the effects on intestinal epithelial cells seem to be largely dose-dependent.

Fibre and protein fermentation and intestinal health

Protein fermentation in the GIT can lead to intestinal disorders including post-weaning diarrhoea. Diets higher in protein (21% vs 13%) have been reported to predispose piglets to enteropathogenic *E. coli* infections and post-weaning diarrhoea (Prohászka and Baron 1980). Consequently, a common strategy to reduce the risk of post-weaning diarrhoea is reducing the dietary protein level (Heo *et al*. 2013). For example, feeding low (13%) versus high (23%) dietary protein increased the faecal lactobacilli to enterobacteria ratio (Wellock *et al*. 2008). In addition, lower protein diets have been associated with reduced incidence of post-weaning diarrhoea in enterotoxigenic *Escherichia coli*-challenged and also non-challenged piglets (Heo *et al*. 2009; Opapeju *et al*. 2009). In turn, lower protein levels were accompanied with increased levels of bacterial species (*Roseburia/E. rectale*-like), which are specialised for carbohydrate utilisation and butyrate formation (Opapeju *et al*. 2009).

It has to be noted that *E. coli*-induced disease and diarrhoea occurs mainly in the small intestine (Fairbrother *et al*. 2005), whereas the site of excessive protein fermentation is the large

intestine. A higher CP level in the diet may change the buffering systems in the upper GIT, thereby favouring the proliferation of enterobacteria or clostridia (Prohászka and Baron 1980). Protein-derived metabolites formed by proteolytic bacteria such as clostridia may impair barrier function, which in turn, may reduce the ability for fluid re-absorption, which otherwise could mask small intestinal hypersecretion. Interestingly, diets high in fermentable protein reduced the activity of the large intestinal epithelial ameloride-sensitive sodium channel, which was associated with more liquid faeces in piglets (Richter *et al*. 2014).

The GIT bacteria may switch from fermentation of carbohydrates to AA as substrates to derive energy. Consequently, a variety of fermentable carbohydrates may reduce harmful protein fermentation in the porcine GIT (de Lange *et al*. 2010). In fact, RS, cereal fibres and SBP have been shown to decrease the abundance of protein fermentation products (Awati *et al*. 2006; Bikker *et al*. 2006; Nyachoti *et al*. 2006; Pieper *et al*. 2012*b*, 2014). Diets rich in fibrous compounds can thereby also promote a shift of nitrogen excretion from urine to faeces (Bindelle *et al*. 2009). However, less clarity exists about the optimum fermentable protein/fermentable carbohydrate ratio with respect to GIT microbial ecology and especially host responses. For example, although dietary carbohydrates (wheat bran, SBP) reduced the formation of protein-derived metabolites in the large intestine of piglets, blood urea nitrogen was higher in high protein diets regardless of carbohydrate level (Pieper *et al*. 2012*b*; Stumpff *et al*. 2013). In addition, this was accompanied with increased inflammatory conditions, altered permeability of the epithelium towards macromolecules and changed secretory responses (Pieper *et al*. 2012*b*; Kröger *et al*. 2013; Richter *et al*. 2014). Linking the site of dietary protein fermentation with the effectiveness of different carbohydrate sources (insoluble, partly soluble), an experiment with a 2×3 factorial design was conducted with two different levels of protein (18% vs 24%) and inclusion (or not) of two different carbohydrate sources (cellulose, SBP). The SBP mainly increased SCFA and lactate and decreased protein-derived metabolites in the large intestine, whereas cellulose was partly fermented in the distal large intestine and reduced mainly phenols, indoles and cadaverine, but not NH_3 (Villodre Tudela *et al*. 2015). In the same study, it was shown that some protein-derived metabolites (i.e. NH_3, putrescine) were negatively correlated with the gene expression of MCT1 in the GIT of pigs (Villodre Tudela *et al*. 2015), and MCT1 expression was reduced in high protein diets regardless of dietary carbohydrate inclusion. Further analyses revealed that NH_3 (likely through TNFα-mediated signalling) leads to a downregulation of the MCT1 gene linking impaired protective effects of butyrate with increased pro-inflammatory conditions in the colon of pigs.

Summary and outlook

The porcine GIT is colonised by a highly diverse microbial community, which is increasingly recognised for its role in nutrient utilisation and influence on host health. Our understanding of this ecosystem has emerged during the past years but some aspects including the complex nutrition–microbe–host interactions have received less attention. Targeting the GIT microbial ecosystem by means of nutritional manipulation may help to maintain or improve GIT functionality, improve food safety and animal welfare. This includes the use of certain soluble and insoluble DF sources and protein sources and levels. Although some results seem to be promising to effectively manipulate the microbial ecosystem, results related to host response are to date not as clear. Aspects that have not been touched on in this review such as dietary particle size, hydrothermal treatments, viscosity and water-holding capacity or buffering capacity need to be addressed in conjunction with the selection of DF sources and diet composition. With respect to prevention of pathogen-induced diseases by manipulating the dietary carbohydrate and protein level, it seems that this depends largely on pathogens to be targeted (e.g. pathogenic *E. coli*, Salmonella, detrimental clostridia, Brachyspira). Most studies showing beneficial effects on putative pathogens have used high levels of fibre. High fibre diets might reduce ileal nutrient digestibility and can reduce feed intake. Experimental diets with certain levels of functional fibres may accomplish the abovementioned goals in pigs. The requirement for DF for swine also depends on age. Deriving recommendations for optimal inclusion rates of DF for weaning, growing or sows is difficult at this stage due to many factors influencing the mode of action of GIT fibre utilisation. However, defining minimum required levels for DF and maximum dietary CP levels in swine diets could be formulated in the future.

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Functional short-chain carbohydrates (prebiotics) in the diet to improve the microbiome and health of the gastrointestinal tract

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Abstract. Advancement in technologies to identify and quantify bacterial species in the gastrointestinal tract has escalated interest in its microbiome worldwide. There is enormous interest in understanding the roles that bacterial species play in gastrointestinal health and overall wellbeing. What constitutes a 'healthy gut microbiome' includes: favourable fermentation-dependent characteristics such as butyrate supply to all regions, minimisation of putrefaction of proteins, and adequate laxation. The relative abundance of specific bacterial species with certain functional characteristics is also important and include: traditional prebiotic bacteria – *Bifidobacteria*; strongly butyrate-producing – *Clostridium coccoides* and *Faecalibacterium prausnitzi* as well as a mucus-associated bacterium *Akkermansia muciniphila*. Manipulation of diet and dietary factors may be essential to favourably influence these fermentation-dependent parameters and select for growth of beneficial bacterial species. In this regard, this laboratory has identified indigestible oligosaccharides with prebiotic effects and now has an extensive database that quantifies indigestible oligosaccharides in a wide variety of foods including whole grains, cereals, legumes, seeds, nuts, fruits and vegetables. Future research in this area should consider the role of dietary components that best establish and maintain a 'healthy gut microbiome'.

Additional keywords: gut microbiome, fermentation.

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Introduction

The importance of gastrointestinal tract (GIT) microbiota to the health of the intestine and the health of the organism in general has been escalated from relatively little interest to a major research focus of scientists and clinicians around the world (Guarner *et al*. 2013).

One of the major reasons for this has been the introduction of accessible and affordable means to characterise the bacteria and other microbes by rRNA and DNA technologies (Weinstock 2012).

The importance of understanding how dietary components influence the structure of the gut microbiome and its function via, for example sacchrolytic (carbohydrate) fermentation and putrefaction (proteolytic fermentation), has been highlighted (Tremaroli and Backhed 2012; Scott *et al*. 2013). As diet has such strong influences on the gut microbiota (defined as the microbiome and its metabolic activities), concepts of what comprised a 'healthy diet' for 'wellbeing' have been greatly expanded from maintaining nutritional adequacy with adequate fibre for laxation to considerations of what effect diet has on the gut microbiota, described further in the next section.

The nutraceutical industry has been a strong advocate for the use of purified supplements of indigestible oligosaccharides and inulin as specific stimulators of health-promoting bacteria and suppression of potentially pathogenic bacteria with subsequent putative health benefits. The term 'prebiotic' is used to describe this class of indigestible oligosaccharides (Roberfroid *et al*. 2010). This has led to the fortification or supplementation of many foods with pure prebiotics for such potential health benefits. Despite this, there has been little or no attention to prebiotics that are naturally present in food. Interventional studies of prebiotic therapies have not addressed prebiotic intake in the background (habitual) diet, which is heterogenous across the populations studied, and knowledge of prebiotic content of food has been either absent or ignored.

Indigestible oligosaccharides have now been quantified in a wide variety of foods including whole grains, cereals, legumes, seeds, nuts, fruits and vegetables (Muir *et al*. 2007, 2009; Biesiekierski *et al*. 2011). More research attention needs to be paid to natural prebiotics and role their modulation may play in maintaining gut health and prevention of disease.

The pig as a model for human nutrition and concept of 'GIT health'

The pig is considered an excellent model for studying gut function relevant to human GIT health. Both pigs and humans are hindgut fermenters and pigs have the closest physiology and intestinal similarities to humans (Heinritz *et al*. 2013). The pig has been used extensively to study the fermentation-dependent characteristics of different types of indigestible carbohydrates

(e.g. dietary fibre, resistant starch) (Topping *et al*. 1997; Govers *et al*. 1999) – effects that were later confirmed in studies involving humans (Muir *et al*. 2004).

The concept of 'health' and a 'healthy GIT' is relevant to both humans and pigs and could be considered to involve; the optimal functioning of the GIT including absorption of adequate nutrients to support growth and nutritional requirements, good laxation (to ensure removal of waste and putrefactive factors) and the absence of infection, inflammation and gastrointestinal disease.

The major objective of this present review, however, is to summarise what constitutes a healthy GIT microbiota and bowel function in humans. This review does not cover specifically pig science or pig nutrition, which has been adequately and extensively reviewed elsewhere (Wenk 2001; Bindelle *et al*. 2008; Rist *et al*. 2013), nevertheless many of the observations made in humans may be highly relevant to pig nutrition in the future.

Determinants of a healthy gut microbiome

Although it is not clear what constitutes a 'healthy gut microbiome', several aspects of the GIT structure and function together with colonic propulsive motility are known or are emerging as important for health (see Tuohy *et al*. 2005; Quigley 2011; Flint *et al*. 2012; Tremaroli and Backhed 2012). These aspects include the abundance or density of bacteria in the colonic lumen and mucus, which appear to be critical to the ability to produce butyrate and other important substances (see Tuohy *et al*. 2005; Quigley 2011; Flint *et al*. 2012; Tremaroli and Backhed 2012). Favourable fermentation-dependent characteristics include:

- Efficient fermentation to all regions of the large bowel such that the butyrate supply is provided to all regions (Govers *et al*. 1999; Muir *et al*. 2004) and not just proximally, where too much butyrate can be detrimental (epithelial injury, Lin *et al*. 2005), visceral hypersensitivity (Bourdu *et al*. 2005), loss of anti-carcinogenic effect (Scheppach *et al*. 1995).
- Minimisation of putrefaction of proteins, the rate of which appears more dependent upon delivery of carbohydrates for fermentation as the amount and type of protein consumed (Birkett *et al*. 1995).
- Colonic transit that permits adequate laxation. The relationship of this to the microbiota is known to be via the bulking effect of bacteria, and the delivery of short-chain fatty acids (SCFA) to promote motility, sodium and water absorption (Brownlee 2011).

The relative abundance of specific bacteria with important metabolic activities. These include:

- Abundant prebiotic bacteria, especially *Bifidobacteria*;
- Strongly butyrate-producing bacteria such as *Clostridium coccoides* and *F. prausnitzi*, for their supply of butyrate as the principal energy source for colonic epithelium and for its differentiative, anti-neoplastic and anti-inflammatory effects;
- *Akkermansia muciniphila*, an important mucus-associated bacterium that provides acetate for butyrate production for other species of bacteria such as *C. coccoides*. This 'cross-feeding' appears to be critical for a healthy mucus barrier; and
- *Ruminococci*, which are also mucus-degrading bacteria like *A. muciniphila*, but appear to have no beneficial effects and are found in greater abundance in many pathological conditions of the colon (Hoskins 1993).

When putrefaction of dietary proteins predominates in the colon as a result of increasing intake of dietary or resistant protein, toxic metabolites such as ammonia, N-nitroso-, phenolic and indolic compounds and the highly toxic hydrogen sulfide (Blachier *et al*. 2007) are produced. These end products can exert the following effects in the colon:

(1) Promotion of an unfavourable ecology of the luminal microbiota. Unlike carbohydrate fermentation, protein fermentation lowers concentrations of total and individual SCFA (butyrate, acetate and propionate) (Duncan *et al*. 2007). Both caecal and faecal pH become significantly elevated whereas higher concentrations of branched-chain fatty acids are found (Russell *et al*. 2011). Additionally, in animals fed a diet high in protein, greater growth of *Escherichia coli* and *Salmonella* spp., *Clostridium* spp. and *Bacteroides* spp. were induced (Pieper *et al*. 2012) whereas, in another study, a reduction in *Lactobacillus* spp. and *Bifidobacteria* spp. was observed (Bedani *et al*. 2010).
(2) Negative effects on the colonic epithelium. Increased exposure to ammonia, hydrogen sulfide and phenols have been shown to cause disruptions in absorptive function, growth and metabolic activity of colonocytes (Blachier *et al*. 2007), particularly in the cellular oxidation of butyrate for energy production (Blachier *et al*. 2007).

Supplementing a high protein diet with fermentable carbohydrates appeared to reverse these putrefaction-induced changes in the colon (Pieper *et al*. 2012). One approach to encourage the growth of the healthy microbiome, as we have defined in the previous section, appears to be with indigestible oligosaccharides (i.e. not degradable by host enzymes and available for fermentation). To date, however, the majority of research in this area in man and animals has concentrated on using purified supplements or 'functional fibres' of fructo-oligosaccharides (FOS)/inulin (mostly purified from chicory root) and galacto-oligosaccharides (GOS), and none have examined the potential influence of increasing the dietary intake of food that are naturally rich sources of prebiotic carbohydrates.

Natural prebiotics in food

Encouraging the growth of a healthy microbiota using whole foods requires knowledge about the levels of natural prebiotics to be found in food. The major class of natural prebiotics are indigestible oligosaccharides – fructans (FOS/inulin) and GOS (Muir *et al*. 2007; Biesiekierski *et al*. 2011). Publications from this laboratory list the quantities of natural occurring indigestible oligosaccharides, including total fructans and GOS (Muir *et al*. 2007, 2009; Biesiekierski *et al*. 2011) (see Fig. 1). These short-chain carbohydrates are present in a wide variety of foods, but are particularly high in certain cereals, grains, legumes, vegetables, some fruits and nuts. This food composition knowledge is now a key component of 'The Monash University Low FODMAP diet', a diet now used worldwide as a therapy for improving gastrointestinal symptoms in patients with irritable bowel syndrome (IBS). The term 'FODMAP' is an umbrella term and stands for Fermentable, Oligosaccharides, Disaccharides,

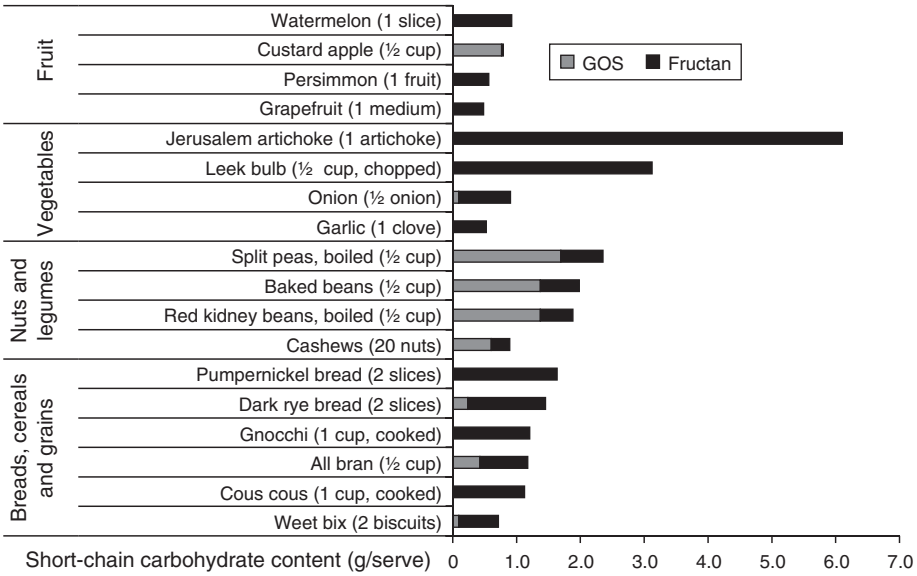

Fig. 1. Natural prebiotic-rich foods (i.e. rich in fructans and GOS) (Muir *et al*. 2007; Biesiekierski *et al*. 2011).

Table 1. The mean daily total, absorbed and fermentable carbohydrate information of provided low and typical Australian FODMAP diets (Halmos *et al*. 2015)

n.s., not significant

Per day	Typical Australian diet	Low FODMAP diet	*P*-value
Total carbohydrate (g)	219 [180–259]	215 [181–249]	n.s.
Sugars (g)	120 [103–137]	122 [106–139]	n.s.
Starch (g)	94.0 [52.8–135]	95.4 [59.7–131]	n.s.
Total dietary fibre (g)	29.7 [23.9–35.7]	30.4 [24.2–36.5]	n.s.
Fibre (g)	25.9 [21.3–30.6]	23.4 [18.7–28.2]	n.s.
Resistant starch (g)	3.74 [1.85–5.63]	6.93 [3.56–10.3]	n.s.
Total FODMAP (g)	23.7 [16.9–30.6]	3.05 [1.86–4.25]	<0.001
Oligosaccharides[A] (g)	5.49 [2.34–8.65]	1.57 [0.47–2.66]	0.009
Polyols (g)	4.21 [2.57–5.85]	0.20 [–0.04–0.44]	0.002
Lactose (g)	1.35 [0.20–2.49]	0.05 [–0.01–0.10]	0.033
Fructose in excess of glucose (g)	12.7 [8.06–17.3]	1.24 [0.41–2.07]	0.001

[A]Oligosaccharides = total fructans (FOS and inulin) and GOS.

Monosaccharaides And Polyols (Halmos *et al*. 2014). In patients with IBS, FODMAP can trigger gastrointestinal symptoms including gas production and associated gut distension (Ong *et al*. 2010; Halmos *et al*. 2015) as well as promote the movement of water into the lumen via osmotic effects (Barrett *et al*. 2010).

Manipulating the gut microbiome with diet

Recent evidence from this laboratory shows that lowering FODMAP in the diet can affect the numbers of certain bacteria including *Bifidobacteria* spp. (Staudacher *et al*. 2012). Work in patients with IBS has shown that levels of faecal bacteria in patients with IBS (n = 23) can be lowered while following a low FODMAP diet when compared with a typical Australian diet (see Table 1 for the dietary intake) (Halmos *et al*. 2015). In these studies (see Fig. 2), absolute bacterial abundance in butyrate-producing species – *C. leptum*, *F. prausnitzii*, *C. coccoides*, *Roseburia* spp. – and the key mucus-associated bacterium, *A. muciniphila* and *Bifidobacteria* spp., was significantly decreased ($P < 0.005$), whereas relative abundance of *C. coccoides* and *A. muciniphila* was markedly reduced, and *R. torques* increased ($P < 0.006$) (Halmos *et al*. 2015).

The 'dose' of indigestible oligosaccharides required to produce these changes in faecal bacteria was ~4 g/day (Table 1). Table 1 shows the difference between the 'typical Australian diet' and the 'low FODMAP diet' in the content of fructan plus GOS. The dose of pure prebiotics that have been used in provocation studies to produce physiological responses typically ranges from 4 to 15 g per day (Williams *et al*. 1994; Gibson *et al*. 1995; Rao 1999).

Prebiotic carbohydrates co-exist with other indigestible carbohydrates

The major benefits of long-chain indigestible carbohydrates (dietary fibre and resistant starch) are well understood and include bulking and laxation, raised luminal SCFA including butyrate, and lower luminal pH (Scheppach *et al*. 2001). Intriguingly, foods that tend to be naturally high in prebiotics –

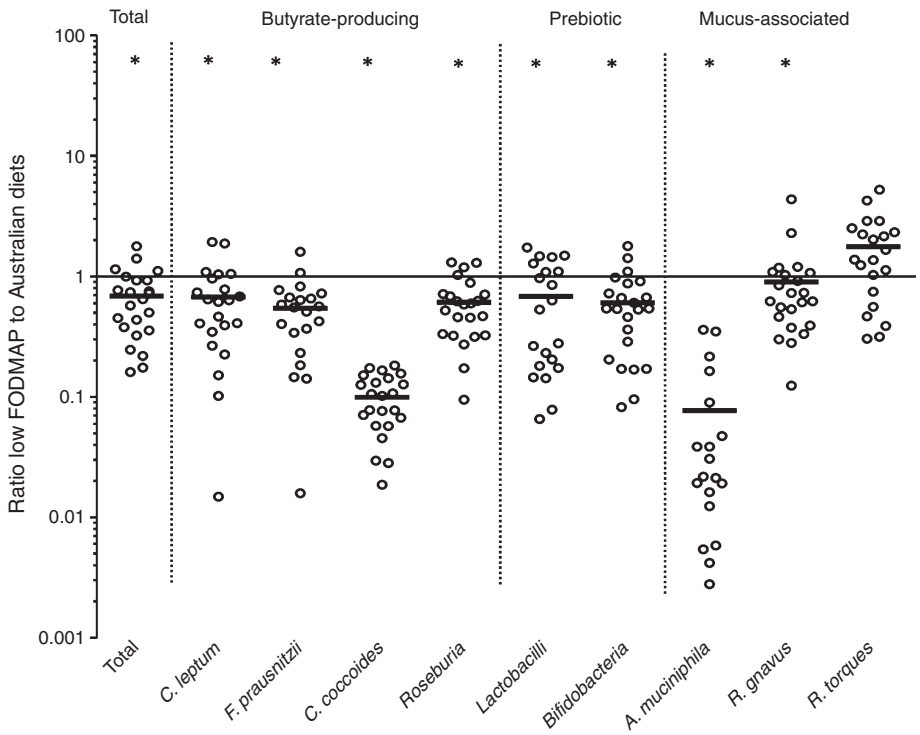

Fig. 2. Changes in absolute abundance of faecal bacteria in irritable bowel syndrome patients (n = 23) following the low FODMAP diet versus changes with a typical Australian diet. Data is expressed as a ratio of low FODMAP to a typical Australian diet and was analysed using Wilcoxon matched-pairs signed rank test. The dark horizontal lines represent median values. Legend: *C. leptum*, *Clostridium leptum* group; *F. prausnitzii*, *Faecalibacterium prausnitzii*; *C. coccoides*, *Clostridium coccoides* group; *A. muciniphila*, *Akkermancia muciniphila*; *R. gnavus*, *Ruminococcus gnavus*; *R. torques*, *Ruminococcus torques.* * represents statistically significant differences between diets, $P \leq 0.005$ after Bonferroni correction were obtained for (Halmos *et al*. 2014).

fructans and GOS – also tend to be high in dietary fibre and resistant starch (i.e. they co-exist with other major classes of indigestible carbohydrates). It follows that any dietary approach that concentrates on raising 'natural prebiotics' in the diet will have the added advantage of consuming other health-promoting food components (dietary fibre and resistant starch) and as such have considerably advantages over added pure prebiotics supplements to the food supply.

Conclusion

Understanding the role the GIT microbiome plays in GIT health is a rapidly expanding area of research worldwide. It is well accepted that certain types of dietary carbohydrates play a role in determining the types and distribution of bacterial species present in the GIT. Studies using prebiotics – FOS/fructans and GOS – supplied as purified supplements have been extensively researched. Few studies, however, have been undertaken using foods that are naturally high in these prebiotic carbohydrates. Our laboratory has a growing database that lists the quantities of indigestible oligosaccharides (fructans/GOS) present in food, and has recent evidence that manipulation of the diet containing these natural occurring prebiotic carbohydrates can have a major impact upon populations of bacteria in the gut. The implications of these changes for health and diseases are unknown. Future research needs to consider whole diets that naturally contain prebiotic carbohydrates. Such research may hold to the key to better understanding how to both establish and maintain a 'healthy gut microbiome' for both humans and pigs.

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Chapter 3: Symposia – The impact of heat stress on performance and health of pigs

Physiological consequences of heat stress in pigs

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Abstract. Heat stress negatively influences the global pork industry and undermines genetic, nutritional, management and pharmaceutical advances in management, feed and reproductive efficiency. Specifically, heat stress-induced economic losses result from poor sow performance, reduced and inconsistent growth, decreased carcass quality, mortality, morbidity, and processing issues caused by less rigid adipose tissue (also known as flimsy fat). When environmental conditions exceed the pig's thermal neutral zone, nutrients are diverted from product synthesis (meat, fetus, milk) to body temperature maintenance thereby compromising efficiency. Unfortunately, genetic selection for both increased litter size and leaner phenotypes decreases pigs' tolerance to heat, as enhanced fetal development and protein accretion results in increased basal heat production. Additionally, research has demonstrated that *in utero* heat stress negatively and permanently alters post-natal body temperature and body composition and both variables represent an underappreciated consequence of heat stress. Advances in management (i.e. cooling systems) have partially alleviated the negative impacts of heat stress, but productivity continues to decline during the warm summer months. The detrimental effects of heat stress on animal welfare and production will likely become more of an issue in regions most affected by continued predictions for climate change, with some models forecasting extreme summer conditions in key animal-producing areas of the globe. Therefore, heat stress is likely one of the primary factors limiting profitable animal protein production and will certainly continue to compromise food security (especially in emerging countries) and regionalise pork production in developed countries. Thus, there is an urgent need to have a better understanding of how heat stress reduces animal productivity. Defining the biology of how heat stress jeopardises animal performance is critical in developing approaches (genetic, managerial, nutritional and pharmaceutical) to ameliorate current production issues and improve animal wellbeing and performance.

Additional keywords: epigenetics, metabolism, reproduction, swine.

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Introduction

Abiotic stress and heat stress, in particular, severely impairs efficiency at every stage of the production cycle. Pigs are particularly sensitive to heat stress because they lack functional sweat glands and despite decades of intense genetic selection, still have a thick layer of subcutaneous adipose tissue that acts as an effective insulation layer. The swine industry prioritises production efficiency and as a result, has achieved rapid improvements in the lean growth of market pigs and reproductive efficiency over the past several decades. The bulk of heat stress-induced financial burden occurs through reduced and inconsistent growth and poor sow reproductive performance but is also realised through increased mortality and morbidity, and decreased carcass value. Consequently, heat stress is one of the largest economic barriers to the USA pork industry (St-Pierre *et al.* 2003). Although currently a large impediment, heat stress will likely become more of a production hurdle in the future if traditional production traits continue to be genetically emphasised, as selection for improved lean tissue accretion rates and reproductive capacity (piglets born and weaned) are both accompanied with increased basal heat production (Brown-Brandl *et al.* 2004). This reduced thermal tolerance may be partly mediated by altered body composition (i.e. increased lean tissue), as synthesising and maintaining skeletal muscle generates metabolic heat (Brown-Brandl *et al.* 2001). Thus, genetic selection for economically important production traits will likely further decrease tolerance to heat stress (Nienaber and Hahn 2007; Baumgard and Rhoads 2013). The focus of this review is to evaluate our current understanding of the physiological basis for compromised growth and reproduction in pigs as a result of heat stress. Deepening our understanding of the physiological consequences of heat stress in pigs is essential to developing strategies to mitigate the deleterious effects of current heat stress and future climate change on the global swine industry.

Heat-stress effects on swine production

Accurately determining the heat-induced economic loss is difficult, but a recent estimate suggests poor sow performance alone (not including reduced offspring growth, carcass quality) costs the USA swine industry $450 million annually (Pollmann 2010). Even if optimal heat-stress abatement strategies were implemented by all pig producers at all stages of production, heat stress is estimated to cost the USA swine industry millions

annually (St-Pierre *et al*. 2003). The combination of climate change forecasts, increased pork production in tropical and subtropical regions of the globe, and improved genetic capacity for lean tissue accretion and fecundity, all point to increasingly negative impacts of heat stress on pork production efficiency and quality in the future.

Heat stress impacts feed intake

Reduced nutrient intake during a thermal load is a highly conserved response across species and presumably represents an attempt to decrease metabolic heat production (Baumgard and Rhoads 2013). Additionally, a meta-analysis of publications (1970–2009) revealed the effect of heat stress on feed intake and growth in pigs to be more pronounced in recent years, supporting the posit that genetic selection for growth and carcass traits increases pig thermal sensitivity (Renaudeau *et al*. 2011). Traditionally, the detrimental effects of heat stress on production have been solely attributed to inadequate feed intake. However, recent findings from this laboratory challenge this dogma as it has been repeatedly demonstrated that, given the same plane of nutrition, production responses differ between thermal neutral and heat-stress environments in both cattle and growing pigs (Baumgard and Rhoads 2013; Pearce *et al*. 2013*a*). This led to the hypothesis that heat stress has both direct and indirect (via reduced feed intake) effects on animal productivity.

Identifying how much of the decreased productivity is caused by heat-induced reductions in feed intake is difficult. This is primarily because the composition of tissue accretion is not taken into consideration when measuring gross changes in bodyweight gain. For example, heat-stressed sows (Prunier *et al*. 1997) do not lose as much bodyweight and body condition as do their pair-fed thermal neutral counterparts; this holds true also for growing pigs (Pearce *et al*. 2013*a*; Sanz Fernandez *et al*. 2015). Therefore, reduced feed intake may appear to explain a majority of the decreased performance in growing animals, but the direct effects of heat may be altering the hierarchy of tissue synthesis.

Heat stress impacts carcass composition

In addition to the aforementioned reduced productive measures, heat stress also alters carcass composition (more fat and less lean). It is well known that pigs reared in heat-stress conditions have reduced muscle mass and increased adipose tissue (Close *et al*. 1971; Verstegen *et al*. 1978; Heath 1983; Bridges *et al*. 1998; Collin *et al*. 2001). Although there are some inconsistencies in the literature regarding the effects of thermal stress on carcass composition (Nienaber *et al*. 1987; Le Bellego *et al*. 2002), these differences are explained by dissimilar environmental conditions and experimental animal size as heat stress has little effect on carcass tissue mass in young pigs but markedly increases adipose tissue accretion and reduces carcass nitrogen content in heavier pigs (Christon 1988). This phenomenon is not unique to pigs, as heat stress also alters carcass composition similarly in rodents (Schmidt and Widdowson 1967; Katsumata *et al*. 1990) and growing poultry (Geraert *et al*. 1996; Yunianto *et al*. 1997). This metabolic shift in heat-stressed animals is energetically interesting as animals in thermal neutral conditions consuming a restricted diet will deposit protein at the expense of lipid accretion (i.e. the carcass lipid to protein ratio decreases meaning the carcass becomes leaner), and the quantity of lipid deposited per unit of energy consumed decreases (Le Dividich *et al*. 1980; Van Milgen and Noblet 2003; Oresanya *et al*. 2008). Hence, the reduced feed intake to body composition relationship is exactly opposite in pigs reared in heat-stress conditions and is independent of the plane of nutrition (Baumgard and Rhoads 2013). Therefore, it is clear that heat stress alters the hierarchy of normal nutrient partitioning and this unusual metabolism is not conducive to profitable pig production.

Metabolic consequences of heat stress in pigs

The aforementioned production data suggest that heat stress alters metabolism differently than would be expected based upon calculated whole-body energy balance. Surprisingly, we and others have demonstrated that basal plasma non-esterified fatty acid levels are typically reduced in heat-stressed rodents (Sanders *et al*. 2009), pigs (Pearce *et al*. 2013*a*), and cattle (Shwartz *et al*. 2009) despite marked reductions in feed intake, and especially when compared with pair-fed thermal neutral controls (Rhoads *et al*. 2009; Sanz Fernandez *et al*. 2015). Furthermore, this laboratory has recently reported that both heat-stressed cows and pigs have a decreased non-esterified fatty acid response to an epinephrine challenge compared with pair-fed thermal neutral counterparts (Baumgard *et al*. 2011; Sanz Fernandez *et al*. 2015). The blunted lipolytic capacity of adipose tissue is especially unusual as heat-stressed animals are severely nutrient restricted, which is an energetic state typically associated with elevated circulating non-esterified fatty acid levels (Bauman *et al*. 1988).

Heat stress alters insulin circulation

Despite hallmarks traditionally associated with hypoinsulinemia such as (1) marked reductions in feed intake, (2) calculated negative energy balance and (3) rapid bodyweight loss, it has been demonstrated that basal insulin concentrations gradually increase in lactating heat-stressed cows (Wheelock *et al*. 2010), growing calves (O'Brien *et al*. 2010) and pigs (Pearce *et al*. 2013*a*; Sanz Fernandez *et al*. 2015). The increase in insulin, a potent anabolic hormone, during heat stress, an intensely catabolic condition, is seemingly a biological paradox. The reason for this counter-intuitive physiological occurrence is not clear although may involve insulin's role in the activation of cellular stress responses (Li *et al*. 2006). Regardless, increased plasma insulin concentrations in these experiments agree with data from other heat-stressed ruminant reports (Itoh *et al*. 1998), a malignant hyperthermic pig model (Hall *et al*. 1980), and a heat-stressed rodent model (Torlinska *et al*. 1987). In addition, and in response to a glucose tolerance test, heat-stressed cows and calves have an increased insulin response compared with pair-fed thermal neutral controls, whereas glucose disposal is quicker or remains unchanged (O'Brien *et al*. 2010; Wheelock *et al*. 2010). It is also demonstrated, using the hyperinsulinemic-euglycemic clamp technique, that insulin sensitivity is improved in growing heat-stressed calves (Rhoads *et al*. 2009) and growing pigs (Sanz Fernandez *et al*. 2015), as heat-stressed animals required more glucose to maintain euglycemia. Similarly, growing pigs appear to be more insulin sensitive based on the insulin to glucose response to a glucose tolerance test (Sanz Fernandez *et al*.

2015). Whole-body glucose utilisation appears to increase during heat stress; however, the contribution of the different tissues to this net effect remains unknown. The immune system is a potential glucose utiliser that, as described below, might be stimulated due to the deleterious effects of heat stress on intestinal health. Once activated, immune cells become obligate glucose utilisers, and this altered hierarchy of fuel requirements may trigger a whole body shift in nutrient partitioning in order to spare glucose for the immune system (Baumgard and Rhoads 2013). In this scenario, adipose and muscle become refractory to insulin whereas activated immune cells become insulin sensitive, and the immune system's glucose utilisation may exceed that of systemic tissue.

Heat stress compromises intestinal health

Mechanisms responsible for altered nutrient partitioning during heat stress are not clear, however, heat-stress effects on gastrointestinal health and function might mediate them. The small intestine is one of the first tissues upregulating HSP during a thermal load (Flanagan *et al*. 1995). During heat stress, blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat (Lambert *et al*. 2002), leading to intestinal hypoxia (Hall *et al*. 1999). Enterocytes are particularly sensitive to oxygen and nutrient restriction (Rollwagen *et al*. 2006), resulting in ATP depletion and increased oxidative and nitrosative stress (Hall *et al*. 2001). This contributes to tight junction dysfunction, and gross morphological changes that ultimately reduce intestinal barrier function (Lambert *et al*. 2002; Pearce *et al*. 2013*b*). As a result, heat stress increases the passage of luminal content [e.g. lipopolysaccharide (LPS) from Gram-negative bacteria] into the portal and systemic blood (Hall *et al*. 2001; Pearce *et al*. 2013*b*). Increasing evidence suggests that LPS directly or indirectly increases pancreatic insulin secretion as infusing LPS into the mammary gland increased (~2-fold) circulating insulin in lactating cows (Waldron *et al*. 2006). In addition, intravenously infusing LPS into growing pigs and calves demonstrated a >10-fold increase in circulating insulin (Rhoads *et al*. 2009; Stoakes *et al*. 2015).

Reproductive consequences of heat stress in pigs

In North America, the effects of heat stress on swine reproduction are also quite apparent with pregnancy detection rates on Day 28 of gestation reaching their lowest levels in August into October and reduced farrowing rates in November and December (based on inseminations conducted in June–September). Although this repeated observation, which is referred to as 'seasonal infertility', could arguably be related to factors such as photoperiod, seasonal infertility in pigs is associated with periods of excessive heat, and heat stress has been repeatedly demonstrated to negatively impact reproductive efficiency, particularly due to lost embryonic development (Tompkins *et al*. 1967; Omtvedt *et al*. 1971).

Heat stress compromises boar fertility

Although the effects of heat stress on female pig reproduction are notable, boars are also negatively affected by heat stress, which are explained primarily by poor semen production and quality. In comparison to boars in thermal neutral environments, exposing boars to heat stress for 90 days (34.5 and 31.0°C for 8 and 16 h/day, respectively), reduced sperm motility and increased sperm abnormalities within 2 weeks from heat-stress initiation (Wettemann *et al*. 1976). Furthermore, utilisation of semen from heat-stressed boars reduced the number of fetuses on Day 30 post-insemination (Wettemann *et al*. 1976). Similarly, boars exposed to heat stress had increased presence of abnormal sperm within 2–3 weeks following induction of heat-stress exposure (Cameron and Blackshaw 1980). Therefore, the boar is obviously an important part of a successful summer reproductive management plan, and efforts to minimise the negative effects of heat stress on male fecundity are imperative.

Heat stress compromises female fertility

Heat stress decreases fertility in sows and gilts that is typically manifested during seasonal infertility (Love 1978; Prunier *et al*. 1994). Increased ambient temperature lowers farrowing rates and is postulated to delay the onset of puberty (Bertoldo *et al*. 2009). Tolerance to heat stress and heat stress-induced exacerbation of infertility appears to occur differentially in different genetic lines. For example, Bloemhof and colleagues (2008) demonstrated that sows selected for increased farrowing rate were more sensitive to heat stress (i.e. reduced litter size and total number born) and that farrowing rate per first insemination was compromised compared with those lines not selected for increased farrowing rate (Bloemhof *et al*. 2008). How exactly heat impacts female fertility is not well understood, although it most likely involves compromised production of gametes and embryos capable of development. Heat stress has been associated with reduced developmental competence, and induction of apoptosis in *in vitro* fertilised and parthenogenetically activated pig embryos (Isom *et al*. 2007*b*; Bertoldo *et al*. 2010; Pennarossa *et al*. 2012). Although the heat shock protein (HSP) machinery is constitutively expressed in the somatic cells of the ovary, there is only a change in abundance in the oocyte in response to whole-ovary heat stress (Pennarossa *et al*. 2012), suggesting that regulation in response to hyperthermia may occur within the porcine oocyte.

Heat stress during oocyte and embryo development impairs reproductive success

An inability to maintain a healthy body temperature has significant implications for the production of gametes capable of yielding developmentally competent embryos. Ewes exposed to heat stress before oestrus or after insemination have suppressed reproductive ability, including reduced ability to demonstrate behavioural oestrus despite ovulation and suppressed pregnancy rates (Sawyer 1979*a*, 1979*b*; Sawyer *et al*. 1979). Interestingly, it appears a sizeable portion of seasonal infertility could be explained by the thermal sensitivity of early stage embryos, as early stage bovine embryos are unable to mount an effective and sufficient heat shock response (Sakatani *et al*. 2012). Consequently, early stage embryos are incredibly sensitive to subtle increases in body temperature. *In vitro* oocyte maturation mimics the *in vivo* oocyte development that occurs during late proestrus and during the first part of behavioural oestrus before ovulation, concomitant with the first service, as ovulation occurs at ~55–60% of the way through the behavioural oestrus (Soede and Kemp 1997). We have developed an *in vitro* oocyte maturation model to investigate effects of heat stress on oocyte

development. Oocytes subjected to heat stress during *in vitro* maturation have an impaired ability to survive beyond the 4-cell stage of development, despite being fertilised and cultured in thermal neutral conditions (E. C. Wright and J. W. Ross, unpubl. data).

The impact of heat stress during oocyte maturation and early embryonic development is evidenced in that sows exposed to hyperthermia for 5 days following breeding have a significantly reduced number of viable embryos after Day 27 of gestation, with control pigs possessing an average of 11.0 (68.8% survival) viable embryos and heat-stressed sows containing only 6.8 (39.1% survival) viable embryos (Tompkins *et al*. 1967). In this study, heat stress was administered following breeding, which generally occurs before ovulation and complete oocyte maturation, as pigs typically ovulate in the mid to latter half of oestrus (Soede and Kemp 1997). The severity of negative effects of heat stress during pregnancy in pigs appears to depend on the stage of gestation. Omtvedt *et al*. (1971) demonstrated this by exposing pregnant gilts to heat stress for 8 days during different stages of gestation. Heat stress (37.8°C for 17 h and 32.2°C for 7 h) beginning either on Day 0 or Day 8 of gestation compared with thermal neutral conditions (constant 23.3°C) reduced the number of viable embryos by Day 30 of gestation. Interestingly, the same heat-stress conditions administered on Days 53–61 did not affect farrowing performance whereas heat stress during late gestation (Days 102–110) resulted in a significantly increased number of dead piglets born and a four-piglet reduction in the number born alive (Omtvedt *et al*. 1971). However, a more moderate cyclic heat stress, on bred gilts beginning on Day 3 and extended to either Days 24 or 30 of gestation, did not impact embryo survival (Liao and Veum 1994). Thus, there are specific reproductive stages that are sensitive to heat stress and identifying those phases and mechanisms involved are of both academic and practical interest.

Due to the difficulty for such studies *in vivo*, characterisation of heat-stress effects during oocyte growth and maturation and early embryonic development in pigs has been demonstrated using *in vitro* oocyte maturation and embryo culture systems. Some evidence of *in vitro* heat-stress models during the transition between germinal vesicle breakdown and the 4-cell stage of development demonstrates the susceptibility of this stage to heat stress. Culture of pig embryos at 42°C for 9 h following porcine *in vitro* fertilisation significantly reduced blastocyst formation rate (Isom *et al*. 2007*a*), and heat shock of 41.5°C following *in vitro* maturation also reduced oocyte development (Tseng *et al*. 2006). The impact of *in vitro* heat stress during oocyte maturation and its impact on subsequent developmental competency have also been demonstrated. Oocytes exposed to heat stress (41°C) for the first half (21 h) or the duration of (42–44 h) of *in vitro* maturation demonstrated impaired ability to reach metaphase II arrest whereas heat stress during only the second half (21 h) of *in vitro* maturation did not impact maturation rate (E. C. Wright and J. W. Ross, unpubl. data). Metaphase II arrested oocytes following heat stress during *in vitro* maturation demonstrated impaired developmental competency compared with oocytes matured at 39°C, as measured by their ability to develop to the blastocyst stage following *in vitro* fertilisation and culture in thermal neutral conditions. This model has subsequently been used to demonstrate differences in gene expression in developing 4- to 8-cell embryos as a result of heat-stress conditions during *in vitro* maturation (E. C. Wright and J. W. Ross, unpubl. data).

LPS-induced signalling influence on ovarian function

As already mentioned, intestinal integrity is compromised by heat stress and is associated with increased circulating endotoxin. Elevated endotoxin may be a mechanism through which heat stress compromises ovarian function. From a reproductive perspective, LPS-induced poor fecundity is a phenomena reported throughout the literature (as summarised in Fig. 1). Interestingly, follicular fluid that surrounds and nourishes the maturing oocyte contains LPS levels reflective of the systemic circulation. Thus, LPS appears to reach the ovary via the systemic circulation having the ability to directly interact with the oocyte (Herath *et al*. 2007). In cattle, LPS contact with ovarian cortical explants was associated with reductions in the number of primordial follicles simultaneous with increased atresia of the ovarian reserve (Bromfield and Sheldon 2013). In rodents, LPS exposure *in vivo* also reduced primordial follicle number, potentially mediated via Toll-like receptor 4 signalling as Toll-like receptor 4 null mice were resistant to LPS-mediated primordial follicle depletion (Bromfield and Sheldon 2013). In addition to compromising the follicular pool, LPS alters anterior pituitary hormone secretion. In anestrous ewes, LPS infusion suppressed luteinising hormone release while having a stimulatory effect on prolactin and cortisol levels. Furthermore, mRNA for luteinising hormone and luteinising hormone receptor was suppressed by LPS infusion, although follicle stimulating hormone (FSH) and FSH receptor as well as prolactin and prolactin receptor genes were elevated (Herman *et al*. 2010).

Elevated insulin alters ovarian function

Elevated insulin secretion has repeatedly been observed in response to heat stress and hyperinsulinemia likely alters ovarian function. One mechanism is through insulin's ability to activate phosphatidylinsoitol-3 kinase pathways (Kasuga 1996), which in turn can regulate oocyte recruitment and activation. During specific physiological states such as polycystic ovary syndrome and obesity, where insulin levels are elevated, associated reproductive problems including reduced fecundity and increased pregnancy loss also exist. Nteeba *et al.* (2015) demonstrated increased expression of

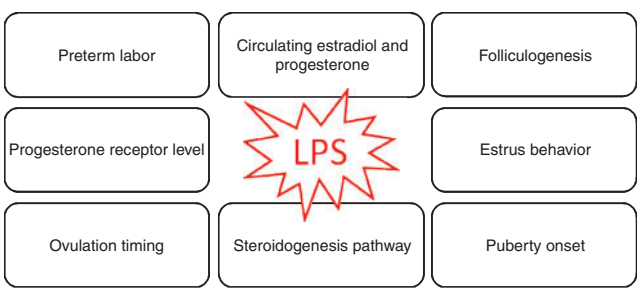

Fig. 1. Summary of reproductive areas that can be impacted by lipopolysaccharide (LPS) exposure. Based upon a wide variety of literature sources from different species, LPS has been demonstrated to induced pre-term labour, affect gene expression, impact ovarian sex steroid hormone production, and influence puberty onset. Many of these phenotypic consequences of ovarian LPS exposures are observed in heat-stressed sows and gilts.

the gene encoding the insulin receptor in ovaries of gilts exposed to heat stress, indicating ovarian sensitivity to increased insulin during heat-stress conditions. Additionally, heat stress increased genes encoding the ovarian steroidogenic enzymes, suggesting the potential for altered oestradiol synthesis during heat-stress conditions. Furthermore, the composition of follicular fluid, essential for providing an important microenvironment for maintaining oocyte competency, can also be altered by environmental conditions (Gosden *et al*. 1988; Fortune 1994). It is our postulate that elevated insulin signalling during heat stress in female pigs is associated with insulin-induced altered signalling in the ovary that compromises the production of viable oocyte capable of fertilisation and full-term development.

Autophagy-induced signalling in the ovary

Autophagy is emerging as an additional mechanism through which heat stress alters cellular function. Autophagy is the process by which somatic cells recycle energy through the reutilisation of cellular components, and is activated in somatic cells by a variety of stressors. There are three major types of autophagy: chaperone-mediated autophagy, microautophagy, and macroautophagy. Macroautophagy accounts for the largest amount of energy reacquisition of the three different types (Klionsky 2005). Autophagy is the sequestration of cytoplasm into a double-membraned cytosolic vesicle, the autophagosome, which fuses with a lysosome to form an autolysosome for degradation by lysosomal hydrolases (Klionsky and Emr 2000). The steps of autophagy can be broken down into induction, autophagosome formation, autophagosome-lysosome fusion, and degradation (Pyo *et al*. 2012). These processes are marked by the formation of large protein complexes, and much of the regulation occurs at the post-translational level (Mizushima 2010; Mizushima *et al*. 2011). Both basal and stress-induced autophagy have been observed in the embryo and oocyte. Deficiencies in autophagy-related genes negatively affect both early- and late-stage embryonic development (Zeng *et al*. 2006; Fimia *et al*. 2007; Qu *et al*. 2007; Cecconi *et al*. 2008). Embryos also respond to external stressors by the induction of autophagy (Adastra *et al*. 2011; Xu *et al*. 2011). In the oocyte, autophagy-related gene 5 knockout mice fail to develop past the 4-cell embryo stage (Tsukamoto *et al*. 2008). Furthermore, microtubule-associated protein 1 light chain 3 (LC3)-II, an autophagy marker, is detectable during initial culture of pig oocytes (Lee *et al*. 2014), and the autophagy protein, Beclin1 (BECN1), has been observed in the mouse oocyte (De Felici *et al*. 2008).

It has been well documented that regulation between autophagy and apoptosis is highly coordinated (Mukhopadhyay *et al*. 2014). In this way, B-cell lymphoma 2 (BCL2) is known to interact with both BECN1 and Bax, and inhibit the activation of both proteins. Initial phosphorylation of BCL2 decreases its interaction with BECN, therefore activating BECN and inducing autophagy (Pattingre *et al*. 2005). Though the mechanism is not well understood, it is hypothesised that BCL2 can be hyper-phosphorylated, causing it to no longer interact with Bax and induce apoptosis (Oltval *et al*. 1993; Ling *et al*. 1998). This has led investigators to hypothesise a model where different levels of stress affect the level of phosphorylation of BCL2 and act as a regulator between autophagy and apoptosis in response to an increasing gradient of environmental stress (Levine *et al*. 2008; Mukhopadhyay *et al*. 2014).

To investigate the effects of heat stress-induced autophagy signalling in the pig ovary, synchronised follicular development in a group of gilts occurred using Matrix (DPT Laboratories, San Antonio, TX, USA), for which the active component, altrenogest, functions as a progesterone receptor agonist. For 5 days following Matrix withdrawal, during follicular development, gilts were subjected to cyclical heat stress or thermal neutral conditions. After 124 h of cyclical heat stress and the emergence of the dominant follicle pool, gilts were sacrificed and whole ovaries collected. Using whole-ovary extract, it was determined that cyclical heat stress increased the abundance of BECN1 and cleavage of LC3-I to LC3-II (B. J. Hale and J. W. Ross, unpubl. data), key markers of autophagy induction. The protein BECN1 has a known role in autophagosome formation and its ability to react to a wide variety of regulators (Liang *et al*. 1998; Wurmser *et al*. 1999; Funderburk *et al*. 2010; Pyo *et al*. 2012) whereas LC3-II is a key component of the conjugative system necessary for the expansion of the autophagosome membrane. Interestingly, it has also been shown that the increase of BECN1 abundance correlates with an increase in phosphorylation of BCL2 at the threonine 56 (B. J. Hale and J. W. Ross, unpubl. data).

Epigenetics of heat stress

Epigenetics is translated to mean ‘above the genome’, and is the study of DNA modifications outside of base-pair sequence information that are capable of impacting gene expression. Multiple modifications, such as DNA methylation, histone regulation, chromatin state, and miRNA expression, to name a few, all have the ability to impact the epigenetic code. Importantly, environmental influences occurring both pre-natally and post-natally can influence cell specific imprinting of the epigenetic profile and result in altered responses to environmental conditions or stimuli.

Epigenetic mechanisms

DNA modification via methylation is a stable DNA modification that can be either inherited or acquired throughout an animal’s lifetime, although is very dynamically regulated during early development (Rivera and Ross 2013). Methylation of DNA is achieved through the enzymatic actions of DNA methyltransferases capable of transferring a methyl group from the methyl donor, S-adenosyl methionine, commonly to the cytosine of CpG dinucleotides located upstream of gene promoters. Hyper-methylation of these CpG islands in promoter regions of genes will most often result in suppression of gene expression through recruitment of DNA-binding proteins capable of interfering with transcription factor function. Alternatively, methylation of DNA in non-CpG can result in more variable regulation patterns. *De novo* or maintenance methylation is accomplished via two classes of DNA methyltransferases (Klose and Bird 2006). Copying existing methylation patterns during replication and development is referred to as maintenance methylation whereas *de novo* methylation introduces new methylation patterns. Induction of *de novo* methylation in response to environmental stress is one potential mechanism for observations of an imprinted response to stress.

Developmental imprinting

Following fertilisation, both parental genomes experience global demethylation producing a single cell totipotent zygote. After several rounds of holoblastic cleavages, the embryonic cells undergo dynamic reorganisation and genetically identical cells begin differentiation leading them towards specific cell lineages (Reik 2007). The mechanism controlling the molecular programming of these cells is not well understood but is thought to be largely attributed to differences in DNA methylation of CpG islands as well as alterations in histone methylation and acetylation patterns (Kelly and Trasler 2004). Continued differentiation and programming of cells during pre-natal development is largely the result of continued modifications of DNA methylation patterns. During cellular differentiation, some epigenetic modifications can be changed whereas others persist, such is the basis of numerous disease statuses as the result of abnormal epigenetic imprints. Functionally, epigenetic modifications can improve the plasticity of the genome to improve responses to specific environmental conditions or can permanently impair an individual's ability to cope or respond to particular environmental conditions. Classical examples of gestational imprinting have been observed in multiple species. One example is the lifelong consequences of humans exposed to the Dutch famine *in utero* (Roseboom *et al*. 2006). Individuals exposed to the famine *in utero* demonstrate lifelong consequences as a result of the intrauterine environment created by the famine with exposure during the first trimester having significant impairments with respect to metabolic syndrome (Roseboom *et al*. 2006). More recently, the metabolic alterations in response to the famine incurred during gestation have been demonstrated to be in part due to altered DNA methylation profiles (Tobi *et al*. 2014).

Epigenetic programming due to maternal stress

In addition to decreased fertility, pre-natal stress can also result in intrauterine growth retardation (IUGR) that may result in further losses as piglets born during periods of maternal stress have diminished performance in post-natal life. In agricultural species, the post-natal effects of pre-natal stresses are still being characterised. The majority of research on epigenetic regulation in response to maternal stressors has been conducted in pigs and sheep. Although there is little direct evidence suggesting that heat stress *in utero* confers an epigenetically mediated response to heat in later life, there is ample evidence of stress-induced epigenetic changes in these species.

A variety of intrauterine events can have extensive and permanent effects on post-natal pig performance (Foxcroft *et al*. 2009). Experimental models, such as IUGR, indicate growth/development and lifetime piglet performance is impaired and is associated with alterations in skeletal muscle phenotype (Bee 2004; Foxcroft *et al*. 2006, 2009; Cerisuelo *et al*. 2009). In pigs, IUGR has lasting effects on growth potential and carcass quality (Foxcroft *et al*. 2006, 2009). In addition to IUGR, maternal diet has been demonstrated to impact on post-natal performance of piglets. Supplementing gestating sow diets with omega 3 fatty acids improved glucose uptake in offspring (Gabler *et al*. 2009). Moreover, maternal stress may also result in an imprint on immune response. Exposure to maternal restraint stress, for example, caused a greater inflammatory response to endotoxin challenge in offspring (Collier *et al*. 2011).

Similar to IUGR, metabolic imprinting describes a lasting epigenetic imprint in response to the pre-natal metabolic environment (Waterland and Garza 1999). This imprint is characterised by changes in organ and tissue structure, cell number, and differentiation. Csaba *et al*. (1984) demonstrated the phenomenon of metabolic imprinting by injecting parental rats with insulin, which in turn altered the insulin-binding response in the offspring in a sex-dependent manner. Metabolic and hormonal imprinting has since been demonstrated through the study of obesity-prone offspring born from diabetic mothers in rodents and humans (Poston 2011).

Epigenetic programming and hormonal changes in IUGR animals are somewhat similar to that observed in animals exposed to pre-natal heat stress. This change is likely a result of alterations in metabolism, uterine blood flow, and reproduction caused by heat stress. The effects of maternal heat stress may alter a variety of physiological parameters in offspring later in life. For example, in mice and guinea-pigs, pre-natal exposure to heat stress resulted in reduced post-natal weight gain and smaller brain weights that lasted into maturation (Jonson *et al*. 1976; Shiota and Kayamura 1989). The cause of suboptimal post-natal performance in mice exposed to pre-natal heat stress has been suggested to be due to interference with establishment of the hypothalamic-pituitary-axis. In mouse embryos, a gene-specific DNA methylation imprint of heat was observed following heat stress (Zhu *et al*. 2008).

Epigenetic programming in response to heat stress

In some species, epigenetic conditioning following exposure to heat results in thermal tolerance to heat stress later in life. This has been demonstrated in chickens where heat stress at 3 days of age resulted in protection against acute heat stress-related mortality during adulthood (Yahav and McMurtry 2001), and is thought to be mediated by modifications to the histone code thereby enabling an epigenetic memory (Kisliouk *et al*. 2010). Chicks acutely heat stressed 3 days post-hatching and again 1 week later had increased H3K9 acetylation and H3K9 dimethylation. Chronic heat stress has also been reported to alter histone modifications in rodents resulting in elevated expression of HSP-70 and increased HSP-90 protein in response to heat acclimation and re-acclimation (Tetievsky and Horowitz 2010). Additionally, mice fibroblasts exposed to a conditioning heat load had improved survival in response to what would normally be a lethal heat load (Luft *et al*. 2001).

How pre-natal heat stress exposure impacts post-natal performance is poorly understood but is likely a result of epigenetic programming. Epigenetic programming is significantly influenced by differences in DNA methylation of CpG islands (Klose and Bird 2006), that when negatively impacted during development can have lasting implications on gene expression (Bernal and Jirtle 2010) and is thought to occur in pigs having a potentially significant impact on lifetime production (Foxcroft *et al*. 2009). These epigenetic modifications in chromatin structure, (which can last short periods or lifelong) influence the condensation of the DNA reducing the recruitment and ability of DNA-binding proteins, such as RNA polymerases, to interact with and transcribe

genes from the genome. Intrauterine modifications via DNA methylation can occur in temporal- and tissue-specific manners (Schneider *et al*. 2010). Because the biological underpinning of the pig heat-stress response involves the coordinated interactions between adipose, hepatic and muscle tissues, an objective is to understand which molecular responses in these tissues are associated with pre-natal epigenetic programming through DNA methylation.

Gestational heat stress in pigs impacts the offspring

Boddicker *et al*. (2014) tested the hypothesis that *in utero*, heat-stress exposure alters future piglet performance through some measure of epigenetic imprinting. Interestingly, gilts exposed to heat stress during the first half of gestation produced piglets that tended to have greater back-fat depth at 12 weeks of age and had increased circulating insulin at 19 weeks of age. This study was replicated and examined the growth performance of pigs produced from dams exposed to heat-stress conditions for the entire length of gestation. Interestingly, during the lipid accretion phase of growth (60–90 kg), exposure to heat stress *in utero* resulted in a propensity to accrete adipose tissue more efficiently than piglets gestated in dams in thermal neutral conditions (Johnson *et al*. 2015*c*), although this was not the case with piglets during the lean tissue accretion phase (30–60 kg) (Johnson *et al*. 2015*b*). In addition to gestational heat stress repartitioning nutrient priorities during offspring growth and development, it also appears that *in utero* exposure to heat stress impacts thermoregulatory capabilities of offspring (Johnson *et al*. 2013). In particular, post-natal pigs exposed to heat stress *in utero* have an increased body temperature (~0.3°C) during both thermal neutral and heat-stress conditions (Johnson *et al*. 2013; J. S. Johnson and L. H. Baumgard unpubl. data). This small difference in body temperature could have large implications on feed efficiency if this due to increased basal heat production, as this thermal energy would have been derived from feed energy (Johnson *et al*. 2015*a*). To investigate the mechanisms by which these phenotypes (body composition and body temperature) occur, Boddicker *et al*. (2015) utilised RNA sequencing on adipose tissue, liver and *M. longissimus dorsi*, and demonstrated differential expression of hundreds of mRNA transcripts as a result of exposure to heat-stress conditions *in utero*.

Conclusions

Genetic selection for rapid, lean tissue accretion and fecundity in the swine industry is associated with an increased susceptibility to heat-induced suppression of production efficiency. These production losses primarily occur through compromised production during the finishing phase and through seasonally compromised losses in reproductive efficiency. Epigenetic imprinting during *in utero* exposure to heat stress is another mechanism through which heat stress compromises swine production. Offspring from gilts exposed to heat stress during gestation had increased insulin levels, fatter carcasses, and had increased body temperature during post-natal life. These altered phenotypes represent an underappreciated consequence to heat stress, and combined with the well documented effects of heat stress on growth and reproduction, create a massive impediment to efficient global pork production.

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Nutritional strategies to alleviate heat stress in pigs

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Abstract. Pigs are comparatively less heat tolerant than other species of production animals, which poses challenges for stock productivity and management during seasonal heat waves that occur in summer. The issues surrounding heat and pig production are predicted to increase, based on the actions of climate change increasing the intensity, frequency and duration of heat waves. Furthermore, future growth areas of pig production are going to be in tropical regions such as South-east Asia and Latin America. Efforts by the pig to dissipate excess body heat come at a cost to health and divert energy away from growth, compromising efficient pig production. Management of heat stress requires multiple strategies, and recent research is improving the understanding of the application of nutritional strategies to ameliorate the effects of heat stress. In particular the use of feed additives is an important, flexible and economical method to alleviate heat stress and the intensive nature of pig production lends itself to the use of additives. Some specific examples include antioxidants, betaine and chromium, which have been proved effective or being tested in mitigating some certain impacts of heat stress in pigs. The aim of this review is to summarise recent advances in the nutritional management of heat stress in pigs.

Additional keywords: hyperthermia, nutrition.

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Introduction

Heat stress (HS) causes tremendous economic loss to the global pig industry. For example, in 2003 in the US the annual economic loss in sow productivity alone was estimated to be $US113 million (St-Pierre *et al*. 2003). These estimates were calculated from increased days open due to HS. For growing and finishing pigs, the loss is almost twice as great largely as a result of lowered growth rate and increased mortality due to HS. A survey in the publication St-Pierre *et al*. (2003) showed that in North Carolina and Iowa the reduction in growth rate was ~2.0 and 2.7 kg per head and mortalities due to heat waves were 1.6 and 1.1 per 1000 pigs, respectively. The economic loss of piglets caused by HS has not been calculated. Young pigs do not tend to be directly affected by HS as much as larger pigs by virtue of their smaller size and higher zone of thermal comfort. Instead the effects of HS on the piglet tend to be indirect, principally through reductions in sow milk production (Black *et al*. 1993). When forecasts for increases in global temperature, increased expansion of tropical pig production and selection for more highly productive genotypes that produce greater metabolic heat (Brown-Brandl *et al*. 2004) are factored in, it is clear that HS is not only a current but an emerging issue for pig production.

Mitigation strategies against HS can be achieved through various means. Existing heat abatement technologies such as improved shade, ventilation and spray cooling will remain cornerstones of any piggery. However, improvements in the accuracy of weather forecasts are allowing the development of pre-emptive strategies based on feed additives and nutritional modification. Improved weather forecasting will allow piggeries to be better informed and prepared as to the timing and severity of heat events, allowing for more targeted delivery of summer rations to alleviate HS.

Combating the effects of HS is metabolically expensive for production animals. Existing NRC guidelines are based upon requirements for temperatures below 30°C, and the NRC arbitrarily states that the maintenance requirements could be increased by 7–25% during HS conditions (Baumgard and Rhoads 2013). Therefore, increased energy and micronutrient utilisation coupled with reduced feed intake during HS can induce micronutrient deficiencies and altered metabolism, culminating in poorer growth performance. Pig production is globally competitive industry and the ability to manage HS in pig herds will increasingly be a driver for profitability. Some economically viable nutritional strategies to mitigate HS as it

pertains to dairy and beef production have been reviewed elsewhere (Dunshea *et al*. 2013; DiGiacomo *et al*. 2014), and the aim of this review is to investigate recent advances in the field of nutritional interventions for ameliorating HS in pigs.

Physiology of heat stress in pigs

Body heat is produced by metabolism and can also be gained from the environment; in order to maintain a constant body temperature any excess heat must be dissipated. The primary mechanisms by which this occurs are through radiant and evaporative heat loss. Radiant heat loss is achieved by the redistribution of blood flow from the body core to the periphery, allowing for the dissipation of excess heat to the atmosphere. As pigs lack functioning sweat glands evaporative heat loss is achieved by a combination of panting (Fig. 1) and drooling. Under conditions of high or extended heat load pigs may enter a 'heat-stressed' state where heat dissipation is insufficient, resulting in elevated core body temperature, thermal injury and if unchecked progress to heat stroke. The consequences of HS on swine production include reduced feed intake, growth rate, increased mortality and in sows, reduced fertility, lactation weight loss, milk yield and litter weight gain (Fig. 2; Verstegen *et al*. 1973; Hall *et al*. 2001; Baumgard and Rhoads 2013).

The process of redistributing blood flow requires a commensurate reduction in blood flow to other organs or tissues. During HS, blood flow is redistributed away from the splanchnic bed, that is, away from the gastrointestinal tract (GIT) (Bell 1993; Hall *et al*. 2001). The energy expenditure of the GIT is disproportionately high, comprising only 5% of bodyweight yet accounting for 25% of whole-body O_2 consumption (Yen *et al*. 1989; Yen 1997). When hepatic O_2 consumption is factored in this contribution may be as high as 50%, and this predisposes visceral organs to hypoxia when blood perfusion is restricted. As demonstrated by Hall *et al*. (1999) in a murine model, increasing core temperature from 37°C to 41°C increased the incorporation of [^{3}H]-misonidazole by 29% in the intestine and 80% in the liver. Misonidazole undergoes bio-reduction and is then retained within hypoxic cells, and therefore this experiment underscores not only the relative degree of hypoxia but intestinal and hepatic free radical generation during HS. Increased hypoxia and free radical damage then results in increased permeability to macromolecules such as endotoxins (Pearce *et al*. 2012, 2013*b*), inflammation (Lambert 2009) and villus damage. As the villus is the digestive apparatus of the intestine, HS can reduce nutrient digestion and absorption. For example, Kiefer *et al*. (2012) reported exposure to 31°C caused an increase in faecal protein, phosphorus, calcium and some trace element excretion in 45-kg barrows. Similarly, 14 days of 30°C heat exposure reduced nitrogen retention in pigs (Brestenský *et al*. 2012). Therefore, HS can compromise digestive function and limit nutrient absorption and retention and precipitate endotoxaemia due to reduced barrier function (reviewed in more detail by Gabler and Pearce 2015).

The elevated respiration rate associated with panting and evaporative heat loss increases blood pO_2 and reduces pCO_2. In turn, this results in reduced buffering of CO_2 with bicarbonate and an increase in blood pH, which can result in respiratory alkalosis. As the normal response to reduce respiratory alkalosis is to lower respiration rate, HS places a premium on thermal dissipation at a cost to acid-base balance. HS results in increased renal buffering via H^+ excretion and urine acidification (Patience *et al*. 2005), increasing maintenance energy expenditure (Baumgard and Rhoads 2013).

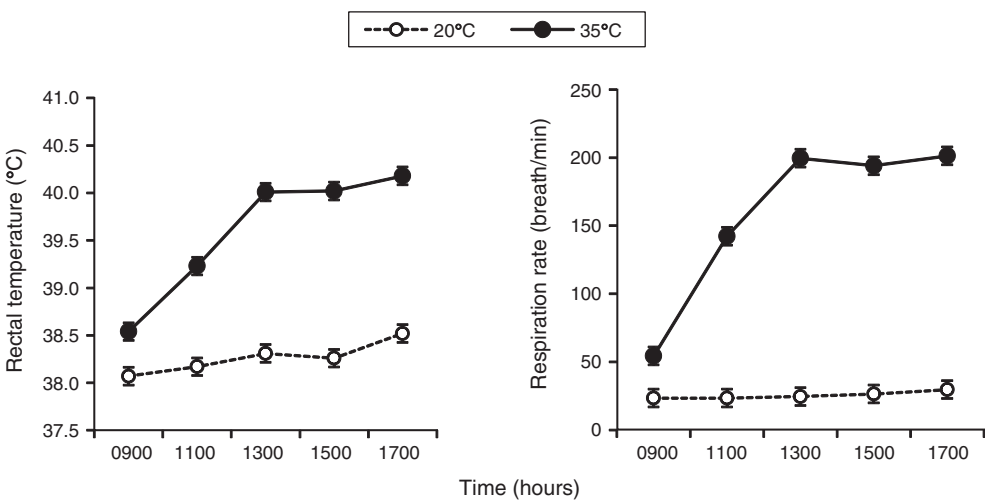

Fig. 1. Rectal temperature and respiration rate of growing pigs exposed to either a thermoneutral condition (20°C, relative humidity 35%) or a cyclic heat stress condition (35°C, 0900–1700 hours, 28°C overnight, relative humidity 30–35%) in climatic controlled rooms. To mimic the summer environment, the heat stress room started to increase room temperature from 28°C at 0900 hours and reached 35°C at 1000 hours, then maintained at 35°C until 1700 hours. Thereafter, room temperature started to drop to 28°C in the heat stress room. Physiological measurements were taken at five time points for two consecutive days. Data were analysed by linear mixed model with fixed effects including temperature, time and day. Pigs were used as a random effect. The plots were expressed as mean of temperature × time and s.e.d. ($n = 24$). The P-values for effects of temperature, time and their interaction are all <0.001 (F. Liu, unpubl. data).

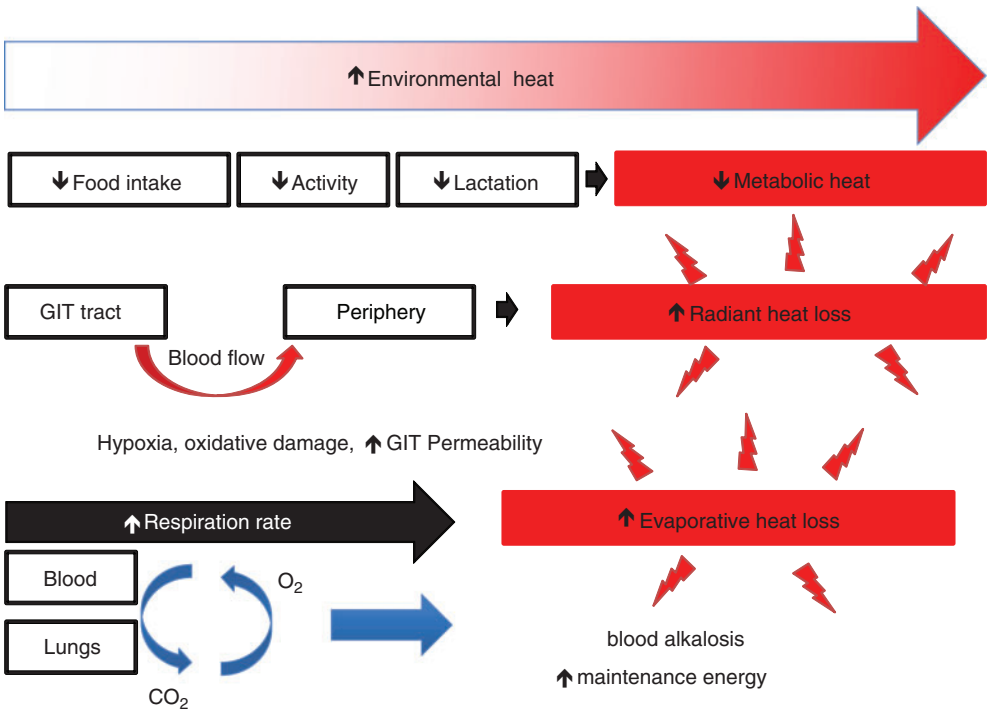

Fig. 2. Overview of heat management in the pig. To increase thermal dissipation during heat stress pigs reduce activities that produce metabolic heat, and increase radiant and evaporative heat loss. The resultant shift in the physiological and metabolic status of the pig places a premium on heat dissipation at the expense of processes beneficial for efficient pig production.

Influence of heat stress on porcine metabolism

The normal response to reduced feed intake is to spare glucose and increase fat mobilisation. During HS pigs reduce feed intake in an effort to reduce metabolic heat production (Fuller 1965; Peng and Heitman 1974). Counter-intuitively, HS increases fatness in pigs (Stahly *et al*. 1979; Christon 1988; Katsumata *et al*. 1996) due to reduced lipid mobilisation, as evidenced by lower circulating non-esterified fatty acid (NEFA) concentrations compared with pair-fed pigs housed in thermoneutral conditions (Kouba *et al*. 2001; Pearce *et al*. 2013*a*). As plasma NEFA concentrations are highly correlated with lipid mobilisation in pigs (Dunshea *et al*. 1992), these experiments imply that lipid mobilisation is attenuated by HS, even when feed intake is inadequate or after lipolytic challenges with adrenaline or clenbuterol (Katsumata *et al*. 1990; Sanz Fernandez *et al*. 2015*a*). Moreover, in order to compensate for inadequate energy availability for maintenance or glucose production, muscle proteins and amino acids are catabolised. In other words, heat-stressed animals lack the ability to mobilise fat as an energy source.

One putative explanation for the attenuated lipid mobilisation is the upregulation of insulin secretion. Insulin is an anti-lipolytic hormone and increased responses during glucose tolerance tests have been observed in heat-stressed ruminants (O'Brien *et al*. 2010; Wheelock *et al*. 2010; Baumgard *et al*. 2011; Baumgard and Rhoads 2012). However, investigations into the effect of HS on insulin sensitivity have proved inconsistent between ruminants and pigs. HS has been observed to increase basal insulin concentrations, implying reduced insulin sensitivity (Pearce *et al*. 2013*a*). However, in another study, a lower insulin response to a glucose challenge was observed despite increased concentrations of C-peptide (Sanz Fernandez *et al*. 2015*a*). In another study no change was observed (Williams *et al*. 2013). Recently, Sanz Fernandez *et al*. (2015*b*) investigated these conflicting results in more detail using a euglycaemic clamp model for measuring insulin sensitivity. This model demonstrated the normal homeorhetic response to spare glucose utilisation by reducing insulin sensitivity in pair-fed thermoneutral pigs compared with *ad libitum*-fed pigs. However, HS pigs retained insulin sensitivity at *ad libitum*-fed levels despite a 30% reduction in feed intake, demonstrating that HS increased insulin sensitivity in pigs.

Without substantial lipid mobilisation during HS, pigs must rely increasingly on carbohydrate utilisation. Although plasma lactate concentrations increase in humans exercising under hot conditions (Fink *et al*. 1975; Febbraio 2001) and in pigs during hyperthermia (Hall *et al*. 1980), there is no overt increase in pigs with HS (F. Liu, unpubl. data). In other species, HS increases muscle glycogenolysis (Febbraio *et al*. 1994) and gluconeogenesis. Also, hepatic glucose release is increased in the athletes during HS (Hargreaves *et al*. 1996), even after carbohydrate ingestion (Angus *et al*. 2000).

In summary, under high environmental heat load pigs reduce feed intake to minimise metabolic heat production. The primary mechanisms of excess heat dissipation are via blood flow redistribution to the periphery for radiant heat loss and increased respiration and drooling for evaporative loss. The cost to the pig of these heat management strategies is the propensity to predispose the GIT and liver to ischaemic damage, and may be mediated in

part through oxidative damage. This reduces barrier and digestive function and influences hepatic metabolism. Increased respiration can trigger respiratory alkalosis, which increases maintenance energy and impair acid-base balance. A key component of HS is an increase in insulin sensitivity and reduction in lipid metabolism, resulting in pigs favouring carbohydrate metabolism, counter to the normal homeorhetic response to reduced feed intake.

Overview of nutritional strategies to alleviate heat stress

Nutritional strategies to manage HS include manipulating the amount of protein and fibre in the diet. Increasing dietary fibre has been observed to reduce the bodyweight of heat-stressed sows (Renaudeau *et al*. 2003) and increased dietary fat improves grower and finisher weight gain during hot periods (Spencer *et al*. 2005). High protein diets generate a greater post-prandial thermogenic response and so lowering the protein content of the diet may provide an option during HS (Dunshea *et al*. 2007). With regard to feeding a low crude protein diet (12% vs 16%), pigs showed reduced growth performance and carcass fattening, but supplementing lysine, tryptophan, and threonine in a low crude protein (12%) diet could reduce heat production without detrimental effects on body fat composition and growth performance during HS (Kerr *et al*. 2003). Although metabolic heat production is essential for thermogenesis, high protein and fibre diets that have a higher heat increment than fat and starch are not recommended for summer rations (Patience *et al*. 2015).

Nutritional strategies based on ingredients and nutrients that ameliorate specific physiological responses to HS offer important intervention approaches for the pig producer. Given the physiological difference in heat-stressed pigs, a summer ration should be tailored to satisfy the pig's requirement during periods of elevated temperature. The most recent edition of 'Nutrient Requirements of Swine' released in 2012 includes software which features a function to adjust the nutrient requirements of pigs as the temperature increases up to a limit of 30°C. However, this function is mainly developed to compensate for reduced nutrient intake by predicting the reduction of the feed intake at a given temperature. Admittedly, the reduction in average daily feed intake (ADFI) is probably the biggest factor, which decreases the productive performance of pigs during HS. However, a series of recent pair-feeding experiments in pigs have indicated that the impacts of the HS on intestinal barrier integrity, nutrient digestion and transport (Pearce *et al*. 2013*b*), and metabolism (Pearce *et al*. 2013*a*), are not solely due to the reduction in ADFI, but also that HS itself can directly alter these parameters. Therefore, the ideal nutritional requirements during HS are still to be elucidated and can only be achieved by understanding the impacts of HS at the cellular, tissue, organ and whole-body levels. Based on the physiological responses outlined above, it is possible to highlight some functional feed additives including antioxidants, chromium (Cr) and betaine, which have either proved effective or present a logical basis for being investigated for their capacity to mitigate HS in pigs.

Dietary antioxidants

The link between HS and oxidative stress (OS) has been established in dairy cows (Bernabucci *et al*. 2002), sheep (Chauhan *et al*. 2014*a*, 2014*b*), poultry (Mujahid *et al*. 2005; Mujahid 2011) and pigs (Montilla *et al*. 2013, 2014; Liu *et al*. 2015*a*). Oxidative stress arises because of an imbalance between reactive oxidant species (ROS) production and antioxidant capacity, and supplementation with antioxidants during HS has been used as a strategy to alleviate oxidative damage. Generally, antioxidants can be classified as non-enzymatic antioxidants and antioxidant enzymes that synergistically form an antioxidant defence system. The non-enzymatic antioxidants are the group of compounds that poses reducing ability such as uric acid, glutathione, vitamin E (Vit. E), vitamin C, and polyphenols. Antioxidant enzymes are a group of enzymes that can neutralise free radicals or their metabolites and include catalase, superoxide dismutase, and glutathione peroxidase. The application of various antioxidants have been extensively studied in poultry but not sufficiently reported in pigs, although supplementing antioxidants to reduce HS is a theoretically feasible method of alleviating HS.

Recently, this laboratory has investigated the actions of Vit. E and selenium (Se) in heat-stressed pigs, as they are essential micronutrients for animal health and production (McDowell *et al*. 1996; Hefnawy and Tortora-Perez 2010; Suttle 2010; Willshire and Payne 2011; Chauhan *et al*. 2014*c*). Metabolic functions of Se and Vit. E are closely linked and they act synergistically. Vit. E is the most lipid-soluble antioxidant, which stops pro-oxidants' propagation, which very efficiently can scavenge ROS and lipid hydroperoxides converting them into non-reactive forms (Hidiroglou *et al*. 1992). Alternatively, Se is an important core component of glutathione peroxidase (Rotruck *et al*. 1973) and protects against oxidative damage by catalysing the reduction of H_2O_2 to water (along with the oxidation of glutathione) and similar reduction of lipid hydroperoxides. Thus, the major function of both Se and Vit. E is to prevent the oxidative damage of biological membranes by neutralising oxidants or free radicals. When the generation of free radicals exceeds their neutralisation by the antioxidant system, oxidative balance is perturbed and results in OS (Machlin and Bendich 1987; Lykkesfeldt and Svendsen 2007). Therefore, the levels of antioxidants normally supplemented may be insufficient to scavenge the excessive oxidants generated during environmental challenges such as HS, particularly given the reduction in ADFI. Animals are more likely to be affected by HS, especially when under an attenuated antioxidant defence and with greater metabolic activity, hence a sufficient supply of dietary antioxidants may alleviate the negative outcomes from HS (Finch and Turner 1996; Bottje and Carstens 2009).

As mitochondria are the principal cellular site of O_2 consumption, they are also one of the principal sites of ROS production. Indeed mitochondrial ROS production is increased during HS (Flanagan *et al*. 1998; Katschinski *et al*. 2000). This may arise due to reduced protein expression, thermal deactivation of regulatory proteins or antioxidant enzymes. For example, thermal damage can inactivate superoxide dismutase activity (Yang and Lin 2002), which is the principal mitochondrial antioxidant pathway during HS (Belhadj Slimen *et al*. 2014). Furthermore, thermal damage reduces expression of uncoupling proteins, which prevent ROS production by uncoupling of the electron transport chain (Mujahid *et al*. 2006). The consequences for the cell are reduced efficiency of oxidative phosphorylation, mitochondrial damage and may result in apoptosis (Qian *et al*. 2004).

Recent research has shown that supplementation with Vit. E, or Se, or their combination, alleviated the physiological response to HS in pigs (Liu *et al*. 2014), sheep (Alhidary *et al*. 2012; Chauhan *et al*. 2014*a*, 2014*b*) and dairy cows (Calamari *et al*. 2011). Supplementation of Vit. E and Se at supra-nutritional levels counteracted the effects of HS, lowering respiration rate, rectal temperature and improving feed intake and oxidative balance in sheep (Chauhan *et al*. 2014*a*). At the cellular level, protection against HS is achieved by induction of Heat Shock Proteins (HSP) (Collier *et al*. 2008), which are highly conserved molecular chaperons (Gething and Sambrook 1992). Supra-nutritional levels of dietary Vit. E and Se supplementation mitigates HS in sheep by upregulation of HSP mRNA expression and downregulation of pro-inflammatory cytokine and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-B) transcription factor, enhancing protection against hyperthermia and the resultant OS (Chauhan *et al*. 2014*b*). Supra-nutritional antioxidants successfully scavenged ROS thereby preventing activation of NF-B and leading to reduced tumour necrosis factor-α (TNF-α) expression, causing improved responses during HS. Antioxidants modulate the abundance of HSP in skeletal muscle in sheep (Chauhan *et al*. 2014*a*), and have also been reported to alter several signalling pathways including transcription factors, NF-B and activator protein-1 in poultry (Sahin *et al*. 2013) during HS. Furthermore, a growing body of evidence indicates crosstalk between OS and inflammation in ruminants (Sordillo and Raphael 2013), which might have implications for HS responses. However, the effects of antioxidants have not been sufficiently studied in heat-stressed pigs. Montilla *et al*. (2014) reported that HS leads to OS but not to inflammation in pig skeletal muscle exposed to HS for 1 and 3 days, indicating a possible role for supra-nutritional antioxidants. Despite increased lipopolysaccharides, TNF-α and ROS in muscle tissues of pigs, an inconsistent array of NF-B signalling was found in oxidative and glycolytic skeletal muscle. Therefore, further investigations are required in pigs to understand the association between OS and inflammatory responses triggered during HS.

Recent studies have found that supplementation for 14 days of supra-nutritional levels of dietary Se (1.0 ppm) and Vit. E (200 IU/kg) protected small intestinal barrier integrity, as evidenced by a prevention of reduction in trans-epithelial electric resistance, in growing pigs during cyclic HS (Fig. 3). Additionally, the diets increased intestinal glutathione peroxidase activity and reduced oxidised glutathione, indicating improved antioxidant buffering and reduced oxidative damage (Liu *et al*. 2015*b*, 2015*c*). A similar result was achieved in rats fed Se-enriched mushrooms and Vit. E during HS (Maseko *et al*. 2014).

In separate experiments, supplementation with Se or Vit. E individually provided less consistent results than when supplemented together. Supplementation with Se or Vit. E resulted in inconsistent effects on ameliorating the respiration rate and rectal temperature in HS pigs, which has differed to results obtained from sheep. For example, Liu *et al*. (2014) found that during 7 days of cyclic HS, Se ameliorated increases in skin and rectal temperature in pair-fed pigs. Vit. E but not Se ameliorated blood bicarbonate loss, indicating reduced respiratory alkalosis during HS. In a shorter study of 2 days' duration, no improvements in bicarbonate buffering were observed (Liu *et al*. 2015*b*), which may reflect the slow response for urinary excretion of bicarbonate (Patience *et al*. 2005). Putting aside the differences in duration of HS applied in the two studies (2 vs 7 days), the benefits of co-administration of antioxidants needs to be further investigated, particularly against a backdrop of increased regulatory oversight of micronutrients such as Se.

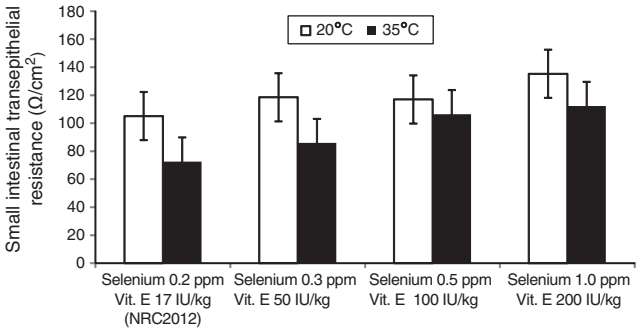

Fig. 3. Small intestinal trans-epithelial resistance (as an indicator of intestinal barrier integrity) in growing pigs fed on different dietary levels of selenium-yeast and vitamin E combination under either thermoneutral conditions (20°C, humidity 35–45%) or cyclic heat stress conditions (35°C, humidity 35–45%, 0900–1700 hours; 28°C overnight). Results were analysed by linear mixed model with fixed effects of temperature, diets, sites (jejunum and ileum), and their interactions, heat stress reduced the trans-epithelial resistance ($P = 0.03$), whereas combined supplementation of selenium and vitamin E improved the trans-epithelial resistance ($P = 0.02$). The P-value for the interaction between temperature and diet is 0.75. The bars represents the mean of temperature × diet and s.e.d. ($n = 6$). Values were pooled from the measurements of ileum and jejunum (Liu *et al*. 2015*c*).

Chromium

The involvement of insulin to the HS response has been suspected after the observations of increased mortality of diabetic patients during heat waves (Schuman 1972; Semenza *et al*. 1999). The specific mechanism whereby insulin counteracts the effects of HS are not fully known, but it is postulated to be due in part to improved regional blood flow, which augments radiant heat loss. Therefore, it stands that compounds that improve insulin sensitivity may be beneficial during HS.

Chromium is an essential mineral and has been conditionally included in pig diets to improve growth performance and carcass traits. The Cr augments receptor binding of insulin, augmenting insulin sensitivity via the Cr containing protein chromodulin (Davis *et al*. 1997). Recent studies in this laboratory suggest that Cr can be added in summer rations to ameliorate the impact of HS (Hung *et al*. 2010, 2015). For example, Hung *et al*. (2014) conducted a study to determine the impact of nano-sized chromium picolinate (nCrPic) on the growth performance of finisher gilts during summer. A total of 60 finisher Large White × Landrace gilts were fed either a control finisher diet (wheat-based diet containing 13.8 MJ digestible energy per kg and 0.56 g available lysine/MJ digestible energy) or a control diet containing 400 ppb Cr as nCrPic. Dietary nCrPic supplementation increased ADFI by 6% over the entire 4-week study ($P = 0.05$). In particular, dietary nCrPic increased ADFI by 8% ($P = 0.02$) during the final 2 weeks of the study. A similar result was observed in sheep where ADFI was increased by

dietary nCrPic supplementation in sheep under HS conditions (average temperature 40.4°C, average relative humidity 54.0%, and average thermal heat index 36.7) (Hung 2014). Other studies have reported similar results in heat-stressed quails (Sahin *et al*. 2002*a*, 2002*b*, 2005), broilers (Sahin *et al*. 2003; Samanta *et al*. 2008) and cows (Al-Saiady *et al*. 2004), whom had greater feed intake when consuming diets fortified with Cr. The improved feed intake during heat load indicated that dietary Cr might have ameliorated the negative impacts of HS on aspects of animal physiology.

A recent experiment in this laboratory showed that Cr supplementation could mitigate HS in growing pigs under a cyclic HS condition (Liu *et al*. 2015*d*). Briefly, this study involved 36 gilts (Large White × Landrace) that were subjected to two diets (0 or 400 ppb Cr as Cr picolinate) and two temperature regimes (thermoneutral; 20°C or cyclic HS; 35°C) for 8 days. HS increased plasma glucose and decreased NEFA area under the curve during an oral glucose tolerance test, indicating that insulin sensitivity was reduced in pigs during HS. Pigs fed with Cr had lower rectal temperature (40.2°C vs 39.9°C) and respiration rate (173 vs 136) under HS and Cr also reduced the glucose 'basal to peak' increment under thermoneutral conditions (4.15 vs 2.55 mM) but did not alter the glucose area under the curve (AUC) during HS treatment. Moreover, Cr tended to increase NEFA recovery (20–90 min) rate and AUC in HS treatment, indicating that Cr could facilitate lipid mobilisation when pigs were under HS.

Reduced plasma cortisol is a typical metabolic response to Cr supplementation in livestock, especially when animals are under stress (Chang and Mowat 1992; Samanta *et al*. 2008; Zha *et al*. 2009; Hung *et al*. 2014). It is indicated that Cr-regulated insulin action may be via altered cortisol concentrations (Borgs and Mallard 1998), because cortisol secretion increases when the animal is under stress and cortisol acts as an insulin antagonist. In this context, cortisol increases plasma glucose concentrations and slows down glucose utilisation by peripheral tissues, which is indicative of an induced insulin resistance. The maintenance of plasma glucose homeostasis is a complex process involving many hormones and genes. A proper response of the insulin signalling pathway genes is crucial for maintaining insulin sensitivity. Hung (2014) indicated that the Jun N-terminal kinases (JNK) expression in sheep skeletal muscle was downregulated by dietary nCrPic supplementation. The JNK is believed to cause insulin resistance by causing the phosphorylation of serine phosphorylation of insulin receptor substrate-1 (IRS-1) at serine residue (Hilder *et al*. 2003). Chen *et al*. (2009) also indicated that the improvement of insulin signalling by Cr was associated with decreased IRS-1-Ser307 phosphorylation and JNK activity.

A high ambient temperature may trigger several signalling pathways, some which facilitate cell survival and some that initiate cell death. Gabai and Sherman (2002) reported that HS can activate either protein kinase B (Akt; cell survival) or JNK (cell death) pathways, and HSP are the key regulators to control the direction towards cell survival or death pathway. Several *in vitro* and *in vivo* studies demonstrated that HSP72 can inhibit JNK, and lower levels of HSP72 result in JNK activation (Chung *et al*. 2008). In turn, activity of HSP is regulated by Akt through inhibition of glycogen synthase kinase-3β, a negative regulator of heat shock factor-1 (Xavier *et al*. 2000). The inhibition of glycogen synthase kinase-3β means increased glycogenesis, and therefore helps animals maintain a normal glucose homeostasis. Hung (2014) observed that dietary CrPic reduced JNK mRNA expression. Moreover, CrPic was also able to maintain glucose homeostasis because animals fed with nCrPic-supplemented diets had a lower glucose AUC after glucose infusion. Furthermore, dietary nCrPic is able to decrease the rectal temperature and increase feed intake (Hung 2014). Taken together, these results suggest that the dietary nCrPic supplementation ameliorated the negative physiological responses in heat-stressed sheep by decreased JNK expression in skeletal muscle and consequently, prevention of cell apoptosis.

Betaine

Trimethyl glycine, also known as betaine due to first being isolated from the juice of sugar beets, is accumulated in some animal, plant and microbial sources and is accumulated in cells undergoing osmotic stress (Sizeland *et al*. 1993; Suzuki *et al*. 2003; Lever and Slow 2010). In mammalian tissues, betaine has three known functions: (1) as an organic osmolyte, it helps to retain cell volume under osmotic stress and can accumulate in molar concentrations (Burg 1995); (2) it can protect against protein denaturation (Caldas *et al*. 1999); and (3) it can act as a methyl donor (Zeisel and Blusztajn 1994).

It has never been formally quantified but is generally accepted that betaine is rapidly and nearly fully absorbed (Kettunen *et al*. 2001). Endogenous betaine synthesis is catalysed by the cytosolic enzyme betaine:homocysteine methyltransferase, which is expressed in high concentrations in the liver and kidney (Schwahn *et al*. 2003), which along with the intestine are major sites of exogenous betaine distribution (Kettunen *et al*. 2001). Elimination of betaine occurs via metabolism rather than excretion, even at relatively high doses of 100 mg/kg (Schwahn *et al*. 2003). Metabolism of betaine occurs principally as a methyl donor in liver and kidney mitochondria via transmethylation in the methionine cycle, sparing methionine, detoxifying homocysteine and yielding S-Adenosylmethionine (Barak *et al*. 1996).

When not catabolised, betaine can act as an organic osmoprotectant (Fernández *et al*. 1998; Huang *et al*. 2007) and is transported into the cell by both active and passive means (Craig 2004). Urea also accumulates in cells in response to hyperosmolarity, but as urea is a potent destabiliser of proteins it is not desirable for long-term use as a feed additive for monogastrics. Alternatively, betaine can alleviate osmotic stress without affecting protein structure and function (Yancey and Burg 1990), making betaine particularly useful as a long-term osmolyte (Kempson *et al*. 2003). It has been proposed that the action of betaine as an osmolyte may have an energy sparing effect by reducing the activity of cellular ATPase pumps required to maintain cellular ion gradients. It is thought that this would be most beneficial to tissues with high burdens for osmotic regulation, such as those from the GIT (Moeckel *et al*. 2002; Eklund *et al*. 2005). This is indicated in feed-restricted pigs where the small intestine weight increased (by 11%) when pigs were supplemented with 0.125% betaine (Fernández-Figares *et al*. 2002).

Dietary betaine supplementation can act as a carcass modifier by increasing carcass muscle and decreasing fat depths in pigs (Fernández-Figares *et al*. 2002; Huang *et al*. 2008). As betaine can act as a methyl donor during its conversion into glycine, it can provide substrates for re-methylation into methionine and thus replace a portion of an animal's methionine requirement and provide additional substrates for protein synthesis (Matthews *et al*. 2001*a*). Additionally, betaine can enhance carnitine concentrations in liver and muscle tissues. As carnitine is required for fatty acid transport through mitochondrial membranes, betaine supplementation may improve fatty acid oxidation thereby reducing carcass fat (Eklund *et al*. 2005). Finally, betaine supplementation can increase basal and mean serum growth hormone concentrations and increase protein deposition, serum urea nitrogen and total protein concentrations (Huang *et al*. 2006, 2007). Furthermore, insulin-like growth factor-1, free thyronine, free thyroxine and insulin levels all increased by 40–50% in response to betaine supplementation (Huang *et al*. 2006). However, the inherent variability between experiments and lack of studies exploring different doses of betaine supplementation in pigs makes it difficult to make conclusive remarks about the efficacy of betaine supplementation as a carcass modifier. Matthews *et al*. (2001*b*) found a quadratic decrease in 10th rib fat thickness in pigs fed increasing levels of dietary betaine, whereas Dunshea *et al*. (2009) demonstrated a subtle but positive (5%) increase in lean tissue deposition in pigs supplemented with 0.15% betaine. However, Dunshea *et al*. (2009) ascribed this increase in lean tissue deposition to an increase in energy being made available for lean tissue deposition in energy-restricted pigs. According to Wray-Cahen *et al*. (2004), there was no variation in lean gain and feed efficiency responses between pigs supplemented with betaine at 0.125% versus 0.5% of the feed, whereas Fernández-Figares *et al*. (2002) showed betaine supplemented at 0.5% of the feed was the most effective dose for improving lean gain in pigs compared with 0.125% and 0.25%. This increase in lean gain was accompanied by a concurrent linear decrease in carcass fat with increasing betaine levels, although this only occurred in male feed-restricted pigs (Fernández-Figares *et al*. 2002). Nevertheless, a meta-analysis of 13 studies concluded that overall betaine decreases back fat and 10th rib fat thickness in finishing pigs (Sales 2011).

A decrease in fat thickness is particularly important in times of heat exposure as an increased subcutaneous fat depth can impair heat dissipation (Brown-Brandl *et al*. 2006; Gaughan *et al*. 2010). This was demonstrated in growing chicks exposed to heat where betaine increased weight gain and decreased rectal temperature in a dose-dependent manner, where 1 g was more effective than 0.5 g per kg of feed (Attia *et al*. 2009). The osmotic actions of betaine may also be important in times of heat exposure, particularly in renal tissues where compensation of respiratory alkalosis occurs to maintain acid-base homeostasis as increased respiration rates can lead to changes in blood pH as well as electrolyte and acid-base imbalances (Collier *et al*. 1982). Porcine muscle tissue accumulates betaine when it is supplemented in the diet, suggesting that these tissues would have increased water retention and perhaps a reduction in osmotic stress associated with heat dissipation methods (Matthews *et al*. 2001*b*). Additionally, as an osmolyte, betaine can reduce energy expenditure by reducing the need for cellular ion pumps and can thus be energy sparing (Caldas *et al*. 1999; Craig 2004). Furthermore, the capacity of betaine to utilise osmotic gradients rather than ion pumps in intestinal tissues can decrease the energy required for Na^+/K^+ pumps by >60% (Moeckel *et al*. 2002), potentially allowing whole-body energy savings of ~8% (Cronje 2005) and reducing metabolic heat production. In pigs that have the potential to use this additional energy such as those restrictively fed and/or in porcine somatrotopin-treated boars, the additional energy can be used for lean tissue deposition (Suster *et al*. 2004). However, if the pigs have already reached their inherent potential for protein deposition, any additional energy will be deposited as fat.

Comparatively less is known about the benefits of betaine supplementation in pigs during heat exposure than other species. Under thermoneutral conditions, betaine supplementation (0.125%) does not alter feed intake or growth in pigs, however once exposed to mild HS conditions (36°C for 6 h), pigs fed betaine had lower respiration rates than pigs fed a control diet (Gabler *et al*. 2013). Moreover, dietary betaine supplementation was able to mitigate mild HS-induced increases to intestinal permeability in pigs as demonstrated by a reduced permeability in the ileum, although this was not noted in the caecum or colon (Gabler *et al*. 2013). In one study the heat production from the portal-drained viscera tended to be lower in pigs fed betaine and was 58% lower in those fed a combination of betaine and conjugated linoleic acid (0.5% and 1% of diet, respectively), whereas the O_2 consumption of the portal-drained viscera was lower in both betaine and betaine plus conjugated linoleic acid-supplemented pigs (Rojas-Cano *et al*. 2013). Visceral tissues have a high metabolic rate and therefore a large energy requirement, suggesting that a decrease in heat production, (which perhaps indicates an increase in efficiency or decrease in metabolic activity) elicited by betaine and conjugated linoleic acid supplementation may spare energy that can be used by other tissues, as supported by the demonstrated decrease in O_2 consumption (Rojas-Cano *et al*. 2013). Total heat production was lower in pigs fed an energy-limiting diet supplemented with betaine (1.25 g/kg feed) and housed in thermoneutral (20°C, 65% relative humidity) conditions, which can be attributed to either an improvement in efficiency or a decrease in maintenance energy requirements (Schrama *et al*. 2003). In late-finishing pigs housed in high environmental temperatures (32°C), dietary betaine supplementation (0.2%) did not alter carcass composition but reduced ADFI and ADG, whereas betaine supplemented at 0.0625%, 0.125% and 0.1875% elicited a quadratic decrease in carcass yield and therefore did not improve pig performance (Mendoza *et al*. 2015). The promising effects of betaine supplementation in ruminants, as reviewed by Dunshea *et al*. (2013), highlights that betaine may improve production in animals exposed to high environmental temperatures. Although amelioration of heat-induced production losses with betaine supplementation is promising, further research, particularly in pigs, is required.

Other nutritional strategies

Cinnamon is the dried inner bark and twig in the Lauracea family that is native to Sri Lanka and India but is cultivated extensively in the tropical regions around the world. Cinnamon is widely added

to food and beverages to improve taste and flavour and has been used for centuries in Chinese medicine. Research has suggested that cinnamon may have pharmacological benefits for treatment of diabetes by improving insulin sensitivity (Akilen *et al*. 2012). For example, Mang *et al*. (2006) found that cinnamon extracts can reduce fasting plasma glucose concentrations in diabetic patients with poor glycaemic control, whereas several studies showed that cinnamon extracts can improve insulin sensitivity in mice and in adipocyte cell cultures (Roffry *et al*. 2006; Sheng *et al*. 2008). Cinnamon extract has been reported to reduce circulating glucose, insulin, triglycerides and cholesterol concentrations in fructose-fed rats through enhancement of insulin signalling mediated via the regulation of the expression of multiple genes related to carbohydrate and lipid metabolism in adipose tissue (Qin *et al*. 2004, 2010). Cao *et al*. (2007) also reported that cinnamon increased the concentration of proteins involved in insulin signalling such as glucose transporter-4, and the anti-inflammatory response in mouse 3T3-L1 adipocytes. Recently, diet supplementation with 1.25% cinnamon in the finisher phase of pigs improved insulin sensitivity (Hung *et al*. 2012), which may also be beneficial for the management of HS.

Glutamine is the major nutrient for the intestinal epithelial cell and is able to upregulate HSP70 expression to ameliorate thermal damage. An experiment in rodents showed that glutamine supplementation alleviated the increase of core temperature and intestinal permeability, and decreased the bacterial translocation during acute heat exposure (Soares *et al*. 2014). Similarly, glutamine supplementation protected broilers from acute HS-induced intestinal OS by upregulating HSP70 production (Gu *et al*. 2012; Hao *et al*. 2012). To date there are no published data on the effects of glutamine supplementation in heat-stressed pigs.

Conclusions

Improvements in the understanding of the responses and impact on pig physiology, nutrition and metabolism are enabling the development of feed additives indicated for the amelioration of HS. Due to the intensive nature of pig farming feed additives are ideal for the integration into existing production systems. Recent strategies for improving productivity in heat-stressed pigs have included improving antioxidant defence, insulin sensitivity, osmolytes and reducing maintenance energy. Each of these pathways can be targeted with low cost feed additives suitable for use in pig production.

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The impact of heat stress on intestinal function and productivity in grow-finish pigs

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Abstract. Heat stress is a physiological condition when animals can no longer regulate their internal euthermic temperature. When livestock such as pigs are subjected to this environmental stress, it can be detrimental to performance, health and well-being, and if severe enough even death. Growing pigs are particularly susceptible to heat stress and one of the major organs first affected by heat stress is the gastrointestinal tract. As a result, reductions in appetite, intestinal function and integrity and increased risk of endotoxemia can modify post-absorptive metabolism and tissue accretion. These changes in intestinal integrity may be a result of altered expression of tight junction proteins, increased circulating endotoxin concentrations and markers of cellular stress (heat shock and hypoxia response), which is evident as early on as 2 h after heat-stress onset. Due to restricted blood flow, the ileum is more severely affected compared with the colon. Interestingly, many of the negative effects of heat stress on intestinal integrity appear to be similar to those observed with pigs reared under reduced nutrient and caloric intakes. Altogether, these depress pig performance and health, and extend days to market. Despite this impact on the gastrointestinal tract, under heat-stress conditions, intestinal glucose transport pathways are upregulated. This review discussed how heat stress (directly and indirectly via reduced feed intake) affects intestinal integrity and how heat stress contributes to decreased growth performance in growing pigs.

Additional keywords: heat shock proteins, hypoxia, nutrient transport.

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Introduction

The health and homeostasis of the gastrointestinal tract (GIT) of pigs is affected by multiple factors including disease, environment, and genetics that all interact to predispose pigs to physiological, metabolic, or immunological stresses. Collectively, these stresses can lead to reductions in well-being, growth, reproduction and lactation performance, chronic illness or ultimately death if severe and prolonged. Certain types of stressors such as hypoxia, inflammation, and oxidative stress can be particularly elicited by environmental hyperthermia. Environmental heat stress or hyperthermia can adversely affects animals (Bouchama *et al*. 2007; Bernabucci *et al*. 2010; Renaudeau *et al*. 2011; Pearce *et al*. 2013*a*; Chauhan *et al*. 2014). The global economic impact of heat stress to animal agriculture is immense (St-Pierre *et al*. 2003) and this value is likely to increase with the elevated threat of global warming and increased incidence of severe weather events (EPA 2014). For pig production, despite recent advances in heat abatement strategies, heat stress continues to cause increased days on feed, health problems, reduced growth, reduced reproductive performance and may lead to increased mortality.

Physiologically, the intestines are highly sensitive to heat stress in most species. Heat stressed-animals redistribute blood to their periphery via vasoconstriction in the GIT in an attempt to maximise radiant heat dissipation (Lambert 2008). However, the reduced blood and nutrient flow to the intestinal epithelium compromises integrity of the intestinal barrier (Yan *et al*. 2006; Pearce *et al*. 2013*c*; Montilla *et al*. 2014). Tight junction protein complexes in the intestine are necessary for normal barrier function and integrity. Alterations in these proteins and their interaction with one another is implicated in certain types of stress (including heat stress), which can lead to increased intestinal permeability and reduced integrity. This increased permeability can elevate pathogen loads and endotoxemia, which can antagonise anabolic processes and lead to multi-organ system failure if severe enough (Hall *et al*. 2001). This review will discuss how the early onset of heat stress alters intestinal integrity and function in growing pigs and the contribution this has on reducing growth performance.

Pig performance and heat stress

Heat stress suppresses feed intake and growth rates of grow-finish pigs (Renaudeau *et al*. 2011). An immediate heat-stress phenotype response consistently observed in pigs is a decrease in feed intake (Collin *et al*. 2001; Renaudeau *et al*. 2011, 2012; Song *et al*. 2011; Pearce *et al*. 2013*a*; Boddicker *et al*. 2014; Johnson *et al*. 2015*a*, 2015*b*). Reduced appetite is presumably a strategy to minimise metabolic heat production and this was recently confirmed in respiratory chambers with pigs subjected to heat stress (Renaudeau *et al*. 2013). The reduction in feed

intake appears to be controlled by the hypothalamus and changes in neuropeptide hormones such as gastric inhibitory peptide, ghrelin, and cholecystokinin, which cause reduced appetite and increased gastric motility (Pearce *et al*. 2014). Changes in feed intake and gastric processes are likely to contribute to the reduced bodyweights (BW) consistently observed in research completed by Pearce *et al*. (2013*a*). Even with short durations of high ambient heat exposure (2–24 h), pigs lose a significant amount of BW (Pearce *et al*. 2013*a*, 2013*b*, 2014; Boddicker *et al*. 2014). These losses in BW may partially be explained by a combination of reduced feed intake, increased basal metabolic rate and a presumed increase in defecation and urination. Although water intake was not measured in this research, hematocrit data suggested hydration status was not a significant factor in this BW loss. Song *et al*. (2011) has shown water intake in heat-stressed pigs to increase compared with their thermal neutral counter parts.

In the short-term, both feed intake and BW losses can be quite dramatic as a result of heat stress. In growing pigs, 24 h of heat stress caused a 50% reduction in feed intake and BW loss of almost 3 kg (Pearce *et al*. 2013*a*). Pigs reared at 33°C lost almost 1 kg of BW over a 6-day period and feed intake was reduced by 300 g (Collin *et al*. 2001). In a longer-term study, pigs heat stressed at 32°C for 3 weeks had a reduction in feed intake of 771 g, which equated to a 32% decrease compared with thermal neutral animals (Renaudeau *et al*. 2013). In a diurnal pattern of heat stress (27−37°C) grow-finish pigs had a reduction in average daily gain (0.87–0.58 kg) and a 26% reduction in feed intake over a 28-day period (Song *et al*. 2011). Also in grow-finish pigs (~60 kg BW), Johnson *et al*. (2015*b*) reported that 6 weeks of constant heat stress (34°C and ~54% humidity) reduced average daily gain, protein and fat whole-body tissue accretion rates, feed intake and attenuated feed efficiency compared with the thermal neutral control pigs. However, in younger pigs (30 kg BW), these authors reported no differences in feed efficiency and protein accretion rates, although heat stress (34°C and ~49% humidity) reduced average daily gain, whole-body tissue fat accretion rates and feed intake (Johnson *et al*. 2015*a*). In a recent heat-stress study, Cruzen *et al*. (2015) reported carcasses from heat-stressed barrows also had less carcass separable fat and backfat compared with thermal neutral barrows, even after controlling for hot carcass weight. These authors concluded that heat stress (constant 32°C) during finishing results in longer times to reach market weight and a leaner carcass once market weight is achieved. Although data is limited in pigs, heat stress-induced changes in meat quality include, but are not limited to: changes in meat colour, lower carcass and organ weights, increased lipid oxidation, and changes in fat depots (Toghyani *et al*. 2012; Zeferino *et al*. 2013).

Few studies have examined the impact heat stress has on grow-finish pig nutrient digestibility and retention. Nitrogen retention rates are decreased when growing pigs were subject to 3 weeks of heat stress (Renaudeau *et al*. 2013). Nitrogen excretion in urine and faeces was decreased when pigs were heat stressed. These results contradict those of Collin *et al*. (2001) who showed increased apparent total tract digestibility coefficients for energy, nitrogen and dry matter in pigs reared at 33°C. However, if corrected for equal feed intake (heat-stressed animals ate less) there was no effect of climate on digestibility. Brestensky *et al*. (2012) reported that nitrogen excretion was decreased and nitrogen retention was unaffected, due to decreased feed intake resulting in a decrease in daily nitrogen intake.

Heat shock proteins, hypoxia, inflammation and heat stress

Heat stress evokes a rapid heat shock response involving the activation of heat shock proteins (HSP) and this is particularly evident in the intestinal tract in pigs within 2–4 h of heat stress (Pearce *et al*. 2014). HSP are a large family of stress proteins that are highly conserved across species and are aptly named according to their molecular weight. Their sizes and functions cover a wide range, but they generally serve as molecular chaperones or stabilisers of protein denaturation. These proteins can be upregulated due to heat, hypoxia, oxidative, and nutritional stress. Their activity is prevalent within several hours of the onset of severe stress and lasts for a few days (Horowitz 2002). Expression of these proteins is mediated by activation of heat shock factor 1 that is a transcription factor, which binds to heat shock elements and initiates transcription of several heat shock genes (Singh and Hasday 2013).

One of the major inducible HSP family members is HSP 70–72 kDa, that aids in protein folding, ubiquination, renaturing proteins, and providing protection during times of cellular stress (Petrof *et al*. 2004). HSP 70 has also been shown to regulate toll-like receptor (TLR)-4 signalling at the level of the intestine by targeting TLR-4 for degradation and subsequently diminishing the immune response (Afrazi *et al*. 2012). Another important HSP is HSP 25/27, which is involved in thermal tolerance and protein ubiquitination, (which targets proteins for degradation). HSP 25/27 is also an inducible protein, which helps maintain cytoskeletal structure, and protects cell architecture during stress (Petrof *et al*. 2004). Studies in renal epithelial cells have shown that HSP 25/27 co-localises and proteins the actin cytoskeleton (Van Why *et al*. 2003). In the intestine, HSP 70 and HSP 25/27 are integral in protecting the mucosal barrier under both normal physiologic, pathophysiological and stressful conditions (Pearce *et al*. 2013*b*). These are most abundant in the lower small intestine as well as stomach and large intestine (Petrof *et al*. 2004).

Most of the biological function of HSP occurs in the cytosol. However, HSP are also found extracellularly and in the mitochondria (Grubbs *et al*. 2013). Traditionally HSP were thought to be located exclusively in intracellular compartments of the cell. However, a little over 10 years ago researchers showed that HSP 70 was released after tissue necrosis and was involved in the immune response (Basu *et al*. 2000). Other HSP such as HSP A5, HSP A9, HSP 60 and HSP 90 have also been found in extracellular spaces (De Maio and Vazquez 2013). Export of HSP from the cell may primarily be a result of necrosis, or by a secondary type of unconventional secretory, active transport mechanism, or lysosome-endosome pathway (Nickel and Seedorf 2008). However, the mechanism by which HSP are exported remains unclear and under investigation. Collectively, HSP are rapidly upregulated in pigs during heat stress. This increase is seen within 2–4 h of heat exposure and is long lasting (Pearce *et al*. 2013*a*, 2014, 2015).

Heat stress induces hypoxia in visceral organs due to changes in blood flow and oxygen supply (Hall *et al*. 1999). Hypoxia is a condition within the body where the body is deprived of

adequate oxygen supply. The master regulators of the hypoxic response in cells and tissues are the hypoxia inducible factor (HIF) regulatory subunits HIF-1α and HIF-2α. These are transcription factors regulated by oxygen-sensing prolyl hydroxylases (Eltzschig and Carmeliet 2011). Both HIF activate hypoxia-related genes in a similar manner; however, most studies emphasise HIF-1α as being most important in gene transcription in response to hypoxia and HIF-2α is important for endothelial cells (Loboda *et al*. 2010).

When oxygen is readily available, HIF-1α subunits are hydroxylated, which causes it to be marked for degradation. During hypoxic stress, the activity of prolyl hydroxylases is reduced and HIF-1α is stabilised, allowing it to act as a transcription factor for many genes involved in adaptive and survival functions (Majmundar *et al*. 2010). Over 100 genes are regulated by HIF-1α including genes involved in erythropoiesis and iron metabolism in order to increase delivery of oxygen to tissues (Ke and Costa 2006). Angiogenesis is also upregulated by HIF, specifically upregulation of vascular endothelial cell growth factor, which is important for endothelial cell recruitment to hypoxic/avascular areas (Greer *et al*. 2012). During hypoxia, cells divert glucose metabolism to anaerobic glycolysis and allow cells to increase ATP generation by increasing glucose uptake. Therefore, HIF transcribe many glycolytic genes, as well as glucose transporters (Ke and Costa 2006). Genes involved in cell proliferation and survival are also increased such as insulin-like growth factor-2 and transforming growth factor-α as well as MAPK and PI3K pathways (Semenza 2007). Conversely, apoptosis can also be increased due to hypoxia involving the activation of several caspases and release of cytochrome c.

Several conditions have been shown to increase expression of HIF including ischemia, cancer, kidney disease, and inflammation, among others (Semenza 2007) and most of these involve negative consequences of HIF overexpression. However, stabilisation of HIF during intestinal ischemia or inflammation increases adenosine signalling pathways, which promotes restitution and wound healing (Grenz *et al*. 2012). Research has consistently shown HIF-1α protein and gene abundance increased with heat stress in the small and large intestine of pigs (Pearce *et al*. 2012, 2013*b*, 2013*c*, 2014).

Inflammation and proinflammatory cytokines are often associated with stress events and immune system activation that antagonise pig growth (Mani *et al*. 2013). However, growing pigs exposed to heat-stress conditions appear to have a reduced pro-inflammatory cytokine response, yet still mount an acute phase response. Interestingly, we have observed in growing pigs little to no increase in circulating proinflammatory cytokines such IL-8, IL-1β, and TNF-α in pigs subjected to 2–6 h (Pearce *et al*. 2014), 12 h (Pearce *et al*. 2015), 24 (Pearce *et al*. 2013*b*) or 3–7 days (Pearce *et al*. 2013*c*; Montilla *et al*. 2014) of constant heat stress. This is contrary to other species including poultry, rodents, humans, and cattle, in which heat stress has been shown to orchestrate an inflammatory response (Leon *et al*. 2006; Lim *et al*. 2007; Lambert 2009). Additionally, Campos *et al*. (2014) reported in growing pigs that compared with thermal neutral conditions (24°C), heat stress (30°C) alleviated the inflammatory response and growth suppression resulting from a lipopolysaccharide (LPS) challenge.

Intestinal integrity and heat stress

Functional integrity of the intestine is dependent upon coordinated regulation of the mucus layer, tight junctions, epithelial cells, as well as the enteric immune system (Groschwitz and Hogan 2009). The intestinal epithelium is selectively permeable through two pathways; transcellular and paracellular (via tight junctions). Adhesive junctional complexes contain proteins, which link adjacent epithelial cells to the actin cytoskeleton through scaffolding proteins. Adherens junctions and desmosomes are important for mechanical linking of cells whereas tight junctions are responsible for regulating selective solute transport. Both sets of complexes are also important for cellular proliferation and differentiation (Groschwitz and Hogan 2009). From a morphological standpoint, heat stress causes villous atrophy and augmented villi autolysis in pigs (Pearce *et al*. 2012, 2013*b*). By 4–6 h of heat stress, intestinal villi are sloughing and undergoing autolysis, indicating severe intestinal epithelial damage (Pearce *et al*. 2014). This agrees with the increased incidence of intestinal lesions in heat-stressed mice as found in Leon *et al*. (2006).

Although tight junctions are important barrier defences, perhaps the most important and largest barrier defence for the GIT is the mucus layer coating the length of the GIT. This mucus layer provides the first line of defence against intestinal injury. Intestinal goblet cells are responsible for secretion of several mucins (MUC) including MUC 2, MUC 1, MUC 4, MUC 3, MUC 12, MUC 13, and MUC 17 (Johansson *et al*. 2013). They are large glycosylated glycoproteins with several O-linked oligosaccharide side chains, which create a gel-like structure. MUC are the major secreted product of goblet cells, however, there are several other molecules secreted by goblet cells (Kim and Ho 2010). The intestine is colonised by many species of commensal and pathogenic bacteria, which can become trapped within the mucus layer and removed via peristalsis or activate the immune response. The mucus layer allows for nutrient transport while at the same time preventing microbial attachment and colonisation (Kim and Ho 2010). Several factors including microbes, microbial products, cytokines, toxins, and more regulate expression of MUC genes. The mucus layer also contains other products of goblet cell secretion including trefoil factors, antimicrobial peptides (β-defensins, lysozymes) and secretory IgA, along with several other molecules. Resistin-like molecule β is also secreted by goblet cells and induces goblet cell hyperplasia as well as functions as an immune effector molecule in response to a nematode infection. With that, Fc-γ-binding protein (also known as IgG Fc-binding protein) binds IgG antibodies and participates in stabilisation of the mucus layer (Johansson *et al*. 2009).

Tight junction proteins such as zonula occludin, claudins, and occludins re-distribute or re-localise during times of stress and play a key role in regulating intestinal barrier integrity (Turner 2006, 2009; Zhang *et al*. 2012). Zonula occludin-1 is thought to primarily regulate paracellular permeability in the intestine, although the role of occludin in paracellular permeability is still not fully understood (Raleigh *et al*. 2011). Re-distribution of tight junction proteins has previously been shown due to oxidative stress (Musch *et al*. 2006) in a c-Src kinase-dependent manner (Basuroy *et al*. 2003). Phosphorylation of occludin by multiple kinases and phosphatases is thought to contribute to tight junction

regulation and modification (Dörfel and Huber 2012). These kinases include the Src-family kinases that are thought to be involved in tight junction assembly and intestinal integrity. Casein kinase II-α activation plays a key role in occludin phosphorylation and acts as an important regulator of zonula occludin-1, claudin-1, and claudin-2 tight junction protein complex dissociation resulting in impaired barrier function (Raleigh *et al*. 2011). Interestingly, no differences in c-Src expression were observed due to heat stress (Pearce *et al*. 2013*b*).

Intestinal barrier dysfunction increase transcellular permeability of enteropathogenic bacteria (Barreau and Hugot 2014). Intestinal viruses such as astrovirus (Moser *et al*. 2007), rotavirus (Jacobi *et al*. 2013) and transmissible gastroenteritis (Egberts *et al*. 1991) also have been shown to increase intestinal permeability. In rodents, heat stress significantly affects small intestinal permeability to macromolecules (Lambert *et al*. 2002). Even within a short time period (60 min) heat-stressed rodents at 41.5−42°C core temperature had increased macromolecule permeability. Decreased transepithelial resistance (TER; electrophysiological measure of barrier integrity) has also been observed in other heat-stress cell culture and rodents models (Prosser *et al*. 2004; Dokladny *et al*. 2006).

In an attempt to study the localisation of these proteins by western blot, Pearce *et al*. (2013*b*) observed an overall (membrane plus cytosolic) increase in claudin 3 and occludin protein expression due to heat stress. Interestingly, the distribution of tight junction proteins differed, in that claudin 3 protein expression was higher in the membrane fraction, whereas occludin was more highly expressed in the cytosolic or detergent-insoluble fraction compared with the thermal neutral samples. Although puzzling, this occludin data agrees with previous 24-h intestinal heat-stress research in which occludin protein was upregulated (Dokladny *et al*. 2006, 2008). Increased expression of these tight junction proteins may indicate a barrier enhancement effect during heat stress in an attempt to compensate for increased permeability. However, more conclusive immunohistochemistry analysis is required to better understand these protein–protein interactions and their localisation during heat stress.

Regulation of the intestinal epithelial cell cytoskeletons is also critical in tight junction physiology and intestinal integrity. This cytoskeleton regulation is largely mediated by myosin light chain kinase (MLCK) and post-translational modifications to myosin light chain (Turner 2006). Increased expression and activation of MLCK and subsequent phosphorylation of myosin light chain has been observed during heat stress and associated with increased intestinal permeability (Yang *et al*. 2007). This agrees with our data, as heat stress increased MLCK protein expression and reduced intestinal integrity (Pearce *et al*. 2013*b*). Interestingly, MLCK is known to be activated by oxidative stress, hypoxia and HIF 1-α (Qi *et al*. 2011). Therefore, there appears to be a strong link between heat stress-induced intestinal oxidative stress and hypoxia, and changes in intestinal epithelial integrity, as heat stress increases intestinal markers of oxidative stress and hypoxia.

Intestinal integrity declines within the first 2–4 h in the ileum, whereas the colon remains unaffected at these time points (Pearce *et al*. 2014). Between 6 and 12 h, TER and transport of the fluorescently labelled macromolecule FITC-Dextran 4 kDa (FD4) integrity markers rebounds, as TER increases and FD4 transport decreases by 12 h (Pearce *et al*. 2014, 2015). However, ultimately TER decreases again at 24 h under heat stress (Pearce *et al*. 2012, 2013*b*). This rapid transient increase in permeability was similar to work done in a cell culture model (see Dokladny *et al*. 2006). It is well documented that heat stress causes blood endotoxin concentrations to increase, presumably through a reduction in intestinal integrity (Bouchama *et al*. 1991; Hall *et al*. 2001; Yan *et al*. 2006; Pearce *et al*. 2013*c*). In support of this and as a consequence of reduced intestinal integrity, an increase in circulating endotoxin was also observed by 6 h (Pearce *et al*. 2014), which continues to increase dramatically by 12 h (Pearce *et al*. 2015). These responses are accompanied by a decrease in LPS-binding protein from 2 to 12 h. Lipopolysaccharide-binding protein (LBP) is an acute phase protein, which interacts and binds LPS molecules, subsequently presenting them to CD14 to initiate an immune response (Fang *et al*. 2002). It may there be hypothesised that a free form of circulating LBP is decreased over time as endotoxin increases and is bound by LBP molecules. Collectively, these changes in intestinal integrity and blood biomarkers of intestinal integrity to heat stress within 2–24 h of exposure in growing pigs is summarised in Fig. 1.

A compromise in intestinal integrity as a result of heat stress was also accompanied by an increase in ileum MUC 2. Ileal MUC 2 (protein and gene expression) was increased by 6 h of heat stress and remained elevated through 12 h of heat stress

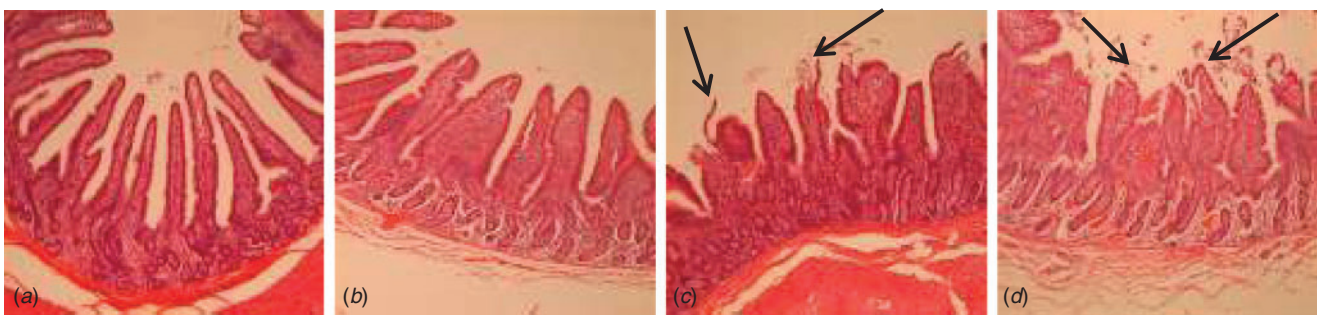

Fig. 1. Heat stress (HS; 37°C; ~40% humidity) induced changes in morphology of the ileum. (*a*) 0-h HS exposure, (*b*) 2-h HS, (*c*) 4-h HS, and (*d*) 6-h HS exposure. Note the villous atrophy and autolysis of the villi tips as the duration of heat stress increases. The black arrows indicate areas of autolysis. Adapted from Pearce *et al*. (2014).

compared with the thermal neutral pigs (Pearce *et al*. 2014). MUC 2 is a secretory MUC, which is secreted from intestinal goblet cells and acts as a protective barrier for the intestine. During ischemia, MUC addition to intestinal epithelial cells has been shown to improve intestinal permeability in the rodent small intestine (Chang *et al*. 2012) and in heat-stressed chickens, MUC-producing goblet cells are increased in the ileum (Ashraf *et al*. 2013). Heat stress-induced changes in intestinal integrity and morphology appear to be orchestrating the increase in MUC production in pigs. It may be speculated that this increase in MUC expression may be another compensatory mechanism to endogenously protect the intestinal epithelial barrier. Further, endogenous MUC production may also be contributing to a reduction in nitrogen balance (Brestensky *et al*. 2012; Renaudeau *et al*. 2013). This may have large implications on amino acid metabolism and endogenous loses of serine, cysteine, and threonine, as these amino acids are highly abundant in MUC proteins (Corfield 2015).

Many heat-stress studies in pigs have not attempted to distinguish the differences between the direct and indirect effects (i.e. reduced feed intake) of heat. Therefore, a pair-fed thermal neutral model was utilised in order to accurately determine if environmental heat is independently causing the unwanted heat-stress phenotypes (Pearce *et al*. 2013*a*, 2015). Utilising this pair-feeding model has allowed for more accurate understanding of the physiology related to either caloric restriction or high ambient heat, and will aid in the further development of better heat-stress mitigation strategies. Previous research has shown that pair feeding for 7 days (Pearce *et al*. 2013*c*) explains a large majority of the negative effects on the intestine. Pair feeding has also been shown to explain a significant reduction in milk production in heat-stressed dairy cattle (Wheelock *et al*. 2010). Utilising a pair-fed group, it was hypothesised that reduced feed intake would account for many of the negative consequences to the GIT during heat stress. Pair feeding for as little as 12 h has a similar impact as heat stress on growing pig intestinal integrity, decreased TER, increased FD4 and a moderate increase in blood endotoxin. Further, histologically, pair-fed animals exhibit villi shortening and increased villi width and this is accompanied by an increase in MUC 2 protein expression. However, it was observed that minimal villi tip autolysis, laminar separation and structural damage compared with the heat-stress treatment (Pearce *et al*. 2013*c*, 2015). Nevertheless, it can be concluded that reduced feed intake is playing a significant role in intestinal barrier dysfunction.

Nutrient and glucose transport

Under heat stress, pigs have maintained or enhanced intestinal glucose transport compared with their thermal neutral counterparts (Pearce *et al*. 2013*b*). Ileum active glucose transport measured *ex vivo* in modified Ussing chambers was augmented. Further supporting these data were the increase in glucose transporter 2 expression and Na^+/K^+ ATPase activity, as well as blood glucose concentrations (in the face of reduced feed intake). However, there was also a significant decrease in brush border digestive capacity (sucrase, maltase and aminopeptidase activities) (Pearce *et al*. 2013*b*). This was similar to previous work in heat-stressed poultry done by Garriga *et al*. (2006). It may be speculated that this increase in glucose uptake and blood glucose in the presence of reduced feed intake relates to post-absorptive fuel selection being more towards glycolytic metabolism under stress (Seppet *et al*. 2009; O'Neill and Hardie 2013). Further, the increase in intestinal glucose transport is a possible mechanism for cellular protection and hydration during heat stress. Intestinal sodium dependent glucose transporter 1 (SGLT-1)-mediated glucose uptake has been shown to protect intestinal epithelial cells against LPS and Giardia-induced apoptosis via targeting mitochondrial dependent and independent pathways (Yu *et al*. 2006, 2008). The increase in glucose transport due to heat stress may also be a reflection of increased water transport. Water can be co-transported along with Na^+ and glucose through SGLT-1 (Wright and Loo 2000).

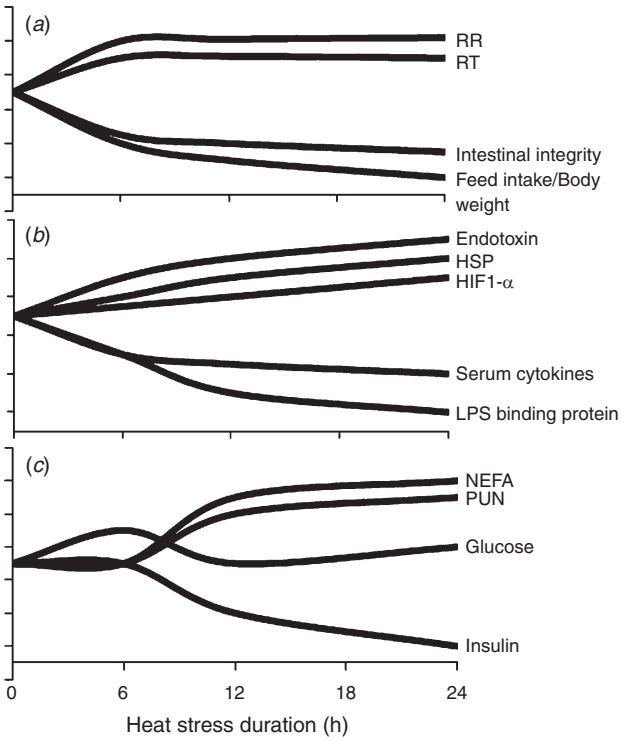

Fig. 2. The temporal changes in a growing pig's physiology in response to heat stress. (*a*) Rectal temperature (RT), respiration rate (RR), feed intake (FI), and bodyweight (BW). (*b*) Tissue hypoxia-inducible factor 1-α (HIF 1-α), heat shock protein 70 (HSP 70), serum cytokines, serum endotoxin, and plasma LPS-binding protein (LBP). (*c*) Changes in blood glucose, blood insulin, plasma urea nitrogen (PUN), and non-esterified fatty acids (NEFA) first 24 h of heat stress.

Conclusion

Collectively, changes in the phenotypic, cellular stress, and metabolic responses to heat stress within 2–24 h of exposure in growing pigs is summarised in Fig. 2.

Over time, pigs increase their core temperature and respiration rate under heat stress. As a result, there is depression in feed intake and reduction in BW gain. With that, the cellular stress responses increase and blood endotoxin concentration is increased. Interestingly inflammatory markers and the acute phase protein, LPS-binding protein, are decreased in a time-dependent manner.

Metabolically, major changes in markers of catabolism (non-esterified fatty acids, blood urea nitrogen) have not been observed until after 6 h of heat stress, after which they sharply increase. This is consistent with time course LPS challenge work in nursery pigs (Webel *et al*. 1997). Further, blood glucose concentrations fluctuate in response to heat stress, but are maintained in the face of reduced feed intake, whereas insulin begins to decrease after ~12 h of heat stress. Understanding how pigs and other mammals first respond to heat stress will provide novel insights into the development of better mitigation strategies to alleviate the negative impacts of heat stress on pig performance and health.

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Chapter 4: Reviews

An opportunity to revolutionise sow management

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Abstract. Research in any area of animal production can provide the opportunity to change how the system operates and is managed. The reliance on having to wean lactating sows to re-mate them has limited the commercial options for sow management. The desire to limit lactation length to maximise the litters per sow per year concurrently creates major challenges for such-aged piglets weaned abruptly. These issues are discussed in the review. This management system also fails to recognise that sows have the potential to spontaneously ovulate in lactation even when housed in farrowing crates. Inhibition of luteinising hormone release is the basis of lactational anoestrus with the suckling stimulus providing the strongest afferent signal to this inhibitory system. Any management strategy that reduces this inhibition has the potential to trigger lactational oestrus. In this review, group housing of sows, boar exposure and intermittent suckling are identified as strong stimuli that can promote lactational oestrus. Removing the need to wean sows to mate them offers further opportunities to change the way lactating sows are managed. One option is a two-stage lactation system in which the sows are housed in farrowing crates for the first 10–14 days and then moved to group accommodation for the remainder of lactation. This system provides welfare benefits for the litter in the early stage of lactation and then the benefits of less confinement for the sows in later lactation. Group lactation would also lend itself to the implementation of stimuli to assist the mating of sows in lactation, such as piglet separation and/or boar exposure. It also accommodates the mating of sows that spontaneously ovulate in lactation. Removing the need to wean sows to re-mate them provides the opportunity to increase weaning age and implement a gradual weaning, helping to attenuate the post-weaning growth check and potentially limiting antimicrobial use in weaner pigs.

Additional keywords: intermittent suckling, lactation, oestrus, weaning.

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Introduction

Change in the Australian pig industry has accelerated in recent years with consumers, welfare organisations and in particular retail markets, having influenced these changes. The most significant recent on-farm change has been to accommodate ways of reducing sow confinement. The Australian industry has committed to reducing the time sows spend in gestation stalls. However, further pressure to change has to be expected and it is highly probable, that in time, the housing of sows in farrowing crates could be limited to only part of the lactation period.

With the probable drive to further reduce sow confinement, the pig industry will need to evaluate new approaches to sow management. Following the decision to remove or reduce the amount of time sows spend in a gestation stall from the production system, research entities have worked at finding solutions to the problems associated with group housing during gestation. Such foresight should be extended to identifying management options limiting the use of farrowing crates during lactation. In this regard, the welfare concerns associated with sow confinement in gestation and lactation have received extensive consideration in other countries, but especially in the European Union. The European Union made it compulsory to house sows from 4 weeks after mating to 1 week before farrowing in groups (Einarsson *et al*. 2014). In Sweden, sows are required to be loose-housed during both gestation and lactation. Einarsson *et al*. (2014) made an extensive review of group housing of lactating sows, especially in Swedish housing systems. Some key points made in the review about loose housing in lactation were: the variable oestrus occurrence before weaning, a higher pre-weaning mortality, the potential for some sows to experience stress especially in large groups, and a higher return to re-breeding rate after mating.

It has been proposed that for lactation housing, there often exists a conflict between sow and piglet welfare and the profitability of sow performance (Cain and Guy 2006). The reliance on having to wean sows to re-mate them limits the options available in managing the breeding herd. The current practice is for producers to limit lactation length to maximise the number of litters per year. The necessity to wean sows to re-mate them is based on the long held principle that sows are naturally anoestrus during lactation. The basis of lactational anoestrus is the lack of luteinising hormone (LH) support for pre-ovulatory ovarian follicle development and so sows fail to come into oestrus and ovulate (Armstrong *et al*. 1988). The paradigm that sows remain anoestrus during lactation needs to be challenged. Recent evidence (discussed later) indicates that some sows spontaneously ovulate in lactation

and, under some circumstances, the higher than anticipated rate could require a rethink of how sows are managed in the peri-weaning period.

In proposing a model to account for anoestrus in lactation, the concept of an inhibition threshold is fundamental (Fig. 1). An analogy would be to consider it as a barometer with the threshold being the level above which neural inhibition limits hypothalamic-pituitary regulation of LH secretion. There are a range of factors that impinge on the threshold and while these collectively maintain the inhibition above the threshold level, sows will remain anoestrus. The central role of the suckling stimulus to this inhibition is obvious, when this is removed at conventional weaning (Quesnel and Prunier 1995). However, the threshold for effective neural inhibition of LH release is modified by other factors such as genotype, individual responsiveness, parity, the season, sow metabolic state, sow feed intake, group housing and boar exposure. It is the collective inputs of these factors, whether positive or negative, which decide what the level of inhibition on LH release would be.

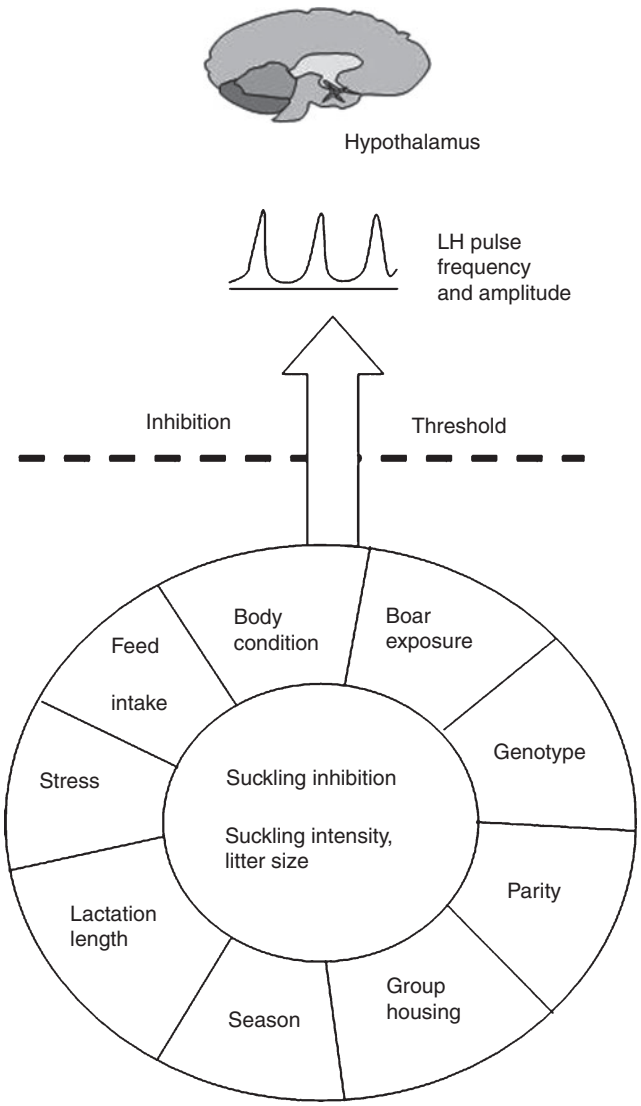

Fig. 1. A diagrammatical representation of the inhibition threshold above which luteinising hormone (LH) pulse frequency and amplitude is limiting and results in lactational anoestrus in the sow. A range of factors in concert act to determine the degree of inhibition and if this is above the threshold sows remain anoestrus. Central to the degree of inhibition is the suckling stimulus.

If the need to wean sows to re-mate them could be removed, some of the management opportunities would be:

(1) Wean at a later age and avoid some of the negative effects associated with 'weaner setback' and reduce or help eliminate antimicrobial use in weaner pigs.
(2) Introduce a gradual weaning strategy rather than abrupt weaning.
(3) Identify and mate sows that spontaneously ovulate in lactation by using positive stimuli to synchronise a group lactational oestrus.
(4) Use of a two-stage lactation system that includes the benefits of a farrowing crate in early lactation, and then the welfare benefits of reduced sow confinement in groups during late lactation.

The value of the farrowing crate to early piglet survival remains relevant but probably for only part of the lactation period. The conundrum for the pig industry is whether the drive for reduced sow confinement can be achieved while maintaining herd productivity, including maximising the annual number of litters per sow. There are other reasons (discussed later) that may also force a rethink of how sows are managed. For example, abrupt early weaning of piglets is a major challenge to their growth and health (Pluske *et al*. 1997) and sometimes requires the use of antibiotics or where possible, effective bioactive non-antibiotic alternatives (Lallès *et al*. 2009).

The overall purpose of this review is to evaluate the potential for changing sow management, by removing the need to wean sows to have them re-mated, and discuss how this could be incorporated into new housing systems that allow for an extended lactation period, a more gradual piglet weaning, and a reduction in sow confinement.

Piglet performance after weaning

A shorter lactation and an abrupt weaning strategy create major problems for the piglet. The dramatic change in diet results in a lower nutrient intake, poorer growth rate, changes in gut morphology and function and stress, collectively increasing the susceptibility to gastrointestinal disease (Van Beers-Schreurs *et al*. 1992; Pluske *et al*. 1997; Weary *et al*. 2008), and sometimes an associated increased antimicrobial use (Kil and Stein 2010). The probability that in-feed antibiotic use will be limited by legislation as in Europe, or even retail supply chain constraints, provides further impetus to change current weaning practices.

In nature, weaning is completed over a period of 3–4 months (Bøe 1991), and during this gradual weaning process the sow has control over the suckling frequency (Jensen and Recen 1989). As this occurs the sow gradually escapes from the suckling-induced inhibition of LH release and returns to oestrus and is mated. Although the pork industry cannot be expected to delay weaning for an excessively long period, there may be opportunities to change lactation management by

weaning somewhat later than currently practiced to avoid some of the problems associated with abrupt weaning at a younger age.

The consumption of creep feed before weaning has been shown in some studies to influence gastrointestinal function of piglets at weaning, and Pluske *et al*. (1995) identified a positive relationship between villous height of the small intestine mucosa and weight gain after weaning. The function of the epithelium is affected by the atrophy of villi, and post-weaning diarrhoea is a response due in part to higher bacterial pathogen loads (e.g. *E. coli*) causing damage to the epithelium (Pluske *et al*. 1996). Pre-weaning creep feed intake has been found to correlate positively to post-weaning villous height in some studies (Van Beers-Schreurs *et al*. 1998) but not others (Hampson 1986). Using an intermittent suckling (IS) protocol of 12 h/day from Days 16 to 27 of lactation, the net absorption in the small intestine was found to be significantly higher in piglets that consume creep feed compared with those who had not consumed creep feed (Kuller *et al*. 2007*b*). Creep feed intake during a conventional short lactation is low and very variable (Appleby *et al*. 1992), and an older weaning age increases post-weaning feed intake and helps attenuate the reduced growth rate seen following abrupt earlier weaning (Leibbrandt *et al*. 1975). Intermittent suckling in combination with an extended lactation, could act to promote a gradual increase in creep feed intake and may reduce intestinal dysbiosis and allow the epithelium to adjust to the solid diet before weaning (Berkeveld *et al*. 2009).

In general, although the benefits associated with increasing pre-weaning creep feed intake are likely at any age they are more likely to be realised with weaning ages of 4 weeks or older and are not compatible with an abrupt weaning at an early age. An older weaning age and gradual weaning are management strategies that can be used to stimulate pre-weaning creep feed.

Group housing of sows in lactation

Group housing and lactational oestrus

It has been known for more than 50 years that group housing of sows resulted in lactational oestrus (Eames 1964). A reduced suckling intensity is likely to be part of the reason for this response, but other factors involved could be sow feed intake and body condition, time of grouping, season, litter creep feed intake and boar exposure. Rowlinson and Bryant (1981) grouped sows at 10, 15, 20 and 25 days of lactation with boars being introduced at the same time. All but one sow displayed lactational oestrus with the time from grouping to oestrus being similar when grouping was at 10, 15 or 20 days (range 9.2–12.2 days), but was shorter when grouping occurred at 25 days (7.3 days average). Later grouping (Day 25) reduced the time to lactational oestrus suggesting that as lactation progresses the inhibition threshold on LH release is attenuated.

In the studies of Hultén *et al*. (1995), under commercial farm conditions, sows were farrowed in individual pens and then housed as groups (11–22 sows) from 2 to 3 weeks until weaning on Day 38 of lactation. On another farm, 21 sows were housed in individual pens throughout lactation and weaned on Day 34 of lactation. In both systems there was no boar contact. Using a rise in faecal progesterone as the measure of ovulation occurrence, the frequency of ovulation varied between groups (0–54%) and the rate was largely dependent on sow parity, with no primiparous sows ovulating and 48% of sows being parity five or greater having ovulated. Only 65% of sows identified to have ovulated in lactation showed post-weaning oestrus within 10 days. By contrast, 87% of group-housed sows and 100% of individually housed sows that were anoestrus in lactation were mated within 10 days of weaning.

Again under commercial conditions, Hultén *et al*. (1998) reported that when multiparous sows were grouped (8–12 sows) at 2 weeks in lactation and compared with individually penned sows, and with both groups weaned at 5–6 weeks, the percentage of sows mated 10 days after weaning was lower on farms using group housing, and the difference was most evident in sows with parities less than five. Repeat breeding and failure to farrow were common reasons for culling on the group farms. The supposed breeding failure could be due to lactational oestrus stimulated by grouping and the subsequent long weaning to oestrus intervals.

The results from these studies highlight an essential problem with sows that spontaneously ovulate during lactation. These sows will have a long return to oestrus interval after weaning and this variation would create an obstacle for batch farrowing. Additional stimuli of group-housed sows could be used to reduce the spread in timing of oestrus.

Group housing, boar exposure and lactational oestrus

In an experiment to explore the effects of group housing and boar exposure on the incidence of oestrus in lactation, commercial lactating sows (23 primiparous and 157 multiparous) were housed singly until group-housed (5–7 sows per group) at 3 weeks and then weaned at 6–7 weeks (Rowlinson *et al*. 1975). Boars were introduced around the time of grouping. All 180 sows showed lactational oestrus at an average of 11.1 ± 0.3 days after grouping. With the interval from farrowing to oestrus being 35.5 ± 0.5 days, a total of 2.35 litters per sow per year would be possible indicating that the extended lactation and later weaning was still compatible with good average annual sow productivity. With the unsupervised boar mating, the conception rate was 84.9%.

In a later study, sows were placed in groups of five on Day 15 of lactation with suckling litter size varying from 7 to 13. Boars were introduced at the time of grouping and weaning was on Day 42 of lactation (Bryant *et al*. 1983). Oestrus was recorded in 77% of sows at 10.2 ± 3.3 days after grouping. Piglet creep feed intake accounted for 44% of the variation in the incidence of lactational oestrus, whereas sow weight change from grouping to weaning and sow weaning weight both accounted for 33% of the variation in the incidence of lactational oestrus. Sows that took longer to show oestrus had heavier piglets at grouping and more piglets at weaning. The sows which gained more weight to weaning and whose litters consumed more creep feed were more likely to display oestrus than sows that gained less weight and whose litters consumed less creep fed. Only 53% of the sows mated in lactation actually farrowed on time with 18% returning to oestrus at a normal time (18–23 days), and 29% returned outside the normal expected range. The sows were mated during Days 21–28 of lactation and weaned on Day 42. This meant that

for individual sows the weaning would have occurred over Days 14–21 of the subsequent gestation. The stress of weaning at the time pregnancy is being established could be a concern and a possible reason for the high percentage of sows returning to oestrus (Einarsson *et al*. 1996), as the majority of embryonic death occurs before Day 18 of pregnancy (Lambert *et al*. 1991).

Group housing, sow metabolism and lactational oestrus

The role that lactation nutrition has on sow reproductive performance has received considerable attention (Dourmad *et al*. 1994; Thaker and Bilkei 2005), but the role it has on lactational oestrus is less well documented. The role sow feed intake in lactation had on the rate of oestrus induction in multiparous sows was reported by Rowlinson and Bryant (1982*b*). Sows were group-housed at Day 20 of lactation and fed to appetite (8.1 kg/day), to requirements (6.1 kg/day), or restricted to 0.70 of requirement (4.2 kg/day). For sows on these feeding regimens the lactational oestrus rates were 68%, 73% and 18%, respectively. Although only small numbers of sows were used in the study, the results suggest that a high feed intake and limited weight loss is associated with a high incidence of lactational oestrus. Sow feeding behaviour is likely to be modified in group-housed lactating sows but the degree would be influenced by social position, group size, space allocation, and parity (van Nieuwamerongen *et al*. 2014). There is some evidence that when group-housed lactating sows are faced with some of these obstacles they can modify their behaviour in an effort to maximise feed intake and meet their nutrient needs. When small groups of sows ($n = 4$) were housed in pens with a single feeder, and allocated a large pen space (8.6 m^2 per sow) or a small space (1.34 m^2 per sow), the daily feed intakes were similar (Burke *et al*. 2000). Sows in the smaller pens made less visits to the feeder, had a lower total feeding time, but consumed larger meals when compared with the sows in the larger pens.

Sow-controlled housing

In this type of housing system, also referred to as ‘get-away systems’, sows have access to a communal area and can dictate the time they spend with their litter. In such systems, there is a large individual variation in the time sows spend with the litter (Pajor *et al*. 2000). Rantzer *et al*. (1995) recorded the time that sows and piglets were separated in a sow-controlled housing system, to be 25% at Week 2 of lactation and increased to 75% at Week 4. In a sow-controlled housing system, piglets were found to have higher creep feed intake and post-weaning feed intake (Weary *et al*. 1999). This type of housing can also benefit the sows because although they ate less they have a similar weight loss and weaning to oestrus interval as sows housed conventionally (Weary *et al*. 2002). In any group lactation housing, but especially sow-controlled housing, there is the potential for sows to self-wean and in multi-suckling systems where litters are grouped, for piglets to cross suckle.

Piglets competed for milk by cross suckling when sows had a communal area and piglets were allowed to mingle from Day 7 of age, and this was found to encourage sows not to nurse their litters and to self-wean (Pedersen *et al*. 1998). When mixing was started later at 14 days, the cross-suckling rate was found to be low (Weary *et al*. 2002). It is possible that the maternal bond and sow-litter recognition are better established by Day 14 of lactation. Sows that were group-housed from Day 5 to Day 26 of lactation had the same suckling frequency as sows individually housed in pens (Bohnenkamp *et al*. 2013). However, there was a reduction in suckling frequency as lactation progressed and this occurred earlier in primiparous sows (Day 18) than in multiparous sows (Day 22). The issues of a sow self-weaning and suckling disruption could be expected to have effects on litter performance. Hultén *et al*. (1997) measured litter performance when sows were group-housed or individually housed. No difference in litter growth rate was recorded at weaning or at 2 weeks after weaning. Depending on their age, piglets may compensate for any reduction in milk supply by increasing creep feed intake.

In summary, there is sufficient evidence to indicate that group housing during lactation can stimulate spontaneous ovulation in sows. However, the rate can be highly variable. From the information available, factors such as sow parity and metabolism (feed intake and bodyweight changes), litter suckling intensity (litter size and stage of lactation), litter creep feed intake and boar exposure are all known to have an influence. It is likely that any group lactation system may need to include a strategy to manage spontaneous ovulation.

Limited nursing: intermittent suckling

The concept of limiting the period of suckling to induce oestrus is not new. It was initially referred to as restricted nursing but more recently as IS. Intermittent suckling is a practice whereby the sows and piglets are separated for a period of each day during part of the lactation.

Sow reproductive performance

Smith (1961) used parity two sows and showed they would display oestrus 6–7 days after implementing IS for 12 h/day in lactation but not when it was only 8 h/day. Stevenson and Davis (1984) reported that 65% and 50% of sows had lactational oestrus when daily separation was for 6 h and 12 h, respectively. Newton *et al*. (1987) found in one trial that 65% of sows showed oestrus after 3 h/day IS and this increased to 79% when the separation was for 6 h/day. In a second trial the success rate was only 16% after separation for 6 h/day.

Various researchers have examined different aspects of IS. Soede *et al*. (2012) compared sows subjected to IS treatment (12 h/day) for 7 or 14 days starting on Day 19 of lactation with weaning on Day 26 or Day 33, respectively, or 7 days of IS starting on Day 26 with weaning on Day 33. Sows had no boar exposure but were subjected to boar auditory sounds. When IS was started on Day 19, lactational oestrus rate was 50% for 7 days of treatment and 64% when it was 14 days of treatment. When implemented for 7 days starting on Day 26 of lactation, the oestrus rate was 61%. There were parity effects with oestrus rate in first parity sows being 23%, second parity sows 85%, and older parity sows 61–65%. Sow weight loss, back fat loss, litter weight and litter feed intake had no effect on the rate of lactational oestrus. In this study, sow weight loss was 7.5% for parity one sows, 5.5% for parity two sows, and 6.3% for older parity sows, but in each case these were less than the suggested 10–12% weight loss needed to affect fertility (Thaker and Bilkei 2005).

Mating sows in lactation and continuing IS treatment for a further 9 days after mating had no effect on subsequent litter size. When the IS duration was extended for 23 days after mating, embryo survival rate (54% vs 70%) was lower (Gerritsen *et al*. 2008*b*). Although this suggests that prolonging IS could create concerns it would need to be confirmed because in this study the IS was started on Day 20 of lactation and the comparable control sows were weaned on Day 27 and mated later. A later study by Gerritsen *et al*. (2009) reported that pregnancy rate and farrowing rate tended to be higher when sows were weaned immediately after being mated in lactation compared with sows that continued to suckle for 20 days after mating.

Highly prolific genotypes with different breeding goals have been used in some IS studies. When commercial TOPIGS-40 sows (Topigs, Helvoirt, The Netherlands) were exposed to IS treatment for 12 h/day during Days 14–45 of lactation, oestrus was observed in 100% of the sows and 93% of these had ovulated (Gerritsen *et al*. 2008*a*). Interestingly, although 17 of 21 control sows (74%), which were weaned on Day 21 of lactation showed oestrus after weaning, five of the remaining six sows (21%) were reported to have ovulated before weaning on Day 21. Furthermore, the rate of oestrus induction after 12 h/day of IS treatment during Days 14–28 of lactation, with half the sows having fence-line boar contact during the period of separation, was investigated in TOPIGS-20 sows (Langendijk *et al*. 2009). Over the 14 days of IS treatment only 32% of the sows had a lactational oestrus and the boar exposure had no effect. Close to one-third of the IS-treated sows that remained anovulatory had large ovarian follicles similar to the sows that had ovulated. However, the plasma oestradiol concentrations increased in the ovulatory sows but not the anovulatory sows, suggesting that in the latter group, the ovarian follicles might be in the process of atresia. Although the differences between these two TOPIGS lines could be due to their genetics, the absence of a control conventionally weaned comparison group in the later study leaves this open to question.

Research interest by the author's group in lactational oestrus was derived from an effort to improve weaner performance and limit the post-weaning growth check and use of antimicrobials in weaner management. Many of the issues associated with weaner 'set-back' are due to abrupt weaning at an early age. Increasing weaning age and gradual weaning would help reduce these problems. It was clearly understood that producers would be reluctant to increase weaning age at the expense of reducing annual sow productivity. The only obvious solution to accommodating both would be to mate in lactation.

The initial induction protocol involved giving a PG600 (Intervet, Australia Pty Ltd, Bendigo, Vic., Australia) injection to sows and then physically separating the sow and piglets in the farrowing crate for 16 h overnight and exposing sows to fence-line boar exposure twice daily until oestrus was detected and the sows mated. The concept was tested in a commercial setting and started on Day 20 of lactation with piglets weaned at Day 35 (Downing *et al*. 2009). The performance was similar to sows conventionally weaned at Day 20 of lactation with 20 of 23 (87%) sows mated in each treatment.

Using the initial induction protocol, Downing *et al*. (2011) established that induction could be started as early as Day 14 of lactation but the limit for commercial acceptance (>85% mated) was probably Day 16. The abbreviated results are shown in Table 1. Twenty-one percent of the sows having fence-line boar exposure showed lactational oestrus, and it was first thought that boar exposure alone had an influence. However, two of these sows were in oestrus when the boar treatment was started, indicating they had spontaneously ovulated in lactation. Furthermore, for all the treatments, 100% of the sows were mated in lactation or within 5 days of weaning. This suggested that the failure to have 100% of sows mated by 7 days after conventional weaning could be due to a percentage of sows spontaneously ovulating in lactation. The long period of separation had a negative effect on piglet growth rate and so further consideration was given to what degree of sow and piglet separation would be needed for successful oestrus induction.

These further studies identified that the PG600 injection was not necessary in multiparous sows as piglet separation alone gave similar results. Also, the period of separation could be limited to 3 days of 16 h/day (Downing *et al*. 2012). As part of these studies, an on-farm depopulation of the sow herd provided the opportunity to determine the role of fence-line boar exposure and the rate of spontaneous ovulation in lactating sows. Two groups of sows were conventionally weaned and then transported to an abattoir where the ovaries were collected and the number of functional corpora lutea recorded. Ovaries were collected from 59 sows from replicate one (weaned on Day 29) and 60 sows from replicate two (weaned on Day 25). Of the 119 sows, 10 from replicate one (16.9%) and seven from replicate two (11.7%) had mature

Table 1. The reproductive performance of multiparous lactating sows exposed to fence-line boar exposure and an oestrus induction protocol involving a PG600 injection and physical separation of the litter for 16 h/day (1530–0730 hours) starting on Days 14, 16 or 18 of lactation until oestrus or weaning on Day 26 of lactation

Within rows, values with different letters are significantly different ($P < 0.05$) (Downing *et al*. 2011)

Treatment	Control boar only	Day induction started			*P*-value
		14	16	18	
Sows treated	29	29	28	29	–
Mated in lactation (%)	20.7b	79.3a	92.9a	89.7a	<0.001
Mated within 10 days of weaning (%)	79.3a	17.2b	7.1b	10.3b	<0.001
Total mated (%)	100.0	96.5	100.0	100.0	–
Mated in lactation and farrowed (%)	50.0	71.4	84.6	62.9	0.31
Total born (±s.e.)	13.30 ± 0.53	11.00 ± 0.62	12.24 ± 0.69	12.05 ± 0.56	0.18
Piglet average daily gain Days 14–23 (g) (±s.e.)	230 ± 11a	122 ± 14b	112 ± 11b	127 ± 15b	<0.001

functional corpora lutea at the time of ovary collection. From these observations, 15.1% of the sows had spontaneously ovulated during the period of lactation. A further two batches of lactating sows were provided with twice daily fence-line boar exposure only, starting on Day 15 of lactation. Of the 60 sows that had fence-line boar exposure, 24.6% displayed standing oestrus and had ovulated in lactation. Although there was a boar effect, at this level of exposure it was probably small when consideration is given to the rate of spontaneous ovulation in the sows having no boar exposure. A role for a greater level of boar exposure in any induction protocol became of interest for future studies.

In an effort to refine the induction protocol to its minimal intervention, multiparous sows were allocated to one of four treatments (McDonald *et al*. 2013). Control sows were conventionally weaned on Day 21 of lactation and moved to an adjacent mating shed. In the remaining three treatments, sows were: physically separated in the farrowing crate from their respective litters for three overnight periods of 16 h; separated from their litter for three periods of 8 h by day; or not separated but removed from the farrowing crates and walked to a mating pen where they had direct exposure to boars for 30 min daily. All sows were checked for oestrus twice daily using fence-line boar exposure. Sows were mated by AI at their first oestrus. All induction treatments started on Day 21 of lactation and piglets weaned on Day 28. The condensed results are given in Table 2.

Separation of piglets and sows for 16 h overnight allowed 82% of sows to be mated within 7 days of which 92% successfully farrowed. These results were similar to those of the sows conventionally weaned on Day 21. The shorter separation period of 8 h by day or full physical boar contact were not sufficient to achieve commercially acceptable farrowing rates. The sows separated for 16 h overnight farrowed similar numbers of piglets as the conventionally weaned sows but it tended to be lower for sows separated for 8 h by day.

The negative impact of 16-h separation on piglet growth rate is a concern. Although there has been no research initiated to solving this problem there are potential strategies that could be used. For example, in the author's work, piglets had access to creep feed only from the time of separation. Earlier provision of creep feed, from Day 10 of lactation for instance, could help to limit any effect of separation on growth rate of piglets. An extended weaning age and gradual weaning could also allow piglets time to compensate for any reduced growth rate during the separation period. Although 16-h separation was used in the study of McDonald *et al*. (2013), because it suited management of the labour available, it might be possible to reduce this separation time. Also, although 3 days of separation was used, investigations as to whether less days of separation could be feasible have not been conducted.

In summary, this research has:

(1) Identified that three overnight periods of sow and piglet separation for 16 h, starting on Day 21 of lactation and weaning on Day 28, resulted in acceptable lactation mating and farrowing rates. However, a disadvantage of the 16-h separation is the reduced growth rate of the litter.
(2) Identified that when the separation is for 8 h during the day, separation for 3 days is not sufficient to achieve acceptable mating and farrowing rates.
(3) Shown that a percentage of sows are likely to spontaneously ovulate in lactation.
(4) Shown that boar exposure is a stimulus for lactational oestrus induction but this is minimal when it is fence-line contact; however, it is more extensive when it is direct contact but by itself does not seem to give commercially acceptable farrowing rates. In combination with other stimulants such as group housing or split weaning, boar exposure might be a valuable strategy for oestrous induction.

Intermittent suckling and litter performance

Most IS treatments have used conventional weaning ages of 21–28 days and piglet growth rate is reduced when the separation period is greater than 6 h/day. The lactation weight gain of piglets following IS for 12 h/day for 11 days before weaning on Day 27 was found to be 92% of that of continuously suckled piglets (Kuller *et al*. 2004). The IS litters consumed more creep feed, and after weaning the IS piglets had higher (255 vs 177 g/day) average daily gain (ADG), higher average daily feed intake (ADFI) and, by the end of first week, the post-weaning liveweights were similar. The IS sows lost less

Table 2. The reproductive performance of multiparous lactating sows weaned at Day 21 of lactation (control) or physically separated from the piglets in the farrowing crate for three overnight periods (1530–0730 hours) at Days 21–24, or 3 days during the daytime (0730–1530 hours) at Days 21–24, or sows removed from their farrowing crate to a mating pen and given 30 min of full direct boar contact daily at Days 21–28

All sows had fence-line boar contact twice daily. Within rows, values with different letters are significantly different ($P < 0.05$) (McDonald *et al*. 2013)

	Treatments				*P*-value
	Control[A]	Three nights of 16-h separation	Three days of 8-h separation	Full boar exposure	
Number treated	47	50	47	48	–
% of sows mated in lactation	96.3a	82.0ab	53.2c	75.0b	0.002
Days to first mating (±s.e.)	4.3 ± 0.2b	5.5 ± 0.2a	5.0 ± 0.3ab	5.1 ± 0.3ab	0.01
% of sows mated that farrowed	79.5	92.3	71.4	73.5	0.1
% farrowed of total sows treated	73.8ab	75.0a	32.6c	54.3b	<0.001
Total litter size born (±s.e.)	13.5 ± 0.4	12.4 ± 0.6	10.81 ± 0.9	12.7 ± 0.6	0.06
Piglet average daily gain Days 21–28 (g) (±s.e.)	70 ± 10c	191 ± 10b	220 ± 8ab	244 ± 9a	<0.001

[A]For the control the performance measures are for sows conventionally weaned on Day 21 of lactation.

bodyweight during lactation (10 vs 16 kg) although this was not evident in parity one and two sows.

Individual creep feed intake is, however, highly variable. In one study it ranged from 8 to 1056 g/piglet when litters were exposed to 12 h/day IS treatment over Days 14–24 of lactation and only 9–513 g/piglet for litters continuously suckled, both being weaned at Day 25 (Kuller *et al*. 2007*a*). The IS-weaned piglets had a higher ADFI in the first 2 weeks after weaning but it was similar to controls from this point until slaughter. The IS piglets were weaned 1 kg lighter but they had higher post-weaning ADG and by the end of the first week, had compensated for the lower weight before weaning.

Using chromium oxide as a faecal marker, piglets were identified as 'eaters' and non-eaters' of creep feed to examine the impact of IS on creep feed consumption (Kuller *et al*. 2007*a*). Interestingly, similar percentages of 'eaters' were seen in IS- and continuously suckled litters (23% vs 19%, respectively). The IS treatment failed to stimulate more piglets to eat creep feed but increased the amount eaten by those predisposed to eating. For those piglets designated as 'eaters' the ADG was higher by Day 52 of age and they remained heavier to slaughter. To attenuate reduced piglet growth rate during IS treatment, an answer might be to find a way to encourage so-called 'non-eaters' to become 'eaters' of creep feed.

Although a single episode of fasting for 6 or 12 h has no effect on gut morphology (Hartke *et al*. 2005), IS-treated piglets are subjected to repeated periods of fasting. Nevertheless, IS treatment for 8 h/day over Weeks 3–5 of lactation had no effect on post-weaning gut function (Nabuurs *et al*. 1993), and 2 weeks of IS treatment with creep feeding before weaning on Day 32 prevented post-weaning villous atrophy (Nabuurs *et al*. 1996). A 7–14-day period of IS treatment combined with an extended 33-day lactation prevented the villous atrophy on Day 2 after weaning compared with conventionally weaned piglets (Berkeveld *et al*. 2009).

Collectively, these data suggest that extending the lactation length needs to be associated with increased creep feed intake if benefits are to be realised for pigs after weaning. The data also suggest that any reduction in ADG during IS treatment is rapidly compensated for after weaning.

Intermittent suckling, behaviour and welfare

Some detractors of using piglet separation to induce an oestrus in lactation have questioned the welfare implications for the sows and piglets, especially the potential for udder damage from excessive piglet attention after rejoining. Berkeveld *et al*. (2007) found that on the first day (Day 14 of lactation) of IS treatment, where the sows were removed from the farrowing pens, total piglet activity and vocalisation was at its highest. The authors proposed that this was likely due to the sudden separation of the sows from the piglets. By Day 16 of lactation the total piglet activity had decreased and stabilised for the remainder of the lactation, with the 24-h pattern of piglet activity being synchronised with the sow's presence or absence. The decrease in total activity over the first 2 days could be viewed as the piglets becoming more accommodating to absence of the sows.

Kluivers-Poodt and colleagues (2010) measured plasma cortisol in sows conventionally weaned at Day 21 and sows subjected to IS for 12 h/day (IS12) over Days 14–21 of lactation. Plasma cortisol was determined for the day before weaning or start of IS treatment, and then on Days 2, 7 or 14 after treatment started. There was a change to the normal circadian rhythm in cortisol concentrations in both treatments. Instead of the normal decrease expected during the daytime, there was a significant increase after weaning or the start of IS treatment. The normal rhythm was quickly restored by Day 2 in the treatments. The authors concluded that IS separation for 12 h/day caused a short acute stress response similar to that experienced by sows following weaning.

Kearns *et al*. (2011) investigated the behaviour of sows and piglets when separated during IS treatments. In one study, piglets were separated from their sows overnight between 1600 hours and 0800 hours for 5 days. The multiparous sows and litters were housed in individual farrowing crates and the piglets had access to a communal area behind four adjacent farrowing crates. Litters were restricted to this communal area during separation and had full access to the communal area and all four farrowing crates during the non-separation period. Alternatively, a physical 'within-pen' separation was achieved in four further farrowing crates by placing a board in the crate between the sow and piglets. Video cameras were set up over each pen and a range of behaviours was recorded, as were the relationships between piglet behaviours and sow activity. During the period of separation, and on all days, there was a high probability that the piglets spent their time lying passively and this was not influenced by whether the sow herself was active or inactive (Fig. 2). In general, both methods of overnight separation showed similar results and indicated that separation was not associated with any behavioural patterns indicative of piglet or sow distress.

In a recent report, Downing *et al*. (2015) provided details of a further evaluation of piglet and sow behaviour during IS treatment. In this work there were two treatments: a control group of sows, which had a conventional lactation, and a group of sows that were subjected to IS for 7 days (8 h/day) before weaning at 28 days of age. The piglets were physically separated from the sow in the farrowing crates. Saliva samples were collected from each sow and from four focal piglets in each

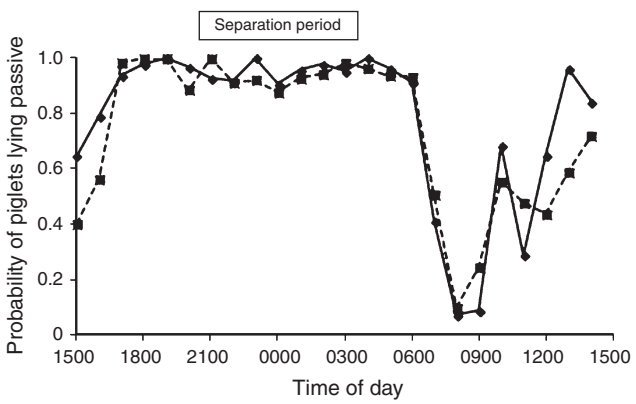

Fig. 2. The probability that piglets would be lying passively when sows were active (■) or not active (◆) while separated (1600–0800 hours) or together (0800–1600 hours) over 5 days. The sow's level of activity had no association with the piglet activity (Kearns *et al*. 2011).

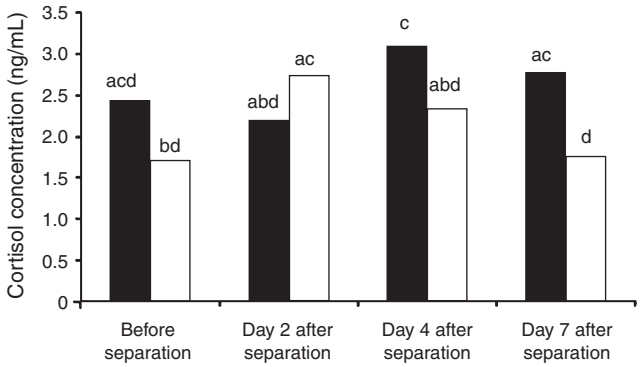

Fig. 3. The mean salivary cortisol concentration from control (■) and intermittent suckled (IS) (□) piglets. Control piglets remained with the sows throughout from Day 21 of lactation until weaning on Day 28. In the IS treatment, the sow and piglets were separated in the farrowing crate from 0800 hours to 1600 hours on Day 21 to Day 28 of lactation. A basal sample was taken on Day 21 (0600–0700 hours) before the start of treatments (before separation) and then at 1200–1300 hours on Days 2, 4 and 7 after the start of separation. The treatment × day interaction was significant ($P = 0.01$). Between and within treatments the values without common letters are significantly different ($P < 0.05$) (Downing *et al*. 2015).

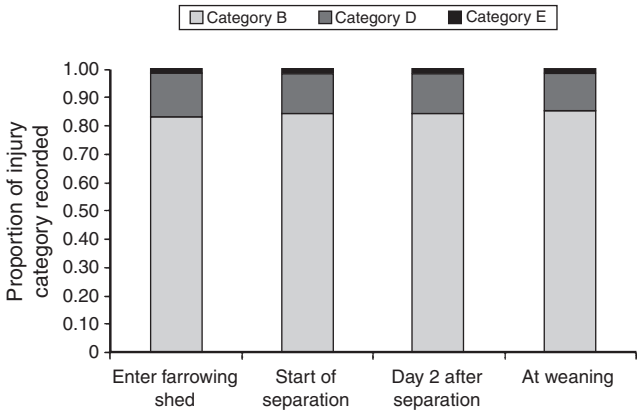

Fig. 4. The mean proportions of different udder injury scores for sows separated from their litters in the farrowing crates from 0800 hours to 1600 hours each day on Days 21–28 of lactation. The injury to each mammary gland was evaluated on the day the sows entered the farrowing shed (Days 110–112 of gestation) at the start of the intermittent suckling (IS) (Day 21), 2 days after the start of IS (Day 24), and then at weaning on Day 28 of lactation. Udder scoring consisted of counting the number of functional teats and identifying and recording the wounds present. Wounds were categorised as: A, fresh scratches or cuts; B, old scratches or cuts; C, bruising or welling; D, having a damaged teat but still functional; and E, having a damaged teat and not functional. The proportion of category A injuries is not shown as this was less than 0.45% of the total injuries, and no category C observations were recorded. The majority of injuries were category B (~84%), followed by category D (~14%) and a small proportion of category E (~1%). These proportions did not change from the start of IS until weaning (Downing *et al*. 2015).

litter before separation and on Days 2, 4 and 7 after separation started. Udder injury scoring of all sows was carried out when the sows entered the farrowing crate and then before the start of piglet separation, on Day 2 after separation and at the time of weaning. For piglets, the IS treatment had an effect on salivary cortisol (Fig. 3), but this changed with time as the interaction with day was significant ($P = 0.01$). On Day 2, the IS piglet cortisol concentration was significantly higher than the concentration before separation. The concentrations on Days 4 and 7 were similar to the concentration before the start of separation. Cortisol concentrations for control piglets were similar to IS piglets at all sampling times except on Day 7 when it was significantly higher, but this could be accounted for by piglets having been handled to administer an oral vaccination before sampling. There were no differences in sow salivary cortisol concentrations and on all days these were less than 1 ng/mL. The pattern of cortisol concentrations in the IS piglets suggested that although the separation had caused some degree of acute stress in the first 2 days this was not evident by Day 4 or Day 7 after the start of separation. No differences in udder injury were observed from the start of separation until weaning (Fig. 4).

A concern for the IS procedure is that limiting the number of sucklings in lactation could result in udder engorgement and discomfort to the sows, however Downing *et al*. (2015) provided no evidence to support this and based on the physiology of sow lactation, it is not likely. The epithelial cells of the alveoli secrete the milk components into the alveoli and when the lumen is filled the cells shrink and their secretory activity ceases. Shortly after the milk is removed, the cells increase secretory activity until the alveoli is again filled (Frandson *et al*. 2003). Milk production by the sow is closely related to the number of functional mammary glands, level of suckling intensity and resultant milk withdrawal from individual mammary glands (Hartmann *et al*. 1997). It is thought that local autocrine inhibitory mechanisms control the rate of milk synthesis within individual glands (Peaker 1995), and this depends on how much milk the piglet is able to remove from the gland in the limited let down period. This helps to explain the linear relationship between litter size and the increase in milk production following an increased suckling stimulus (Auldist and King 1995). Under these regulatory mechanisms it is not likely that milk production continues unabated when piglets are separated from the sow during IS treatment.

Use of boar contact as a stimulus to induce lactation oestrus

Split weaning, the practice of reducing the number of piglets suckling for some period before weaning, has been used to enhance pre-weaning ovarian follicle development (Zak *et al*. 2008). Removing part of the litter 2–7 days before weaning reduces the weaning to oestrus interval (WOI). Using parity one and two sows, reducing the litter size to six for the 7 days before weaning on Day 28 of lactation lowered the WOI from 5.6 days to 5.1 days with the difference more obvious in parity two sows (5.4–4.6 days) than in first parity sows (5.9–5.6 days) (Vesseur *et al*. 1997). Matte *et al*. (1992) made a statistical evaluation of the relevant data in the literature and found that most of the variation in WOI interval after split weaning was due to the number of piglets left to suckle, and the shortest re-breeding interval would be achieved by leaving three piglets on the sow for 4.7 days before weaning.

Terry *et al*. (2013) investigated the rate of lactational oestrus in sows following different degrees of split weaning combined

with fence-line boar exposure. On Day 18 of lactation piglets were removed from litters so sows continued to suckle litters of 10, 7, 5 or 3 piglets. From Day 18 of lactation sows were physically removed from their farrowing crates and moved to a mating area where they were given 15 min of fence-line boar exposure. Sows were weaned on Day 30 of lactation. Sows suckling three, five or seven piglets had lactational oestrus rates of 83%, 89% and 94%, respectively, with 56% of the sows suckling 10 piglets having lactational oestrus. This suggests there are probably two components to reducing the inhibition on oestrus, the reduction in suckling intensity and the boar exposure.

Members of the same research group further investigated the effect of boar exposure on lactational oestrus induction. On a commercial piggery large numbers of multiparous and primiparous sows were exposed to full boar contact during lactation (Terry *et al*. 2014). Sows were given 15 min of full physical boar contact or no boar contact starting on Day 18 of lactation with weaning on Day 30. A third treatment was included in which sows had the suckling litter size reduced to seven piglets and were given daily physical boar contact. With boar exposure only, the rate of lactational oestrus was 76% for multiparous sows and 47% for primiparous sows. The values were significantly higher, 89% for multiparous and 61% for primiparous sows, when litter size was reduced to seven piglets. Based on plasma progesterone concentrations, 24% of multiparous and 8% of primiparous sows with no boar exposure had ovulated in lactation. It is clear again that both boar exposure and reduced litter size had an effect. However, a third component needs further investigation. In the work of McDonald *et al*. (2013), the oestrus induction treatment where sows were moved from their farrowing crate to a mating pen and provided with direct boar contact, was observed to cause significant distress to the sows. Sows' movement, mixing and relocation might disturb the suckling pattern in the litters and could help to account for the high percentage of oestrus in multiparous sows (24%) moved from their crates but having no boar contact (Terry *et al*. 2014).

Parity as a constraint to lactational oestrus induction

An obstacle to implementation of any IS protocol is the variation in response between multiparous and primiparous sows. Soede *et al*. (2012) found that when sows were exposed to IS treatment (12 h/day) for 7 days before weaning, the lactation oestrus response for primiparous sows was only 23% compared with 85% for second parity sows and 60–65% for older parity sows. Terry *et al*. (2014) stimulated oestrus by removing sows from their farrowing crates and providing them with daily direct boar contact, over Days 18–30 of lactation. This level of boar exposure resulted in 76% of multiparous sows and 47% of primiparous sows achieving lactational oestrus. Interestingly, when litter size was reduced to seven and boar exposure used, the oestrous rates increased to 89% for multiparous sows and to 61% for primparous sows. Hultén *et al*. (2006) group-housed sows during 3–7 weeks of lactation, and found that parity had a significant effect on the rate of lactational ovulation with it being 17%, 50%, 44% and 83% for parities one, two, three and four, respectively. For multiparous sows a single stimulus might be sufficient to induce lactational oestrus but with primiparous sows, multiple stimuli might be needed to achieve acceptable oestrus results. The response of primiparous sows is currently an issue that is likely to limit implementation of mating in lactation.

Could spontaneous ovulation in lactation be limiting sow productivity?

As mentioned previously, the collection of ovaries from recently weaned sows indicated that around 15% had ovulated during a 25- or 29-day lactation. There are other reports of similar events occurring in group-housed sows. Hultén *et al*. (2006) found that the rate of spontaneous ovulation was 4.7% in the third week of a 7-week lactation. In Weeks 4, 5, 6 and 7 of lactation the rate was 6.1%, 2.9%, 10.8% and 24%, respectively. Rowlinson and Bryant (1982*a*) found that 17% of sows housed in individual pens had ovulated during Days 20–42 of lactation.

With Swedish Yorkshire × Swedish Landrace gilts, ovarian activity was monitored over the first four parities while being housed in groups of four, during Weeks 3–7 of lactation (Hultén *et al*. 2006). The sows and litters were housed outdoors during spring and summer and indoors during autumn and winter. Of the 124 sows recorded, 52 had ovulated once during the 3–7 weeks of grouping and eight sows had two ovulations during the same period, and this together caused 47% of the sows to ovulate in lactation. Sow parity had a significant effect on the rate of lactational ovulation with it being 17%, 50%, 44% and 83% for parities one, two, three and four, respectively. Another major influence was the week of lactation with the distribution of those sows ovulating being 10%, 13%, 6%, 22% and 49% in Weeks 3, 4, 5, 6 and 7 of lactation, respectively. Sows which had ovulated in lactation had a longer WOI than the sows which remained anoestrus (12.6 ± 10.4 vs 5.0 ± 3.8 days). Other factors reported to have a significant association with the rate of lactational ovulation were season (lower in summer than winter), a decrease in the number of piglets per suckling and a higher piglet ADG from Day 14 of lactation until to weaning. Litter size, litter weight change and suckling behaviour were found to have had no association with the rate of lactational oestrus.

Spontaneous ovulation in lactation is not likely to be a major issue in sow herds where weaning is before 21 days of lactation, However, any effort to increase weaning age beyond 3 weeks is likely to be associated with a higher percentage of sows spontaneously ovulating in lactation, and this could be a limit to sow productivity. For two lactational oestrus studies (Downing *et al*. 2011, 2012), the WOI of the sows allocated to the research and weaned at Day 26 of lactation has been plotted (Fig. 5). Sows were allocated into three groups. There was a small number of sows that had very long WOI, and were identified as sows having been transferred from one lactation to the next to function as surrogate dams for fostered piglets. Next, there was a large group of sows considered to have a normal WOI of less than 12 days. Finally, a third group that had WOI of between 13 and 22 days was observed. If a sow had spontaneously ovulated between Days 18 and 26 of lactation she would be expected to return to oestrus between Days 13 and 22 after weaning. In the two examples this rate was found to be

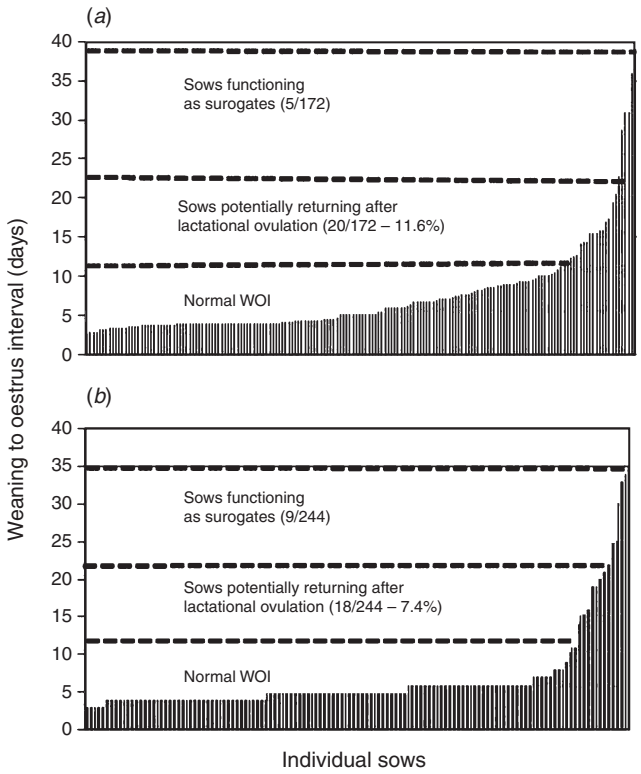

Fig. 5. The individual weaning to oestrus interval (WOI) for two groups of sows (A and B) used in lactational oestrus studies (Downing *et al*. 2011, 2012). For each batch the sows were divided into three groups based on their WOI. The largest group was sows that had a normal WOI of less than 12 days. A second group are sows that had a very long WOI and were identified as having been moved from one lactation batch to the next lactation batch and used as surrogate dams for fostered piglets. In between there is a group of sows that are classed as having a longer than normal WOI, and potentially could be sows which have returned to oestrus after weaning but has possibly spontaneously ovulated in lactation.

11.6% and 7.4%. Although this is speculation, if a producer was to find a persistently high proportion of sows having an extended WOI, than the possibility that these were spontaneously ovulating in lactation needs consideration. Producers' should therefore be aware that it could be a possible reason for sows having a long WOI following a conventional weaning.

A two-stage sow lactation management system

Eliminating the need to wean sows by mating in lactation offers a range of new strategies for sow management. A two-stage lactation management system provides an opportunity to attenuate some of the negative aspects of early abrupt weaning. It would also enable the industry the opportunity to better meet the pressures to reduce sow confinement. Any system needs to accommodate both piglet survivability and behaviour needs of the sow (Guy *et al*. 2012).

The two stages would most likely comprise the farrowing of sows in crates and holding them there for 10–14 days after parturition, and then moving the sows and their litters to group lactation pens for the reminder of lactation. Confining the sows in crates for the early part of the lactation limits their freedom and behaviour but affords welfare benefits for the piglets, with lower mortality (Marchant *et al*. 2000; Baxter *et al*. 2012; Hales *et al*. 2014; Chidgey *et al*. 2015). It also allows for easier management of the litters after farrowing when fostering, routine procedures and intensive care of smaller and less viable piglets is needed. In the later stages of lactation when piglets are more mature and robust to avoid sow overlay, the welfare benefits of reduced confinement of the sow can be taken advantage of.

Recently, McDonald *et al*. (2015) investigated a system where sows were housed in farrowing crates or PIGSAFE farrowing pens for the first 2 weeks of lactation and then moved to group lactation pens for the following 2 weeks before weaning. Based on several measures, 16–20% of sows spontaneously ovulated in lactation. The group lactation would accommodate the mating of sows spontaneously ovulating but also allows for other stimuli, such as piglet separation and boar exposure, to be reasonably easily implemented. By mating in lactation weaning age could be extended and allow for a more gradual weaning. The benefits of older weaning age and gradual weaning have been discussed previously.

Just as the transition from gestation stalls to group housing during pregnancy required extensive research to determine how this could be managed to maintain sow productivity, the same will be needed if a two-stage lactation system and extended lactation is to be a commercial option to the current use of farrowing crates alone. Some of the concerns that need to be investigated include:

- The best time to transfer sows from the crate to the group lactation pen.
- How primiparous sows would perform under this system.
- The effect of the second phase (group housing) on litter performance.
- Whether group housing in lactation increases the risk for piglet mortality.
- Whether spontaneous ovulation could be a constraint on sow productivity.
- If sows could be successfully mated in lactation as part of an extended lactation.

Conclusions

Current sow management is essentially dominated by the need to wean sows to then re-mate them. This is based on the long held paradigm that sows are anoestrus in lactation. This principle has encouraged producers to wean early to maximise the number of litters per sow per year, but early (and abrupt) weaning creates significant negative consequences for piglets. In this review, several strategies that stimulate sows into oestrus and ovulation in lactation provide producers with opportunities to access new sow management options. In the future, the impetus to change lactation sow management may come from external sources just as it has for the change to gestation sow management. Although there are good reasons for the use of farrowing crates to protect piglet welfare, this really only holds for the early stages of lactation. This review puts forward the proposition for a two-stage lactation system as an option for reaping the benefits of farrowing crates in early lactation and then the benefits to sows of reduced confinement by group

housing in later lactation, and for piglets with potentially enhanced creep feed intake and better post-weaning performance and health. Group lactation also provides an opportunity to use stimuli proven to induce lactational oestrus. Intermittent suckling, boar exposure and group lactation are identified as strong stimuli supporting oestrus in lactation and could be implemented readily in the proposed two-stage lactation system. Mating in lactation will remove the need to wean piglets early and allow not only older age weaning but also gradual weaning.

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Review

Under what conditions is it possible to produce pigs without using antimicrobials?

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Abstract. Antimicrobials are commonly used in pig production to control bacterial infections. However, there is increasing pressure from supermarkets and consumers and other sectors to reduce or eliminate the use of antimicrobials in animal agriculture. Banning the use of antimicrobials in some countries has led to increased disease and welfare problems, so it is important to know under what conditions pigs can be produced without the use of antimicrobials. In this review, practices that can prevent disease, and therefore reduce the need for antimicrobials were researched from published experimental challenge trials, field studies and risk factor analyses. Disease prevention practices were examined from pathogen survival and transmission studies, vaccine and disinfectant efficacy studies and nutrition trials. From these studies we collated the important practices that manage or prevent disease and improve pig health. We also reviewed new diagnostic assays and technologies to better monitor the pig and its environment at the herd level. Many of the conditions necessary to produce pigs without antimicrobials have been known and understood for a long time. The application of high standards of biosecurity and hygiene is crucial for creating the conditions for reduction of antimicrobial use. Factors important in preventing disease include eradication or elimination of pathogens, minimising mixing of pigs, cleaning and disinfection of pens and sheds, ventilation to improve air quality, reducing stocking density and eliminating potential vectors of disease. Improving the health of pigs also relies on vaccination and improved consistency of nutrition. The development of diagnostic technologies that correlate with disease and production will enable the detection of potential disease problems at the individual or herd level before disease outbreaks occur and before antimicrobials are needed. The development of vaccination technologies for prevention of disease and diagnostic technologies that can be used on-farm to predict disease outbreaks are integral to safely moving towards antimicrobial-free pork. Pig production without the use of antimicrobials is not simply a matter of substituting conventional antimicrobials with alternative antimicrobial substances and expecting the same result. Any move to antimicrobial-free production requires an acknowledgement that pig production costs may increase and that many pig production practices must change. Such changes must also ensure that animal welfare and food safety and quality standards are maintained or improved, and that reliable markets for the product are found. This paper does not seek to argue the science or opinion of reasons behind the desire to reduce antimicrobial use in animal agriculture, but rather discuss the circumstances under which reduction or elimination of antimicrobial use in pig production is possible.

Additional keywords: biosecurity, diagnostics, hygiene, prophylaxis, therapy.

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Introduction

Definitions

The word ‘antibiotic’ from the Greek anti (against) bios (life) refers to a substance produced by a microorganism that has activity against other microorganisms. This is not synonymous with the term ‘antimicrobial’, which refers to any substance, naturally occurring or synthetic, that acts against a type or types of microorganism (bacteria, virus, fungus, protozoa) (EPRUMA 2013). It is important to clarify that the common usage of the term ‘antibiotic’ can be misleading, in that it could naively exclude reference to the use of many substances used to control microorgansims, and could also aggregate alternative substances to conventional antimicrobials. It is necessary to define these terms and that any distinction between terms is understood if animal industries are to pursue notions of ‘antibiotic-free’ or ‘reduction of antibiotic usage’.

For the purpose of this discussion and to limit semantic argument, when referring to ‘antibiotic’ or ‘antimicrobial’ the intent is reference to naturally occurring, semi-synthetic and synthetic compounds with antimicrobial activity that can be administered orally, parenterally or topically (Phillips *et al*. 2004). Such compounds that are commonly used in swine production in Australia belong to the antimicrobial classes of aminocyclitols, aminoglycosides, β-lactams, ionophores, lincosamides, macrolides, polypeptides, quinoxalines, streptogramins, tetracyclines, thiamphenicols and trimethoprim-sulfonamides.

Alternative antimicrobial substances are commonly used in pig production. These alternatives include acidifiers, enzymes, essential oils, heavy metals, disinfectants, sanitisers, immune modulators as well as probiotics and competitive exclusion products. Such substances are generally not referred to as 'antibiotics' or 'antimicrobials'. This discussion focuses on reduction or elimination of use of the former group of substances, not the latter. It specifically focuses on antimicrobial use, disease management and prevention on commercial pork production farms in Australia.

Reasons for antimicrobial usage

Antimicrobials are commonly used in pig production for therapy, prophylaxis, metaphylaxis and growth promotion. Antimicrobial use for therapy is the administration of an antimicrobial to an animal or group of animals that exhibit clinical disease (EPRUMA 2013). Prophylaxis is the treatment of an animal or a group of animals before clinical signs of disease, in order to prevent the occurrence of disease or infection (EPRUMA 2013). Metaphylaxis is the treatment of a group of animals after the occurrence or diagnosis of clinical disease in part of the group, with the aim of treating the clinically sick animals and controlling the spread of disease to animals in close contact and at risk, which may already be (sub-clinically) infected (EPRUMA 2013). Antimicrobial use for growth promotion is the use of sub-therapeutic levels of antimicrobials to enhance the growth (average daily weight gain and feed efficiency) of animals. Specifically, through an unknown mechanism, an animal given sub-therapeutic doses of antibiotics will, on a lesser quantity of feed, gain an equal amount of weight as an untreated animal.

In terms of the intention of use of antimicrobials, it may be argued that there is little distinction between prophylaxis and metaphylaxis. The use of antimicrobials for these purposes is to reduce or eliminate the occurrence of expected diseases in an individual or within a population. In many ways the reference to antimicrobial use as 'growth promotion' could be argued to be prophylactic or metaphylactic. One argument for this is that the precise mechanism for growth promotion action is unclear (Giguere *et al*. 2013). Several antimicrobials used in pig production are known to have specific activity against particular production-limiting pathogens (e.g. macrolides and *Lawsonia intracellularis*); however, it is often difficult to draw a distinction between types of use of antimicrobials. The macrolides and lincosamides are effective for the treatment and prevention of proliferative enteropathy (McOrist *et al*. 1996, 1997; Moore *et al*. 1997), but can also increase growth rate and feed intake (Pauling *et al*. 1999; McOrist *et al*. 2000).

Therapeutic use of antimicrobials is the use of antimicrobials in response to clinical bacterial disease. Such antimicrobial use is less desirable for good production or welfare outcomes for an individual animal or group of animals because disease has already occurred. Antimicrobial treatment is required to prevent the worsening of disease and to assist recovery from disease. Much of the focus and application of pig medicine is the prevention of disease in order to maximise production and optimise animal welfare. Currently in pig production, the use of antimicrobials in prophylaxis and metaphylaxis is one method of enhancing this aim. However, even with good preventative programs in place (including but not limited to antimicrobial use), clinical bacterial disease still occurs, making therapeutic use of antimicrobials necessary to maintain individual and herd production and welfare.

The consequences of antimicrobial removal on pork production

If antimicrobials for therapeutic, preventative and growth-promotion purposes are simply removed from most pig production systems there is very likely to be costs in terms of reduced production and poorer animal welfare outcomes (Baker 2002). Significant clinical disease problems occurred in the first 4 years after Sweden banned the use of antimicrobial growth promoters (AGP) in 1986, which required increasing antimicrobial usage in up to 75% of piglets (Wierup 2001). The level of use of antimicrobials was halved by 1993 due to improved management on-farm and antimicrobial consumption continued to decrease with the increasing use of alternatives to antimicrobials.

Danish pig veterinarians reported an increased prevalence of proliferative enteropathy after the ban on AGP, which led to an increase in the use of therapeutic antimicrobials and a reduction in average daily gain (ADG) of between 18 and 50 g per day (Jensen 2006). Along with increased weaner mortality, this cost to the industry was estimated to be equivalent to US$1–3 per pig. Over the longer term, the ban on sub-therapeutic antimicrobial dosing and all AGP in 1994 and 2000, respectively, led to decreased antimicrobial consumption in Denmark from 100 mg/kg of pig produced in 1992 to 31 mg/kg in 1999, then up to 49 mg/kg in 2008 (Aarestrup *et al*. 2010). Ultimately, ADG increased for weaning and finisher pigs from 1992 to 2008, but mortality rates for weaning and finishing pigs did not change significantly over this time.

A survey of 73 small herds in Finland (median of 56 sows per herd), following the ban on olaquindox and carbadox as growth promoters in 1999, showed no increase in the incidence of post-weaning diarrhoea (PWD) and no overall increase in the level of antimicrobials used to treat PWD. However, 14% of the herds did increase the level of antimicrobial use to treat diarrhoea, which was predominantly caused by *E. coli* (Laine *et al*. 2004). There was also a trend towards reduced numbers of piglets weaned per sow per year in herds monitored for 12 months after removal of AGP. The absence of other pathogens and the relatively small size of the Finnish herds may help explain the limited impact of antimicrobial withdrawal on PWD incidence and antimicrobial use, with 82% of herds free from *Mycoplasma hyopneumoniae.* Larger herds are routinely associated with increased risk of respiratory disease (Stärk 2000).

Current restrictions on antimicrobial use

There are restrictions and controls on the use of antimicrobials in pig production. Antimicrobials registered in food animals are restricted to the classes or compounds mentioned above. Much of the use of these products is under veterinary control through prescription and dispensing. Non-prescription products' use is controlled through the labelling of products, whereby use is required to be as per the registered labelling of the product (Bond 2005). If the use of non-prescription products differs

from the registered label instructions, this use must be under the direction of a veterinarian. These conditions place considerable responsibility for the use of antimicrobials on the veterinarian. This responsibility extends to veterinarians having a thorough understanding of swine diseases and their epidemiology (Karricker 2011), as well as the reasons for use of antimicrobials. Therefore, the need for a thorough understanding of the pharmacology and efficacy of antimicrobials in pigs is axiomatic. Consequently, veterinarians are required to have genuine veterinary, client and patient relationships whereby the use of antimicrobials on-farm is closely monitored and understood.

Apart from these restrictions on the use of antimicrobials, there are also the considerations of the appropriateness of use and cost. This is where therapy versus prevention becomes important. If therapy is sought for bacterial disease episodes, the pig production enterprise and its attending veterinarian are confronted with ensuring that the choice of antimicrobial is appropriate, will be effective and economical. Effective therapy for bacterial clinical disease is most commonly achieved using antimicrobials, therefore, management of bacterial disease must focus on prevention as well as the use of alternatives to antimicrobials if the industry aims to reduce or eliminate antimicrobial use.

Disease prevention

Hygiene and management of the environment

Many pig-raising facilities do not maintain a high standard of hygiene and would have considerable pathogen loads present in the environment on placement of stock. The role of hygiene is particularly important in multifactorial enteric and respiratory diseases, where the environment also has a major impact on disease severity (Madec 2005). Under these circumstances hygiene practices need to reduce pathogen load, but also have to reduce environmental factors that exacerbate disease expression including production of excessive ammonia in the environment, dust and other pathogens. Commonly, antimicrobials are used to maintain production or limit disease in the face of generalised infections due to poorer hygiene. Improving hygiene will decrease bacterial exposure and reduce the opportunity for compromise of stock that can lead to disease or compromise production. However, the health benefits of improved hygiene can rarely be attributed to a single factor. Stocking rate, herd size, air volume and quality, continuous production, temperature and humidity have all been identified as risk factors for lesions of the respiratory tract in experimental challenge studies and epidemiological surveys (Pointon *et al*. 1985; Straw 1991; Maes *et al*. 2000; Stärk 2000).

Air quality

Optimising ventilation to maintain air quality and air flow rates appropriate to the class of stock will help to manage temperature and pathogen load (Eisenmenger 2006). Elevated concentrations of ammonia and hydrogen sulfide gases and dust, skin and airborne bacteria can all negatively affect the health and growth of pigs (Robertson *et al*. 1990; Cargill and Skirrow 1997; Stärk 2000). Atmospheric ammonia and organic dust both can increase the severity of atrophic rhinitis in pigs exposed to *Pasteurella multocida* or *Bordetella bronchiseptica* (Drummond *et al*. 1981; Hamilton *et al*. 1999). The total and respirable dust levels in piggeries can be measured with personal gravimetric samplers, and ammonia and other gases can be measured with standard gas tubes (Cargill and Skirrow 1997). When dust and ammonia levels increase, ventilation can be used to improve air quality. The load of bacteria in aerosols can be monitored by either traditional culture techniques or by the recently developed quantitative polymerase chain reaction assays that can also detect non-culturable bacteria.

Cleaning and disinfection

Readily available technologies such as hygienograms and organic matter quantification and air quality assays should be routinely used to monitor hygiene standards (Venkateswaran *et al*. 2003; Vangroenweghe *et al*. 2009). This monitoring can then be used to correlate production and disease outcomes with and without the use of antimicrobials. Cleaning and disinfection between batches of pigs is critical for reducing pathogen load and the associated disease risks. Removing organic matter before disinfecting pens is critical in reducing the load of *L. intracellularis*, enterotoxigenic *E. coli*, and *Brachyspira* spp. (Lofstedt *et al*. 2002; Thomson *et al*. 2007; Collins *et al*. 2013). Poor hygiene is a significant risk factor for Salmonella shedding at the end of the finisher period and includes failure to empty pits below slatted floors, infrequent removal of sow faeces and the presence of residual Salmonella on pen floors and pen dividers (Belœil *et al*. 2004). Disinfection of pen fixtures, such as drinkers, is especially important in reducing the risk of Salmonella shedding in herds with bowl drinkers (Bahnson *et al*. 2006). Aerial disinfection by fogging has been reported to reduce loads of both bacterial and viral respiratory pathogens in sheds while pigs are still resident (Fotheringham 1995). More recently, electrostatic particle ionisation sanitation of air in pig sheds has been trialled to help reduce the load of swine influenza virus and porcine reproductive and respiratory syndrome virus (Alonso *et al*. 2014*a*, 2014*b*).

Temperature

Temperature has been identified as a critical factor in the prevention of PWD. Pigs subjected to cold stress (20°C) before challenge with enterotoxigenic *E. coli* suffered increased severity of diarrhoea and reduced weight gains relative to pigs housed at thermoneutral temperatures (Wathes *et al*. 1989). The importance of automatic temperature control to reduce variation in ambient temperature was demonstrated in a Finnish survey of 74 herds, where automatic temperature control was associated with a reduced risk of PWD (Laine *et al*. 2008). Draughts in pens of weaner pigs can also reduce ADG, and increase sneezing, coughing and diarrhoea in herds with evidence of endemic pleuropneumonia, Aujeszky's disease virus and swine influenza (Scheepens *et al*. 1991). Controlling temperatures to be in thermal comfort zones for the different classes of stock will assist with reduction of stressors that predispose pigs to disease.

Stocking rates

Stocking rates are calculated based on available floor space for the predicted number and weight of pigs upon exit of a facility.

Avoiding or eliminating over-stocking will reduce stressors (Brumm 2005). Provision of optimal feeder space and drinker availability is likewise related to improved disease control through the reduction of stressors. For respiratory disease, the available air quantity and quality, as well as floor space, impact on disease risks associated with stocking density. Recommendations of more than 3-m^3 air space per pig were developed based on the incidence of respiratory lesions at slaughter (Lindquist 1974).

Consistent nutrition

Consistency of nutrition will also have an impact on the production of a pig herd (Tokach *et al*. 2008). Much focus is placed on reducing feed costs and improving feed efficiency, however it can occur that this strategy is not always the most cost effective for a farm (Tokach *et al*. 2008). Least-cost feeding commonly means sourcing ingredients from different locations with differing ingredient types (grains, proteins). Diet changes are a common factor in precipitating disease or producing transitory production deficits (Shurson and Johnston 1998). Anecdotally, prophylactic or metaphylactic use of antimicrobials is commonly employed to manage groups of pigs through a transition of diet. Therapeutic antimicrobials may also be used if a group succumbs to resident pathogens subsequent to a change of diet ingredients or formulation. This also emphasises the need for all advisors to pig farms to cooperate towards a common goal (Patience *et al*. 2008). By managing the impact of major elements such as diet and ingredients on pig health, the whole management system can be more attuned to reduction of antimicrobial use.

Biosecurity

Both internal and external biosecurity are important aspects in preventing the transmission of disease and hence reducing the need for antimicrobials. External biosecurity focuses on preventing pathogens from entering the herd through pigs, people, feed, equipment or other animals. Internal biosecurity focuses on prevention of pathogen transfer between batches of pigs within the same farm. A biosecurity scoring system was developed to investigate the relationship between biosecurity, production and treatments needed for disease control (Laanen *et al*. 2013). External biosecurity was scored by measuring management practices that prevented pathogens entering a herd, including purchase and transport of animals, supply of feed, water and equipment, vermin control and entry of visitors. Factors that reduce the spread of pathogens within a herd were scored for internal biosecurity and included disease management, cleaning, disinfection, and management of pigs in the farrowing, nursery and fattening units. The study of 95 Belgian breeder-to-finisher herds found that higher internal and external biosecurity scores correlated positively to ADG and negatively to both feed conversion ratio and disease incidence (Laanen *et al*. 2013), suggesting that improving biosecurity may reduce the use of prophylactic antimicrobials and increase production.

All-in-all-out production and consistency of production flows

Internal biosecurity extends to concepts of batch integrity and all-in-all-out (AIAO) production. Batch integrity refers to the keeping together of animals with a common age and history. This is a means to help ensure a common immune status and experience of expected diseases within a group of pigs. It also extends to the rapid removal of pigs affected by disease from the batch. The best practice application of these ideas begins from the farrowing house (McCaw 2000), and continues with AIAO production for the life of the pig.

All-in-all-out production and maintaining batch integrity is a well-established technology for improving the health of pigs (Scheidt *et al*. 1995). Ensuring batches of pigs have the same environment (air, water, feed), and minimising the spread of ages within the group is critical for the success of these systems. True AIAO production is difficult to accomplish successfully, especially when variation in production can result in variable numbers of pigs produced and may increase the proportion of 'tail-ender' pigs. Continuous flow production and frequent mixing of pigs have been identified as risk factors for proliferative enteropathy (Bane *et al*. 2001; Bronsvoort *et al*. 2001; Collins and Love 2003). Managing production for consistency of flow will enable more reliable AIAO batch production. Controlling other inputs and aspects of the environment will allow consistent numbers placed at consistent ages with operations occurring at consistent times, ultimately helping to achieve more consistent production targets. In this regard, transmission of disease from older animals to weaners is prevented by segregating weaners using AIAO production, disinfection of pens between batches, strict biosecurity and avoiding mixing animals of different ages together (Whiting and Pasma 2008).

Assisted reproduction technologies such as synchronised or induced oestrus and timed insemination, may help make achieving production targets more consistent (Knox 2014). This consistency of production is critical to making optimum use of facilities and therefore more easily availing the production system of AIAO technologies and batch integrity. This in turn improves the overall health of the herd and reduces the need for antimicrobial use. However, the pig production industry must be aware that just as there is community or regulatory pressure to reduce or eliminate antimicrobial usage in pig production, there could well be similar pressures on other technologies readily available to improve production, such as the use of hormones and genetic modification.

Biosecurity – introduction of livestock and genetic material

The fundamental principle of having high health herds must be to keep them that way (Alexander and Harris 1999). This means controlling or eliminating the introduction of novel infectious agents to the herd. Maintaining the highest standards of biosecurity necessitates having a good understanding of the means by which infectious agents can be introduced to a herd. The primary means of introducing pig pathogens is with pigs, meaning introduction of live animals to the herd. The most expedient way to reduce this biosecurity risk is to close the herd. Commonly this means having no introduction of live animals to the herd, but can be extended to include no introduction of semen as well.

If herds cannot be closed, then systems for the introduction of replacement stock that minimises disease transmission risks

must be used. These systems include using a single source of replacement stock, using quarantine periods before introduction of livestock and minimising the size and frequency of such introductions. Frequently introducing more stock or bringing in more than 100 pigs at a time increases the odds of introducing *Actinobacillus pleuropneumoniae* to a herd by 10.87- and 6.89-fold, respectively (Rosendal and Mitchell 1983). Love and Love (1977) recommended separating and medicating replacement stock from the main herd for three weeks to prevent proliferative haemorrhagic enteropathy. Embryo transfer is another technology that allows the introduction of genetic material to a herd with much reduced risk of transfer of pathogens (Thibier 2011). The expense and difficulty of applying this technology to pig production has largely excluded it from common usage. However, the technology is available and would allow the introduction of new or improved genetics to herds without the disease transmission risk associated with introduction of live animals. By avoiding introduction of new or different swine pathogens, a herd can remain stable to resident bacterial flora and thus have a reduced risk of disease events.

Eradication or elimination of pathogens

Prevention of disease in swine production systems is based on elimination of pathogens, prevention of pathogen introduction, fortification of individual and herd immunity and the elimination of conditions that predispose to disease. Elimination of pathogens includes starting with high health or specific pathogen-free stock. Pigs obtained surgically, through 'snatch-farrowing' or medicated early weaning, can be free from common pathogens including *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Brachyspira hyodysenteriae*, and external and internal parasites (DAFF 2010). A management system called 'Isowean' reduces the potential for transmission of endemic infections to newly weaned pigs by separating the weaned pig from the breeder and grower herd (Alexander and Harris 1999).

However, there are bacterial pathogens unable to be excluded from farms with current technology. Numerous attempts to eradicate *L. intracellularis* with depopulation, cleaning and medication have all failed in the long term (Flø *et al*. 2000; Johansen *et al*. 2002; Ellegaard *et al*. 2008), partly because the pathogen survives for at least 2 weeks in the environment (Collins *et al*. 2000) and can be easily re-introduced through rodents (Collins *et al*. 2011). This is where stability of the individual and herd immunity to resident pathogens must be a priority, as well as control or elimination of the conditions that predispose to disease.

Vaccination

Vaccine and vaccination technology also need to be considered as alternatives for disease prevention. Factors to consider are development of vaccines for known pathogens not yet well controlled, as well as economical and efficacious vaccine delivery systems (Royer *et al*. 2006; Chase 2008) that can improve vaccination compliance and effectiveness. Improved vaccination leads to improved disease prevention and control and thus encourages the reduction or elimination of antimicrobial use. It is probably true that pork production systems have become 'lazy' with reliance on antimicrobial use for control of commonly occurring bacterial diseases. If farms and their advisors can have confidence in the effectiveness and robustness of vaccines, combined with more convenient delivery systems to pigs, this will provide further avenues to improved disease control and reduction of antimicrobial usage.

Segregation of gilt and sow progeny

Segregation of gilt progeny from sow progeny has also been proposed as a way to reduce antimicrobial use by targeting medication to the pigs at highest risk of disease. Gilt progeny have a higher incidence of pre-weaning diarrhoea and increased concentrations of acute phase proteins that indicate inflammation (Morales *et al*. 2006; Miller 2008). After weaning, increased mortalities and medications in gilt progeny continue to suggest an increased exposure to pathogens from gilts and reduced transfer of passively acquired immunity against endemic pathogens from gilts (Miller 2008). The health and survival of gilt progeny could be improved by targeting vaccination programs in gilts to improve the transfer of pathogen-specific immunity to their progeny and stabilisation of herd immunity (Miller 2008). This improvement in overall herd immunity by targeting specific groups within the herd is fundamental to improving herd health and reducing antimicrobial use.

Diagnostics for monitoring pathogens at the herd level

Many diagnostic assays are based at the individual animal level, rather than the herd level. Diagnostic technologies that collect and interpret samples at the herd level will help increase understanding of the dynamic nature of disease within a herd will help elucidate methods to control the influence of pathogens without the use of antimicrobials. Pooling of samples from pigs within a pen or shed may enable a broader overview of disease at the herd level, but pooling samples will also reduce the sensitivity of the assay, especially in herds with low disease prevalence and in pigs with low pathogen numbers. In pigs clinically affected with proliferative enteropathy, a single positive sample could be detected in a pool of nine negative pigs (Collins and Barchia 2013). However, more than 60% of pigs needed to be positive in the same size pool when pigs were sub-clinically affected. Oral fluids can be used to sample groups of animals to monitor either pathogen loads by quantitative polymerase chain reaction or antibody titres by ELISA (Prickett *et al*. 2008). Oral fluid collected on cotton ropes in pens over 20–30 min provide a sub-sample of ~70% of the pen population (Seddon *et al*. 2012).

Together with improved diagnostic technology must come confidence in the accuracy and relevance of diagnostic testing and improvement in the rapidity or convenience of the testing. Pen-side or on-farm tests for common disease syndromes that are prognostic rather than diagnostic as well as non-pig-based testing (such as of the environment or feed or water) are all on the production veterinarians' wish list. Diagnostics that demonstrate strong correlations between pathogen loads and disease or production losses can be used for routine herd monitoring to avoid disease outbreaks. Critical thresholds of both porcine circovirus type 2 and *L. intracellularis* have been established that indicate clinical outcomes and production losses (Brunborg *et al*. 2004; Olvera *et al*. 2004; Collins and Barchia 2014). Routine testing for production and disease issues, so that conditions can

be modified to avoid disease events, is part of the whole farm consultancy that advisors and managers seek.

Diagnostic assays that can quantify numbers of commensal or protective bacteria in the intestine may help predict disturbances to intestinal health and reduce the reliance on antimicrobials to control enteric pathogens. Quantifying lactate- and butyrate-producing bacteria, which are able to prevent pathogen colonisation through competitive exclusion and excretion of bacteriocins (Gueimonde *et al*. 2006; Lim and Ahn 2012; Liu *et al*. 2013), may provide an indicator of protection against pathogens and improved intestinal health. An increased relative abundance of butyrate-producing bacteria was associated with the absence of diarrhoea in weaners (Bowring *et al*. 2015), and with reduced shedding of Salmonella in experimentally challenged pigs (Bearson *et al*. 2013).

Conclusion

Pork production without antimicrobials is not simply a matter of substituting antimicrobial products with non-antimicrobial products and expecting the same result. The concept must be approached as a different method of pork production (EIP-AGRI Focus Group 2014). This infers that markets or customers have a different expectation of production practices; however, it is likely that all minimum standards of animal care and resource usage will not be relaxed. Similarly, staff training should be changed to alter expectations at the farm level, and it is likely that different attitudes to welfare on-farm are necessary. This is both to manage animal welfare in the absence of routine or familiar treatments and also for economic considerations (Morrow *et al*. 2004). Education of producers and advisors must be ongoing to consider pork production without the use of antimicrobials. The pig industry must be willing to learn from other industries and other disciplines that are not necessarily directly involved in pork production (Waddell 2010). The industry must also recognise that some farms operate successfully now without the use of antimicrobials, and to be willing to learn from them (Kohler *et al*. 2008). Changes in attitude to management of farms, and more attention to detail in critical management and production factors (Becton 2006), will be fundamental to successful implementation of antimicrobial-free pork production.

It is worth recognising that many of the conditions necessary to assist with production of antimicrobial-free pork have been known and understood for a long time. The application of high standards of biosecurity and hygiene is crucial to creating the conditions for reduction of use of antimicrobials. It is indicative of the pressures of pork production that systems stray from these conditions in an effort to increase or improve pork production. It is also indicative of the economics of use of antimicrobials that they are commonly relied on to sustain many production practices and conditions in Australia.

Any discussion on antimicrobial-free pork production must recognise that a move to antimicrobial-free production must involve definite aims for farms, markets and consumers. There is currently no regulatory imperative to produce antimicrobial-free pork, so any decision to pursue such production systems must have clear benefits; that is, it would be voluntary on the part of farms. Farms that move towards antimicrobial-free production will usually do so because of specific markets and a possible sale premium. If this continues to happen, or if the demand for such product increases, there needs to be definitions of what is expected from 'antimicrobial-free pork'. There would also be a commensurate need to have verification systems around antimicrobial-free pork production, and changes to on-farm auditing of production practices. If such changes were market or customer driven, there would need to be clear expectations from these markets and customers and clear guidelines for farms to follow.

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Review

Antimicrobial stewardship: what is it, and how does it work?

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Abstract. Antimicrobial stewardship is emerging as a vital management tool in the efforts to contain antimicrobial resistance and retain the efficacy of available agents. It is based on a set of concepts about antimicrobial use and resistance that have been developed over the past 70 years. There are seven basic requirements for a stewardship program to function at a local level, including (1) 'executive' ownership of the issue, (2) consensus prescribing guidelines, (3) a local formulary with various levels of restricted access, (4) a local champion (or champions) who is a trusted peer, (5) authority to intervene in prescribing and/or dispensing, (6) authority for measurement of use, audit and feedback, and (7) access to reliable laboratory services and their cumulative resistance data. Stewardship programs are most advanced in larger public hospitals, but there is considerable interest and need for developing programs tailored to a wide range of settings in human and animal health, each with their own particular characteristics of access to antimicrobials and potential controls. The potential value of stewardship in food animal production is now recognised globally, and Australia has taken the first steps towards surveillance and stewardship in this sector, supported by a recently released national One Health strategy on the containment of antimicrobial resistance.

Additional keywords: antimicrobial resistance, authority for measurement, authority to intervene, prescribing guidelines.

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Introduction

Antimicrobial stewardship (AMS) is a new term for an old idea. In human medicine, the idea of attempting to instill and ensure rational prescribing of antimicrobials (see below) has a decades-long history in Australia, starting with the initiative to come up with consensus prescribing guidelines in metropolitan Melbourne in the mid-1970s (Harvey *et al.* 2003). The principal aim of AMS is to reduce unnecessary and (or) irrational use of antimicrobials and, thereby, reduce selection pressure for antimicrobial resistance. There are some important concepts behind AMS that must be understood by everyone to make it work. These are principles that have emerged for the evidence and experience gained through the long history of antimicrobial use following the introduction of penicillin in the early 1940s. They apply to all settings where antimicrobials are used and where resistance can be driven by use (Woolhouse *et al.* 2015):

- Resistance selection is one of the most important adverse effects of antimicrobials.
- Resistance is doubly contagious,
  - resistant strains of bacteria can be amplified and spread to other individuals, to groups and to the environment, and
  - resistance genes and gene complexes can be spread from one bacterium to another, including to other genera and species.
- Antimicrobial resistance will emerge whenever, wherever and whichever antimicrobials are used.
- Resistance can be a 'one-way street'; it can emerge, be amplified and be reduced, but often never completely eliminated.
- In exposed individuals, resistance is much more commonly selected 'silently', i.e. in normal flora than it is in the targeted pathogen.
- Longer (and more frequent) antimicrobial exposures lead to higher resistance levels in an individual.
- Higher proportions of antimicrobial exposure in a population lead to higher resistance levels in a population.
- A significant amount of antimicrobial use is unnecessary: it is either of no or very limited individual benefit, be it used for treatment or prophylaxis.
- Reducing exposure through shorter courses or avoiding unnecessary use results in reduced levels of resistance.
- Most antimicrobial 'prescribers' are well meaning but are motivated to care for individuals rather than whole populations, be they human or animal.
- Effective antimicrobials are becoming a scarce resource, and need to be preserved for those most in need.
- Stewardship systems improve prescribing practices and reduce antimicrobial resistance selection pressure, ultimately stabilising or reducing resistance.

It is also possible to institute stewardship concepts at levels beyond those of a single institution, for instance at the regional, jurisdictional and national level. The shapes of stewardship at these levels is still in their infancy, and clearly have to be tailored to the requirements at those levels.

This review focusses primarily on the principles and elements of antimicrobial stewardship as they are currently implemented in

human medicine. It is recognised that many of those principles and elements can be found in other areas of antimicrobial use, including veterinary medicine. Hence, the review also addresses how the elements of stewardship are emerging in pig production.

The seven basic requirements of AMS

Antimicrobial stewardship is the adoption of a systematic approach so as to optimise antimicrobial use in the relevant setting (Table 1). Each setting has its own particular characteristics and challenges. However, there are some features that should be common to all AMS programs, including (1) 'executive' ownership of the issue, (2) consensus prescribing guidelines, (3) a local formulary with various levels of restricted access, (4) a local champion (or champions) who is a trusted peer, (5) authority to intervene in prescribing and/or dispensing, (6) authority for measurement of use, audit and feedback, and (7) access to reliable laboratory services and their cumulative resistance data. Each of these plays a vital role in the success.

Working examples of each of these are provided below, on the basis of the successful development of AMS programs in Australian hospitals that has been driven by the Australian Commission on Safety and Quality in Health Care (ACSQHC). The ACSQHC has an AMS committee that created the first working manual on stewardship for Australian hospitals (Duguid and Cruickshank 2010). Subsequently, AMS was included in one of the hospital accreditation standards, and the Commission created a national clinical care standard for AMS to which every Australian hospital is expected to subscribe (Australian Commission on Safety and Quality in Health Care 2014). Antimicrobial stewardship is one of the seven key elements of the national strategy for containing antimicrobial resistance in Australia, which has a One Health focus (Department of Health 2015).

'Executive' ownership

The problems of inappropriate antimicrobial use and the worsening antimicrobial resistance, and benefits of AMS in addressing these issues, need to be owned at the highest levels of the organisation or business. In Australian hospitals, it is expected that the Chief Executive Officer, who takes ultimate responsibility in matters of accreditation, also takes AMS responsibility. In large institutions, that task is often delegated to dedicated AMS committees and teams, or to safety and quality committees. In smaller institutions, that task often falls to individuals such as infectious diseases specialists, pharmacists or infection control practitioners. Obviously, it will be a different person or people in other settings. For instance, in a group general practice, it would be assumed that at least one of the senior partners would assume ownership of AMS in that practice.

Consensus prescribing guidelines

Australia is very fortunate in having long-standing and highly respected national antimicrobial prescribing guidelines, i.e. Therapeutic Guidelines: Antibiotic (Antibiotic Expert Groups 2014). These guidelines were first drawn up in 1978 and went Australia-wide in 1990. The guidelines are written using the latest published evidence by a raft of national experts, and are updated every 3–4 years. The 'antibiotic guidelines', as they are commonly referred, are considered the reference standard to which all prescribing is compared, and are essential to AMS programs in hospitals. Their uptake in community medical practice has been more limited. It is always possible to deviate from these guidelines in clinical practice, but if it is in the treatment of an individual, the prescriber is expected to document the rationale in the medical record. In some instances, it is reasonable for a hospital or a jurisdiction to develop additional or modified guidelines designed to deal with prevalence of local

Table 1. Established and potential settings for antimicrobial stewardship

Sector		Setting	Progress
Human health	Hospital	Public	Advanced
		Private	Advanced
		Day procedure clinics	Yet to commence
	Community	Primary care	Yet to commence
		Specialist practice	Yet to commence
		Residential aged care	Under development
		Aboriginal health services	Advanced in some places (e.g. NT)
		Pharmacy: over-the-counter	Yet to commence
Animal health	Hospital	University	Yet to commence
		Private	Yet to commence
	Community	Primary companion animal pactice	Yet to commence
		Specialist companion animal practice	Yet to commence
		Large animal practice	Yet to commence
	Food producing	Intensive meat animal production	Yet to commence
		Extensive meat animal production	Yet to commence
		Egg production	Yet to commence
		Dairy production	Yet to commence
		Aquaculture	Yet to commence
Other	Food producing	Apiculture, orchards	Yet to commence
	Other	Racehorses, zoos, exotic pets	Yet to commence

resistances or to enhance the detail of the national document in the local context (SA Health 2015; Antimicrobial Guidelines).

Veterinary antimicrobial prescribing guidelines have been developed and published in Australia in the past (Cooper 1994, 2000), but never seem to have been widely recognised or used. Recently, there has been renewed interest in developing national antimicrobial prescribing guidelines that might more closely parallel those available for human medicine (Australian Veterinary Association, pers. comm.). Some international organisations are taking the initiative in guideline development, such as the International Society for Companion Animal Infectious Diseases (Weese *et al.* 2011; Hillier *et al.* 2014). While such moves are welcome, they highlight the difficulty of developing guidelines when clinical practice and drug availability vary across countries.

Local formulary

One of the most effective weapons in the control of unnecessary prescribing and resistance containment has been to apply restricted access to certain antimicrobial classes, such as those considered 'last-line' and those associated with a high capacity to drive resistance. In the hospital setting, this requires the development of a local formulary, a set of restricted antimicrobials (which can be restricted in different ways), and a set of procedures for accessing restricted agents. The local champion or champions then have the role of 'gatekeeper' for those agents that are restricted. In recent times, software programs have been developed that can undertake this role on behalf of the champions (Guidance MS 2015).

In the community setting, the Pharmaceutical Benefits Scheme has the capacity to restrict access to certain antimicrobial agents or even whole classes (Department of Health, Commonwealth of Australia 2015). It can do this by, first, classifying them as a 'Restricted benefit', designed to alert the prescriber to the only reasonable indications for an agent. A second method is that of an 'Authority required', with a specific list of approved indications that require a phone call to gain approval to prescribe, or for certain indications the addition of a specific code. Alternatively, the Pharmaceutical Benefits Scheme can choose not to list the agent at all.

Local champion or champions

The AMS programs cannot function in the absence of a local champion or champions to drive change. These need to be teams or individuals working at the local level whose opinion is respected. Similar to the establishment of infection control in public hospitals in Australia, it can take a long time for the institution to find such a person or people, or provide them sufficient training and expertise to earn the respect of their peers. In human medicine, there has been the steady expansion of the speciality of infectious diseases, as the health care system comes to terms with the breadth and complexity of infection management. Where there are infectious disease physicians, then these are the natural champions of AMS. In larger hospitals, these physicians are now being supplemented with infectious disease pharmacists, and with other relevant staff including trainees, forming stewardship teams. In smaller institutions, AMS has to be delivered by other physicians, pharmacists or infection control practitioners.

Authority to intervene at the time of prescription

The value of 'executive' ownership is that it can provide the champion of champions with the necessary authority to intervene at the time of prescription. Prescription interventions are most effective at the time of prescribing, and can take the form of preventing the use of an antimicrobial when it is deemed to be not required, directing the prescriber away from an inappropriate to an appropriate agent, convincing the prescriber to order an investigation where it will provide useful guidance, promoting the earliest switch to oral therapy when initial therapy has been parenteral, and stopping antimicrobials that would otherwise be continued without additional benefit (Duguid and Cruickshank 2010).

Authority for measurement of use, audit and feedback

Antimicrobial stewardship programs benefit enormously from local data on antimicrobial use and resistance. The executive sponsor must both endorse and resource these activities. Having a complete understanding of usage patterns enables the champions to detect trends and anomalies and undertake interventions where necessary. One tool that has proven extremely valuable to hospital AMS programs has been the development of the National Antimicrobial Prescribing Survey, and a web-based program enabling an AMS team to conduct an at least an annual audit of prescribing in their hospital within available resources. The audit is conducted nationally, often on a single day, and entered into the web-based tool, and provides the user with access to their data as well as the ability to compare their practices with their peers. The results of 2014 survey, representing findings from 248 hospitals across Australia, have been published and have provided valuable information on where there are important priorities for intervention, such as unnecessarily prolonged surgical prophylaxis and inappropriate antimicrobial choices for some lower respiratory tract infections (Table 2) (Australian Commission on Safety and Quality in Health Care 2015).

Access to reliable laboratory services and their cumulative resistance data

Antimicrobial stewardship programs also need access to quality laboratory services. Laboratories provide essential information on the results of microbiological investigations on individual patients that assist in antimicrobial decision-making at the point-of-care. They also provide ongoing evidence of changes in susceptibility patterns of the key pathogens, and should be capable of generating so-called antibiograms, which provide stewardship teams with overall prevalence of resistance for the major pathogens and their associated antimicrobial agents (Australian Commission on Safety and Quality in Health Care 2013). Antibiograms inform teams on when adjustment to local formularies are required.

What is rational prescribing?

The principles of rational prescribing are simple to describe but harder to put into practice. In essence they are as follows:

(1) *Start only if you have to*; prescribe an antimicrobial only where the evidence of benefit is demonstrable and substantial

Table 2. Commonest antimicrobial prescribing problems in Australian hospitals, 2014

Indicator	% of prescriptions inappropriate[A]	Commonest problem
Surgical prophylaxis	40.2	Duration too long (>24 h)
Infective exacerbation of chronic obstructive lung disease	36.8	Spectrum too broad
Cholecystitis	27.8	Spectrum too broad
Community-acquired pneumonia	25.0	Spectrum too broad

[A]Appropriateness judged against set written criteria.

(there are good examples in human medicine where the evidence of benefit is minimal even for some bacterial infections, such as acute sore throat (Spinks *et al.* 2013).

(2) *Stop as soon as you can*; antimicrobial therapy should cease as soon as there is clinical evidence of recovery, while being mindful of evidence about what constitutes a minimum course.
(3) *Choose wisely*; use the narrowest-spectrum agent that will cover the likely pathogens.
(4) *Dose wisely*; ensure that the dosing regimen is adequate and safe for the patient and the condition being treated.
(5) *Use the laboratory*; whenever it is feasible, collect a specimen for microbiology laboratory testing before starting, and modify therapy when results are available, aiming for a narrower spectrum if possible, and, if necessary, make a switch to oral therapy if clinically safe to do so.
(6) *Use prophylaxis prudently*; surgical prophylaxis can be a single dose in almost all circumstances; medical prophylaxis should be used only when the evidence shows that the benefit is substantial (Antibiotic Expert Groups 2014).

Stewardship beyond human healthcare

Antimicrobial agents are used widely outside the human healthcare systems. As such, there is a compelling reason to consider developing AMS programs in settings for non-human use. Options are now emerging for AMS in small animal practice (Guardabassi and Prescott 2015; Weese *et al.* 2015) and agriculture (Prescott *et al.* 2012). The science in these areas is in its infancy, but with the application of the use and resistance principles, and those of rational prescribing, much can be achieved in a short time. The issues of emerging resistance and running out of effective antimicrobials are just as relevant to the animal health and agricultural sectors. The future is bright for better management of the valuable resource that antimicrobials afford society as we develop the range of AMS programs required.

Antimicrobial stewardship in food-animal production

The use of antimicrobials in food-animal production introduces some special challenges for stewardship. The political and regulatory landscape is quite different from human health. Antimicrobial use in food-animal production has been under intense scrutiny since the late 1990s. The evidence for the selection and amplification of antimicrobial resistance in food-animal production, and its subsequent transmission to humans, has slowly accumulated to the point where few would deny that it is happening (Marshall and Levy 2011).

In Australia, the first national approach to the issue was taken by the Joint Expert Technical Advisory Committee on Antimicrobial Resistance report (JETACAR 1999). Many of the recommendations in its report as they relate to the use of antimicrobials in intensive animal production have been implemented completely or partially. Most important was the transfer of almost all antimicrobials to Schedule 4, which put the onus on veterinarians to prescribe antimicrobials, and, by implication, prescribe responsibly. Further guidance was provided by the Antimicrobial Resistance Standing Committee through the release of its 'Importance Ratings' document (Antimicrobial Resistance Standing Committee 2014), which rates different antimicrobials and their classes according to their importance in the treatment of human disease. The intent of the document is to signal which antimicrobial agents and classes should be used sparingly or not at all in other fields of human endeavour.

Internationally, there has been a lot of activity in establishing guidelines for responsible antimicrobial use as they apply to food-animal production (Page 2011). Most active has been the Responsible Use of Medicines in Agriculture Alliance in the United Kingdom. (www.ruma.org.uk, verified August 2015), who have produced a broad range of 'responsible use' prescribing guidelines for the intensive industries including pig production (Responsible Use of Medicines in Agriculture Alliance 2013). As expected, these guidelines put strong emphasis on the application of good animal housing and husbandry practices, as well as vaccines, to prevent infection and, thereby, reduce the need to prescribe antimicrobials.

However, stewardship is more than producing and promulgating guidelines. Rather, guidelines should support a program of audits and interventions, which will identify areas of suboptimum antimicrobial use and working with prescribers to improve use. How such a program is conducted depends on the setting, but always involves the use of adequately respected and trained personnel. Building antimicrobial stewardship programs for food-animal production should be a co-operative venture among regulators (state and federal), peak bodies, pharmacies (including compounding pharmacies) and veterinarian prescribers. When conducted effectively, the establishment of such stewardship programs can actually pay their way through savings on antimicrobials, although this should never be the primary motive. The primary motive for stewardship should always be the reduction of antimicrobial resistance selection pressure, thereby prolonging the effectiveness of antimicrobials in that setting.

Experience with stewardship programs in food-animal production is quite limited at present. There is much less

published information about the extent of inappropriate use, or what interventions are effective in moving to responsible use. The establishment of good-quality surveillance programs on use and resistance are an essential first step. Otherwise, the effect of any interventions cannot be measured. Fortunately, the first steps towards a national surveillance program in food-animal production in Australia are being taken, with the first work to start this year in pig production. Further research is also essential into the structure and function of stewardship programs in food-animal production. Recently, the National Health and Medical Research Council has funded the establishment of a National Centre for Antimicrobial Stewardship at the University of Melbourne (https://ncascre.wordpress.com/, verified August 2015). This Centre has included research into stewardship in food-animal production, in recognition of the One Health nature of antimicrobial use and resistance.

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Review

Selection for productivity and robustness traits in pigs

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Abstract. Pig breeding programs worldwide continue to focus on both productivity and robustness. This selection emphasis has to be accompanied by provision of better-quality environments to pigs to improve performance and to enhance health and welfare of pigs. Definition of broader breeding objectives that include robustness traits in addition to production traits is the first step in the development of selection strategies for productivity and robustness. An approach has been presented which facilitates extension of breeding objectives. Post-weaning survival, maternal genetic effects for growth as an indicator of health status and sow mature weight are examples of robustness traits. Further, breeding objectives should be defined for commercial environments and selection indexes should account for genotype by environment interactions (GxE). Average performances of groups of pigs have been used to quantify the additive effects of multiple environmental factors on performance of pigs. For growth, GxE existed when environments differed by 60 g/day between groups of pigs. This environmental variation was observed even on well managed farms. Selection for improved health of pigs should focus on disease resistance to indirectly reduce pathogen loads on farms and on disease resilience to improve the ability of pigs to cope with infection challenges. Traits defining disease resilience may be based on performance and immune measures, disease incidence or survival rates of pigs. Residual feed intake is a trait that quantifies feed efficiency. The responses of divergent selection lines for residual feed intake to various environmental challenges were often similar or even favourable for the more efficient, low residual feed intake line. These somewhat unexpected results highlight the need to gain a better understanding of the metabolic differences between more or less productive pigs. These physiological differences lead to interactions between the genetic potential of pigs for productivity and robustness and the prevalence of specific environmental conditions.

Additional keywords: breeding objective, disease resilience, environmental variation, genotype by environment interactions, residual feed intake.

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Introduction

Selection for efficiency and productivity has been the long-term focus of pig breeding programs worldwide leading to considerable genetic gains in production levels of pigs. These genetic improvements in efficiency and productivity, however, have high physiological demands, which may have unfavourable consequences for the robustness of animals (e.g. Knap and Rauw 2009; Prunier *et al*. 2010). Robustness has recently been described as a central concept in reconciling productivity and feed efficiency with health, adaptation, welfare and reproduction (Phocas *et al*. 2014). This general description of robustness provides an overview of the concept of robustness and requires development of specific applications in animal breeding. In pig breeding, robust pigs were defined by Knap (2005) 'as pigs that combine high production potential with resilience to external stressors, allowing for unproblematic expression of high production potential in a wide variety of environmental conditions'. Knap (2005) provided examples of robustness traits including pre-weaning survival of piglets and rebreeding success of sows. For growing pigs, additional robustness traits may be extended to include survival of growing pigs, disease incidence and possibly maternal genetic components that improve growth and health status of growing pigs. Further, the concept of environmental sensitivity mentioned by Knap (2005) in the definition of robustness can be applied to a wide range of environmental descriptors including the incidence of disease and pathogen load to better describe specific infection challenges for the definition of disease resilience.

A wide range of research continues to focus on aspects of robustness worldwide. In Australia, the development of healthy, robust pig genotypes is the aim of one research program of the Cooperative Research Centre for High Integrity Australian Pork. This review will provide an overview of selection for both productivity and robustness in pigs. This is a very extensive research topic and not all aspects can be covered. In particular, genomic selection is often mentioned as a selection strategy for health and robustness traits because these traits are difficult to

measure and are often not available for selection candidates before selection. However, genomic selection still requires accurate definition of phenotypes, which has been the focus of this overview of research currently underway in Australia.

Rate of genetic improvement

Selective breeding leads to genetic improvement of animals and is based on quantitative genetics, which was described by Nicholas (1997) as 'sufficiently mathematical to strike fear into the hearts of many practical pig breeders'. However, Nicholas (1997) also pointed out that 'pig breeders around the world have been at the forefront of the practical application of quantitative genetics in pig improvement programs.' This statement is also true for Australian pig breeders who adopted genetic technologies based on Best Linear Unbiased Predictions in the early 1990s to identify genetically superior animals more accurately. The initial selection emphasis was on growth, backfat and feed conversion ratio (FCR) whereas litter size was considered in selection decisions slightly later when breeders were more familiar with Best Linear Unbiased Prediction-based selection procedures. In regard to selection for FCR, it is ideal if information about feed intake of individual pigs is available from environments that represent on-farm conditions. Recording of feed intake in group-housed pigs required development of electronic feeders (Eissen *et al*. 1998; McSweeny *et al*. 2001; Casey *et al*. 2005), which are not used by all breeders. The development of juvenile insulin like growth factor 1 (IGF-1) as a selection criterion for efficiency and leanness in the late 1990s, as summarised by Bunter *et al*. (2005), aided genetic improvement of FCR. Selection for litter size has unfavourable consequences for piglet survival and Australian pig breeders adopted various strategies for genetic improvement of piglet survival (Hermesch 2001; Bunter 2009). The list of traits considered in selection decisions continues to grow, which demonstrates the ability and willingness of pig breeders to adopt research findings about new traits with economic and societal importance.

The rate of genetic improvement is quantified by the genetic trend, which is calculated as the mean of estimated breeding values (EBV) of animals born each year. The genetic trends of five traits from 2000 to 2005 were summarised by Hermesch (2006) using data from 28 Australian pig populations. The average annual genetic gain was 5.02 g/day for growth rate, –0.15 mm for backfat, –0.01 kg : kg for FCR, 0.05 mm for muscle depth and 0.07 piglets for litter size during this time period (Table 1). These genetic trends were then compared with phenotypic trends, which describe the change in observed performance over time due to changes in genetic and non-genetic factors. Phenotypic trends were summarised for a subset of these populations representing eight herds. Average annual phenotypic improvements were similar in magnitude to genetic trends with annual improvements in performance of 3.80 g/day for growth rate, –0.10 mm for backfat and 0.09 for litter size. There was, however, substantial variation in phenotypic trends between herds and phenotypic performances differed substantially between years within herds. Therefore, changes in environmental conditions may fully override genetic gains. This highlights the need to monitor environmental conditions on farm more accurately in order to provide improved and more consistent environments to pigs. Optimising environmental conditions on farms is of paramount importance because it not only improves performance, it also enhances health and welfare of pigs.

Genetic gains of traits are usually expressed in the unit of each trait. Therefore, a comparison of genetic gains across traits is not directly possible, even for what might seem to be the same trait. For example, a comparison of genetic gains of growth rate between studies based on the actual unit of the trait (i.e. g/day) may not always be meaningful because growth traits may differ between studies in regard to recording procedures and the models used in genetic analyses. These differences in trait definitions may lead to differences in additive genetic variances, which determine the rate of genetic gain possible for traits. This limitation is overcome if genetic gains are expressed relative to the genetic standard deviation (s.d.) of each trait, making a comparison of genetic gains across traits, studies and even species possible.

The mean annual genetic gains summarised by Hermesch (2006) represented 3–15% of the genetic s.d. for each trait, whereas genetic gains achieved in the top 25% populations varied from 13% to 22% of the genetic s.d. of each trait. These rates of genetic gain were similar to genetic gains reported for other pig populations (e.g. Knap and Wang 2012) or for the Angus beef population in Australia (Barwick and Henzell 2005). Australian Angus breeders included a substantially higher number of traits in their breeding programs and were still able to achieve genetic gains of 2–19% of the genetic s.d. for individual traits. Further, rate of genetic gain in profitability had increased

Table 1. Mean annual genetic trends of 28 pig populations in Australia along with annual genetic gains of the top 25% populations achieved from 2000 until 2005 (Hermesch 2006)

Trait	Mean	Mean of top 25% ranked on breeding objective	Mean of top 25% ranked on each trait separately
Growth rate (g/day)	5.02	7.520	9.590
Backfat (mm)	–0.15	–0.260	–0.280
Feed conversion ratio (kg : kg)	–0.01	–0.027	–0.028
Live muscle depth (mm)	0.05	0.014	0.200
Number born alive (piglets)	0.07	0.120	0.180
Breeding objective[A] ($/pig)	1.06	1.920	–

[A]Breeding objective was defined as: $0.049 * EBV_{ADG} - 2.05 * EBV_{BF} - 21.1 * EBV_{FCR} + 1.0 * EBV_{LMD} + 3.56 * EBV_{NBA}$ (Cameron and Crump 2001), where EBV is estimated breeding value, ADG is growth rate, BF is backfat, FCR is feed conversion ratio, LMD is live muscle depth and NBA is number of piglets born alive and $ represents Australian dollar.

from 1985 until 2005 by extending the number of traits over time while maintaining genetic gains in existing traits. The breeding objective used in this beef example included carcass, meat quality and cow reproductive traits. This shows that it is possible to achieve genetic gains simultaneously in multiple traits and inclusion of additional robustness traits in pig breeding programs is expected to increase gains in breeding objectives.

Definition of breeding objectives

Breeding objectives combine all economically important traits in a single economic index, which is the basis for selection decisions of animals. Various authors have proposed to include traits describing vitality, uniformity, welfare, and health of animals in pig breeding objectives (Kanis *et al*. 2005; Knap 2005; Merks *et al*. 2012). These traits are important to society and describe aspects of robustness. The range of traits affecting profitability of pork production is increasing and seedstock suppliers require greater flexibility in the establishment of company-specific breeding objectives (Barwick *et al*. 2011). The approach of Amer *et al*. (2014) and Hermesch *et al*. (2014*a*) to derive economic values of traits from an independent sub-model for each trait provides flexibility to pig breeders in setting up breeding objectives. Economic values quantify the change in profit when a trait is changed by one unit, and they are the basis for the economic weights used to combine all economically important traits in breeding objectives.

Economic values are shown for performance and robustness traits of growing pigs in Table 2. Relative to the genetic s.d. of each trait, the magnitude of economic values varied from $0.47 to $6.95 (Australian dollar) per pig between traits. Well-managed breeding programs can achieve genetic gains of 10–20% of the genetic s.d. of each trait on average as outlined above. This implies that the proposed breeding objective, which considers both productivity and robustness traits of growing pigs, has the potential to achieve annual rates of genetic gains of about $2 to $4 per pig.

Post-weaning survival, a robustness trait, was the most important breeding objective trait for growing pigs given the assumptions about additive genetic s.d. No information was found in the literature for the genetic s.d. of post-weaning survival, which was derived assuming a survival rate of pigs of 97% after weaning and a heritability of 0.05. Post-weaning survival was estimated to be lowly heritable and genetically correlated with pre-weaning survival (Kim Bunter, pers. comm.). In contrast, Dufrasne *et al*. (2014) found no genetic association between post- and pre-weaning survival. Obviously it is important to obtain accurate genetic parameters for post-weaning survival of pigs in order to consider this trait in pig breeding programs more effectively.

Maternal genetic effects represent the genes of the dam affecting the performance of the progeny. Although maternal genetic effects only influence performance of growing pigs indirectly, they may offer opportunities for genetic improvement that so far have been overlooked because the low estimate of maternal genetic effects were regarded as unimportant (Solanes *et al*. 2004*b*). However, the genes of the dam affect all progeny in the litter and the economic value for maternal genetic effects of a trait is obtained by multiplying the economic value of the direct genetic effects of the trait of interest with the number of pigs per litter surviving until slaughter (Amer *et al*. 2014). Maternal genetic effects are expressed per farrowing and represent a trait of the sow that is relevant for maternal lines. Estimates of maternal genetic effects are higher at birth with values of about 0.20 for piglet weight (Hermesch *et al*. 2001; Solanes *et al*. 2004*a*) and decrease continuously for weights of pigs after weaning as the pig matures. Estimates of maternal genetic effects varied from 0.00 to 0.09 for growth and from 0.00 to 0.07 for backfat recorded shortly before slaughter between breeds in different studies (Johnson *et al*. 2002; Solanes *et al*. 2004*b*; Akanno *et al*. 2013; Hermesch *et al*. 2014*b*). These estimates indicate that maternal genetic effects offer opportunities to increase genetic gains in multiple performance traits that describe productivity of pigs. Further, it should be explored whether maternal genetic effects are becoming more important as litter size continues to increase.

Maternal genetic effects may also enhance genetic improvement of robustness traits because the dam is known to provide immunological support to piglets. Maternal genetic effects for immune parameters may be difficult to obtain because estimation of maternal genetic effects requires records from multiple generations. However, growth has been used as a

Table 2. Economic values[A] of breeding objective (BO) traits of growing pigs
GSD, genetic standard deviation

Trait	Unit	GSD	$[B]/trait unit	$/GSD
Feed conversion ratio (FCR)	kg feed/kg weight gain	0.150	–27.44	–4.11
Daily feed intake (DFI)	kg feed/day	0.094	–36.12	–3.39
Growth rate (with FCR in BO)	g/day	30.000	0.09	2.70
Growth rate (with DFI in BO)	g/day	30.000	0.16	4.80
Post-weaning survival (cost-saving approach)	pig survival/pig weaned	0.038	169.74	6.45
Post-weaning survival (lost-revenue approach)	pig survival /pig weaned	0.038	182.88	6.95
Carcass fat depth	mm	1.000	–1.70	–1.70
Loin weight	kg	0.680	3.60	2.45
Belly weight	kg	0.390	1.20	0.47
Growth rate maternal[C]	g/day per farrowing	20.000	0.83	3.83

[A]Based on Hermesch and Jones (2010), Amer *et al*. (2014) and Hermesch *et al*. (2014*a*).
[B]Australian dollar.
[C]Growth rate maternal is only part of a breeding objective for maternal lines. The $/GSD was multiplied by two to account for the fact that sows contribute only half of the genetic component to efficient lean meat growth of commercial pigs.

proxy of health status of pigs and moderate maternal genetic effects for weight traits recorded around weaning may be used to select pigs that are better able to cope with the weaning process.

Finally, maternal genetic effects can be estimated from existing data and do not require any additional information to be recorded. Given the assumed genetic s.d. for growth traits outlined in Table 2, maternal genetic effects were of similar importance to direct genetic effects for growth in maternal breeding objectives. Therefore, maternal genetic effects offer opportunities to increase genetic gain in the breeding objective without the need for any additional investments in recording data.

Robustness traits of sows

Economic weights for robustness traits of sows include sow longevity, farrowing and pre-weaning survival of piglets (Knap 2005; Amer *et al*. 2014). Further, sow mature weight may be regarded as a robustness trait of sows when environmental conditions are disadvantageous for larger sows with higher nutritional and housing requirements. The economic weight for sow mature weight includes four economic value components which quantify the effects of (a) energy requirements of gilts, (b) sow maintenance cost, (c) sow capital costs and (d) sow mature weight cull value on profit (Amer *et al*. 2014). Sow mature weight was the second most important maternal trait after litter size. Selection for growth in pigs results in heavier gilts with heavier piglets and higher lactation feed intake capacity (Bunter *et al*. 2010). Further, regression of sow weights observed across parities on farm on estimated breeding values for growth of pigs indicated that a genetic gain of 100 g/day was associated with an increase in sow weight of 30 kg (Hermesch *et al*. 2010). However, the pattern of residual and phenotypic correlations estimated by Bunter *et al*. (2010) also indicated environmental limitations to performance of gilts with high genetic potential for growth. Overall, these findings highlight the need to modify environmental conditions continuously to accommodate the rapidly changing requirements of sows due to selection for lean meat growth and the need to consider sow mature weight in selection decisions.

Variation in environmental conditions

The environment experienced by pigs is defined through multiple characteristics including temperature, floor space, air quality, nutrition, feeding or vaccination and general health status of pigs. Each one of these environmental characteristics may lead to an environmental stressor when conditions are suboptimal. Hyun *et al*. (1998) showed that multiple environmental stressors affect growth rate of pigs in an additive manner. It is therefore generally beneficial to remove a single known environmental stressor even when other potentially unknown environmental constraints may still be present.

Specific information about infection challenge on farm is required for the development of selection strategies to improve disease resilience of pigs (Doeschl-Wilson and Lough 2014; Hermesch 2014). The infection challenge experienced by a group of pigs may be based on the average of various immune parameters or pathogen levels of the pigs in a group. Alternatively, infection challenge may be derived from measuring pooled samples of faeces or saliva collected from a group of pigs. Examples of on-farm measurements of pathogen load and their associations with performance and disease were described in the review by Collins (2014). Infection challenge experienced by pigs on farms is not only affected by the amount of potentially interacting pathogens and their virulence, but also by environmental factors such as air quality, temperature and humidity. Collins (2014) suggested that monitoring air quality may provide a better indicator of pig health and growth than monitoring individual pathogen loads because air quality affects growth and health of pigs. Various devices and measurement techniques are now readily available to monitor environmental conditions on farms regularly. These devices and techniques should be implemented by pig producers to provide the best possible environment for pigs raised indoors. Further, these devices offer opportunities for pig breeders to monitor environmental conditions on farm more precisely for the evaluation of genotype by environment interactions (GxE).

Use of performance records

Information about specific environmental factors such as air quality or specific information about pathogen load, however, is often not available for all groups of pigs. Instead, the average performance of a group of pigs housed together may be used as an environmental descriptor. Groups of pigs may represent farms, sheds, or pigs housed in the same building at the same time period. The time period may consist of weekly batches or may include groups of pigs that started (or finished) the test in the same month. Information available for any trait recorded on farms can be used to obtain an environmental descriptor for genetic analyses or for evaluation of management procedures. Such information has also been used to identify periods of disease prevalent on farms. For example, reproductive records have been used to identify outbreaks of Porcine Reproductive and Respiratory Syndrome (PRRS) on farms (Lewis *et al*. 2009; Rashidi *et al*. 2014).

Studies conducted in Australia and France found considerable environmental variation for growth and backfat in herds with good management and health status (Table 3). This demonstrates that it is not possible to fully control environmental variation.

Table 3. Number of herds (N-H) and groups (N-G), standard deviations (s.d.) and maximum difference between monthly estimates of the environmental variable (Range) based on growth (E-ADG) or backfat (E-BF)

Study	E-ADG (g/day)				E-BF (mm)		
	N-H	N-G	s.d.	Range	N-G	s.d.	Range
Gilbert *et al*. (2014)	1	80	25.0	110	44	2.0	6.9
Hermesch *et al*. (2015)	1	72	13.9	67	72	1.8	6.1
Li and Hermesch (2015)	9	950	31.0	150	950	1.0	5.0

The exact causes of this environmental variation are not known, however, heat stress is an important environmental factor, especially in Australia. Further, the range of average performances of monthly groups of pigs was 0.41 kg/day for daily feed intake and 0.32 kg : kg for FCR (Hermesch *et al*. 2015). Differences in average performances of groups were multiplied by the economic value of each trait leading to a maximum economic difference between monthly groups of pigs of $17.41 per pig based on an economic index that considered daily feed intake and $11.78 per pig for the index that included FCR (Hermesch *et al*. 2015). These economic differences between groups are expressed per pig and need to be multiplied with the number of pigs per group to obtain total economic differences between groups. It follows that considerable investments to improve environments on farms with good health and management status may still be profitable and should be considered to further improve environmental conditions for pigs on farm.

Performance and health status of pigs are affected by multiple environmental factors. Guy *et al*. (2012) discussed the mechanisms of resistance and tolerance of pigs to disease and environmental challenges. The use of data routinely collected on farms was emphasised to model and predict selection for disease resistance and disease tolerance. Further, Guy *et al*. (2012) concluded 'that a simple one-dimensional reaction norm, with pathogen burden as the only explanatory variable, cannot be used. A number of factors need to be taken into account simultaneously, including not only genotype and disease variables, but also descriptors of the environment, as well as any potential interactions'. This aspect may be addressed by using principal component analyses, which have been applied to combine individual environmental variables (e.g. Haskell *et al*. 2007). Further, Huquet *et al*. (2012) proposed a definition of the environment based on the local production environment of monthly test days using multiple factor analyses to form environmental clusters. This approach offers the possibility to investigate GxE either via multi-trait analyses, based on a distinct cluster of environments, versus more complex reaction norm models, which require continuous environmental parameters available from the first axis of the factor analysis.

Genotype by environment interactions

Differences between genotypes in their responses to environmental variation represent GxE, which may be evaluated with multi-trait analyses by defining a trait like growth rate as a different trait in each distinct environment. In pig breeding, GxE have been identified for (a) test stations, nucleus farms and commercial farms; (b) purebred and crossbred pigs; and (c) *ad libitum* and restricted feeding regimes (e.g. Merks 1989; Lutaaya *et al*. 2001; Hermesch 2004). Environments, however, may be defined more accurately with specific measurements that describe environmental conditions on a continuous scale. In these cases, reaction norm models, which fit a separate regression coefficient for each genotype on an environmental trajectory, may be used to model GxE (Falconer and Mackay 1996). In pig breeding, reaction norm models have been used to quantify the response of genotypes to varying environmental conditions using information from multiple herds for litter size (Knap and Su 2008; Herrero-Medrano *et al*. 2015) or growth and backfat (Li and Hermesch 2012). Each study found genetic differences in the responses of sows or progeny of sires to variation in environmental conditions.

Aspects of these two approaches to quantify GxE were combined by Li and Hermesch (2013), who divided the environmental trajectory based on least squares means for growth rate into seven environmental classes leading to seven growth rate traits along the environmental trajectory. Therefore, the continuous environmental scale was divided into separate environmental classes to define individual growth traits. Differences were found in variance components and heritabilities for growth rate across the environmental trajectory. Further, genetic correlations between these seven growth traits varied from 0.61 ± 0.16 to 0.99 ± 0.02 and decreased as the difference between environments increased (Fig. 1). A genetic correlation of below 0.80 is generally regarded as biologically significant and traits should be treated as two separate traits in genetic evaluations. Fig. 1 indicates that growth rate recorded in environments differing by about 60 g/day or more may be regarded as a different trait in genetic evaluations in order to account for GxE. This variation in environmental conditions observed in nucleus herds may be used to select pigs more suited to commercial conditions. For example, traits expressing GxE may be defined as separate traits for inferior and superior environments observed in nucleus herds to select pigs better suited for the specific needs of commercial herds with inferior or possibly superior environments.

Considering environmental sensitivity in the breeding objective

The breeding objective should be defined for the environment that is relevant for commercial, crossbred pigs. Selection decisions, however, are made on purebred animals in nucleus farms and environmental conditions may differ between commercial production environments and environments of a nucleus farms due to differences in husbandry, housing and health status. If genotypes differ in their response to variation in environmental conditions, then the genetically superior genes selected for in the nucleus environment may not confer the same gains in commercial conditions with differing environments. Knap

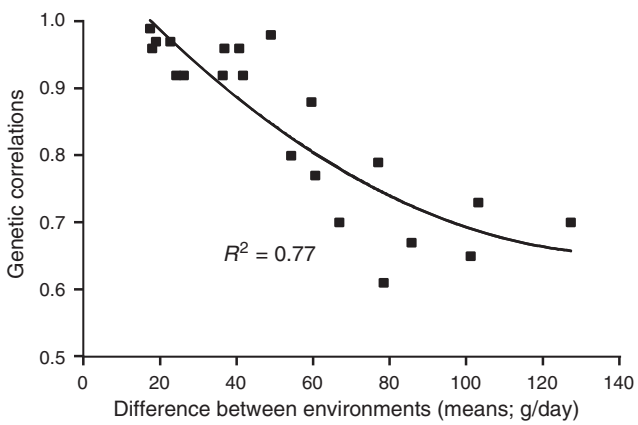

Fig. 1. Genetic correlations for growth rate defined as a separate trait in each environment declined as the difference in mean growth rate between two environments increased (Li and Hermesch 2013).

(2005) presented an approach to derive the economic value for reaction norms of days to reach market weight, assuming that pigs were selected in a superior environment typical for nucleus herds, while production was at an inferior environment representing the average customer farm with lower performance. Economic values for reaction norms were based on the economic value of the underlying performance trait, for example days to market or growth rate. This economic value was multiplied by the difference in the environmental variable of the selection and production environment. It follows that the magnitude of economic value for reaction norms depends on the difference between the selection and production environments and the economic value of the trait of interest. Further, economic values for reaction norms may be negative or positive depending on whether the production environment is below or above the selection environment (Hermesch and Amer 2013). This last point is of interest for Australian pig breeders who often select purebred pigs in very hot environments, which in fact may be inferior to commercial conditions experienced by pigs overseas with more temperate environments and may also be inferior to highly controlled environments prevalent on some commercial farms in Australia.

Hermesch and Amer (2013) compared the relative economic importance of environmental sensitivity of growth (i.e. reaction norms or slope of the reaction norm model) with the economic importance of the level of growth rate (i.e. intercept of the reaction norm model). The comparison demonstrated relatively low economic importance of environmental sensitivity because economic values of reaction norms were only 6% or 8% of the economic value for the intercept for growth when compared on the basis of genetic variation of each trait. This comparison assumed that the selection and the average production environment differed by 30 g/day for growth and 0.9 mm for backfat. The importance of environmental sensitivity depends on this difference between commercial and selection environment in regard to definition of breeding objectives as well as magnitude of GxE, which can only be estimated reliably if performance records from all environments are available and good genetic links between environments exists.

Disease resilience

Disease resilience was defined as the ability of a host to maintain a reasonable level of productivity when challenged by infection (Albers *et al*. 1987). This definition of disease resilience includes productivity and as such affects profitability of livestock production directly. Therefore, it might be more profitable to breed for low production losses due to infection rather than for high resistance to pathogen burden *per se* (Bisset and Morris 1996). This approach focuses on reducing the effects of infection rather than reducing the infection itself following the earlier work by Clunies-Ross (1932) who made the distinction between 'resistance to infection' and 'resistance to the effects of infection'.

Disease resilience is assessed via the difference in performance of an individual or a family group (e.g. sire family) between environments with different pathogen burden (Bisset and Morris 1996). Often specific measures of pathogen challenge are not known and various performance traits, immune parameters, and veterinary records may be used to estimate infection challenge prevalent for a specific environment (Hermesch 2014). Infection has been defined as the colonisation of a host by a pathogen and disease as the side effects of infection (Bishop and Stear 2003). In this context, infection challenge is the result of the pathogen burden experienced by a group of pigs, which may include multiple pathogens, and the effects of multiple environmental factors that enhance or inhibit the effects of pathogen burden on performance, health and survival of pigs.

Selection for disease resilience uses data available on farms to improve health of animals. Infection challenges experienced by individual animals and epidemiology processes of a disease outbreak are usually not considered. Ignoring different stages of infection of individuals when performance records are collected may bias estimates of disease resilience. This potential bias may be reduced by taking repeated measurements of host performance over a sufficiently long time period to capture the full impact of the infection on performance of individual pigs (Doeschl-Wilson and Lough 2014). In particular, repeated weight and feed intake records available from electronic weight scales and feeders offer opportunities to develop selection strategies for disease resilience because poor growth and reduced feed intake are observed in many diseases where clinical signs are present. Further, even in sub-clinical infections, energy is being directed away from growth towards tissue repair and immune activation (e.g. Collins 2014).

Resistance and tolerance improve disease resilience

Traits to describe disease resilience used information about pathogen burden in environments (e.g. Bisset and Morris 1996) because information about pathogen levels within a host is usually not available. Therefore, disease resilience defined in this way does not provide information about disease resistance and disease tolerance of the host. Disease resistance is the ability of the host to exert control over the parasite or pathogen life cycle whereas disease tolerance has been defined as the net impact of an infection on performance of a host (e.g. Bishop 2012).

Direct measures of disease resistance relate to the intrinsic ability of animals to fight pathogens and are often based on accurate laboratory analyses of faecal egg count, viraemia or bacterial load to quantify the infection load due to nematodes, viruses or bacteria. It is therefore not surprising that genetic variation has been found for traits describing disease resistance. For example, there is ample evidence for genetic variation in pigs in viral load of the porcine reproductive and respiratory syndrome virus (e.g. Lunney and Chen 2010), and genetic factors including genetic markers affecting susceptibility of pigs to atrophic rhinitis, *Escherichia coli* infection and *Trichinella sprialis* were outlined in the review by Crump (1999).

Selection for direct measures of disease resistance reduces within-host pathogen burden, which reduces the overall pathogen burden on farms. The review by Bishop (2012) listed experimental studies in sheep that demonstrated epidemiological benefits arising from populations with improved resistance to nematode infections. This observation provides a strong argument to implement selection strategies for improved disease resistance with beneficial health and welfare consequences for groups of animals, because selection for disease resistance improves environments by reducing pathogen load.

Disease tolerance may be quantified by changes in performance with increasing pathogen burden. Genetic variation in tolerance implies that genotypes differ in their response to pathogen burden, which again can be quantified with reaction norm models that describe the response of a genotype to varying pathogen burden. However, this requires large datasets with individuals adequately quantified for pathogen burden. Doeschl-Wilson *et al*. (2012) provided a mathematical framework to quantify tolerance for an individual more precisely based on within-host pathogen burden. Such an approach requires information about (a) repeated measures of host performance and within-host pathogen burden over time for each animal, (b) information about the performance potential of an animal in a pathogen-free environment, and (c) information about other factors influencing performance over time. Although it may be possible to obtain sufficient data to fulfil (b) and (c) from farm data, repeated measures of host performance and pathogen burden for individual animals are currently not routinely available from commercial populations. Therefore, it may not be possible to quantify disease tolerance and to distinguish disease tolerance and disease resistance in practical breeding programs. Group measures of pathogen burden, however, do present an estimate of the overall infection burden prevalent on-farm and may be used to develop new traits for disease resilience, which does not distinguish between disease resistance and disease tolerance.

Immune response and competence

Pig genotypes have been shown to differ in their response to disease challenges (e.g. Schinckel *et al*. 1999; Doeschl-Wilson *et al*. 2009) possibly due to differences in immune parameters, which have been shown to be moderately to highly heritable (e.g. Clapperton *et al*. 2005, 2008; Henryon *et al*. 2006; Flori *et al*. 2011). The wide range of immunity traits was grouped by Flori *et al*. (2011) into traits describing global immunity, cell-mediated adaptive immunity, humoral-mediated adaptive immunity, innate immunity and other haematological traits. So far, there is no consensus among scientists about the use of specific immunity traits in pig breeding programs. Bishop *et al*. (2002) pointed out that heritability estimates tend to increase from traits describing general disease category to traits quantifying specific disease resistance and traits measuring specific immune response. This was also observed by Kerr *et al*. (2005), who found higher heritability estimates for gene-expression traits when extreme observations were included in genetic analyses. These extreme observations may have been due to a disease incidence for specific pigs. However, overall heritability estimates were low for these gene-expression traits that were based on records obtained in a commercial herd. Kerr *et al*. (2005) mentioned technical and logistical challenges of data collection on-farm and indicated that an acceptable stress challenge that can be applied on farms before collection of blood samples may be required in order to identify genetic differences in immune parameters based on commercial data.

Recently, Hine *et al*. (2014) reviewed selection strategies for immune competence. The authors concluded that selection for resistance to a specific disease carries the potential risk of increasing the susceptibility to other diseases. This risk is reduced by selection for general immune responsiveness as an alternative or complementary selection strategy to selection for a specific disease. Differential blood counts describe global and innate immunity (Flori *et al*. 2011), and haptoglobin has been recommended as an important marker of herd health in pigs (Petersen *et al*. 2004). Maternal effects influence health of progeny and maternal immunoglobulins may be used to quantify the effect of the dam on progeny health (Collins 2014). Overall, these studies indicate that immune parameters offer opportunities for selection to improve health and disease resilience of pigs. However, the consequences of using specific immune parameters in selection decisions have to be monitored.

Unexpected and unfavourable consequences of selection for immune response can be avoided by focusing directly on disease resilience, survival and low incidence of clinical and sub-clinical diseases. In pigs, Henryon *et al*. (2001) found genetic variation for clinical and sub-clinical disease in pigs that were based on veterinary records from the central test station in Denmark. In addition, genetic variation was found for a simple disease incidence score based on routine veterinary observations on non-specific digestive disorders in a commercial rabbit population (Garreau *et al*. 2008). These results are noteworthy because simple disease incidence scores were derived from routine veterinary records and genetic variation for disease scores was found in good health and housing conditions. Despite these promising results, selection for improved health remains challenging and information should be collected from as many environments as possible including commercial environments that may have higher incidence of disease than nucleus farms.

Residual feed intake and robustness

The resource allocation theory developed by Beilharz *et al*. (1993) defines fitness as a product of separate component traits each requiring environmental resources that are additively allocated to individual processes. Resources available from a specific environment determine the genotype selected in each environment that maximises phenotypic fitness at that environmental level. This matching of genotypes to different environmental resources leads to GxE if environmental resources vary from the resources that were available in the selection environment (Beilharz and Nitter 1998). The equation presented by Beilharz and Nitter (1998) to allocate resources to individual processes is very similar to the linear regression of feed intake on individual components of performance that defines residual feed intake (RFI) as outlined by Rauw (2007), who suggested that 'the similarity between these models implies that calculation of RFI can be used to quantify the amount of 'buffer' resources available to an animal for example physical activity and the ability to cope with unexpected stresses'.

The RFI of pigs can be estimated as the residual of a model for feed intake that includes growth rate, backfat and possibly metabolic bodyweight as covariates (Mrode and Kennedy 1993). In 2000, divergent selection lines for RFI were established in Iowa (USA) and France. The comparison of divergent selection lines at each location showed that selection for lower RFI resulted in more efficient, leaner and slower growing pigs (Dekkers and Gilbert 2010). More recently, pigs from these selections lines have been compared in different environments and have been exposed to various challenges including heat stress and PRRS

Table 4. Significance (*P*-value; ****: $P < 0.0001$) of the effects of line, environment and line × environment interaction, and least square means (LSMEANS) of the line × environment interaction (Gilbert *et al.* 2012)

Letters indicate values different at $P < 0.05$ within traits. E, environment; FE, France; Europe; FWI, French West Indies; HRFI, high residual feed intake (RFI) selection line; LRFI, low RFI selection line; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; BF, backfat; wk, weeks

Statistic	Effects	ADG (g/day) 11–15 wk	ADFI (g/day) 15–23 wk	ADG (g/day) 15–23 wk	FCR (kg : kg) 15–23 wk	BF (mm) 23 wk
P-value	Line	****	****	0.33	****	****
	E	****	0.22	0.62	0.69	0.0008
	Line × E	0.04	0.71	0.02	0.008	****
LSMEANS	HRFI × FE	797a	2290a	776ab	2.98a	18.4a
	LRFI × FE	770a	2043b	788ab	2.61b	15.9b
	HRFI × FWI	583b	2345a	806a	2.92a	15.7b
	LRFI × FWI	510c	2080b	773b	2.69b	16.0b

virus challenge (Gilbert *et al*. 2012; Campos *et al*. 2014; Dunkelberger *et al*. 2015).

Performance of pigs from the French selection lines was compared in the temperate environment prevalent in France, Europe and the tropical environment of the French West Indies (Gilbert *et al*. 2012; Table 4). In France, growth rate was similar between the two lines during the earlier and later growth phases. In comparison, line differences were larger in the French West Indies for the earlier growth phase from 11 to 15 weeks of age compared with the period from 15 to 23 weeks. The low RFI line grew on average 73 g/day less in the earlier period than the high RFI line, whereas the difference was 33 g/day in the later growth period. Overall, these results indicate a higher depressive effect of the tropical environment on growth of low RFI pigs in the early stages of growth that was not fully compensated during the later growth period. However, the line difference was limited for FCR and the low RFI pigs remained more efficient in the tropical environment.

Average daily feed intake of each selection line was similar in each environment in the study by Gilbert *et al*. (2012). This is unexpected because reduction in feed intake is a main response to heat stress, which has also been observed for these lines in experiments with controlled temperature challenges (Renaudeau *et al*. 2013; Campos *et al*. 2014). In the study by Renaudeau *et al*. (2013), both selection lines had a similar reduction in feed intake resulting from increased temperature whereas the decrease in feed intake due to raised temperature was larger for the high RFI line in the study by Campos *et al*. (2014). Further, it was found that the low RFI line adapted to the raised temperature more quickly. There were no differences between lines in their response to controlled heat stress in regard to rectal or skin temperature and respiratory or heart rate, confirming results by Renaudeau *et al*. (2013) that thermal acclimatisation of both selection lines to heat stress was similar. Therefore, selection for feed efficiency based on RFI did not decrease the ability of pigs to withstand heat stress. This finding is relevant for Australia with a hot climate where pigs are exposed to heat stress regularly.

In a subsequent study, environmental sensitivity of these selection lines was investigated by comparing the response in growth to environmental variation observed on farm in France (Gilbert *et al*. 2014). Effectively, a reaction norm was fitted for each selection line on the estimate of monthly growth environments. Contrary to expectations, it was the high RFI line that had higher environmental sensitivity for growth whereas no line differences were found for backfat. The lower environmental sensitivity in growth of the low RFI line, however, corresponds to findings by Dunkelberger *et al*. (2015) who compared growth and viral load of the RFI selection lines in Iowa following a challenge with PRRS virus. There was a tendency for pigs from the low RFI line to have a lower viral load ($P = 0.09$), a greater growth rate ($P = 0.10$) and a greater chance of surviving the PRRS virus challenge ($P = 0.06$). Further, the joint analysis of challenged and non-challenged pigs showed a significant interaction between RFI line and challenge status for growth. Growth of the low RFI line was less affected by the PRRS virus challenge than growth of the high RFI line. These results question the hypothesis that high RFI provides a buffer for animals to face stresses and may indicate a more complex relationship between available resources and individual metabolic processes than indicated by the resource allocation theory. Gaining a better understanding of these relationships is important for genetic improvement of productivity and robustness of pigs.

Conclusions

Pig breeding programs around the world continue to improve both productivity and robustness by extending selection emphasis to a wider range of traits. No trait group can be seen in isolation. Further, genetic improvement itself cannot be viewed in isolation and needs to be accompanied by improvement in management strategies. Selection and management strategies will both lead to continued improvements in performance, health and welfare of pigs. The main conclusions of this review are:

(1) Improving environmental conditions on-farm is the first priority. Genetic analyses disentangle genetic from environmental effects and provide descriptors of environmental conditions in the absence of explicit environmental measures. Estimates of environmental descriptors from genetic analyses could be used to monitor environmental conditions on-farm, which depend on multiple specific environmental factors including the incidence of disease. Furthermore, new technologies in precision agriculture and veterinary practice offer new opportunities to quantify environmental and pathogen challenges better.

(2) A flexible approach has been presented that facilitates extension of breeding objectives to include further traits that describe productivity and robustness of animals. The level of performance affects the economic importance of some breeding objective traits and breeding objectives should be defined for the commercial environments of the production of pork.
(3) Defining traits for breeding programs to improve health status of pigs remains challenging. The rate of genetic improvement increases as more sources of phenotypic information and genetic information, via marker-assisted selection or genomic selection, are incorporated in genetic evaluations. Information about repeated measures of growth and feed intake, survival of pigs, disease incidence and medication records as well as immune parameters will aid genetic improvement of disease resilience.
(4) Selection for improved health of pigs should incorporate disease resistance traits for infectious diseases such as *Escherichia coli* infections because selection for improved disease resistance reduces pathogen load on farm and therefore improves environmental conditions for all pigs. The specific infection pathways of each pathogen have to be considered in selection strategies for each specific disease resistance trait.
(5) It is possible to improve productivity and robustness simultaneously. The responses of divergent selection lines for RFI to challenging environments, or controlled heat or PRRS challenges were often similar or even favourable for the more efficient, low RFI line. Further research is required to evaluate why the associations between productivity and robustness traits have been variable between studies. This requires better understanding of the metabolic differences between more or less productive pigs to comprehend the interactions of the genetic potential of pigs for productivity and robustness and the prevalence of specific environmental conditions.

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Scouring weaner pigs have a lower abundance of butyrate-producing bacteria

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Scouring caused by pathogenic bacteria leads to poor weight gain, dehydration and (or) sudden death in newly-weaned pigs (Fairbrother *et al.* 2005). Commensal bacteria, including butyrate producers, are thought to reduce scouring by preventing colonisation of enterotoxigenic *E. coli*, whilst improving growth performance and intestinal function through increased villous height (Wen *et al.* 2012). This study hypothesised that scouring weaner pigs would have a lower abundance of butyrate-producing bacteria in faeces than non-scouring pigs.

Individual faecal samples classified as either non-scouring (n = 47) or scouring (n = 26) were submitted from pigs 2 to 3 weeks after weaning from six Australian piggeries; four medicated and two non-medicated. Faecal DNA was extracted using the MagMAX Pathogen RNA/DNA Kit and bacteria were sequenced using universal 16S rRNA primers V4/5 (515F and 806R). Sequences were analysed using the QIIME pipeline with appropriate quality controls and bacterial groups were expressed as abundance relative to total bacteria. The impact of scouring and farm factors on the relative abundance of bacterial taxa was assessed using canonical correspondence analysis (CCA) approaches (R, version 3.1.2). Microbial groups in the upper right quadrant are more abundant in scouring weaners, whereas those in the lower left are more abundant in non-scouring weaner pigs (Fig. 1).

Faecal microbial communities from scouring and non-scouring pigs clustered separately (Fig. 1B), despite a farm effect (Fig. 1A). The faecal samples from scouring pigs were dominated by *Clostridium* (#21), *Lactobacillales* (#14), *Enterobacteriaceae* (#53) and *E. coli* (#54), whereas a higher abundance of butyrate-producing bacteria such as *Pseudobutyrivibrio* (#27), *Roseburia* (#28) and *Veillonellaceae* (#39) were recovered from the non-scouring pigs (Fig. 1C). Faecal samples from Farm 1 contained more *Ruminococcaceae*, Farm 5 had higher numbers of *Lactobacillales* and *Actinobacteria*, and Farm 6 had a greater abundance of *Porphyromonadacea*e and *Erysipelotrichaceae* (data not shown). The pigs at the remaining farms shared a similar faecal bacterial composition.

This study demonstrated an increased abundance of butyrate-producing bacteria and reduced *E. coli* and *Enterobacteriaceae* in non-scouring pigs, suggesting that butyrate plays an important role in gastrointestinal tract health, as described previously (Wen *et al.* 2012). The high abundance of *Lactobacillales* in scouring pigs could reflect increased antagonistic activity of *Lactobacilli* against *Enterobacteriaceae* (Looft *et al*. 2014). Further studies would help to separate the impact of scouring from farm factors, including diet, antimicrobial use, hygiene and genetics.

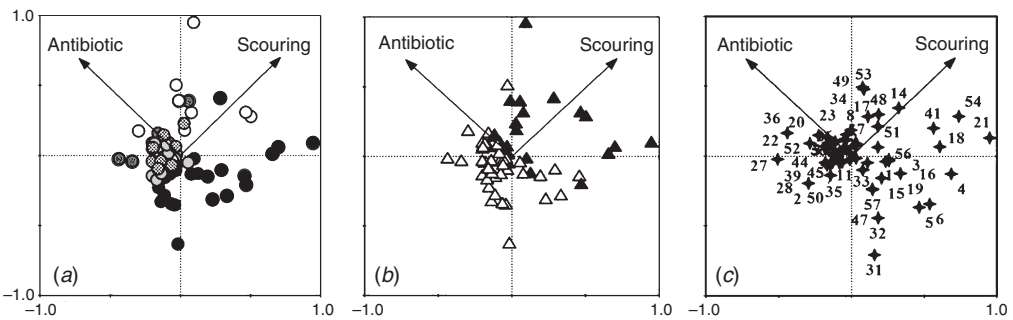

Fig. 1. Canonical correspondence analysis showing the influence of farm effects (A) and scouring (B) on pig faecal microbiota and individual taxa distributions (C), where plots represent: ● Farm 1, ● Farm 2, ● Farm 3, ○ Farm 4, ● Farm 5, ○ Farm 6, ▲ scouring, △ non-scouring and ✦ microbial groups.

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A comparison of inflammation models in weaner pigs

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Inflammation models are used to compare the effectiveness of anti-inflammatory agents. Subcutaneous injections of turpentine have been used in the past to cause an acute phase response in pigs (Lampreave *et al*. 1994; Eckersall *et al*. 1996). It has been suggested that some vaccines may be used as models for inflammation due to the sickness-like behaviour they elicit (Fangman *et al*. 2011), but there have been no controlled studies to investigate this claim. In this study it was hypothesised that the administration of Improvac® and Neovac® would provide an inflammation response similar to the administration of turpentine.

This trial involved 24, 7-week-old male Landrace x Large White weaner pigs (n = 6/treatment). Pigs were housed in pens of four (one per treatment group). Inflammation was induced by a single subcutaneous injection behind the right ear with one of the following: physiological saline (2 mL, 0.9%), Improvac® (2 mL; Zoetis, Sandton, South Africa), Neovac® (2 mL; Zoetis, Rhodes, NSW, Australia), or pure turpentine (0.2 mL/kg) on d 1. Inflammation was assessed by measuring haptoglobin and C-reactive protein (CRP) concentrations in blood collected on d 0, 2 and 4 after injection using Tridelta® Phase™ Range assays. Infrared eye temperatures (IET) were collected from images taken daily (d 0 – d 4) 45 cm from the left eye and eye temperature determined by dot point analysis. Tear staining areas were measured from photographs taken daily of the left eye and analysed using the freeware Image-J software (NIH; Rockville, MD, USA). Haptoglobin, CRP and IET data were analysed using a linear mixed model (LMM) (Genstat, 17th Edition; UK). Tear staining data were log transformed and analysed using LMM.

The administration of turpentine, Improvac® and Neovac® resulted in increases in haptoglobin ($P < 0.001$) and CRP concentrations ($P < 0.001$) relative to saline controls. Turpentine-treated weaner pigs had higher eye temperatures compared to all other treatment groups ($P < 0.05$). Pigs administered Neovac® had lower amounts of tear staining than pigs administered Improvac® or turpentine ($P < 0.05$) (Table 1).

The increases in haptoglobin and CRP concentrations indicated that the subcutaneous administration of Improvac®, Neovac® and turpentine caused an inflammatory response in weaner pigs. Pigs administered turpentine showed a severe behavioural pain response (data not shown), and so this is not recommended for future work. Pigs treated with Improvac® showed an acute phase response similar to turpentine, without the associated pain, which indicates that this model may be suitable for testing the efficacy of analgesic/anti-inflammatory drugs.

Table 1. Haptoglobin and CRP concentrations, IET and tear staining area after a subcutaneous injection of either saline, Improvac®, Neovac® or turpentine. Values are mean ± SE

Variable	Saline	Improvac®	Neovac®	Turpentine
Haptoglobin (mg/ml)	1.3 ± 0.1[a]	2.2 ± 0.1[c]	1.7 ± 0.1[b]	2.3 ± 0.1[c]
CRP (ng/ml)	802 ± 166.9[a]	1805 ± 166.9[c]	1235 ± 166.9[b]	1705 ± 166.9[c]
IET (°C)	33.7 ± 0.22[a]	33.8 ± 0.22[a]	33.9 ± 0.22[a]	34.4 ± 0.22[b]
Tear staining (cm^2)	0.06 ± 0.018[ab]	0.08 ± 0.022[a]	0.03 ± 0.009[b]	0.08 ± 0.022[a]

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

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Investigation into the occurrence of newly recognised agents of swine dysentery in Australian pig herds

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Swine dysentery (SD) is a severe mucohaemorrhagic colitis classically described as resulting from infection of the caecum and colon with the anaerobic intestinal spirochaete *Brachyspira hyodysenteriae*. Swine dysentery can severely depress feed conversion efficiency in the grower/finisher phases and represents an animal welfare issue. In addition, control of SD requires considerable antimicrobial use.

Historically, *B. hyodysenteriae* has been believed to be the sole causative agent of SD, however outbreaks of bloody diarrhoea indistinguishable from SD have been documented since 2007 in grower-finisher pigs in Canada and the USA in farms where *B. hyodysenteriae* could not be identified. Investigation of these cases led to the recognition of novel, strongly β-haemolytic *Brachyspira* isolates, for which the name '*Brachyspira hampsonii*' has been proposed (Chandler *et al*. 2012). Experimental inoculations of pigs have established the pathogenic potential of this new species (Rubin *et al*. 2013*a*). In addition to North America, cases of SD caused by *B. hampsonii* have been recorded in pigs in Europe in 2013 (Mahu *et al*. 2014), and the species has been isolated from migratory waterbirds in Canada and in Spain. The latter species are thought to be reservoirs of the pathogen (Martínez-Lobo *et al*. 2013; Rubin *et al*. 2013*a*, 2013*b*). A distinct agent called '*Brachyspira suanatina*' that causes a swine dysentery-like disease also has been described in feral waterbirds and pigs in Scandinavia (Råsbäck *et al*. 2007).

In Australia, cases of colitis associated with 'atypical' strongly β-haemolytic *Brachyspira* strains also have been observed, although these have not been further investigated. Although Australian pig veterinarians are well aware of the importance of '*B. hampsonii*' and related species, their prevalence amongst and within Australian herds is still not known. The lack of availability of diagnostic tools capable of identifying '*B. hampsonii*' is undoubtedly a contributing factor to this lack of data.

The aim of this study was to determine to what extent novel pathogenic *Brachyspira* species, including the recently described '*B. hampsonii*', are present in Australian pig herds. Diagnostic polymerase chain reactions for the direct identification of '*B. hampsonii*' and '*B. suanatina*' were developed and applied to samples collected from pigs with signs consistent with SD, or where the SD status was uncertain.

To date, 372 faecal samples and 239 colon samples have been received and tested. A total of 83 isolates (13.6%) of *B. hyodysenteriae* have been recovered from these samples. In addition, 64 isolates (10.5%) of *Brachyspira pilosicoli* (the agent of porcine intestinal spirochaetosis) and 56 isolates (9.2%) of *Brachyspira intermedia* (a species of uncertain pathogenicity) have also been identified. However, no isolates of '*B hampsonii*' or '*B. suanatina*' have been recovered. The results suggest that if isolates of the new pathogenic *Brachyspira* species are present this would likely be at a low prevalence, and hence they should not be a major issue for the Australian industry at the present time. Nevertheless, further surveillance is justified.

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Relationships between diets different in fibre type and content with growth, *Escherichia coli* shedding, and faecal microbial diversity after weaning

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Insoluble non-starch polysaccharides (iNSP) can decrease enterotoxigenic *E. coli* (ETEC) shedding in the gastrointestinal tract (GIT) and reduce post-weaning diarrhoea (PWD), whilst higher levels of soluble NSP (sNSP) have been associated with increased PWD (Pluske *et al*. 2002). A number of mechanisms such as reduced retention time, inhibition of mucosal *E. coli* adhesion and proliferation of butyrate-producing bacteria have been suggested to explain the beneficial effects of more iNSP in the diet (Lindberg 2014). However associations between dietary iNSP levels, specific microbial species and effects on production and ETEC shedding after weaning have not been explored in detail. The hypothesis tested was that pigs fed iNSP would have a higher abundance of butyrate-producing bacteria that in turn is correlated to indices of production and ETEC shedding.

An experiment having a 2 × 4 factorial arrangement of treatments using 48 individually-housed male weaner pigs (initial body weight 8.8 ± 0.05 kg; mean ± SEM) was conducted, with factors being low and high sNSP (7 versus 28 g soluble arabinoxylan/kg) and four levels of iNSP added as Opticell® (equivalent to 5.5, 19.0, 34.5 and 51 g iNSP/kg). Faecal samples were collected pre- (day 5) and post- (day 9) infection with ETEC. Faecal β-haemolytic *E. coli* shedding (after Heo *et al*. 2009) and average daily gain (ADG) was measured. Extracted faecal DNA was quantified, amplified by polymerase chain reaction and sequenced. All sequencing data was analysed using the QIIME pipeline and the relationship between dietary fibre, microbial diversity and production indices was explored using linear regression analysis (R: Free Software Foundation's GNU General Public License).

Increasing dietary iNSP improved growth performance and reduced *E.coli* shedding (Fig. 1*a*). It was also associated with an increased relative abundance of *Christensenellaceae* (a butyrate producer) and decreased abundance of *Lactobacillaceae* (a lactate producer) (Fig. 1*b*). In contrast, increasing dietary sNSP significantly decreased abundance of *Christensenellaceae* (data not shown). *Christensenellaceae* play a key role in maintaining GIT structure and function by forming syntrophic partnerships with *Methanobrevibacter* (the main methanogen in the GIT). *Christensenellaceae* alters host gene expression and reduces inflammation during *E. coli* infection, and has been associated with lean and healthy humans (Guilloteau *et al*. 2010). Increasing iNSP content in the diet altered the balance between butyrate and lactate producing taxa that in turn increased ADG and decreased ETEC count.

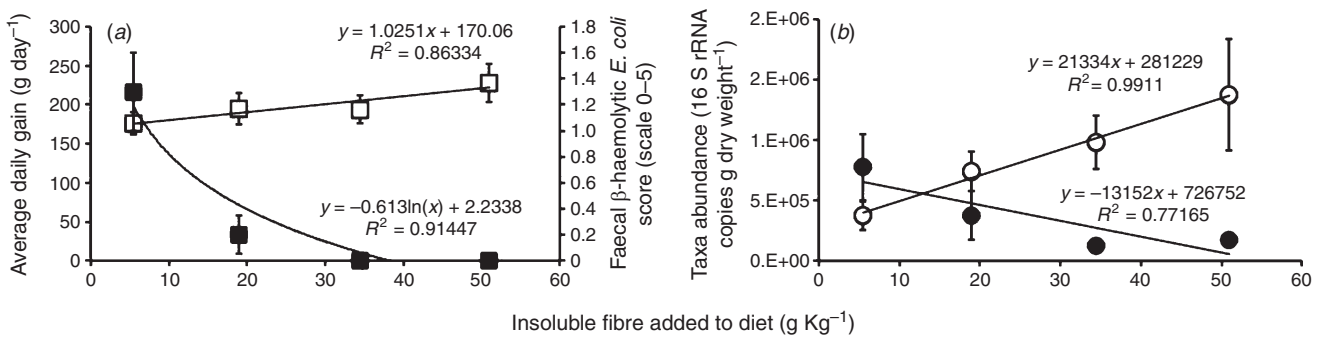

Fig. 1. The influence of increasing insoluble fibre diet intake (supplied as Opticell®) on (*a*) average daily gain (□) and faecal β-haemolytic *E. coli* score assessed form 1-5 (■), and (*b*) the relative abundance of *Christensenellaceae* (○) and *Lactobacillaceae* (●) in the first 2 weeks after weaning following infection with enterotoxigenic *E. coli* (mean ± SEM; n = 3).

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Aeration of anaerobic pig slurry for ammonia oxidation

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Anaerobic ponds (AP) are common practice at Australian piggeries for the treatment of pig waste, with effluent discharge from ponds often reused on-farm (APL, 2010; Tucker *et al*. 2010; Buchanan *et al*. 2013). The present study was conducted as part of a larger project looking to boost pig effluent as an alternative sustainable resource and improve the pork industry's environmental status. This will be achieved through the conversion of greenhouse gas emissions to a renewable energy source via the growth of microalgae in integrated piggery wastewater treatment systems (WWTS) (Buchanan *et al*. 2013). Effluent from AP is rich in ammonia (NH_3), pathogenic organisms, and high-suspended solid (SS) loads. High levels in pig effluent, if untreated, could inhibit algal growth or pose a potential risk to pig health when reused as shed flushing material, which are concerns for reuse (Buchanan *et al*. 2013). Aerobic treatment for the oxidation of NH_3 to nitrate (NO_3) is a potential method to alleviate the adverse effects of NH_3 on algal growth and to reduce the concentrations of SS and pathogens. The objective of this preliminary experiment was to determine, at laboratory scale, NH_3 oxidation within an aerobic reactor fed AP effluent at an aeration level of 10% saturation (0.7 mg O_2/L) and a 5-day theoretical hydraulic retention time (THRT).

Anaerobic pig slurry (ANPS) collected from an AP at a local South Australian piggery, was pumped intermittently through a bench top aerobic reactor over a 25–30 day period to achieve the desired 5-day THRT. The ANPS was aerated by blowing air intermittently through the reactor to maintain a dissolved oxygen level of 0.7 mg O_2 /L. Influent and effluent samples were collected and analysed at 4–5 day and 2–3 day intervals, respectively. Table 1 summarises the results of a series of chemical analyses performed on inlet and outlet slurry post aerobic treatment, using standard wastewater analysis methods (APHA 1995) to assess the oxidation potential (nitrification) of the system under these conditions.

The mean inlet ammonium (NH_4-N) concentration at the start of the experiment was 1.5 ± 0.7 g/L (mean $\pm$ SD) and that of suspended solids was 0.8 ± 0.1 g/L (Table 1). Post-aerobic treatment showed mean NH_4-N and SS levels had decreased by 28.8% and 52.2% respectively. This, in conjunction with increased NO_2-N and NO_3-N from zero in the inlet effluent to 0.2 ± 0.1 g/L and 0.1 ± 0.0 g/L detected in the outlet effluent, demonstrated that NH_3oxidation had occurred. Reducing NH_4-N to its non-toxic form NO_3-N can lead to lower disease potentials associated with NH_3 exposure and improved water quality. Both are vital and beneficial for reuse on-farm.

Findings from this preliminary experiment suggest aeration of ANPS to be a positive candidate for the treatment of piggery waste. Ammonia oxidation did occur, however the conversion of NH_4-N to NO_3-N was relatively low. Further research will assess the oxidation capability of the integrated system under different conditions of DO and THRT to best identify optimal operating conditions to achieve maximum nitrification.

Table 1. Mean nutrient levels in anaerobic pig slurry before and after aerobic treatment at 10% saturation and a 5-day THRT. Values are mean ± SD

	NH_4-N[A] (g/L)	NO_2-N (g/L)	NO_3-N (g/L)	TN (g/L)	SS (g/L)	TOC (g/L)	TC (g/L)	IC (g/L)
Inlet	1.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 0.2	0.8 ± 0.1	0.8 ± 0.3	2.7 ± 0.4	1.9 ± 0.1
Outlet	1.0 ± 0.5	0.2 ± 0.1	0.1 ± 0.0	1.3 ± 0.3	0.4 ± 0.0	0.5 ± 0.1	1.4 ± 0.3	0.9 ± 0.2

[A]Chemical analysis performed: Ammonium (NH_4-N); Nitrite (NO_2-N); Nitrate (NO_3-N); total nitrogen (TN); total organic carbon (TOC); total carbon (TC); inorganic carbon (IC); suspended solids (SS).

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Bioprospecting microalgae for growth on undiluted anaerobic digestate of piggery effluent

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Microalgae cultures do not generally compete with agricultural food crops except for their reliance on fertilisers (Borowitzka and Moheimani 2013). Comparatively, some agricultural wastes such as anaerobic digestion of piggery effluent contain very high concentrations of nitrogen and phosphorous (Buchanan *et al*. 2013). The use of microalgae culture for the treatment of anaerobic digestate of piggery effluent offers attractive advantages over current wastewater treatment systems used by piggeries. This effluent is very high in ammonium, which at high pH is toxic to most organisms (Buchanan *et al.* 2013). If microalgae can recover nutrients from anaerobic digestion of piggery effluent in the form of biomass, this could potentially be used as a source of feed or bioenergy. If the undiluted anaerobic digestion of piggery effluent is treated by selective microalgae, this can also improve water recycling and economic returns (Buchanan *et al*. 2013). This study utilised bioprospecting strategies (indoor and outdoor) incorporating the selection and culture of microalgae that were capable of growing on undiluted, untreated anaerobic digestate of piggery effluent.

Detailed bioprospecting was conducted to isolate suitable microalgal species capable of growth on anaerobic digestion of piggery effluent (Ayre 2013). As a result, *Chlorella*, *Scenedesmus* and a pennate diatom were isolated using a synthetic medium with up to 500 mg NH_3-N/L.

The next step involved the culture of isolated species in outdoor paddle-wheel-driven raceway ponds over a course of 20 weeks with ammonia concentrations of up to 1,600 mg NH_3-N/L. Maintaining a steady culture density in the raceway ponds over the course of cultivation demonstrated the potential for on-going long-term nutrient removal using microalgae and translation to large-scale applications.

The highest ammonium removal rate achieved was equal to 83.3 mg NH_3- N/L/d. Under the batch mode, the phosphorus (P) and carbon (C) removal rates were 5.2 mg P/L/d and 562 mg IC/L/d, respectively. The average biomass productivity of 25.6 mg dry matter or ash-free dry weight/L/d was achieved. It was also found that CO_2 addition could significantly ($P < 0.05$) enhance microalgae growth (repeated measure one-way ANOVA). This proof-of-concept study illustrated the potential for culturing microalgae in untreated and undiluted anaerobic digestion piggery effluent having high ammonium content.

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Co-digestion of pig slurry with an algae-rich municipal wastewater sludge

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Increasingly, covered anaerobic lagoons are being considered by the Australian pork industry to manage greenhouse gas (GHG) emissions and recover methane (CH_4) for energy production. Algal biomass produced in high-rate algal ponds (HRAP) treating piggery wastewater removes CO_2, contributing to GHG mitigation, and is an additional source of biomass energy that could be released via co-digestion with pig slurry (Buchanan *et al.* 2013). The objective of this study was to investigate an optimum feed ratio for co-digestion of wastewater grown algal biomass with pig slurry for CH_4 production.

Algae-rich sludge (ALBAZOD; a mixture of algae, bacteria, zooplankton and detritus) was collected from a dissolved air flotation plant and a pig slurry sample was collected from a piggery in South Australia. Experiments were established in 30 L plastic batch anaerobic digester vessels, which were seeded with 20 L of anaerobically digested sludge obtained from the two sites described. The reactors were purged with N_2 gas and digested under room temperature (17−25°C) for 3 months with manual mixing by rotating the vessels once per day. Six experimental groups were studied as follows: 100% pig slurry (PS); 96.5% PS + 3.5% ALBAZOD (A); 92.9% PS + 7.1% A; 85.4% PS + 14.6% A; 67.8% PS + 32.2% A; and 100% A. All experiments were performed with triplicate analysis (n = 3) and the ALBAZOD percentages were calculated based on volatile solids (VS) per g of dry weight (APHA 1995). The results were statistically analysed by independent samples T-Test (95% confidence interval, $P \leq 0.05$).

The highest CH_4 production was observed from the 96.5% PS + 3.5% A mixture (Fig. 1), with a production of 0.344 L/g VS removed and a slightly lower production of 0.339 L/g VS removed from 100% PS. However, no significant difference was found on CH_4 production compared to the 100% PS. The CH_4 production decreased as the ratio of ALBAZOD increased in the mixture. When the ALBAZOD ratio was beyond 7.1% A, the CH_4 production decreased to below 0.200 L/g VS removed. The lowest CH_4 (L/g VS removed) was observed from the 100% A control experiment with an average of 0.040 L/g VS removed over the first 73 day period, that then rapidly increased up to 0.174 L/g VS removed at d 91.

The results suggested that although there was a slightly increase in overall CH_4 production with the optimum ALBAZOD mixture, the ratio is crucial in order to achieve optimum CH_4 production between pig slurry and ALBAZOD because it is known as poorly degradable. In conclusion, anaerobic digestion and co-digestion can capture energy in the form of CH_4 which can be converted into electrical energy further enhancing the sustainability of the pork industry (Miao *et al.* 2014; Astals *et al.* 2015). Further investigations of pre-treatment with ALBAZOD to increase its biodegradability would seem warranted to optimise this research.

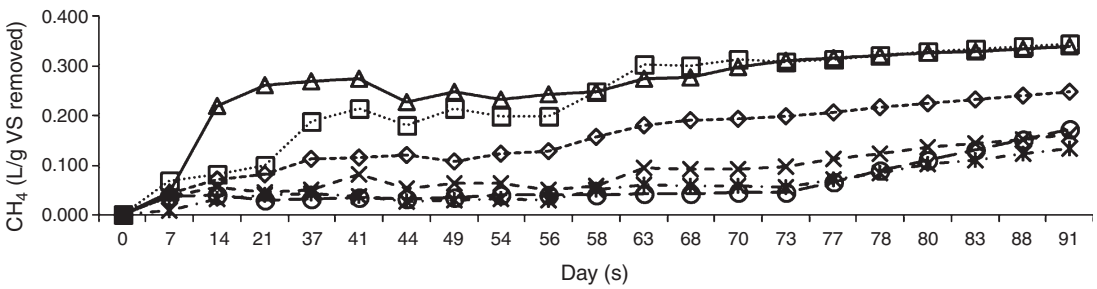

Fig. 1. Cumulative methane (CH_4) production calculated based on per gram of volatile solid (VS) removed from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91-day period. Values are means ± SE (n = 2). △: 100% PS; □: 96.5% PS + 3.5% A; ◇: 92.9% PS + 7.1% A; ×: 85.4% PS + 14.6% A; ✳: 67.8% PS + 32.2% A; ○: 100% A.

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A novel separation system removes solids from pig effluent more effectively than other systems in common use

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About 40% of Australia's piggeries use anaerobic lagoons (ponds) to manage liquid waste, but associated maintenance and infrastructure costs can be significant. Also, methane from treatment ponds accounts for as much as 60–70% of greenhouse gases emitted across the Australian pork supply chain (Wiedemann *et al.* 2009). Lastly, ponds can be a significant odour source. For these reasons, there is interest in pondless effluent management systems that can recover manure as solids prior to substantial fermentation, thereby producing a solid cake for co-composting and a low-strength liquid waste (filtrate) for treatment in smaller less costly ponds or for direct recycling as flush water, thus creating a closed-loop system. The aim of this study was to evaluate a potentially pondless system consisting of a novel de-watering/filtering system, the Z-Filter (Z-Filter Pty Ltd, WA), on farm at a commercial pig shed housing 1,200 pigs aged 10 to 22 weeks.

For each filtration test run, an entire flush from any one of four flush lanes was collected in a 10 kL holding tank, to which 0.8 to 1.0 L of coagulant (Floquat FL 2949, SNF-Australia Ltd) was added while mixing. The flush manure was pumped from the holding tank, through a static mixer where a flocculant solution (a 0.5% solution; FlopamTM, SNF-Australia) was added at 38–45 mL/L, after which it passed through a floccule-maturator to grow floccules and then onto the Z-Filter. Filtrate was pumped into another holding tank before being recycled back to a flush tank for a following day's flush, thus creating a closed loop. Flushing frequency varied from 2–3 times/week at the start of the pig batch to daily at the end. The Z-Filter works continuously with a fabric filter called a 'sock', which follows a triangular path closing it into a tube containing slurry, pressing it with rollers to remove water through its porous sock and then re-opening it to discharge dewatered solids. Eleven samples, each of flush manure (from the holding tank), filtrate and separated solids were collected over 11 weeks representing the four flush-lanes and the pig growth batch. These samples were collected from 20 L containers holding aggregates of 15 sub-samples, which were stirred/mixed to ensure homogeneity. All samples were stored at –20°C and air-freighted frozen on dry ice to Brisbane for analysis. Upon receipt (still frozen) the samples were further stored at –20°C prior to analysis. Samples were analysed (Gopalan *et al.* 2013) for total solids (TS), volatile solids (VS), volatile fatty acids (VFA, by gas chromatography), phosphate, oxidised nitrogen and ammonium nitrogen (ammonia N, by flow injection analysis), and total Kjeldahl nitrogen (TKN) and phosphorous (Total P). Minerals were analysed by ICP-OES after nitric acid digestion (Tait *et al.* 2009).

Removal extents achieved by the Z-Filter were higher than for other similar solids separation systems in common use. Despite significant variation in TS of the flush manure over the trial period (flush manure into the Z-filter contained 1.3–2.4 wet mass % TS), the Z-filter sustained removal extents at around 58%. Other removal extents averaged 73% for VS, 35% for TKN and 50% for total P. However, the Z-Filter (as with other mechanical systems) was unable to remove colloidal and dissolved compounds, with removal extents for ammonia nitrogen (14%), potassium (10%) and VFA (16%) being low. Therefore, further treatment would be required for the filtrate of the Z-filter in onsite ponds, albeit with estimated 60% smaller pond sizes. The separated solids had an average dry matter content (TS) of 22 wet mass %, and were stackable with minimal seepage and easily transportable.

The present Z-filter trial produced a solid cake suitable for co-composting and a low-strength liquid waste (filtrate) for treatment in smaller/less costly onsite ponds. However, filtrate recycled as flush water over extended periods would require some further treatment to remove soluble compounds. Preliminary economic modelling for a 2,000 sow farrow-to-finish conventional piggery estimated capital and operating costs for a Z-Filter to be around $50 and $132/t TS processed, or $0.04 and $0.12 per kg of dressed finisher weight sold/y for low and high flush volumes, respectively. These costs currently are similar to conventional pond systems, but opportunity exists to reduce chemical costs of the Z-filter. Further work is required to quantify other potential benefits of pondless systems, such as enhanced use of nutrients, reduced water use, reduced odour, and site constraints that may limit the use of conventional pond systems.

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Spatial modelling to estimate the risk of feral pigs to pig farm biosecurity in south-eastern Australia

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Freedom from many high priority diseases is a key competitive advantage for the Australian pork industry (Brookes *et al.* 2014). Despite a strict quarantine system, exotic diseases may be introduced and establish in wild or feral animal populations. Pearson (2012) showed that some production-limiting pathogens are already endemic to feral pigs around piggeries in Australia. Pearson (2012) also found that the risk of pathogen transmission from feral pigs coming into contact with domestic herds is low but not negligible. This scoping study aimed to investigate whether spatial modelling can help to identify 'farm biosecurity hotspots', where the risk of exposure by domestic pig herds to diseases carried by surrounding feral pig populations is greatest.

The study area, south-eastern Australia, contains almost 90% of the national domestic pig herd (ABS 2015). Relative risk of exposure was defined as the proportion of land within estimated risk zones around piggeries that coincided with suitable feral pig habitat. Habitat suitability was modelled using a participatory approach adapted from Murray *et al.* (2014) that combined expert knowledge, probabilistic modelling and spatial analysis. The model was calibrated for southern Queensland and extended to the broader study area. As suitability was influenced by the variable availability of key resources such as water, food and cover, seasonal (summer or winter) and climatic (above or below average rainfall periods) scenarios were analysed in this study. Only highly suitable habitat (probability > 0.5) was considered. Location data (partly based on post code) was obtained for 1,908 commercial piggeries. Following Pearson (2012), a circular zone within 0–100 m around piggeries was considered high risk and within 100–500 m moderate risk of exposure.

Results were aggregated by state to show broad spatial trends in both habitat suitability and risk of exposure. The model predicted on average that 32.9% of the study area was suitable feral pig habitat. However, this varied by scenario and state from 6.4% under drought conditions in NSW to 81% during wet periods in Victoria. Consequently, relative risk of exposure to feral pigs also differed considerably across scenarios and states (Table 1). Risk was highest during the winter growing season in Victoria (>94%) and lowest during arid summer conditions in NSW (<13%). Averaged across all states and scenarios, the proportion of high and moderate risk zones coinciding with feral pig habitat was 46.4% and 47.1% respectively. The results from this scoping study indicated that across the study area many piggeries are located in the vicinity of highly suitable feral pig habitat, particularly when abundant resources allow feral pigs to extend their range. To confidently assess risk of exposure at the property level, modelling would benefit from more comprehensive piggery location data as well as information on farm types, existing biosecurity measures and disease prevalence in feral pigs. Results of the habitat suitability model also need to be validated in the farming systems of Victoria.

Table 1. Relative risk of exposure (percentage of high/moderate risk zones around piggeries coinciding with suitable feral pig habitat) by scenario and state

Climate:	Below average rainfall period				Above average rainfall period			
Season:	Summer		Winter		Summer		Winter	
Risk zone:	High	Moderate	High	Moderate	High	Moderate	High	Moderate
NSW (%)	12.3	12.3	36.2	35.3	16	16	45.6	44.3
VIC (%)	58.7	56.3	94.4	94.3	68.8	66.9	94.6	94.7
QLD (%)	15.5	15	64.2	62.6	38.1	38.1	86.4	86.1
SA (%)	14.5	14	56.6	54.7	18.2	17.2	61.6	60.4
Total (%)	24.1	23.5	59.8	58.9	34.4	33.9	70	69.3

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Porcine haptoglobin levels measured at 7–14 days after weaning were independent of age, weight or gender

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Acute phase proteins (APP) are cytokine-induced plasma proteins produced mainly in the liver in response to infection, inflammation and stress. The levels of some APP have been used as diagnostic indicators for a number of diseases (Petersen *et al.* 2004) and for health monitoring. Haptoglobin (Hp) is an APP responsible for collecting and recycling free haemoglobin. The high level of variation in Hp levels of apparently healthy pigs has been attributed to factors such as age, gender, source herd and pig husbandry (Piñeiro *et al.* 2009). This study investigated the range in Hp levels from a single breed of pigs, 7–14 days after weaning and from a single piggery under the same housing and management conditions. The hypothesis tested was that age, collection date (season), gender and (or) weight would affect Hp levels.

A single serum sample was taken 10 days (10.5 ± 2.7: mean ± SD) after weaning from 810 pure bred Large White pigs of mixed sex (49% female and 51% male), housed at The University of Queensland Gatton piggery. Haptoglobin levels were determined by ELISA using a standard validated on a commercial kit. The ELISA antibody set included rabbit anti-human Hp (capture), mouse anti-human Hp (detection) and rabbit anti-mouse IgG-AP (Sigma-Aldrich. Missouri, USA.). Each serum was tested at dilutions of 1 : 30,000, 1 : 1000 and 1 : 50 and Hp levels were calculated from plate specific standard curves using four-parameter logistic fit (4PL) analysis (SoftMax® Pro 5 software). Sera were collected over different seasons in the first (summer), second (autumn) and final (spring) quarters of the calendar year (2013). Data were grouped according to collection quarter (1, 2 and 4) and by pig age at the time of collection (32–37, 38–42 and 43–47 days of age). The descriptive statistics of the observed Hp concentrations were determined using Microsoft Excel® (Table 1). Distribution and ANOVA analysis (R: Free Software Foundation's GNU General Public License) were used to determine the effect and significance of collection date, age, weight and gender of the pigs on the log-transformed Hp levels.

The distribution of log transformed Hp level was bimodal after allowing for differences in the main effects of age and collection date (season). Within the sample population, 2% of pigs had Hp concentrations within the acute range of 3,000–8,000 μg/mL (PHASE™ Tridelta Development Ltd. Ireland), while the majority of pigs (54%) had Hp levels <1.0 μg/mL (0.4 ± 0.2: mean ± SD μg/mL). No significant difference was observed between the Hp levels in females (336 ± 38.9: mean ± SD μg/mL) and males (361 ± 39.5: mean ± SD μg/mL). Haptoglobin levels decreased with increasing pig age in quarters 1 and 4 but increased with increasing pig age in quarter 2 (Table 1). Significantly higher Hp levels were observed in pigs sampled in the 4th quarter ($P < 0.001$). However, ANOVA analysis also determined that pig age, weight and gender did not have a significant effect on Hp level. Despite controlling for breed, time after weaning, source herd, housing and management, sizeable variation in Hp levels were observed. The relative elevation of Hp levels observed in quarter 4 and the bimodal distribution of response indicated other factors, such as season, pathogen loads and other stressors, should be considered in future studies.

Table 1. Descriptive statistics of serum haptoglobin (Hp) levels in pigs, during the second week after weaning, grouped by collection dates into quarters of the year (2013) and into three age groups

	Haptoglobin Concentration (μg/ml)								
Collection Period	1st Quarter			2nd Quarter			4th Quarter		All quarters
Age group[A]	1	2	3	1	2	3	1	2	4
Mean ± SEM[B]	162 ± 94	124 ± 30	45 ± 24	94 ± 56	110 ± 36	169 ± 150	716 ± 64	417 ± 77	348 ± 28
Median	0.34	0.51	0.27	0.42	0.43	0.36	271.4	194.5	0.68
Maximum	4637	2977	339	1304	3987	1959	6229	2834	6229
n	52	176	16	32	180	13	282	58	810

[A]Age groups: 1, 32–37 days; 2, 38–42 days; 3, 43–47 days; 4, 32–47 days. [B]SEM, standard error of the mean.

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Multiple treatments targeting the immune system of commercially-reared weanling pigs

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Pigs exposed to conventional housing systems with high microbial loads grow around 20% more slowly than gnotobiotic pigs or pigs in 'clean' environments (Black and Pluske 2011). In-feed antibiotics reduce microbial numbers and modulate the immune system, but result in concerns about microbial resistance to antibiotics in human health (Collignon 2003). Black and Pluske (2011) suggested using a multi-targeted approach to reduce microbial load in pigs, reduce release of pro-inflammatory cytokines and subsequent production of prostaglandin E_2 (PGE_2), which causes anorexia, fever and decreases protein synthesis. The hypothesis was that multiple treatments targeting the immune system of pigs would improve performance.

Treatments initially identified were: i) fatty acids monolaurin and monomyristin (2:1), which are toxic to most microbes; ii) a *n*-6:*n*-3 ratio <4:1, to reduce pro-inflammatory cytokine release; iii) aspirin, to reduce pro-inflammatory cytokines and inhibit PGE_2 formation; and iv) meloxicam, to inhibit COX-2 action and restrict PGE_2 synthesis. Due to regulatory and product availability constraints, aspirin, meloxicam, and monomyristin could not be evaluated in this study. Male (M) and female (F) pigs (1,240 of PrimeGro™ Genetics, initial weight 8.2 ± 1.25 kg, mean ± SE) were allocated to five treatments in a designed experiment with nine replicates in three sheds and 13-14 pigs/pen. The treatments, offered feed *ad libitum*, were: (A) monolaurin at 2% of a weaner diet (≈15.3 MJ/kg digestible energy (DE), 216 g/kg crude protein (CP), 13.1 g/kg available lysine); (B) fish-safflower oils with a *n*-6:*n*-3 ratio of 2 : 1 at 6%; (C) treatments A and B combined; (D) negative control diet with no antibiotics, zinc oxide and a *n*-6:*n*-3 ratio of 20.7:1 at 6%; and (E) positive control, being the weaner diet with sulphatrim (0.1%). Pigs were initially weighed individually, then in pens after 14 days and individually at the end of the experiment (28 days). Pen feed intake was measured from d 0-14 and 14-28 and averaged for the number of pig-days in each pen. A linear mixed model, analogous to ANOVA, was fitted to average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR). The experimental unit was the pen and the observational unit was a pig in a pen. Pig initial weight, pig gender, shed, pen and pen row were included in the model. Treatment did not affect (P>0.05) ADFI, but ADG tended to be greater (results not shown) and FCR lower (P < 0.05) for treatment C (Fig. 1).

Multiple treatments aimed at modifying microbial load and the immune response may allow removal of antibiotics from the diets of young pigs. It is anticipated that FCR would be further enhanced if aspirin, COX-2 inhibitors and monomyristin were included in diets aimed at modifying the immune response.

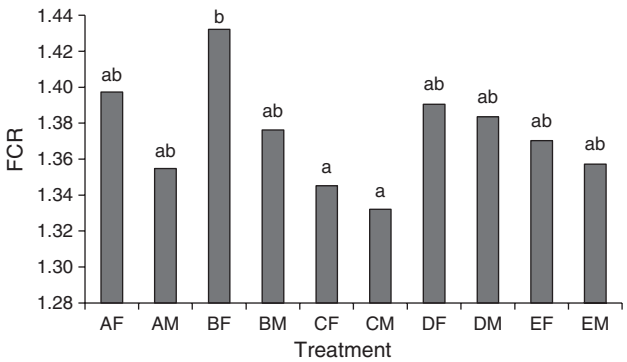

Fig. 1. Statistical model-predicted effect of treatment (A–E) and pig gender (M–F) on feed conversion ratio (FCR) over the 28-d experiment. [a,b]Means between columns not having the same superscript are significantly different (P < 0.05).

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Application of sorbers to mitigate greenhouse gas emissions from land-applied pig litter

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Nitrous oxide is the foremost greenhouse gas (GHG) generated by land-applied manures and chemical fertilisers (Australian Government 2013). This research project was part of the National Agricultural Manure Management Program and investigated the potential for sorbers (i.e. specific naturally-occurring minerals) to decrease GHG emissions from spent piggery litter (as well as other manures) applied to soils. The sorbers investigated in this research were vermiculite and bentonite. Both are clays with high cation exchange capacities, of approximately 100–150 cmol/kg (Faure 1998). The hypothesis tested in this study was that the sorbers bind ammonium in soil solution thereby suppressing ammonia (NH_3) volatilisation and in doing so, slowing the kinetics of nitrate formation and associated nitrous oxide (N_2O) emissions.

A series of laboratory, glasshouse and field experiments were conducted to assess the sorbers' effectiveness. The laboratory experiments comprised 64 vessels containing manure and sorber/manure ratios ranging from 1 : 10 to 1 : 1 incorporated into a sandy Sodosol via mixing. The glasshouse trial involved 240 pots comprising manure/sorber incubations placed 5 cm below the soil surface, two soil types (sandy Sodosol and Ferrosol) and two different nitrogen (N) application rates (50 kg N/ha and 150 kg N/ha) with a model plant (kikuyu grass). The field trial consisted of 96, 2 m × 2 m plots on a Ferrosol site with digit grass used as a model plant. Manure/sorber mixtures were applied in trenches (5 cm below surface) to these plots at increasing sorber levels at an N loading rate of 200 kg/ha. Gas produced in all experiments was plumbed into a purpose-built automated gas analysis (N_2O, NH_3, CH_4, CO_2) system. In the laboratory experiments, the sorbers showed strong capacity to decrease NH_3 emissions (up to 80% decrease). Ammonia emissions were close to the detection limit in all treatments in the glasshouse and field trial. In all experiments, considerable N_2O decreases (>40%) were achieved by the sorbers. As an example, mean N_2O emission decreases from the field trial phase of the project are shown in Fig. 1*a*.

The decrease in GHG emissions brought about by the clays did not negatively impact agronomic performance. Both vermiculite and bentonite resulted in a significant increase in dry matter yields in the field trial (Fig. 1*b*). Continuing work will optimise the sorber technology for improved environmental and agronomic performance across a range of soils (Vertosol, Dermosol in addition to Ferrosol and Sodosols) and environmental parameters (moisture, temperature, porosity, pH).

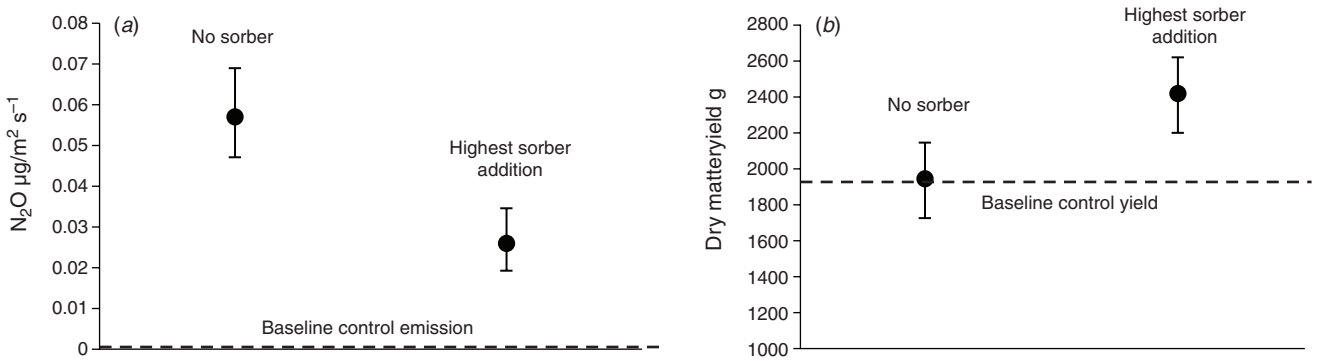

Fig. 1. Average: (*a*) N_2O emissions; and (*b*) dry matter yield (DMY) from spent litter applied to Ferrosol in the field trial; upper and lower standard error values included. Vermiculite and bentonite results are combined in the figures. Highest sorber addition level corresponds to 1 : 1 ratio to dry weight manure mass. Differences are significant at $P < 0.05$ for N_2O decreases and DMY increases.

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Greenhouse gas emission abatement in Australian piggeries

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As part of a national strategy to understand and reduce greenhouse gas (GHG) emissions from Australian piggeries, pork producers with a variety of production systems voluntarily participated in on-farm studies aimed at calculating their existing piggery baseline emissions and possible emissions reductions. For 55 Australian piggeries, representing 24% of Australian pork production, the PigGas Calculator (Kruger *et al.* 2013; Mills and Kruger 2014) was used to calculate total on-farm baseline GHG emissions and emissions' intensities using Australia's GHG accounting factors. The on-farm 'business' emissions boundary used included energy but excluded other pre-farm or post-farm emissions. Individual farm data relating to energy use, pig production parameters, manure management systems, land application practices and pork sales were collected from piggery records and observations at each farm were used in emissions calculations. In consultation with individual pork producers, the PigGas Calculator was then used to model feasible GHG abatement options for each farm. Abatement scenarios included changes in feed efficiency, housing, waste treatment methods, effluent and manure reuse and energy use.

On-farm baseline GHG emissions, average on-farm emissions intensities and potential abatements for the 55 piggeries were grouped by pig production system (Table 1). Total GHG emissions abatements ranged from 0–84% of the baseline on individual piggeries. Highest abatements of 75–84% were achieved on piggeries using covered anaerobic ponds to capture and burn methane in cogeneration systems. Abatement of 10% was achieved by improving feeding efficiency. Modifying waste treatment and reuse systems resulted in 15–25% abatement. Housing pigs in deep litter sheds resulted in about 40% abatement compared with housing in conventional flushed sheds.

On-farm baseline emissions calculated from 24% of Australia's pork production totalled 260,481 t CO_2-e/y with potential abatement of 54%, or 141,232 t CO_2-e/y. On a whole industry basis, maximum potential abatement is 588,467 t CO_2-e/y. It is also possible to reduce baseline emissions intensities by 51% from an industry average of approximately 3.9 to 1.9 kg CO_2-e/kg HSCW. These data provide evidence of the Australian pork industry's capacity to reduce GHG emissions as it moves into a carbon-constrained future.

Table 1. On-farm total greenhouse gas emissions, average emissions intensities and potential abatements on 55 Australian piggeries

Pig production system	Total emissions (t CO_2-e/y)[B]		Average emissions intensity (kg CO_2-e/kg HSCW)[C]	
	Baseline	Abated scenario	Baseline	Abated scenario
Farrowing only – conventional (5)[A]	6,576	4,224	8.7	3.6
Farrow to weaner – conventional (1)	2,211	205	6.0	5.4
Farrow to pork – conventional (1)	1,880	1,579	6.4	1.0
Farrow to finish – conventional (19)	112,991	72,236	4.0	1.8
Grow out – conventional and deep litter (2)	7,131	2,005	3.5	1.9
Grow out – conventional (5)	23,757	15,410	3.2	0.8
Farrow to finish – conventional and deep litter (20)	102,444	45,488	2.9	1.8
Farrow to finish – outdoor farrow, deep litter grow (2)	3,491	85	1.4	1.4
Total [average]	260,481	141,232	[3.9]	[1.9]

[A]Number in parentheses refers to number of each type of piggery studied. [B]CO_2-e represents the global warming potential of combined nitrous oxide and methane emissions expressed as carbon dioxide equivalents. [C]HSCW, hot standard carcase weight.

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Alternative low-cost solid media for scrubbing of hydrogen sulphide from piggery biogas

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Australian pig producers are increasingly using biogas from on-farm manure management for shed heating and electricity generation. However, hydrogen sulphide (H_2S), a toxic and corrosive gas ingredient in raw piggery biogas, is currently impeding further adoption of biogas technology. To remove H_2S, biogas is typically passed through a packed column containing a commercial solid medium with an active ingredient (such as iron) which reacts with and sequesters H_2S while allowing the treated biogas to pass to the point of use (Skerman *et al*. 2012). However, periodic replacement of media (when spent) represents a significant operating cost for pig producers using biogas, consuming as much as 20% of the financial benefit of using the biogas. The aim of this laboratory-scale study, batch H_2S sorption/reaction was to evaluate and compare the performance of a commercial scrubbing medium with that of several low-cost, agricultural and industrial by-products.

Experiments involved passing a pre-humidified standard gas (Encore Automation Pty Ltd; WA) with 2,000 ppm H_2S in high purity nitrogen, through the various media, suspended on stainless steel mesh, in a PVC pipe canister (internal diameter 29.8 mm). In-line sensors (Alphasense H2S-BE, Great Notley, CM77 7AA; UK), which had been cross-calibrated with standard gases, were used to measure H_2S concentration in the treated gas discharged from the canister over time. The media tested in the experiments included cg_5® commercial iron-oxide pellets (Clean-Gas, ACP Technologies Inc, USA), and the alternative media: granular steel furnace slag (<5 mm), red soil (krasnozem/red ferrosol, Toowoomba, QLD), commercial compost (Naturegrow – Amgrow Pty Ltd, QLD), composted feedlot manure (Kerwee feedlot, Jondaryan; QLD) and biochar (Green waste 550, Pacific Pyrolysis Pty Ltd, NSW). The alternative media were passed through a 2 mm sieve prior to testing, to remove any coarse fragments.

A measured H_2S of 0 ppm indicates that the media had effectively removed all of the H_2S in the canister inflow, while H_2S >0 ppm (following breakthrough) indicates that a portion of the H_2S in the canister inflow had not been absorbed/removed by the media and had been emitted through the canister exit (Fig. 1). The results showed that the cg_5® commercial iron-oxide pellets vastly outperformed the alternative media, with a substantially higher H_2S loading before breakthrough of H_2S occurred. However, the red soil showed noteworthy performance. These results suggested that, because of the lower loading capacity, the alternative media would probably require a significantly larger scrubbing column and more frequent medium replacement, compared to a commercial medium. The red soil medium appeared to warrant further investigation, especially for use as a secondary polishing step to treat lower residual levels of H_2S following primary biological scrubbing.

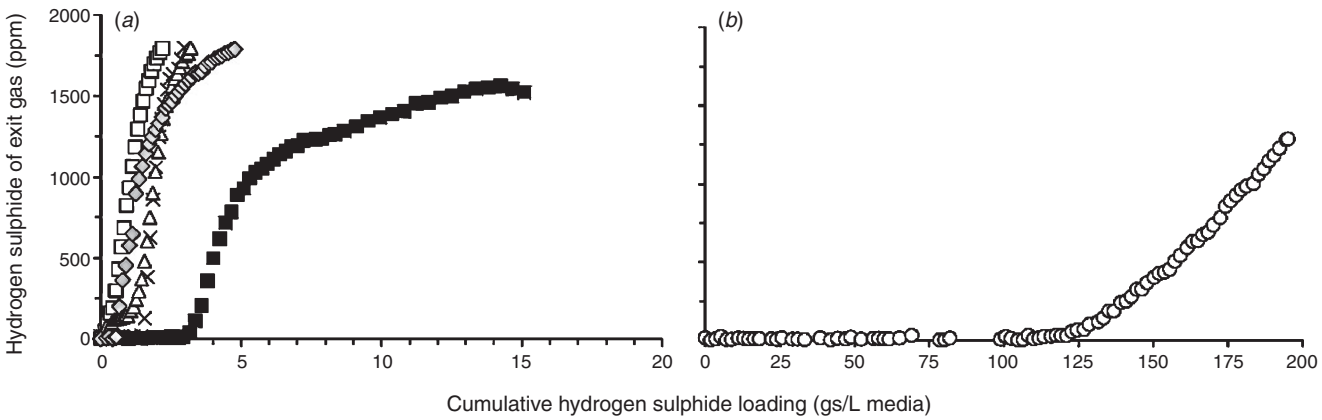

Fig. 1. Experimental data for: (*a*) various alternative media, red soil (■), composted feedlot manure (□), biochar (×), commercial compost (△), granular slag (◆); and (*b*) for the commercial media cg_5®, showing the measured H_2S concentrations in the treated gas exiting the scrubber canister vs cumulative H_2S fed into the canister. Note the different extents of scale on the horizontal axes.

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Inhibition resilience of microbes in pig effluent lagoons

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A recent inhibition test protocol (Astals *et al.* 2015), which has been optimised for cost and speed, allows pork producers to quantify microbial inhibition in piggery effluent lagoons. This is important, because effective effluent treatment relies on healthy microbial activity in effluent lagoons. The inhibition test measures the KI_{50} value, which is the concentration of a specific inhibitor at which the activity of exposed microbes are reduced by 50% (Astals *et al.* 2015). Accordingly, if the inhibitor concentration in flush manure fed to a lagoon is less than the KI_{50}, then the lagoon may be uninhibited. The aim of this study was to determine if inhibition test data also provided information about the relative tolerance of microbes, in a piggery lagoon, to unavoidable periodic increases in inhibitor concentrations that are below the KI_{50}.

Ammonia (NH_3) was selected as the model inhibitor because flush manure is rich in NH_3 and it is a key inhibitor of anaerobic digestion. For experiments, a sludge sample (containing microbes for which inhibition is to be tested) was collected from an unmixed covered lagoon at a commercial breeder piggery in NSW. The volatile solids (VS), background NH_3 nitrogen and native pH of the sludge sample were measured (Astals *et al.* 2015) at 10 g VS/L, 776 mg N/L and pH 7.2, respectively. Glass vials (160 mL) were loaded with the sludge and different amounts of NH_3 (added as NH_4Cl salt) and with 2 g/L acetate as food source. The vials were sealed and incubated at 37°C. The pH in the vials was 7.08–7.72 depending on the amount of NH_4Cl added. Methane produced by microbes in sludge inside the vials was measured at 1, 2 and 3 days of incubation (Astals *et al.* 2015). Specific methanogenic activity (SMA) was determined as the slope of a linear line fitted to the methane data over time (expressed in units of chemical oxygen demand or COD equivalents, normalized with respect to the amount of VS in the sludge added to each vial). All the experiments were run in triplicate and the error in SMA was estimated at the 95% confidence level (seven degrees of freedom). The SMAs were plotted against NH_3 (symbols, Fig. 1*b*) and KI_{50} was estimated by linear interpolation, corresponding to the NH_3 content at which SMA had been reduced to 50% of the highest measured SMA.

As expected, increasing NH_3 decreased measured microbial activity/SMA (symbols, Fig. 1*b*), likely due to inhibition. The estimated KI_{50} of 3.98 ± 0.7 gTAN/L (given with error at 95% confidence level) was the threshold concentration for NH_3 inhibition of the particular sludge sample being tested. The background NH_3 (776 mgN/L) was noted to be well below this KI_{50} and thus indicated that the lagoon was not likely to be inhibited by NH_3. Further, the shape of the inhibition profile (symbols, Fig. 1*b*) showed a gradual decrease in SMA with increasing NH_3, indicating that the microbes were reasonably tolerant to increases in NH_3, albeit with some decrease in SMA. A stronger threshold-type response was observed for another lagoon sludge (solid line, Fig. 1*b*, Astals *et al.* 2015), with decrease in activity being more drastic around the KI_{50} value. These different shapes of the SMA curves (Fig. 1*b*) suggested differences in tolerance to NH_3. The results in this paper illustrated how inhibition test data can be used to estimate a threshold inhibitor concentration (KI_{50}) as well as to obtain a measure of microbial tolerance to increases in inhibitor concentration.

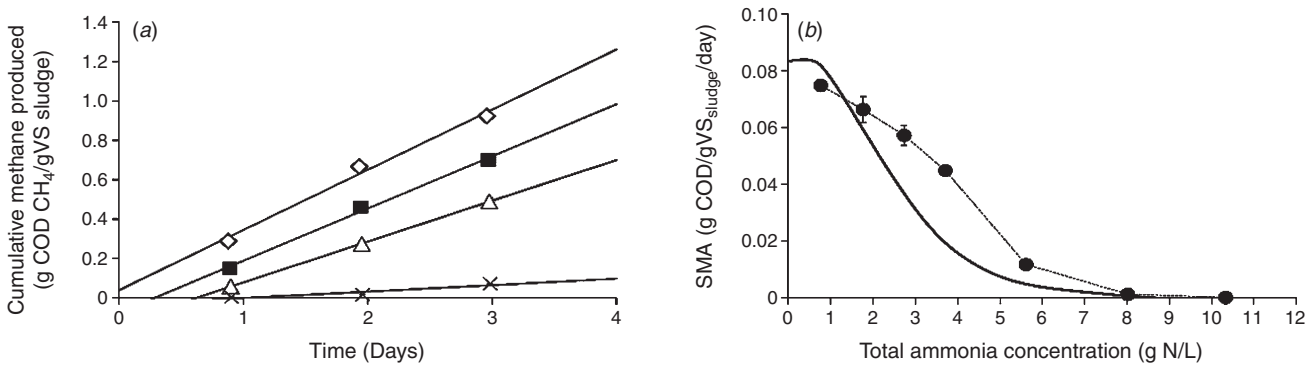

Fig. 1. (*a*) Cumulative methane produced by the lagoon sludge versus time at 0.77 g N/L (◇), 2.74 g N/L (■), 3.71 g N/L (△), 5.61 g N/L (×) ammonia (added plus background). Note: slopes of the linear lines of best-fit are the specific methanogenic activities or SMA. (*b*) SMAs (symbols, estimated from Fig. 1*a*) versus NH_3 content. KI_{50} =3.98 ± 0.67 g N/L. The solid line was derived from data of a different lagoon sludge (adapted from Astals *et al.* 2015).

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Breakdown of electrical energy use during summer and winter at six piggeries

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Energy efficiency is an important performance and sustainability indicator for the Australian pig industry because of the rise in energy costs and increased focus on greenhouse gas emissions. Wiedemann *et al*. (2012) showed a seven-fold variation in electricity usage across naturally-ventilated, farrow to finish piggery unit types, suggesting opportunities for improved energy efficiency. A survey of 21 piggeries showed on that on average the highest energy use component is electricity at 75% (McGahan *et al*. 2014). The aim of this study was to continuously monitor electricity use for a 2-week period in summer and winter at six piggeries, five in Queensland and one in Victoria.

The piggeries included natural and tunnel-ventilated sheds, farrow to finish, finisher and breeder units. Electricity was monitored by current transformers attached to electrical circuits and measured by a Nemo® 72-L power meter and an Environdata® data logger. An Environdata® temperature probe logged ambient temperature. The system was capable of measuring electricity from three circuits simultaneously, allowing high energy use areas to be identified.

Monitoring showed that electrical energy usage is heavily dependent upon ventilation system type and climatic conditions (Table 1). Naturally ventilated farms all used less electricity during summer compared to winter, possibly due to a decrease in operating hours for the farrowing heating system. Electrical energy use at tunnel ventilated farms increased during summer, probably due to warmer temperatures increasing the operating time of the ventilation fans. In naturally ventilated piggeries containing farrowing, the highest electrical consumption was from heat lamps (Tables 2 and 3). These piggeries could improve energy efficiency by reducing the heating area and heat wastage, or by installing a thermostat to automatically switch off heat lamps when the temperature reaches a trigger level. Electrical energy usage in tunnel-ventilated piggeries is driven by the use of ventilation fans to maintain shed climate. These piggeries can improve energy efficiency by ensuring the control system is operating correctly and fans are well maintained. Other ways to improve electrical energy use includes, selecting energy efficient lighting types (compact fluorescent or LED), and ensuring that motors and pumps are correctly sized and well maintained. Energy costs can be reduced through managing and reducing peak energy loads. If possible, peak energy use should be converted to low tariff hours.

Table 1. Average total site daily electricity use in summer and winter at study piggeries (kWh/day)

Farm	Piggery System	Ventilation Type	Location	Winter (kWh/d)	Summer (kWh/d)
1	Farrow to finish	Natural	South QLD	389	371
2	Farrow to finish	Tunnel	South QLD	2223	3504
3	Breeder	Tunnel	South QLD	2592	3768
4	Finisher	Tunnel	South QLD	2069	5480
5	Farrow to finish	Natural	South QLD	187	138
6	Breeder	Natural	Central Vic	921	834

Table 2. Breakdown (% of total) of electrical energy use areas at three farrow to finish piggeries in southern Queensland

Piggery Area	Farm 1	Farm 2	Farm 5
Farrowing	40%	42%	66%
Bore Pump	11%		
Finishing		49%	4%
Feed mill			24%
Workshop/amenities	39%	6%	7%

Table 3. Breakdown (% of total) of electrical use components inside Farm 1 naturally-ventilated farrowing shed

Electrical Component	% Total Use
Heat Lamps	77%
Effluent Pumps	7%
Effluent Agitator	6%
Hose Pump	5%
Reticulation Pump	4%
Feed Motor	1%

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Soil nitrate and phosphorus accumulates rapidly with a non-uniform distribution in two outdoor pig areas

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With an increasing number of pigs in Australia managed in outdoor systems, a greater understanding of the impact of these systems on environmental sustainability is required. Previous research (Galloway and Wiedemann 2011) applied electro-magnetic induction (EMI) technology to measure spatial variability in nutrient distribution across paddocks at two outdoor piggeries, and showed a distinct pattern of elevated nutrient levels in some parts of the paddocks. The present study aimed to extend this research by first, determining the rate of soil profile nutrient accumulation and distribution at rotational outdoor piggeries and second, to measure the impact of changing management practices to improve nutrient distribution.

Soil nutrients were measured over a 3-year period on four paddocks at different stages in rotation. Soil mapping using EMI was applied to determine 12 sampling points annually, based on variability in apparent soil conductivity (EC_a). Nutrient distribution maps were determined from a regression of EC_a and nutrient levels. Four fixed monitoring points were also established in each paddock and sampled annually at 0–10 cm and 20–30 cm depths, and results were analysed using ANOVA between means for each year. Significant differences were determined using the least significant difference (LSD) test.

Mean soil Colwell phosphorus (P) and nitrate N levels increased significantly ($P < 0.05$) between year 1 (the first year of pig occupation) and year 2 (Table 1). Colwell P levels exceeded the upper environmental threshold level of 85 mg/kg in the surface (0–10 cm) in the second year and remained elevated after pigs were removed in year 3.

Nitrate N (Fig. 1) and P (data not shown) were distributed in a non-uniform pattern, corresponding to EC_a (Fig. 1, $R^2 = 0.86$, $P < 0.01$). Areas of highest nitrate (Fig. 1) were 10 times higher than other parts of the paddock, and these hotspots corresponded to the location of shelters, feeders and waterers. Concentrations in hotspot areas were up to six times higher than mean levels for the whole paddock (Wiedemann 2015). Despite the non-uniform distribution of nutrients, minimum nitrate N, and P levels were sufficient for the subsequent crop farming without additional fertiliser. Successful utilisation of nutrients in hotspot areas would require specialist management during subsequent years of the cropping phase. Rotational outdoor farming resulted in a rapid build-up of nutrients in the surface and subsoil, sufficient to exceed environmental thresholds in the first year of pig farming, suggesting that further investigation of the risks of nutrient loss, and approaches to manage this risk, is required.

Table 1. Aggregated mean nutrient levels measured over three years from fixed monitoring points from two outdoor pig paddocks in southern Australia

	Colwell Phosphorus (mg/kg)		Nitrate N (mg/kg)	
	0–10 cm	20–30 cm	0–10 cm	20–30 cm
Year 1	45.2	5.8	22.3	4.7
Year 2	118.3**	21.4**	67.5**	20.3**
Year 3	101.3**	16.5**	25.2	10.7
LSD	32.3	8.3	20	7.2

**Indicates significant difference to year 1 ($P < 0.05$).
Year 1: first year of pig occupation. Year 2: second year of pig occupation. Year 3: following removal of pigs.

Fig. 1. Distribution of subsoil (50–60 cm) nitrate-N (shading light to dark, mg/kg: <5; 5–25; 25–50; >50) at two outdoor pig paddocks in southern Australia.

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Effectiveness of different mitigation strategies to reduce nitrous oxide emissions from pig manure amended soils

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Developing effective mitigation strategies for reducing nitrous oxide (N_2O) emissions from manured soils requires a better understanding of the microorganisms and mechanisms involved (Barton *et al*. 2013; Banning *et al.* 2015). Previous work has indicated that nitrifying microorganisms at the surface (0–10 cm) were largely responsible for N_2O emissions in Western Australian semi-arid soils and these microorganisms responded to targeted mitigation strategies for reducing N_2O (Barton *et al*. 2013). However, the effect of adding pig manure to these soils on the N_2O emitting microbial populations and mitigation remains largely unknown. The aim of this study was to evaluate the effectiveness of different pig manure types (stockpiled, composted and pelletised manure) and application methods (broadcast or incorporated into the soil) at reducing N_2O emissions following manure amendment. It was hypothesised that the amount of nitrified-N_2O could be reduced by a) incorporating manure at depth to avoid ammonia oxidisers in the topsoil, and b) composting or pelletising manure to decrease availability of ammonium (VanderZaag *et al.* 2011; Barton *et al*. 2013).

A soil microcosm experiment having a $2 \times 5 \times 2$ factorial arrangement of treatments in triplicate was conducted using 557-mL glass jars, with factors being sandy or clayey soil (clay contents of 1.6 and 8.2% respectively) (collected from UWA Future Farm, Pingelly), five different amendments applied at 100 kg of N/ha (unamended, inorganic fertiliser, stockpiled, composted or pelletised manure), and two application methods (broadcast or incorporated). The microcosms were adjusted to 40% water holding capacity and incubated at 25°C for 2 weeks. The glass jars were unsealed, except during gas flux measurements when they were sealed with an air-tight lid fitted with a septum to trap the expired gases for 2 hours. The N_2O flux was analysed at 0, 2, 6, 24, 48, 72, 96, 120, 168 and 336 h by gas chromatography.

The N_2O emissions ranged from 0.002 to 0.85 kg/ha/d but were most pronounced in the clayey soil (Fig. 1*b*) and for the stockpiled manure amendment (Fig. 1). Incorporating stockpiled manure in sandy soils caused a 2-fold decrease in N_2O flux compared to broadcast (Fig. 1*a*), but this benefit was lost in the clay soils (Fig. 1*b*). Although the composted manure had the overall lowest emissions on both soils (Fig. 1), the pelletised manure reduced the emissions relative to stockpiled manure and probably offers the best mitigation option for semi-arid soils since it avoids emissions during the composting process and is easier to handle, transport and apply. Composting is more suitable for larger or mixed (piggery and grain) enterprises where there are multiple waste streams to manage. In conclusion, the effectiveness of the greenhouse gas mitigation method depends on both manure type and soil type. Mitigation methods that decrease nitrification and availability of ammonium and nitrate, such as composting, pelletising or incorporating manure, have the greatest potential to reduce N_2O emissions in semi-arid cropping systems.

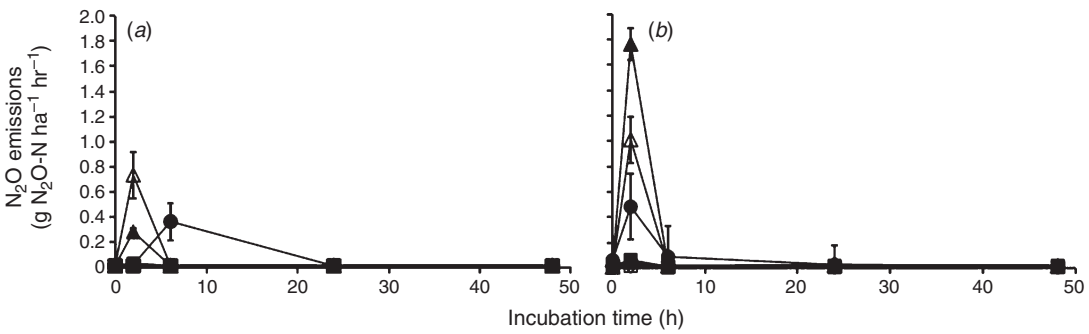

Fig. 1. Nitrous oxide (N_2O) flux during the first 48 hours following the broadcast application or incorporation of different manure types to sandy (*a*) and clayey (*b*) soils (mean ± SEM; n = 3). The treatments are as follows: unamended control (□), mineral fertiliser (■), stockpiled manure broadcast (△) or incorporated (▲), composted manure broadcast (◇) or incorporated (◆) and pelletised manure (●).

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Economic implications of environmental variation observed in a pig nucleus farm in Australia

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The performance of a group of pigs, adjusted for other known systematic and genetic effects, can be used to quantify environmental variation (EnVar) on farms. Using such an approach, Li and Hermesch (2015) found variation between environments for average daily gain (ADG) and backfat (BF) in nucleus herds with good management and high health status that was similar to the genetic variation. In that study, EnVar for daily feed intake (DFI) and feed conversion ratio (FCR) could not be assessed because data for DFI were not available. The economic implications of EnVar may be evaluated by multiplying differences in group means for each trait by the corresponding economic value (EV) (Hermesch *et al*. 2014). An EV for a trait quantifies the change in profit when the trait is changed by one unit. It is independent from other EVs and can be applied to other non-genetic factors. We hypothesised that EnVar exists in a nucleus farm for ADG, BF, DFI and FCR leading to economic differences between environments.

Data were obtained from 90,524 growing pigs from seven lines recorded from 2008 to 2014. The ADG and BF were measured at an average live weight of 96.7 kg. A proportion of pigs (3,045) had DFI records along with the associated traits of test daily gain (TDG) and FCR. An animal model was applied using ASReml (Gilmour *et al*. 2009) and fitting common litter effect as an additional random effect. Fixed effects were birth week or birth month, sex (ADG, BF), line, line by sex interaction (ADG), birth farm and weight at recording as a linear covariable (BF). Variation in weekly or monthly estimates (solutions) may also have been due to systematic changes over time like a change in target market weight. For ADG, birth week or birth month was fitted within two separate time periods to account for differences in market weight. Birth week or birth month estimates, centred on zero for each trait, were the environmental variables describing environmental conditions (EADG, EBF, EDFI, ETDG, EFCR). Using EVs of Hermesch *et al*. (2014), economic indexes ($/pig) were derived to quantify economic implications of EnVar: IDFI is a function of EADG, EBF and EDFI; and IFCR is a function of EADG, EBF and EFCR.

Considerable variation in environmental conditions was observed for all traits (Table 1), which was similar to the results of Li and Hermesch (2015) for ADG and BF. Environmental variables differed more for weekly groups than monthly groups, partly due to better accounting of environmental conditions and partly due to larger sampling effects of weekly groups. Standard errors doubled for EADG and EBF and tripled for EDFI, ETDG or EFCR for weekly versus monthly groups. Environments differed more for EDFI than EFCR, which may indicate that DFI captures differences in environments better. As a result economic indexes including DFI varied more, differing by $17.41/pig for IDFI in comparison to $11.78/pig for IFCR for monthly groups. These differences in economic indexes need to be multiplied by the number of pigs per group to quantify economic implications of variation in environmental conditions for groups of pigs. Results from this study suggest that investing in improvement of environmental conditions on farms, practising good health and management, should be considered by producers.

Table 1. The number of groups (N), standard deviations (SD), average standard errors (SE), and maximum range (Range) of estimates for birth month or birth week for each trait (Etrait) and each economic index

	Weekly groups				Monthly groups			
	N	SD	SE	Range	N	SD	SE	Range
EADG[A] (g/d)	318	16.22	9.77	89.37	72	13.88	4.25	67.25
EBF (mm)	318	1.81	0.31	6.69	72	1.79	0.128	6.08
EDFI (kg/d)	126	0.14	0.17	0.59	28	0.12	0.05	0.41
ETDG (g/d)	127	44.33	69.19	251.67	28	35.09	20.77	143.9
EFCR	126	0.097	0.149	0.461	28	0.08	0.04	0.32
IDFI[B] ($/pig)	126	5.27		25.59	28	4.38		17.41
IFCR[C] ($/pig)	126	3.72		16.23	28	2.55		11.78

[A]Refer to text for trait abbreviations used. [B]IDFI, index with DFI. [C]IFCR, index with FCR.

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Vitamin E does not counteract the shortened shelf life of long-stored pork with increasing levels of intramuscular fat

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The extended storage of red meat products increases lipid oxidation, particularly in high intramuscular fat (IMF) meat (Calnan *et al.* 2014). This decreases retail shelf life, an effect that is minimised in sheep by dietary supplementation with vitamin E (VE; Jose *et al.* 2008). However this has not been tested in long-stored (>14 d) Australian pork. The hypothesis examined herein was that supplementing finisher pigs with VE would enable extended storage of pork without an associated increase in spoilage during retail display.

Thirty two female Landrace × Large White pigs (49.3 ± 0.15 kg, mean ± SD) were housed in individual pens and fed a diet supplemented with either 35, 300, 500 or 700 IU of VE (α-tocopherol acetate) for 6 weeks (n = 8). The pigs were slaughtered at an average live weight of 86.6 ± 1.21 kg. A sample of the *m.longissimus thoracis et lumborum* (loin) was removed from the carcass 24 h after slaughter, divided into two and allocated to one of three aging treatments (0, 14 and 28 days), vacuum packed and stored at 4 °C for the corresponding period of time. The muscle was then cut into steaks 2.5 cm thick, overwrapped and set for retail display under florescent lights at 4 °C. A 5 g sample was removed from each steak at 0, 2, 4 and 6 d of retail display to measure the thiobarbituric acid reactive substances (TBARS). A TBARS number greater than 0.5 mg of malondialdehyde equivalents (MDA eq.)/kg is an indication of off-flavour development (Lanari *et al.* 1995). Both IMF and VE content in the muscle were measured and were used as continuous variables to test the development of TBARS during retail display at different aging treatments. Data were analysed using a linear mixed effects model (SAS®; USA).

Loin VE content increased with increasing supplementation ($P < 0.05$), with values ranging from 2.59 to 8.06 mg/kg (mean of 5.06 ± 0.24 mg/kg). The TBARS increased with days on retail display ($P < 0.001$) and at a greater rate in long-stored product. Increasing VE content decreased the TBARS concentration in the 0 d-stored product by 0.02 g MDA eq./kg for every 1 mg/kg of VE in the muscle ($P < 0.001$). However there was no effect ($P > 0.05$) in the meat stored for 28 d, contrary to the hypothesis. The apparent ineffectiveness of VE may be due to a lack of range in muscle VE concentrations, particularly at the lower end of the scale. In this regard, Jose *et al.* (2008) improved shelf-life in lamb up to a maximum muscle VE concentration of 3.5 mg/kg, a level exceeded with all supplementation rates used in this experiment. There was no effect of increasing IMF concentration on TBARS in 0 d-stored product, however an increase of 1% IMF increased the production of TBARS by 0.085 and 0.12 g MDA eq./kg for the 14 d- and 28 d-stored product, respectively (Fig. 1). This was despite a small range in IMF content (0.1 to 1.9%; mean = 0.61 ± 0.19%). Under long-stored conditions, the high-IMF pork had reached the off-flavour threshold after 4 d of retail display (Fig. 1). Thus, IMF appears to be an important factor limiting the shelf life of long-stored pork.

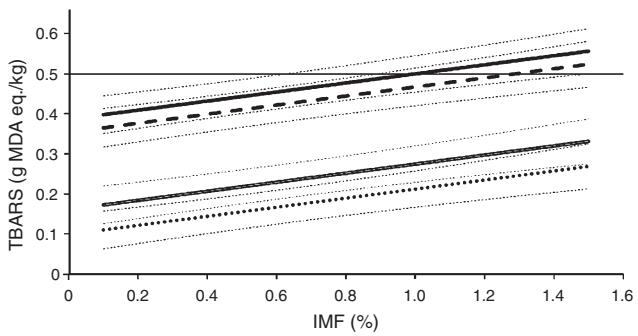

Fig. 1. The impact of IMF content on the production of TBARS in 28 d-stored loin during days of retail display (— Day 0 ···· Day 2 - - Day 4 ▬ Day 6) (±SEM, as thin dashed lines). The horizontal line at 0.5 is the TBARS threshold, indicating off flavour development.

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Pork eating quality was not improved by extended ageing for 14 days

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Industry effort is being directed to establish a pathway-based system for pork to improve its quality and consistency. Channon *et al*. (2003) showed that ageing for 7 days and constant current electrical stimulation (ES) of pig carcases can improve pork tenderness without detrimentally affecting drip loss or colour. However, recent data has suggested that both ageing period and constant current electrical stimulation may not be effective in improving eating quality consistency when commercially used in two different supply chains (Channon *et al*. 2015*a*, 2015*b*). This study aimed to determine the effect of gender, electrical stimulation, ageing for 14 days, and moisture infusion of five cut × cooking method treatments on pork eating quality. It was hypothesised that an extended ageing period of 14 days, rather than 7 days, together with electrical stimulation may be needed for fail rates of less than 10% to be achieved and be comparable to fail rates observed following moisture infusion.

A total of 69 entire male and 68 female Large White × Landrace pigs were managed on-farm, within gender. All male pigs were immunised against gonadotrophin releasing factor (GnRF) using Improvac® (Zoetis Ltd, USA), with injections administered at 13 and 17 weeks of age (IM). At 22 weeks of age pigs were penned with familiar pigs for transport, and held in lairage with access to water within gender groups for 22 hours before slaughter. Pigs, within gender, were randomly selected for electrical stimulation (none or 150 mA applied for 30 sec at 2 min after exsanguination; ES). A total of 25 pigs per gender, within carcass specifications of 60–75 kg (Trim 1) and 8–13 mm P2, were selected within ES treatment in the chiller at 60 min after slaughter and sides were then allocated to ageing period (2 or 14 days) ($n = 10$ sides per treatment). Moisture infusion was only applied to no ES, 2-day-aged cuts at a rate of either 0% (no-MI) or 10% brine solution (MI). Cut × cooking treatments used and overall liking and fail rate was determined as described by Channon *et al*. (2015*a*, 2015*b*). Data were analysed by ANOVA.

The OL of pork from IM and F pigs was comparable (57.7 vs 56.8, respectively; SED 1.50, $P = 0.542$), with an equivalent FR also observed (19.1% for both genders). Ageing for 14 days did not improve OL compared with 2 days (56.0 versus 55.1, respectively; SED 1.58, $P = 0.943$. The response to ES, as well as MI, differed ($P < 0.05$) between cut × cooking method treatments (Table 1). This indicated that the response to pathway interventions imposed is not necessarily consistent between different cut types, even when from the same muscle. Across all cuts evaluated, MI achieved a fail rate of 10.8%. Differences in the effectiveness of ES and ageing on eating quality between this study and those of Channon *et al.* (2015*a*, 2015*b*) highlights that each supply chain may need to consider different pathway interventions to enable consistent production of high quality fresh Australian pork.

Table 1. Electrical stimulation (ES) and moisture infusion (none or 10% infusion) effects on fail rate (%) and overall liking scores[A] of five pork cut × cooking treatments

ES treatment	Moisture infusion	Overall liking score					Fail rate (%)
		Silverside roast	Silverside stir fry	Loin roast	Loin stir fry	Loin steak	
No stimulation	No	45.7[a,e]	54.3[a]	51.3[a,e]	60.4[a]	53.3[a,e]	23.6
Stimulation	No	53.9[f]	55.0[c]	59.8[f]	62.1[c]	60.0[f]	18.9
No stimulation	10% brine	59.0[b]	63.2[b,d]	62.5[b]	71.7[b,d]	63.5[b]	10.8

[A]0 = dislike extremely to 100 = like extremely. [a,b]Means in a column between MI and No stimulation not having the same superscript are significantly different ($P < 0.05$); [c,d]Means in a column between MI and electrical stimulation not having the same superscript are significantly different ($P < 0.05$); [e,f]Means in a column between stimulation treatments not having the same superscript are significantly different ($P < 0.05$).

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Immunisation against gonadotrophin releasing factor reduces pork eating quality fail rates

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The Cooperative Research Centre for High Integrity Australian Pork is aiming to achieve consumer fail rates of less than 10% for pork through the implementation of various eating quality pathways. An issue facing the Australian pork industry is boar taint (Channon and Warner 2011), which is likely to result in higher fail rates in pork from entire male pigs. An effective way to eliminate boar taint is through immunisation of entire male pigs against gonadotrophin releasing factor (GnRF). The hypothesis was that immunisation against GnRF will reduce pork eating quality fail rates compared to entire male pigs at both light and heavy slaughter weights.

Sixty-four Large White × Landrace × Duroc pigs were used in a 2 × 2 × 2 factorial experiment ($n = 8$) with the main treatments being: 1) sex [entire male pigs vs male pigs immunised against GnRF (immunised; Improvac®; Zoetis Australia, Rhodes NSW)]; 2) weight at second immunisation [50 kg (light) vs 80 kg (heavy) live weight (LW)]; and 3) feeding regime (2.5 times maintenance vs *ad libitum*). Pigs were housed individually. The diets were fed for, and the second immunisation of GnRF was given, 28 days before slaughter (68.4 kg LW for light pigs and 106 kg LW for heavy pigs). At 24 hours after slaughter, 2-cm thick steaks were cut from the *Longissimus thoracis* for sensory analysis. Consumers graded the pork steaks into one of five quality/re-purchase intention categories: 1) unsatisfactory/definitely would not buy it; 2) below average/would probably not buy it; 3) average/might buy it); 4) above average/would probably buy it, and 5) excellent/would definitely buy it. Steaks were deemed to have failed if the score was ≤2. Skatole and androstenone concentrations were measured in belly fat using high performance liquid chromatography. Data were analysed by Chi-square and ANOVA (GenStat, 15th Edition; UK).

Fail rates were reduced by 9.1% and 12% for pork from immunised males for quality grade ($P = 0.007$) and re-purchase intention ($P = 0.001$), respectively, compared to pork from entire male pigs (Table 1). Skatole ($P = 0.001$) and androstenone ($P < 0.001$) levels in belly fat were higher in entire male pigs than immunised male pigs, which may in part help to explain the higher fail rates in pork from the entire males compared to the immunised males (data not shown). In addition, 37.5% of the light entire male pigs fed *ad libitum* showed skatole levels that exceeded the sensory threshold of 0.2 μg skatole/g, providing further evidence to the work of D'Souza *et al*. (2011) that boar taint is still an issue at lower carcass weights. This work confirms that immunisation against GnRF is effective in eliminating boar taint and reducing pork eating quality fail rates by approximately 10% compared to pork from entire male pigs.

Table 1. Percentage of consumer scores for quality grade and re-purchase intention for entire male pigs and immunised male pigs ($n = 240$)

Sex	Quality grade					Fail rate	*P* value
	1	2	3	4	5	(% ≤2)	
Entire	5.6	24.2	37.1	27.4	5.6	29.8	0.007
Immunised	0.8	19.8	39.7	31.0	8.6	20.7	
	Re-purchase intention						
	1	2	3	4	5		
Entire	14.5	24.2	24.2	21.0	16.1	38.7	0.001
Immunised	8.6	18.1	30.2	29.3	13.8	26.7	

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Prescription of energy-restricted diets with higher and lower pork protein content achieves weight loss and improved glycaemic control in adults with type 2 diabetes

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Energy-restricted, high-protein diets have shown to be effective for enhancing weight loss and improving glycaemic control in type 2 diabetes (T2DM) (Dong *et al.* 2013). Regular pork consumption has also been shown to improve weight loss and body composition both without energy-restriction (Murphy *et al.* 2012) and during energy restriction (Wycherley *et al*. 2010), but there is little data available on whether benefits are maintained during weight maintenance following initial weight loss. The aim of this study was to compare the effects of a higher pork protein content (HPP) and a lower pork protein (LPP) diet on weight loss and glycaemic control (measured by glycosylated haemoglobin [HbA1c %]) in overweight and obese adults with T2DM during weight loss and subsequent weight maintenance. It was hypothesised that an energy-restricted HPP diet would result in greater reductions in weight and HbA1c than the LPP diet during weight loss, and these improvements would be sustained during subsequent weight maintenance.

Sixty-one overweight and obese adults (aged 37–67 years; body mass index [BMI] 34.3 ± 0.6 kg/m^2 (mean $\pm$ SEM) with moderately controlled T2DM (HbA1c 8.1 ± 0.2%) were randomised to one of two hypocaloric diets: HPP diet (38% carbohydrate, 30% protein, 29% fat) or a LPP diet (53%:21%:23%) for 12 weeks, after which energy was adjusted to maintain a stable weight for a further 12 weeks while preserving the allocated macronutrient profile. Fresh, lean pork consisting of fillet steaks, stir-fry strips or diced pork, was prescribed for four times per week throughout the study (HPP 200-250 g/serves; LPP 100–150 g/serves). At baseline, participants completed a Food Frequency Questionnaire (FFQ) to assess habitual pork intake (frequency and portion size) over the previous 12 months. Daily semi-quantitative food checklists were completed throughout the study to capture dietary compliance. Dietary advice, meal planning and recipe ideas were provided every 2 weeks. Participants performed regular aerobic exercise throughout. Outcomes were measured at baseline and the end of each diet phase (Weeks 0, 12 and 24). Data were analysed using a linear mixed effects model utilising all data collected regardless of study completion (IBM SPSS, Version 21.0; USA).

Forty-four participants completed the study (HPP $n = 23$, LPP $n = 21$). Habitual intakes indicated the participants were infrequent pork consumers prior to entering the study (median, range: HPP 49.0 g/week, 0 to 305 g/week, LPP 52.5 g/week, 0 to 613 g/week, $P = 0.77$). During the weight loss phase, average pork consumption was 720 ± 29 g/week for the HPP diet and 384 ± 31 g/week for the LPP diet ($P < 0.001$). This indicates a 90 ± 3% and 94 ± 3% compliance with the prescribed pork intake for the diet groups respectively. There was a small decrease in compliance during the weight maintenance phase but this did not reach significance for time ($P = 0.06$) or between diet groups ($P = 0.71$). At the end of the 12-week weight loss phase, both groups showed reductions ($P < 0.001$) in weight (HPP -8.0 ± 0.8 kg; LPP -7.6 ± 0.8 kg) and improvements in HbA1c (HPP -1.5 ± 0.2%; LPP -1.3 ± 0.2%), with no differences between diets ($P > 0.05$). Following the 12-week weight maintenance phase, weight and HbA1c remained stable ($P > 0.05$).

Both diets achieved substantial weight loss and improvements in glycaemic control following energy-restriction that was sustained during weight maintenance. These data suggest lean pork can be included as part of a weight loss program for overweight and obese individuals with T2DM to achieve benefits for glycaemic control.

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Selenohomoalanthionine improves muscle selenium deposition in pigs

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Selenium (Se) is an essential trace element for pigs with its biological effects exerted as part of selenoproteins. There are over 30 identifiable selenoproteins in the body that play key roles in detoxification, immunity and reproduction. Traditional animal feed supplementation of Se has been as sodium selenite, however there is increasing use of organic Se sources such as Se yeast that is predominately selenomethionine. Organic selenium has been shown to significantly increase the deposition of Se into loin muscle (Mahan *et al*. 1999). The development of new organic Se sources such as selenohomoalanthionine (SeHLan) from different yeast strains has been postulated to be more efficient at incorporation of Se into animal tissue (Tsuji *et al*. 2010). The hypothesis tested in this experiment was that the incorporation of Se into muscle will be more efficient by the supplementation of SeHLan in the diet of finisher pigs than Se yeast (SeMet) or sodium selenite (SSe), and increased with higher levels of organic Se.

Sixty Primegro commercial immunocastrated male pigs were selected at 16 weeks of age (74.7 kg ± 5.41 kg; mean ± SEM) and housed in individual pens with feed and water available *ad libitum*. All pigs were offered a commercial grower diet during a 7-day acclimatisation period, after which pigs were individually weighed and randomly allocated to one of five test diets ($n = 12$) for the next 42 days. All diets contained 13.5 MJ of digestible energy and 7.2 g of standardised ileal digestible lysine. Sodium selenite was added to the first treatment diet to provide 0.3 ppm of added Se. Selenium yeast was added to the second and third treatment diet to provide 0.3 ppm and 0.6 ppm of Se, respectively. SeHLan was added to the fourth and fifth dietary treatments to provided 0.3 ppm and 0.6 ppm of added Se, respectively. Pigs were slaughtered in a commercial abattoir and hot standard carcass weight (HSCW) trim 13 and fat depth at the P2 site were recorded. A 20 g sample of liver was obtained at evisceration and a 50 g sample of loin was taken 24 hours later at boning from each carcass for Se analysis. Data were analysed by ANOVA (IBM SPSS, Version 22.0; USA) with the individual pig as the experimental unit.

There was no difference ($P > 0.05$) in HSCW or backfat depth at the P2 site between treatments (Table 1). The liver Se levels for pigs fed the 0.3 ppm SSe diet were higher ($P = 0.026$) than organic sources of Se at the 0.3 ppm inclusion level and similar to the 0.6 ppm inclusion of organic Se sources. The loin Se level was lowest ($P < 0.001$) for the SSe treatment. The loin Se level increased with the addition of SeMet at the 0.3 ppm level and increased further for the 0.6 ppm of SeMet. The 0.6 ppm SeMet and the 0.3 ppm SeHLan treatments had a similar level of loin Se and was higher by 43% when 0.6 ppm of SeHLan was fed to the pigs.

Organic Se was incorporated into the loin tissue of the pig more effectively than SSe, and SeHLan was at least 30% more effective than SeMet when included at either 0.3 or 0.6 ppm in finisher diets. The SeMet generally mimics methionine in its metabolism whereas SeHLan may have a different metabolic pathway to incorporation of Se into the muscle which is potentially more efficient.

Table 1. Hot standard carcass weight (HSCW), backfat depth and selenium (Se) levels in the liver and loin from pigs fed Se from three different sources and at two different levels for the organic sources

	Source of added Se						
	SSe[B]	SeMet[B]	SeMet	SeHLan[B]	SeHLan	SEM[A]	*P* value
Diet Se (ppm)	0.3	0.3	0.6	0.3	0.6		
HSCW (kg)	95.2	96.7	92.5	96.2	97.0	0.96	0.582
P2 backfat depth (mm)	14.4	13.4	13.9	13.4	15.4	0.42	0.552
Liver Se (mg/kg)	1.26[a]	0.70[b]	1.25[a]	0.88[bc]	1.13[ac]	0.07	0.026
Loin Se (mg/kg)	0.18[a]	0.23[b]	0.29[c]	0.30[c]	0.43[d]	0.01	<0.001

[A]SEM, standard error of the mean. [B]SSe, sodium selenite; SeMet, selenomethionine; SeHLan, selenohomoalanthionine. [a,b,c,d]Means in a row not having the same superscript are significantly different.

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Immunisation against gonadotrophin releasing factor increases fat deposition in finisher pigs

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Immunisation against gonadotrophin releasing factor (GnRF) is associated with an increase in backfat and carcass fatness and a decrease in lean meat content (Batorek *et al.* 2012; Dunshea *et al.* 2013). However, the timing of the increase in fatness and how it is impacted by feed intake and the weight at which the second immunisation of GnRF is given needs to be further explored to enable strategies to be developed to minimise the increase in carcass fatness. The hypothesis was that pigs that have been immunised against GnRF at heavy live weights (80 kg) and fed *ad libitum* would deposit more fat than those immunised at light live weights (50 kg) or fed restrictively.

Sixty-four individually-housed male Large White × Landrace × Duroc pigs were used in a 2 × 2 × 2 factorial experiment with the main factors being: sex (entire male pigs and immunised male pigs); live weight (LW) at second immunisation against GnRF [50 kg (light) and 80 kg (heavy), Improvac® (Zoetis Australia, Rhodes NSW)]; and feeding regime [2.5 times maintenance (restricted E_m (kJ/d) = 444 kL × $LW^{0.75}$, where E_m = energy maintenance) and *ad libitum*]. Diets were formulated to contain 13.5 MJ digestible energy (DE)/kg and 0.59 g standardised ileal digestible lysine/MJ DE. The experimental treatments were implemented 28 days before slaughter (68.4 kg LW for light pigs and 106 kg LW for heavy pigs). Pigs were scanned for body composition using dual energy X-ray absorptiometry (Suster *et al.* 2004) on d 0, 14 and 28 after the second immunisation against GnRF. Data were analysed with ANOVA (Genstat, 15th Edition; UK).

The heavy immunised male pigs deposited 135 g/d less lean tissue than entire male pigs during days 15–28 ($P = 0.022$) with no difference between sex in the light pigs (Table 1). Fat deposition was not affected by sex during d 0–14 ($P > 0.05$) but during d 15–28, the immunised male pigs deposited nearly 50% more fat than entire male pigs ($P = 0.025$). Immunised male pigs fed *ad libitum* deposited 87.1 g/d more fat during d 15–28 compared to entire male pigs ($P = 0.036$) with no difference between sex when fed restrictively. The majority of fat deposition occurred during the second 2-week period after the second immunisation against GnRF. However, the increase in fat deposition did not occur in those fed the diet restrictedly. Future research should target ways to decrease carcass fatness in immunised male pigs, particularly during the two to three weeks after the second immunisation.

Table 1. The effects of sex (S), live weight (LW) and feeding regime (F) on lean and fat deposition for the periods d 0-14 and d 15-28 after the second immunisation against GnRF ($n = 8$)

F		Restricted		*Ad libitum*			*P*-value[A]		
LW	Sex (S)	Light	Heavy	Light	Heavy	SED[B]	S	F	W
LD[E] 0-14	E[C]	467	679	747	887	77.7	0.566	<0.001	<0.001
(g/day)	I[D]	433	565	749	944				
LD 15-28	E	689	762	849	972	71.6	0.168	<0.001	0.650
(g/day)	I	597	688	1010	783				
FD[F] 0-14	E	19	56	88	139	30.0	0.937	0.035	<0.001
(g/day)	I	32	132	31	112				
FD 15-28	E	12	139	94	125	39.0	0.025	<0.001	0.002
(g/day)	I	46	100	184	211				

[A]Significant interactions are discussed in the text. [B]SED, standard error of difference between means. [C]Entire male pigs. [D]Male pigs immunised with GnRF. [E]Lean deposition. [F]Fat deposition.

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Response to different pathway interventions to improve pork eating quality consistency

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For consistent delivery of high quality, boar-taint-free pork cuts in Australia, additional data is needed to quantify interactions between different pathway factors. Few studies have been conducted comparing eating quality traits of different pork cuts from entire male pigs immunised against gonadotrophin releasing factor (GnRF) (IM) with females, in combination with other pathway interventions, including ageing for 7 d and electrical stimulation (ES) previously shown to improve pork eating quality (Channon and Warner 2011). This study aimed to validate the effect of ES, ageing (A) and moisture infusion (MI) on eating quality attributes of five different pork cuts from female (F) and IM.

Large White × Landrace pigs (F, n = 50; IM, n = 50), raised from weaning in straw-based eco-shelters with access to feed on an *ad libitum* basis, were slaughtered at 21 weeks of age. For three weeks before slaughter, ractopamine hydrochloride was included in the feed at a rate of 5 ppm/tonne. Entire male pigs were vaccinated with Improvac® (Zoetis Ltd., USA) at 10 and 15 weeks of age. Pigs were separated according to gender prior to transport and slaughtered at a commercial abattoir. Pigs were randomly allocated within gender to the ES treatment (control or 150 mA for 30 sec at 2 min after slaughter). A total of 25 pigs per gender were selected from the larger group in the chiller at 60 min after slaughter based on carcass specifications of 60–75 kg (Trim 1) and 8–13 mm P2. At 24 hours after slaughter, roast and stir fry portions were obtained from loin and silverside muscles from both carcase sides and steaks from the loin only. Ageing (2 or 7 days) was allocated to muscles from each carcase side (n = 10 sides per treatment) and frozen prior to thawing for sensory analysis. In addition, MI (no, or 10% extension) was applied to no-ES, 2-day-aged loin and silverside muscles from 10 sides per gender. Consumers (n = 400) rated 2,000 samples for quality grade (1 = unsatisfactory to 5 = excellent). Fail rate was determined (expressed as a percentage of evaluations achieving a quality grade score of 1 or 2). Data were analysed by ANOVA.

Ageing for 7 days increased ($P < 0.05$) quality grade scores across all cut × cooking method treatments compared with 2 days (3.38 versus 3.26; SED 0.046), with larger improvements in quality grade scores found due to MI (3.54 vs. 3.32, for MI and no-MI cuts respectively; SED 0.058; $P < 0.05$). Across all cut × cooking methods, neither gender nor ES influenced ($P > 0.05$) quality grade scores and no interactions across cut × cooking methods were found (data not presented). At the cut level, loin roasts from IM had higher quality grade scores ($P = 0.034$) and had lower fail rates than F (Table 1). Differences in quality grade scores between cuts evaluated in this study were greater in magnitude compared with other interventions imposed.

As quality grade scores (and fail rates) of different pork cuts from IM were either comparable, or better, than those from F, this suggests that pork from IM may be included into any future eating quality system. Font i Furnols *et al.* (2008) also reported no differences in tenderness and juiciness of pork loin from F and IM pigs. Further investigations to understand mechanisms impacting on the ability of pork loin and silverside muscles to age are needed as ageing for 7 days after slaughter only caused very minor improvements in quality grade scores. The lack of response to ES in this study suggests that alternate options need to be explored for individual supply chains to enable an eating quality system for pork to be successful.

Table 1. Quality grade scores (and fail rate, %) of pork from female (F) and entire male pigs immunised against GnRF (IM) for five cut × cooking treatments

	Quality grade score (Fail rate, %)				
Cut	Silverside		Loin		
Cooking method	Roast	Stir fry	Roast	Stir fry	Steak
F	3.38 (19.5)	3.25 (18.5)	3.08 (26.0)	3.58 (11.5)	3.24 (22.5)
IM	3.57 (14.0)	3.25 (17.5)	3.38 (16.0)	3.67 (10.5)	3.23 (21.0)
P value	0.086	0.96	0.034	0.29	0.93

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Aitchbone hanging or moisture infusion, but not ageing, influenced eating quality of pork cuts

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The Australian pork industry is working to develop a cuts-based, non-prescriptive eating quality system for pork. Channon *et al.* (2011) identified that as the majority of studies have utilised the loin muscle, more data is needed to quantify the impact of different production and processing factors, as well as cooking methods, on eating quality of different pork cuts. For this system to be non-prescriptive, different supply chains need to have flexibility in determining which pathway interventions may be implemented to deliver high quality pork to consumers. This study aimed to validate the effect of hanging method, ageing period, moisture infusion, cut and cooking method, and their interactions, on eating quality attributes of pork from female (F) and entire male pigs immunised against gonadotrophin releasing factor (GnRF) (IM) pigs. It was hypothesised that pork eating quality can be improved to result in a fail rate of less than 10% by the implementation of a combination of pathway factors known to influence eating quality.

Large White x [Landrace x (Duroc x Large White)] entire male and female pigs ($n = 36$ per gender) were managed on-farm, within gender, until slaughter at 22 weeks of age. All males were immunised against GnRF using Improvac® (Zoetis Ltd, USA), with the vaccine administered at 10 and 17 weeks of age. A total of 25 pigs per gender was selected from the larger group based on carcase specifications (60–75 kg Trim 1; P2 8–13 mm) at 60 min after slaughter and carcases were randomly allocated to hanging method [Achilles (AH), or aitchbone (ABH)]. Within hanging treatment, sides were randomly allocated to ageing period (2 or 7 days). Moisture infusion was only applied to AH 2 day aged cuts at a rate of either 0% (no-MI) or 10% brine solution (MI)). Cut x cooking treatments used and fail rate was determined as described by Channon *et al.* (2015). Consumers ($n = 400$) rated 2,000 samples for overall liking (OL) (0 – dislike extremely to 100 – like extremely). Data were analysed by ANOVA.

Ageing for 7 days did not improve ($P > 0.05$) OL scores and pork from IM and F carcases was comparable for eating quality (data not presented). For OL, interactions between hanging method and between MI and hanging method were observed within cut (Table 1). Within each cut type, ABH improved OL scores for loin stir fry ($P = 0.004$) and roast ($P = 0.028$) and silverside stir fry ($P = 0.005$) compared with AH, indicating positive opportunities to improve pork eating quality. Across all cuts, ABH reduced fail rates by 9.6% compared with AH. The OL scores were improved ($P < 0.05$) by MI, compared with all non-MI cuts obtained from AH and ABH carcases (except ABH silverside stir fry). Across all treatments, only MI loin stir fry, roasts and steaks and ABH loin stir fry achieved fail rates of <10% (data not presented). Significant challenges to both identify and commercially implement cut-based strategies that reduce the fail rate of pork cuts to <10%, in addition to MI, remain. Given that ageing for 6 to 10 days after slaughter has a positive effect on eating quality (Ngapo and Gariepy 2008), further work to understand why ageing was not an effective intervention for pork in this supply chain is needed.

Table 1. Hanging method and moisture infusion (none or 10% infusion) effects on fail rate (%) and overall liking scores [A] of five pork cut x cooking treatments

Hanging Method	Moisture infusion	Overall liking score					Fail rate (%)
		Silverside roast	Silverside stir fry	Loin roast	Loin stir fry	Loin steak	
Achilles	None	$50.0^{a,e}$	$53.0^{a,c}$	55.4^{a}	$57.4^{a,e}$	54.0^{a}	27.1
Aitchbone	None	$56.8^{c,f}$	61.9^{f}	58.9^{c}	$66.8^{c,f}$	57.9^{c}	17.5
Achilles	10% brine	$65.8^{b,d}$	60.5^{b}	$79.4^{b,d}$	$76.9^{b,d}$	$66.4^{b,d}$	13.8

[A]0 = dislike extremely to 100 = like extremely. [a,b]Means in a column between MI and Achilles hanging not having the same superscript are significantly different ($P < 0.05$). [c,d]Means in a column between MI and aitchbone hanging not having the same superscript are significantly different ($P < 0.05$). [e,f]Means in a column between hanging treatments not having the same superscript are significantly different ($P < 0.05$).

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Piglets born with a high degree of meconium staining display altered behaviour throughout lactation

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Piglets may experience asphyxia during parturition. In some, this results in anoxia and the piglet is still born. For others, the degree of asphyxia is less severe and the piglets are born alive, but may suffer organ damage. The brain is particularly susceptible to hypoxia, and induced asphyxia at birth has been shown to alter cognitive ability in guinea pigs (Becker and Donnell 1952). Hence, hypoxic piglets may display behavioural deficiencies. In the present study it was hypothesised that piglets exposed to birth hypoxia would be more anxious than normoxic piglets.

Piglets were identified as normoxic or hypoxic by assessing the meconium stain score (Mota-Rojas *et al*. 2002) following birth [score 0 (n = 18) and 3 (n = 17) respectively]. On d 11 and 21 of age, each piglet was placed inside a holding box for 1 min, and then a door was removed revealing an open arena. Emergence time from the holding box, and then behaviours (listed in Table 1) were recorded via real time observations and video camera during the arena test. Non-normally distributed data were natural-logarithmically transformed and when this occurred, the back-transformed means are presented in parenthesis. Data were analysed using a general linear model (ASReml, 3rd Edition; UK).

There was no significant effect of the interaction between level of hypoxia and day, so only main effects are reported in Table 1. There was a strong trend for piglets with a meconium stain score of 3 to take longer to emerge from the holding box ($P = 0.059$) than pigs with a meconium stain score of 0 (Table 1). Piglets with a meconium stain score of 3 displayed fewer squeals ($P < 0.05$) and fewer grunts ($P < 0.05$) than piglets with a meconium stain score of 0.

Whilst effects of hypoxia on peri-natal behaviour appear commonly in the literature (Herpin *et al.* 1996), the present data support the notion that piglets born with a high degree of meconium staining display altered behaviour for at least 21 d during lactation. The increase in emergence time and reduction in low pitched grunts in high meconium stained piglets may represent a decreased willingness to interact with a new environment. This supports the hypothesis in part and, in turn, warrants the need for further research into the long term effects of hypoxia on pig behaviour.

Table 1. The effect of hypoxia (as indicated by meconium score) on piglet behaviour. Values are mean ± SEM

	Level of hypoxia		
	Meconium score 0	Meconium score 3	*P* value
Emergence time (sec)	2.3 ± 0.4 (10.0)	3.4 ± 0.6 (30.0)	0.059
Number of walking events	12.2 ± 1.4	14.3 ± 2.5	0.96
Time spent walking (sec)	4.9 ± 0.1 (134.3)	4.7 ± 0.1 (109.9)	0.92
Number of lines crossed	3.1 ± 0.2 (22.2)	3.1 ± 0.4 (22.2)	0.44
Number of freezing events	8.5 ± 1.4	10.6 ± 2.4	0.93
Time spent frozen (sec)	3.2 ± 0.4 (24.5)	3.8 ± 0.8 (44.7)	0.61
Number of squeals	1.4 ± 0.5 (4.1)	0.1 ± 1.0 (1.1)	<0.05
Number of grunts	158.7 ± 19.1	132.0 ± 32.7	<0.05
Escape attempts	0.7 ± 0.3 (2.0)	0.4 ± 0.5 (1.5)	0.90

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Inclusion of $MgSO_4$ in the diet of sows before farrowing improves measures of piglet colostrum ingestion

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Colostrum ingestion is one of the most important factors contributing to piglet survival (Devillers *et al*. 2011). Hypoxia during parturition may reduce the amount of colostrum consumed, and neuro-protective agents could provide a simple method of increasing the viability of piglets that have suffered oxygen deprivation during the birth process. Magnesium ions reduce cell death after a hypoxic event (Marret *et al*. 2007), hence magnesium sulphate ($MgSO_4$) may be a suitable candidate for inclusion in a sow diet before farrowing. It was hypothesised that sows supplemented with $MgSO_4$ would give birth to piglets with improved vitality and increased colostrum ingestion.

Sows (parity 2 to 9) were fed 3.0 kg/d of a control lactation diet (Control; n = 30) or a diet supplemented with 21 g/d of $MgSO_4$ (Mg; n = 31) from 5 d before farrowing. Piglet measurements (n = 758) collected from sows that farrowed between 0600 and 2200 h included meconium stain score to indicate hypoxia (0: no staining, normoxic to 3: severe staining, hypoxic), vitality score (0: no movement, no breathing after 15 sec to 3: good movement and breathing, piglet attempts to stand within 15 sec), first 24-hour weight gain, blood glucose concentration and estimated piglet serum IgG content (immunocrit; Vallet *et al*. 2013) at 24 hours of age. Non-normally distributed data were transformed and subsequently analysed using a linear mixed model (Genstat, 16th Edition; UK) with sow identification fit as the random term. Data presented are least square means ± SEM.

There was a tendency ($P = 0.08$) for all piglets from the Mg treatment to display a higher vitality score immediately after birth (1.6 ± 0.1) than Control piglets (1.4 ± 0.1). Piglets from the Mg treatment tended ($P = 0.07$) to have higher blood glucose levels (5.9 ± 0.3 mmol/L) than Control piglets (5.3 ± 0.4 mmol/L) at 24 hours of age, but immunocrit measurement remained unaffected ($P > 0.05$). Piglets from the Mg treatment that received a meconium stain score of 2 or 3 gained weight over the first 24 hours ($P < 0.01$) whilst Control piglets within these scores effectively did not gain weight (Fig. 1).

Given that weight gain in the first 24 hours is indicative of colostrum uptake in piglets (Devillers *et al.* 2011), our data show that the inclusion of $MgSO_4$ in a pre-farrow sow diet assists meconium stained piglets to consume colostrum. There was also some evidence of improved vitality and energy levels across all $MgSO_4$-treated piglets. Further work should determine if these improvements result in increased piglet survival.

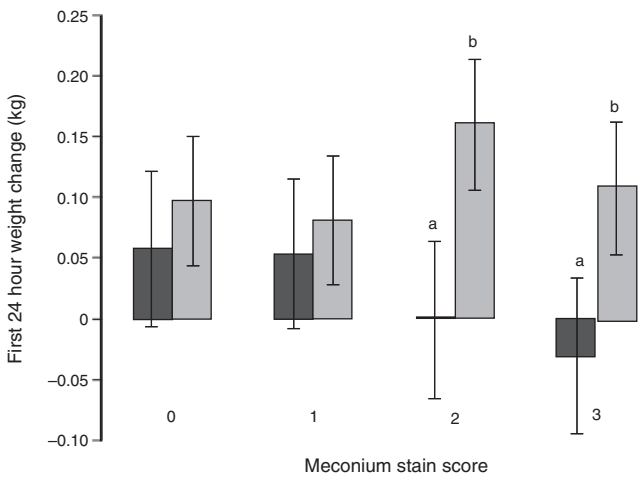

Fig. 1. Bodyweight change (kg; mean ± SE) in the first 24 hours of life for piglets from sows fed a standard lactation diet (Control) (■) and those supplemented with 21g/sow/day of $MgSO_4$ (Mg) (▩) prior to farrowing for piglets ranging from a meconium score of 0 (normoxic) to 3 (hypoxic).

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Neonatal split suckling improves survival of small piglets

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Split suckling (SS) is a management technique that could provide more sufficient colostrum to all piglets within large litters, thereby improving the chances of survival. The absorption of colostral IgG is essential for immune system development as there is no placental transfer of antibodies between sow and piglet *in utero* (Nguyen *et al.* 2013). This is achieved by reducing competition at the sow's udder, which allows smaller piglets to consume adequate colostrum (Vallet 2013). This trial aimed to identify if two SS treatments would improve piglet survival under commercial conditions. It was hypothesised that split suckling will increase colostrum ingestion and reduce mortality in piglets.

The experiment was conducted at a commercial piggery using parity 0–7 litters (n = 423). Each litter was assigned to one of the following three treatments (n = 141 litters per treatment): control (no SS); rotational (half litter SS hourly for 4 hours); or SSam (separation of the largest piglets in a litter, allowing the smallest to suckle for 2 hours in the morning). Prior to any cross-fostering, piglets were tagged, weighed [piglets were classed as small (<0.85 kg), normal (0.86–2.07 kg) and large (>2.08 kg)], and subjectively scored for vigour (0–3 scale; adapted from Herpin *et al.* 1996). The SS treatments were then applied. On d 1 after farrowing, a blood sample was taken from four piglets (two heaviest and two lightest piglets) from each litter. Piglets were then re-scored for vigour, thereby enabling the change in vigour to be calculated. The blood sample was used for the estimation of colostrum ingestion (immunocrit technique; quantification of IgG in serum) (Vallet *et al.* 2013). Piglet mortality from birth to weaning was recorded to determine survival between the treatment groups. Traits were analysed with a generalised linear mixed model (SAS®; USA), with birth and rearing sow fitted as a random effect. Fixed effects included sex, piglet birth weight, litter size, sow parity, and SS treatment. Binary traits (survival) were analysed with a logistic transformation.

Survival of small piglets from the SSam treatment was 13% greater than small piglets in the control and rotational treatments (Fig. 1; $P < 0.05$). Change in vigour from d 0 to d 1 in small piglets from the SSam treatment was different by half a score from small piglets in the control and rotational SS groups (Fig. 2; $P < 0.05$). Colostrum absorption was similar among treatments and size classifications ($P > 0.05$). In conclusion, the data provides evidence that SSam improves the survival of small piglets through enhancing their vigour, though there was no overall effect of treatment on colostrum ingestion.

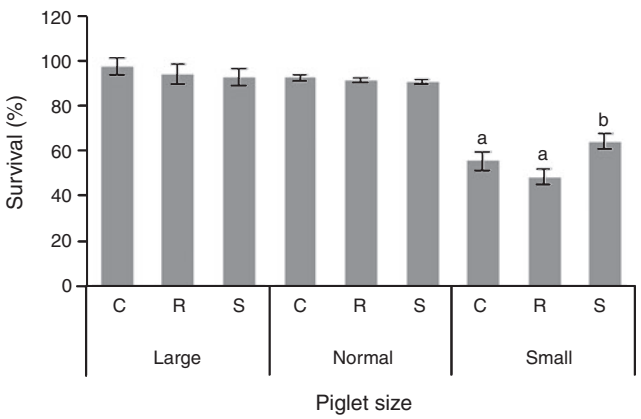

Fig. 1. Mean (± SEM) pre-weaning piglet survival for treatment groups [Control (C), Rotational (R) and SSam (S)] grouped on weight [small (<0.85 kg); normal (0.86–2.07 kg); large (<2.08 kg)]. [a,b]Superscripts are significantly different ($P < 0.05$).

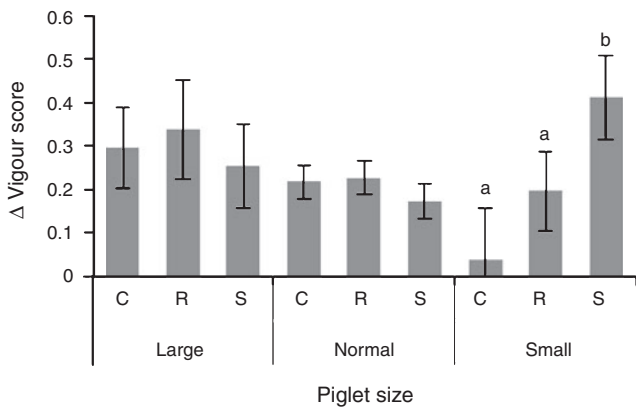

Fig. 2. Mean (± SEM) change (Δ) in vigour from d 0 to d 1 for treatment groups [Control (C), Rotational (R) and SSam (S)] grouped on weight [small (<0.85 kg); normal (0.86–2.07 kg); large (<2.08 kg)]. [a,b]Superscripts are significantly different ($P < 0.05$).

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A 'two-stage' farrowing and lactation system: piglet survival and growth performance

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Loose farrowing systems that meet the biological maternal needs of the sow have been developed (i.e. the PigSAFE system), and minimum pen design criteria for loose farrowing systems have been recommended based on behavioural needs and basic body dimensions of sows and piglets (Baxter *et al*. 2011). However, any effective pen design requires extra floor space compared to conventional farrowing crates and, consequently, adds capital costs. An additional system is a two-stage system that maximises the throughput of sows by allowing sows to farrow loose in individual pens or farrowing crates then grouping sows and litters into a more cost effective system at approximately 2 weeks after farrowing (i.e. group lactation (GL) systems). This experiment tested the hypothesis that piglet survival and growth performance would be the same in farrowing crates, PigSAFE and a 'two-stage' farrowing and group lactation system.

A total of 360 mixed-parity sows (Large White x Landrace, PrimeGro™ Genetics) over six time replicates was studied. Sows were randomly allocated to one of four treatment groups: 1) Farrowing crates (FC): sows housed in farrowing crates until weaning; 2) GL_{FC}: sows housed in farrowing crates then moved into GL 14 days before weaning; 3) PigSAFE (PS): sows housed in the PigSAFE loose farrowing system until weaning; and 4) GL_{PS}: sows housed in the PigSAFE system then moved to GL 14 days before weaning. The housing treatments were located in three adjacent buildings, all similar in terms of ventilation and construction material. The buildings were open-sided with shutters and heating which enabled temperature control. All sheds were managed by the same stockpeople. The experiment began in March and finished in November 2014. The total number of piglets born (born alive, still born and mummified piglets), number of piglet deaths and number weaned were recorded for each litter. Piglet live born mortality (%) (from birth to weaning) was calculated for each litter. Individual live weight of piglets was recorded at birth, 14 days before weaning and at weaning (25 ± 2.7 days; mean ± SD). Univariate GLM analysis (IBM SPSS, Version 21.0; USA) was undertaken using each sow/litter at the start as the experimental unit with replicate as a random factor in the design.

There was no difference ($P > 0.05$) in the number of piglets born alive or number weaned between housing treatments (Table 1). There was however a trend for higher live born mortality in the PS systems compared to FC systems ($P = 0.094$). Piglets in the GL_{FC} and GL_{PS} housing treatments had a lower ($P < 0.001$) rate of gain in the GL period compared to piglets that remained in the FC and PS housing treatments, which may be attributed to increased socialisation, piglet activity and cross-suckling. The outcomes from this study support the need for further development of loose farrowing systems for Australian conditions and suggest piglet growth performance may be reduced in group lactation systems. Further research is warranted to determine the impact of the GL system on post-weaning and lifetime performance of these piglets.

Table 1. Piglet survival and average daily gain (ADG) from birth to weaning, and from mixing to weaning, in the different housing treatments

	FC[A]	GL_{FC}	PS	GL_{PS}	SEM[B]	*P* value
Litters farrowed	141	36	142	36	–	–
Average number piglets weaned[C,D]	9.2	9.7	9.2	8.9	0.10	0.385
Live born mortality (%)[D]	16.6	14.6	19.9	20.3	0.823	0.094
Piglet ADG (g, birth to weaning)[E]	218[ab]	193[c]	221[a]	206[bc]	0.002	0.001
Piglet ADG (g, mixing to weaning)[F]	264[a]	156[c]	248[b]	168[c]	0.003	< 0.001

[A]Refer to text for treatment details. [B]SEM, standard error of the mean. [C]Included fostered piglets. [D]Number of piglets born alive used as a covariate. [E]Piglet birth weight used as a covariate. [F]Piglet pre-mix weight used as a covariate [a,b,c]Means in a row not having the same superscript are significantly different.

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Sows with high milk production had a high feed intake and high body mobilisation

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The modern high-producing sow has undergone major genetic improvements during the last decades resulting in larger and leaner animals and larger litter sizes. Milk production is affected positively by feed intake during lactation (Vadmand *et al*. 2015), but many sows turn catabolic during the lactation period to maintain their milk production, because they are unable to increase the feed intake at the same rate as milk production is increasing (Hansen *et al.* 2012). It was hypothesised that sows having the highest litter gains during lactation would stand out from other sows when comparing feed intake, body mobilisation during lactation and the subsequent reproduction.

The data for the current evaluation was obtained from a nutritional study of the effects of increasing dietary valine-to-lysine ratio on the performance of litters and sows during lactation. There were no effects ($P > 0.30$) of the dietary treatments on any of the measured parameters (Strathe *et al*. 2015) and therefore the data were pooled for this evaluation. The data from 565 sows (parity 2.5 ± 1.0; mean ± SD), where litters had been standardised to 14 piglets at d 2 post-partum and piglets were weaned at d 26, were used in the analysis. Sow body weight (BW), backfat thickness (BF) and litter weight were registered for all sows at d 2 and at weaning. Milk yield was calculated using equations by Hansen *et al*. (2012). Pearson's correlations (R Core Team, Austria) were calculated to test for correlations between the measured variables.

On average, the sows weaned 13.0 ± 1.1 piglets (mean ± SD), had a litter gain of 2.9 ± 0.5 kg/d and had an estimated milk yield of 11.3 ± 1.4 kg/d. The feed intake of the sows was 6.1 ± 0.7 kg/d and the sows lost 22.5 ± 12.7 kg BW (9.1 ± 5.1% of their BW at d 2) and 2.9 ± 1.7 mm BF from d 2 post-partum until weaning. The weaning-to-estrus interval (WEI) was 5.3 ± 6.1 days and in the next litter, the sows gave birth to 18.2 ± 3.8 total born piglets. Feed intake ($r = 0.46$, $P < 0.001$), BF loss ($r = 0.42$, $P < 0.001$) and BW loss ($r = 0.48$, $P < 0.001$) during lactation had a positive effect on litter gain (Fig. 1). Increasing feed intake in lactation reduced the WEI ($r = –0.16$, $P < 0.001$) and increased total born piglets in next litter ($r = 0.14$, $P < 0.01$), but BW loss only had an effect on total born piglets in next litter ($r = –0.10$, $P < 0.05$) and no effect on WEI ($r = –0.04$, $P = 0.30$) (Fig. 1).

Increasing average daily feed intake during lactation with 1 kg improved daily litter gain by 340 g [Litter gain (kg/d) = 0.85 + 0.340 × Feed intake (kg/d)]. A sow BW loss of 1 kg heightened daily litter gain with 20 g [Litter gain (kg/d) = 2.48 + 0.020 × BW loss (kg)], whereas a BF loss of 1 mm enhanced litter gain with 130 g/d [Litter gain (kg/d) = 2.55 + 0.130 × BF loss (mm)]. In conclusion sows with high litter gains both had a high feed intake and BW loss during lactation, but the high body mobilisation had a negative effect on the size of the next litter.

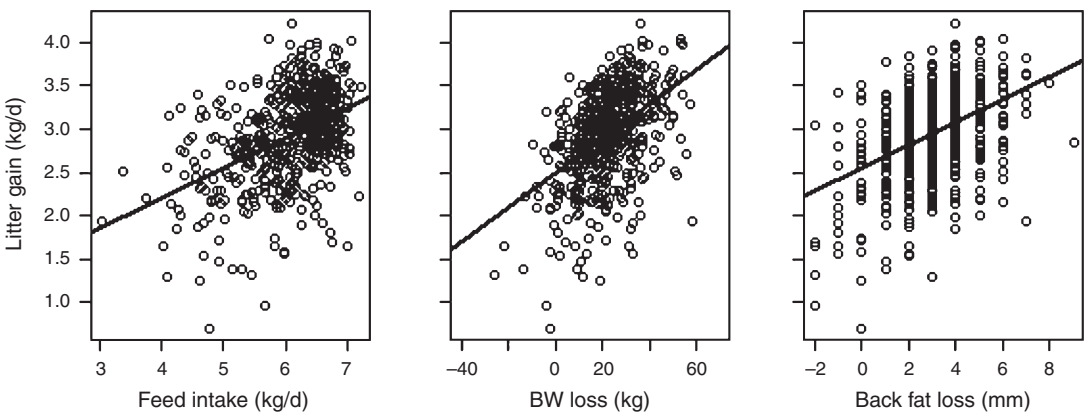

Fig. 1. The influence of feed intake ($r = 0.46$, $P < 0.001$), body weight (BW) loss ($r = 0.48$, $P < 0.001$) and backfat (BF) loss ($r = 0.42$, $P < 0.001$) of the sow during lactation on litter gain.

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Intermittent suckling causes a stress response in piglets that is attenuated over time

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Intermittent suckling (IS), where a sow and her piglets are separated for a period of time each day before weaning, can induce oestrus in lactation (Downing *et al.* 2007). However, the effects of repeated maternal separation on aspects of the piglets' stress response and the welfare implications of this require examination. This study tested the hypothesis that piglets subjected to IS in the week before weaning would show changes in cortisol, neutrophil : lymphocyte ratios (N : L) and injury scores indicative of a stress response in the peri-weaning period.

Gilt litters (n = 21) were allocated to one of two weaning regimes: conventional weaning (CW), where piglets had continuous access to the sow until weaning at d 29 ± 2.3 (mean ± SD), and IS, where piglets were separated from the sow for 8 h per day (0700 to 1500 h) starting at d 22 ± 1.3 for a week before weaning (d 29 ± 1.3). Creep feed was provided *ad libitum* from d 14 of lactation. At weaning, litters were mixed within treatment and housed in pens according to sex and size (24–25 pigs per pen, approximately 0.23 m^2 per piglet). Blood samples were taken from two randomly selected piglets per litter at 1 and 7 days before weaning and six randomly selected pigs per pen at 1 and 7 days after weaning. Blood sampling started at noon and each sample took approximately 90 sec to collect. Samples were not collected from the same piglets at each time point due to ethics requirements. Injury scores adapted from Widowski *et al.* (2003) were also recorded the day after weaning. Plasma cortisol, N : L ratios and injury scores were compared between treatments using GLM procedures (IBM SPSS, Version 21.0; USA).

Cortisol levels were higher ($P = 0.01$) in IS piglets 7 days before weaning (i.e. the day after IS began) (Table 1). However the N : L ratio, another measure of the stress response (Davis *et al.* 2008), tended to be higher ($P = 0.07$) in CW piglets 7 days before weaning. There was no treatment effect for cortisol or N : L at the other time points. This lack of treatment effect was also reflected in post-weaning injury scores ($P = 0.26$ for redness and $P = 0.32$ for scratches). Apart from a peak in cortisol at the start of IS, piglets subjected to IS did not display physiological or behavioural indicators indicative of a stress response the day before weaning and 1 and 7 d after weaning, suggesting that short periods of maternal separation (such as, 8 h/day) do not appear to compromise piglet welfare over the peri-weaning period.

Table 1. Mean total plasma cortisol and neutrophil : lymphocyte (N : L) ratios before and after weaning for conventionally weaned (CW) and intermittently suckled (IS) piglets

	Before weaning				After weaning			
	Cortisol (ng/mL)		N : L ratio[B]		Cortisol (ng/mL)		N : L ratio	
Day[A]	–7	–1	–7	–1	+1	+7	+1	+7
IS	37	22	0.8 (0.6–1.1)	1.1 (0.7–1.8)	21	18	2.3 (1.5–3.4)	3.9 (2.7–5.7)
CW	16	20	1.3 (0.3–1.8)	1.8 (1.1–2.9)	20	18	2.0 (1.3–3.1)	4.9 (3.4–7.2)
SEM[C]	5.1	3.4			4.0	2.6		
P value	0.01	0.75	0.07	0.16	0.80	0.88	0.67	0.38

[A]Indicates day in relation to weaning. [B]Data were logarithmically transformed for GLM and then back-transformed and expressed as least-square means with 95% CI (in parentheses). [C]SEM, standard error of mean.

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Teat order influences piglet performance after weaning

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The position of piglets on the udder during sucking can influence growth rate (Pluske and Williams 1996) and the interaction between intermittent suckling and teat order has been explored previously (Berkeveld *et al.* 2007). However, it is not known whether a gradual reduction in sow contact has differing impacts on piglets from anterior, middle and posterior sucking positions. It was hypothesised that piglets sucking from posterior teats would show improved pre-weaning creep consumption and therefore growth after weaning, but that reduced sow contact in lactation would remove this effect of teat order.

An incremental reduction in sow contact was achieved by separating the sow from piglets (n = 30 litters) for 5 h/d (d -18 to -13 relative to weaning), 7 h/d (d -13 to -8), and 9 h/d (d -8 to -1). Control sows (n = 20) remained in full contact with the litter to weaning that occurred at 28 ± 1 d of age (mean ± SEM). Piglets were observed during sucking events to determine teat order (anterior, middle and posterior locations). Creep feed was provided from d -17, and consumers were identified (creep feed dyed using indigo carmine with piglet faeces assessed for colour using rectal swabs) and weights recorded on d -17, -12, -7, -1, 1, 2, 7 and 14 relative to weaning. Blood samples were collected on d -1 and 1 relative to weaning for plasma free cortisol concentration analysis. Non-normally distributed data were transformed prior to analysis (IBM SPSS, Version 20.0; USA) using repeated measure analysis. Creep feed intake was analysed using a binomial regression analysis with logit function (ASReml, 3rd Edition; UK).

There was no interaction between gradual weaning treatment and teat order for all traits examined ($P > 0.05$). Anterior positioned piglets were consistently heavier than middle and posterior piglets (Table 1; $P < 0.001$). Average daily gain was also influenced by teat position on the days after weaning, with posterior and middle piglets gaining more weight compared with anterior positioned piglets ($P < 0.05$). The number of piglets consuming creep feed was similar for all piglets across most time points except for d 2, when a reduced proportion of anterior piglets were recorded to be consuming creep than those from middle or posterior teats ($P < 0.05$). Plasma cortisol concentration tended to be lowest in piglets sucking posterior teats (back-transformed mean: 41.6 nmol/L) when compared with piglets sucking middle teats (55.9 mmol/L), with piglets sucking the anterior teats being intermediate (46.1 nmol/L) in response to weaning (P=0.07). Piglets from posterior teat locations had a lesser post-weaning growth check. Incremental reduction in sow contact prior to weaning did not change the performance of piglets based on their position in the teat order.

Table 1. Piglet weight, average daily gain (ADG) and creep consumers for piglets sucking from anterior, middle and posterior teats. Values are mean ± SEM

Day relative to weaning	1	2	7	14	*P* value Day	Teat Order	Day x Teat Order
			Body weight (kg)				
Anterior	7.7 (0.1)[a]	7.9 (0.1)[a]	8.5 (0.1)[a]	11.0 (0.1)[a]	<0.001	<0.001	<0.001
Middle	7.3 (0.2)[b]	7.6 (0.2)[b]	8.4 (0.2)[ab]	10.7 (0.2)[b]			
Posterior	7.1 (0.2)[b]	7.4 (0.2)[b]	8.2 (0.2)[b]	10.5 (0.2)[b]			
			ADG (g)				
Anterior	–53 (23)[a]	–58 (25)[a]	88 (23)[a]	299 (23)	<0.001	NS	<0.05
Middle	22 (24)[b]	93 (25)[b]	157 (21)[b]	300 (23)			
Posterior	11 (25)[b]	81 (27)[b]	158 (25)[b]	322 (25)			
			Creep consumers (%)				
Anterior	27.3 (3.9)	44.2 (4.1)[a]	99.1 (4.2)	98.4 (4.7)	<0.001	<0.10	<0.05
Middle	39.4 (4.1)	55.0 (4.2)[b]	98.9 (4.3)	98.2 (4.8)			
Posterior	33.1 (4.2)	63.9 (4.5)[c]	99.4 (4.5)	99.6 (4.9)			

[abc]Means in a column (within parameter) not having the same superscript are significantly different.

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Neonatal split suckling has no impact on pre- and post-weaning piglet growth

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Split suckling (SS) is a management technique used when there is a risk that neonatal piglets will not consume adequate amounts of colostrum. The technique involves reducing competition at the udder by removing the larger first-born piglets, thus allowing smaller piglets' better access to colostrum during and shortly after parturition. Whilst there is evidence that SS improved colostrum ingestion and, subsequently, piglet survival (Vallet 2013), the effects on growth remain to be elucidated. It was hypothesised that split suckling will improve the growth performance of piglets before and after weaning under commercial conditions.

The experiment was conducted at a commercial piggery using parity 0–7 litters (n = 423). Each litter was assigned to one of the following three treatments (n = 141 litters per treatment): control (no SS); rotational (half litter SS hourly for 4 hours, so that each half received 2x1 hour suckling opportunities); or SSam (separation of the largest piglets in a litter, allowing the smallest to suckle for 2 hours in the morning). Prior to fostering on d 0, piglets were tagged and weighed, after which the SS treatments were applied. On d 1, a blood sample was taken from four piglets (two heaviest and two lightest piglets) and used for the estimation of colostrum ingestion (immunocrit technique; quantification of IgG in serum; Vallet *et al*. 2013). Individual piglets were weighed on d 0 and d 21 relative to farrowing. At weaning, piglets were weaned into treatment group pens (n = 29 pens/treatment; n = 35 pigs/pen), and pen weights were taken on d 0, 10 and 35 relative to weaning. Pre-weaning traits were analysed with a generalised linear mixed model (SAS®; USA), with birth sow and rearing sow fitted as a random effect. Fixed effects included sex, litter size, sow parity, and SS treatment after adjustment for birth weight. Post-weaning fixed effects included replicate, sex and adjustment for initial pen weight.

During the pre-weaning stage, there was a negative effect of litter size on average daily gain (ADG; $P < 0.001$). Piglets from gilt litters gained less weight than those from sow litters (Table 1; $P < 0.001$). There was no effect of SS treatment on the growth of piglets before or after weaning (Table 1; $P > 0.05$). The SSam piglets tended to grow faster from d 10-35 and 0-35 after weaning, than rotational SS piglets but not from control piglets. Colostrum ingestion (immunocrit) was not affected by treatment or correlated with pre-weaning ADG ($r = 0.12$ $P > 0.05$). Under commercial conditions, SS neonatal piglets failed to increase the levels of immunoglobulins unlike those previously reported by Vallet *et al*. (2013) and hence, piglet ADG both prior to and following weaning was unaffected.

Table 1. Effects of rotational and SSam split suckling and sow parity on the average daily gain of piglets pre- and post-weaning. Values are mean (± SEM)

	Control[A]	Rotational	SSam	*P* value	Gilt	Sow	*P* value
Immunocrit (proportion)	0.14 (0.0)	0.13 (0.0)	0.13 (0.0)	NS[B]	0.12 (0.0)[a]	0.15 (0.0)[b]	<0.0001
Pre-weaning ADG (g)							
Days 0–21	225 (6.0)	219 (5.8)	220 (5.4)	NS	193 (4.4)[a]	225 (3.4)[b]	<0.0001
Post-weaning ADG (g)							
Days 0–10	222 (5.2)	227 (5.1)	228 (5.3)	NS	209 (4.9)[a]	258 (3.8)[b]	<0.0001
Days 10–35	551 (6.8)	534 (6.7)	554 (6.9)	0.055	543 (6.6)[a]	563 (5.1)[b]	<0.0001
Days 0–35	458 (5.3)	447 (5.2)	462 (5.4)	0.097	447 (4.9)[a]	478 (3.8)[b]	<0.0001

[A]Refer to text for treatment details. [B]Not significant ($P > 0.10$). [a,b]Means in a row within a main effect not having the same superscript are significantly different.

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Evaluation of sow and litter performance with addition of a bio-surfactant to lactation diets

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Sow performance in lactation is a key driver of productivity in commercial pork production. Lactation diets are formulated with high energy [>14 MJ digestible energy (DE)/kg)] and high fat (>8%) components in an attempt to meet the nutritional demands of lactation. Improved utilisation of the ingested energy may assist in improving milk production, minimising body condition loss and maximising subsequent reproductive performance (Whitney 2012). The emulsification process that assists in the digestion of dietary fat may be improved with the inclusion of a natural bio-surfactant such as Lysoforte® (Kemin; Killara NSW). This study tested the hypothesis that the inclusion of Lysoforte® in high fat (6.6% added fat) lactation diets would improve litter weaning weight and help maintain the body condition of sows between farrowing and weaning.

A total of 281 mixed parity sows [Large White x Landrace, PrimeGro™ Genetics, average parity 2.7 ± 0.07 (mean ± SE)] was selected at 15 weeks of gestation and allocated by parity and P2 backfat to one of two dietary treatments: control lactation diet (14.0 MJ digestible energy (DE)/kg, 6.6% added fat and 8.7 g standardised ileal digestible lysine/kg); and control lactation diet plus 0.1% Lysoforte®. All animals were fed a common pre-farrowing diet at 2.5 kg/d from entry to farrowing. After farrowing, sows were fed the allocated treatment diet on a step-up program for 4 days and then were fed *ad libitum*. Sow feed intake was recorded daily. Sow liveweight (LW) and P2 backfat were measured on entry to the farrowing house and the day of weaning (26.6 ± 0.15 days of lactation). Litter weight and size were recorded after cross fostering and again at weaning. The impacts of dietary treatment and parity were tested using two-way ANOVA with the sow (litter) as the experimental unit (Genstat, 15th Edition; UK).

Inclusion of Lysoforte® did not improve piglet weaning weight with treatments A and B averaging 7.57 kg and 7.44 kg, respectively ($P = 0.73$), nor was there a difference in the number of piglets weaned ($P = 0.60$). Piglet average daily gain (ADG) increased as sow parity increased ($P < 0.001$). However the effect of treatment on ADG tended to be variable between parities ($P = 0.087$). Parity had a significant influence on the P2 backfat response to dietary treatment ($P = 0.033$), with parity 1 and 2 sows displaying reduced P2 backfat loss with Lysoforte® inclusion (Fig. 1A). There was no difference between treatments in P2 backfat loss over all parities ($P = 0.68$). Sows offered the Lysoforte® diet tended to have a reduced loss in LW from entry to weaning ($P = 0.077$) (Fig. 1B) however, the interaction between treatment and parity was not significant ($P = 0.51$). Average daily feed intake for sows offered Lysoforte® was higher than the controls ($P = 0.037$), and there was no interaction between treatment and parity ($P = 0.40$).

Lysoforte® inclusion in diets for lactating sows had an effect on the maintenance of sow body condition, particularly in parity 1 sows whose change in P2 backfat loss differed by 3.8 mm between treatments. There was no difference between treatments in wean to oestrus interval ($P = 0.91$). The outcomes from this study suggest Lysoforte® inclusion in lactation diets may increase voluntary feed intake and minimise body condition loss in younger parity sows with minimal effect on piglet weaning weights.

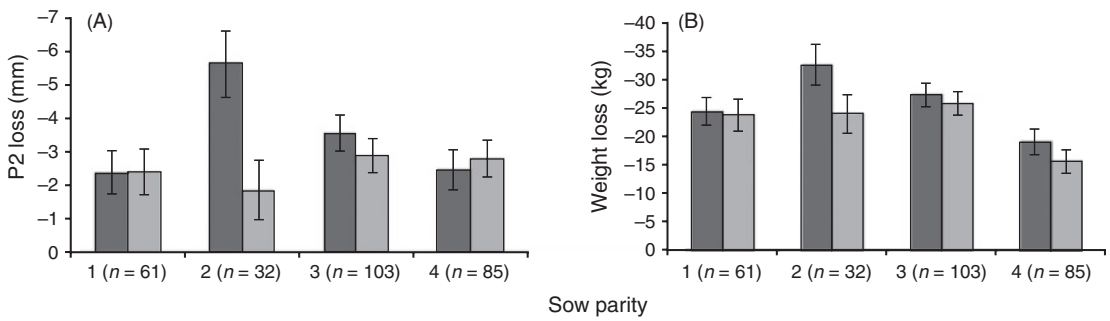

Fig. 1. The effect of Lysoforte® (▭) fed during lactation vs a control diet (■) on the change in sow P2 backfat loss (A) and LW (B).

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Saliva cortisol and heart rate measurements of nurse sows during lactation compared to control sows

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Nurse sows are used in piggeries with hyper-prolific sows to manage large litters. It is not known if nurse sows experience prolonged stress by having to stay in farrowing crates beyond the normal weaning time (Baxter *et al*. 2013). Our aim was to quantify long-term saliva cortisol as a measurement of stress of nurse sows compared to sows weaning their piglets at d 25 of lactation (control) and compare heart rate responses to d 20. A method called 'cascade fostering' using two lactating sows is normally performed in Denmark. In this method, the first nurse sow (N1) has her own piglets removed after a week and receives surplus newborn piglets that she fosters until weaning. The second nurse sow (N2) weans her own litter after 21 days and receives the litter from N1, which she rears until weaning. It was hypothesised that N1 and 2 sows would have increased saliva cortisol throughout lactation compared to control sows.

In total, 60 sows (n = 20) were randomly allocated to become a control, N1 or N2 sow in the same section over two time periods (summer 2013 and winter 2013/2014). Saliva was collected on d 6, 13, 20 and 24 at 1000 h, 1300 h and 1600 h for all sows and pooled on a daily basis for analysis. Additional saliva samples were taken on d 31 for N1 and N2 and d 38 for N2 for long-term measurements. Saliva samples were analysed for cortisol using a Salivary Cortisol kit (Salimetrics, UK). Pulse belts (model RS800CX, Polar Electro Oy, Finland) were placed around the chest of the sow from Monday to Wednesday to measure heart rate. Recordings were measured from the morning and continued to late afternoon. Specific time points (1000 h, 1300 h, 1600 h and 1900 h) were chosen to compare mean heart rate (HR), in 5 min intervals. Data were analysed using PROC MIXED (SAS®; USA). Cortisol data was not normally distributed and therefore logarithmically transformed before analysis. Results presented here are arithmetic back-transformed data.

Results showed that there was no effect of treatment on saliva cortisol, but an effect of day ($P < 0.001$) with saliva cortisol declining throughout lactation (Fig. 1*a*). The N1 sows tended to have lower cortisol values (8.3 nmol/l) on d 31 than on d 24 (11.5 nmol/l; $P = 0.08$), and N2 sows had lower cortisol values on d 38 (7.4 nmol/l) and on d 31 (7.5 nmol/l) than on d 24 (11.1 nmol/l; $P < 0.05$). Heart rate values increased throughout lactation ($P < 0.001$) but remained unaffected by treatment (Fig. 1*b*).

These data indicated that saliva cortisol levels declined throughout lactation with no differences in saliva cortisol levels between control and NURSE sows. Heart rate increased throughout lactation to d 20 probably due to the increase in milk production. Salivary cortisol levels as indicators of stress, suggested no additional long-term effects of being selected as a nurse sow.

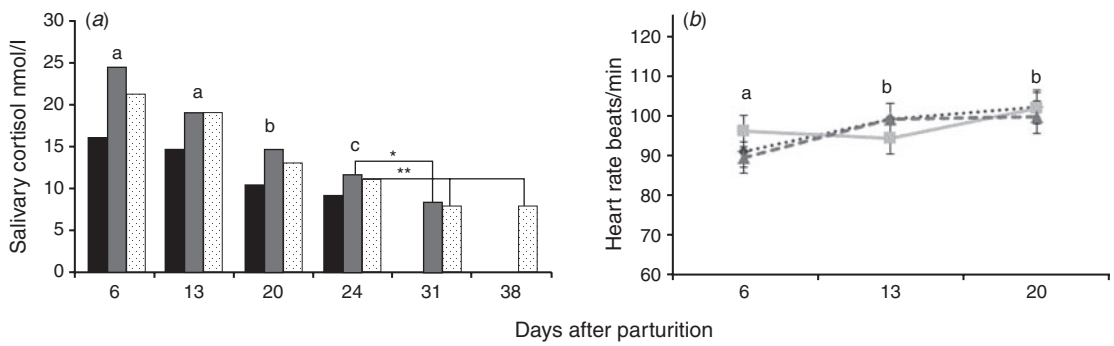

Fig. 1. (*a*) Pooled salivary cortisol for control (black), N1 (grey) and N2 (dotted) sows presented as arithmetic back-transformed values and sows differing significantly on day *($P < 0.05$), **($P < 0.01$) when compared to themselves. (*b*) Mean heart rate (± pooled SE) during lactation, for control (◆), N1 (■) and N2 (▲) sows. Letters denote effect of time. [a,b]Significant effect of time ($P < 0.05$).

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Nitrous oxide for piglet gas euthanasia

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The neonatal stage is a critical time in the life of a pig, when they are prone to become sick or weak. This is the stage at which most euthanasia procedures are required for pigs. The search for humane methods to euthanize piglets is critical to address public concern that current methods are not optimal. Blunt force trauma is humane but aesthetically unpleasant. Carbon dioxide (CO_2) gas is used but aversive to piglets (Rault *et al*. 2013). This previous research suggested that nitrous oxide (N_2O; 'laughing gas') is less aversive than CO_2 for piglets. This research sought to: evaluate the aversiveness of inhaling N_2O using an approach-avoidance test relying on the piglet's perspective; and validate its humaneness to induce loss of consciousness by electroencephalography (EEG), a neurobiological technique that provides insight into brain processes and state of consciousness (Murrell and Johnson 2006). It was hypothesised that exposure to N_2O is less aversive to piglets than exposure to CO_2.

The gas mixtures tested were: N_2O and air (90%:10%; '90N'); N_2O, oxygen and air (60%:30%:10%; '60N'); and, for experiments 2 and 3, CO_2 and air (90%:10%; '90C') as a control. All piglets were the progeny of Yorkshire × Landrace dams bred to Duroc × Hampshire sires. Data were analysed using mixed models or Kruskal-Wallis tests (SAS®; USA). Experiment 1 allowed 16, 2-week-old female piglets to walk freely between one chamber filled with air and another prefilled with 60N or 90N, using a previously validated behavioural paradigm (Rault *et al*. 2013). All piglets exposed to 60N finished the 10 min test whereas all piglets exposed to 90N had to be removed within 5 min (mean ± SE: 255.4 ± 65.5 sec) because they fell recumbent and non-responsive and then started to flail. Hence, N_2O could be used as a sedative agent for piglets. Experiment 2 performed the same test using 24 female piglets except the gas chamber held N_2O prefilled at 25%, 50%, or 75%; or CO_2 prefilled at 7%, 14%, or 21%. The test was shorter at higher concentrations ($P < 0.001$). Time spent disoriented was greater in the middle concentration gradients ($P < 0.002$). Flailing behaviour (e.g. erratic movements, jumps) tended to correlate with increasing concentrations of CO_2 ($r = 0.40$, $P = 0.06$), but not N_2O ($r = 0.28$, $P = 0.19$). Overall, these data supported our hypothesis that exposure to N_2O is less aversive to piglets than exposure to CO_2. Experiment 3 used the minimal anaesthesia model (Murrell and Johnson 2006) on 15, 10-day-old male piglets to record EEG. Both 90N and 90C induced isoelectric EEG (Table 1), equivalent to brain death, but not 60N over 15 min, which then had to be euthanised using 90C for ethical reasons.

The EEG results supported the behavioural findings by demonstrating differences in terms of effects on the brain. This means that the behavioural changes seen reflect differences in the piglet's perceptive experience of the treatments rather than, for example, alterations in motor function. The EEG data strengthen the link between the behavioural results and the implications for animal welfare, namely that N_2O is less aversive than CO_2, taking 13 s longer to induce full loss of consciousness in our settings. This project also demonstrated that 90% N_2O can kill piglets.

Table 1. Latency (sec) and range of latency (sec; in brackets) to the onset of transitional and isoelectric EEG in Experiment 3. Values are means ± SE

Variables	90% N_2O	90% CO_2	90% CO_2 after exposure to 60% N_2O	*P* value
Transitional EEG	62.10 ± 4.80[y]	45.49 ± 5.23	41.82 ± 5.18[x]	0.07
	[40–87]	[39–54]	[15–51]	
Isoelectric EEG	71.49 ± 7.47	58.66 ± 8.14	48.83 ± 8.07	0.19
	[55–94]	[46–68]	[33–73]	

[x,y]Means in a row not having the same superscript show a trend for being significantly different ($P < 0.10$).

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Two different strategies for housing gilts after mating did not affect the proportion of gilts culled

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Gilts and young sows often have the lowest rank in a group of sows mainly because rank to a certain extent is defined by bodyweight (Hoy *et al*. 2009). It is often difficult for animals with low social status to access the electronic sow feeder because they are fed later than sows with a higher status and tend to be displaced from the feeder queue more often. Sows with low social status were also observed less often in the lying area (O'Connell *et al*. 2003). At the same time, gilts and young sows are at risk of being culled from the herd before their economic potential is fully exploited (Anil *et al*. 2005). The aim of this study was to compare two different strategies for housing gilts after mating on the proportion of gilts culled throughout the first gestation and lactation period. The two strategies were: mated gilts in dynamic groups with gilts mated in the previous weeks versus mated gilts in stable groups with sows.

A total of 1355 gilts (Landrace × Yorkshire) in two different herds with group housing and electronic sow feeding were included in the study. Both herds had approximately 1100 sows. The gilts were on average 275 days old when they were introduced to either a dynamic groups with gilts mated in the previous weeks or stable groups with sows. In both herds the gilts were introduced to the pen in groups of 12–15 gilts at 4 weeks after mating. All gilts were trained in using the feeding station before mating. The total area per gilt was the same in both herds and pen types (1.7–1.9 m^2 per gilt).

Data was collected on farm by a technician from the Danish Pig Research Centre (PRC). On three occasions all gilts were inspected for lameness; just before mating, two weeks after grouping in the gestations unit, and just before moving to the farrowing unit. Lameness was assessed using the following scale: no lameness, slightly lame, severely lame and not able to stand. Each week at 9 am a scan of the pens was made and it was recorded if the gilts were resting in the lying area or in the activity area. The proportion of gilts culled throughout the first gestation and lactation period was collected. Further, the individual feed intake was measured by collecting data from the feeding stations (Skiold Datamix).

The proportion of gilts culled throughout the first gestation and lactation period and the proportion of gilts with lameness was analysed using a Fisher's Exact Test. Lying behaviour and individual feed intake was analysed by mixed-model (SAS®; USA) with weeks in the pen, number of gilts in the pen and group as fixed factors and pen as random factor. The herds were analysed separately.

There was no significant difference regarding the proportion of gilts culled throughout the first gestation and lactation period (Table 1). Also there was no significant difference regarding the proportion of gilts with lameness between the two housing strategies (herd 1: $P = 0.62$; herd 2: $P = 0.18$). Further, the strategies did not show any significant differences in regard to feed intake (herd 1: $P = 0.90$; herd 2: $P = 0.14$).

In both herds the proportion of gilts in stable groups lying in the activity area was higher compared with gilts in dynamic groups ($P < 0.001$). The explanation for this finding could be that the pens with stable groups were smaller and had fewer "lying nests" than the pens with dynamic groups. In conclusion, group composition did not influence either the proportion of gilts culled, lameness or feed intake. A further effort to reduce the proportion of culled gilts is needed and it could be relevant to focus on socialisation on young gilts, pen design and new strategies for mixing gilts and sows.

Table 1. Proportion of gilts culled throughout the first gestation and lactation period (%)

Strategy	Herd 1	Herd 2
Group size (dynamic/stable group)	100/55	180/90
Gilts in dynamic group with gilts	12	13
Gilts in stable groups with sows	15	11
P value	0.29	0.56

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Validity of modified methods to assess three welfare indices for use in on-farm pig welfare monitoring

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The Australian Pork Industry Quality Assurance Program (APIQ✓®) requires producers to be annually audited against a set of standards and performance indicators, but does not provide them with the opportunity to monitor and (or) benchmark the welfare status of their animals over time. Although the assessment of animal welfare at farm level remains an on-going challenge, the literature demonstrates the opportunity to develop a practical welfare assessment tool, using valid and reliable animal-based welfare indices (Winckler *et al*. 2003; Knierim and Winckler 2009). Whilst extensively employed within the literature and more recently within on-farm assessment schemes, the validity of animal-based welfare indices modified from experimental settings for practical use in on-farm welfare monitoring has not been robustly examined. This study aimed to examine, in a commercial setting, the validity and intra- and inter-observer reliability of modified methodologies (M) of three commonly used animal-based pig welfare indices: body condition score (BCS), lameness score (LS) and injury score (IS).

To improve on-farm practicality and reduce observer subjectivity, validated methods of assessment (V) for the three welfare indices were simplified to create the modified methodologies (BCS: Patience and Thacker 1989; LS: Karlen *et al*. 2007; IS: De Koning 1985). For example, BCS was modified from a 5-point visual and tactile assessment of the pig's condition performed outside of the group-pen, to a 3-point visual assessment of the pig conducted within the group-pen. The validity and reliability of the M measures of BCS, LS and IS were investigated in group-housed sows and grower pigs at a large Australian commercial piggery, over a 6-week period. Four trained observers sampled 240 group-housed pigs over six 2-day periods (120 sows in weeks 1–3 and 120 grower pigs in weeks 4–6); each observer assessed 20 focal animals for BCS, LS and IS on d 1 using both the M and V methodologies, and 40 focal animals using the M methodologies on d 2. Whilst not blind to the group on d 2, observers were blind to the individual animal.

The validity of the M methodologies was investigated using Spearman's rho correlations (ρ) to examine the strength of the relationship between the assessment scores from the M and V measures, and Kappa statistics (κ) to determine the level of agreement between the two measures. The reliability of the M methodologies was investigated using a test-retest assessment that used ρ to examine the similarity between measures collected on an animal at two different time points (intra-observer), and κ to investigate the agreement between measures taken on an animal by multiple assessors (inter-observer).

Moderate to substantial levels of agreement (κ = 0.61 to 1.00) confirmed the intra- and inter-observer reliability of the M methodologies in group-housed sows and grower pigs. However, validity testing only indicated a moderate relationship (ρ = 0.30 to 0.49) with slight to fair levels of agreement (κ = 0.21 to 0.60) between the M and V methodologies. Given that the V indices underwent only minor modification, greater correlation and agreement between the measures were expected. These results may be due to the homogeneity of the data due to a lack of variation in the condition of the animals sampled, rather than a genuine lack of validity of the M methodologies. The lack of variation in the sample means that the minor inconsistencies that are commonly found between observers/observations are enough to substantially reduce the level of agreement between the measures. Whilst the correlations and level of agreement between the measures were not as strong as expected, the current findings do not refute the validity of the M methodologies as on-farm measures of BCS, LS and IS in group-housed sows and grower pigs.

Given these findings, further testing in populations with greater variation is required to confirm the validity of the M methodologies. Confirming the validity of these measures is vital if they are to be used effectively by producers to monitor and benchmark pig welfare over time.

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A study of agonistic strategies after mixing in group housed sows

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Group-housing systems offer sows more physical space, and the opportunity for exploration and social interaction. However, sows in groups may have to adopt different agonistic strategies to deal with the social environment, and research has shown distinct differences between these agonistic strategies in associated injuries and stress in sows (Mendl *et al*. 1992; Verdon *et al*. 2013). The aim of the present study was to examine some behavioural strategies that sows may adopt to cope with aggression at mixing. It was hypothesised that submissive sows will have less access to feed, will perform less aggressive and more avoidance behaviours, and will have less fresh skin injuries compared with other sows.

This study was conducted at a commercial piggery in Victoria, Australia. Over three replicates (one replicate per week), 155 recently-weaned sows (parity1 to 8) were allocated to one of two mixing pens. Pen 1 housed an average of 27 sows per week (range 24–30 sows) at an average space allowance of 2.7 m^2/sow (range 2.4–3.0 m^2/sow), whereas Pen 2 housed an average of 24 sows per week (range 21–27 sows) at an average space allowance of 2.7 m^2/sow (range 2.4–3.1 m^2/sow).

During each replicate, behavioural observations were made on 10 focal animals randomly selected from each pen. All behavioural observations were made by a single observer using video records. Focal sows were observed for a total of 45 min in the first, third and fifth hours after mixing on d 1 and for 45 min after each feed drop at d 2 (0730 and 1300 h). Agonistic behaviours (threat, parallel pressing, head and body knocking, bites, fights, submission and displacements) were continuously observed for a total of 45 min for each observation period. All aggressive interactions delivered and received by the focal sows were recorded to calculate an 'aggression index' using the formula: aggression delivered/(aggression delivered + aggression received) (after Verdon *et al*. 2013). Sows were then classified into three aggression categories according to the calculated ratio [dominant (D), subdominant (SD) and submissive (S)]. In addition, 1-min point sampling was used to record time spent feeding (TF) and resting (TR) and the area of the pen where the sows were located. Areas of the pen that provided food and bedding were classified as preferred resting areas, and less preferred areas were defined as those with no food and bedding materials. The TF and TR were analysed as a proportion over the total observation time (225 min), and those sows culled for lameness after the experiment were not included in the statistical analysis for TR. Skin injuries were measured in focal sows using the method described by Karlen *et al*. (2007), at 1500 h on d 2. Data were appropriately transformed when the assumption of normality was not fulfilled. One-way ANOVA was used to compare differences in agonistic behaviour, space utilisation and skin injuries of D, SD and S sows. Multiple comparisons between means were performed using the least significant difference test (SAS®; USA).

Significant differences existed between the aggressive interactions delivered ($P < 0.001$), the submissions performed ($P = 0.015$) and the aggression received ($P = 0.016$) between the three aggression categories. The D and SD individuals delivered significantly more aggression than S sows, the D sows performed less submission, and the SD sows received more aggression than the other two categories. The SD sows also presented more skin injuries (old and fresh) ($P = 0.009$) compared with the D sows. Significant differences also existed in relation to space utilisation: S animals spent less time feeding ($P = 0.031$) and more time resting in less preferred areas of the pen ($P = 0.021$) than D sows.

In conclusion, each aggression category appeared to have costs and benefits with regard to aggression received and delivered, injury, and resource access. The D and SD sows delivered similar levels of aggression, but SD were more persistent in displaying aggressive behaviour regardless of defeat, and thus had higher numbers of skin injuries (old and fresh) compared with D sows. In addition, S sows may have experienced difficulties in gaining access to resources such as feed and preferred lying areas of the pen. Further research on features of a mixing pen, such as provision of a barrier and increased floor space, is required to examine opportunities to minimise aggression and safeguard the vulnerable individuals.

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Potential for use of physiological and physical measurements to monitor sow muscle catabolism during lactation

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Protein loss from skeletal muscle catabolism during lactation, whether a result of reduced feed intake during lactation or high demand from suckling piglets, appears to have the largest influence on subsequent reproductive performance (Clowes *et al.* 2003). The capacity to routinely identify significant muscle catabolism during lactation could therefore be a very useful management tool if productivity is to be optimised. Apart from physical measures of catabolism such as loin muscle depth (LM) and backfat (BF) loss, metabolic products of muscle catabolism such as creatinine, (Crea) and 3-methylhistidine (3MH) may hold potential. The aim of this experiment was to restrict feed intake in lactating sows to promote muscle catabolism, and then measure plasma (Crea, 3MH), whole blood (Crea) and physical (LM, BF) parameters that may reflect this catabolism. We hypothesised that plasma Crea and 3MH and whole blood Crea would increase with decreasing sow feed intake while LM and BF would decrease.

Four levels of feeding were offered to sows in lactation to induce muscle catabolism, with each treatment being composed of an equal mix of parity two and three sows (n = 10 per treatment). Sows were fed a commercial diet [14.5 MJ digestible energy (DE)/kg, 0.55 g standardised ileal digestible lysine/MJ DE] with feeding levels increased to achieve a plateau intake 10 days after farrowing. Control sows were fed to achieve 9 kg/d, R1 sows were restricted to 8 kg/d, R2 sows were restricted to 7 kg/d, and R3 sows were restricted to a peak intake of 6 kg/d. On d 0, 14 and 20 (weaning), BF and LM were measured by ultrasound 7 cm from the midline, at the head of the last rib. Sows were bled on these same days and plasma was analysed for levels of circulating 3MH (plasma amino acid quantitation) and Crea (general chemistry). Whole blood Crea was also measured using a hand-held Nova StatSensor Creatinine Meter (RHCG NSW, Australia). Data were analysed using the GLM procedure and a simple linear regression analysis (Genstat, 15th Edition; UK).

Measurement of whole blood Crea using a hand-held meter revealed a significant difference ($P < 0.05$) between sows fed 9 kg/d and sows fed 8 kg or less per day (Table 1). There were also significant differences in BF between treatments but this did not reflect the treatments and may have been influenced by the initial body condition of the sows. Correlations also existed between whole blood Crea and measures of BF (r = 0.22, $P = 0.02$; n = 39) and LM (r = 0.26, $P = 0.006$; n = 39). Plasma 3MH and Crea and LM were not responsive to feeding level. An increase in whole blood Crea is consistent with the hypothesis, and the significant correlation with LM depth suggests potential as a measure of muscle catabolism. It should be noted, however, that as a sow fails to meet her energy requirement through feed, reduced water intake might be concurrently reducing kidney function causing Crea levels to rise (Butani *et al.* 2002). As a consequence, further research is required to ascertain whether whole blood Crea reflects muscle loss or reduced water intake. Regardless, it appears whole blood Crea measured using a hand-held meter has potential as a useful management tool for lactating sows either as an indicator of muscle catabolism or as a measure of sub-optimal feed and water intake.

Table 1. Mean levels of plasma 3-methylhistidine (3MH) and creatinine (Crea; μmol/L), whole blood Crea (μmol/L), backfat (BF) depth (mm) and loin muscle (LM) following graded levels of feed restriction in a 20 d lactation

Measurement	3MH	Crea (Plasma)	Crea (Whole)	BF	LM
		Treatment			
Control (9kg/d)	45.4	178.5	98.5[b]	17.62[b]	48.12
R1 (8kg/d)	43.8	187.1	113.8[a]	21.26[a]	51.11
R2 (7kg/d)	48.8	176.6	124.9[a]	14.92[b]	47.60
R3 (6kg/d)	45.5	170.1	138.0[a]	17.12[b]	48.74
SED[A]	4.82	17.48	13.54	1.93	2.14
P value	0.756	0.783	0.029	0.021	0.382

[A]SED, standard error of difference. [a,b]Means in a column not having the same superscript are significantly different.

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A 'two-stage' farrowing and lactation system: assessing the impacts of group lactation on the incidence of lactational oestrus and reproductive performance

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Piglet removal for 16 hours per day from d 16 after parturition onwards, combined with fence-line boar exposure, stimulated a high proportion of crated sows to exhibit a synchronous ovulatory response during lactation (Downing *et al*. 2011). However, it is still unclear whether the natural separation of sows and piglets in a group housing system would induce a similar response (van Nieuwamerongen *et al*. 2014). This study tested the hypothesis that an increase in follicular development and spontaneous oestrus would be observed amongst group-housed sows during lactation compared to crated- and PigSAFE-housed sows.

Mixed-parity sows (Large White x Landrace, PrimeGro™ Genetics; n = 160) over six time replicates were allocated to one of four treatment groups 14 days before weaning: Farrowing crates (FC): sows housed in farrowing crates until weaning; GL_{FC}: sows housed in farrowing crates then moved into group lactation 14 days before weaning (six sows/replication); PigSAFE (PS): sows housed in the PigSAFE loose farrowing system until weaning; and GL_{PS}: sows housed in the PigSAFE system then moved to group lactation 14 days before weaning (six sows/replication). In the 14 days before weaning, 24-hour daily video footage was recorded over the two group lactation pens. Signs of sexual behaviour, including mounting and ano-genital sniffing, were recorded. Ovarian follicle development was measured once 7 days before weaning and once at weaning, using rectal ultrasound (focal sows, n = 36/treatment). Blood was collected from focal sows 4 days after weaning for analysis of progesterone concentration. Sows were mated on their first return to oestrus after weaning. Data were analysed using Chi-square and post-hoc Bonferroni (IBM SPSS, Version 21.0; USA).

The average WRI showed a weak tendency ($P = 0.155$) to be shorter in FC sows, and more FC sows were mated within 4 days of weaning ($P = 0.049$) (Table 1). The progesterone concentration data, together with the WRI, indicated no statistical difference ($P > 0.05$) that sows in PigSAFE and group lactation systems experienced a higher incidence of lactational oestrus and ovulation compared to farrowing crates. The combination of oestrus behaviour signs, ovarian follicular size, increased WRI and progesterone concentrations suggest that between 3.1 and 20.7% of sows across all treatments experienced lactational oestrus and perhaps ovulation, possibly as a result of shifting suckling patterns. In a 'two-stage' lactation system, strategies to manage for spontaneous ovulation seem essential and could be achieved by further stimulating the sows through piglet separation and (or) boar exposure, so that the majority of sows can be mated during lactation.

Table 1. Reproductive performance of sows in two-stage lactation systems, crates and PigSAFE. Values are mean ± SD (where indicated)

	FC[A]	GL_{FC}	PS	GL_{PS}	*P* value
WRI[B] (d)	5.2 ± 3.2	8.3 ± 6.6	7.4 ± 6.1	7.6 ± 6.5	0.155
Sows with WRI ≤ 4 d (%)	75.0[a]	56.3[b]	53.1[b]	45.5[b]	0.049
Sows successfully mated post-wean (%)	88.9	88.9	91.4	91.7	0.711
Subsequent no. piglets born alive	12.1 ± 3.1	12.0 ± 2.8	12.8 ± 2.4	11.4 ± 1.5	0.443
Counts of oestrus behaviour (counts/treatment – cumulative total)	–	45	–	42	0.961
Sows with large follicles ≥4 mm (pre-weaning) + WRI >7 d (%)	3.1	12.5	6.3	9.1	0.732
Sows with WRI >7 d + progesterone concentration >2.5 ng/mL (%)	10.3	16.1	19.4	20.7	0.674

[A]Refer to text for treatment details. [B]WRI, weaning to re-mating interval (number of days after weaning until the sow showed signs of standing oestrus and was mated). [a,b]Means in a row not having the same superscript are significantly different.

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Increasing dietary valine-to-lysine ratio for lactating sows had no effect on litter performance or sow tissue mobilisation

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The correct ratio between lysine (Lys) and other essential amino acids is needed for optimal utilisation of dietary protein. The effects of the dietary valine to lysine ratio (Val:Lys) for lactating sows on litter growth and sow body mobilisation is equivocal (e.g. Richert *et al.* 1996; Gaines *et al.* 2006), however these studies were conducted in sows suckling around 10 piglets. Given the hyperprolificacy of modern sow genotypes with added demands for higher milk production, the hypothesis tested in the current study was that increasing the dietary Val:Lys ratio would improve litter growth and reduce sow tissue mobilisation.

A total of 565 sows (DanBred hybrids) were randomly allocated to one of six diets (n = 93) with analysed total Val:Lys ratios of 83.9, 86.4, 88.0, 90.5, 95.3, and 99.1% [calculated standardised ileal digestible (SID) Val:Lys ratios of 75.8, 79.0, 82.0, 85.0, 91.0, and 97.0%, with 7.1 g SID Lys/kg in all diets] in a complete block design from d 2 post-partum, at which point litters were standardised to 14 piglets per sow. The sows were fed semi-*ad libitum* twice per day until d 10, after which time feeding was increased to three times per day. Sow body weight (BW), backfat (BF) thickness and litter weight were recorded at d 2 and at weaning (d 26). On a random subsample of 12 second parity sows per dietary group, litter weights were recorded weekly. A milk sample was obtained and the BW and BF of sows registered at d 17. Prior to milk sampling the litter was removed from the sow for 45 min, after which an intramuscular injection with 2 mL oxytocin (Orion Pharma, Denmark) was given. Milk samples were analysed for dry matter (DM), lactose, fat, protein and urea. Statistical analysis was performed with the individual sow as the experimental unit (R: Free Software Foundation's GNU General Public License). Milk composition, feed intake, average daily gain (ADG), BW loss and BF loss were analysed in a model testing the effects of Val:Lys, random effect of block and with BW, BF or litter weight at d 2 as a covariate.

Average daily feed intake (6.1 ± 0.7 kg, mean ± SD; $P = 0.23$) of the sows, litter size at weaning (13.0 ± 1.1, $P = 0.23$), ADG of the litter (2.93 ± 0.53 kg; $P = 0.84$; Table 1), and litter weight at standardisation ($P = 0.30$), d 10 ($P = 0.29$), d 17 ($P = 0.06$) and at weaning ($P = 0.73$), was similar among all dietary treatments. The loss of BW and BF from d 108 of gestation to d 2 post-partum (32.7 ± 10.9 kg and 0.9 ± 1.1 mm), from d 2 to weaning (22.1 ± 12.7 kg and 2.9 ± 1.7 mm; Table 1), from d 2 to d 17 (17.9 ± 11.7 kg and 2.6 ± 1.6 mm), and from d 17 to weaning (8.0 ± 7.9 kg and 0.7 ± 1.5 mm), were also all unaffected by the dietary Val:Lys ratio ($P > 0.05$). Milk yield (11.3 ± 1.4 kg/d; $P = 0.49$), and the DM ($P = 0.33$), lactose ($P = 0.05$), protein ($P = 0.90$), fat ($P = 0.37$) and urea ($P = 0.35$) concentrations of milk, were similarly not affected by dietary treatments. In conclusion and contrary to expectations, there was no effect of increasing the total dietary Val:Lys above 83.9% on litter performance and sow body mobilisation.

Table 1. Effect of the calculated dietary valine-to-lysine ratio on the average daily gain (ADG) of the litter and sow body weight and backfat loss during lactation

	Calculated Val:Lys ratio							
	83.9	86.4	88.0	90.5	95.3	99.1	SE	*P* value
ADG of litter (d 2-26) (kg)	2.85	2.93	2.93	2.89	2.88	2.92	0.060	0.84
BW loss (d 2-26) (kg)	22.0	22.8	23.2	20.6	21.4	23.5	1.36	0.21
Backfat loss (d 2-26) (mm)	2.8	3.0	3.0	2.8	2.6	3.1	0.18	0.11

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A 'two-stage' farrowing and lactation system: sow behaviour and injuries

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Loose farrowing systems that meet the biological needs of the sow have been developed (Baxter *et al*. 2011). A 'two-stage group lactation' system, where the sow farrows in either a loose farrowing pen (e.g. PigSAFE system) or farrowing crate and is then moved into a group lactation (GL) system approximately 14 days after farrowing, is being investigated. The PigSAFE loose farrowing system allows visual and physical 'fenceline social contact' between sows which could maintain social bonds between sows and piglets and may reduce aggression and enhance maternal behaviour when mixed into group lactation systems. This experiment tested the hypothesis that sow behaviour and injuries would differ when sows are mixed into group lactation from either farrowing crates or a PigSAFE system.

A total of 360 mixed-parity sows (Large White x Landrace, PrimeGro™ Genetics) were studied over six time replicates. Sows were randomly allocated to one of four treatment groups: 1) Farrowing crates (FC): sows housed in farrowing crates until weaning; 2) GL_{FC}: sows housed in farrowing crates then moved into GL 14 days prior to weaning; 3) PigSAFE (PS): sows housed in the PigSAFE loose farrowing system until weaning; and 4) GL_{PS}: sows housed in the PigSAFE system then moved to GL 14 days prior to weaning. The housing treatments were located in three adjacent buildings with similar ventilation and construction material. The buildings were open-sided with shutters and heating which enabled some temperature control. All sheds were managed by the same stockpeople. Sows had access to an *ad libitum* feeder in the GL pen. The behaviour of the sows in GL_{FC} and GL_{PS} pens was recorded for 4 hours immediately after mixing and the day before weaning (from 0800 h) using HD Sports cameras. Aggressive behaviour (parallel pressing, head knocks and bites) was observed for 1 hour and suckling behaviour for 4 hours during the observation period, with all data recorded by one observer using a scan sampling technique. The time for the sow to first suckle a litter was recorded upon entry to the group lactation pens. Sow skin injury (assumed to be caused by aggression) was assessed on all sows according to Karlen *et al*. (2007) at 13 days before weaning (after mixing in the group lactation pens) and at weaning (25 $\pm$ 2.7 days; mean $\pm$ SD). The injury data were transformed prior to analysis. Univariate GLM analysis (IBM SPSS, Version 21.0; USA) was used to analyse the injury scores using each block of six FC, six PS and GL pens (6 sows/pen) as the experimental unit with replicate as a random factor in the design. An independent two-sided T-test was used for analysis of the behaviour data.

Fresh skin injuries were lower in sows housed in the FC and PS systems compared to either of the GL systems (Table 1). There was no difference in skin injuries or aggression between sows mixed into GL from either the FC or PS systems. Sows that had previously been housed in the PS pen showed a shorter latency to first suckle after mixing into GL ($P < 0.05$) compared to sows from the FC (35 vs 53 min. $\pm$ 5.4 min; mean $\pm$ SEM, GL_{PS} and GL_{FC} treatments, respectively). This suggests that aspects of sow behaviour immediately after mixing into GL can differ depending on the farrowing environment. Further research is warranted to fully assess the welfare of sows and piglets in GL systems, particularly the impact of age at mixing.

Table 1. Average sow skin injuries in different housing treatments[B]

	FC[A]	GL_{FC}	PS	GL_{PS}	SEM[C]	*P* value
13 days prior to weaning	0.50[a] (–0.30)	5.11[b] (0.71)	0.93[a] (–0.03)	3.52[b] (0.55)	0.543	<0.001
At weaning	1.74 (0.24)	1.41 (0.15)	1.04 (0.02)	1.33 (0.12)	0.228	0.601

[A]Refer to text for treatment details. [B]Values are presented as back-transformed means (transformed means presented in parenthesis). [C]SEM standard error of the mean. [a,b]Means within a row not having the same superscript are significantly different.

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Ranking for fight lesion scores is not consistent over time

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Aggressive behaviours such as fighting compromise the welfare of group-housed gilts and sows. The extent of lesions that result from fighting is a simple measure of the aggression received by individual animals within a group, and is a potential measure of individual behaviour (Turner *et al*. 2006). However, group dynamics and individual behaviour can change over time. The hypothesis investigated in this study was that for individual gilts regrouped within parity over time, post-selection and pre-farrowing lesion scores might not be a consistent indicator of aggressive behaviours.

A subset of gilts (n = 3,238) selected at 170 ± 3.3 (mean ± SD) days of age, housed in temporary groups of 20–40 gilts/pen, were scored (0–3) for fight lesions on each quadrant of the body separately, 24 hours after mixing post-selection. Each scoring increment represented an additional five lesions. Total scores for the anterior or posterior regions (0–6) or over the whole body (0–12) were accumulated. Non-zero values were re-scaled to create 0–3 score categories, representing a range of 0–30+ lesions for anterior and posterior scores, or 0–60+ lesions over the whole body. Gilts were subsequently allocated to single parity groups of up to 10 sows post-mating for their gestation period, and rescored (n = 1,929 at 342 ± 15.1 days of age) for fight lesions (0–3) over the whole body upon transfer to farrowing accommodation using the same scoring increment. The range in lesion count across scores was therefore relatively lower pre-farrowing, from 0–15+. Sows removed from groups prior to transfer were not scored pre-farrowing. Associations between anterior and posterior lesion score categories recorded post-selection, and between post-selection and pre-farrowing lesion scores, were examined using a Chi-square test.

With regard to gilts, 5.5% had no lesions 24 hours after mixing whereas 28.7% of sows had no lesions pre-farrowing, indicating a large change in social dynamics between these time points and the visible evidence of fighting amongst sows (Table 1). A reduction in lesion scores was expected, since the development of a social hierarchy within a stable gestating group should reduce measures of antagonistic interactions between sows (Arey 1999). However, the presence of lesions pre-farrowing suggests that some source(s) of motivation for agonistic behaviour within gestation groups (e.g. competition for food) were present. The percentage of sows with high lesion scores post-selection was higher for anterior compared to posterior scores (15.3% vs 4.1%), indicating most fight injuries were received on the front of the sow. There was an association ($P < 0.0001$) between anterior and posterior scores. Sows without anterior lesions were unlikely to have posterior lesions (<3% of sows) and sows actively engaged in fighting (high anterior scores) also had high posterior lesion scores. In contrast, there was no association ($P > 0.05$) between lesion scores of gilts recorded post-selection with their lesion scores pre-farrowing. Engagement in fighting post-mixing as gilts was not a predictor of individual engagement in fighting within a new group at a later stage. This is consistent with the results of Tönepöhl *et al*. (2013), who found no relationship between a sow's behaviour for initiating aggression with their own lesion scores recorded 10 weeks later.

Table 1. The percentage distribution of anterior and posterior lesion score groups recorded post-selection (n = 3,238) and their associations with a pre-farrowing score of 0 (from n = 1,595)

		Score				
		0	1	2	3	Pre-far 0
Anterior (%)		8.3	37.6	38.9	15.3	28.7
Posterior (%)		15.7	52.3	27.9	4.1	28.7
		Posterior score (%)				
		0	1	2	3	Pre-far 0
Anterior	0	5.5	2.7	0.15	0.0	25.4
Score	1	9.3	24.7	3.6	0.0	29.7
(%)	2	0.9	21.4	15.9	0.8	27.4
	3	0.03	3.6	8.3	3.4	28.8
	Pre-far 0	28.6	27.5	29.6	29.3	

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Provision of novel materials reduced knocks and injuries and increased play in sows following mixing

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Aggression between sows at mixing is unavoidable. Although a natural behaviour, aggression between unfamiliar pigs is a welfare concern due to the associated injury and stress. Management around the time of mixing can mitigate the short-term nature of this stress response (Arey and Edwards 1998). One parameter that can be managed to reduce aggression and stress in sows is enrichment or addition of novel materials. The aim of this experiment was to determine the effect of the presence of novel materials on the aggression between sows at mixing. It was hypothesised that if the novel materials were a source of interest for the sows and not a limiting resource, then they would decrease aggression following mixing.

The experiment used 144 multiparous, Large White x Landrace sows. Following artificial insemination, sows were mixed into groups of 12 and allowed space of 2 m^2/sow. Sows were allocated to either a standard pen or a novel environment pen, with the latter having eight hanging ropes, two hanging yellow disks and two hanging rubber mats. The sows remained in these pens until ultrasound scanning for pregnancy, after which all sows were moved into a shelter (approximately d 30). Injury counts and behaviours (6 hours, including eating, fighting, knocks, bites, rest and exploration) were measured on d 0, 1, 4, 7 and 20 relative to mixing. Data were analysed using a linear mixed model (IBM SPSS, Version 20.0; USA) with sow identification fit as a random effect, and replicate, sow parity, day of measure and treatment as fixed effects. For injury counts, the d -1 measure was fitted as a covariate. Data are expressed as least squares means ± SEM. Where statistical transformations occurred, the non-transformed means have been presented in parentheses.

The number of knocks delivered was significantly altered by treatment and day ($P < 0.05$; Fig. 1), with fewer knocks in the novel pen than the standard pen on d 1 (4.9 versus 3.0) and d 20 after mixing (4.4 vs 3.1). The total numbers of injuries was decreased by the presence of novel materials on both d 4 [5.0 ± 0.2 (27.6) vs 5.5 ± 0.2 (35.0)]) and d 20 [3.5 ± 0.2 (15.0) vs 4.2 ± 0.2 (20.6)]) after mixing ($P < 0.01$). The percentage time that sows spent excitedly playing, a behaviour not recorded in the standard pens, increased significantly over the experimental period in the novel pens, with sows playing with the materials more on d 7 and 20 than on d 0, 1 and 4 ($P = 0.02$).

The presence of novel materials decreased aggression and injuries, both of which could indicate welfare benefits. Given that the amount of time that sows spent playing with the novel materials increased over the 20-day study period, it can be concluded that the enrichment successfully engaged the sow's attention, and habituation to the materials did not occur over this time frame. Therefore, increased play elicited by enrichment, suggest that the presence of the materials may have improved sow welfare, if only minimally affecting aggression at mixing.

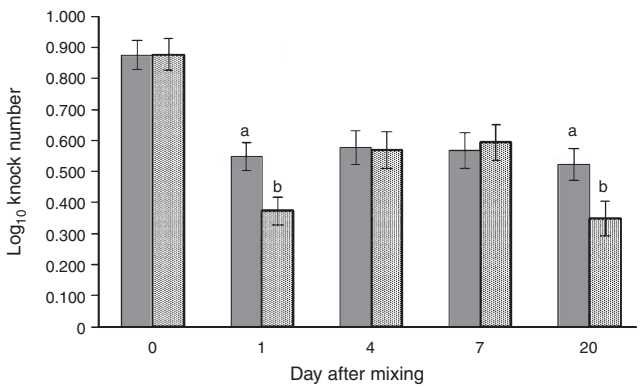

Fig. 1. The number of knocks delivered by group housed sows in standard pens ■ and pens containing novel materials ⬚ following mixing. Data are log_{10}-transformed means ± SEM. Significant differences between treatment within day are highlighted using superscripts ($^{a,b}P < 0.01$).

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Feeding behaviour, aggression and dominance in group-housed sows

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Group-housed sows are required to share resources and competition for feed is of paramount importance both for production and sow welfare (Verdon *et al.* 2015). We hypothesised that there are relationships between aggression, dominance status and feeding behaviour after mixing in gestating sows.

Over two replicates on a commercial farm, 100 Landrace × Large White primiparous sows were randomly mixed within 1 week of insemination into pens of 10 with a floor space allowance of 1.8 m^2/sow. Sows were fed a daily allowance of 2.5 kg/sow of a pelleted food over four feeding bouts (0730, 0900, 1100, 1500 h) via two overhead drop feeders placed 2 m apart. Sows were individually marked and observed through video recording on d 2 and 9 (i.e. 1 week later) after mixing for the first and third feeding bouts. The presence of individuals in the area under each feeder was observed using instantaneous point sampling at 30 s intervals for 30 min after feed delivery. Aggression delivered and received by each sow at feeding was observed continuously for the same period. Individual sow aggression level and the resulting index were calculated according to Rault *et al.* (2014), with sows classified as dominant (D) if they delivered more aggression than they received, subdominant (SD) if they received more than they delivered, and submissive (S) if they never delivered aggression. Data were analysed using a mixed model with Tukey adjustments for post-hoc comparisons or Spearman rank correlation test if not normally distributed (SAS®; USA).

The interaction of day and feeding bout was significant ($P = 0.03$). Sows were present in the feeding area less often during the third feeding bout on d 2 than during the first bout on d 2 and during the first and third bouts on d 9 (all $P \leq 0.02$). There was a weak correlation between aggression level and overall presence at the feeder ($r = 0.16$, $P = 0.001$) that held true on d 2 ($r = 0.23$, $P < 0.001$) but not on d 9 ($r = 0.07$, $P = 0.35$). Using the aggression index, 37% of sows were classified as D, 29% as SD and 34% as S. Both D and SD sows were present more often in the feeding area than S sows on d 2 ($P \leq 0.007$) but not on d 9 ($P > 0.05$) (Table 1). Dominant sows were present more often in the feeding area than S sows during the first bout ($P = 0.001$) but less frequently during the third ($P = 0.03$), whereas SD and S sows had similar presence at the first and third feeding bouts ($P > 0.05$). There was no individual sow preference for left or right side feeders ($P > 0.05$).

Presence at feeding differed more on d 2 than on d 9 after mixing. Dominant sows were seen less often at the third bout, suggesting they may be satiated after the first feeding bout of the day. In agreement, Verdon (2014) found that D sows gained most weight between d 2 and 100 after mixing in that system. However, SD sows were present as frequently in the first and third bouts. Multiple feed drops may therefore provide SD sows increased opportunities to access feed in later bouts. Verdon (2014) also found that aggression received by SD and S sows reduced with subsequent feeding bouts. Nonetheless, S sows were seen less often in the feeding area on d 2, possibly because there was more competition from D sows for the first bout and then from SD sows for the third bout. In conclusion, feeding sows over four bouts may reduce competition for feed on d 2 after mixing, benefiting SD but not S sows. The challenge remains to allow sows at the bottom of the hierarchy sufficient access to feed to safeguard production and welfare of group-housed sows, although drop feeding is recognized as a feeding system with intense feeding competition.

Table 1. Sow presence in the feeding area by aggression index, day and feeding bout. Values are least-squares means ± SE (unit is the average count over 60 intervals per feeding bout)

Aggression index	Day 2		Day 9	
	1st feeding bout	3rd feeding bout	1st feeding bout	3rd feeding bout
Dominant	28.5 ± 1.7	22.1 ± 1.7	26.5 ± 1.5	23.9 ± 1.8
Subdominant	26.0 ± 1.9	22.2 ± 1.8	23.9 ± 2.0	25.6 ± 2.0
Subordinate	19.2 ± 1.8	16.1 ± 1.9	21.9 ± 2.0	22.4 ± 1.6

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Inducing satiety in sows through nutritional manipulation of gastrointestinal tract volume and volatile fatty acid production

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Failure to meet satiation causes feeding motivation to increase. Frustration may be redirected into replacement behaviours (Lawrence and Terlouw 1993) that over time may become stereotypic behaviours or cause aggression. Satiety may be attained by increasing the bulk of the diet. Also of interest is the role of hindgut fermentation of non-starch polysaccharide rich diets, with the production of volatile fatty acids (VFA) from this fermentation having a glucose sparing effect (de Leeuw *et al.* 2005). Alternative dietary sources that may show effects on satiation include sugar beet pulp (SBP), guar gum, Opticell® (Agromed Austria GmbH, Kremsmünster, Austria) and magnesium oxide (MgO). This study aimed to investigate the effects of dietary inclusions, aimed to induce satiety through the manipulation of gastrointestinal tract (GIT) volume and (or) VFA production, on blood glucose and behavioural variables in sows.

Fifteen (15) mixed-parity sows (non-pregnant; Landrace X Large White) were housed in individual stalls and offered dietary treatments in a crossover design, such that each sow received each treatment over time. Diets were isoenergetic and isonitrogenous [12.8 MJ digestible energy (DE)/kg, 0.40 g standardised ileal digestible lysine/MJ DE] taking into account experimental inclusions. There were five diets offered: Control diet including no additions; SBP included at 20% of total diet; guar gum (0.5%); Opticell® (4.0%); and MgO (0.1%). Diets were given for 2 weeks comprising a 1-week period of diet acclimation and then a 1-week period of diet provision, replicated five times, until all sows had received all treatments. Sows were fed twice daily, receiving 60% (1.5 kg) at 0700 h and 40% (1.0 kg) at 1400 h. Behavioural measurements were recorded by scan sampling (1 min) and consisted of individual video monitoring during the 5 min before the first feed, 25 min before the second feed and 45 min after completion of the first and second feed. Behavioural data were grouped into abnormal (oral-nasal) behaviours and postures. The glucose sparing effects of VFA production were monitored through blood glucose measurements (Accu-Chek Performa, Roche, Castle Hill, NSW) which occurred 15 min prior to feed one and two, then at 0.5, 1, 2, 3, 5 and 7 hours after both feeding times. Data were analysed using the GLM procedure (Genstat, 15th Edition; UK).

Blood glucose measurements showed significant effects of dietary inclusions on blood glucose levels (Table 1). The inclusion of guar gum in diets for sows reduced fasting blood glucose levels with significantly lower blood glucose levels before the first feeding. Immediately after feeding, the inclusion of guar gum and Opticell® resulted in higher circulating blood glucose concentrations ($P < 0.05$), possibly a result of delayed GIT emptying and (or) reduced insulin sensitivity. Glucose levels of sows receiving guar gum diets returned to fasting levels by 7 hours after feeding. There was no significant effect of treatment on behavioural observations, however time spent displaying abnormal behaviours increased over time ($P < 0.05$; data not shown) as sows habituated to their stalled environment. Whilst SBP inclusion level was lower in this study, the lack of effect on feeding motivation was unexpected given prior positive effects (Meunier-Salaün *et al.* 2001). Results of blood glucose sampling suggest all four treatments were able to influence blood glucose levels but the lack of behavioural effect suggests inducing satiety warrants further investigation of economically viable inclusion levels used in this study, in a more stable environment.

Table 1. Mean blood glucose levels (mmol/L) 15 min prior to, and 30 min and 7 hours after the first feed, in sows fed a control diet or a diet containing 0.5% guar gum, 4% Opticell®, 0.1% magnesium oxide (MgO) or 20% sugar beet pulp (SBP)

	Treatment						
Feed event	Control	Guar gum	Opticell®	MgO	SBP	SED[A]	*P* value
15 min prior	4.3[b]	4.0[a]	4.3[b]	4.1[b]	4.1[b]	0.10	0.050
30 min post	4.1[a]	4.6[c]	4.5[bc]	4.2[ab]	4.1[a]	0.19	0.011
7 hours post	4.6[c]	4.1[a]	4.5[bc]	4.3[ab]	4.3[ab]	0.14	0.010

[A]SED, standard error of difference between means. [a,b,c]Means in a row not having the same superscript are significantly different.

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The response of group-housed sows to dietary inclusion of magnesium oxide and sugar beet pulp

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Mixing unfamiliar sows during early gestation can often lead to injury and lameness as a result of inter-sow aggression which can be accentuated by an increased motivation to feed in systems where a restricted amount of feed is delivered once or twice daily. Nutritional satiety may be achieved in group-housed sows through the addition of fibre, such as sugar beet pulp (SBP; Danielsen and Vestergaard 2001). Magnesium oxide (MgO) has been suggested to play a role in insulin resistance (Barbagallo *et al.* 2003), resulting in stabilised insulin levels that in turn stabilise blood glucose levels, leading to satiation (Bo and Pisu 2008). It was hypothesised that the inclusion of SBP and (or) MgO in the diet of sows at mixing would lead to reduced inter-sow aggression.

Thirty-six multiparous sows (Landrace X Large White) were used across this study, re-randomised into four treatment groups (n = 6) for each of six replicates. Twenty-four sows were used in each replicate, with 12 sows off test, to allow for completely unfamiliar groups at each replicate. Each replicate ran for 7 days with sows being housed initially in individual stalls for the first 4 days and offered allocated treatment diet. At 0700 h on d 5, sows were shifted to their respective group pen. Daily data collection began on d 5 (day of mixing). Measures taken during each 3-day observation period included aggressive behaviour (push, chase, attack, bite and threat) and posture observations. This use of short-term assessment is suited due to the 1–2 days after mixing that are associated with dominance aggression at mixing (Arey and Edwards 1998), yet takes into account the extended length that sows recognise each other (Spoolder *et al.* 1996). All diets were formulated to be isoenergetic and isonitrogenous [12.9 MJ digestible energy (DE)/kg, 0.40 g standardised ileal digestible lysine/MJ DE] and were fed at 2.3 kg/d. The four diets offered over the 7 day period were: control diet; a diet including 20% SBP; a diet including 0.2% MgO; and a diet including both 20% SBP and 0.2% MgO. Data were analysed using the Univariate GLM procedure (Genstat, 15th Edition; UK) with the experimental unit being the pen.

The inclusion of SBP and (or) MgO in the diet had no significant effect on sow behaviour and no significant effect on aggressive behaviour (Table 1). However, a time effect was seen for some behavioural observations. Chase behaviour increased the day after mixing (d 6) before falling again the following day ($P < 0.05$), whilst threat behaviour increased over time. There was a trend ($P < 0.10$) for the time sows spent fighting to reduce after the first 24 hours of mixing (from d 5 to d 6). Salivary cortisol levels (data not shown) increased over time, which appears in conflict with the decline in fight time. Whilst these dietary interventions were not able to influence behaviour, this study did show that fighting behaviours are short-lived. The increase in threat behaviour over the period contrasting with the decreased fighting behaviour suggests a rapid establishment of hierarchal positions in the first days of mixing.

Table 1. Time (min) sows spent engaged in behaviours 1 h after feeding, for diets containing 20% SBP and (or) 0.2% MgO and for all treatments over the experimental period

Treatment	Control	SBP	MgO	SBP+MgO	SED[A]	*P* value
Chase	0.12	0.12	0.22	0.09	0.06	0.281
Threat	0.19	0.13	0.22	0.17	0.08	0.717
Fight time (s)	2.99	2.97	1.66	5.15	1.89	0.331
Day[B]	5	6	7			
Chase	0.11[b]	0.22[a]	0.08[b]		0.06	0.031
Threat	0.08[b]	0.10[b]	0.35[a]		0.07	<0.001
Fight time (s)	5.34	1.69	2.55		1.64	0.075

[A]SED, standard error of difference of means. [B]Day: d 1–4, non-experimental period and sows held in individual stalls; d 5, day of mixing and commencement of daily observations (d 6 and 7). Fight time (s), mean length of fighting bout. [a,b]Means in a row not having the same superscript are significantly different.

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Use of a nutritional lick block and higher feeding levels to reduce aggression and provide enrichment for sows in groups

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There is evidence that providing enrichment may reduce aggression and fighting between sows at mixing (Schaefer *et al.* 1990), whilst the lack of substrate to allow opportunity for foraging and feel satiated once established in group housing can accentuate ongoing inter-sow aggression (Danielsen and Vestergaard 2001). It was hypothesised that the provision of a higher feeding level or the use of enrichment in the form of a supplemental block would reduce aggression at time of mixing.

A commercial dry sow diet [12.9 MJ digestible energy (DE)/kg, 0.40 g standardised ileal digestible lysine/MJ DE] was fed to all treatments which consisted of a control group fed at 2.3 kg/sow/d, a block enrichment group fed at 2.3 kg/sow/d and provided a 30 kg poured supplemental block (hard block, comprised of a range of ingredients including molasses, sugar beet pulp and magnesium oxide), and a group fed at 4.0 kg/sow/d. All treatments were floor fed once daily at 0700 h. Thirty-six multiparous sows (Landrace X Large White) were used across this study, re-randomised into three treatment groups (n = 6) for each of six replicates. Eighteen sows were used in each replicate, with 18 sows off test, to allow for completely unfamiliar groups at each replicate. This short-term assessment was appropriate given the 1–2 day timeframe associated with dominance aggression at mixing (Arey and Edwards 1998), and accounts for the period that sows can recognise each other (Spoolder *et al.* 1996). Each experimental replicate ran for 7 days with sows being housed initially in individual stalls for the first 3 days. At 0700 h on d 4 sows were shifted to their allocated group pen (1.5 m^2/sow). Daily data collection began on d 4 after mixing. Measures taken during each 4-day observation period included the supplemental block weight, aggressive behaviours (push, chase, attack, bite and threat) and posture observations for 1 hour after feeding. Data were analysed using the Univariate GLM procedure (Genstat, 15th Edition; UK).

The presence of either the supplement block or higher feeding level had a significant positive effect on chase behaviour (Table 1). Sows fed the high feed level or provided with a supplemental block spent more time lying (P = 0.038) and less time standing (P = 0.006), and they also tended to spend less time involved in foraging behaviour than the control treatment (P = 0.084). The provision of a supplement block or a higher feeding level of 4.0 kg/d appears to provide a method to modify the behaviour of the sow at mixing, increasing the time spent at rest (lying) and reducing the exhibition of foraging behaviour.

Table 1. Mean time (min) sows' spent engaged in behaviour and posture 1 h after feeding over the 4 d of observation, for sows receiving 2.3 kg/d (Control), sows receiving a high-feeding level (4.0 kg/d, High Feed), or sows receiving a supplement block in addition to 2.3 kg feed/d (Block)

Activity/Posture	Treatment			SED[A]	*P* value
	Control	Block	High feed		
Push	0.09	0.08	0.10	0.24	0.868
Chase	0.29[a]	0.08[b]	0.11[b]	0.47	0.019
Attack	0.40	0.42	0.36	0.58	0.811
Bite	0.10	0.12	0.06	0.25	0.392
Threat	0.13	0.11	0.10	0.27	0.736
Foraging	28.48[x]	25.67[xy]	25.15[y]	9.76	0.084
Lying	9.13[b]	13.30[a]	13.66[a]	11.30	0.038
Standing	50.63[a]	45.91[b]	45.26[b]	10.85	0.006

[A]SED, standard error of difference between means. [a,b]Means in a row not having the same superscript are significantly different. [x,y]Means in a row not having the same superscript indicate a trend for a significant difference (P < 0.10).

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The key indicators of stockpersonship and their relationship with independent behavioural observations and supervisor assessments of stockpeople

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Stockpeople are a vital component of animal production systems (English *et al*. 1992) but despite this recognised importance in regard to animal welfare, very few animal welfare audits assess stockpersonship. The aims of this study were to identify the key indicators of stockpersonship, establish the validity of these measures by correlating them with stockperson behaviour and supervisor assessments of the stockperson and to establish the reliability of these measures using (i) Cronbach's alpha coefficient to establish internal consistency and (ii) test-retest correlations to establish the repeatability of these measures.

Stockperson self-report and supervisor questionnaires were developed. Questionnaire development was based on focus group information and relevant literature. Following data collection, the questionnaires were refined using Principal Component Analysis (PCA) to identify the underlying commonalities of the questions. This process produced twelve separate stockperson subscales: *recognition and relationships*; *job enjoyment*; *responsibility and independence*; *positive interaction beliefs*; *physical effort beliefs*; *husbandry beliefs*; *negative attitudes towards pigs*; *positive attitudes towards pigs*; *empathy (attribution)*; *empathy (affect)*; *citizenship; and knowledge*. Cronbach's alpha coefficients ranged from 0.69 to 0.90, indicating moderate to strong reliability, for the stockperson subscales. Principal Component Analysis produced four separate supervisor subscales: *reliable*; *proactive*; *committed*; and *conscientious*. Supervisor subscales obtained Cronbach's alpha coefficients ranging from 0.71 to 0.95.

The behavioural observation protocol was created using expert opinion, focus group information and literature. Fifteen piggeries across Australia were involved in the study. A total of 117 stockperson questionnaires, 138 supervisor surveys and 132 behavioural observations were completed. This resulted in 79 complete datasets with corresponding stockperson questionnaires, supervisor reports and behavioural observations completed. A number of the stockperson subscales significantly correlated with supervisor assessments or with behavioural observations. Mild or positive behaviours positively correlated with *empathy (affect)* ($r = 0.29$, $P < 0.01$), *empathy (attribution)* ($r = 0.24$, $P < 0.05$) and *positive attitudes towards pigs* ($r = 0.25$, $P < 0.05$). These findings indicated that the greater the stockpersons' empathy and positive attitude towards pigs, the greater the frequency of mild or positive behaviours. Negative behaviours were negatively correlated with *citizenship* ($r = –0.26$, $P < 0.05$), *empathy (affect)* ($r = –0.28$, $P < 0.01$) and *husbandry beliefs* ($r = –0.25$, $P < 0.05$). This suggested that as citizenship (or allegiance to the company), empathy and husbandry beliefs scores decreased, the frequency of negative behaviours increased. Negative stockperson behaviours were positively correlated with *negative attitudes towards pigs* ($r = 0.24$, $P < 0.05$) indicating that stockpeople with negative attitudes towards pigs were more likely to engage in negative interactions during animal handling. Knowledge was related to several other stockperson subscales including *citizenship* ($r = 0.30$, $P < 0.01$), *empathy (attribution)* ($r = 0.36$, $P < 0.01$), *positive attitudes towards pigs* ($r = 0.28$, $P < 0.01$), *positive interaction beliefs* ($r = 0.22$, $P < 0.05$) and *responsibility and independence* ($r = 0.24$, $P < 0.05$). These results suggested that knowledge about pig health and welfare was related to citizenship, empathy, positive beliefs and attitudes towards pigs and handling pigs as well as being responsible and independent at work.

The supervisor subscale, *proactive* correlated positively with *citizenship* ($r = 0.21$, $P < 0.05$) and negatively with *negative attitudes towards pigs* ($r = –0.20$, $P < 0.05$) indicating that stockpeople assessed as proactive by their supervisor were more likely to have higher levels of citizenship and were less likely to hold negative attitudes towards pigs. The *conscientious* subscale was positively correlated with *empathy (attribution)* ($r = 0.22$, $P < 0.05$), suggesting that stockpeople assessed as conscientious by their supervisor were more likely to have higher levels of *empathy attribution*.

The test-retest correlations for the questionnaire data were all significant, ranging from $r = 0.35$ to $r = 0.78$. These results provided evidence for validity and reliability of the questionnaire as a measurement tool for monitoring stockpeople and the attributes of stockpersonship.

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Boar contact and seven hours of interrupted suckling improved sow performance

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In the majority of lactating sows, piglet sucking plus the metabolic demands of milk production prevent the post-partum resumption of oestrous cyclicity until after weaning. Therefore, lactation length ultimately determines farrowing frequency, with high suckled litter sizes and lactation body reserve loss also impairing subsequent reproductive performance. Sow-piglet separation (interrupted suckling; IS) for 12 h stimulated ovarian follicle growth and ovulation prior to weaning (Kemp and Soede 2012); however, the effect of a shorter period of IS on the timing of ovulation post-partum and subsequent reproductive output and efficiency is less clear. The current study tested the hypothesis that seven hours of IS would increase the incidence of lactation ovulation and improve the reproductive efficiency of sows remaining anoestrus until weaning.

From d 17 ± 0.2 post-partum (mean ± SEM), 32 Large White x Landrace sows (parity 4.1 ± 0.12) suckling 9.6 ± 0.24 piglets, experienced 3 days of zero (0IS) or 7 (7IS) hours of piglet separation (n = 16 sows/treatment). Average parity did not differ between treatments. In the 7IS treatment, separation was between 0800 and 1500 h, and was achieved through the use of a board placed between the sow and the creep area. From the start of IS until weaning (day 27 ± 0.2 post-partum), sows in both treatment groups received 5 min of nose-to-nose contact with a mature boar through the open door of their farrowing crate. During the night, a boar was housed in a farrowing crate within the farrowing shed. Sows were checked daily for oestrus in the presence of the boar, and inseminated at first detection of oestrus. The timing of oestrus, farrowing rates and the subsequent total litter size were recorded. An ANOVA model was used to determine treatment effects on the timing of oestrus and subsequent litter size (Genstat, 10th Edition; UK), with differences between proportions analysed by Chi-square.

Treatment (7IS versus 0IS) tended ($P < 0.1$) to increase the incidence of lactation oestrus and reduce the interval from parturition to first oestrus (Table 1). The parity of sows ovulating during lactation was higher ($P < 0.05$) than those which did not (4.4 ± 0.23 vs 3.9 ± 0.15). Subsequent litter size was unaffected by the timing of ovulation; however, the farrowing to farrowing interval was shorter ($P < 0.05$) for sows mated during, as opposed to after, lactation: 139.4 ± 0.39 vs 147.3 ± 0.29 days. The total number of piglets produced per 100 sows entering the 0IS and 7IS treatments was calculated to be 1125 and 1310.

These data provide preliminary evidence that seven hours of sow-piglet separation for only three days during late lactation may increase reproductive efficiency. This management strategy could be used to improve reproductive output when environmental conditions are unfavourable (i.e. during summer) or body tissue mobilisation is high, particularly for high parity sows.

Table 1. Effect of seven (7IS) vs zero (0IS) hours of interrupted suckling (IS) between d 17 and 20 of lactation on the timing and incidence of oestrus and subsequent reproduction of sows weaned at 27 d

Item	Proportion of oestrus sows		Interval from parturition to oestrus			FR[A] (%)	Total born
	In lactation	Post-weaning	In lactation	Post-weaning	All		
0IS	0.19*	0.81*	23.0[a]	30.9[b]	29.4*	93	12.1
7IS	0.50*	0.50*	21.9[a]	31.6[b]	26.8*	100	13.1
Pooled SEM[B]			0.42	0.27	0.80		0.49

[A]FR, farrowing rate. [B]SEM, standard error of the mean. [a,b]Means in a row and within item not having the same superscript are significantly different ($P < 0.01$). Within columns, *$P < 0.1$.

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Effect of different feed density during gestation for group housed and fed sows on litter size and farrowing rate

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Many studies have focused on creating the best feeding strategy for sows in the gestation period. The conclusions show that the feeding strategy must follow the daily needs for maintenance, adjusted to the body condition of the sow (Quesnel *et al*. 2010; Athorn *et al*. 2011). In Europe, loose housing systems based on small, stable groups with floor feeding during gestation are common, because these systems are cheap and easy to manage. However, this leads to aggressive behaviour during feeding. One possible way to reduce aggressive behaviour is to increase the daily feed intake or the eating time with low-density diets. It was hypothesised that feeding sows with a low density commercial pelleted diet during the gestation would increase farrowing rates but not affect the litter size.

The study took place in one production herd, where sows were housed in pens of 13–14 from immediately after mating to farrowing and fed twice daily on the floor with commercial pelleted feed. A total of 1556 multiparous DanAvl sows were assigned to two groups blocked by parity: Low (11 MJ digestible energy (DE)/kg) and High (13 MJ DE/kg) energy density. The sows were weighed and scanned for backfat depth at the P2 site just after mating and just before farrowing. Sows followed the same feeding strategy based on MJ DE/day, but it was possible to shift the feeding curve parallel up/down for each pen depending on the average P2 backfat depth just after mating. The aim of the feeding strategy was to have the same average P2 backfat depth at farrowing in all the pens.

The difference in MJ DE/kg was achieved by increasing the level of oats in the Low diet. The diets used in the two groups had different levels of crude fiber but the same content of minerals, vitamins and protein per MJ DE and followed common standards for nutrients for gestating sows. Therefore, sows in the Low Group had to eat 15% more feed daily to receive the similar intake of DE and nutrients as the sows in the High group (based on the Danish feed evaluation system). At farrowing the number of total born piglets per litter was recorded per sow, but the averaged litter size from all the sows in each pen was used in the statistic model. The farrowing rate was calculated as percentage of sows in each pen transferred to the farrowing.

Litter size, body weight (BW) and backfat P2 gain to farrowing were analysed in a linear model by ANOVA under the GLM procedure, while farrowing rate was analysed by logistic regression in the MIXED procedure (SAS®; USA). The covariates were pen, parity, body weight (BW) and P2 at mating.

The Low- and High-density diets resulted in the same ($P > 0.05$) increased BW gain and P2 backfat gain (Table 1). Using the Low-density diet caused a higher ($P = 0.04$) litter size, but there was no difference ($P > 0.05$) in farrowing rate between groups. In conclusion, in this study the density of the diet (based on change in crude fiber content) was detrimental to litter size, but not to the farrowing rate.

Table 1. Effect of two diets of different density fed during gestation on litter size and farrowing rate. Values are mean ± SE (per pen)

Dietary treatment (DE/day)	Low	High	*P* value
N	57	57	
Average number of sows in each pen	13.6	13.7	
Average parity	3.0	2.9	
BW at mating (kg)	210 ± 42	208 ± 42	
BW, gain to farrowing (kg)	72 ± 38	62 ± 40	0.17
Backfat P2 at mating (mm)	12.6 ± 2.03	12.5± 2.00	
Backfat P2 gain to farrowing (mm)	4.2 ± 1.48	3.4 ± 1.79	0.17
Total born piglets per litter	18.3 ± 1.61	17.9 ± 1,59	0.04
Farrowing rate (%)	86.8 ± 9.2	84.7 ± 10.4	0.19

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A comparison of suckling reduction strategies along with boar exposure to induce oestrus in lactating sows

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Breeding sows during late lactation offers pig producers the potential to uncouple weaning from re-mating. Recent research (Frobose *et al.* 2013; Terry *et al.* 2014) demonstrated that combining boar exposure and reduced suckling allowed lactational oestrus and fertility comparable to conventionally weaned sows. However, questions remain about the most practical method to apply treatments on farms. The objective of this study was to prove the hypothesis that different suckling reduction strategies will vary in the incidence of lactational oestrus and result in different effects on sow fertility and piglet growth.

A total of 135 sows (PIC 1050), from parity one to five (2.6 ± 1.4; mean ± SD), was used in five consecutive farrowing groups (Feb to Aug). Litter size was equalised by parity (11.5 ± 1.1 piglets; mean ± SD) at d 2 after farrowing. At d 18, sows were assigned to one of five treatments (n = 26 to 28) based on parity, farrowing date, and suckled litter size. Treatments were: Control; ALT (sows placed in adjacent pairs, on d 18 all but the five lightest piglets were weaned and remaining piglets combined and alternated between sows at 12 h intervals from d 18 to 25; SEP (piglets separated for 12 h/day from d 18 to 25); Split-wean (SW; all but the five lightest piglets weaned on d 18); and 24HR (piglets separated from sows for 24 h on d 18). Controls were weaned at d 21, with all other treatments weaned at d 25. All sows were provided nose-to-nose contact with a mature boar for 5 min/d from d 18 until weaning without removing them from farrowing crates. Creep feed and water access was provided from d 14 to weaning. Offspring average daily gain (ADG) was recorded to market for two farrowing groups. Data were analysed using GLIMMIX (binomially) or MIXED procedure (SAS®; USA) (normally distributed).

Sow backfat and BW losses during lactation were similar across treatments. Of 106 sows subjected to suckling treatments, 80 (76%) expressed lactational oestrus. The SEP and 24HR sows were in oestrus earlier ($P < 0.05$) than SW sows (Table 1). A tendency for reduced conception rate in SEP and 24HR sows was observed ($P < 0.10$) versus control and SW sows. Creep feed disappearance was greatest ($P < 0.01$) for SEP and 24HR litters and pig ADG from d 18 to 32 was reduced ($P < 0.05$) for these treatments. No negative effects ($P > 0.05$) on final BW or carcass composition were observed for the reduced suckling treatments. Altered suckling treatments differ in their ability to induce lactational oestrus and impact on gain immediately post-weaning. However, no evidence was found of negative effects on growth to market weight.

Table 1. Effects of suckling reduction strategy and boar exposure on the incidence of lactational oestrus, interval to oestrus, and pig growth to market weight

	Control	ALT[A]	SEP	SW	24HR	SEM[B]	$P \leq$[C]
Lactating sows inseminated[D] (%)	0.0	77.8	73.1	85.2	62.9	0.09	0.318
Day 18 to insemination (d)	–	5.0[ab]	4.7[a]	5.5[b]	4.4[a]	0.33	0.036
Conception rate (%)	–	80.4	62.8	87.9	59.8	0.14	0.133
Sows inseminated post-wean (%)[D]	100.0	22.2	26.9	14.8	37.0	0.09	0.318
Wean to oestrus (d)	3.49	3.76	4.54	3.60	4.27	0.754	0.131
Day in oestrus after farrowing	24.5	24.3	24.6	24.4	25.0	0.66	0.868
All sows conception rate (%)	96.7[b]	78.3[ab]	75.0[ab]	92.0[b]	66.3[a]	0.08	0.094
Creep feed disappearance (g/piglet/d)[E]	10.8[a]	13.5[a]	28.6[b]	11.6[a]	24.4[b]	1.69	0.001
Offspring ADG d 18 to 32 (g)[E]	222[b]	215[b]	164[a]	217[b]	165[a]	9.9	0.001
Offspring ADG d 32 to 170 (g)[E]	894	879	890	868	879	14.6	0.715
Offspring d 170 BW (kg)[E]	132.5	130.2	131.0	128.8	129.6	2.09	0.734

[A]Refer to text for treatment details. [B]SEM, pooled standard error of mean. [C]Overall significance set at $P < 0.05$ for individual treatment comparisons. [D]Controls excluded from these analyses due to lack of variance. [E]Piglet growth reported from two farrowing groups (n = 54 litters), adjusted using d 18 body weight (BW) as a covariate. [a,b]Means in a row not having the same superscript are significantly different.

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Split suckling versus intermittent suckling with primiparous sows: skip-a-heat effects on oestrus during lactation and reproductive performance

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The stimulation of lactational oestrus with subsequent viable mating outcomes opens up the possibility of increasing lactation lengths and thus the weaning age of piglets without significant losses in sow productivity. Results from previous work in multiparous (MP) sows have resulted in >80% of sows being mated in lactation through stimulation techniques such as piglet separation and boar exposure (McDonald *et al*. 2013). Compared to MP sows, primiparous (PP) sows face extra metabolic challenges during lactation that may compromise subsequent reproduction. It was hypothesised that mating PP sows at the subsequent oestrus following their first oestrus during lactation (skip-a-heat) would improve reproductive outcomes when combined with either an intermittent suckling or split suckling oestrus induction protocol.

Primiparous sows (Large White × Landrace, PrimeGro™ genetics; n = 138) were allocated to one of three treatments: Control (C28), where piglets were weaned at d 28 of lactation; Intermittent suckling (IS21), where all piglets were separated from the sow for 8 h each day from d 21 of lactation until weaning at d 28; and Split suckling (SS21), where only half of the litter suckled at any one time from d 21 of lactation until weaning at d 28. All sows in the IS21 and SS21 treatments received twice-daily boar exposure whilst in the farrowing crate throughout the entire separation period. The IS21 and SS21 sows were mated at either lactational oestrus, or at the subsequent oestrus following lactational oestrus (skip-a-heat). The C28 sows and any IS21 or SS21 sows that did not experience a lactational oestrus (non-responders) were mated at their first post-weaning oestrus. Data were analysed using univariate GLM analysis or a Chi-square test (for farrowing rate) (IBM SPSS, Version 21.0; USA).

Approximately 40% of PP sows in the IS21 and SS21 treatments displayed oestrus during lactation, which was lower than in previous studies (Chen *et al*. 2013). Farrowing rates and litter size did not differ ($P > 0.05$) between treatments or between sows mated at lactational oestrus and those mated at the subsequent oestrus following lactational oestrus (skip-a-heat) (Table 1). Reproductive performance of PP sows mated during lactation was comparable to sows mated after weaning. Furthermore, skip-a-heat mating compared to mating at the lactational oestrus did not significantly improve reproductive outcomes in PP sows. These data suggest PP sows have a lower response rate to the induction of lactational oestrus compared to MP sows, which needs to be taken into consideration when implementing lactational oestrus induction protocols. However, PP sows that do respond can be mated at their first induced lactational oestrus with no negative effect on subsequent reproductive outcomes.

Table 1. Lactational oestrus, farrowing rates, and second litter size of primiparous sows mated at lactational oestrus (first heat), at the subsequent oestrus following lactational oestrus (skip-a-heat), or at normal post-weaning oestrus (non-responders). Values are mean ± SEM

Treatment[A]	C28 (n = 33)	IS21 (n = 58)			SS21 (n = 47)		
Lactational oestrus (%)	–	38 (22/58)			43 (20/47)		
		First heat	Skip-a-heat	NR[B]	First heat	Skip-a-heat	NR
Farrowing rate (%)	94 (30/33)	100 (13/13)	100 (9/9)	86 (30/36)	89 (8/9)	100 (11/11)	96 (24/26)
Total born	12.3 ± 0.54	13.1 ± 0.82	13.7 ± 0.98	12.0 ± 0.54	11.7 ± 1.04	13.5 ± 0.88	12.8 ± 0.60
Born alive	11.3 ± 0.53	12.2 ± 0.80	13.1 ± 0.96	11.4 ± 0.52	11.4 ± 1.02	12.6 ± 0.87	11.4 ± 0.59

[A]Refer to text for treatment details. [B]NR = Non-responders.

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Sow aggression in early gestation is decreased by greater space allowance in the first four days following mixing

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Group housing of sows is preferable to the use of stalls as it allows for higher social interaction and movement (Seguin *et al.* 2006). One disadvantage of group housing is that the mixing of sows, and therefore aggression, is unavoidable. Aggression between domestic sows is highest when sows are first introduced to each other and hierarchies are formed. The aim of this study was to determine the effect of a mixing pen involving increased space allowance at the point of mixing followed by restricted space after hierarchy formation on sow aggression. It was hypothesised that aggression at mixing would be negatively correlated to space allowance, and that space restriction after hierarchy formation would result in no detrimental effects.

The experiment used 132 multiparous, Large White x Landrace sows. Following artificial insemination sows were mixed into groups of six. Australian standards state sows must be housed at 1.4 m^2/animal or greater but recent research suggests this figure is too low (Hemsworth *et al.* 2013), and so this experiment allowed 2 m^2/sow (LOW), 4 m^2/sow (MED) or 6 m^2/sow (HIGH). The sows remained in these pens until d 4 after mixing, at which point all pens were equalised to 2 m^2/sow. Behaviours (6 h, including eating, fighting, displacements, rest and exploration) were measured on d 0, 1, 3 and 4 relative to mixing. Data were analysed using a linear mixed model (IBM SPSS, Version 20.0; USA) with sow identification fit as a random effect, and replicate, sow parity, day of measure and treatment as fixed effects and sow as the experimental unit. Data are expressed as least squares means ± SEM. Where transformation of data occurred, the non-transformed means have been presented in the text.

The LOW group sows had a greater fight number than HIGH sows on both d 0 and 1 after mixing (LOW = 6.1, MED = 4.1, HIGH = 3.0, $P < 0.05$; Fig. 1). HIGH sows were involved in more fights than MED sows when the pens were decreased on d 4 (LOW = 1.9, MED = 1.7, HIGH = 2.5, $P < 0.05$; Fig. 1). When the change in aggression from d 3 to d 4 (after pen size was standardized) was analysed, there were no treatment effects ($P > 0.05$).

In line with previous reports (Weng *et al.* 1998), results from this study support the notion that providing sows with large space allowances is an effective method to reduce aggression. A novel finding of the current investigation was that space can be reduced after hierarchy formation with little impact on the number of fights per sow. As space is often cited as a limiting resource on farms, this could be an attractive methodology for producers in order to limit the effects of aggression between sows at mixing.

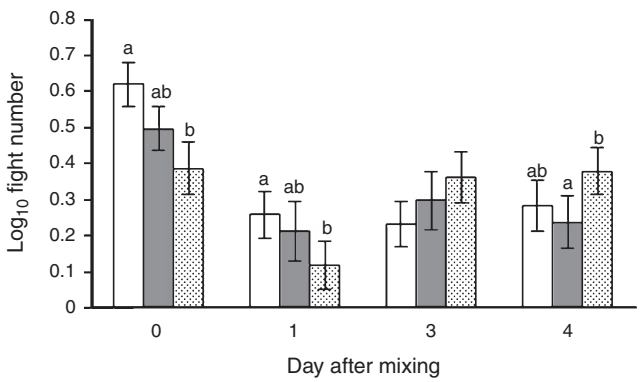

Fig. 1. The effects of 2 m^2/sow (LOW □), 4 m^2/sow (MED ■) or 6 m^2/sow (HIGH ▩) in group-housed sows on fight number per day/sow (on d 4 treatments were standardised to 2 m^2/sow). Data are presented as $\log_{10}$-transformed means ± SEM; significant differences between treatments, within day, are highlighted using superscripts ($^{a, b}P < 0.01$).

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Maternal dietary energy rather than lysine intake during late gestation positively influences piglet birth weight

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Increasing the feed allowance in late gestation for gilts and sows is known as 'bump feeding'. However, the effect of bump feeding on piglet birth weight, particularly in herds of high prolificacy (>14.5 total piglets born/sow), is unclear. Further, the relative contributions of dietary amino acids and energy on potential improvements in piglet birth weight remain unclear. Preliminary results (Gonçalves *et al*. 2015) showed that compared to high-energy intake, low energy during late gestation significantly decreased body weight (BW) gain with a greater magnitude in sows than in gilts. Additionally, there was no difference between bump feeding and the control in the number of total piglets born or in total litter weight. The objective of the current study was to evaluate the effects of lysine (Lys) and energy intake during late gestation on individual piglet birth weight and on subsequent reproductive performance of gilts and sows. It was hypothesised that both maternal dietary Lys and energy in the late gestation period would affect piglet birth weight.

A total of 1105 females (PIC 1050; d 90 of gestation until farrowing) were blocked by parity (P1 or P2+). Females within each parity group were housed in pens, blocked by weight within each pen and individually assigned to dietary treatments consisting of combinations of two standardised ileal digestible lysine (Lys) intakes (10.7 or 20.0 g/day) and two energy intakes (18.8 or 28.3 MJ net energy (NE)/day). Diets were corn-soybean meal-based. Data were analysed using generalised linear mixed models (SAS®; USA) with pen as the experimental unit for parity and the individual female as the experimental unit for dietary treatments.

Individual born alive birth weight was approximately 30 ± 8.2 g heavier (mean ± SEM, P=0.01; Fig. 1) in high energy intake compared to low energy intake females, regardless of Lys intake. Overall, piglets born from sows were approximately 97 ± 9.5 g (mean ± SEM) heavier ($P < 0.001$) than those born from gilts. There was no evidence for dietary differences ($P > 0.17$) on the coefficient of variation for birth weight within a litter, and neither on litter size after cross-fostering ($P = 0.46$). Pre-weaning mortality was reduced ($P = 0.03$) by 1.2 percentage points in piglets suckling from high Lys intake females, regardless of energy intake. There was no evidence for differences ($P > 0.10$) between dietary treatments on wean-to-oestrus interval, percentage of females bred until 7 days after weaning, and subsequent performance (farrowing rate, total born, and born alive). These data support a positive dietary energy intake effect, but no evidence for any Lys intake effect, on piglet birth weight under commercial conditions in a high prolificacy herd. No evidence for any dietary effects on subsequent reproductive performance of either gilts or sows was apparent. Thus, the positive effect of bump feeding on individual piglet birth weight is due to energy rather than lysine intake. While females gained weight regardless of dietary treatment, this suggests 18.8 MJ NE/day could be below their total energy requirement in late gestation.

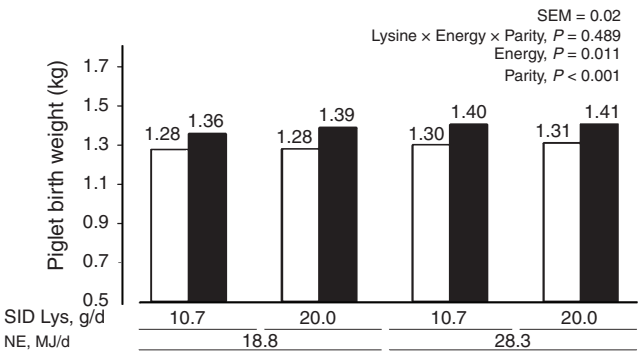

Fig. 1. Effects of lysine and energy intake during late gestation on individual piglet birth weights of gilts (□) and sows (■).

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Oocyte quality and embryo survival are impaired when sows mated in lactation lose more than five percent of their body weight

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The later stages of sow ovarian follicle growth and ovulation are normally inhibited by piglet suckling and the high metabolic demands of milk production during lactation (Quesnel 2009). Stimulating a fertile oestrus in lactation presents an opportunity to increase piglet weaning age without impairing farrowing frequency. Although lactation body weight (BW) loss is known to affect oocyte quality, follicular development and sow fertility after weaning (Quesnel 2009), no studies have investigated the effect of high BW loss on the quality of oocytes shed during an oestrus in lactation. This study tested the hypothesis that a high BW loss during lactation would reduce the capacity of sow oocytes collected on d 21 of lactation to develop *in vitro* and reduce embryo survival *in vivo* when sows were mated in lactation.

A total of 98 Large White × Landrace multiparous sows (parity 3.3 ± 0.2, mean ± SEM) was studied, with sows slaughtered at one of two time points; d 21 post-partum (prior to expected lactation oestrus expression in some proportion of sows; n = 39), or d 30 after being bred at their lactational oestrus (n = 47). Twelve sows (20%) did not express lactational oestrus and were returned to the breeding herd. On d 1 and 21 of lactation and at the first sign of lactation oestrus, sow BW was recorded. From d 18 until slaughter on d 21, or until expression of lactational oestrus and breeding, sows received 15 min of full physical boar contact daily. Ovaries were collected from sows slaughtered on d 21 and all follicles larger than 4 mm were aspirated. Recovered cumulus-oocyte complexes were matured and fertilised *in vitro*. Cleavage rate was recorded 28 h after fertilisation, and the stage of embryonic development was assessed on d 6 after fertilisation. All other sows were artificially inseminated (AI) at first detection of oestrus in lactation. On d 30 after AI, sows were slaughtered, ovulation rate was recorded, and embryo survival was calculated as the number of embryos as a proportion of the number of corpora lutea. Sow BW loss was calculated as the percentage of d 1 BW lost at either d 21 post-partum or at lactational oestrus. Data were analysed using a univariate general linear model with sow as the experimental unit (IBM SPSS, Version 20.0; USA).

The percentage BW loss did not affect the time taken for sows to express lactational oestrus (22.7 ± 0.24 days). However, sows that lost more than 5% of their BW had reduced blastocyst development *in vitro* and poorer embryo survival *in vivo* (Table 1). Data collected from the present study suggest that greater BW loss over lactation reduced oocyte quality and embryo survival, without affecting follicle size and ovulation rate, when sows were mated before weaning. This supports previous studies (Quesnel 2009) that showed higher BW loss during lactation consistently results in reductions in early embryo survival when sows are mated after weaning. This is likely the result of an impaired follicular environment in which the oocyte matures.

Table 1. The effect of sow body weight (BW) loss over lactation on embryo cleavage and blastocyst development *in vitro*, and ovulation rate and embryo survival *in vivo*. Values are means ± SEM

	Lost more than 5% BW	Lost less than 5% BW	*P* value
N[A]	22	17	
Mean follicle size	6.2 ± 0.2	6.2 ± 0.3	NS[C]
% cleaved	69.7 ± 6.4	71.9 ± 7.2	0.066
% blastocyst/total	31.8 ± 3.8	42.2 ± 4.3	0.006
N[B]	33	14	
Ovulation rate	22.9 ± 0.8	22.0 ± 1.3	NS
Embryo number	12.7 ± 0.7	13.7 ± 1.0	NS
Embryo survival (%)	57.0 ± 3.4	64.5 ± 5.2	0.034

[A]N, number of sows slaughtered on day 21 of lactation. [B] N, number of sows slaughtered on d 30 after AI. [C]NS, not significant.

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The influence of cumulus cells on porcine oocyte maturation in the presence of L-carnitine

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Porcine *in vitro* maturation (IVM) enables large numbers of oocytes to be salvaged from ovarian follicles and cultured *in vitro* to a stage at which they are capable of facilitating fertilisation and subsequent embryonic development. However, resultant embryos have reduced viability compared to those produced *in vivo* (Kikuchi *et al.* 2006). The relative contributions of carbohydrate and lipid metabolism during IVM and the effect of cumulus cells on lipolysis are still poorly understood. The objective of this study was to determine the influence of cumulus cells on porcine oocyte maturation in the presence of L-carnitine (LC), a lipid metabolism stimulant, under reduced carbohydrate conditions. It was hypothesised that the LC treatment would increase cellular energy generation, thereby improving the maturation of oocytes.

Cumulus-oocyte complexes (COCs) were recovered from 3–6 mm follicles of abattoir-derived ovaries, and either kept as intact COCs or denuded of their cumulus cells (CCs). Groups of COCs and denuded oocytes (DOs) were matured separately or co-cultured together to assess any indirect influence of CCs (DOs + CCs). Modified porcine oocyte medium (POM; Yoshioka *et al.* 2008) containing a low concentration (1.5 mM) of glucose and no pyruvate and lactate was supplemented with either 0 or 12 mM LC. Nuclear maturation was assessed at 44 h of IVM. The intra-oocyte concentration of ATP was also measured in oocytes in the presence (+PL) and absence (-PL) of pyruvate and lactate at 0, 22 and 44 h. Data were analysed using ANOVA and Fisher's unprotected lease significant difference test (GENSTAT, 16th Edition; UK).

Supplementing LC had no significant effect on the proportion of oocytes that were mature at 44 hours (Fig. 1). As expected, significantly greater proportions of oocytes cultured in the presence of cumulus cells were mature when compared to DOs. An interaction was observed between time and treatment on the ATP concentrations observed per oocyte (Fig. 2). Mean ATP concentrations were increased ($P < 0.05$) in oocytes matured in the presence of pyruvate and lactate for 44 h compared to all other treatments across all time points. The results indicated that under low carbohydrate conditions LC does not enhance oocyte nuclear maturation, irrespective of the presence of CCs, nor does it significantly increase ATP production. Thus, the hypothesis that LC treatment would improve oocyte maturation by increasing cellular energy generation was rejected. Further studies are required to elucidate whether cumulus cells play a role in porcine oocyte lipid metabolism.

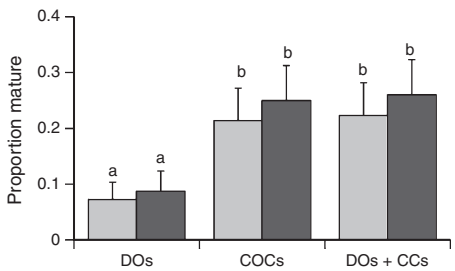

Fig. 1. Effect of L-carnitine (LC) on oocyte maturation (mean ± SEM) at 44 hours of IVM. Oocytes were matured with □ 0 mM and ■ 12 mM LC. Different letters indicate differences between treatments ($P < 0.05$). Denuded oocytes (DOs), cumulus-oocyte complexes (COCs), cumulus cells (CCs).

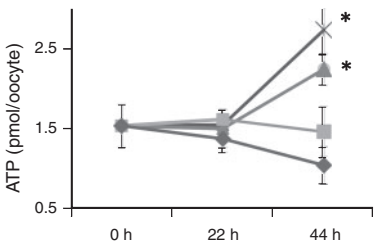

Fig. 2. Effect of L-carnitine (LC) on intra-oocyte ATP concentrations (mean ± SEM) at 0, 22 and 44 hours IVM. Oocytes were matured with (+PL) or without (-PL) pyruvate and lactate, and treated with 0 (-LC) or 12 mM (+LC) L-carnitine (×+LC/+PL, ▲-LC/+PL, ■+LC/-PL, ◆-LC/-PL). Values with an asterisk are significantly different ($P < 0.05$).

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Administration of human chorionic gonadotropin in early pregnancy increases ovarian activity in sows

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Reproductive performance is affected by variables such as nutrition and season, which can have a negative effect on ovarian function (Greer 1983; Stalder *et al.* 2004). Human chorionic gonadotropin (hCG) increases ovarian follicle growth in gonadotropin-treated gilts and increases steroid production in these gilts and in pregnant sows by virtue of its luteinising hormone-like activity. Literature, regarding an hCG effect on follicle growth in mated sows, is not evident. However, there is potential to use hCG after mating in early pregnancy if follicle growth occurs and increases oestrogens, as this could reinforce the embryonic signal for maternal recognition of pregnancy. This could then improve pregnancy maintenance and subsequent litter size. The aim of this pilot study was to test the hypothesis that administration of hCG on day 12 after mating would induce ovarian follicular growth during early pregnancy.

During lactation, 36 sows were assigned to receive a restricted feed intake of 4 kg/d for parity one, and 5 kg/d for parity two or three during the final 10 days of a 28 day lactation, with the objective of simulating lower feed intakes associated with summer. At 12 days after mating, 17 sows received an intramuscular injection of 1,000 IU hCG [Intervet (Pty.) Ltd]; this dose was based on Tilton *et al.* (1989). The diameters of the 10 largest ovarian follicles were measured for each sow by transrectal ultrasound on d 12, 16, 20, 24, and 28 after mating. Differences between days for mean follicle diameters were compared using the GLM procedure with treatment, day, treatment × day interaction, and parity as fixed effects (SAS®; USA).

Follicle size was increased ($P < 0.01$) on d 16 to 28 after mating by hCG treatment (Fig. 1). Maximum follicle size occurred on d 20 for hCG-treated sows at 9.1 mm compared to 2.8 mm for non-treated sows. Thereafter, follicle size decreased for the hCG treated group. There were no detrimental effects on reproductive performance in the next parity (data not shown). Sows that did not receive hCG did not exhibit any follicular growth during this period.

The increased follicle growth attributed to the hCG treatment could prove beneficial in early pregnancy if this follicle growth occurs with production of oestrogen, potentially reinforcing the signal for maternal recognition of pregnancy. Additionally, by d 12 of gestation corpora lutea respond to luteinising hormone stimulation with increased progesterone production potentially improving individual embryo survival. Further work should explore endocrine effects and whether these results translate to increased farrowing rates and litter sizes.

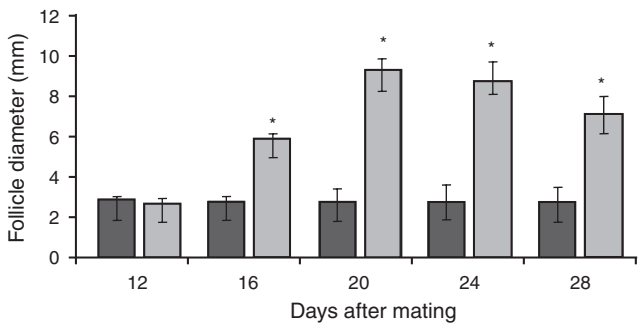

Fig. 1. Follicle diameter (mean ± SEM) for sows given restricted feed (n =19), or restricted feed + hCG (n = 17) on d 12, 16, 20, 24, and 28 after mating. (*$P < 0.01$). Restricted Feed (■); Restricted Feed +hCG (▨).

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Fighting of gilts after mixing is associated with early removals, altered litter sex ratio and lower piglet survival

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Aggressive behaviour can compromise the welfare of group-housed gilts and sows and affect reproductive performance (Spoolder *et al*. 2009), but the extent of individual aggression is frequently unknown. Lesions resulting from fighting indicate the intensity and extent of aggressive encounters between sows (Bunter and Boardman 2015). The aim of this study was to investigate if lesions resulting from fighting amongst gilts were associated with subsequent reproductive outcomes for group-housed sows.

Gilts (n = 3238) scored for the extent of lesions resulting from fighting at 24 hours after mixing post-selection and again before farrowing (n = 1929) were used in this study. Lesion scoring and their grouping were described in Bunter and Boardman (2015). Gilts were also scored for pre-farrowing condition and locomotion. Condition scores represented under- to over-conditioned sows (scored: –1, 0, 1) while locomotion was scored on a four point scale, from 0 (normal) to 3 (very poor). Gilts removed from the herd after selection without a farrowing event were identified (n = 881), and farrowed sows were recorded for litter size and average piglet birth weight. Data were also available from a smaller subset of litters (n = 915) at the time of analyses, to investigate sex-ratio of live born piglets within litters and piglet survival until weaning. The association between lesion score categories (Bunter and Boardman 2015) and removals without a farrowing event was assessed using logistic regression, submitting score groups (anterior, posterior, or whole body) separately to the analysis, after accounting for selection date (61 levels) and breed (two levels). Implications of lesion scores as covariates for reproductive traits were examined using linear models with ungrouped scores (SAS®; USA).

The extent of fight lesions 24 hours after mixing was highly associated ($P < 0.001$) with selection date and breed, but not gilt weight. Breed differences in lesion scores were no longer evident for sows rescored before farrowing. Relative to other score groups, there was an increased tendency for selected gilts with high anterior lesion scores (group 3) recorded after selection to be removed from the herd without a farrowing event (31.1 vs 26.3%, $P = 0.026$), but removals for a specific reason (e.g. feet and leg problems, stale, not in pig) were not statistically significant. There were no significant associations between lesion scores of gilts after selection and their pre-farrowing condition or locomotion scores, or between lesion scores (either after selection or before farrowing) with sow reproductive traits, such as litter size or average piglet birth weight. In contrast, sows with more fight lesions scored before farrowing had reduced pre-farrowing condition score ($P = 0.005$), poorer locomotion scores ($P < 0.001$) and a slightly shorter lactation length ($P = 0.004$). Higher anterior, but not posterior, lesion scores recorded on gilts after selection were also linearly associated ($P = 0.041$) with an increasing ratio of female : male piglets. This equates to a maximum change in sex ratio of 4.2% across a seven-score range in lesions (0 to 30+ fight lesions), and implies that engagement in fighting had physiological consequences that directly or indirectly affected the sex-ratio of offspring born almost 6 months later. A sex-ratio biased towards females has been repeatedly demonstrated in guinea pigs subjected to an unstable social environment and was accompanied by reduced maternal androgens, which also affect fertility (Kemme *et al*. 2009). Increasing posterior ($P = 0.019$), whole body ($P = 0.021$) or to a lesser extent anterior lesion scores ($P = 0.057$) recorded on gilts 24 hours after mixing were also associated with a decreased proportion of piglets that survived from birth until weaning.

Overall, lesion scores resulting from fighting explained little of the variation in gilt removals ($R^2 < 0.5\%$) or reproductive traits (typically $R^2 < 1$–2%), even when associations identified were statistically significant. Therefore, individual variation in engagement in fighting is just one of many, frequently unidentified factors contributing to variation in sow wastage and reproductive outcomes under group housing. Moreover, lesion scores are non-specific descriptors for individual behaviours and (or) stress relating to aggression at the time of scoring, and thus may have limited predictive utility.

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Locomotion scores in early gestation of younger parity sows are associated with fight lesions and body condition

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Lameness in gilts and breeding sows is a major cause of premature removal or culling throughout the Australian pork industry, leading to economic and production losses (Dewey *et al.* 1992; Anil *et al.* 2005). Although lameness can have many causes, the injuries acquired as a result of negative interactions and aggression between sows can be common in group-housing systems (Heinonen *et al.* 2013). The aim of this study was to investigate if lameness in gestating sows was associated with sow condition and the negative interactions between sows, such as fighting.

In total, 1,975 gestating gilts (P0) and parity one (P1) and two (P2) sows of Large White, Landrace and Duroc origins were recorded at a single site. The P0 sows were kept in pens of two, seven or 11, while older parity sows were moved to mixed parity groups of 11 in different sheds, after mating. All pens consisted of half concrete slats and half solid concrete flooring. Observations for all traits were taken at five weeks of gestation, by the same observer. Sow locomotive abilities were scored when sows were encouraged to stand and walk around their pen, ranging incrementally between 0 = normal movement (no evidence of lameness) and 3 = non-weight bearing on affected limb or an inability to walk. Sow condition was scored as average, over- or under-conditioned. Fight lesion scores were used to describe the extent and number of injuries present, ranging between 0 = no scratches present to 3 = > 10 scratches present, and lesions were classified as new or old. Sows were also noted as willing or unwilling to move, depending on whether encouragement to move was required: if encouragement was needed, the sow was classed as unwilling to move. Date of scoring, breed, and a term for parity/shed (gestation accommodation) were accounted for in the analyses as nuisance factors when required ($P < 0.05$), using linear models to identify associations between scores; treating one score as a dependant variable and the second as a class effect.

Over the complete study 87 sows exhibited some degree of lameness at five weeks of gestation, with locomotion scores of 1 or 2. No sows suffered from severe lameness (score 3). Concurrent scores for fight injuries, condition and willingness to move were all found to be significantly associated with locomotion score ($P < 0.001$). Sows with higher scores for fight lesions, over-conditioned or those unwilling to move had poorer locomotion scores (Table 1). However, age of the fight lesion (old vs new) was not associated ($P > 0.05$) with locomotion score. Date of recording was the only factor significant ($P < 0.001$) for locomotion score, as parity/shed and breed effects were not statistically significant. Over-conditioned sows were more likely ($P < 0.001$) to have fight lesions. Neither scores for condition or fight injuries were significantly associated with a sow's willingness to move ($P > 0.05$). Date, parity/shed and breed significantly affected the incidence of fight injuries ($P < 0.001$), and to a lesser extent (and excluding date), sow condition ($P < 0.05$). None of these nuisance factors appeared to be associated with a sow's willingness to move. The presence of fight injuries, over-conditioned sows and a lack of willingness to move were associated with the incidence of lameness in sows in early (five weeks) gestation. Developing strategies to reduce fighting and manage nutrition may have favourable outcomes for locomotion and condition of group-housed sows in early pregnancy.

Table 1. The associations between fight lesions, condition, willingness to move and locomotion score

Locomotion score	Fight lesions				Body condition			Willingness to move	
	0	1	2	3	Under	Av.	Over	Yes	No
0	1178	479	220	11	93	1681	114	1861	27
1	33	25	14	2	3	61	10	68	6
2	8	1	4	0	0	9	4	9	4
Lame sows (%)	3.4%	5.6%	7.6%	15.3%	3.1%	3.9%	10.9%	3.9%	27.0%
P value		<0.001				<0.001		<0.001	

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Temporary confinement of sows for four days after farrowing has little influence on postural changes

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Confinement of loose-housed sows for a few days after farrowing where piglets are at greatest risk of dying can potentially reduce piglet mortality. Moreover, sow behaviour in early lactation is characterised by prolonged lateral lying (Baxter *et al*. 2011), indicating that the physical restriction imposed by confinement might not be as detrimental for sow welfare in this period of time compared to other, more active periods. This study aimed at investigating if confinement for 4 days after farrowing influenced sow behaviour, with the hypothesis examined that loose-housed sows had more postural changes than loose-housed sows.

The study was conducted in a Danish piggery with SWAP (Sow Welfare And Piglet protection) farrowing pens. Sows were randomly allocated to one of three treatments: loose-loose (LL: loose from placement in the farrowing unit to weaning; n = 20); loose-confined (LC: loose from entry to end of farrowing and confined to d 4 after farrowing; n = 19); and confined-confined (CC: confined from d 114 of gestation to d 4 after farrowing; n = 19). All sows were loose housed from d 4 of lactation to weaning, after 4 weeks. Behavioural observations of sow postures (standing, sitting, lying sternally and lying laterally) were obtained from video recordings on days 1, 2 and 3 after farrowing in the time intervals 0400–0600 h, 1000–1200 h, 1600–1800 h, and 2200–2400 h. Data were statistically analysed by use of linear models (SAS®; USA) (PROC MIXED).

Regardless of treatment, sow behaviour was characterised by a low frequency of postural changes (<12 postural changes in 2-h bouts) and a large proportion of time spent in lateral recumbency (80–120 min of 2-h bouts), especially on d 1 and 2 after farrowing. Postural changes increased during the day in all treatments but more so in LL than LC and CC ($P = 0.02$) (Fig. 1*a*). Similarly, the frequency of rolling (changes between lateral and sternal postures) increased from d 1 to d 3 after farrowing in all treatments, but LL had a greater increase than LC and CC ($P < 0.001$). Time spent lying laterally was similar across treatments ($P = 0.66$) (Fig. 1*b*). Sows generally spent more time standing during daytime intervals than night-time intervals, but the diurnal pattern was dissimilar in the three treatments ($P < 0.01$) and differed in the three days ($P < 0.01$).

Loose-housed sows displayed a different behavioural pattern than sows that were confined to d 4 after farrowing (treatment LC and CC). Differences however were mainly seen on d 3, indicating that sow behaviour was only marginally affected by confinement in the first days of lactation. In conclusion, the results suggested that confinement for 4 days after farrowing had little influence on sow behaviour.

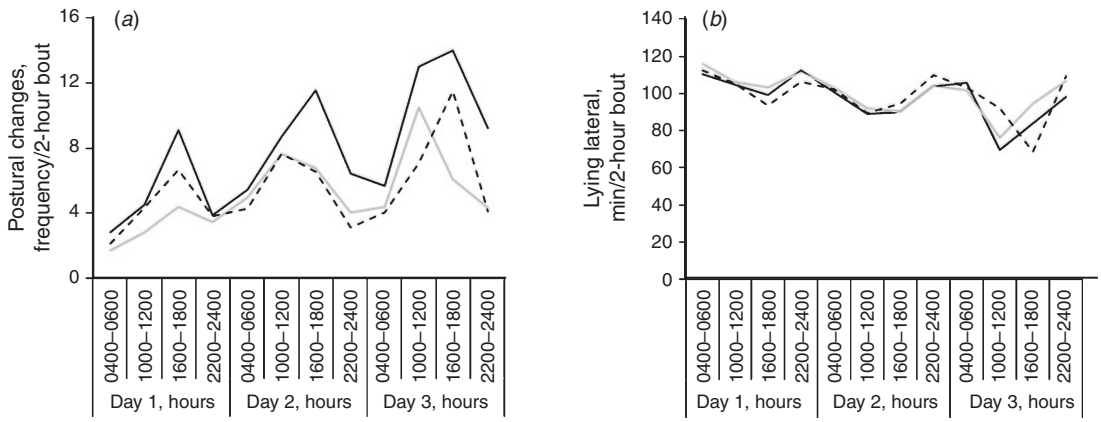

Fig. 1. Postural changes (*a*) and time spent lying laterally (*b*) in 2-h observation bouts at d 1 to 3 after farrowing for loose-housed sows (LL –), sows that were confined from the end of farrowing to day 4 after farrowing (LC –), and sows that were confined from gestation d 114 to d 4 after farrowing (CC - -).

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Short and long-term repeatability of individual sow aggressiveness

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Published literature on the repeatability of individual sow aggression over multiple feeding bouts in a day or over the longer term does not exist, despite its implications for sow welfare (Verdon *et al*. 2015). This study tested the hypotheses that the aggressive behaviour of individual sows early after mixing into groups will (1) be consistent over multiple feeding bouts, and (2) be related to aggressive behaviour within the first gestation and (3) between the first and second gestations.

For the purpose of this paper, recently inseminated gilts were classified as sows. A total of 275 Landrace × Large-White sows was randomly mixed into uniform parity groups of 10 (1.8 m^2/sow) within 7 days of insemination for their first and second gestations (200 sows per gestation with 126 sows observed in both gestations). Incidents of aggression delivered and received by individuals were observed for 30 min after four daily feeding bouts (0730, 0900, 1100, and 1500 h) at the day after mixing (d 2) and at d 9 and 51 of the first gestation, and at d 2 of the second gestation. At d 2 of the first gestation, sows were classified as 'Submissive' (SM) if they delivered little or no aggression, 'Subdominant' (SD) if they received more aggression than delivered, and 'Dominant' (D) if they delivered more aggression than received. At d 2 of both gestations, the aggression index for each sow [i.e., aggression delivered/(aggression delivered + aggression received)] was also calculated. An ANOVA for repeated measures examined the effects of SM, SD and D classification as well as the effects of feeding bout number on aggressive behaviour at d 2 of the first gestation. Data were square-root transformed prior to this analysis. The repeatability of the sow aggression index from d 2 to d 9 and 51 of the first gestation, and from d 2 of the first gestation to d 2 of the second gestation were tested using Spearman rank correlations (IBM SPSS, Version 17.0; USA).

The aggression index at d 2 of the first gestation correlated to that at d 9 ($r = 0.69$, $n = 197$, $P < 0.001$) and d 51 ($r = 0.53$, $n = 137$, $P < 0.001$) of the first gestation as well as to that at d 2 of the second gestation ($r = 0.50$, $n = 125$, $P < 0.001$). The between-gestation correlation was weaker than within-gestation relationships. Aggression delivered by SM and SD sows at d 2 of the first gestation was relatively constant regardless of feeding bout, but aggression delivered by D sows declined over subsequent bouts (classification × bout, $F_{6,318} = 9.96$, $P < 0.01$; Fig. 1). Consequently, aggression received by all sows reduced over the same period ($F_{3,318} = 25.6$, $P < 0.001$) although D sows received the least aggression ($F_{2,106} = 5.5$, $P < 0.05$; Fig. 1).

While genetics is likely to contribute to sow aggression, the reduced strength of the between-gestation correlation suggests that social experience and group composition may also influence the aggressive phenotype. Multiple bouts may provide SD and SM sows with increased opportunity to access food in later feeding bouts with reduced risk of aggression and injury, but will not prevent them from receiving aggression.

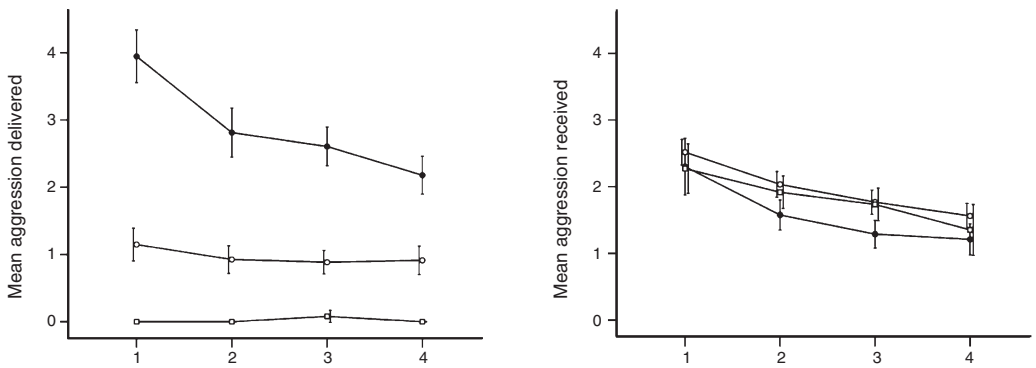

Fig. 1. Mean (± SEM) aggression delivered and received by Dominant (●), Subdominant (○) and Submissive (□) sows over four feeding bouts (x axis) at day 2 post-mixing for the first gestation.

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Effect of sow confinement and non-confinement during parturition on piglet viability

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Sows housed in farrowing crates have previously been shown to have prolonged farrowing durations compared to sows housed in confinement-free systems (Oliviero *et al*. 2008). An increase in farrowing duration can increase the degree of hypoxia in the piglet and consequently decrease piglet viability at birth (Herpin *et al*. 1996). This study tested the hypothesis that measures of piglet viability would be improved when sows farrowed in confinement-free compared to confinement housing systems.

One hundred and fifty-four piglets were born from gilts that were housed in a swing-sided pen with the sides of the pen open (OPEN; n = 69 piglets) or with the sides closed (CLOSED; n = 85 piglets) for the entire experimental period. At the birth of each piglet, a mixed blood sample (sow and piglet blood) was collected from the umbilical cord and plasma glucose was measured. The times taken from birth to stand, reaching the udder of the sow and sucking on a teat were recorded for each piglet. Two hours after birth, rectal temperature and body weight were recorded for each piglet. Data were analysed using a general linear model with farrowing treatment and replicate as fixed effects and total litter size as a covariate (IBM SPSS, Version 21.0; USA). Data were considered significant at $P < 0.05$.

The number of total and live born piglets was not different ($P > 0.05$) between treatments, and averaged 12.1 ± 0.7 and 11.4 ± 0.7 piglets per sow, respectively. Piglets born from sows housed in OPEN pens took less time to stand, reach the udder and suck on a teat compared to piglets from sows housed in CLOSED pens (Fig. 1). There was no difference between treatments in umbilical cord glucose concentration (OPEN, 3.9 ± 0.1 vs CLOSED, 3.7 ± 0.1 mmol/L; $P = 0.82$), rectal temperature at 2 hours after birth (OPEN, 37.0 ± 0.2 vs CLOSED, 37.4 ± 0.2°C; $P = 0.85$) or weight at 2 hours after birth (OPEN, 1.4 ± 0.03 vs CLOSED, 1.4 ± 0.04 kg; $P = 0.16$). These results indicate that allowing sows to farrow in a confinement-free environment can improve certain aspects of piglet viability, which could lead to potential improvements in piglet performance in these systems.

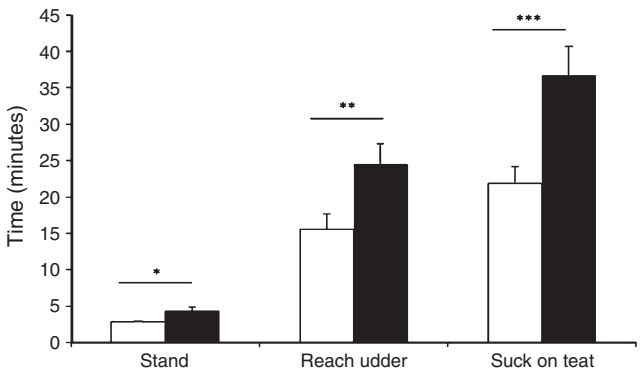

Fig. 1. Time measured from birth for piglets to stand, reach the udder and suck on a teat from sows housed in swing-sided pens with the pen open (□) or closed (■) during parturition. Horizontal line within a trait indicates statistical difference between treatments (* = $P < 0.1$, ** = $P < 0.05$, *** = $P < 0.01$). Data are means ± SEM.

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Pre-partum straw-directed behaviour by sows in farrowing pens is positively associated with piglet survival

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Non-confinement farrowing is generally associated with higher piglet mortality than farrowing crates, including increased risk from overlying by the sow (Baxter *et al*. 2012). These are significant welfare and economic issues that will undoubtedly influence producers' consideration of adopting lower confinement housing for sows at farrowing and during lactation. However, public interest in achieving non-confinement during all stages of production is nevertheless a constant driver for change that cannot be ignored by industry. Large between-sow variation in piglet mortality in farrowing/lactation pens has been reported. For example, Andersen *et al.* (2005) investigated individual sow differences in pre-partum interaction with straw bedding to explain some of this variation. While research has identified the importance of straw in farrowing pens to promote maternal behaviour, the occurrence of variation also highlights the opportunity for selection of sows better-suited to farrowing in pens. Andersen *et al*. (2005) and Westin *et al*. (2015) reported positive associations between pre-partum straw-directed behaviour and careful behaviour by sows in the peri-partum period which indirectly was associated with lower mortality. In the present experiment we investigated the association between self-selection of straw by sows prior to farrowing in pens, performance of pre-partum sow behaviour and piglet survival. The hypothesis examined was that straw-directed behaviour would be positively associated with improved piglet survival in farrowing pens.

The pre-farrowing behaviour of 40 Large White-Landrace sows (parity 1–6) was collated from digital video records [M. Šafro & Co. Ltd. (MSH), Latvia]. Sows farrowed in pens measuring 2.4 m by 3.3 m. Each pen contained two areas: a 'nest area' (2.4 × 1.7 m) and a 'non-nest' area (2.4 × 1.6 m), separated by a 0.27 m high metal step-over barrier. The nest area incorporated internal sloped panels on the rear and one side wall to assist sow posture changing behaviour; the rear of the nest area also formed a heated piglet creep. A wire basket attached on the opposite sidewall was filled with 4 kg straw each morning before sows farrowed. The nest area floor consisted of a thin layer of wood shavings on a 30-mm thick rubber mat over solid concrete, sloped towards the barrier. The non-nest area contained the sow feeder and drinker, and the floor comprised both solid and slatted flooring. Four farrowing pens were located in a non-heated, partially insulated room. The study was conducted over 13 replicates in time, with 1–4 sows observed per replicate. The timing of piglet deaths was recorded, with cause of death confirmed by necropsy.

The video record of each sow's farrowing event was collated using one-zero (binomial) sampling to record whether the sow performed any of 10 behaviours (see below) during 144, 10-min periods from 24 h pre-partum to the birth of the first-born piglet in the litter. Data were expressed as the mean probability that the specified behaviour was observed during any 10 min interval 24 h pre-partum, and analysed using correlation analysis (Genstat edn. 14.1; VSN International, UK). The listed behaviours were: (1) Take straw from rack; (2) Carry straw in mouth; (3) Root/nose straw on floor; (4) Paw at straw; (5) Root/nose pen walls; (6) Root/nose bare floor; (7) Feed; (8) Drink; (9) Defaecate; and (10) Urinate. Piglet mortality in the litters averaged 3.0 ± 2.71 (mean ± SD) piglets and ranged from 0 to 10 deaths per litter (0 to 83.3% of those born alive). There was an inverse association between the combined straw-directed behaviours (1 to 4) and piglet mortality ($r = -0.328$, $P < 0.05$), and piglet mortality due to overlying by the sow on d 1 post-partum ($r = -0.352$, $P < 0.05$). However, there were no associations between pre-partum nesting behaviour of the sow and piglet mortality due to overlying after d 1 post-partum ($P > 0.05$). Sows that spent more time in the nest area during 24 h pre-partum tended to have fewer piglet losses due to small/weak/chilled ($r = -0.299$, $P = 0.061$), and correspondingly, sows that spent more time outside the nest area pre-partum tended to have more piglet losses due to small/weak/chilled ($r = 0.302$, $P = 0.059$).

The results support the hypothesis and the findings of Andersen *et al.* (2005) and Westin *et al*. (2015), that increased straw-directed behaviour by sows in the 24 h pre-partum was associated with reduced piglet mortality in lactation, and specifically, due to reduced overlying by the sow within the first day of life.

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Prenatal and neonatal gilt management and anti-Müllerian hormone: effects on the ovary and response to the boar

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Sub-optimal sow reproductive performance constrains breeding herd efficiency and causes premature sow culling. In cattle, the capacity of ovaries to respond to gonadotrophins and produce high quality embryos is determined in the neonatal period and is positively related to circulating concentrations of anti-müllerian hormone (AMH) (Ireland *et al*. 2011). Further, more gilts reared in small compared to large litters survived to parity six (Flowers 2012). The current study had two objectives: first, to determine the effect of prenatal and neonatal environment on ovarian development and response to boar stimulation, and second, to determine relationships between plasma AMH and ovarian characteristics, and response to boar stimulation.

A total of 101 gilts (Camborough 29 × PIC 400) was selected from small or large birth litters. At 12–24 h after birth, male pigs were cross-fostered into small litters to achieve suckled litter sizes of 9 or 12 piglets creating a 2 × 2 factorial arrangement of treatments with main effects being gestated litter size (≤9 vs ≥12 piglets; Small and Large, respectively) and suckled litter size (9, Small vs 12, Large). A plasma sample was collected at weaning (20 ± 0.1 days; mean ± SEM) and at 20 weeks of age, and assayed for concentrations of AMH using a pig AMH ELISA kit (CUSABIO Biotech, China). From 20 weeks of age, gilts received daily exposure to a mature boar for 14 days. Thereafter, gilts were marketed at 102 ± 0.5 kg and 169 ± 1.5 d of age, and ovaries recovered. The number of corpora lutea (CL) and surface antral follicles <1 mm were recorded for a subset of gilts (Table 1). Puberty attainment was defined as the presence of CL. Gilts were allocated to a high or a low weaning or 20 week AMH group with the cut off being the median value for the population. Treatment and AMH group effects were analysed using ANOVA (Genstat, 15th Edition; UK). Differences between proportions were analysed by Chi-square.

Total surface follicle number and the number of CL were not statistically influenced ($P > 0.05$) by gestated or suckled litter size (Table 1). Puberty attainment showed a trend ($P < 0.1$) to be higher in the Large-Small compared to the Small-Small treatment group (Table 1). Gilts reared in a small litter had higher ($P < 0.05$; main effect) AMH concentrations at weaning (Table 1). There was a weak trend ($P < 0.2$) for puberty attainment in gilts with high (>8.3 ng/mL) compared to low (< 8.3 ng/mL) AMH at weaning (62% vs 45%, respectively). Within the large gestated litter size treatment, puberty attainment was higher ($P < 0.05$) for gilts with a high compared to low AMH concentration (74% vs 42%, respectively) (data not shown).

These data tend to suggest that higher concentrations of AMH at weaning are associated with improved capacity to ovulate in response to boar contact, with this relationship stronger for gilts born into a large litter. If earlier puberty indicates greater potential fertility, the effect of gestated and reared litter size on puberty attainment suggests a possible impact of the Large-Small litter combination on subsequent fertility.

Table 1. Interaction effects of gestated litter size (Small or Large) and suckled litter size (Small or Large) on ovarian characteristics at 169 d of age, puberty attainment and plasma AMH levels at weaning and 20 weeks of age. Values are mean ± SEM

	Litter size treatments (Gestated–Reared)			
	Small-Small	Small-Large	Large-Small	Large-Large
No. gilts	18	19	26	29
No. antral follicles	125.6 ± 14.96	134.2 ± 13.72	126.8 ± 12.38	104.1 ± 12.37
No. corpora lutea	11.2 ± 1.38	12.9 ± 1.19	13.1 ± 0.90	13.3 ± 0.98
Pubertal gilts (%)	39%*	47%	65%*	48%
Plasma AMH, weaning (ng/mL)	9.0 ± 0.58[b]	7.3 ± 0.56[a]	8.4 ± 0.46[b]	7.8 ± 0.45[a]
Plasma AMH, 20 weeks (ng/mL)	6.5 ± 0.43	6.2 ± 0.38	5.9 ± 0.34	6.5 ± 0.34

[a,b]Means is a row not having the same superscript or * are significantly different ($P < 0.05$) or show a trend to be significantly different ($P < 0.1$), respectively.

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A specific carbohydrate diet fed in late lactation to enhance post-weaning fertility in primiparous sows

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In primiparous lactating sows, feed intake is generally insufficient to meet energy requirements for milk production, causing excessive mobilisation of body reserves, and potentially compromising post-weaning reproductive performance. Besides feed intake, the dietary energy source during lactation can also influence post-weaning reproductive performance through luteinising hormone secretion and insulin production (van den Brand *et al*. 2001). Chen *et al*. (2013) showed that glucose and insulin secretion were elevated and subsequent litter size increased by feeding a supplement of carbohydrates rather than fat during the last week of lactation. The object of this study was to use a fully formulated carbohydrate diet (CHO) to increase gonadotrophin by stimulating insulin and glucose secretion in late lactation to improve subsequent litter size in a commercial piggery.

The study was conducted on a commercial production unit in South Australia. Eight days before weaning, primiparous sows (n = 119) weighing 200 ± 6.4 kg (mean ± SD) were allocated based on suckled litter size to a CHO diet (14.3 MJ digestible energy (DE)/kg, 198 g/kg crude protein) or a standard lactation diet (Control; 14.2 MJ DE/kg, 195 g/kg crude protein). The CHO diet was to provide glucogenic content (wheat extruded, dextrose and sugar) instead of fat, and without changing total dietary energy. Only litters with 10 or more piglets remaining 8 days before weaning were included in the study. Feed allowance was increased gradually from farrowing until maximum feed intake was achieved. Feed intake in lactation was recorded daily. Sows and piglets were weighed after litters had been standardised to ≥11 piglets at beginning of lactation, and at weaning. Mating dates, pregnancy status, sow removals and second litter size were recorded. All statistical analyses were performed using the GLM procedures (SAS®; USA).

Body weight loss was less (Table 1) than generally reported (around 10%) for primiparous sows during lactation (Schenkel *et al*. 2010). For sows that were mated within 10 days of weaning, the weaning-mating interval was reduced by half a day ($P < 0.05$) by feeding the CHO diet. However, conception rate and subsequent litter size did not differ between treatments. In conclusion, providing an enriched CHO diet fed in late lactation did not improve subsequent reproductive performance in the present study. This may due to there was no second litter syndrome in those primiparous sows and, therefore, there was little margin to improve fertility. However, there were physiological effects on post-weaning gonadotrophins from the CHO diet in terms of a shorter weaning-mating-interval.

Table 1. Body weight loss and energy balance during lactation, and post-weaning reproductive traits, in primiparous sows fed either a Control diet or a high carbohydrate diet (CHO). Values are mean ± SEM

	Control (n = 60)	CHO (n = 59)
Litter size at allocation	11.1 ± 0.1	11.1± 0.1
Body weight loss (kg)	–7.7 ± 1.4	–5.8 ± 1.2
Energy balance (MJ ME[A]/d)	–11 ± 2[a]	–5 ± 2[b]
Anoestrous (%)[B]	15.5	13.5
Wean-mating-interval (d)	4.8 ± 0.1[a]	4.3 ± 0.2[b]
Average daily feed intake during treatment (kg)	5.5 ± 0.1[a]	5.9 ± 0.1[b]
Conception rate (%)	88	90
Total born second litter	12.6 ± 0.4	12.0 ± 0.5
Born alive second litter	11.8 ± 0.5	11.5 ± 0.5
TB[C]	11.4 ± 0.8	12.0 ± 0.5

[A]ME, metabolisable energy. [B]Sows mated >10 d after weaning or not mated were considered anoestrous. [C]TB, total born. [a,b]Means in a row not having the same superscript are significantly different ($P < 0.05$).

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The relationship between mitochondrial DNA haplotype and litter size in commercial pigs

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The mitochondrial genome (mtDNA) is associated with a number of traits, which include tolerance to heat (Wallace *et al*. 2003), growth and physical performance (Nagao *et al.* 1998), meat and milk quality (Brown *et al*. 1989; Mannen *et al*. 2003), and fertility (Sutarno *et al*. 2002). A key region of mtDNA is the D loop, which is widely used to determine maternal lineages. Maternal lineages cluster into mtDNA haplotypes that have evolved over billions of years (Ruiz-Pesini *et al*. 2004). In this study, we aimed to determine if: pig fertility is directly related to the sow's mtDNA haplotype; sows with mtDNA haplotypes favourable to increased litter size produce more developmentally competent oocytes; and their developmentally competent oocytes have higher mtDNA copy number, which according to Spikings *et al.* (2007) is associated with successful fertilisation.

The D-loop region for 368 sows from four Australian commercial breeders was sequenced to determine their maternal lineages. Litter size was determined for each haplotype. Developmentally competent cumulus-oocyte-complexes (COCs) were selected using the dye, brilliant cresyl blue (BCB), to determine the ratio of developmentally competent (BCB^+) to incompetent (BCB^-) COCs. Oocyte quality was also assessed by quantifying mtDNA copy number. Developmental potential of BCB^+ COCs was assessed by *in vitro* maturation, fertilisation and embryo culture. Statistical differences were determined using ordinary one-way ANOVA followed by parametric multiple comparison post-hoc tests.

In this study, we identified five mtDNA haplotypes (A to E) in the commercial pig breeding population in Australia. Haplotypes C, D and E had significantly larger litter sizes than haplotype A but when live births were assessed only C and E were significantly larger. In addition, fewer sows from haplotype A produced $\geq$15 piglets per pregnancy than C ($P < 0.05$), D ($P < 0.01$) and E ($P < 0.05$). The ratio of BCB^+ to BCB^- COCs per ovary was similar for each haplotype. However, mtDNA copy number for BCB^+ oocytes was higher for haplotype D oocytes than for haplotypes B ($P < 0.01$) and E ($P < 0.001$). The proportion of oocytes progressing to metaphase II following *in vitro* maturation was lower for haplotype C oocytes compared with haplotypes A ($P < 0.001$), B ($P < 0.01$) and E ($P < 0.05$). Following insemination of BCB^+ oocytes and culture to the blastocyst stage, fewer oocytes from haplotype C fertilised and cleaved than A ($P < 0.05$), B ($P < 0.01$) and E ($P < 0.01$). However, there was no difference ($P > 0.05$) in blastocyst development rates amongst the haplotypes. Although haplotype C produced proportionally fewer developmentally competent oocytes, the resultant embryos had the same potential to develop to blastocyst as embryos from other haplotypes. This highlights a more pronounced selection process during gametogenesis for haplotype C.

The results demonstrated that haplotypes C and E produced significantly larger litter sizes. However, each haplotype had different rates of oocyte maturation and fertilisation. This suggested that each haplotype has very different mechanisms for generating their respective litter sizes. These findings could lead to a simple genotyping test for the selection of sows with better reproductive capacity, which would enhance economic breeding values.

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Multi-suckling and sow-piglet separation: effects on lactation oestrus

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Piglet sucking is the primary cause of lactation anoestrus in sows. It has been proven, separately, that enforced, protracted periods (12 h) of sow-piglet separation and daily boar contact can reliably stimulate a high incidence of lactation oestrus (Kemp and Soede 2012). Within multi-suckling systems, the frequency of suckling reduces as lactation progresses and has been associated with low, but unpredictable, incidences of lactation oestrus (Lindgren *et al*. 2013). The objective of the current study was to determine whether 6 h of sow-piglet separation of sows housed individually or in groups, increases the incidence of lactation oestrus in response to supervised, daily, boar contact.

Large White × Landrace sows (parity 1.2 ± 0.09; mean ± SEM) suckling 10.3 ± 0.22 piglets were used in a 2 × 2 factorial arrangement of treatments to compare the effect of two housing systems [farrowing crates (n = 23) vs multi suckling (n = 24)] and two periods of sow and piglet separation [zero (n = 23) versus 8 (n = 23) h]. Treatments commenced on d 18.4 ± 0.15 of lactation and ended at weaning on d 27.0 ± 0.15 post-partum. The multi-suckling treatment consisted of three sows and their litters housed together with 4.86 m^2 of space per sow and litter. The sow-piglet separation involved removing sows from their litters for 6 hours (0800 to 1400 h). From day 18.4 ± 0.15 to weaning or the end of lactation oestrus, whichever came first, sows received 20 min of full, boar contact in a detection mating area. Sow and piglet liveweight (LW) were measured at the start of treatment and at weaning. The timing and incidence of lactation oestrus and piglet mortalities were recorded. Data were analysed using a general ANOVA model, with litter size at the start of treatment included as a covariate (GenStat, 15th Edition; UK). Differences between proportions were analysed by Chi-square. There were no interactions between treatments, so main effects only are presented.

There was no difference in the expression of oestrus in lactation when sows and piglets were housed together as opposed to individually in farrowing crates (70.8% vs 52.2%, $P < 0.2$). Sows housed in groups took longer ($P < 0.05$) to express oestrus in lactation (Table 1). The weight and number of piglets at weaning was unaffected by treatment (Table 1). However, more piglets died in group housing compared to farrowing crates between d 18 and weaning (3.9% versus 0.4%, $P < 0.05$). Piglet mortality rate during late lactation was similar ($P > 0.05$) in the zero (2%) and 6-h (2%) separation groups.

Group housing of sows and litters reduces suckling intensity and increases lactation oestrus in the absence of any additional stimuli (Lindgren *et al*. 2013), which may explain why lactation oestrus expression appeared to be higher in group housed sows in our study. It is plausible that less fertile sows may ovulate when housed in groups as opposed to individually, as they experience more positive inputs into the hypothalamic-pituitary-ovarian axis, thus explaining the increase in the mean interval to lactation oestrus. Strategies to prevent increased piglet mortalities are required before group lactation housing is a viable option. The apparent increase in lactation oestrus in groups housed sows requires validation using more replicates.

Table 1. Effect of two lactation housing systems and two periods of sow-piglet separation from day 18 to 27 of lactation on the expression of oestrus in lactation, piglet weaning weight and sow weight change. Values are means ± SEM

	Sow-piglet separation		Housing system	
	Zero hours	6 hours	Farrowing crate	Group pen
Sows with oestrus in lactation (%)	60.9 (14/23)	62.5 (15/24)	52.2 (12/23)	70.8 (17/24)
Days to lactation oestrus	5.4 ± 0.42	5.2 ± 0.42	4.3 ± 0.47[a]	6.1 ± 0.41[b]
Piglet weight at weaning (kg)	8.0 ± 0.20	7.7 ± 0.20	8.0 ± 0.21	7.8 ± 0.20
Litter size at weaning	10.1 ± 0.25	10.1 ± 0.25	10.3 ± 0.26	9.9 ± 0.25
Sow LW change, day 18 to weaning (kg)	–2.2 ± 2.53	1.3 ± 2.53	–0.1 ± 2.63	–0.8 ± 2.63

Means in a row and within main effect not having the same superscript are significantly different ($P < 0.05$).

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Intermittent suckling with primiparous sows: skip-a-heat effects on oestrus during lactation, reproductive performance and embryo survival

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Research in Europe and Australia has demonstrated that oestrus can be evoked during late lactation by periodic separation of sows and piglets combined with boar contact, and that mating during lactation results in pregnancy rates and subsequent litter sizes comparable to that of conventionally weaned multiparous (MP) sows (Soede *et al*. 2012; McDonald *et al*. 2013). However, there is limited data available on primiparous (PP) sows, and because this is a category of sows that is generally challenged metabolically during lactation (more so than MP sows), this may have negative effects on lactational oestrus response rates and subsequent reproductive outcomes. Therefore, it was hypothesised that mating PP sows at the subsequent oestrus following their first oestrus during lactation (skip-a-heat) would improve reproductive performance and embryo survival when combined with an intermittent suckling oestrus induction protocol.

Primiparous sows (Large White × Landrace, Hypor genetics; n = 76) were allocated to either a Control treatment (C28), where piglets were weaned at d 28 of lactation, or an intermittent suckling treatment (IS21), where all piglets were separated from the sow for 8 h/d from d 21 of lactation until weaning at d 28. The IS21 sows were housed in group pens during the separation period and received twice-daily fence-line boar exposure in a detection mating area. Sows were mated at either lactational oestrus, or at the subsequent oestrus following lactational oestrus (skip-a-heat). The C28 sows and any IS21 sows that did not experience a lactational oestrus (non-responders) were mated at their first post-weaning oestrus. At approximately d 30 of gestation the sows were slaughtered on site to examine embryo characteristics. A mixed model was used to analyse effects of treatment on reproductive parameters (SAS®; USA). Pregnancy rate was analysed separately using the generalised logit function of SAS.

Ovulation rate and embryo survival between PP sows mated at their first oestrus during lactation and at the subsequent oestrus following lactational oestrus (skip-a-heat) differed ($P < 0.05$), however this did not cause a difference in the number of viable embryos at d 30 ($P > 0.05$; Table 1). Interestingly, mating at the first oestrus during lactation reduced ($P < 0.05$) placental development and embryonic weight at d 30 compared to C28 sows, with skip-a-heat sows and non-responder sows being intermediate (Table 1). Overall, skip-a-heat mating compared to mating at the lactational oestrus did not significantly improve reproductive performance or embryo survival in PP sows. However, the effect of lactational oestrus on subsequent litter development requires further examination.

Table 1. Lactational oestrus and subsequent reproductive outcomes in primiparous sows mated at lactational oestrus (first heat), at the subsequent oestrus following lactational oestrus (skip-a-heat), or at normal post-weaning oestrus (non-responders). Values are the least-squares mean ± SEM

Treatment[A]	C28 (n = 19)	IS21 (n = 57)		
Lactational oestrus (%)	–	61 (35/57)		
		First heat	Skip-a-heat	NR[B]
Mating rate (%)	79 (15/19)	100 (18/18)	100 (17/17)	77 (17/22)
Pregnancy rate (%)	100[a] (15/15)	83[ab] (15/18)	100[a] (17/17)	76[b] (13/17)
Ovulation rate	23.4 ± 0.90[a]	23.6 ± 0.87[a]	19.6 ± 0.82[b]	22.2 ± 0.94[ab]
Number of live embryos	16.4 ± 1.04 (n = 14)	17.5 ± 1.15 (n = 10)	17.1 ± 1.07 (n = 12)	18.5 ± 1.09 (n = 12)
Embryonic survival (%)	70.8 ± 4.49[a]	76.8 ± 4.92[ab]	88.8 ± 4.63[b]	85.3 ± 4.71[b]
Embryonic weight (g)	1.52 ± 0.039[a]	1.33 ± 0.045[b]	1.46 ± 0.039[ab]	1.49 ± 0.042[a]
Allantochorioic fluid volume (ml)	230.1 ± 7.78[a]	187.0 ± 9.98[b]	196.3 ± 8.08[b]	210.1 ± 8.83[ab]

[A]Refer to text for treatment details. [B]NR, non-responders. [a,b]Means in a row not having the same superscript are significantly different ($P < 0.05$).

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Predictive modelling of *Salmonella* spp. inactivation in pork burger patties of varying fat contents

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Salmonellosis is the second most common reported cause of foodborne illness annually in Australia with pork products implicated in outbreaks (Pointon in press). Pork burgers are a serving option for pork mince and can potentially have foodborne pathogens internalised during grinding. Predictive models exist for the thermal inactivation of *E. coli* O157:H7 in beef patties (Juneja *et al.* 1997), but analogous models for *Salmonella* spp. in pork patties could not be found. This study aimed to create such a model and determine if fat content or serovar affect *Salmonella* survival.

Pre-packaged pork minces marked as 'Regular' and 'Extra Lean' were purchased from a retail chain in Adelaide, inoculated with one of three *Salmonella* serovars (*S.* 4,[5],12,i:–, *S.* Senftenberg and *S.* Typhimurium; all isolated previously from porcine sources), and formed into pork burger patties. Before inoculation, samples of the mince were taken for fat content determination by fatty acid extraction. Patties were then cooked to various internal endpoint temperatures on an electric skillet, bagged and rested for 3 min before being submerged in ice. Patties were then homogenised in buffered peptone water, with serial dilutions of the homogenate plated on Xylose Lysine Deoxycholate agar plates, incubated at 37°C for 22 ± 2 h and typical colonies counted. In total, 144 patties were formed, 126 were cooked and 18 uncooked controls over 18 experiments. Data on the internal endpoint temperature (°C), fat content of the mince and *Salmonella* serovar, were fitted to the three parameter logistic regression model of Wadley (1949) scaled to the concentration in the raw patties to generate a predictive model for *Salmonella* concentration (CFU/g). The overall mean fat content was 6.11%, but two distinct groups were observed: <5% fat (mean 2.99%) and >10% fat (mean 12.35%), i.e. the fat content of mince samples did not correspond to the nomenclature used on the packages; some batches had lower fat contents than indicated. Separate models were developed for each of these groups and, within each group, fat content was treated as a continuous variable. Interactions between the temperature and fat content influenced *Salmonella* survival (P = 0.043). The difference between fat groups disappeared as the temperature approached 62°C (Fig. 1). For pork mince with mean fat contents of 2.99% and 12.35%, *Salmonella* survival was predicted to decrease by 0.227 and 0.268 $\log_{10}$ CFU/g respectively for a 1°C increase in temperature. For both fat groups, a 5-$\log_{10}$ reduction in the *Salmonella* concentration was predicted to occur at 63°C. There were no significant differences in the inactivation kinetics between the three serovars ($P > 0.05$).

S. 4,[5],12,i:– is an emerging serovar of public health interest (Pointon in press) and as revealed in this study, it appears to have a similar inactivation kinetics to other serovars. A novel predictive model was developed for inactivation of *Salmonella* spp. in pork burgers, using the model by Wadley (1949), not previously used in this context. Reduced fat in pork burger patties may decrease the risk of salmonellosis when cooked to a lower degree of 'doneness'. This work provides industry with knowledge that can be used in marketing to inform consumers about pork burger cooking and in food service to validate safe cooking processes.

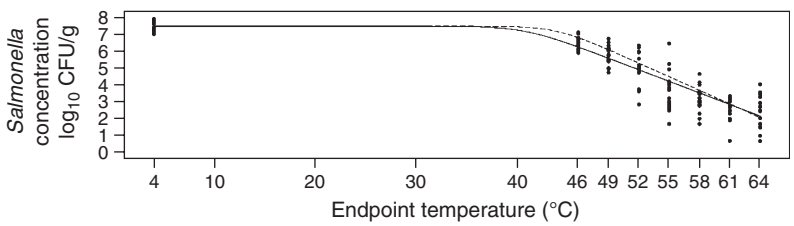

Fig. 1. *Salmonella* spp. concentrations at each endpoint cooking temperature. The predictive model for the means of the two groups of mince (<5% fat, solid line and >10% fat, dashed line) is also depicted.

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Antibiotic resistance in *Escherichia coli* isolated from pre- and post-weaned piglets: a snapshot survey of Australia

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The Australian pig industry experiences outbreaks of pre- and post-weaning diarrhoea caused by *Escherichia coli*, which is linked to reduced growth rates, high medication costs and high levels of mortality and morbidity (Fairbrother *et al.* 2005). Antibiotics are often used for treatment at weaning but *E. coli* can develop resistance over time. This is concerning for effective control of *E. coli* disease as well as abundance of antibiotic resistant strains in both humans and animals. The aim of this study was to isolate *E. coli* from healthy and sick piglets to determine resistance to antibiotics used in human medicine.

A snapshot survey was conducted from September 2013 to May 2014 in 22 commercial piggeries located in South Eastern Australia (New South Wales n = 9; Victoria n = 10; and South Australia n = 3). Faecal samples were collected from each herd (10 from pre-weaned and 40 from post-weaned piglets) and spread onto sheep blood agar (SBA) and CHROMagar orientation to isolate *E. coli*. A total of 325 *E. coli* isolates (15 from each herd) were tested for resistance to 27 human antibiotics using the BD Phoenix Automated Microbiology System (BD Diagnostics) according to human Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

Resistance to antibiotics was found in pre-weaning piglets despite greater exposure to antibiotics after weaning, suggesting possible colonisation by resistant bacteria from sows or the farrowing environment. Chloramphenicol (no longer used in the Australian pig industry) showed a significant increase ($P < 0.001$) in resistant *E. coli* from pre- to post-weaned, suggesting co-selection for the resistance phenotype due to exposure to other antibiotics (Fig. 1). Resistance to human third-generation cephalosporins (ceftriaxone and ceftazidime) was less common (Fig. 1), although continued monitoring for emerging resistance to these antimicrobials is essential, considering their importance in human therapeutics. Multi-drug resistance (resistant to $\geq$3 classes of antibiotics; Magiorakos *et al.* 2012) was observed in 34% of isolates in this study including drugs important for human health requiring further investigation. Surveillance of *E. coli* resistance in both healthy and diseased piglets is necessary to anticipate any potential threat to both animal and public health.

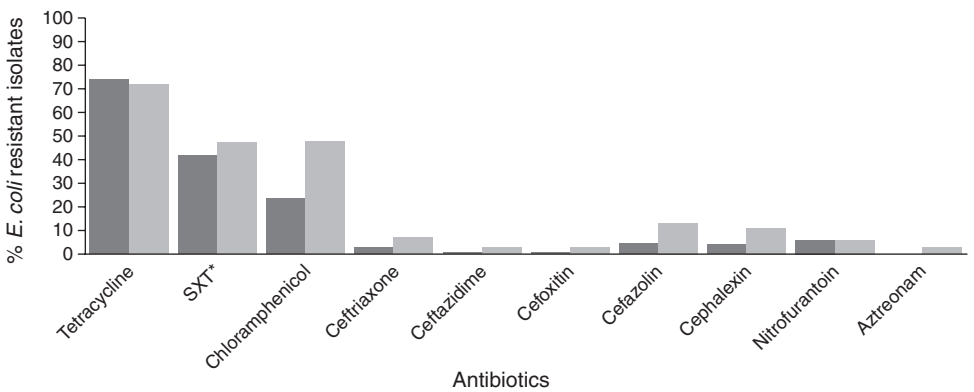

Fig. 1. Antimicrobial resistance of *E. coli* isolated from pre-weaned (■) and post-weaned (■) piglets in South Eastern Australia. All other *E. coli* resistant isolates were < 2%. *SXT – trimethoprim-sulfamethoxazole. Chloramphenicol: significant increase ($P < 0.001$) in resistant *E. coli* from pre- to post-weaned.

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Preliminary verification of molecular techniques to more accurately assess the risk from *Toxoplasma gondii* in pork

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Toxoplasma gondii is a two-host meat borne protozoan parasite, with felines being the primary host and all other warm-blooded animals (including humans) as secondary hosts, in which it causes lifelong infection (Tenter *et al.* 2000). Secondary hosts can become infected from either faeces of an infected cat or from consumption of undercooked infected meat (including pork). The ability of *T. gondii* to cross the placental barrier and infect the foetus, as well as its ability to emerge from hibernation within muscle cysts during periods of immune suppression and its association with schizophrenia, has led to increasing public health concern (Flegr 2013). Traditional serological diagnosis of infection has proved problematic when attempting to assess the risk of human exposure through the consumption of undercooked meat, with different tests giving widely varying results (Dubey 2009). Molecular methods such as polymerase chain reaction (PCR) have low sensitivity, particularly in pigs/pork, as there is a low density of cysts in muscle tissue. This preliminary study investigated a method to (1) concentrate the diffuse *Toxoplasma* bradyzoites in meat for identification by both nested and qPCR, followed by (2) a bioassay to determine both the accuracy of quantification, and (3) the continued infectivity of the concentrated bradyzoites. The aim of this project was to trial and verify methods developed overseas for use in Australia on meat, to support assessment of the risk of consumer exposure.

Infected brain material was obtained from Swiss-Webster mice injected subcutaneously 12 weeks earlier with an inoculum of tissue culture *Toxoplasma* tachyzoites. The PCR and qPCR estimated a concentration of 2.7×10^5 *T. gondii*/20 mg of brain. A 50 g sample of previously frozen pork mince was spiked with 300 mg of mouse brain, then digested with pepsin, filtered, centrifuged and re-suspended in 5 mL of 0.9% saline as described by Dubey (1998). After quantification by qPCR, three pairs of fresh mice were then subcutaneously injected with an estimated 10^5, 10^3 or 10^1 *T. gondii*. Clinical signs and cysts were observed in both the 10^5 and 10^3 tachyzoite-infected mice, and one of the 10^5 infected pair died. The five surviving mice were euthanised after 12 weeks. Infection was confirmed in the 10^5 and 10^3 infected mice by qPCR with estimated levels of 2.3×10^3 and 4×10^3 *T. gondii*/20 mg of brain, respectively, but not in the 10^1 mouse, suggesting the infective mouse dose for this post-digestion *Toxoplasma* pig strain lay between 10^1 and 10^3 organisms.

An opportunistic preliminary estimate of the recovery rate of *T. gondii* from spiked pork mince following acid/pepsin digestion was conducted by preparing two 50 g pork mince samples: one as a control, and one spiked with an estimated 4.6×10^4 *T. gondii* organisms. Both mince samples were processed by pepsin digestion/centrifugation and the re-suspension examined by qPCR. The re-suspension from the control mince contained no detectable *Toxoplasma* DNA, while that from the spiked mince contained an estimated 1.1×10^5 *T. gondii*. Despite the acknowledged lack of replicates, this single result suggests recovery of the majority of the spiking organisms is possible.

In conclusion, a refined molecular test was established to concentrate and detect *T. gondii* in meat samples. The Dubey (1998) digestion/centrifugation technique was verified to improve sensitivity and the *T. gondii* recovery rate investigated. An effective mouse bioassay was developed to enable verification of the continued infectivity of *T. gondii* detected in meat samples following the digestion-PCR method, and multiplication of detected strains to allow future genotyping if required. Standard test methods and laboratory procedures that cover extraction and detection of *T. gondii* in meat samples are now available to allow the pork industry to determine risks to human health associated with the consumption of undercooked pork products that has not undergone a kill step for *T. gondii*.

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Dietary lactulose supplementation improves grower-finisher pig performance and indices of gastrointestinal tract function

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Concerns regarding the use of antibiotics in the pig industry have increased the interest in possible alternatives to antibiotics including prebiotics such as non-digestible oligosaccharides (Branner *et al.* 2004). Lactulose (4-O-β-D-galactopyranosyl-D-fructose) is metabolised in the colon by the saccharolytic microbiota (Bird *et al.* 1990), and can influence the intestinal microbiota by stimulating the growth of *Lactobacillus* spp. in the gastrointestinal tract (GIT). Previous studies show that lactulose elicits a prebiotic effect (increased counts of *Bifidobacteria* and *Lactobacillus*) in pigs (Konstantinov *et al.* 2004). The hypothesis tested in this experiment was that lactulose supplementation in diets could improve grower-finisher pig performance and bacterial counts.

This study was conducted to evaluate the effects of lactulose on growth performance, diet component digestibility and faecal microbial shedding in grower-finisher pigs. A total of 80 (Landrace × Yorkshire × Duroc) pigs with a bodyweight (BW) of 20.8 ± 3.20 kg (mean ± SD) and aged 10 weeks was randomly allotted to four dietary treatments with four replicate pens per treatment and five pigs (three gilts and two barrows) per pen. Dietary treatments included: Control (CON), pigs fed a basal diet; L05, CON + 0.05% lactulose (L); L10, CON + 0.10% lactulose; and L15, CON + 0.15% lactulose. The experiment included two stages: grower (0 to 6 weeks) and finisher (6 to 18 weeks). All pigs were fed diets mixed with 0.2% chromium oxide to calculate the coefficient of total tract apparent digestibility (CTTAD) of DM, nitrogen (N) and gross energy (GE). At the end of experiment, faecal samples were collected directly by massaging the rectum of pigs randomly selected from each pen (one gilt and one barrow) from which a 1 g sub-sample was diluted with 9 mL of 10 g/L peptone broth to evaluate faecal microbiota (i.e. *Lactobacillus*, *E. coli*, *C. perfringens*, and *Bifidobacteria*). All data were subjected to statistical analysis via a randomised complete block design using GLM procedures (SAS®; USA). Duncan's multiple test was used to compare the means of the treatments.

Pigs fed L10 and L15 diets had greater average daily gain (ADG) throughout the overall period when compared with the CON diet (793, 801 vs 778 g, respectively, $P < 0.05$). Pigs fed L10 and L15 diets increased faecal *Lactobacillus* and reduced *E. coli* counts compared with CON pigs ($P < 0.05$, Table 1). The CTTAD of DM was greater for the L10 and L15 treatments than CON pigs (0.76 and 0.77 vs 0.72; 0.81 and 0.81 vs 0.76, $P < 0.05$, respectively) at weeks 12 and 18.

Results from this study indicated that L10 and L15 supplementation improved performance in growing-finishing pigs. Cho and Kim (2014) suggested that the improved growth performance observed in response to dietary lactulose supplementation occurs through an increased nutrient digestibility and improved faecal microbiota. Lactulose cannot be hydrolysed by digestive enzymes but fermented to short chain fatty acids in the lower gut and reduces pH of the ileal environment, and promotes growth of beneficial types of bacteria. These include *Bifidobacterium, Eubacterium*, and *Lactobacillus*, as well assuppressed *E. coli* counts in the large bowel (Boguslawska-Tryk *et al.* 2012), which could be used to explain the increased *Lactobacillus* and decreased *E. coli* seen in this study.

Table 1. Effect of lactulose supplementation on faecal microbiota counts in growing-finishing pigs

Faecal microbiota, $^{log}_{10}$ cfu/g	CON	L05	L10	L15	SEM[A]
Lactobacillus	6.78[b]	6.84[b]	7.52[a]	7.61[a]	0.01
E. coli	6.65[a]	6.51[ab]	5.86[b]	5.85[b]	0.05

[A]SEM, standard error of the mean. [a,b]Means in a row not having the same superscript are significantly different ($P < 0.05$).

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Piglet growth performance is improved using a low protein starter feed or by fortifying conventional starter feed with spray dried porcine plasma and (or) functional fibre

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The composition of piglet starter feeds is an important determinant for the growth and health of weaned piglets (de Lange *et al.* 2010). Factors such as lowering dietary protein level and strategic use of specific ingredients such as spray-dried porcine plasma (SDPP) have been found to reduce disease incidence and promote growth (de Lange *et al.* 2010). Other ingredients, such as the functional fibres β-glucan (βG) and xylooligosaccharide (XOS), are also used commercially to improve piglet growth and health. This study tested the hypothesis that a low protein starter diet would support similar piglet growth and survival when compared to conventional diets with and without functional fibre sources and SDPP.

Eight hundred and forty piglets (PrimeGro™ Genetics, Corowa, NSW) were weaned at 26 days of age (8.9 ± 0.21 kg; mean ± SE) and housed in commercial pens (14 pigs/pen) with feed and water available *ad libitum*. Piglets were blocked by sex and pen and randomly allocated to one of four dietary treatments (n = 15): a low protein starter diet [LPS; 175 g/kg crude protein (CP), 14.25 MJ digestible energy (DE)/kg and 0.87 g available lysine (AvL)/MJ DE]; a conventional starter diet (CS; 205 g/kg CP, 15.3 MJ DE/kg, 0.92 g AvL/MJ DE); CS with functional fibres [0.05% XOS® (Longlive Biotechnology) and 0.05% Fibosel® (Selko Feed Additives)]; and CS with functional fibres and 2.5% SDPP. All starter diets were fed for 12 days before all pigs were weighed and fed a common weaner 1 and weaner 2 diet (210 g/kg CP, 15 MJ DE/kg, 0.90 g AvL/MJ DE; and 210 g/kg CP, 14.5 MJ DE/kg, 0.85 g AvL/MJ DE, respectively). Pig weights and feed consumption were recorded on a pen basis at 0, 12 and 35 days after weaning. All deaths and removals were recorded. Performance data were analysed using ANOVA and mortality data were analysed using Chi-square analysis (Genstat, 16th Edition; UK).

In the starter period, 0–12 days after weaning, piglets fed CS ate less and grew slower than piglets fed LPS and CS supplemented with functional fibres and SDPP (Table 1). Furthermore the pigs fed CS + XOS + βG + SDPP also grew faster than those fed the LPS and CS XOS + βG diets. However by 35 days after common weaner diets had been fed, these differences had diminished so that only pigs fed the CS from 0–12 days had lower feed intakes and growth rates than those fed CS + XOS + βG + SDPP. Pigs fed CS exhibited a trend for an increased mortality ($P = 0.07$), while mortality on diets LPS and CS with functional fibre sources tended to be lower. These results demonstrated that weaned pig growth and mortality is improved through use of low protein starter feeds or addition of SDPP and (or) functional fibre to conventional starter feeds.

Table 1. Performance from 0 to 12 days and 0 to 35 days after weaning and mortalities at 35 days after weaning in pigs fed either a low protein starter diet (LPS) or conventional (CS) starter diets with a range of ingredients in the first 12 days following weaning

	Average daily gain (g)		Average daily feed intake (g)		Mortality (%)
	0–12	0–35	0–12	0–35	
LPS	238[b]	484[ab]	300[b]	661[ab]	3.81
CS	195[a]	460[a]	259[a]	622[a]	7.18
CS + XOS[B] + βG[C]	262[bc]	489[ab]	284[ab]	659[ab]	1.91
CS + XOS[B] + βG[C] + SDPP[D]	294[c]	507[b]	311[b]	689[b]	4.78
SED[A]	0.018	0.013	0.019	0.020	
P value	<0.01	0.01	0.04	0.02	0.07

[A]SED, standard error of difference of the mean. [B]Xylooligosaccharide. [C]β-glucan. [D]Spray-dried porcine plasma. [a,b,c]Means in a column not having the same superscript are significantly different.

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Additional dietary tryptophan and methionine improves feed conversion efficiency and markers of inflammation in weaner pigs infected with *Escherichia coli*

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Activation of the innate immune system after weaning leading to inflammation of the gastrointestinal tract (GIT) has been linked to compromised GIT barrier function, increased risk of enteric disorders, and poorer performance (Gallois *et al*. 2009). To counteract these effects, the dietary requirement for some amino acids such as tryptophan (Trp) and (or) sulphur amino acids (SAA) may increase. The present experiment tested the hypothesis that additional dietary Trp and (or) SAA would improve growth performance and ameliorate indicators of inflammation in weaner pigs experimentally infected with *Escherichia coli*.

Male pigs (n = 76) (Landrace × Large White) with an initial body weight (BW) of 6.2 ± 0.78 kg (mean ± SD) were stratified into one of four treatments according to a 2 × 2 factorial arrangement, with the factors being ratios of: 0.16 or 0.24 standardised ileal digestible (SID) Trp:Lysine (Lys); and 0.52 or 0.60 SID SAA:Lys (using SID coefficients from Sauvant *et al*. 2004). Diets were formulated to contain 11.2 MJ net energy/kg, 14 g SID Lys/kg and 198 g/kg crude protein, and were fed to pigs in meal form *ad libitum* for 2 weeks after weaning. Pigs were infected with 6, 8 and 10 mL of an enterotoxigenic strain of *E. coli* (3.44×10^8 colony forming units/mL; serotype O149:K98:K88; toxins LT, ST, and STb) on d 5, 6 and 7 after weaning, respectively. Blood samples were taken on d 8 after weaning and measured for C-reactive protein (C-RP), pig major acute-phase protein (PigMAP), apolipoprotein (APO-A1) and interferon-gamma (IFN-γ). An acute phase protein index (APP Index) was calculated as follows: APP Index = (C-RP × PigMAP)/APOA1 (Heegaard *et al.* 2011). Data were analysed using GLM procedures (IBM SPSS, Version 21.0; USA).

Pigs fed a higher level of Trp tended to increase ADG ($P = 0.080$) (Table 1). Pigs fed more Trp ($P = 0.036$) and SAA ($P = 0.028$) had better FCR, and higher levels of both Trp and SAA tended to improve FCR (interaction; $P = 0.092$). Pigs fed more SAA had a lower APP Index ($P = 0.045$), while increasing Trp in the diet tended to decrease the APP Index ($P = 0.075$). An interaction occurred for IFN-γ, with pigs fed low Trp and high SAA having lower levels of IFN-γ, and pigs fed either low Trp and low SAA or high Trp and high SAA having higher levels of IFN-γ ($P = 0.027$). These data suggest that both Trp and SAA play important roles in mediating the inflammatory responses of pigs after weaning. Additional supplementation of Trp and SAA (as Met) improved performance in the 2-week period after weaning.

Table 1. Growth performance, the acute phase protein index and plasma interferon-gamma (IFN-γ) levels in pigs fed low and high levels of tryptophan (Trp) and sulphur amino acids (SAA) and experimentally infected with *E. coli* after weaning

SID Trp:Lys	0.16		0.24		SEM[A]	*P* value		
SID SAA:Lys	0.52	0.60	0.52	0.60		Trp	SAA	Trp × SAA
Day 0–14								
ADG[B] (g)	68	93	96	103	10.8	0.080	0.140	0.420
ADFI[C] (g)	168	175	163	175	14.1	0.872	0.492	0.852
FCR[D] (g:g)	2.96	2.06	2.09	1.96	0.228	0.036	0.028	0.092
APP Index	0.50	0.12	0.14	0.07	0.109	0.075	0.045	0.173
IFN-γ (pg/mL)	6681[c]	2235[a]	4202[ab]	4621[bc]	1065.4	0.965	0.065	0.027

[A]SEM, standard error of the mean. [B]ADG, average daily gain. [C]ADFI, average daily feed intake. [D]FCR, feed conversion ratio. [a,b,c]Means in a row not having the same superscript are significantly different.

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A preliminary survey examining the effect of oral health on feeding behaviour and efficiency in culled sows

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Although the effect of oral disease in growing pigs on weight gain has been recognised and the incidence of dental disease has been observed to be high in sows (Knauer *et al.* 2007), the relationship between oral disease and feeding efficiency in pigs has not been examined. As the rate of sow culling has been linked to inadequate nutritional intake (Hughes *et al.* 2010), the role of dental disease should be considered. The high level of sow wastage has been identified as an important limitation on production. The hypothesis tested in this study was that oral disease of culled sows is related to feeding behaviour and efficiency.

Thirteen commercial-strain sows selected for culling at a large piggery in Victoria were examined in this study. Sows ranged from parity two to parity four. A feeding test was given in the piggery on the day prior to slaughter: 475 g of feed was placed inside a 25 by 25 cm square in a designated floor space, and each sow was video-taped until it consumed the meal in its entirety or left the area. The time taken to consume was recorded (feeding time) as well as the amount of feed remaining (if applicable). If eating stopped for 10 s then subsequent feeds were considered as a separate event. The rates of chewing (the number of mandible chewing motions per minute) with the head down and raised were calculated from the video data.

The oral cavities of the sows were examined post-mortem (at the abattoirs) (data presented in Table 1). The maxilla and mandibular lengths were recorded. The number of teeth that were chipped or cracked, displaced, missing or not erupted, and broken were recorded. The degree of periodontal disease, determined by the degree of gingival disruption around the tooth (mild or not present, n = 0 moderate, n = 4; advanced, n = 9) and calculus accumulation (mild or not present, n = 0 moderate, n = 4; advanced, n = 9) was estimated. A Dental Wear Index (DWI) was determined (the number of teeth with signs of wear multiplied by the severity of wear; from 0, not worn, to 3, severe). The relationships within the data were examined for significance using regression analysis and Fisher's exact test (Minitab®, Version 16.0; USA).

Missing or non-erupted teeth was negatively correlated to DWI ($R^2 = 0.73$, F-test = 29.9, $P = 0.023$). Sows with advanced calculus accumulation were more likely to have advanced periodontal disease (Fisher's exact test; $P = 0.014$). The number of chipped or cracked teeth was positively correlated to feeding time ($R^2 = 0.48$, F-test = 4.83, $P = 0.032$).

It is concluded that there is a high incidence of dental degenerative degree occurring over the life of the sow and that dental abnormalities affect the efficiency of feed intake. This study was not able to take into account the factors that may predispose sows to dental issues, although it appears morphological factors such as jaw alignment and size may be important. As there has been little selective pressure for jaw and teeth morphology the high incidence of abnormalities identified was not surprising. The small numbers of sows limits the analysis of the correlation of the measurements. Further investigation of the time course of the degenerative processes may indicate when these abnormalities have an impact on feeding efficiency. The relationship of feeding efficiency to growth and production needs to be investigated. To be fully evaluated, the relative importance of the described dental abnormalities on sow growth, health and welfare needs further study.

Table 1. Survey of dental abnormalities and feeding behaviour in culled sows. Values are mean ± SE

Dental values		Feeding values	
Bite discrepancy (cm)	0.18 ± 1.29	Feeding time (min)	4.9 ± 0.39
Maxilla length (cm)	21.1 ± 2.2	Feed remaining (g)	255 ± 107
Mandible length (cm)	23.4 ± 2.5	Chews/min	175 ± 18.2
Chipped/cracked teeth (No.)	2.2 ± 1.16	Chews/min when head raised	20 ± 17.9
Displaced teeth (No.)	0.7 ± 0.83	No. of feeding events	3.1 ± 1.95
Missing/non erupted teeth (No.)	2.6 ± 3.03		
Broken teeth (No.)	0.2 ± 0.59		
Dental Wear Index	61 ± 6.6		

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Numbers of selected bacterial species in pig faeces do not accurately represent their numbers in the ileum

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Although it is known that the microbial population of faeces differs from that of the gastrointestinal tract (GIT) (Looft *et al*. 2014), monitoring the microflora of the pig GIT requires euthanasia. Identifying correlations between selected bacterial species in faeces and ileal mucosa would allow extrapolation of GIT bacterial numbers from faeces. This study tested the hypothesis that numbers of selected bacterial groups would differ between the ileum and faeces, and that they would be significantly correlated. *Clostridium perfringens* and *Escherichia coli* (part of the *Enterobacteriaceae* family) were selected as prominent pathogens, whilst Lactobacilli are important commensal bacteria.

Paired faecal and ileal mucosal scrapings (without intestinal contents) were collected from three nursery and nine weaner pigs. Bacterial numbers were determined by quantitative polymerase chain reaction (qPCR) on extracted DNA (MagMAX Pathogen Kit), as previous studies demonstrated a good correlation with culture techniques (Castillo *et al*. 2006). The qPCRs targeted the 16S or 16S-23S rRNA intergenic region of selected and total bacteria (Collins and Bowring 2014). The percentage of selected bacterial groups relative to total bacteria was calculated to overcome variation in water content of samples, and then $\log_{10}$ transformed for normality. Bacterial numbers and percentages were analysed using the paired t-test and Pearson correlations were performed on percentages (Genstat, 17th Edition; UK).

Differences in *Cl. perfringens*, Lactobacilli and total bacterial numbers were observed in ileal mucosa and faeces ($P < 0.025$) (Table 1). However, when bacterial numbers were expressed as percentages of total bacteria only *Cl. perfringens* remained significantly different ($P = 0.015$), along with a trend towards a reduced percentage of Lactobacilli in mucosa ($P = 0.076$). Adhesion to the intestinal mucosa is a characteristic feature of pathogenic *Cl. perfringens*, which may partly explain the increased percentage of *Cl. perfringens* in mucosa and its underrepresentation in faeces. Looft *et al*. (2014) also observed an increased relative abundance of *Cl. perfringens* in ileal mucosa compared to faeces using microbial sequencing. Linear correlations between bacterial numbers in faeces and ileal mucosa were not demonstrated, suggesting that other factors may affect the relative abundance of bacteria.

Expressing selected bacterial numbers as a percentage of total bacteria was critical for comparing the two different sample types, which varied in their water content. The absence of significant correlations between percentages of selected bacteria in faeces and mucosa may be explained by the small sample size and the dramatic changes occurring in the GIT associated with weaning and disease. Larger sample sizes are needed to identify correlations between bacterial numbers in faeces and mucosa. Good correlations between bacterial numbers in faeces and mucosa would enable approximation of bacterial numbers in the ileum, avoiding animal sacrifice and allowing repeated sampling over time. Regardless of correlations, faeces remain a valuable, non-invasive sample for quantifying pathogen excretion and potential disease transmission.

Table 1. $\log_{10}$ numbers of *Cl. perfringens, E. coli, Enterobacteriaceae*, Lactobacilli and total bacteria (mean ± SE), and percentages of selected bacteria in ileal mucosa and faeces

Bacterial group	$\log_{10}$ bacteria in ileal mucosa	$\log_{10}$ bacteria in faeces	Percentage bacteria in ileal mucosa relative to total	Percentage bacteria in faeces relative to total	Pearson correlation coefficient (R)
Cl. perfringens	7.04 ± 0.44^{a}	5.42 ± 0.37^{b}	1.35 ± 0.82^{c}	0.03 ± 0.02^{d}	0.205
E. coli	7.38 ± 0.67	7.54 ± 0.31	12.99 ± 6.48	1.14 ± 0.50	0.021
Enterobacteriaceae	8.08 ± 0.68	8.22 ± 0.35	69.87 ± 38.46	7.05 ± 3.75	0.081
Lactobacilli	5.28 ± 0.61^{a}	6.80 ± 0.41^{b}	0.27 ± 0.20	0.43 ± 0.29	0.423
Total bacteria	9.30 ± 0.21^{a}	10.03 ± 0.12^{b}	–	–	–

[a,b,c,d]Mean $\log_{10}$ numbers or percentages in a row not having the same superscript are significantly different ($P < 0.05$).

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Mycoplasma vaccination responses in immunodepressed weanling pigs supplemented with *S. cerevisiae boulardii*

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Mycotoxins are known for their immunodepressive properties in animals by affecting non-specific and acquired immunity (Oswald *et al*. 2005). As a consequence, immunity acquired through vaccination is also impaired by mycotoxin ingestion. *Saccharomyces cerevisiae boulardii* (SCB) is extensively documented for its immune-modulatory benefits in animals and in man (Kelesidis and Pothoulakis 2012). This study focused on the interaction between mycotoxin and yeast by examining in particular the vaccination response and small intestinal histomorphometry as ways of assessing pigs' responses to the challenge.

Twenty-four castrated 6-week-old pigs (13.6 ± 1.80 kg; mean ± SD) were involved in a 4-week study. Pigs were individually housed and randomly allocated to one of four diets: Control (C); Fumonisin B1 (FB1) at 12 ppm; SCB CNCM I-1079 at $5x10^9$ cfu/kg feed; and FB1 + SCB. At d 0 and 8, the pigs were vaccinated using a commercial vaccine allowing subsequent specific immunoglobulin (Ig) titration as a model vaccine (*Mycoplasma hyopneumoniae*, Stellamune mono-injection; Pfizer, France). Weekly blood samples were taken for measurement of Ig content by ELISA (Bethyl, TX, USA for IgA, IgG, IgM; Kit IDVET for *M. hyponeumoniae* specific Ig). Pigs were necropsied at the completion of the study and samples of jejunum and ileum processed for morphometry (Bracarense *et al*. 2012). Data were analysed per time point by ANOVA and differences between means separated by Tukey's post-hoc test (XLSTAT®; USA).

No treatment effect ($P > 0.05$) was depicted for IgA, IgM and IgG. However, specific Ig levels against *Mycoplasma* increased from d 15 in C whereas it was still minimal for FB1 (Fig. 1). Interestingly, FB1-SCB reached a similar Ig titer than SCB after 29 d, suggesting an inhibition of a deleterious effect from FBI. Histologically, the intestine was affected by FB1 and addition of SCB restored ($P < 0.05$) intestinal lining in the ileum compared to FB1 alone (12.0, 9.7, 15.3, 11.0 for C, SCB, FB1 and FB1-SCB, respectively). Villous height increased ($P < 0.05$) in both jejunum and ileum for FB1-SC vs FB1 to become comparable to C (342, 354, 280, 345 µm for C, SCB, FB1 and FB1-SCB, respectively). No treatment effect ($P > 0.05$) was found for crypt depth.

In conclusion, the FB1 immune challenge model given to pigs notably reduced the specific antibody response. The use of SCB (strain I-1079) increased the challenged pigs' vaccination response to *M. hyopneumoniae*. Measures of small intestinal morphometry were positively improved with SCB reaching villi similar in height to non-challenged animals. However, the findings of this pilot study require confirmation in larger scale studies to assess the impact on animal performance and lungs lesions.

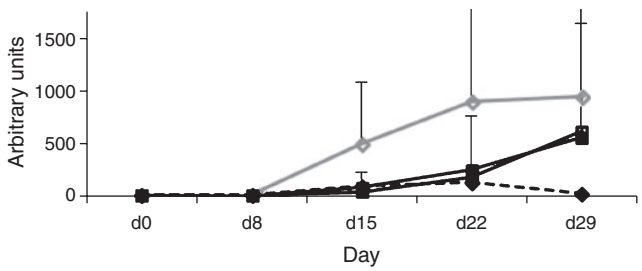

Fig. 1. Specific antibody titers of *Mycoplasma hyopneumoniae* according to the dietary treatments (C: control (◇), SCB (□): *Saccharomyces cerevisiae boulardii*, FB1 (◆): Fumonisin B1; FB1+SCB (■). Titers were normalised before vaccination.

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A comparison of three anti-inflammatory drugs in weaner pigs using Improvac® as an inflammation model

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Analgesic/anti-inflammatory drugs must be effective to ensure appropriate treatment of sick/injured animals. Wilson *et al*. (2014) reported that meloxicam is the anti-inflammatory drug most frequently used on pig farms in Australia. However, it appears that ketoprofen may have a greater analgesic effect in young pigs than meloxicam (Fosse *et al*. 2011*a*, 2011*b*). It was hypothesised that ketoprofen would have a greater analgesic/anti-inflammatory effect than meloxicam and dexamethasone in weaner pigs.

This experiment used 32, 10-week-old male Landrace x Large White weaner pigs [n = 8/treatment; body weight 34.5 ± 0.51 kg (mean ± SE)]. Pigs were housed in pens of four (one per treatment group). Inflammation was induced using a single subcutaneous injection of Improvac® (2 mL; Zoetis, Sandton, South Africa) behind the right ear on d 1. Pigs were injected intramuscularly daily for 3 d with physiological saline (2 mL, 0.9% NaCl), ketoprofen (3 mg/kg Ketofen®; Merial, North Ryde, Australia), meloxicam (0.04 mg/kg Metacam®; Boehringer Ingelheim Vetmedica, North Ryde, Australia) or dexamethasone (1 mg/10 kg Dexason®; Illium, Glendenning, Australia). Inflammation was assessed by measuring haptoglobin and C-reactive protein concentrations (CRP) in blood samples collected on d 0, 2 and 4 after Improvac® treatment using Tridelta® Phase™ Range assays. Rectal temperatures (RT; MC-246, Omron Healthcare, Australia) were measured daily. Haptoglobin, CRP and RT data were analysed using linear mixed models (Genstat, 17th Edition; UK).

The administration of ketoprofen and meloxicam caused a decreased RT ($P < 0.05$) compared to control animals. Haptoglobin concentrations were lower in ketoprofen-treated pigs compared to all other treatment groups ($P < 0.001$, Table 1). No treatment effects were evident for CRP, however a day effect was evident where CRP concentrations increased from 2028 (± 330.8) to 6612 (± 330.8) and lowering to 4436 (± 330.8) ng/mL ($P < 0.001$).

The haptoglobin responses suggest that ketoprofen may be a more effective analgesic agent than meloxicam or dexamethasone in weaner pigs. Further research needs to be completed using a larger range of responses to inflammation, for example, behaviour and feed intake of individual pigs, before it can be conclusively determine that ketoprofen is a more effective analgesic/anti-inflammatory agent.

Table 1. Haptoglobin and C reactive protein (CRP) concentrations, and the rectal temperature (RT), after treatment for inflammation with either saline, ketoprofen, meloxicam or dexamethasone. Values are mean ± SE

Item	Saline	Ketoprofen	Meloxicam	Dexmethasone
CRP (ng/mL)	4585 ± 369	4048 ± 369	3895 ± 369	4306 ± 369
Haptoglobin (mg/mL)	1.9 ± 0.13^{bc}	1.4 ± 0.13^{a}	1.8 ± 0.13^{b}	2.1 ± 0.13^{c}
RT (°C)	39.5 ± 0.13^{b}	39.2 ± 0.13^{a}	39.2 ± 0.13^{a}	39.3 ± 0.13^{ab}

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

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Aerosol disinfection from weaning: a pilot study to assess the impacts on clinical signs of *Actinobacillus pleuropneumoniae*

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Actinobacillus pleuropneumoniae (APP) is a respiratory disease causing ill thrift, acute deaths and carcass damage. The pathogen is difficult to control in large populations, with disease severity impacted by numerous factors including the number of pigs in an airspace (Cargill and Banhazi 2001). Previous research suggests that air quality is improved with aerosol disinfection (fogging) (Costa *et al.* 2014), however the impacts on clinical signs of APP are unclear. This study tested the hypothesis that fogging during the weaner period would reduce coughing, improve survival and decrease carcass pleurisy when pigs were subsequently housed in a fogged finisher facility.

A total of 3829 pigs (Large White x Landrace; PrimeGro™ Genetics) was selected at weaning and housed in one of four weaner rooms (955–957 pigs/room). Weaner rooms were allocated to one of two treatments: Control weaners, no aerosol disinfection during the weaner period (CW); and Fogged weaners, aerosol disinfection during the weaner period (FW). Fogging was achieved using Ozmist Patiomist Pedestal fans, with four fans in each weaner room. Timers were used and the fans were set to fog for 30 min every 2 h, 24 h/day. At 9 weeks of age, 3623 pigs were moved to the one finisher facility, with the CW pigs housed at one end of the facility and the FW pigs housed at the other end. The entire finisher facility was aerosol disinfected using a fixed high-pressure system (750 psi and 10 micron nozzles), with the system running 10 min every 2 h outside of working hours and then on a restricted schedule during the day (10 min on; 10 min off; 10 min on during staff breaks). Virogard (quaternary ammonium compounds; Chemetall Pty Ltd; Bayswater North, Victoria), at a rate of 1 : 1000, was used as the disinfectant in both fogging systems. Pigs were offered *ad libitum* access to commercial diets from weaning through to slaughter (21 weeks of age). Growth performance was measured on a pen basis (45 pens/treatment) from 9 to 21 weeks of age, while the prevalence of coughing was assessed during this period every 4 weeks on five pens per treatment. The protocol for cough scoring involved waking the pigs and immediately counting the number of coughs per pen in the subsequent 3 min. Pigs were slaughtered in a commercial abattoir and a pleurisy score (increasing scale from 0 to 3) obtained for each carcass. Growth performance, cough score and slaughter data were analysed for treatment effects using ANOVA (Genstat, 16th Edition; UK), with the finisher pen as the experimental unit. The impact of fogging on mortality was analysed using Chi-square.

Individual pedestal fans delivered an average of 17.6 L/d during the weaner period. Weaner mortality and removal rates were similar between treatments to 9 weeks of age ($\chi^2 = 0.27$, $P = 0.60$). Post mortems were conducted on 98.5% of all deaths from 9 weeks of age, with lung lesions associated with APP present on 78% of pigs autopsied. Between 9 and 14 weeks of age, the number of deaths and destructions tended to be greater in CW pigs compared to counterparts previously fogged as weaner pigs (3.2% and 2.2% of the population, respectively; $\chi^2 = 0.21$, $P = 0.06$). This was primarily due to a 3-week delay in the first APP outbreak in the FW pigs. There was no treatment effect on total deaths and destructions over the entire period (9–21 weeks of age, $\chi^2 = 0.51$, $P = 0.48$). Cough score during the grower/finisher period was similar between treatments ($P = 0.38$) as was daily weight gain ($P = 0.94$). Average pleurisy score at slaughter was similar between treatments (2.62 and 2.56 for the CW and FW respectively, $P = 0.12$), however pleurisy scores improved in the FW pigs with increased distance in the shed from the CW ($P = 0.003$).

The delay in the first APP outbreak in the FW treatment group was encouraging, suggesting early fogging may reduce clinical disease. An eventual APP outbreak in the FW was expected considering that half of the pigs in the total finisher airspace were CW. The outcomes from this pilot study suggested that the use of aerosol disinfection from weaning may provide a tool for reducing the clinical impacts of APP in large populations. Further investigations are aimed at quantifying the benefits when whole batches of pigs are fogged from weaning in combination with a tight age spread (<7 d), vaccination and 800–1000 psi fogging systems.

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A preliminary study of the molecular epidemiology of *Brachyspira hyodysenteriae* isolates in Australia

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Swine dysentery (SD) is a mucohaemorrhagic colitis of grower-finisher pigs. Affected pigs have faeces ranging from soft, yellow-grey to mucoid, bloody diarrhoea. Swine dysentery is one of the most economically significant enteric diseases of pigs in Australia due to its effect on growth rate, feed efficiency, mortality and the associated medication control costs. The classical causative agent is a strongly β-haemolytic anaerobic intestinal spirochaete *Brachyspira hyodysenteriae.* Diagnosis of SD requires bacterial isolation and (or) identification using polymerase chain reaction (PCR). A number of PCR methods have been described for identifying *B. hyodysenteriae*. In this study, a multiplex PCR for *B. hyodysenteriae*, *B. pilosicoli*, *L. intracellularis* and Salmonella *spp*. including primers described by Elder *et al*. (1997) was compared with the PCR targeting NADH oxidase (*nox*) as described by La *et al*. (2006). Multi-locus sequence typing (MLST) was used to determine the relatedness of the *B. hyodysenteriae* isolates (La *et al*. 2009). The hypothesis was that isolates that were test-negative using the multiplex PCR but test-positive using the simple PCR were related but different to those positive on both PCR tests.

A total of 11 *B. hyodysenteriae* isolates from grower pigs from 11 farms having clinical signs of SD and collected over the period 2010–2014 was tested. Isolates were cultured to demonstrate pure cultures for MLST. The pigs originated from three genetic sources (Sources A, B and C). Isolates 1–3 were from three different farms supplied by Source A. Isolates 4, 5 and 6 were from three farms supplied by Source B. Isolates 7–11 were from five different farms supplied by Source C.

The multiplex PCR detected six (55%) of the *nox* PCR positive isolates. There was no clear relationship between the enteric PCR positive and negative isolates (Fig. 1). Isolates from different farms that obtained pigs from the same source generally were closely related, with isolates 1–3 (Source A) and isolates 7–11 (Source C) being identical or nearly identical, but different from those recovered elsewhere.

This study showed that there were no consistent strain-related patterns among multiplex PCR negative or positive isolates. Isolates from pigs from the same sources were similar in MLST, demonstrating that this method can reliably be used to map the movement of *B. hyodysenteriae* isolates between farms.

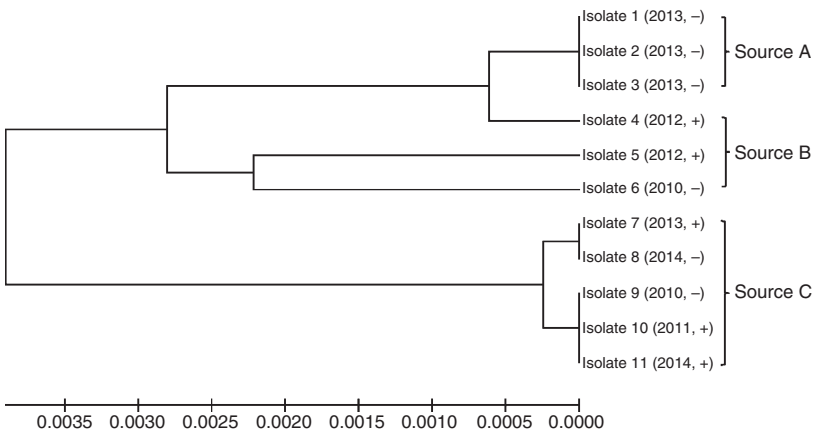

Fig. 1. A multi-locus sequence typing tree of the 11 *B. hyodysenteriae* isolates cultured from pigs from 11 farms. The year of isolation is indicated in parentheses, as well as the positive (+) and negative (–) multiplex PCR results for each isolate. The source of the pigs for each farm is indicated. The scale bar represents 5 nucleotide substitutions in 1000 base pairs of the sequenced gene fragment.

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Detection of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* among pigs in different stages of production

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Several European studies have found different levels of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) prevalence in pig farms ranging from 3 to 60% (EFSA 2009). A number of studies have found an association between the presence of *S. aureus* strains (MRSA and MSSA) on farm and carriage of these organisms by humans on those farms (van Cleef *et al*. 2014). The risk of acquisition of MRSA in human is considered to be directly related to frequency and type of contact with animals that are carrying the pathogen (Graveland *et al*. 2011). However, limited studies have been conducted regarding carriage of these pathogens in Australian pigs. The aim of this study was to detect and identify potential risk factors for MRSA and MSSA carriage among pigs at various stages of production in farms where persistent outbreaks of human MRSA has been reported among piggery employees.

A cross-sectional study was performed at a commercial pig farm in NSW. Swabs were collected from the internal nares and ear skin of individual animals. A questionnaire was also completed by the piggery manager. The questionnaire collected information on various aspects of farm practices, animal health, hygiene and biosecurity. The piggery had seven sheds that included two dry sow sheds, two grower sheds and a single shed for farrowing, weaners, and finishers. The number of animals varied between 1000 and 3000 pigs per shed. Each shed was divided into 8–10 rooms. From each shed 60 animals were randomly chosen resulting in a total of 420 animals being swabbed. Ear and nose swabs were taken from individual animals. Ear and nose swabs of 10 animals were pooled into one to give a total of six pool samples per shed. In addition, five environmental samples from shed walk ways, pen floors, feeders, fences and walls were also collected from each shed and pooled into one sample. All samples were processed within a week of collection commencing with pre-enrichment in Mueller-Hinton (MH) broth containing 6.5% sodium chloride for 18 h at 37°C. After the pre-enrichment stage, two separate procedures were used for MRSA and MSSA screening. For MRSA detection, a selective enrichment was performed in Tryptone Soya Broth (TSB) containing 3.5 mg/L cefoxitin and 75 mg/L aztreonam. Subsequently, a loop of the selective enriched cultured was inoculated onto chromogenic MRSA agar and blood agar. For MSSA screening, inoculum was directly streaked on mannitol salt agar and blood agar after the pre-enrichment stage. Presumptive colonies of MRSA and MSSA were subjected to further confirmatory tests including staining by Gram's method, catalase testing, *S. aureus* Protein A latex agglutination testing, and tube coagulase tests. The susceptibility of all MRSA and MSSA isolates were tested to 28 different antimicrobial agents using the disc diffusion method following the Clinical and Laboratory Standards Institute protocols (CLSI 2014). The detection of *S. aureus* including MSSA and MRSA among pigs of different age groups was compared. The associations between farm practices, considered potential risk factors, and the presence of MRSA in pigs at different production stages were considered.

MRSA was found in pooled samples in every stage of production in this piggery (n = 40). Forty of the 42 pooled samples returned positive results. The MRSA was also identified in the environment of this piggery. However, no disease in pigs related to MRSA was identified in this piggery. MSSA was also found among dry sows, growers, and finishers. The environmental samples were also positive for these sheds at the same time. A total 10 out of 42 pooled samples were positive for MSSA. Weaner and farrowing sheds were negative for MSSA. Antimicrobial susceptibility panel testing was performed on all MRSA and MSSA pig as well as environmental isolates. A diverse antibiogram pattern was found amongst the isolates. MSSA isolates were resistance to fewer antibiotics compared to MRSA.

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Haemophilus parasuis – virulence genes and serovars

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Glässer's disease, caused by *Haemophilus parasuis*, is a significant disease of pigs worldwide, causing polyserositis, polyarthritis and meningitis. There are 15 known serovars of *H. parasuis* (Olvera *et al*. 2006). The correlation between pathogenic and non-pathogenic isolates based on serovar, originally established by Kielstein and Rapp-Gabrielson (1992) through inoculation of pigs with the serovar reference strains, has been challenged (Olvera *et al*. 2006). This has prompted research into virulence genes and other genotyping methods to be able to predict virulence. The hypothesis of the current study was that there is a correlation between six potential virulence genes and the known virulence of the 15 serovar reference strains.

The reference strain for each of the 15 recognised serovars was examined via polymerase chain reaction (PCR) for the presence of the following potential virulence genes: *vtaA* (virulence-associated trimeric autotransporter), *hhdAB* (putative hemolysin operon), *lsgB* (lipopolysaccharide sialytransferase gene), *fhuA* (ferric hydroxamate receptor) and *capD* (polysaccharide biosynthesis protein). The virulence of each of these strains has already been determined by Kielstein and Rapp-Gabrielson (1992).

The results of the presence or absence of these virulence genes for all 15 reference strains are shown in Table 1. No single gene was present in all 10 pathogenic strains and was absent in all five non-pathogenic strains. The best correlation with any single gene was for *vtaA*, which was present in nine out of 10 pathogenic strains and absent in three out of five non-pathogenic strains. The next best correlation occurred with the *hhdAB* gene, which was present in six out of 10 pathogenic strains and absent in all 5 non-pathogenic strains. Overall, none of the tested genes by themselves or in combination were adequate to distinguish between pathogenic and non-pathogenic strains. Current studies were focussed on alternative typing technologies such as enterobacterial repetitive intergenic consensus sequence-based PCR and multi-locus sequence typing to see if these technologies are more useful in predicting pathogenicity in combination with the yes/no approach of the current work. Further, identification of the genes responsible for the high minimal inhibitory concentration (MIC) levels to some antimicrobials recently found in some Australian *H. parasuis* isolates (Dayao *et al*. 2014) is being sought.

Table 1. Results of PCR assays for virulence genes for the serovar reference strains. The virulence is given as highly virulent ++, moderately virulent +, not virulent (according to Kielstein and Rapp-Gabrielson 1992)

Serovar	Strain	Virulence	Presence of[A]					
			vtaA	*hhdA*	*hhdB*	*lsgB*	*fluA*	*capD*
1	NR 4	++	–	–	–	–	–	–
2	SW 140	+	+	–	–	–	–	–
3	SW114	–	–	–	–	–	–	–
4	SW124	+	+	–	–	–	–	–
5	Nagasaki	++	+	+	+	+	+	+
6	131	–	–	–	–	–	–	–
7	174	–	+	–	–	–	–	–
8	C5	+	+	–	–	–	–	–
9	D74	–	–	–	–	–	–	–
10	H367	++	+	+	–	–	+	+
11	H465	–	+	–	–	–	–	–
12	H425	++	+	+	+	+	+	–
13	IA – 84 –17975	++	+	+	–	–	+	–
14	IA – 84 –22113	++	+	+	+	–	+	–
15	SD – 84 –15995	+	+	+	–	–	+	–

[A]Potential virulence genes: *vtaA* (virulence-associated trimeric autotransporter), *hhdAB* (putative hemolysin operon), *lsgB* (lipopolysaccharide sialytransferase gene), *fhuA* (ferric hydroxamate receptor) and *capD* (polysaccharide biosynthesis protein).

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Clinical signs of a European highly-pathogenic strain of China/US porcine epidemic diarrhoea 2a

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Porcine Epidemic Diarrhoea (PEDv) was first recognised in the United Kingdom in 1970 (Wood 1977). In 2010 a variant of PEDv was recognised in China that resulted in severe mortality in piglets less than 10 days of age (Sun *et al.* 2012). In May 2013 this strain was subsequently isolated in the USA and resulted in a major outbreak from 2013 to 2015 in the Americas (Stevenson *et al.* 2013). It was suspected that this virus was present in Europe in 2014. To confirm this suspicion a specific property in the Ukraine was the focus of this study. It was expected that epidemiology methods would confirm that PEDv was present on this property in 2014.

The selected farm practiced weekly batches of 240 farrowing places weaning 3000 piglets per batch. The farm was specific pathogen free to Porcine Reproductive and Respiratory Syndrome virus, *Mycoplasma hyopneumoniae*, Aujeszky's Disease, *Brachyspira hyodysenteriae*, *Sarcoptes scabiei* var *suis*, Toxigenic *Pasteurella multocida*, Transmissible Gastroenteritis Virus (TGE), and OIE pathogens. There were no clinical signs of Swine Influenza. The farm was conscious of biosecurity. The following infectious routes were ruled out: people; transport (feed wagons or slaughterhouse trucking) and feed. The infection was suspected to have been introduced, via the air, from a farm 1.5 km away and under different ownership. Examination of wind data demonstrated this explanation was feasible given the change in the wind direction directly between the two farms for two days prior to the first clinical signs appearing on the property under consideration.

Within hours of the believed introduction, the clinical signs of vomiting and profuse watery yellow diarrhoea spread around the farm in all age groups. The morbidity and mortality of pigs less than 10 days of age was 100%. An abortion outbreak in 36% of sows, 20 to 30 days of pregnancy occurred. A clinical diagnosis of PEDv was made following clinical and postmortem examination of the piglets. On-site testing using a lateral flow device (Antigen Rapid PED/TGE Ag Test Kit; Bionote, Korea) indicated the presence of PEDv antigen in the faeces. These findings were confirmed at the Animal and Plant Health Agency (UK), using an in-house PEDv polymerase chain reaction (PCR) and a commercially available PEDv/TGEv qRT-PCR kit (Qiagen, Hilden, Germany). The BLAST search of the 160 nucleotides RT-PCR amplicon revealed the highest similarity (99%) to several PED viruses from USA and China. The PEDv RNA was then subjected to DNase digest and converted to cDNA for preparation of sequencing libraries using a Nextera XT kit (Illumina; Illumina, San Diego, USA). Paired end sequencing was performed on an Illumina MiSeq. The consensus sequence was obtained by *de novo* assembly using Velvet 1.2.10 of the sequence reads that mapped to the NC003426 reference.

The discovery of this isolated PEDv virus was the first time that the China/US PED 2a strain had been isolated in Europe. The virus was catalogued as Ukraine/Poltava01/2014 strain genome (GenBank accession no. KP403954) and is 27,823 nucleotides in length (excluding the 3' poly A tail). Nucleotide analyses of the full genome of the virus showed the highest similarity to PEDv strains reported recently from the USA, specifically strains USA/Kansas29/2013 (GenBank accession no. KJ645637.1) and USA/Colorado30/2013 (GenBank accession no. KJ645638.1) with 99.8% nucleotide identity.

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China's village pig industry: training influences handling of sick pigs and awareness of medication withdrawal periods

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According to Korves (2015), China produces half of the world's pork and imports the largest volume of pork globally. Gale *et al*. (2012) reported that China's pork industry continues to be volatile and whilst there are a growing number of large, modern producers, the bulk of production still takes place on millions of farms in China's villages. China's swine industry has been affected in recent years by disease outbreaks and food safety scandals that have not only generated volatile price swings but also have negative impacts on the image of the industry and on consumers' trust in pork products. These incidents not only affect farmers' profits and overall efficiency of the industry, but also adversely affect the reputation of the industry and its products. The purpose of this paper is to report the findings from a survey that aimed to provide an understanding of the impact that farmer (defined as the person managing and handling the pigs) training had on current practices of managing sick pigs, the use of medication at the village level, and opportunities to improve herd health and food safety in China's pork industry.

In 2013 a face-to-face survey was conducted with 557 swine producers (139 small, 279 medium and 139 large) across 90 villages in five provinces (Guangzhou, Sichuan, Hubei, Shandong, and Jilin). The results presented in this paper focus on aspects of disease management and are a sub-set of the data collected in the larger survey of village pork producers.

Farmers with training were more likely to know and employ basic health and safety practices than farmers without training (Table 1). As the production of pigs increased the percentage of famers quarantining sick pigs and their awareness of medication withdrawal periods increased. However, the practice of marking sick pigs was less prevalent on the larger herds.

The key outcome of this survey was that regardless of size, training had an impact on food safety practices such as quarantining sick pigs and knowledge of withdrawal periods albeit a diminishing effect as herds sizes increased. Education and reinforcement needs to continue to occur in China in order to improve the safety and thus consumer confidence of domestically produced pork.

Table 1. Handling practices for sick pigs varies depending on training

Practice	If farmer had training			If farmer had no training		
Production (hd/yr)	1–49	50–499	>500	1–49	50–499	>500
			Do you quarantine sick pigs?			
Yes[A] (%)	84.6	89.8	96.8	72.8	77.9	89.5
No (%)	15.4	7.8	3.2	27.2	22.1	10.5
			Do you mark sick pigs?			
Yes (%)	74	76.8	71.4	57.4	59.2	68.4
No (%)	26	23.2	28.6	41.9	40.8	31.6
			Do you know withdrawal period of medication used?			
Yes (%)	84.6	88.9	96.8	77.4	87.4	94.7
No (%)	15.4	11.1	3.2	22.6	12.6	5.3

[A]The percentage of respondents answering 'yes' or 'no' does not necessary equal 100 because respondents could answer 'don't know' or not respond.

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Chapter 10: Contributed papers – Nutrition, growth, gastrointestinal tract and general production

Vitamin E but not selenium alleviates heat stress compromised metabolism in growing pigs

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Pigs raised in a hot environment have an increased carcass fat to protein ratio (Christon 1988). As heat stress (HS) attenuates lipid mobilisation in growing pigs (Pearce *et al*. 2013), it is hypothesised that the increased adiposity is due to elevated insulin, an anti-lipolytic hormone. We have previously observed that supra-nutritional supplementation of selenium (Se) or Vitamin E (VE) could alleviate the physiological response to HS in growing pigs (Liu *et al*. 2014). Therefore, the aim of this study was to investigate the effects of HS and antioxidant supplementation on insulin related metabolism.

Thirty-six gilts (Large White × Landrace) weighing 28.0 ± 4.1 kg (mean ± SD) were fed a Control diet (0.24 ppm Se, 17 IU/kg VE; NRC 2012), a high Se diet (1.0 ppm Se yeast, 17 IU/kg VE), or a high VE diet (0.24 ppm Se, 200 IU/kg α-tocopherol) diet for 14 days. Pigs were then exposed to either a thermoneutral (TN; 20°C) or cyclic HS (35°C from 09:00–17:00 h; 28°C overnight) for 8 d (n = 6 per treatment group). All pigs were restrictedly fed 80% of *ad libitum* over the trial. Pigs were fasted for 18 h from 1800 h on d 7 of thermal exposure and received a simplified oral glucose tolerance test (OGTT; 2 g/kg dextrose) on d 8. Plasma samples were obtained at –1, 30, 60 and 120 min in relative to dextrose intake for insulin, glucose and non-esterified fatty acids (NEFA) measurement. Data were analysed by REML in Genstat (15th edn.).

Feed intake was not different ($P > 0.05$) amongst treatment groups. During the OGTT the HS pigs had a higher glucose concentration at 30 min (6.9 vs 7.9 mM for TN vs HS, $P < 0.01$) and lower insulin concentrations at 30 min and 60 min (101 vs 93 and 23 vs 13 μU/mL for TN vs HS at 30 and 60 min, respectively; $P < 0.05$ for both comparisons) compared to TN conditions. This suggests that HS might have compromised insulin secretion. While glucose levels were not affected by dietary antioxidants, pigs fed on higher Se exhibited higher insulin concentrations ($P = 0.01$) 60 min after the OGTT during TN conditions, suggesting that overloaded Se might potentiate insulin resistance in normal condition. During HS pigs had lower fasting NEFA levels than those measured under TN condition (250 vs 141 μM, $P < 0.05$), indicating HS reduced lipid mobilisation. The pigs fed high VE had higher NEFA concentrations at 120 min than Control pigs in HS ($P < 0.05$), suggesting high VE facilitated lipid mobilisation in HS (Table 1).

In conclusion, the observed decrease in lipid mobilisation during HS was not due to hyperinsulinemia, but rather a decrease in insulin secretion was noted. The attenuated lipid metabolism during HS needs to be further explored. Reduced lipid mobilisation during HS was ameliorated by dietary VE, but not by Se, suggesting that VE may prevent increased adiposity experienced during hot seasons.

Table 1. Effects of heat stress and Se or VE on plasma metabolites in growing pigs during an OGTT[A]

OGTT parameters	20°C			35°C			SED	*P* value			
(time, min)	NRC[B]	Se[C]	VE[D]	NRC	Se	VE		T[E]	Diet	T × Time	T × Diet
Glucose 0 (mM)	5.5	5.3	5.2	5.3	5.9	5.1	0.90	0.11	0.37	<0.01	0.79
Glucose 30 (mM)	7.7	7.1	6.2	7.9	8.1	7.4					
Insulin[F] 0[D]	6.3	3.3	1.8	2.3	3.1	4.4	6.69	0.13	0.01	0.05	0.27
Insulin 30	96	101	108	94	94	93					
Insulin 60	16	36	16	12	15	12					
NEFA 0 (mM)	195	270	321	156	120	146	62.1	0.14	0.02	<0.01	0.41
NEFA 120 (mM)	117	75	176	58	55	226					

[A]OGTT: oral glucose tolerance test. [B]Diet contains 0.24 ppm selenium, 17 IU/kg Vitamin E. [C]Diet contains 1.0 ppm selenium, 17 IU/kg Vitamin E. [D]Diet contains 0.24 ppm selenium, 200 IU/kg Vitamin E. [E]Temperature. [F]Insulin is expressed as μU/mL.

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Pig feed ingredients affect enzyme diffusion coefficients

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Pig diets are mainly manufactured from grains that are milled to various particle sizes. Particle size affects grain and feed digestibility (Huang *et al.* 2015; Nguyen *et al.* 2015), and although animal studies typically use complete feeds (milled grains and ingredients), *in vitro* studies have concentrated on milled grains to calculate the enzyme diffusion coefficients. These are proposed to control particle size-digestibility relationships (Al-Rabadi *et al.* 2009; Tinus *et al.* 2012). Ingredients in feed supplement grains to achieve a balanced nutritional profile, and can affect diet digestibility. This study examined how ingredients affected enzyme diffusion coefficients, with the hypothesis that the coefficients would be unchanged in pig diets.

Sorghum (var. *MR43*) and field pea (var. *Walana*) were each milled and mixed to different particle sizes in duplicate to make 20 diets. The sorghum diets contained 50% of milled sorghum, with dehulled oats (10%), whey powder (10%), lupin kernels (5%), canola meal (5%), soybean meal (5%), meat meal (7%), fish meal (3%), blood meal (2%), tallow (2%), and mineral/vitamin mixes as the ingredients. The field pea diets contained 30% of milled pea, and the ingredients were soft wheat (29%), barley (23%) meat- and bone-meal (7%), soybean meal (7%), tallow (2%), and mineral/vitamin mixes. *In vitro* digestion and geometric mean particle size diameter (D_{gw}) of the milled grains and diets, were analysed (Nguyen *et al.* 2015). Based on Tinus *et al.* (2012), the digestograms were described by a modified first-order kinetic model to obtain the rates of starch (K_{ST}) and protein (K_{PR}) digestion. Diffusion coefficients (D_{IFF}) were obtained from relationships $1/K_{ST}$ α (D_{gw}^2/D_{IFF}) and $1/K_{PR}$ α (D_{gw}^2/D_{IFF}) by regression. Minitab™ statistical procedures were used.

The ingredients did not materially change ($P > 0.05$) the D_{gw} of the diets, which was within 10% of that of the milled grains (field pea: $D_{gw\text{-}grain} = 1.07\ D_{gw\text{-}diet}$; sorghum: $D_{gw\text{-}grain} = 0.91\ D_{gw\text{-}diet}$, $R^2 > 0.4$, $P < 0.01$). However, the starch and protein contents (g/100g; mean ± SD) were for the sorghum: grain - 13 ± 0.3 protein, 58 ± 0.6 starch, diet - 23 ± 0.5 protein, 33 ± 0.7 starch, and for the field peas: grain - 22 ± 0.1 protein, 42 ± 0.1 starch, diet - 20 ± 0.4 protein, 38 ± 1.1 starch. The kinetic model adequately described ($R^2 > 0.9$; $P < 0.001$) the digestograms, and $1/K_{PR}$, $1/K_{ST}$ and D_{gw}^2 were significantly ($P < 0.01$) related (Fig. 1). For the diets and milled grains, the protein digested faster than the starch, and the inverse square relationship is consistent with digestions being rate-limited by enzyme diffusion within particles. However, the ingredients changed the diffusion coefficients ($cm^2\ s^{-1}$) by 30-400% (sorghum: grain – 270 for protein, 3 for starch, diet – 210 for protein, 6 for starch; field peas: grain – 530 for protein, 2 for starch, diet – 110 for protein, 4 for starch). The ingredients reduced the protein coefficients and increased the starch coefficients. It is suggested that the protein-containing ingredients digested more slowly than the field pea or sorghum protein, whereas the starch-containing ingredients digested faster than the field pea or sorghum starch. Starch-protein interactions exist in field peas and sorghum, and limit starch digestion (Tinus *et al.* 2012; Nguyen *et al.* 2015). While the inverse square relationship between particle size and rate of digestion holds for diets and grains, pig feed ingredients affect the values of apparent enzyme diffusion coefficients.

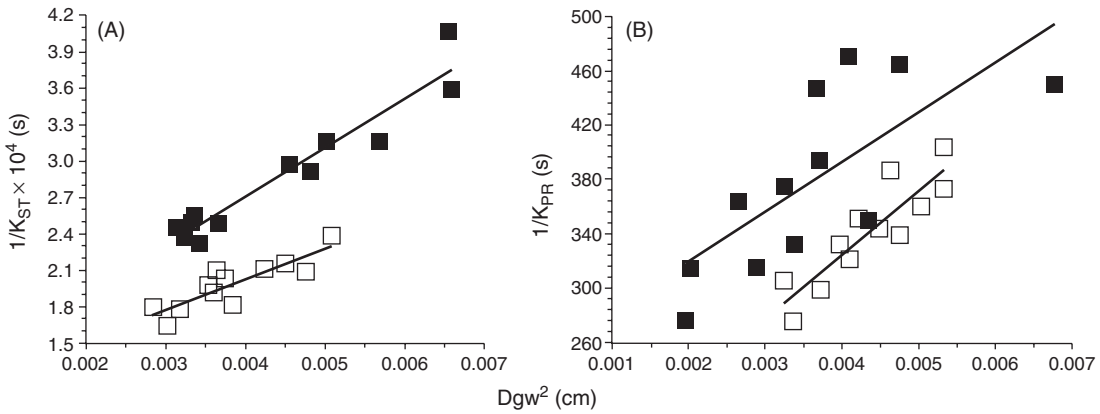

Fig. 1. Typical relationships between the rates of digestion and particle sizes of the milled grains (■) and diets (□) showing starch digestion in the field peas (A) and protein digestion in the sorghum (B). Error bars were omitted for clarity (predicted –).

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Subtilisin protease increases digestible energy content but not protein digestibility in sorghum- and wheat-based diets

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Protease increases *in vitro* protein digestibility (Sopade and Lu, unpublished data) of cereals and protein meals that have an apparent ileal digestibility of 90% or less. Subtilisin has also been shown to improve sorghum protein digestibility (Finn 2011) and the growth performance of pigs offered sorghum-based diets (Cadogan and Finn 2011). The hypothesis of this experiment was that the protein and energy digestibility will be enhanced by the protease in sorghum-based diets containing less digestible protein sources as opposed rations containing high quality proteins such as soybean meal (SBM).

The study was $2 \times 2 \times 2$ factorial design with the factors being: grain (sorghum or wheat); protein source [(soybean meal (SBM) + expeller canola meal (CSM) or Peas+Meatmeal (MM)]; and protease (0 and 350 ppm Subtilisin, Dupont, Marlborough, UK). The diets were formulated to contain 14.0 MJ digestible energy (DE)/kg and 0.72 g available lysine (Lys) per MJ DE. Ileal digesta and faecal samples were collected from male pigs (PIC Australia; ~35 kg n = 14) fitted with a simple T-piece cannula 15 cm anterior to the ileo-caecal valve (van Barneveld 1999). Digesta and faecal samples were pooled and subsampled, freeze dried, and analysed for acid insoluble ash (as an indigestible marker), gross energy, crude protein (CP) and Lys, methionine (Met) and threonine (Thr), for subsequent calculation of their coefficient of total tract apparent digestibility (CTTAD). Data were analysed by ANOVA using SPSS (PASW® Statistics 18: USA).

The SBM + CSM protein source had a higher ileal ($P < 0.001$) and faecal ($P < 0.001$) DE content than the Peas + MM. There was no effect ($P > 0.05$) of grain type on ileal DE content, however wheat had a higher faecal DE compared to sorghum ($P < 0.001$). The protease had a greater effect on sorghum-based diets ($P = 0.002$), particularly in the presence of SBM + CSM, where ileal and faecal DE contents were increased by 1.15 and 0.61 MJ/kg, respectively (Table 1). Protease produced an improvement in faecal DE content ($P = 0.002$), but had no influence on ileal DE or the CTTAD of CP, Lys, Meth, or Thr. There was a significant interaction between protease and protein meal ($P < 0.001$) with the greater enzyme response on SBM + CSM. Sorghum exhibited an inferior CTTAD of CP ($P < 0.001$) to that of wheat, and Pea + MM had a lower protein digestibility ($P = 0.003$) compared to SBM + CSM. The hypothesis was not fully supported, as the protease had a greater positive effect in diets containing SBM + CSM, although the sorghum was more responsive to the enzyme than wheat.

Table 1. Effects of protease on the coefficient of total tract apparent digestibility (CTTAD) of energy, crude protein and selected amino acids in pigs fed diets based on different grains and protein meals

Grain	Protein source	Protease	Ileal DE (MJ/kg)	Faecal DE (MJ/kg)	CTTAD (ileum) CP	Lys	Meth	Thr
Sorghum	SBM + CSM	–	11.53	13.96	79.7	87.5	90.8	78.8
Sorghum	SBM + CSM	+	12.68	14.57	77.9	86.8	91.2	78.1
Sorghum	Peas + MM	–	11.00	13.54	74.1	85.0	90.5	76.1
Sorghum	Peas + MM	+	11.20	13.81	75.6	85.6	90.2	76.3
Wheat	SBM + CSM	–	11.80	14.20	80.6	82.2	88.3	75.7
Wheat	SBM + CSM	+	11.72	14.46	82.5	84.3	91.5	79.4
Wheat	Peas + MM	–	10.94	14.07	78.7	82.8	89.5	75.2
Wheat	Peas + MM	+	11.36	14.20	82.1	86.0	90.2	80.0
SEM[A]			0.253	0.084	0.70	0.68	0.49	0.87
P values								
Grain			NS	<0.001	0.004	NS	NS	NS
Protein			<0.001	<0.001	0.003	NS	NS	NS
Protease			NS	0.002	NS	NS	NS	NS

[A]SEM, Standard error of the mean; NS, Not significant.

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Increased growth performance in weaned pigs fed a diet supplemented with graded amounts of two phytases

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Phytase addition to swine diets generally causes a marked increase in mineral utilisation and bone strength with inconsistent effects on performance (Selle and Ravindran 2008). The aim of this study was to evaluate the effects on performance of a *C. braakii*-(Ronozyme HiPhos) and an *E. coli*-(Quantum Blue) derived 6-phytase at one, two and three times their commercial recommended feed inclusion levels in weaned pigs. The study tested the hypothesis that dosages of high phytase content in diets will give additional benefit in pigs by improving growth performance.

An experiment with 96, 28-day-old weaned pigs (Large-White x Redon) having an initial body weight of 7.9 ± 0.73 kg (mean ± SE) was performed. Pigs were randomly allotted into eight groups of 12 animals each. They were fed *ad libitum* for 42 days with diets based on corn, soybean meal and rapeseed meal. Diets were a positive control diet (PC) formulated to meet the animal requirements according to NRC (2012) [total P: 0.66%; total Ca: 0.80%; crude protein: 192 g/kg; metabolisable energy (ME): 14.2 MJ], or a matrix control diet (MC) with reduced nutrient content [total P: 0.55%; total Ca: 0.63%; crude protein: 188 g/kg; ME: 14.0 MJ]. The MC diets were supplemented with Ronozyme HiPhos at 1000 (H1000), 2000 (H2000) and 3000 U/kg (H3000), and with Quantum Blue at 500 (Q500), 1000 (Q1000) and 1500 U/kg (Q1500). Growth performance parameters were recorded throughout the study and average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. Data were examined by ANOVA and differences between groups were determined by Fisher's least significant difference multiple-range test (significant at $P < 0.05$).

For the first period (d 0 to 14), the ADG was improved ($P < 0.05$) in the H2000 group in comparison to the PC and MC groups but also to the H3000, Q500 and Q1000-fed pigs (Table 1). In the second period (d 15 to 42), all phytase-fed pigs except those in group Q500 performed better ($P < 0.05$) in ADG than the MC-fed pigs. Overall, ADG was improved ($P < 0.05$) and ADFI increased ($P < 0.05$) in H1000, H2000, H3000 and Q1500 treatments in comparison to MC-fed pigs.

Similar effects on performance with graded amounts of phytase have been previously reported (Kies *et al.* 2006; Guggenbuhl *et al.* 2012*a*, 2012*b*). In the present experiment, both phytases tested improved ADG similarly compared to the MC treatment group. These effects were not dose dependent. In conclusion, high dosages of phytase had beneficial effects on performance compensating for reduced nutrient levels.

Table 1. Growth performance in weaned pigs fed graded amounts of two different phytases

Treatments	Period (d)	MC	PC	Phytase (FYT/kg)						SEM[A]	*P* value
				H1000	H2000	H3000	Q500	Q1000	Q1500		
ADG (g)	0–14	154^{a}	168^{a}	186^{ab}	226^{b}	173^{a}	162^{a}	154^{a}	202^{ab}	6.3	0.043
	15–42	430^{a}	482^{abc}	511^{bc}	542^{c}	509^{bc}	442^{ab}	505^{bc}	530^{c}	9.5	0.024
	0–42	336^{a}	375^{abc}	400^{bcd}	432^{d}	394^{bcd}	347^{ab}	385^{abcd}	418^{cd}	7.4	0.015
ADFI (g)	0–14	256	261	279	331	280	251	250	288	7.4	NS[B]
	15–42	807	853	918	953	927	848	888	943	14.7	NS
	0–42	606^{a}	639^{ab}	697^{bc}	739^{c}	706^{bc}	644^{ab}	671^{abc}	720^{bc}	11.8	0.041
FCR	0–14	1.69	1.61	1.57	1.43	1.71	1.63	1.80	1.47	0.074	NS
	14–42	1.86	1.75	1.80	1.89	1.79	2.06	1.74	1.89	0.036	NS
	0–42	1.86	1.76	1.77	1.85	1.76	2.01	1.73	1.82	0.034	NS

[A]SEM, standard error of the mean. [B]NS, not significant. [a,b,c,d]Means is a row not having the same superscript are significantly different.

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Comparative efficacy of a blend of multiple enzymes and an in-feed antibiotic on growth performance and apparent digestibility of energy and protein in nursery pigs

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Traditionally, in-feed antibiotics have been used as growth stimulants and to control gastrointestinal tract pathogens in pigs (Pluske *et al*. 2002). However, because of the perceived risks posed to human health by animal agriculture via the emergence of antibiotic-resistant bacteria, evaluation of alternatives to antibiotics is a topical area of research. There is accumulating evidence that added feed enzymes may aid the pig overcome digestive and enteric health challenges associated with weaning (Pluske *et al*. 2002; Kiarie *et al*. 2013). Few studies have investigated comparative effects of an antibiotic and feed enzymes on post-weaning performance. It was hypothesised that an antibiotic and a multi-enzyme blend (ME) will result in similar growth performance of nursery pigs. The objective of the study was to investigate the comparative efficacy of an antibiotic and ME on growth performance and apparent total tract digestibility in weaned pigs fed a corn-soybean meal based diet.

Seventy-two pigs (6.6 ± 0.1 kg; mean ± SD) were used in a two-phase trial (Phase 1, d 0–14; Phase 2, d 15–42). Diets were: Positive control (PC) + antibiotic (0.5% Mecadox; Phibro Animal Health, Fairfield, NJ); Negative control (NC, no additives); and NC + ME (ME, 4000 U of xylanase, 150 U of β-glucanase, 500 U of protease and 1000 U/kg of amylase per kg of feed; Danisco UK Ltd). The PC and NC basal diets were based on corn and soybean meal and were formulated to meet NRC (2012) specifications except that the NC diet had 5% less digestible energy (DE). In PC, DE and standardised ileal digestible lysine contents were 14.8 MJ/kg and 14 g/kg, respectively, in Phase I, and corresponding specifications for Phase 2 were 14.7 and 12.5 g/kg, respectively. All diets had 0.3% acid insoluble ash, 500 FTU of phytase/kg, and were fed in mash form. Each diet was allotted to eight pens with three pigs per pen. Pigs had free access to feed and water. Feed intake and body weight (BW) were measured weekly to determine average daily feed intake (ADFI), average daily gain (ADG) and gain to feed (G : F). Grab samples of faeces were collected 3 days at the end of each phase to determine the coefficient of total tract apparent digestibility (CTTAD). Data were analysed using PROC GLM procedures (SAS®; USA).

Pig fed PC and ME had higher ($P < 0.05$) ADG than NC fed pigs in Phase 1 (Table 1). In Phase 2, pigs fed PC had higher ($P < 0.05$) ADG than ME which was in turn similar ($P > 0.05$) to that of NC-fed pigs. Pigs fed PC were heavier ($P < 0.05$) at the end of the trial (BW 42) than NC. Treatments did not affect ($P > 0.05$) ADFI whilst PC fed pigs had higher ($P < 0.05$) G : F in Phase 1. In both phases, pigs fed PC and ME diets had higher ($P < 0.05$) CTTAD of GE and CP than NC-fed pigs. A supplemental multi-enzyme blend caused similar performance and CTTAD of GE and CP to pigs fed an antibiotic in the early phase of weaning, suggesting that a feed enzyme mix such as that used in the present study could be a tool for managing the growth performance challenges immediately after weaning.

Table 1. Effects of feeding an antibiotic and a multi-enzyme blend on growth performance and coefficients of total tract apparent digestibility (CATTD) in nursery pigs

Item	Phase 1, days 0–14					Phase 2, days 15–42					
	Performance			CTTAD		Performance				ATTD	
	ADG	ADFI	G : F	GE[B]	CP[C]	BW42	ADG	ADFI	G : F	GE	CP
PC	264[a]	327	0.82[a]	0.80[a]	0.75[a]	28.9[a]	664[a]	989	0.67	0.73[a]	0.70[a]
NC	217[b]	320	0.68[b]	0.73[b]	0.70[b]	27.2[b]	626[ab]	996	0.64	0.70[b]	0.66[b]
ME	265[a]	372	0.71[b]	0.78[a]	0.74[a]	27.7[ab]	618[b]	983	0.63	0.74[a]	0.71[a]
SEM[A]	15.5	18.4	0.02	1.41	1.85	0.54	14.0	29.6	0.01	0.66	1.25

[A]SEM, standard error of means. [B]GE, gross energy. [C]CP, crude protein. [a,b]Means within a column not having the same superscript are significantly different ($P < 0.05$).

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Feeding caffeine to sows in gestation reduced stillbirths

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The incidences of piglets born dead or with low viability remains unacceptably high in the pig industry, and is likely to increase with continuing selection for high total litter size (Kerr and Cameron 1995; Rootwelt *et al.* 2012). Caffeine promoted breathing respiration in premature human babies (Benowitz 1990) and administration of caffeine to sows 24 h prior to an induced parturition improved aspects of neonatal piglet performance (Superchi *et al.* 2013). The current study hypothesised whether 3 days of oral caffeine ingestion by sows prior to a natural parturition would reduce the number of still births and improve piglet behaviour immediately post-partum.

Sixty-four multiparous (parity 3.2 ± 0.14; mean ± SE) Large White × Landrace sows were moved into farrowing crates at least 5 days prior to their farrowing due date. Treatments commenced 3 days prior to the farrowing due date, with sows receiving either 2 g of caffeine with their daily feed ration three times per day (Caffeine, n = 34), or no caffeine (Control, n = 30). Treatments continued up until the commencement of farrowing. During farrowing, piglets were tagged and the times taken to stand, reach the udder and begin suckling were recorded. The total numbers of piglets born, born alive, born dead and mummified were also recorded. For statistical analysis, piglets were grouped by birth order (first, 1–4; middle, 5–8; last, >8), with the data analysed using a univariate general linear model (IBM SPSS Statistics 21) with birth order, treatment, parity, pen and room as fixed effects and litter size as a covariate. Behaviour data were not normally distributed, and were log transformed prior to analyses. Data are presented as mean ± SE of the mean.

There were no treatment effects ($P > 0.05$) (Control vs Caffeine) on total born litter size (11.9 ± 0.56 vs 11.8 ± 0.53), piglet survival in the first 24 h (95.1% vs 96.6%) or piglet survival from 24 h to weaning (90.1% vs 90.3%). However, compared to control sows, Caffeine sows gave birth to fewer stillborn piglets (0.29 ± 0.09 vs 0.67 ± 0.15; $P < 0.05$) and had more live born piglets (11.65 ± 0.22 versus 11.01 ± 0.23; $P < 0.05$). The impact of treatment on piglet behaviour immediately post-partum was affected by birth order (Table 1). Piglets born to Caffeine-treated sows, and born last, took longer ($P < 0.05$; Table 1) to reach the udder (14.44 mins) and suckle (15.2 min) compared to piglets born last in Control sows. Piglets born to Caffeine treated sows, and born first, also took longer ($P < 0.05$; Table 1) to reach the udder (12.97 min) compared to first-born piglets in Control sows.

It is suggested that the increased latency to reach the udder and suckle observed in piglets born last to Caffeine-treated sows reflects a reduction in the number of stillborn piglets. Caffeine promotes breathing, and by reducing the incidence of stillbirths, may have increased the incidence of lower viability piglets born at the end of parturition. These lower viability piglets will always have an increased latency to reach milestones such as reaching the udder and beginning to suckle. Overall, the current data provide preliminary evidence that feeding caffeine to peri-parturient sows reduced farrowing-induced piglet mortalities, and therefore has the potential to increase the number of piglets weaned per sow per litter.

Table 1. The effect of birth order (first born = 1–4, middle born = 5–8, last born >8) on time from birth to reach the udder and suckle for piglets born to Control and Caffeine-treated sows (data transformed prior to analyses, with raw means presented)

Birth Order	First		Middle		Last	
Treatment	Control	Caffeine	Control	Caffeine	Control	Caffeine
Time to udder (mins)	16.24[a]	29.21[b]	25.24	27.43	20.37[a]	34.81[b]
Time to suck (mins)	41.81	52.36	49.00	48.24	37.74[a]	52.90[b]

[a,b]Within a row, and Birth Order, means not having the same superscript are significantly different ($P < 0.05$).

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Combined supplementation of boron, vitamin E and omega-3 fatty acids increases tight junction protein mRNA expression in the colon of *E. coli*-infected weaner pigs

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Enhancing intestinal barrier function and protection against epithelial cell damage in weaner pigs challenged by bacterial and (or) environmental stressors can improve health and growth efficiency. Among many nutrients boron (B), vitamin E (VE) and omega-3 fatty acids (*n*-3 FA) are known to reduce bacterial infection-induced responses through regulation of eicosanoid mediators and improved antioxidant capacity (Kim *et al*. 2013). This study tested the hypothesis that dietary supplementation with a combination of B, VE and *n*-3 FA will improve intestinal barrier function in weaned pigs infected with an enterotoxigenic strain of *E. coli* through down regulation of eicosanoid mediators and improved antioxidant capacity.

A total of 35 pigs (Large White × Landrace × Duroc) weaned at 21 ± 3 d of age and weighing 6.2 ± 0.05 kg (mean ± SE) was allocated to a completely randomised block design with five dietary treatments (n = 7): 1) Control; 15 MJ digestible energy (DE)/kg and 0.9 g standardised ileal digestible lysine/MJ DE, 2) B; Control + 7.5 ppm B (as boric acid), 3) B + VE; Control + B + 200 IU VE as *dl*-α-tocopheryl acetate, 4) B + *n*-3 FA; Control + B + 2% *n*-3 FA (as linseed oil), and 5) Control + B + VE + *n*-3 FA. Diets were fed *ad libitum*. Pigs were challenged with *E. coli* serotype O149:K91:K88 at d 7, 8 and 9 after weaning. Blood samples were collected before (d 7) and after (d 10) infection for cell counts, and all pigs were euthanised on d 10 to collect liver and intestinal tissue samples for analysis of tight junction protein gene expression (occludin and ZO-1) in the ileal and colonic epithelium using RT polymerase chain reaction (PCR). The concentrations of prostaglandin E_2 (PGE_2) and total glutathione (tGSH) were analysed using commercial ELISA kits. Data were analysed using one-way ANOVA (Genstat, 15th Edition; UK).

White blood cell counts showed that *E. coli* infection increased ($P < 0.001$) the numbers of leukocytes, lymphocytes, neutrophils and monocytes, indicating successful immune system activation. The concentration of tGSH in the liver tended to be increased ($P = 0.083$) in pigs fed the B + VE and B + *n*-3 FA diets compared with pigs fed the B diet. The concentration of PGE_2 in the ileal epithelium tended to be increased ($P = 0.066$) in pigs fed the B + VE + *n*-3 FA diet compared with pigs fed the Control diet. The relative mRNA expressions of occludin ($P = 0.105$) and ZO-1 ($P < 0.05$) in the colonic epithelium were increased in pigs fed the B + VE + *n*-3 FA diet compared with pigs fed the Control diet (Table 1). The correlation study results indicated that increased mRNA expressions of selected tight junction proteins in the colonic epithelium of pigs fed the B + VE + *n*-3 FA diet were not associated with either *in vivo* biosynthesis of the eicosanoid mediator PGE_2, or the production of the antioxidant tGSH.

Table 1. Effect of boron (B), vitamin E (VE), omega-3 fatty acids (*n*-3 FA) on concentrations of PGE_2, tGSH and relative expression of occludin and ZO-1 mRNA in *E. coli*-infected pigs after weaning

	Control	Boron	B+VE	B+*n*-3FA	B+VE+*n*-3FA	SEM[A]	*P* value
				Liver			
PGE_2[B]	26	32	22	34	25	3.8	0.170
tGSH[C]	3772[ab]	3335[a]	4138[b]	3949[b]	3609[ab]	201.6	0.083
				Ileum			
PGE_2	108[a]	126[ab]	71[a]	105[a]	200[b]	30.0	0.066
tGSH	4226	3812	4004	3704	4279	290.7	0.537
				mRNA expression in the colon			
Occludin	1.00	1.10	0.92	1.09	1.69	0.204	0.105
ZO-1	1.00	1.45	1.09	1.25	1.84	0.196	0.049

[A]SEM, standard error of mean. [B]PGE_2 (μg/g wet tissue), prostaglandin E_2. [C]tGSH (μg/g wet tissue), total glutathione. [a,b,c]Means in a row not having the same superscript are significantly different.

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Some bitter compounds show potential for decreasing feed intake and fat deposition while others improve growth and feed conversion ratio in finishing pigs

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Immunocastration (IC) of pigs has often been related to increased appetite. In addition, finishing pigs divert a significant part of the dietary net energy into lipid stores resulting in excess fat deposition, poor feed efficiency and carcass downgrades. Recent findings of the physiological mechanisms around bitter taste perception have shown the potential of bitter compounds to suppress appetite (Janssen *et al*. 2011; Roura 2011). The current study tested the hypothesis that bitter compounds can reduce feed intake and fat deposition while improving feed conversion in IC finishing pigs.

A total of 175 Improvac®-treated male pigs with a body weight (BW) of 65.2 ± 5.54 kg (mean ± SD) were selected and housed in individual pens. Animals were weighed and randomly assigned to one of the seven experimental diets (25 pigs per treatment). The trial was conducted over two consecutive blocks (13 and 12 pigs per treatment in blocks one and two, respectively). Animals were offered water and experimental feeds *ad libitum*. The seven experimental feeds consisted of a reference diet (14.6 MJ digestible energy (DE)/kg and 0.58 g available lysine/MJ DE) without (Control) or with one of the six bitter supplements: caffeine (at 0.05% inclusion) and the aqueous extracts of rhubarb (*Rheum rhabarbarum* L.), brassica (*Sinapis alba* L.), gentian (*Gentiana lutea* L.), quassia (*Quassia amara* L.) and artemisia (*Artemisia absinthium* L.), all of them at 0.1% inclusion. Average daily feed intake (ADFI) and average daily gain (ADG) were measured over a 5-week test period. Carcass weight, back fat depth, loin muscle depth and carcass yield were measured at slaughter. The results were analysed using the least significant difference test of the GLM procedure (SAS®; USA).

Overall (d 0 to 35), pigs fed the diet with 0.05% caffeine had a lower ($P < 0.01$) ADFI and backfat deposition and tended ($P < 0.1$) to have a lower ADG compared to Control pigs (Table 1). Gentian and artemisia extracts resulted in a higher ($P < 0.05$) ADG without affecting ($P > 0.1$) ADFI. Similarly, rhubarb and quassia tended ($P < 0.1$) to increase ADG. The FCR of the pigs fed with 0.1% rhubarb, gentian and quassia extract was lower ($P < 0.05$) than the Control pigs. None of the treatments with significantly heavier carcass weights showed more back fat than the Control pigs. Furthermore, feeding quassia tended ($P < 0.1$) to increase loin muscle depth relative to the Control group. In summary, the hypothesis that bitter compounds would decrease feed intake was confirmed in the case of caffeine but not the other compounds tested. However, gentian, quassia and rhubarb extracts increased ADG and feed efficiency, a result that warrants further investigation.

Table 1. Effects of bitter taste compounds on performance and carcass traits of finishing pigs

	Treatments[A]							
Item	Con.	Caff	Rhu	Bra	Gen	Qua	Art	SEM[B]
Initial BW (kg)	72.0	72.0	72.0	72.0	72.0	72.0	71.9	0.43
Final BW (kg)	114.0^{ab}	111.7^{b}	116.1^{a}	115.7^{ab}	117.5^{a}	117.1^{a}	117.7^{a}	0.75
Days 0 to 35								
ADG (kg)	1.20^{ab}	1.12^{b}	1.28^{ab}	1.25^{ab}	1.31^{a}	1.29^{ab}	1.31^{a}	0.015
ADFI (kg)	3.03^{a}	2.73^{b}	3.07^{a}	3.15^{a}	3.14^{a}	3.09^{a}	3.20^{a}	0.038
FCR[C] (kg:kg)	2.55^{a}	2.47^{ab}	2.40^{b}	2.55^{a}	2.40^{b}	2.40^{b}	2.45^{ab}	0.021
Carcass characteristics								
Carcass yield (%)	73.8	73.9	73.2	73.6	73.9	74.1	74.0	0.15
HSCW[D] (kg)	84.2^{ab}	82.6^{b}	85.5^{ab}	85.6^{ab}	86.9^{a}	86.8^{a}	87.1^{a}	0.58
Backfat depth (mm)[E, F]	13.5^{a}	11.5^{b}	12.8^{ab}	13.8^{a}	14.3^{a}	13.4^{ab}	13.7^{a}	0.24
Loin depth (mm)[F]	52.9^{ab}	51.9^{b}	54.0^{ab}	54.2^{ab}	53.4^{ab}	56.5^{a}	56.2^{a}	0.53

[A]Con., Control; Caff, caffeine; Rhu, rhubarb; Bra, brassica; Gen, gentian; Qua, quassia; Art, artemisia. [B]SEM, standard error of the mean. [C]FCR, feed conversion ratio. [D]HSCW, hot standard carcass weight. [E]P2 position. [F]Analysed using HSCW as a covariate. [a,b]Means in a row not having the same superscript are significantly different ($P \leq 0.05$).

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Spray-dried porcine and bovine plasma are equally efficacious and superior than ovine plasma when fed to newly weaned pigs

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Spray-dried plasma (SDP) and similar products have been used by the pig industry for over 20 years to support young pigs prior to and after weaning (Tucker *et al.* 2011). To date, the comparison of porcine and bovine SDP has shown porcine SDP to produce superior growth performance and lower mortalities after weaning (Torrallardona 2010). However, there are recorded comparisons of ovine SDP with either bovine, porcine or mixed-species plasma in weaner pigs. This experiment tested the hypothesis that porcine, bovine and ovine SDP would be equally efficacious in improving growth performance of weaned pigs.

Sixty individually-housed weaner pigs (Large White × Landrace) with an initial body weight (BW) of 7.96 ± 0.047 kg (mean ± SD) and weaned at 25 days of age were stratified into blocks of four pigs on weaning weight and allocated to a randomised complete block design experiment to examine the effects of diet type on performance in the weaner period. The diets were: a commercial Control weaner diet (Riverina Stockfeeds, Warwick, VIC); Control diet with 5% bovine SDP; Control diet with 5% ovine SDP; and Control diet with 5% porcine SDP replacing other protein sources. Diets comprised 15.4 MJ digestible energy (DE)/kg and 0.92 g standardised ileal digestible lysine/MJ DE. Pigs were fed *ad libitum* for 21 days with water available on an *ad libitum* basis. Feed refusals were determined daily and pigs were weighed weekly to determine average daily gain (ADG), average daily feed intake (ADFI) and feed : gain (F : G). Data were analysed by ANOVA (Genstat, 15th Edition; UK) using initial BW as a covariate and contrasts made between control and SDPP as well as within SDPP.

During the first week of the study, SDP increased ADG (202 vs 257 g, $P = 0.022$) and improved FCE (0.65 vs 0.82, $P = 0.007$) with no difference between the different SDP sources (Table 1). These responses occurred without any effect on ADFI ($P = 0.28$). When examined over the 21 day study period, SDP increased ADG (329 vs 377 g, $P = 0.007$) and FCE (0.67 vs 0.73, $P = 0.018$). However, there were within SDP effects such that the ADG of pigs fed porcine and bovine SDP had similar ADG which tended ($P = 0.060$) to be greater than those fed ovine SDP which, in turn, tended ($P = 0.060$) to be higher than Control-fed pigs. There were also within SDP effects on F : G ($P = 0.020$) such that the F : G of pigs fed porcine and bovine SDP had the same F : G that were greater than both the pigs fed ovine SDP and the Control pigs. Over the duration of the study, SDP tended to increase ADFI (499 vs 525 g $P = 0.069$) with no differences between the different SDP. In conclusion, porcine and bovine SDP increased growth performance of weaned pigs in a similar manner whereas the effects of ovine SDP were not as great.

Table 1. The influence of feeding different spray-dried plasma (SDP) products on performance for 21 days after weaning

Treatment	Control	SDP			SED[A]	*P* value		
		Bovine	Ovine	Porcine		Diet	Control vs SDP	Within SDP
BW, d 7 (kg)	9.39	9.78	9.64	9.84	0.205	0.13	0.035	0.54
BW, d 21 (kg)	14.9	16.1	15.4	16.2	0.44	0.009	0.005	0.12
				Days 1–7				
ADG (g)	202	259	246	266	28.2	0.12	0.022	0.77
ADFI (g)	297	311	321	309	17.9	0.63	0.28	0.77
F : G	0.65	0.83	0.77	0.87	0.076	0.031	0.007	0.45
				Days 1–21				
ADG (g)	329	388	348	395	20.8	0.006	0.007	0.060
ADFI (g)	499	526	520	530	17.3	0.30	0.069	0.85
F : G	0.67	0.75	0.68	0.75	0.029	0.005	0.018	0.020

[A]SED, standard error of the difference for Diet and within Plasma effects. For SED for Control vs Plasma, multiply by 0.816.

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Improving weaner pig performance through the inclusion of activated medium chain fatty acids

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Alternatives to using antimicrobial compounds in pig production include the use of medium chain fatty acids (MCFA) with a total chain length of 6 to 12 carbon atoms (caproic, caprylic, capric and lauric acid). The MCFA have shown positive effects on intestinal morphology and modification of the gastrointestinal tract microbiota (Dierick *et al.* 2002). Aromabiotic® Pig (Nuscience, Drongen, Belgium) is a proprietary blend of high purity activated MCFA that have been designed for use in pigs. The functional effects of MCFA lead to the hypothesis that the inclusion of Aromabiotic® Pig, with or without the presence of medication, will enhance the performance of weaner pigs.

Newly-weaned pigs aged 23 days and weighing 5.1 ± 0.10 kg (mean ± SE) were housed in pens of 14 and allocated to one of three treatments (n=10) over a 4-week period using a randomised block design with sex, weight and entry time as blocking factors. Pens were weighed weekly, for 4 weeks, with feed disappearance recorded to correspond with weighing events. Pigs had access to feed on an *ad libitum* basis from a three-space stainless steel feeder, and *ad libitum* access to water via nipple drinkers. Wheat-based diets were formulated to contain 15.1 MJ digestible energy (DE)/kg and 0.85 g standardised ileal digestible lysine/MJ DE. Protein and amino acid sources in the diets included soybean meal and soy protein isolates, blood meal, meat and bone meal, fish meal and milk powder. The control diet contained 2.5% spray-dried porcine plasma (SDPP). Treatments 1 (T1) and 2 (T2) contained 2.5% SDPP and 0.2% Aromabiotic® Pig. All diets contained 0.3% fumaric acid. All pigs received 0.25 mL intramuscularly of Draxxin (Tulathromycin 100 mg/mL, Zoetis, NSW) upon entry. Control and T1 pigs also received 65.7 g/1000 kg liveweight (LW) of Sol-u-Mox (Amoxycillin trihydrate 870 mg/g; Bayer, NSW) and 42.9 g/1000 kg LW of Linco-Spectin (Lincomycin hydrochloride 222 mg/g, Spectinomycin sulphate 445 mg/g; Zoetis, NSW) in water for 28 and 21 days, respectively, while T2 was unmedicated. An unmedicated control diet would likely have resulted in active disease becoming a welfare issue, so was not included in this study. Performance data were analysed via GLM ANOVA with time as a blocking factor, with differences determined by least significant difference ($P < 0.05$). Differences in mortality between treatments were analysed by Chi-square analysis (Genstat, 16th Edition; UK).

Sex effects were not significant. Pigs receiving Aromabiotic® Pig in the presence of medication grew faster ($P < 0.05$) than other treatments across the whole experimental period (Table 1), which resulted in heavier pigs at the end of the 4-week period ($P < 0.001$). Including Aromabiotic® Pig in diets improved FCR ($P < 0.001$) and reduced mortality ($P < 0.001$) compared to the control, whilst removing medication was associated with reduced ADFI ($P = 0.003$). In the first week after weaning, Aromabiotic® Pig in the presence of medication improved ADG ($P = 0.008$), primarily a result of improved FCR ($P = 0.002$). The poorer growth response in the unmedicated treatment was associated with a reduced intake. However, mortality data suggested that the protective effects of MCFA were still observed. Given this experiment, Aromabiotic® Pig was found to have a significant positive impact on the performance of weaner pigs.

Table 1. Performance of weaned pigs receiving 0.2% Aromabiotic® Pig in the presence (T1) or absence (T2) of medication compared with a medicated Control diet

	Control	T1	T2	SED[A]	*P* value
Entry weight (kg)	5.1	5.1	5.1	0.27	0.963
Exit weight (kg)	11.9[a]	12.9[b]	11.7[a]	0.18	<0.001
ADG[B] (kg)	0.242[a]	0.279[b]	0.237[a]	0.007	<0.001
ADFI[C] (kg)	0.38[a]	0.39[a]	0.34[b]	0.011	0.003
FCR[D] (kg:kg)	1.56[a]	1.40[b]	1.45[b]	0.032	<0.001
Week 1 ADG (kg)	0.090[a]	0.125[b]	0.094[a]	0.010	0.008
Week 1 ADFI (kg/	0.13	0.14	0.12	0.009	0.087
Week 1 FCR (kg:kg)	1.49[a]	1.14[b]	1.29[b]	0.082	0.002
Deaths	10	0	1	$\chi^2(2, n = 140) = 16.99, P < 0.001$	

[A]SED, standard error of difference between means. [B]ADG, average daily gain. [C]ADFI, average daily feed intake. [D]FCR, feed conversion ratio. [a,b]Means in a row not having the same superscript are significantly different.

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Suppressing the feed intake of finisher pigs: a preliminary study

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Manipulation of voluntary feed intake in finisher pigs is potentially a tool for the Australian pork industry to regulate seasonal impacts on finisher pig growth performance and carcass quality, and allow some control over annual pork supply. The general hypothesis tested in this preliminary study was that the strategic use of selected feed ingredients could decrease feed intake in finisher pigs.

A total of 28 individually-housed female pigs (Large White x Landrace) with a starting body weight (BW) of 66.9 ± 0.19 kg (mean ± SD) was used in a randomised complete block design study to examine the effects of several dietary additives (n = 7 pigs per diet) on performance in the finisher period. The diets were: a commercial Control diet [13.8 MJ digestible energy (DE)/kg, 153 g/kg crude protein, 0.59 g available lysine/MJ DE; Reid Stockfeeds, VIC]; Control diet plus 4% $CaCl_2$+2.2% $Na_5P_3O_{10}$; Control diet plus chenodeoxycholic acid (CDCA; 120 mg/kg body weight); and Control diet plus 5% lauric acid (*LA*). Diets 2 and 4 were prepared in 200 kg batches using a cement mixer to incorporate the additives, whereas the CDCA in diet 3 was given as a daily top-dress to the pigs' feed. Pigs were fed *ad libitum* for 21 days towards the end of the finisher phase. Water was available on an *ad libitum* basis. Data were analysed using GLM (Genstat, 15th Edition; UK), and least significant difference (LSD) at $\alpha = 0.05$ was used to separate treatment means.

There were acceptance issues in pigs offered 4% $CaCl_2$ + 2.2% $Na_5P_3O_{10}$ in the first week, so subsequently the inclusion rate was halved for each additive (to 2% $CaCl_2$ + 1.1% $Na_5P_3O_{10}$). Overall, ADFI was lowest ($P = 0.007$) in pigs fed $CaCl_2$ + $Na_5P_3O_{10}$ (15% lower than Control pigs) but was not different ($P > 0.05$) to pigs fed LA, which in turn was similar ($P > 0.05$) to pigs fed CDCA. This resulted in pigs fed $CaCl_2$ + $Na_5P_3O_{10}$ being the lightest ($P = 0.039$) at the end of the feeding period. Pigs fed LA consumed 10% less feed ($P = 0.007$) than pigs fed the Control diet, but had similar performance. Pigs fed CDCA or LA showed a better FCR ($P = 0.023$) relative to pigs fed $CaCl_2$ + $Na_5P_3O_{10}$ or the Control diet (an average of 9%) over the 21-day period (Table 1). These data indicate that a reduction in feed intake can be achieved in finisher pigs, with the appetite suppressing effects of $CaCl_2$ + $Na_5P_3O_{10}$ possibly working through induction of metabolic acidosis (Yen *et al*. 1981), and those of LA most likely working through appetite suppression mechanisms such as reduced gastric emptying and (or) increased secretion of appetite-suppressing hormones in the gastrointestinal tract (Little *et al*. 2007).

Table 1. The influence of different dietary additives on performance indices in finisher pigs

Treatment	Control	$CaCl_2$+$Na_5P_3O_{10}$	CDCA	LA	LSD	*P* value
			BW (kg)			
d 7[A]	76.0	72.9	76.8	77.5	2.18	0.002
d 14[A]	86.9	81.8	86.7	86.8	3.54	0.018
d 21[A]	93.8	89.8	94.9	93.4	3.53	0.039
			ADFI[B] *(kg)*			
d 0–7	2.80	1.93	2.60	2.81	0.348	<0.001
d 8–14	3.97	3.50	3.63	3.45	0.424	0.078
d 15–21	3.94	3.61	3.83	3.36	0.402	0.036
			Overall performance			
ADG[C] (kg)	1.28	1.09	1.33	1.26	0.168	0.039
ADFI (kg)	3.56	3.01	3.37	3.20	0.299	0.007
FCR[D] (kg:kg)	2.81	2.81	2.52	2.55	0.235	0.023

[A]Day 0 BW used as a covariate. [B]ADFI, average daily feed intake. [C]ADG, average daily gain. [D]FCR, feed conversion ratio.

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Use of trace mineral analysis to quantify the efficacy of mineral supplementation

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Trace minerals, in particular those that are complexed to organic molecules, have been associated with decreased foot lesions and lameness (Anil *et al.* 2010*a*) and have led to increased numbers of pigs born alive and litter birth weights (Anil *et al.* 2010*b*). However, the exact mechanism(s) by which these actions occur is not fully understood. This experiment aimed to assess if trace mineral analysis, via hair, could be used to detect increased uptake of amino-acid-complexed (AAC) mineral supplementation above standard inorganic mineral inclusions, with the hypothesis that AAC minerals will not differ in their deposition.

Twenty mixed-parity sows (Landrace × Large White) housed in free-access stalls were allotted to a control (n = 10) or a treatment group (n = 10) based on parity and size. The control group was fed a diet with an inorganic mineral and vitamin premix incorporated into a standard diet [12.9 MJ digestible energy (DE)/kg, 0.40 g standardised ileal digestible lysine/MJ DE]. The treatment group was fed the same base mineral and vitamin premix formulation but with the addition of AAC minerals (Availa®; Zinpro Corp., Eden Prairie, USA), these being Cu (10 ppm), Zn (50 ppm), Mn (20 ppm) and Se (0.15 ppm). Diets were offered to individual sows at 2.5 kg/d, with sows held in stalls during feeding. Five accessible areas on the sow were shaved at the commencement of the experiment: neck, left and right shoulder, and left and right rump. Hair samples were taken from each individual site at 9, 15 and 21 weeks at a time when hair growth was sufficient for collection after diets were first offered. Data were statistically analysed with the individual sow as the experimental unit using GLM (Genstat, 15th Edition; UK).

Results indicated that trace mineral analysis of hair samples might be used to demonstrate differences in dietary mineral uptake (Fig. 1). The analysis of hair samples showed distinctively different patterns of deposition for Zn and Mn depending on the source of these minerals in the diet, whilst the patterns of deposition for Cu and Se were more uniform (not shown). Interestingly, the inclusion of AAC minerals impacted other minerals important for bone health, with the pattern of deposition of calcium and phosphorus being markedly altered (Fig. 1). These results indicated that the impact of AAC minerals is beyond their direct inclusion as a trace mineral supplement and may affect the deposition of important bone minerals, that warrants further investigation to understand this mechanism.

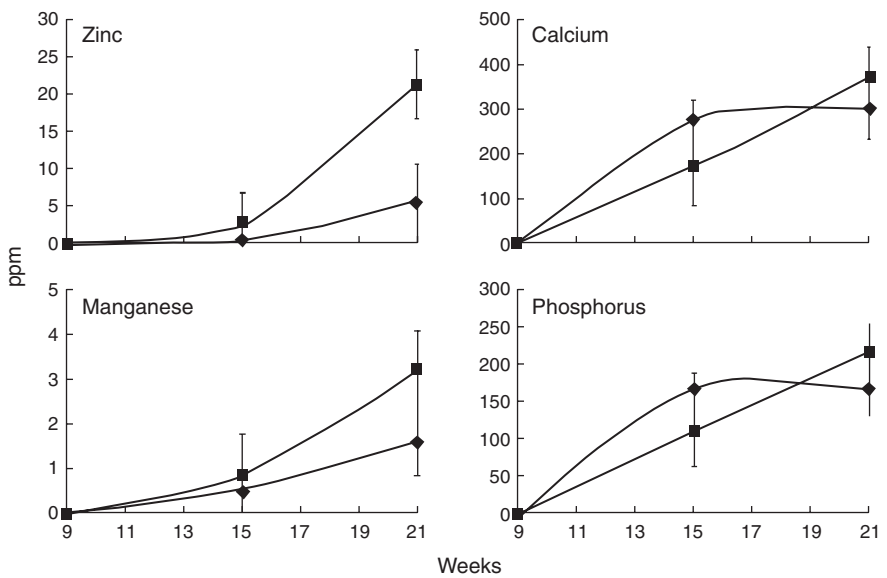

Fig. 1. Changes in mineral content of hair collected 9 15, or 21 weeks after start of feeding of control (♦) or amino-acid-complexed mineral (Cu, Zn, Mn, and Se) treatment (■) diets.

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Growth performance of nursery pigs fed pelleted wheat-based diets containing graded levels of supplemental xylanase

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The nutritive value of wheat for monogastric animals varies due to, among other factors, the fibrous cell wall structure of the grain. For example, correlation of digestible energy (DE) content in 15 Canadian wheat samples with their chemical characteristics revealed that the non-starch polysaccharides' content, specifically the concentration of arabinose and xylose, explained more than 70% of the variation in DE content (Zijlstra *et al*. 1999). Degradation of dietary fibrous components using xylanase stimulated feed intake, nutrient digestibility and digesta short chain fatty acids in weaned pigs fed wheat diets (Walsh *et al*. 2014). However, few studies have examined the effects of higher (>2000) doses of xylanases. It is hypothesised that growth performance of pigs fed wheat-based diets after weaning will be improved in a dose-dependent manner by supplemental xylanase. Therefore, the objective was to provide growth performance data for nursery pigs fed graded levels of supplemental xylanase in wheat-based diets.

A basal diet was formulated to meet or exceed the NRC (1998) nutrient requirements for nursery pigs for a two-phase feeding program: 10 to 20 kg body weight (BW) (Phase I, d 0–21) and 20 to 50 kg BW (Phase II, d 22–42). Wheat, soybean meal, barley, wheat millrun and canola meal were respectively included at 35, 27, 10, 7 and 6% in Phase I diets and 48, 27, 10, 10, and 0.6% in Phase II diets. Diets were fortified with amino acids, vitamins, and minerals to meet nutrient requirements according to NRC (1998). The DE content was 14.1 MJ/kg and 13.9 MJ/kg and true ileal digestible Lys was 13.5 and 12.5 g/kg in Phase I and II, respectively. For each phase, two other test diets were prepared by adding 2000 U or 4000 U of xylanase (XU)/kg of feed. Diets were prepared in pellet form at 70°C. A total of 192 piglets (9.2 ± 0.16 kg BW; mean ± SEM) were weaned and based on their BW assigned in a completely randomised block design to pens containing two barrows and two gilts to give 12 replicate pens per diet. Pigs had free access to feed and water. Feed intake and BW were measured weekly to determine average daily feed intake (ADFI), average daily gain (ADG) and gain to feed (G : F). Data were analysed using linear and quadratic contrasts (SAS®; USA).

Assayed dietary xylanase activities in the control, 2000 and 4000 XU diets in Phase I were <100, 1506 and 3754, respectively; corresponding values for Phase II diets were <100, 1633 and 3782, respectively. Supplemental xylanase tended to improve ADG in a linear fashion ($P = 0.060$) (Table 1). As a result, pigs receiving diets with 4000 XU/kg were 1.5 kg heavier ($P = 0.024$) relative to the control-fed pigs at the end of the experiment. The ADFI was not affected ($P > 0.10$) by feeding treatments. However, supplemental xylanase linearly improved G : F ($P = 0.040$) such that pigs fed 4000 XU/kg exhibited 2.7% greater G : F relative to the control. In conclusion, pigs fed wheat-based diets with xylanase during the initial 42 days after weaning were heavier at the end of the study, and utilised feed more efficiently compared with pigs fed a control diet without addition of xylanase.

Table 1. Effects of graded levels of xylanase on growth performance of nursery pigs fed wheat-based diets after 42 days

Item	Xylanase (Units/kg of feed)			SEM[A]	Contrasts	
	0	2,000	4,000		Linear	Quadratic
Initial BW (kg)	9.19	9.11	9.30	0.091	–	–
Final BW (kg)	39.1	40.5	40.6	0.452	0.024	0.346
ADG (g)	714	745	746	10.81	0.060	0.159
ADFI (g)	1145	1180	1170	20.89	0.409	0.384
G : F (g : g)	0.623	0.632	0.640	0.005	0.040	0.943

[A]SEM, standard error of the mean.

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Cellulase supplementation benefits performance and apparent faecal digestibility of dietary components in lactating sows and their piglets

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During lactation, the demands of milk production and limited nutrient intake can cause catabolic conditions (Kim and Easter 2003). The fibre component of sows' diet is recognised to add an important source of energy to pregnant sows because it is processed through microbial fermentation in the gastrointestinal tract (Schoknecht 1997). However, monogastric animals do not have the enzymes to hydrolyse the dietary fibre contents. Thus, supplementation of exogenous enzyme is necessary to optimise nutrient utilisation. It was hypothesised that a corn-soybean meal based diet, containing high fiber byproducts when supplemented with cellulase, could improve feed intake, nutrient digestibility and reduce backfat loss in lactating pigs and improve performance in their litters.

A total of 15 first parity sows (Landrace × Yorkshire) with their initial body weight (BW) (205 ± 1.6 kg; mean ± SD) and backfat thickness (P2) of 21.6 mm were randomly allocated into one of three treatments with five replicates per treatment. Dietary treatments were as follows: CON (corn-soybean meal-based control); EZ1 (CON + 0.05% cellulase); and EZ2 (CON + 0.10% cellulase). The guaranteed activity of cellulase was 12 000 U/g (AT Life Science Inc., Cheongwon, South Korea). The treatment diets were fed 40 days before farrowing until weaning (25 days after parturition). Sows were fed on a commercial gestation and lactation feed divided into two daily meals in mash form. The calculated metabolisable energy, crude fibre and available lysine content of the gestation diet was 13.38 MJ/kg, 32.1 g/kg and 15.8 g/kg, respectively, and those of the lactation diet were 14.5 MJ/kg, 28.7 g/kg and 14.9 g/kg, respectively. The BW and P2 of sows were measured 4 days before farrowing, and also on d 2 and 25 after birth. Cross-fostering was performed within gestation treatment groups to adjust to 10 piglets per sow. Piglets were not offered creep feed. The average daily gain (ADG) of piglets was measured from d 1 to 25 (weaning). Fresh faecal samples were collected by rectal massage on d 21 to 25 of lactation from all five sows per treatment to determine the coefficient of total tract apparent digestibility (CTTAD) of dry matter (DM), nitrogen (N) and gross energy (GE). Chromium oxide (0.2%) was added to the sow diets as an indigestible marker for a period of 7 days before faecal collection. All data were analysed in accordance with a completely randomised design using the GLM procedure (SAS®; USA). The individual sow or litter of piglets was used as the experimental unit. Differences among the treatment means were determined by using the Tukey's test with $P < 0.05$ indicating statistical significance.

The supplementation of cellulase had no significant effect on BW and feed intake of lactating sows. At weaning, P2 loss decreased significantly ($P < 0.05$) in EZ2 (2.8 mm) compared with CON (4.0 mm). During d 14 to 21, there was an increase in the ADG of piglets from sows fed EZ1 (276 g) than CON (251 g) and during d 21 to d 25, the ADG of piglets increased ($P < 0.05$) in EZ1 (288 g) and EZ2 (275 g) compared to CON (260 g). The CTTAD of DM in EZ2 (0.739) increased ($P < 0.05$) relative to CON (0.726), and that of N also increased ($P < 0.05$) in EZ2 (0.763) compared with CON (0.742), but no improvement in CTTAD of energy was observed (data not shown). In conclusion, it is suggested that 0.01% cellulase supplementation to corn-soybean meal-based diet exerts beneficial effects to sows in improving their backfat thickness at weaning and also helped to improve CTTAD of DM and N but not energy. Dietary lipids may be directly deposited in milk fat. However, if the dietary intake of lipids is greater than the needs for these functions, excess lipids will be stored in the body as body lipids, mainly in adipose tissue. Thus, there is a reduction in P2 backfat loss. Also, piglets born from sows fed enzyme-supplemented diets showed positive effects in improving their ADG. Due to the difficulty in having gestating sows of the same age and body weight at the same time only 15 sows were used in this study. Given this major limitation, further studies with additional sows animals are suggested.

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Effect of dietary anise flavour on performance of sows and their litter at different weaning ages

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Insufficient feed intake by sows during lactation is problematic because sows require large amounts of energy and nutrients for high milk production. A low feed intake during lactation may lead to greater bodyweight (BW) loss, lower milk production, and reproductive problems that may result in early culling of sows (Eissen *et al.* 2000). Feed intake is greatly influenced by the chemical senses of olfaction and taste, and feed flavouring agents can be added to enhance the smell and taste of feed in order to stimulate intake. In addition, Wang *et al.* (2014) reported that flavour increased the average daily feed intake (ADFI) of lactating sows, as well as improving the ADFI and average daily gain (ADG) of weanling pigs. According to Maes *et al.* (2004), the back fat measurements constitute a valuable tool to monitor and improve the productivity and efficiency of high producing pig herds. The objective of the present study was to evaluate the effect of dietary anise flavour (AF) on performance of lactating sows and their litters.

A total of 120 sows (Landrace × Yorkshire, average parity 2.7) with a bodyweight (BW) of 237 ± 1.9 kg (mean ± SE; BW measured at 7 days before farrowing) was allotted into one of four treatments using a 2 × 2 factorial arrangement of treatments with two AF levels (0 or 0.05%) and two weaning ages (21 or 28 days of age). Sows were fed a commercial diet with AF (n = 60) or without AF (n = 60) from d 100 of gestation and throughout lactation. All diets were formulated to meet or exceed the NRC (2012) requirements. The gestation diet had 13.19 MJ metabolisable energy (ME)/kg, 131 g/kg crude protein (CP) and 6.5 g/kg available lysine (AvLys), and the lactation diet had 13.44 MJ ME/kg, 171 g/kg CP and 10 g/kg AvLys. The AF (DadHank Biotechnology Corporation, Chengdu, China) was a non-hygroscopic powder and contained 33.47% eugenol, 11.09% coconut aldehyde, 10.22% linalool, and 9.52% anethole. On the day before farrowing and at weaning, the backfat of sows was measured 6 cm off the midline at the tenth rib using a real-time ultrasound instrument (Piglot 105, SFK Technology, Herlev, Denmark). Data were analysed by using the MIXED procedure (SAS®; USA). Variability of all the data was expressed as standard error (SE) and a probability level of $P < 0.05$ was considered as statistically significant.

Sows fed with AF diets had higher ($P < 0.05$) ADFI and lower ($P < 0.05$) back fat loss than those fed with non-AF diets (Table 1). Sows weaned at d 28 had lower ($P < 0.05$) back fat loss compared with those weaned on d 21, whereas no difference ($P > 0.05$) was observed on weaning BW between piglets in the AF group and non-AF group. In conclusion, the results showed that dietary AF supplementation could increase ADFI and decrease back fat loss of lactating sows. Moreover, early weaning is helpful for reducing back fat loss of lactating sows.

Table 1. Effects of anise flavour (AF) on performance of sows

Treatment	–AF	+AF	SE[A]	*P* value
Parturition back fat (mm)	22.9	23.0	0.5	0.57
Weaning back fat (mm)	17.7	18.5	0.4	0.06
Back fat loss (mm)	5.2	4.5	0.3	0.02
ADFI (kg)	5.0	5.4	0.1	0.002
Treatment	W28[B]	W21	SE	*P* value
Parturition back fat (mm)	22.8	23.1	0.1	0.44
Weaning back fat (mm)	17.8	18.4	0.2	<0.001
Back fat loss (mm)	5.0	4.3	0.1	<0.001

[A]SE, standard error. [B]W28, weaning at d 28; W21, weaning at 21 d.

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Partial fish meal replacement with fermented or enzymatically prepared soybean meal in weaned pig diets

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Fish meal (FM) can be used as a protein source in weaned pig diets in South Korea due to the high digestibility of nutrients, favourable composition of amino acids (AA), and lack of anti-nutritional factors (ANF) that can reduce nutrient availability and negatively affect growth performance of young pigs (Kim *et al.* 2010). Soybean meal (SBM) is cheaper than FM and an important protein source fed to adult pigs because of its excellent balance of essential AA (Wang *et al.* 2011) and low fibre concentration. However, SBM contains ANF and therefore its high use in weaned pig diets is not recommended by nutritionists (Choct *et al.* 2010). On the other hand, fermented or enzymatically prepared SBM, which has a significant reduction in the amount of ANF (trypsin inhibitor <3.02 mg/g; raffinose <0.18%; stachyose <0.54% in this study), has been used to ameliorate the negative effects of the weaning lag (Min *et al.* 2009). The objective of this study was to determine the comparative efficacy of FM versus commercially available, solid state fermented SBM or enzymatically prepared SBM replaced at up to 50% of FM. Parameters of interest included growth performance, nutrient digestibility, and populations of some faecal bacteria. It was hypothesised that treated SBM may be a viable partial replacement for FM in weaned pig diets thereby reducing feed costs without compromising growth performance.

A total of 100 weaned pigs with a body weight (BW) of 6.6 ± 0.29 kg (mean ± SD) was used and were randomly allotted to five groups with four block replicates of five pigs per pen. Diets were formulated to meet or exceed the nutrient requirements by the NRC (2012), and diets were: 5% FM (FF-Skagen); 2.5% FM + 2.5% SoELAB (FEEDUP); 2.5% FM + 2.5% PepSoyGen (Nutraferma); 2.5% FM + 2.5% Soytide (CJ Cheiljedang Bio); and 2.5% FM + 2.5% HP 300 (Hamlet Protein) (as fed basis). Diets were fed for 3 weeks in mash form, and then each group was switched onto a common commercial diet as a crumble for 3 weeks. Growth performance in terms of average daily gain (ADG) and feed intake (FI), nutrient digestibility, and selected microbial population of faecal samples were measured in accordance with the methods described by Jeong and Kim (2015). All experimental data were analysed as a randomised complete block design, with one pen representing an experimental block unit. Data were analysed by GLM procedures (SAS 2001; USA) with Tukey's test to indicate significant differences ($P < 0.05$) amongst means.

Although fermentation generates more free AA to improve nutrient availability, the amount of essential AA (SoELAB 23.7%; PepSoyGen 23.6%; Soytide 24.4%; HP 300 24.5%) was still lacking in comparison to FM (32.3%), which may have important implications. Concerning growth performance, SoELAB (ADG 487 g, FI 691 g) and HP 300 (ADG 494 g, FI 691 g) demonstrated no significant difference compared with FM (ADG 494 g, FI 701 g) after 6 weeks. With respect to nutrient digestibility, SoELAB and HP 300 treatments demonstrated no significant difference compared with FM treatment. Last, none of the SBM preparations demonstrated any significant differences in faecal score ($P > 0.05$), but differentially treated SBM (SoELAB, Soytide and HP 300) increased faecal *Lactobacillus* counts ($P < 0.05$) after 3 weeks while maintaining similar *E. coli* counts ($P > 0.05$) compared with FM treatment. Overall, the results from the present study indicated that treated fermented SBM has potential to serve as a replacement product for FM in diets fed to weaned pigs. The use of fermented SBM did not negatively impact any of the parameters examined. However, using fermented SBM as a complete alternative to FM may not be sufficiently adequate for providing essential AA in the diet. Henceforth, a fermented SBM and FM mixture in the diet is ideal, and can reduce feed costs without any side effects. Further studies should determine ideal mixture ratios in an attempt to maximise costs savings with growth performance.

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Positive effects of protected organic acids on nutrient digestibility and faecal microflora in lactating sows

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Among a variety of candidates for the replacement of antibiotics, organic acids have been broadly applied worldwide with a reasonable success rate (Mroz 2005). Organic acids may influence the physiology of the intestinal mucosa by their action on the villi, by maintaining their integrity, promoting an increase in the number of cells, preventing its flattening, as well as serving as a substrate in the intermediary metabolism of the citric acid-cycle (Partanen and Mroz 1999). Organic acids can also reduce the diets buffering capacity, inhibit the proliferation and decrease colonization of undesirable microorganisms, act on the physiology of the gastrointestinal mucosa by improving the availability of nutrients in the diet, and improve their digestion, absorption, and retention (Costa *et al.* 2011). It was hypothesised that blends of different organic acids with medium chain fatty acids (MCFA) in a matrix coating could play an influential role in improving growth performance, microbial population, nutrient digestibility, blood profiles, and faecal gas emission of lactating sows.

A total of 12 sows with an average initial body weight (BW) of 252 ± 11.7 kg (mean ± SD) were used in a 21-day trial. The protected organic acid consists of MCFA and composite organic acids. The active ingredients were 58.8% stearic acid (palm oil), 17% fumaric acid, 13% citric acid, 10% malic acid, and 1.2% MCFA (capric and caprylic acid). Treatments were: CON, basal diet; POA1, CON + 0.1% protected organic acid; and POA2, CON + 0.2% protected organic acid. The BW and backfat of sows was checked 4 days before farrowing and at weaning day to calculate body weight loss and backfat loss during that period. Chromium oxide was added to diets at 0.2% as an indigestible marker to determinate the coefficient of total tract apparent digestibility (CTTAD) of DM, nitrogen (N) and gross energy (GE). All feed and faecal samples were analysed for DM (method 930.15, AOAC 2007) and crude protein (method 990.03, AOAC 2007). Chromium was analysed via UV absorption spectrophotometry (Shimadzu UV-1201, Shimadzu, Kyoto, Japan). The GE was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, IL). A total of 300 g fresh faecal samples were collected from each sow, and they were transferred to a sealed box and fermented for 48 h at 32°C in an incubator. At d 1, 3, 5, and 7, concentrations of ammonia, thiol, hydrogen sulphide, and acetic acid were measured. Blood from sows were collected via vena cava puncture before feeding at farrowing and weaning (d 21). The concentration of white blood cells (WBC) and lymphocytes in the whole blood samples were determined using an automatic blood analyser (ADVIA 120, Bayer, Tarry town, NY, USA). Whole blood samples were subsequently centrifuged for 15 min at 3000×*g* at 4°C and the harvested serum was used to determine IgG by using nephelometry (Dade Behring, Marburg, Germany). Effects of treatments (Control, POA1, and POA2) were analysed by ANOVA. Results are presented as least square mean and the variability in data was expressed as standard error (SE). Probability values less than $\alpha = 0.05$ were considered as significant.

Protected organic acid (0.2%) diets increased ($P < 0.05$) the CTTAD of DM (4.75%), N (4.83%) and GE (5.77%) over those fed CON diets throughout the experimental period. Dietary supplementation with 0.2% protected organic acid led to a higher ($P < 0.05$) WBC (45.0%) and lymphocyte (6.7%) concentration than the CON treatment at weaning. The IgG concentration was greater ($P < 0.05$) in protected organic acid groups than CON lactating sows. Faecal *Lactobacillus* counts were increased ($P < 0.05$), and *E. coli* concentration was decreased ($P < 0.05$) in sows fed with the diets of protected organic acids at both farrowing and weaning. The faecal H_2S contents were decreased ($P < 0.05$) in protected organic acid groups during farrowing on d 1 compared with CON. It can be concluded from this preliminary study, albeit with a very small number of sows, that dietary supplementation with protected organic acid had some beneficial effects on digestibility and microbial populations in lactating sows.

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Effect of Spanish sweet yacca residue pellet as a replacement for corn on growth performance, nutrient digestibility and haematological profiles in growing pigs

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One of the potential alternative feedstuffs is Spanish sweet yacca (SSY, commonly named cassava, *Manihot esculenta*), an economic energy source in the animal feed industry. However, all SSY organs except seeds contain cyanogenicglucoside (i.e. linamarin and lotaustralin). Spanish sweet yacca should thus be processed in order to reduce cyanogenic potential and phytate content and to preserve their nutritive quality (Salami and Odunsi 2003). The aim of this study was to determine the viability of processed a SSY (i.e. residue pellet) as an alternative to corn. The hypothesis tested in this experiment was that SSY residue pellet replacing corn in growing pigs diet would not cause marked changes in the growth performance.

A total of 84 [(Yorkshire × Duroc) × Landrace] growing pigs (BW of 25.1 ± 2.01 kg, 42-day trial) were allotted to three dietary treatments: CON, Corn-SBM diet; SSY20, replacing corn with 20% SSY; SSY40, replacing corn with 40% SSY. Diets were isonitrogenous and isoenergetic with 178 g/kg crude protein and 13.81 MJ/kg digestible energy, respectively. The experiment consisted of seven replications per treatment and four pigs (two gilts and two borrows) per pen. For the 6-week growth assay, the individual pig weights and feed intake were recorded at d 21 and 42 for the determination of average daily gain (ADG), average daily feed intake (ADFI) and gain : feed (G : F) ratio. The red blood cells (RBC), white blood cells (WBC) and lymphocyte counts of whole blood samples were determined using an automatic blood analyser (ADVIA 120, Bayer, Tarrytown, NY, USA) on d 0, 2, 4, 6 and 42. All pigs were fed diets mixed with 0.2% chromium oxide to calculate the coefficient of total tract apparent digestibility (CTTAD) of DM, nitrogen (N), and gross energy (GE). All data were statistically analysed using the MIXED procedure (SAS®; USA) as a randomised complete block design. Orthogonal polynomial contrasts were used to assess the linear and quadratic effects of increasing dietary concentrations of supplemental SSY.

No significant differences were observed on growth performance among treatments in the whole experiment, while WBC concentration linearly decreased (P = 0.028, Table 1) on d 4. No significant differences were observed on CTTAD (DM, N and GE) among treatments in the whole experiment. Processed yacca meal could be included in the diets of growing pigs up to level of 30% to reduce feed costs without any detrimental effect on performance (Irekhore *et al.* 2006), or up to 60% (total replacement of maize) when maize cost is high (Bawa and Damisa 2007). Enyenihi *et al.* (2009) reported that a diet with yacca led to lower WBC concentrations in laying hens. The observed WBC counts in this study falls within the normal range and therefore it can be concluded that SSY can be used at a level of around 40%, replacing corn in growing pigs diet, without negative effecting growth performance.

Table 1. Effects of feeding different SSY levels on growth performance in growing pigs

Items	CON	SSY20	SSY40	SEM[A]	*P* value	
					Linear	Quadratic
Growth performance (Overall)						
ADG (g)	545	540	529	19.4	0.538	0.896
ADFI (g)	1428	1462	1414	27.2	0.711	0.224
G : F (g : g)	0.38	0.37	0.37	0.013	0.575	0.588
Haematological profiles (d 4)						
RBC ($\times 10^6$/uL)	6.95	6.86	7.21	0.167	0.246	0.271
WBC ($\times 10^3$/uL)	21.4	19.0	18.0	1.03	0.028	0.591
Lymphocyte (%)	57.7	53.1	54.8	2.07	0.332	0.224

[A]SEM, standard error of the mean.

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Low to moderate dietary *n*-6:*n*-3 PUFA ratios do not affect performance of grower-finisher pigs

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Dietary fatty acids are potent mediators of physiological processes related to body composition and development. Previous findings by Wilkinson *et al*. (2014) identified that pigs fed diets with a high *n*-6:*n*-3 (24:1) polyunsaturated fatty acid (PUFA) ratio showed significantly reduced performance and increased health challenges when compared to pigs fed diets with moderate and low ratios of *n*-6:*n*-3 PUFA. Additionally, there is conjecture as to the effect of low *n*-6:*n*-3 PUFA ratios on the feed intake and growth performance of growing pigs. Despite the known physiological importance of *n*-6 and *n*-3 PUFA, no dietary recommendations for the *n*-6:*n*-3 PUFA ratio are available to pig nutritionists. This study investigated the effect of low to moderate *n*-6:*n*-3 PUFA ratios on the performance of grower-finisher pigs. It was hypothesised that feed intake and performance would be similar between groups fed low to moderate *n*-6:*n*-3 PUFA ratios.

A total of 430 gilts (Large White x Landrace x Duroc) with a body weight (BW) of 40 ± 0.2 kg (mean ± SD) were sourced from a high-health-status commercial herd. Pigs were individually ear tagged, weighed and randomly stratified to treatments based on BW. Pigs were housed in groups of seven with 12 replications per treatment and fed a standard commercial diet until an average pen weight of 45 kg was reached. Experimental diets, having, 4:1, 8 : 1 and 12 : 1 *n*-6:*n*-3 PUFA ratios, were fed for approximately 8 weeks. Diets were formulated to contain equivalent digestible energy (DE) (13.5 MJ/kg) and available lysine (0.6 g/MJ DE). Feed disappearance was measured using a Feedlogic system and individual BW was recorded weekly to calculate average daily gain (ADG) and feed conversion ratio (FCR). Carcass weight was recorded and depth of backfat (P2) measured using a Hennessy grading probe on the hot carcass between the 12th and 13th rib. Data were analysed by one-way ANOVA (Genstat, 15th Edition; UK).

There were no treatment differences in feed intake, growth and the carcass measurements ($P > 0.05$; Table 1). In accordance with the hypothesis, diets with *n*-6:*n*-3 PUFA ratios of less than 12 : 1 had no adverse effect on the feed intake and performance of grower-finisher pigs. These results are agreement with those reported by Wilkinson *et al*. (2014) where pigs fed low to moderate *n*-6:*n*-3 PUFA diets (<12:1) were not adversely affected. The effects of feeding higher ratios of *n*-6:*n*-3 PUFA (>12:1 *n*-6:*n*-3 PUFA) in grower-finisher pigs are currently being investigated.

Table 1. Growth performance and carcass composition of pigs fed different ratios of *n*-6:*n*-3 PUFA

n-6:*n*-3 PUFA ratio	4:1	8:1	12:1	SEM[A]	*P* value
		BW (kg)			
Day 1	47.4	47.4	47.6	0.10	0.432
Day 28	78.9	77.9	78.3	0.42	0.490
Day 55	108.2	106.9	106.9	0.72	0.665
		ADG (kg)			
Day 1–28	1.09	1.05	1.06	0.015	0.362
Day 28–55	1.08	1.08	1.06	0.018	0.834
Day 1–55	1.09	1.06	1.06	0.013	0.586
		FCR (kg : kg)			
Day 1–28	2.22	2.23	2.20	0.041	0.995
Day 28–55	2.54	2.55	2.50	0.041	0.186
Day 1–55	2.66	2.62	2.57	0.039	0.586
		Carcass measurements			
HSCW[B] (kg)	73.3	73.4	73.7	0.32	0.638
Dressing %	68.2	68.4	68.8	0.29	0.585
P2 backfat (mm)	13.11	12.94	12.73	0.266	0.222

[A]SEM, standard error of the mean. [B]HSCW, hot standard carcase weight.

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Increasing zinc via an inorganic source (ZnO) in high calcium finisher diets improves growth performance

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Zinc (Zn) is important for protein, carbohydrate and lipid metabolism as it is a component of many enzymes involved in these processes. Because of its role in protein synthesis, Zn is important in the diet of the finisher pig, where fast lean growth is desirable. When formulating pig diets, limestone ($CaCO_3$) is commonly included as a least cost energy diluent, resulting in increased calcium (Ca) levels. However, increasing Ca above 1.5% inclusion can cause a Zn deficiency (Borah *et al*. 2014) in grower pigs, resulting in parakeratosis. The current study was designed to determine if increasing Zn level using either an organic or an inorganic source, is effective in offsetting any potential negative effects of including high levels of Ca (1%) in finisher diets.

Sixty Large White x Landrace (PrimeGro Genetics) immunologically castrated males were selected at 14 weeks of age (49.6 kg ± 0.43; mean ± SEM) and housed in individual pens with *ad libitum* access to feed and water. Pigs were randomly allocated to one of three dietary treatments (n=20) and fed over a period of 35 days. Treatments were: a control finisher diet (13.4 MJ digestible energy (DE)/kg, 0.54 standardised ileal digestible lysine/MJ DE), with 2% limestone ($CaCO_3$) (1% dietary Ca) and basal Zn at 70 ppm Zn; the control diet with Zn increased to 550 ppm using ZnO (0.06%); and the control diet with Zn increased to 550 ppm of Zn from an organic zinc (Bioplex Zn® 0.33%). Pigs were weighed at d 0, 21 and 35, with feed intake measured during these periods. Back fat was measured at the P2 site on d 0 and 35. Hot standard carcass weight (HSCW) and P2 were measured after slaughter. Statistical analysis was conducted using ANOVA (IBM SPSS, Version 21.0; USA).

Inclusion of 550 ppm of ZnO improved FCR compared to other treatments from d 21 to 35 and from d 0 to 35 ($P < 0.05$; Table 1). The ZnO at 550 ppm also improved ADG from d 21 to 35 compared with the other treatments; however ADG was not greater ($P > 0.05$) than for the control diet over the entire finisher period. The HSCW was heavier ($P < 0.05$) in pigs fed diets supplemented with ZnO compared with the organic source, but not different ($P > 0.05$) from that of pigs fed the control diet with 70 ppm Zn inclusion. There was no difference ($P > 0.05$) in P2 between treatments. The outcomes from this study indicate that increasing concentrations of ZnO in finisher diets with high Ca levels improved growth performance. This response may be due to ZnO offsetting any potential negative effects of Ca in the diets or via the anti-microbial properties of ZnO modifying gastro intestinal tract microbiota (Pieper *et al*. 2012). Further research is required to verify the mode of action and determine the efficacy of lower ZnO doses. However, it is important to also consider environmental issues and diet costs when formulating feed rations to offset Zn deficiency and (or) improve growth performance.

Table 1. Influence of feeding a control diet, or diets with 550 ppm inorganic or organic Zn, on performance and carcass measurements. Values are mean ± SEM

Treatment	Control	Control + 550 ppm ZnO	Control + 550 ppm Bioplex Zn
ADG[A] (kg)			
Days 21–35	1.2 ± 0.04[a]	1.4 ± 0.06[b]	1.2 ± 0.03[a]
Days 0–35	1.1 ± 0.03[ab]	1.2 ± 0.03[b]	1.0 ± 0.03[a]
FCR[B] (kg:kg)			
Days 21–35	2.6 ± 0.05[a]	2.3 ± 0.07[b]	2.7 ± 0.07[a]
Days 0–35	2.4 ± 0.05[a]	2.3 ± 0.04[b]	2.5 ± 0.07[a]
HSCW (kg)	67.9 ± 1.29[ab]	69.2 ± 1.33[a]	65.4 ± 1.29[b]
Carcass P2 (mm)	11.4 ± 0.51[a]	12.0 ± 0.55[a]	11.6 ± 0.61[a]

[A]ADG, average daily gain. [B]FCR, feed conversion ratio. [a,b]Means in a row not having the same superscript are significantly different ($P < 0.05$).

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Creatine monohydrate supplementation of sow diets pre-partum improved neonatal piglet characteristics

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Intermittent oxygen deprivation during farrowing reduces the viability and vigour of neonatal piglets. Oxygen deprived piglets which survive parturition take longer to suckle, ingest less colostrum, grow more slowly and are more likely to die before weaning (Herpin *et al*. 1996). Adding compounds to the maternal pre-partum diet which protect the brain of the foetal piglet from the negative impacts of oxygen deprivation (neuro-protectants) may be a simple and effective strategy to improve piglet viability, vigour and survival. In rodents, maternal ingestion of creatine monohydrate (CR) protects the foetal brain from the damage associated with acute hypoxic insults at term (Dickinson *et al*. 2014). Consequently, it was hypothesised that supplementing the diets of gestating sows with CR for 5 days before parturition would increase neonatal vitality of piglets born at the end of the birth order.

Five days prior to the farrowing due date, the diets of 98 Large White × Landrace sows (parity 3.9 ± 0.19; mean ± SEM) were supplemented with either 0%, 2.5% or 5% CR (n = 38, 29 and 31 sows/treatment, respectively). Sows were housed in farrowing crates and received 1 kg of the same diet three times per day (14.2 MJ digestible energy/kg; 17.3% crude protein). The CR was top-dressed onto the diet and divided equally across each feed allocation. Total litter size, number of piglets born alive and still born, neonatal piglet behaviour, piglet liveweight (LW) gain in the first 24 h, piglet plasma glucose and immunoglobulin (IgG) intake (immunocrit; Vallet *et al*. 2013) at 24 h of age were recorded. For statistical analyses, piglets were grouped on birth order (first one to four, middle five to eight, and last > eight). Piglet behaviours were log-transformed prior to analyses. Treatment and birth order effects were analysed using an unbalanced design ANOVA (GenStat, 15th Edition; UK). Actual means are presented for piglet behaviours. Due to the lack of any significant interactions, only main effects are presented.

Treatment did not affect ($P > 0.05$) the total number of piglets born (13.0 ± 0.3), born alive (12.2 ± 0.3) or stillborn (0.8 ± 0.1) (all mean ± SEM). Pre-farrowing supplementation with CR reduced ($P < 0.05$) the piglets' interval to first contact with the udder (Table 1). Compared to the 0% CR treatment, 2.5% CR reduced piglet latency to suckle and increased plasma glucose at 24 h of age (Table 1). Feeding 5% CR doubled piglet LW gain during the first 24 h of life compared to offering no CR ($P < 0.05$; Table 1). Piglets born last in the birth order took longer to suckle and had lower immunocrit at 24 h of age than those born first ($P < 0.05$; Table 1).

It is evident that regardless of piglet birth order adding CR to sow diets for a short time before farrowing improved characteristics of piglets commonly associated with increased pre-weaning survival, and reduced behaviours associated with exposure to intra-partum hypoxia. The effects of maternal CR supplementation on piglet survival and growth to weaning need to be established commercially.

Table 1. Effect of 0%, 2.5% and 5% creatine monohydrate (CR) supplementation for 5 days pre-farrowing and piglet birth order (first one to four, middle five to eight, and last > 8) on neonatal piglet behaviour, piglet weight gain in the first 24 h, and piglet plasma glucose and estimated IgG intake (immunocrit) at 24 h of age

	CR supplementation			Piglet birth order			Pooled
	0.0%	2.5%	5.0%	First	Middle	Last	SEM[A]
Time to udder (sec)	49.1[b]	22.5[a]	28.0[a]	30.3	35.4	32.1	3.90
Time to suckle (sec)	51.0[b]	32.0[a]	42.5[b]	47.9[b]	44.0[ab]	33.5[a]	2.39
24 h piglet LW gain, kg	0.05[a]	0.08[ab]	0.10[b]	0.08	0.07	0.08	0.07
24 h glucose (mmol/l)	5.90[a]	6.77[b]	6.12[a]	6.43	6.18	6.19	0.31
24 h immunocrit	0.124	0.127	0.132	0.137[b]	0.127[ab]	0.120[a]	0.01

[A]SEM, standard error of the mean. [a,b]Means in row and within a main effect not having the same superscript are significantly different ($P < 0.05$).

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Maintaining finisher pig performance without dietary organic copper with a mannan-rich fraction of *Saccharomyces cerevisiae*

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Copper (Cu) has been included in growing pig diets for many decades to improve growth performance and general health (Barber *et. al.* 1955). Copper sulphate ($CuSO_4$) use is still widespread and organic forms of Cu are also common with increased bioavailability facilitating lower inclusion rates (Coffey *et al*. 1994). The potential for co-selection of metal and antibiotic resistance (Baker-Austin *et al.* 2006) as well as environmental build up from pig excretions still raise concern over Cu use. Edwards *et al.* (2014) evaluated the use of Actigen™ [a mannan-rich fraction derived from a strain of *Saccharomyces cerevisiae* (Alltech Inc, Nicholasville, KY, USA)] as a total replacement for $CuSO_4$ in pig diets from 29 kg to sale. The authors reported similar growth performance and survival between the Cu treatment (200 ppm Cu as $CuSO_4$) and Actigen™ (Actigen™ step-down program 400 ppm/200 ppm, 38 days/42 days). The present study aimed to further explore the use of Actigen™, specifically to determine the replacement and additive effects when included in finisher diets containing organic Cu. The study tested the hypotheses that growth performance, survival and carcass weight would be similar with the total replacement of organic Cu with Actigen™, and that no additive effects would occur from the inclusion of both Actigen™ and Cu in the finisher diet.

A total of 697 male pigs (PrimeGro™ Genetics, Corowa, NSW) were housed in commercial finisher facilities in pens of 12–13 pigs. At 16 weeks of age, all pens were weighed (57.9 ± 0.59 kg; mean ± SE) and randomly allocated (18 pens/treatment) to one of three isoenergetic and isonitrogenous (13.0 MJ digestible energy (DE)/kg, 0.56 g available lysine/MJ DE) diets: Control (standard Bioplex Cu diet; 100 ppm Cu proteinate); Actigen™ (200 ppm) plus Cu (100 ppm Bioplex Cu); and Actigen™ (200 ppm). Diets were offered *ad libitum* from 16 weeks of age until slaughter at 22 weeks. Growth performance, feed intake and feed efficiency were recorded on a pen basis. Mortality and morbidity were analysed for association with dietary treatment using Chi-square analysis. All other data were analysed using ANOVA with the pen as the experimental unit (GENSTAT 16th Edition; UK).

Growth performance and measured carcase characteristics were not significantly different between dietary treatments over the entire test period (Table 1). There was a trend for reduced pig mortality and removals for morbidity from the combined Cu plus Actigen™ diet: 6.5%, 3.0% and 7.7% for the three diets, respectively ($\chi^2 = 5.16$, $P = 0.076$). This was primarily due to a trend for a reduction in pigs removed for tail bites during the final four weeks of the study ($\chi^2 = 4.77$, $P = 0.092$).

This study suggests Actigen™ may be considered as a replacement for organic Cu in finisher diets without negative effects on growth performance, feed efficiency or carcass characteristics. No additive effects were apparent with the combination of Cu plus Actigen™ on growth performance, feed efficiency or carcass characteristics. The trend for reduced morbidity with the Cu plus Actigen™ combination of was of interest and may warrant further investigation.

Table 1. Influence of dietary treatment on growth performance and carcass characteristics in grower/finisher pigs grown from 16 weeks to 22 weeks of age

	Control	Cu plus Actigen™	Actigen™	SED[A]	*P* value
Average daily gain (kg)	1.128	1.131	1.117	0.0196	0.76
Average daily feed intake (kg)	2.88	2.85	2.81	0.053	0.46
Feed conversion ratio (kg:kg)	2.56	2.52	2.52	0.033	0.47
Carcass weight (kg)	79.3	79.7	79.3	1.40	0.93
Carcass P2 (mm)	11.7	11.8	11.8	0.26	0.98
Dressing (%)	75.3	75.7	75.7	0.26	0.21

[A]SED, standard error of difference between means.

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Composition of enzyme mixtures influences the faecal digestion of a weaner pig feed

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Estimates of energy release from corn:soy and wheat-based diets of approximately 0.2 MJ/kg were identified in response to the use of xylanase (Feng *et al.* 2013) using a two-phase semi-automated simulated digestion system (SDS) *in vitro* that simulated the gastric and intestinal phases of digestion in the grower pig. The present study sought to extend these findings through examination of mannanase and protease inclusions in enzyme mixtures containing xylanase and other exogenous enzymes in weaner pigs.

Thirty mixed sex, PIC-type pigs [initial body weight (BW) of 12.8 ± 0.59 kg (mean ± SE)] were obtained from a commercial herd and assigned by sex and BW to one of five treatments consisting of either phytase alone (PHY; EC3.1.3.26, 0.1 U/g) or in combination with xylanase (EC3.2.18, 8 U/g), cellulase (EC3.2.1.24, 0.24 U/g) and amylase (EC3.2.1.4, 0.05 U/g). Protease ((EC3.2.23.6) and mannanase (EC3.2.1.78) inclusions were adjusted to provide calculated activities of 0, 0.6, 1.5, 1.8 and 2.4 U/g, for treatments PHY, XCA, XCP, XCM and XMP, respectively. Enzymes were obtained from commercial sources [AsiaPac (Dongguan) Biotechnology]. A pelleted corn: soybean meal-based diet was formulated to provide 13.8 MJ digestible energy (DE)/kg and 9 g/kg available lysine. It contained distillers dried grains with soluble (DDGS), rice bran and rice bran meal at 5%, 3.95% and 3.5% of the diet, respectively. This provided a calculated non-starch polysaccharide (NSP) content of 124 g/kg. Pigs were housed individually in a climate controlled room. Feed and water were available *ad libitum*. Feed use was monitored daily over a 28-day period commencing at d 18 after introduction to the facility. Body weight was recorded weekly. Faecal output was determined by total collection on d 25 to 27 for estimation of the coefficients of total tract apparent digestibility (CTTAD) for dry matter (DM), crude protein (CP) and fibre. Chemical analyses were undertaken using standard laboratory procedures (PONY Laboratories, Shenzhen). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were estimated after the methods of van Soest and Wine (1968). Data were analysed using a GLM procedure (Minitab®, Version 14.0; USA) blocked for treatment and sex. Initial BW was used as a covariate in the analysis of BW and feed intake.

Feed:gain tended to decline ($P = 0.15$) with increased mannanase and protease inclusion (Table 1). The CTTAD of CP increased ($P < 0.025$) with increased protease and mannanase inclusion. All combinations containing the XCA mixture increased the CTTAD ($P < 0.05$) of the ADF fraction above that of the single enzyme (PHY).

The results support those previously obtained *in vitro* with multiple enzyme inclusions producing greater component digestibility. Effects on BW gain were marginal but not unexpected. Small group sizes (n < 10) and the relatively low NSP content of the diet challenge the identification of statistically significant production responses. Further commercial studies will determine whether trends to reduced intake and weight gain with higher inclusions of mixed enzymes can be offset by gains in feed use efficiency.

Table 1. Performance measures and coefficients of total tract apparent digestibility (CTTAD) in young pigs receiving mannanase (M) and protease (P) in enzyme mixtures

Enzyme	PHY	XCA	XCM	XCP	XMP	SE[A]	*P* value[B]
M+P enzyme activity (U/g)	0	0.6	1.5	1.8	2.4		
Feed:Gain (n = 6)	1.86	1.91	1.74	1.74	1.74	0.060	0.15
Final body weight (kg)	32.2	30.9	33.5	33.7	32.7	0.94	NS
			CTTAD (n = 4)				
Crude protein, CP	0.88[a]	0.88[a]	0.89[ab]	0.91[b]	0.90[ab]	0.005	0.024
Acid detergent fibre	0.34[a]	0.47[ab]	0.45[ab]	0.47[ab]	0.50[b]	0.035	0.022
Neutral detergent fibre	0.61	0.63	0.62	0.65	0.65	0.023	NS

[A]SE, standard error. [B]enzyme effect. NS, not significant ($P > 0.1$).

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Enzyme mixtures differentially influence the digestion of nutritional components of a pig grower diet

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Ongoing variability in corn and soybean meal markets has encouraged the investigation of more effective methods for the use of alternative raw materials in the preparation of pig diets. The present study examines the potential for combinations of single enzymes, formulated with different protease and mannanase content, to influence the digestion of a corn : soy diet containing by-products.

Forty castrate male cross-bred pigs [initial body weight (BW) 31.8 ± 0.32 kg mean ± SD] were randomly assigned to one of five treatments consisting of no enzyme (NIL) or one of a combination of xylanase (EC3.2.18, 6 U/g), cellulase (EC3.2.1.24, 0.5 U/g) and amylase (EC3.2.1.4, 0.04 U/g) with differing inclusions of protease (EC3.2.23.6) and mannanase (EC3.2.1.78). Protease (P) and mannanase (M) inclusions were adjusted to provide calculated activities of 0, 0.45, 0.9, 1.8 U/g, for treatments NIL, MP1, MP2, MP3, respectively. Treatment (MP4) consisted of the MP1 formulation with added phytase (EC3.1.3.26, 0.09 U/g). The diet was formulated to provide 13.5 MJ digestible energy (DE)/kg and 9 g/kg available lysine. It contained wheat bran, corn-DDGS and rice bran at 6.4%, 5.0% and 3.5%, respectively. Pigs were individually housed in a climate controlled room. Feed and water were supplied *ad libitum*. Feed use and live weight was monitored over a 21-d period. Faecal samples were collected on d 18–20 for estimation of coefficients of apparent total tract digestibility (CATTD) for dry matter (DM), crude protein (CP) and fibre. Digestibility was estimated by reference to acid insoluble ash as an indigestible marker. Chemical analyses were undertaken using standard laboratory procedures (PONY Laboratories, Shenzhen). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined based on van Soest and Wine (1968). Data were analysed using a GLM procedure (Minitab®, Version 14.0; USA) blocked for treatment and replicate.

Feed intake and feed : gain ratio showed a tendency ($P < 0.1$) to reduced intake and lower feed : gain ratio at the intermediate M and P inclusion (Table 1). Mean BW and BW gains were not statistically influenced by treatment ($P > 0.1$) and averaged 41.8 ± 0.83 kg and 0.955 ± 0.039 kg/day, respectively. Faecal DE was approximately 0.2 MJ/kg higher in pigs receiving the phytase mixture (MP4) than in pigs receiving the higher M + P inclusions. Phosphorus CTTAD increased ($P < 0.012$) with phytase inclusion (MP4) and tended to increase with higher M + P inclusions. The crude protein CTTAD was similar ($P > 0.1$) for all treatments. The CTTAD of NDF was greatest on the highest inclusion of M + P (MP3) while the CTTAD of ADF was greatest on the intermediate enzyme inclusion (MP2). The treatment containing phytase (MP4) showed the lowest CTTAD of ADF and NDF.

The feed intake and feed : gain responses to changes in enzyme composition and concentration reported are at variance with the changes in CTTAD. Interactions between animal performance and enzyme composition of the magnitude observed here warrant further investigation on a commercial scale.

Table 1. Responses to mannanase (M) and protease (P) content of enzyme mixtures in grower pigs

Treatment	NIL	MP1	MP2	MP3	MP4	SEM[A]	*P* value[B]
M+P activity U/g	0	0.45	0.9	1.8	0.45		
Feed intake (g/day)	2124	2001	1932	2087	2142	56.2	0.066
Feed : Gain	2.5	2.6	2.0	2.3	2.6	0.16	0.062
Faecal DE MJ/kg	13.8^{ab}	13.8^{ab}	13.7^{a}	13.7^{a}	13.9^{b}	0.031	0.011
CTTAD (n = 4)							
Crude Protein	0.86	0.86	0.86	0.86	0.87	0.008	NS
Phosphorus	0.47^{a}	0.48^{ab}	0.45^{ab}	0.50^{ab}	0.52^{b}	0.011	0.015
NDF	0.60^{b}	0.62^{bc}	0.58^{ab}	0.63^{c}	0.54^{a}	0.009	0.001
ADF	0.30^{b}	0.35^{b}	0.41^{b}	0.35^{b}	0.23^{a}	0.0208	0.001

[A]SEM, standard error mean. [B]N = 8 for intake, feed : gain and faecal DE.

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Improved mineral utilisation in grower-finisher pigs fed a diet supplemented with graded amounts of two phytases

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Dietary phytase, when used correctly, will prevent possible phosphorus (P) deficiency, reduce the P content in animal waste and maintain animal well-being. The aim of this study was to evaluate the effects on P and calcium (Ca) utilisation, plasma indices and bone strength of a *C. braakii*-(Ronozyme HiPhos) and an *E. coli*-(Quantum Blue) derived 6-phytase at high dosages in grower-finisher pigs. The hypothesis tested was that high phytase inclusion levels would give additional benefit in pigs by improving mineral utilisation.

An experiment was conducted with 64, 70-day-old pigs (Large-White × Redon) having an initial body weight of 23.5 ± 1.96 kg (mean ± SE). Pigs were randomly allotted into eight groups of eight animals each. They were fed *ad libitum* for 84 days with diets based on corn, soybean meal and barley. Diets were a positive control (PC) formulated to meet the animal requirements for the finishing period according to NRC (2012) [total P, 0.47%; total Ca, 0.80%; crude protein (CP), 150 g/kg; metabolisable energy (ME), 13.4 MJ], or a matrix control diet (MC) with reduced nutrient content [total P, 0.37%; total Ca, 0.65%; CP, 145 g/kg; ME, 13.1 MJ]. The MC diets were supplemented with Ronozyme HiPhos at 1000 (H1000), 2000 (H2000) and 3000 U/kg (H3000), and with Quantum Blue at 500 (Q500), 1000 (Q1000) and 1500 U/kg (Q1500). The coefficient of total tract apparent digestibility (CTTAD) of P and Ca, excretion of P and Ca, plasma indices and metacarpal bone characteristics were evaluated at the end of the trial. Plasma *myo*-inositol (INO) was analysed according to Leung *et al*. (2011) and the other parameters using the methods described in AOAC (2012). Data were examined by ANOVA and differences between groups were determined by the Student-Newman-Keuls multiple-range test (significant at $P < 0.05$).

The CTTAD of P was improved ($P < 0.05$) and P excretion reduced ($P < 0.05$) in all phytase groups (Table 1). The Ca excretion was lower ($P < 0.05$) with the phytase and MC treatments in comparison to the PC diet, and was not different ($P > 0.05$) between phytases or inclusion concentrations. Plasma P was increased ($P < 0.05$) in all phytase-fed pigs whereas plasma Ca was higher ($P < 0.05$) in the PC group than in the other groups (Guggenbuhl *et al.* 2012*a*). Plasma INO, the end product of phytate degradation, was increased ($P < 0.05$) in the H100, H2000, H3000, Q1000 and Q1500-fed pigs. Compared to the MC treatment group, bone ash and breaking force were improved ($P < 0.05$) in all phytase groups.

Data from the present study showed similar effects for both enzymes. The highest dosages from each of both phytases had beneficial effects on all measures, thereby compensating for reduced nutrient levels and further reducing P and Ca supplementation in pig diets (Guggenbuhl *et al.* 2012*a*, 2012*b*). The increased plasma INO could be partly involved in the bone strength improvements (Croze and Soulage 2013).

Table 1. Mineral utilisation in grower-finisher pigs fed graded amounts of two different phytases

Treatments	MC	PC	Phytase (FYT/kg)						SEM[A]	*P* value
			H1000	H2000	H3000	Q500	Q1000	Q1500		
CTTAD P	0.35[a]	0.35[a]	0.52[bc]	0.52[bc]	0.54[bc]	0.47[b]	0.51[bc]	0.55[c]	0.010	<0.0001
P excretion (%)	0.25[c]	0.31[d]	0.18[ab]	0.18[ab]	0.17[a]	0.20[b]	0.19[ab]	0.17[ab]	0.005	<0.0001
CTTAD Ca	0.53	0.52	0.60	0.55	0.55	0.59	0.60	0.58	0.008	NS[B]
Ca excretion (%)	0.49[a]	0.62[b]	0.41[a]	0.46[a]	0.48[a]	0.42[a]	0.40[a]	0.43[a]	0.010	<0.0001
Plasma P (mg/dL)	4.0[a]	4.7[a]	5.9[b]	6.3[b]	6.3[b]	6.3[b]	6.0[b]	6.6[b]	0.14	<0.0001
Plasma Ca (mg/dL)	12.9[a]	15.0[b]	12.2[a]	12.4[a]	12.3[a]	11.9[a]	12.0[a]	11.5[a]	0.17	<0.0001
Plasma INO (mg/L)	7.01[ab]	5.70[a]	8.67[bcd]	9.88[cd]	11.1[d]	7.68[abc]	9.01[bcd]	10.90[d]	0.324	<0.0001
Bone ash (%)	48.2[a]	53.0[b]	55.5[bc]	58.1[c]	57.7[c]	56.8[bc]	57.7[c]	58.0[c]	0.55	<0.0001
Break force (N)[C]	146[a]	198[ab]	271[bc]	369[c]	347[c]	273[bc]	293[bc]	293[bc]	12.3	<0.0001

[A]SEM, standard error of the mean. [B]NS, not significant. [C]N, newtons. [a,b,c,d,e]Means in a row not having the same superscript are significantly different.

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Improved mineral utilisation in weaned pigs fed a diet supplemented with graded amounts of two phytases

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The effects of dietary phytase on mineral utilisation in pigs are well known and documented, but less information has been reported when high dietary inclusion levels are used. The aim of this study was to evaluate the effects on phosphorus (P) and calcium (Ca) utilisation, plasma indices and bone strength of a *C. braakii*-(Ronozyme HiPhos) and an *E. coli*-(Quantum Blue) derived 6-phytase at one, two and three times their recommended feed inclusion levels in weaned pigs. The study tested the hypothesis that high phytase dosages will give additional benefit in piglets by improving mineral utilisation.

An experiment with 96, 28-day-old weaned pigs (Large-White x Redon) having an initial body weight of 7.91 ± 0.73 kg (mean ± SE) was performed. Piglets were randomly allotted into eight groups of 12 animals each. They were fed *ad libitum* for 42 days with diets based on corn, soybean meal and rapeseed meal. Diets were a positive control diet (PC) formulated to meet the animal requirements according to NRC (2012) [total P: 0.66%; total Ca: 0.80%; crude protein: 192 g/kg; metabolisable energy (ME): 14.2 MJ], or a matrix control diet (MC) with reduced nutrient content [total P: 0.55%; total Ca: 0.63%; crude protein: 188 g/kg; ME: 14.0 MJ]. The MC diets were supplemented with Ronozyme HiPhos at 1000 (H1000), 2000 (H2000) and 3000 U/kg (H3000), and with Quantum Blue at 500 (Q500), 1000 (Q1000) and 1500 U/kg (Q1500). The P and Ca coefficient of total tract apparent digestibility (CTTAD) and excretion, plasma indices and femur characteristics were evaluated. Plasma *myo*-inositol (INO) was analysed according to Leung *et al*. (2011) and the other parameters using the methods described in AOAC (2012). Data were examined by ANOVA and differences between groups were determined by Student-Newman-Keuls multiple-range test (significant at $P < 0.05$).

The CTTAD of P was improved ($P < 0.05$) and P excretion reduced ($P < 0.05$) in all phytase-fed pigs (Table 1). The CTTAD of Ca was increased ($P < 0.05$) and Ca excretion decreased ($P < 0.05$) in the H2000, Q1000 and Q1500 treatments in comparison to the MC diet. Plasma P was increased ($P < 0.05$) in all phytase-supplemented pigs whereas plasma Ca was only reduced ($P < 0.05$) in the H2000 group compared to the MC group (Guggenbuhl *et al.* 2012). Plasma INO, the end product of phytate degradation, was increased ($P < 0.05$) in H2000, H3000, Q1000 and Q1500-fed pigs. Bone ash and breaking force in all phytase groups, except in Q500 group, were increased ($P < 0.05$) compared to the MC group.

Data from the present study showed similar effects for both enzymes. Phytases had beneficial effects on all measures, thereby compensating for reduced nutrient levels (Guggenbuhl *et al.* 2012). Increased plasma INO could be partly involved in the bone strength improvements (Croze and Soulage 2013). Nevertheless, the benefits of including high phytase dosages were limited in comparison to the low levels tested.

Table 1. Mineral utilisation in weaned pigs fed graded amounts of two different phytases

Treatments	MC	PC	Phytase (FYT/kg)						SEM[A]	*P* value
			H1000	H2000	H3000	Q500	Q1000	Q1500		
CTTAD P	0.28[a]	0.30[a]	0.50[c]	0.55[cd]	0.54[cd]	0.43[b]	0.51[c]	0.59[d]	0.001	<0.0001
P excretion (%)	0.40[d]	0.47[e]	0.28[b]	0.25[b]	0.26[b]	0.31[c]	0.27[b]	0.23[a]	0.009	<0.0001
CTTAD Ca	0.52[b]	0.45[a]	0.59[bcd]	0.65[d]	0.56[bc]	0.52[b]	0.61[cd]	0.64[d]	0.009	<0.0001
Ca excretion (%)	0.47[c]	0.64[d]	0.41[abc]	0.35[a]	0.44[bc]	0.47[c]	0.39[ab]	0.36[a]	0.011	<0.0001
Plasma P (mg/dL)	4.2[a]	6.3[b]	7.8[c]	8.2[c]	8.1[c]	6.7[b]	7.7[c]	8.7[c]	0.16	<0.0001
Plasma Ca (mg/dL)	12.3[b]	13.2[c]	12.3[b]	11.2[a]	11.7[ab]	11.6[ab]	12.2[b]	11.4[ab]	0.10	<0.0001
Plasma INO (mg/L)	7.40[a]	7.53[a]	12.10[abc]	14.60[c]	13.90[bc]	9.12[ab]	13.00[bc]	16.10[c]	0.533	<0.0001
Bone ash (%)	61.2[a]	63.2[b]	65.2[bc]	65.4[bc]	64.3[bc]	63.7[bc]	64.7[bc]	65.9[c]	0.27	<0.0001
Breaking force (N)[B]	141[a]	324[ab]	519[bc]	714[c]	623[bc]	375[ab]	610[bc]	744[c]	37.8	<0.0001

[A]SEM, standard error of the mean. [B]N, newtons. [a,b,c,d,e]Means in a row not having the same superscript are significantly different.

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Assessment of the effects of a serine protease on commercial grower-finisher pig performance in Brazil

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The swine industry in Brazil is constantly looking for reductions in feed costs and use of highly digestible ingredients to improve performance and reduce nutrient excretion. Among alternatives, the use of exogenous enzymes, such as protease, has been intensified in order to improve the digestibility of protein and amino acids (Guggenbuhl *et al*. 2012), reduce the inclusion of protein ingredients and improve performance (Rooke *et al*. 1998). The aim of this study was to evaluate if the inclusion of a serine protease in pigs' diet could reduce the inclusion of protein ingredients without compromising performance.

Ninety-six pigs (PIC × PIC), located on an experimental farm of Cooperativa Central Aurora, were allocated in a completely randomised block design to one of two treatments: a standard Control diet without protease; and a diet with reduced levels of nutrients and supplemented with 200 ppm of RONOZYME ProAct CT (DSM Nutritional Products, Sao Paulo, Brazil). Protease nutrient equivalents at the recommended dose were accounted for in the diet formulation. Diets were mash and based on corn, soybean meal and meat bone meal, and were formulated to meet different phase nutrition requirements [23–36 kg: 14.0 MJ metabolisable energy (ME)/kg, 198 g/kg crude protein (CP), 11.8 g/kg standardised ileal digestible (SID) lysine (Lys); 36–58 kg: 14.0 MJ ME/kg 192 g/kg CP, 11.2 g/kg SID Lys; 58–88 kg: 14.0 MJ ME/kg, 186 g/kg CP, 10.2 g/kg SID Lys; 88–100 kg: 13.8 MJ ME/kg, 164 g/kg CP, 8.5 g/kg SID Lys; 100–120 kg: 13.8 MJ ME/kg, 169 g/kg CP, 10.3 g/kg SID Lys). Each treatment had eight replicates of six pigs (half of each sex) reared from 23.5 ± 0.22 kg (mean ± SD) to 120 kg body weight (BW) over a period of 106 days. Animal performance as average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR), energy and Lys utilisation, and carcass yield parameters were measured. Data were analysed using GLM procedures (SAS®; USA), with $P < 0.05$ accepted as statistical significance.

The reduction in protein and amino acids content and the protease use did not impact ($P > 0.05$) on animal growth (Table 1). Furthermore, when compared to the control diet, the use of protease optimised Lys utilisation, likely due to higher ($P < 0.05$) ADG/CLysd intake, and higher carcass weight. These results are in accordance with Guggenbuhl *et al*. (2012) who found that the use of the same protease improved the digestibility of some essential amino acids in pigs fed a corn and soybean-meal diet. Results suggested that the use of protease enables protein content to be reduced in the diet without compromising pig performance and improving carcass weight.

Table 1. Grower-finisher pig performance, energy and lysine utilisation, and carcass yield measures in response to a protease added to the diet

	Control	Protease	SEM[A]	*P* value
Initial BW (kg)	23.5	23.5	0.05	0.981
Final BW (kg)	120.3	122.1	1.36	0.162
ADFI (kg)	2.02	2.01	0.004	0.239
ADG (kg)	0.92	0.93	0.006	0.162
Feed conversion ratio (at 106 d) (kg : kg)	2.21	2.16	0.016	0.128
Adjusted FCR (for carcass weight of 85 kg)	3.02	2.90	0.027	0.032
ME intake (MJ/kg)	29.7	29.3	0.002	0.239
ADG/ME intake	0.13	0.13	0.002	0.124
Net Energy (NE) intake (MJ/kg)	21.4	21.4	0.001	0.987
ADG/NE intake	0.18	0.18	0.003	0.221
ADG/SID Lys intake[B] (kg/kg)	44.9	46.8	0.895	0.010
Carcass weight (kg)	87.1	89.4	0.55	0.036
Carcass yield (%)	72.1	72.9	0.24	0.110
Lean meat yield (%)	57.8	56.9	0.26	0.095

[A]SEM, standard error of the mean. [B]Dig Lys intake: [(ADFI 23-36 kg*1.18% SID Lys) + (ADFI 36-58 kg*1.12% SIDLys) + (ADFI 58-88 kg*1.02% SID Lys) + (ADFI 88-100 kg*0.85% SID Lys) + (ADFI 100-120 kg*1.03% SIDLys)]/Number of trial days.

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Lysine requirements of modern genotype finisher pigs

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Moore *et al.* (2013) determined the performance responses to dietary lysine concentrations of the modern genotype pig from 50 to 100 kg liveweight (LW) and found that the lysine requirement to optimise growth performance in this weight range was higher by approximately 10% than that being used by the Australian industry. The aim of the current study was to confirm the optimal standardised ileal digestible lysine (SID Lys)/MJ digestible energy (DE) ratio for a modern genotype of entire male and female pigs from 60 to 100 kg LW obtained in a research facility.

A total of 392 pigs (Large White × Landrace × Duroc) was used in a 2 × 7 factorial arrangement of treatments. The treatments were: sex (entire males vs females); and SID lysine concentrations (0.40, 0.46, 0.52, 0.58, 0.64, 0.70 and 0.76 g SID Lys/MJ DE). The diets contained 14.0 MJ DE/kg and were fed for 6 weeks from 63.6 ± 0.44 to 103 ± 0.55 (mean ± SE) kg LW. Pigs were housed in groups of seven and there were four replicates/treatment. The data were analysed using the linear plateau and quadratic models fitted to the treatment means (Nutrient Response Models Version 1.1, Vedenov and Pesti 2008; O'Connell *et al.* 2006). The results from the linear plateau and quadratic models were then averaged to determine the requirement (Williams *et al.* 1984).

For female pigs, the SID Lys concentrations to maximise daily gain and minimise FCR were 0.58 and 0.58 g/MJ, respectively (Fig. 1). For entire male pigs the SID Lys concentrations to maximise daily gain and minimise FCR were 0.64 and 0.63 g/MJ DE, respectively (data not shown). These SID lysine concentrations confirm optimal SID Lys/MJ DE ratio for a modern genotype of entire male and female pigs from 60 to 100 kg LW as they are similar to the results from Moore *et al.* (2013) in a research environment. These values for female pigs are similar to the SID Lys requirement estimated in Australian commercial facilities (Moore *et al.* 2015).

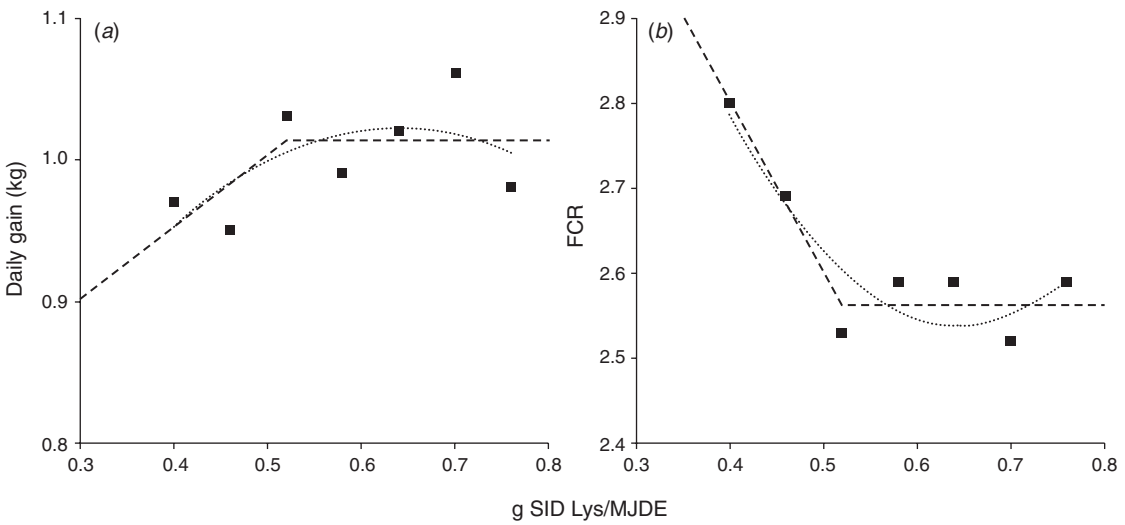

Fig. 1. Effect of dietary SID Lys content on (*a*) daily gain and (*b*) FCR for female pigs from 63 to 98 kg LW (n = 4, mean ± SE). Data has been fitted with linear plateau (- - -) and quadratic (. . .) models.

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Sulphur amino acid requirements of commercially-grown finisher pigs

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Reduced protein utilisation in immune-system-activated pigs is caused primarily by an enhanced rate of protein turnover associated with additional production of immune cells, antibodies and acute phase proteins, which are particularly high in sulphur amino acids (SAA) (Rakhshandeh and de Lange 2011). The current study tested the hypothesis that the SAA requirements of finisher pigs grown commercially will be greater than the current NRC (2012) recommendation of 0.58 standardised ileal digestible SAA to lysine (Lys) ratio (SID SAA:Lys), on the basis that pigs grown commercially are continuously exposed to high microbial loads and environmental challenges.

Two commercial experiments were conducted. A total of 2016 group-housed pigs (Large White × Landrace, PrimeGro Genetics) were selected at 50.1 ± 0.46 kg (mean ± SE) and allocated to diets containing SID SAA:Lys ratios of 0.54, 0.60, 0.63, 0.66, 0.69, 0.75, 0.76, 0.78, 0.80, 0.82, 0.87, 0.89, and 1.02. Experiments 1 and 2 used 12 and 8 replicate pens (containing 14 pigs) per treatment, respectively. Diets used for Experiment 1 and 2 contained 13.8 MJ digestible energy (DE)/kg and 0.55 g SID Lys, and 14.0 MJ DE/kg and 0.56 g SID Lys per kg, respectively. Pigs were fed the experimental diets for 6 weeks and performance was recorded. Data were analysed using REML variance component analysis with the experimental batches and replicate pens set as random factors. Data means were then fitted to the linear-plateau and quadratic plateau models to estimate SID SAA requirements using the Nutritional Response Models 1.1 (Vedenov and Pesti 2008).

The minimum feed conversion ratio (FCR) was achieved at SID SAA:Lys ratios of 0.64 (SE ± 0.29) and 0.71 (SE ± 0.30) for linear-plateau and quadratic-plateau prediction models, respectively (Fig. 1). Increasing the SID SAA:Lys ratio showed no LP or QP responses to growth rate, however average daily feed intake (ADFI) was decreased in a comparable manner to FCR (data not shown). These data suggest that (1) increasing the dietary SID SAA:Lys ratios decreased ADFI but maintained daily growth rate via improvements in FCR, and (2) the requirement for dietary SID SAA:Lys for commercially-grown finisher pigs was not significantly different from the current NRC (2012) estimate, and the high variation in the estimate illustrates the difficulty with conducting research under commercial conditions.

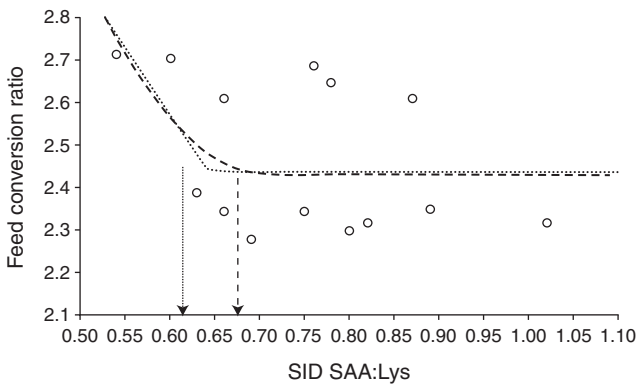

Fig. 1. Requirement for SID SAA in relation to SID Lys content for minimum feed conversion ratio estimated using either a linear-plateau (dotted line, $R^2 = 0.26$, $P < 0.001$, SID SAA:Lys = 0.64 ± 0.29) or a quadratic-plateau model (broken line, $R^2 = 0.25$, $P < 0.001$, SID SAA:Lys = 0.71 ± 0.30) in finisher pigs. The down arrows represent respective SID SAA:Lys requirements estimated by the two models.

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Variation in particle sizes of commercial pig feeds in Vietnam

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Feeds are formulated to supply animals with optimum energy and protein, and minerals and vitamins are added. In order to optimise feed digestibility, feed composition and processing are important. Milling is the first operation in feed processing, during which particle size is reduced and particle size distribution determined (Nguyen *et al*. 2013). Feed mills generate diverse particle sizes, as indicated in a survey of Australian feed mills (Nguyen *et al*. 2013). Previous studies in our laboratory highlighted the dependence of digestibility, and water absorption and solubility indices of grains on particle size and particle size distribution (Nguyen *et al.* 2015), and subsequent animal performance. The aim of this study was to characterise particle size of pig feeds in Vietnam, with a focus on southern regions, where the majority of feed mills are. It was hypothesised that particle size and its distribution would vary widely in Vietnamese pig feeds.

Forty-one mash pig feeds were collected from 11 mills in the Southeast and Mekong Delta regions. As in Nguyen *et al.* (2013) feeds were sieved, in duplicate, on-site using a manual-sieving device. Using methodology detailed in ASABE (2008), the volumes retained in the seven compartments of the device were recorded to calculate the geometric mean particle diameter (D_{gw}) and geometric standard deviation of mean particle diameter (S_{gw}). These results were presented without statistical analyses because feed ingredients were varied and not controlled.

Grains and ingredients were usually hammer-milled together, but the mills used different screen sizes. Irrespective of the feed, D_{gw} did not vary greatly (500–700 µm), and S_{gw} was 400–700 µm (Fig. 1). This observation could be due to the closeness of the screen sizes used by the mills (2.0–3.8 mm). However, 15 to 37% of the particles were larger than 1000 µm, and this could adversely affect feed efficiency. Findings from this study suggested that feed mills in Vietnam should monitor their products to ensure optimum particle size for animal feeds.

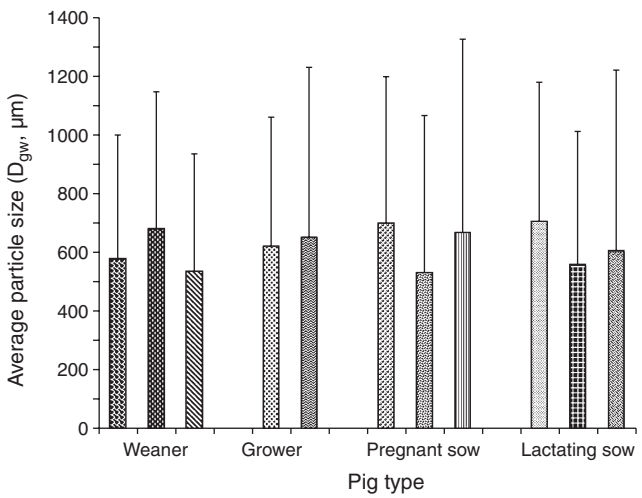

Fig. 1. Average particle size of different commercial feed in selected mills in Vietnam. Geometric standard deviation of mean particle diameter (S_{gw}) shown as error bars.

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Growth performance of weaner pigs fed diets containing grains milled to different particle sizes. I. Sorghum

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Sorghum is the third most important cereal in Australia, in terms of production, and it is a major feed grain (Mahasukhonthachat *et al.* 2010). Mahasukhonthachat *et al.* (2010) and recently, Nguyen *et al.* (2015), revealed particle size and mill type as the primary determinants of *in-vitro* digestion properties of grains. However, there are limited studies on the effects of grain particle size and mill type on animal performance. Using sorghum, this study investigated these effects on performance of weaner pigs, and tested the hypothesis that within an optimum particle size range, pig performance is not affected.

Sorghum (var. *MR43*) was milled with industrial-scale hammer (HM) and disc (DM) mills, in a randomised experiment with two replicates. Four screens (2, 3, 4, and 5 mm) and four disc gaps were used in the HM and DM respectively. Four additional treatments were obtained by mixing the finest (F) and coarsest (VC) sizes from the mills (HM F- DM F, HM F-DM VC, HM VC-DM F, and HM VC-DM VC). Experimental diets [15 MJ digestible energy (DE)/kg, 1 g available lysine/MJ DE, 220 g/kg crude protein, and 350 g/kg starch), consisting 49.8% of the milled sorghum, were fed *ad libitum* to weaner pigs for 21 d. A total of 289 weaner pigs (Large White × Landrace, PrimeGro Genetics) aged 28 days and having a bodyweight of 6.8 ± 0.1 kg (mean ± SD), were individually housed and used in three batches in a randomised block design, with some incomplete blocks. There were 20 diets from the 12 particle size treatments, and 24 pigs were used per treatment. Pigs and feed residues were weighed weekly to calculate average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR). The Rivalea animal ethics committee approved (14N009) the animal experiment. The diets were analysed (Nguyen *et al.* 2015) for geometric mean particle size diameter (D_{gw}) and geometric standard deviation of mean particle diameter (S_{gw}). Statistical methods (ASReml-R) analogous to ANOVA were used (Butler 2009).

The D_{gw} of the milled sorghum ranged from 400–800 μm, with up to 50% of the particles being higher than 1000 μm in size. There was no pronounced ($P > 0.05$) mill effect on S_{gw}, and neither the mill nor particle size affected ($P > 0.05$) the pig growth (Table 1). Irrespective of the mill or particle size, the pigs consumed (ADFI) and grew (ADG) more with age (not shown).

The D_{gw} of the diets (600–750 μm) was not significantly different ($P > 0.05$) from that of the milled sorghum, and, therefore, the ingredients did not influence the D_{gw} of the diets. Hence, the measured animal responses were mainly due to the particle size of the milled sorghum. The absence of significant mill and particle size effects suggests 400–800 μm as the optimum particle size range for sorghum fed to weaner pigs. Feed mills, therefore, need not grind sorghum below 400 μm for milling economy, and particle size above 800 μm might be undesirable for good performance of weaner pigs fed sorghum-based diets.

Table 1. Effects of particle size of milled sorghum on performance of pigs from 0–21 days after weaning

	Disc mill				Hammer mill				Mixtures				SEM[A]
D_{gw}-row (μm)	442	581	659	823	539	570	609	640	450	515	605	689	
S_{gw}-row (μm)	294	453	558	704	317	421	414	482	279	397	487	630	
Particles >1000 μm (%)	7.0	26.8	36.1	51.2	10.9	21.3	23.5	28.9	6.4	17.8	27.0	39.7	
ADFI (g)	719	697	667	654	686	653	690	674	698	630	670	669	0.02
ADG (g)	472	458	459	445	450	436	452	450	460	417	454	442	0.01
FCR (g : g)	1.48	1.48	1.42	1.43	1.49	1.45	1.49	1.47	1.47	1.47	1.44	1.48	0.02

[A]SEM, standard error of the mean.

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Growth performance of weaner pigs fed diets containing grains milled to different particle sizes. II. Field pea

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Various studies have highlighted the importance of grain particle size on growth performance of pigs (Choct *et al.* 2004; Montoya and Leterme 2011). However, the studies concentrated on cereals, used one mill type, or had an insufficient number of treatment levels to probe the performance-size relationships. Field pea is low in anti-nutritional factors, and it is an important protein source in pig feeds (Nguyen *et al*. 2015). Hammer-, disc- and roller-mills are mainly used in pig feed manufacture, and mill types can influence growth performance (Choct *et al.* 2004). Using commercial mills to replicate field situations, this study investigated how weaner pigs responded to diets containing hammer- and disc-milled field peas of different particle sizes. The hypothesis tested was that an optimum particle size range exists, within which, growth performance is independent of particle size.

Field pea (var. *Walana*) was milled, in two replicates, using commercial hammer (HM) and disc (DM) mills, in a randomised design with four screen sizes (2, 3, 4, and 5 mm) and four disc gaps respectively. The finest (F) and coarsest (VC) sizes from the mills were mixed for four additional treatments: HM F- DM F, HM F-DM VC, HM VC-DM F, and HM VC-DM VC. A total of 20 milled grains, but 12 treatments, were used (30%) to formulate the experimental diets [14 MJ digestible energy (DE)/kg; available lysine/DE, 0.09 g/MJ DE; 430 g/kg starch, 190 g/kg crude protein) for weaner pigs [Large White × Landrace, PrimeGro Genetics; 28 days of age and weighing 7.3 ± 0.10 kg (mean ± SD)]. After adaptation for 6 days on a commercial diet, a total of 400 pigs in two batches were individually housed and fed the 20 diets *ad libitum* over a 21-day period using a randomised block design with some incomplete blocks. Hence, there were effectively 20 pigs per diet (or 33 pigs per treatment), and the pigs and feed residues were weighed weekly to calculate average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR). The Rivalea animal ethics committee approved (13N023C) the animal experiment. The diets were analysed for geometric mean particle size diameter (D_{gw}) and geometric standard deviation of mean particle diameter (S_{gw}) as before (Nguyen *et al.* 2015). Statistical methods (ASReml-R) analogous to ANOVA were used (Butler 2009).

Table 1 shows that the D_{gw} of the milled pea ranged from 600–800 μm, and had up to 45% of the particles greater than 1000 μm. The disc-milled pea had a wider D_{gw} range (200 μm) with the mill settings than the hammer-milled pea. With age, the pigs' ADFI and ADG increased (not shown), but their growth performance was not significantly ($P > 0.05$) affected by the mill type and particle size from 0–21 days (Table 1). The D_{gw} of the diets was from 500–700 μm, and not different ($P > 0.05$) from the D_{gw} of the milled pea. Hence, the diet ingredients were not coarser than the milled pea, whose particle size can, therefore, be inferred to solely affect the measured growth of the pigs. In view of the absence of significant effects of the diets on the growth performance of the pigs, it is suggested that the particle size range (600–800 μm) of the milled field pea is an optimum range at the 30% inclusion for weaner pigs. Feed mills should take cognizance of this range to guide their milling operations, during feed manufacture.

Table 1. Effects of particle size of the milled peas on performance of pigs after weaning

	Disc mill				Hammer mill				Mixtures				SEM[A]
D_{gw}-row (μm)	576	709	745	811	569	580	604	675	561	585	694	809	
S_{gw}-row (μm)	443	589	657	737	447	451	479	571	436	478	597	703	
Particles >1000 μm (%)	15.3	35.5	39.5	43.7	16.5	18.0	20.8	29.9	15.3	19.6	31.0	42.2	
ADFI (g)	557	565	586	563	545	540	560	568	575	561	546	562	0.02
ADG (g)	417	415	433	429	403	395	413	416	429	439	405	412	0.02
FCR (g:g)	1.35	1.37	1.35	1.33	1.36	1.39	1.33	1.37	1.35	1.29	1.35	1.37	0.02

[A]SEM, standard error of the mean.

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Rapid changes occur in feed efficiency after infection with *Mycoplasma hyopneumoniae* and *Pasteurella multocida*

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Mycoplasma hyopneumoniae (Mhp) and *Pasteurella multocida (Pm)* are the agents most frequently isolated from lungs affected with enzootic pneumonia. Primary infection with *Mhp* predisposes pigs to opportunistic infection with *Pm*, causing increased lung damage and reduced weight gains and feed intake (Eamens *et al.* 2007). This study tested the hypothesis that infection with multiple pathogens (*Mhp+Pm*) would reduce feed efficiency compared to a single infection of either pathogen or uninfected pigs.

Data from a previous trial using 64 individually-housed hybrid (Landrace x Large White) gilts weighing 24.0 ± 0.53 kg (mean ± SE) challenged with *Mhp*, *Pm*, *Mhp+Pm*, or receiving no challenge, was reanalysed to investigate the effects of single or multiple respiratory pathogens on feed efficiency (Eamens *et al*. 2007). Each treatment group was housed in a separate room. Individual weight gain and feed intakes were calculated weekly for the 4 weeks following challenge. Feed efficiency was calculated as gain : feed (G : F), to allow the comparison of results on a unidirectional scale, as pigs suffered significant weight losses. Differences in G : F were analysed using unbalanced ANOVA (Genstat, 17th Edition; UK).

All challenged pigs developed pneumonia and four pigs (one '*Pm*' and three '*Mhp+Pm*') required treatment. Infection with *Pm*, either alone or with *Mhp*, caused negative G : F ratios which were significantly reduced ($P < 0.001$) in the first week after challenge (Fig. 1). In the absence of repeated challenge or environmental stressors, *Pm* infected pigs recovered rapidly, with no differences ($P > 0.05$) at 2 weeks and higher ($P < 0.001$) G : F in *Mhp+Pm* pigs at 3 weeks. Over 4 weeks, pigs challenged with *Mhp+Pm* had lower ($P < 0.002$) G : F than uninfected pigs or pigs challenged only with *Mhp*.

The large reduction inG : F in *Pm* and *Mhp+Pm* treatments was associated with both reduced average daily feed intake (ADFI) ($P < 0.001$) and reduced average daily gain (ADG) ($P < 0.001$). The rapid recovery in G : F of the *Mhp+Pm* group was associated with higher ADG at week three ($P < 0.001$) relative to all other treatments, as there was no difference in ADFI between pigs with single or multiple pathogens at this time. Carcass composition measures (computed tomography) over this period showed significantly reduced proportions of body fat and increased muscle (by weight) in pigs infected with both *Mhp+Pm* (Eamens *et al*. 2007), suggesting that recovering pigs were largely depositing muscle when ADFI increased.

Calculating G : F ratio on a weekly basis quantified the acute impact of *Pm* infection and also the subsequent rapid recovery in feed efficiency. However, calculations over the total period still showed a negative impact of *Pm* on G : F, and this longer interval is of greater relevance to producers as it indicates the long-term production effects of untreated respiratory disease.

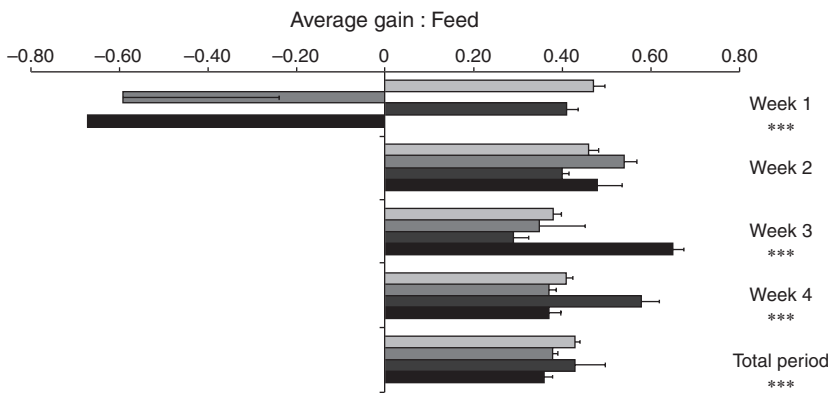

Fig. 1. Gain to feed ratios (+SEM) of uninfected (□), or infected with *P. multocida* (Pm) (▩), *M. hyopneumoniae* (Mhp) (■) or the combined infection (Mhp+Pm) (■) over 4 weeks after challenge. *** denotes significant difference between treatments.

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Postprandial kinetics of bacterial ecology in the terminal ileum of pigs fed soybean meal or differentially processed blue sweet lupins

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Blue sweet lupins (BSL) are considered as an alternative protein source to soybean meal (SBM) in diets for growing pigs. Some recent studies revealed that grinding intensity and hydrothermal processing might improve the nutritional value of BSL (Kim *et al*. 2009; Pieper *et al*. 2015). Altered particle size and viscosity due to processing might also change the bacterial activity in the upper gastrointestinal tract (Kim *et al*. 2009). It is well established that dietary particle size and digesta viscosity may influence the gastric emptying rate in the pig (Rainbird and Low 1986; Gregory *et al*. 1990), but little is yet known to which extent this may influence small intestinal microbial ecology patterns during the postprandial phase. It is hypothesised that digesta flow and particle size are important factors driving the substrate availability to the indigenous bacteria. In the current study, we thus determined postprandial kinetics of bacterial counts and activity at the terminal ileum in growing pigs fed differentially processed BSL.

Blue sweet lupins were processed in a hammer mill passing either a 3 mm sieve (coarsely ground blue lupins; CBL), a 1 mm sieve (finely ground blue lupins; FBL), or ground to pass a 1 mm sieve and subsequently expanded in a modified single screw extrusion-cooker TS-45 (ZMCh Metalchem Gliwice, PL) at 110 to 120°C (expanded blue lupins, EBL). Four experimental diets were formulated based on wheat and barley and either soybean meal or lupins as main protein source. A soybean meal-based diet (SBM) served as control and had similar particle size distribution as the EBL diet. Twelve PIC × Danbred crossbred pigs with an initial body weight of 20 kg were surgically fitted with a simple T-cannula at the terminal ileum and offered the experimental diets twice daily in mash form in a 3 × 4 Latin square design. After a 7-day adaptation period, ileal digesta samples were taken every 2 h over the course of 12 h after the morning meal. Microbial metabolites were determined by HPLC (D-/L-lactate), gas chromatography (short chain fatty acids; SCFA) and colorimetrically (ammonia, NH_3). Total DNA and RNA were extracted from ileal digesta using commercially available kits. The 16S ribosomal DNA and RNA copy numbers were determined by quantitative polymerase chain reaction using primers specific for Lactobacilli, Enterobacteria, Bacteroides-Porphyromonas-Prevotella, and Clostridial clusters I, IV and XIVa. Data analyses were conducted with sampling time and experimental period considered as repeated observations (IBM SPSS, Version 23.0; USA).

Both time point and dietary treatment had statistically significant effects on bacterial metabolites and bacterial DNA and RNA copy numbers. Concentration of SCFA in ileal digesta increased ($P < 0.05$) with SBM and EBL and peaked (20 and 23 mmol/L, respectively) after 4 h, whereas CBL and FBL diets showed only minor effects on SCFA concentration (maximum 9 and 7 mmol/L, respectively). Similar patterns were observed for individual SCFA, although concentration of propionate and butyrate were generally low compared to acetate. In contrast, total lactate was highest (90 mmol/L; $P < 0.05$) after 4 h in CBL-fed pigs, whereas SBM (56 mmol/L) and FBL (54 mmol/L) showed peaks after 6 h, and EBL diets peaked (76 mmol/L) at 8 h after the morning meal. Lactate concentration was correlated to ($R^2 = 0.40$) Lactobacilli 16S rRNA copy numbers and also correlated positively to Enterobacteria counts. Although an antagonistic relationship between Lactobacilli and Enterobacteria would be expected, their 16S rRNA counts showed a positive correlation ($R^2 = 0.42$) revealing that easily accessible substrates are the main driving force for bacterial growth in the pig small intestine. The NH_3 concentration was generally low (<5 mmol/L), and showed only minor responses to dietary treatment as well as during the postprandial phase. The results revealed clear differences in postprandial kinetics of bacterial metabolites and 16S rRNA copy numbers at the ileum of pigs fed diets containing differentially processed lupins. These differences seem to be related to particle size and digesta transit. Thus, choice of sampling time point is crucial for interpretation of microbial ecology in digesta contents.

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A study of nucleotides in weaning pigs challenged with *Escherichia coli* K88

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Nucleotides have also been considered as an alternative to antibiotics (Sauer *et al.* 2011) and have been described to have positive effects on stimulation of systemic immunity (Nagafuchi *et al.* 1997), small intestinal growth (Domeneghini *et al.* 2004) and hepatic composition (Novak *et al.* 1994) in pigs. The objective of this study was to evaluate the effect of different level of nucleotides on growth performance, blood profiles, and faecal scores in weaning pigs challenged with *Escherichia coli* K88. It was hypothesised that different levels of nucleotides may have different effects on weaning pigs.

A total of 140 weaning pigs (Landrace × Yorkshire × Duroc mixed crossbred, n = 35) with an average initial body weight (BW) of 7.2 ± 0.33 kg (mean ± SD) was used in a 42-day feeding trial. Pigs were distributed to four treatments on the basis of BW and sex with five pigs/pen (three barrows and two gilts) and seven pens/treatment. Treatments were: Control (CON), corn-soybean meal diet; R150, CON + 150 mg/kg Rovimax NX (DSM Nutritional Products Philippines, Inc.); R220, CON + 220 mg/kg Rovimax NX; and R275, CON + 275 mg/kg Rovimax NX. According to the manufacturer's fact sheet, experimental diets contained 0, 60, 88, and 110 mg/kg supplemented nucleotides. All diets were formulated to meet or exceed the nutrient requirements recommended by NRC (2012). On d 14 after weaning, two pens were selected from each group and orally dosed with 1.5 mL suspension containing 10^{10} colony forming units/ml of *Escherichia coli* K88. Twenty-four hours after *E. coli* K88 was dosed, blood was collected from two challenged pigs selected randomly per pen and centrifuged at 3000×*g* at 4°C for 15 min to prepare plasma for determination of cortisol, TNF-α, IGF-I, and IL-6. On d 42, the BW of each pig and food consumption per pen were measured to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed (G : F). On d 42, blood was collected from two pigs selected randomly per pen and centrifuged at 3000×*g* at 4°C for 15 min to prepare plasma for IgA and IgM. To assess the faecal score after challenge, faeces from each pig were scored on d 21, 28, 35, and 42 by determining the moisture content, and scored from 1 to 5: 1 = hard faeces, 2 = firm well formed, 3 = soft and partially formed faeces, 4 = loose, semi-liquid faeces, and 5 = watery faeces). Data were analysed as a randomised complete block design using GLM procedures (SAS®; USA). The initial BW was used as a covariate for ADFI and ADG. Differences among the treatment means were determined using the Tukey's multiple-range test with $P < 0.05$ indicating statistical significance.

From d 1 to 42, ADG and G : F of pigs fed the R275 diet was 6.9% and 6.9% higher ($P < 0.05$) than those fed the CON diet. On d 42, pigs fed with the R275 diet had higher ($P < 0.05$) IgA concentrations than other treatments, and IgM was 39.6% higher ($P < 0.05$) in the R275 treatment compared with CON pigs. After challenge, the concentrations of cortisol, TNF-α, and IL-6 in the CON treatment were 53.3%, 16.6%, and 10.3% lower ($P < 0.05$) than the R275 treatment, while IGF-I was higher ($P < 0.05$) in the nucleotide treatments than in CON. On d 21, 28, 35, and 42, CON pigs had higher ($P < 0.05$) faecal scores than the nucleotide treatments.

Nucleotides may maintain a stable microbiota in the ileum, which may lead to improved ADG and G : F. The reduction of diarrhoea could be a direct consequence of an improved intestinal maturation. The increase in immunoglobins after challenge may lead to an improvement in the immune system of pigs fed diets with nucleotides. In conclusion, dietary nucleotide supplementation can improve growth performance, indices of immune function, and decrease faecal score in weaning pigs challenged with *E. coli* K88.

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Differential effects of zinc oxide and a preparation of organic acids and an essential oil on post-weaning diarrhoea

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Weaning of pigs causes a growth check and can render pigs more susceptible to post-weaning diarrhoea (PWD). This condition is associated with proliferation of β-haemolytic strains of enterotoxigenic *E. coli* (ETEC) in the small intestine. Numerous dietary and management strategies to control/mitigate PWD are used, such as zinc oxide (ZnO), with the use of organic acids and (or) essential oils (EO) also reported to aid in the control of PWD (Vondruskova *et al.* 2010). This experiment examined the proposition that supplementation of a commercial product containing organic acids, cinnamaldehyde and a permeabilising substance in a diet for pigs infected with ETEC will decrease the incidence of PWD commensurate to ZnO.

A total of 72 entire male pigs (Large White × Landrace) weighing 7.2 ± 1.02 kg (mean ± SD) and weaned at 22.5 d of age were habituated in pens of four and stratified to a completely randomised block design of three diets and live weight (six pens per treatment). Diet treatments were: base diet without antimicrobial compounds (contained 100 mg ZnO/kg of feed in the vitamin/mineral premix) (Control); Control plus 0.3% ZnO; and Control plus 0.15% OACP [OACP; organic acids (formic, propionic, acetic), cinnamaldehyde, and permeabilising substance; Biotronic Top 3®, Biomin Australia Pty Ltd]. Feed (10.4 MJ net energy/kg, 213 g/kg crude protein, 13.5 g standardised ileal digestible lysine/kg) and water were offered *ad libitum* for 21 d. Pigs were orally infected with 9 mL aliquots of 1.03×10^9 colony forming units/mL of an ETEC (serotype O149:K98:K88) on d 3, 4 and 5 after weaning. Faecal swabs were taken on d 0, 3, 5, 7, 9 and 11 after weaning for assessment of *E. coli* shedding. Faecal consistency (FC; ranging from 1–4, with 1 being firm, well-formed faeces and 4 being faeces of watery consistency) was assessed daily for the first 14 d, and a diarrhoea index (DI) was then calculated. Data were analysed by one-way ANOVA using SPSS (v. 21, IBM). For the *E. coli* score, the effects of time before and after infection with ETEC were tested using repeated-measures ANOVA. Chi-square analysis (IBM SPSS, Version 21.0; USA) was used to compare the percentage of pigs having PWD between the different diets.

The overall infection with ETEC was relatively low, with 14/72 pigs infected having diarrhoea. Approximately 4% of pigs fed ZnO had PWD in the 3 weeks after weaning, which was lower than pigs fed OACP (29%, $P = 0.024$) or the Control (25%, $P = 0.047$) diets (Table 1). There was no difference ($P = 0.745$) in PWD between pigs fed the OACP or Control diets. This corresponded to the DI, which was lower for pigs fed the ZnO diet compared to either the OACP or the Control diet ($P = 0.026$). The *E. coli* score increased after infection ($P < 0.001$), with no difference between treatments ($P = 0.987$). No interaction between day and treatment was detected ($P = 0.442$). Zinc oxide may protect epithelial cells from ETEC by inhibition of bacterial adhesion and internalisation thereby improving intestinal barrier function (Roselli *et al*. 2003), which in the present study may explain the decrease in PWD and DI but not in ETEC shedding.

Table 1. Effects of dietary treatment on post weaning diarrhoea (PWD), the diarrhoea index (DI) and *E. coli* scores before (d 0–3) and after (d 5–11) infection with enterotoxigenic *E. coli*

	Treatment[A]				*P* value[B]		
	Control	ZnO	OACP	SEM[C]	D	T	D × T
% pigs with PWD	25[a]	4[b]	29[a]				
DI (%)[E]	5.06[a]	0.62[b]	6.25[a]	1.526		0.026	
E. coli score[F]							
d 0–3	0.083	<0.01	0.146	0.048	<0.001	0.987	0.442
d 5–11	0.938	1.054	0.906				

[A]Refer to text for treatment details. [B]D, day; T, treatment. [C]SEM, standard error of the mean. [D]PWD was defined as pigs having FC score of 4. [E]Mean proportion of days pigs had diarrhoea (FC = 4) in the 14 d after weaning; [F]Agar plates scored from 0-5 according to numbers of streaked sections containing β-haemolytic *E. coli*, where 0 = no growth and 5 = growth in fifth section of the plate. [a,b]Means in a row not having the same superscript are significantly different.

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Blend feeding or feeding a single diet has no impact on growth performance or carcase value

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Blend-feeding, where two extreme diets are mixed together in varying ratios (allowing the diet to be changed weekly), or feeding the same diet through the grower-finisher period (single), are alternatives to phase-feeding where three or four diets are fed during the grower-finisher period. Blend feeding or feeding a single diet have been found to have no effect on overall growth performance compared to a three-phase feeding program (Edwards 2011; Moore *et al.* 2013). However, there is some concern that the lysine level in the single diet used in these studies was not sufficient and as a result the true effect of feeding the single diet was not realised (Edwards 2011). The lysine requirements of Australian grower-finisher pigs have recently been reported to be approximately 10% higher than that used in these studies (Moore *et al.* 2015). Therefore blend feeding and feeding a single diet throughout the grower and finisher phases were re-examined using the higher lysine requirements. The hypothesis tested was that blend feeding or feeding a single diet will reduce the cost of feeding pigs compared to the phase-feeding system by minimising the excess of nutrients in the diets without adversely affecting pig growth performance and carcase quality.

A completely randomised block design experiment was conducted using 147 female pigs (Large White × Landrace × Duroc; seven pigs/pen and seven replicate pens/treatment) to examine the effect of feeding strategies on performance during the grower-finisher phases. Pigs of a similar age were blocked and randomly allocated to the following feeding strategies on the basis of initial liveweight (LW): Phase-feeding: diets changed when the average LW of pigs in the pen reached 30 kg (14.5 MJ digestible energy (DE)/kg and 0.84 g standardised ileal digestible lysine (SID Lys)/MJ DE), 50 kg (14.0 MJ DE/kg and 0.67 g SID Lys/MJ DE) or 80 kg (13.7 MJ DE/kg and 0.55 g SID Lys/MJ DE); Blend: diets changed weekly to meet the requirements of the average LW of pigs in the pen; and Single: the same diet fed throughout (formulated to meet the requirements of the pig at 60 kg LW; 13.9 MJ DE/kg and 0.65 g SID Lys/MJ DE). The experimental diets were fed for 10 weeks from 30.1 ± 0.33 to 97.3 ± 1.40 kg LW (mean ± SE). All data were analysed by analysis of variance (GENSTAT, 15th Edition; UK).

There was no effect ($P > 0.05$) of feeding strategy on growth performance (Table 1). The SID Lys intake required per kg LW gain was reduced for the Blend and Single feeding strategies compared to the Phase feeding strategy ($P = 0.002$). There was a trend for feed costs for pigs on the Blend and Single feeding strategies to be cheaper (4.36% and 5.05%, respectively) than those fed the Phase feeding program ($P = 0.057$). Single feeding or Blend feeding appears to reduce diet costs with minimal effect on growth performance and carcase value, thus confirming the results from Moore *et al.* (2012).

Table 1. Growth performance, carcase quality and feed costs for female pigs using three different feeding strategies (n = 7)

Item	Phase	Blend	Single	SED[A]	*P* value
Average daily gain (kg)	0.96	0.95	0.97	0.018	0.343
Average daily feed intake (kg)	2.23	2.22	2.22	0.066	0.974
FCR (MJ DE/kg LW gain)[B]	2.32	2.34	2.28	0.048	0.501
g SID Lys intake/kg LW gain	22.2[a]	20.6[b]	20.5[b]	0.297	0.002
DE intake (MJ DE/kg LW gain)	39.3[b]	36.0[a]	38.1[ab]	0.856	0.007
Carcase weight (kg)[C]	68.7	67.1	68.6	1.02	0.204
Dressing percentage	70.6[a]	70.0[ab]	69.8[b]	0.328	0.050
P_2 backfat (mm)	9.43	9.61	9.39	0.395	0.837
Feed costs/kg LW gain ($)	1.01	0.966	0.959	0.018	0.057

[A]SED, standard error of difference between means. [B]FCR, feed conversion ratio. [C]Hot carcase weight: AUSMEAT trim 13-head off, flare off, fore trotters off, hind trotters on. [a,b]Means in a row not having the same superscript are significantly different.

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Reducing variation in finisher growth performance through early post-weaning dietary intervention

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It is well established that pigs with low weaning weights have compromised performance throughout the grower-finisher phase (Gondret *et al.* 2005). There will always be a percentage of pigs that fall below target weaning weights, if steps are not taken to address this poorer performance it will continue into the grower-finisher phase and variation will increase, adding costs to the supply chain (Douglas *et al.* 2014*a*). It was hypothesised that a nutritional intervention in early growth (prior to 35 kg) would enhance the performance of light-weight weaner pigs and result in reduced overall variation in weight at slaughter.

A total of 420 male pigs (Large White, Landrace, Duroc terminal cross) weighing 4.5 ± 0.67 kg (mean ± SD) were received at weaning (19 days of age). Eight pens (14 pigs/pen) were randomly filled with pigs to create the Control group, representing a normal population of pigs of varied weight. The remaining pigs were allocated on weight, to create eight pens (14 pigs/pen) of Low weaning weight pigs, and eight pens (14 pigs/pen) of High weaning weight pigs. Intermediate-weight pigs were removed from the experiment such that the Low and High weight groups were discrete. A starter diet [15.0 MJ digestible energy (DE)/kg, 0.8 g standardised ileal digestible lysine (SID L)/MJ DE] was fed to all groups for the first 4 weeks after weaning. Control and High groups were fed to a program matched to average group weight, as per industry practice. After the starter diet, a series of diets were fed: pigs to 25 kg live weight (14.5 MJ DE/kg, 0.8 g SID L/MJ DE); from 25–50 kg (14.0 MJ DE/kg, 0.7 g SID L/MJ DE); from 50–70 kg (13.8 MJ DE/kg, 0.65 g SID L/MJ DE); from 70–90 kg (12.8 MJ DE/kg, 0.55 g SID L/MJ DE); and from 90 kg (12.6 MJ DE/kg, 0.55 g SID L/MJ DE). The Low group remained on the starter diet until they reached 35 kg live weight, before transitioning into the normal feeding program. Pigs were weighed weekly in the starter phase, before being weighed at diet transitions. Feed disappearance was measured by hand in the starter phase and delivered by a FeedPRO™ system (FeedLogic Corp., Wilmar, MN USA) in subsequent phases. As variation was the main measure of the intervention, all pens ended the experimental period at the same time, when first pigs reached market specifications (95–105 kg live weight), however all pigs were grown out to market weight. Data were analysed via a GLM ANOVA (Genstat, 16th Edition; UK), with differences determined by least significant difference ($P < 0.05$).

Low-weight weaners remained compromised compared to both the High and Control groups, despite the dietary intervention. The Low group ate less feed than the High and Control groups ($P < 0.001$), converted feed to gain at the same rate and thus grew slower ($P < 0.001$), taking 14 days longer to reach market weight (Table 1). Despite reduction in variation due to selection at entry, there was no difference in exit weight CV. These results reflected Douglas *et al.* (2014*a*) in that the Low group were 'too late to catch up', however, the results of Douglas *et al.* (2014*b*) suggested that without our intervention performance may have been poorer. This study showed that producers should reduce impediments that require them to wean pigs at a lighter than optimum weight, as these compromises are conserved throughout the growth phase.

Table 1. Growth performance and coefficient of variation (CV) of a Control population of pigs compared with pigs of Low and High weaning weights across the whole experimental period

	Control (n = 8)	Low (n = 8)	High (n = 8)	SED[A]	*P* value
Entry weight (kg)	4.4[a]	3.8[b]	5.2[c]	0.11	<0.001
Exit weight (kg)	80.2[a]	75.9[b]	84.8[c]	1.10	<0.001
ADG[B] (kg)	0.647[a]	0.617[b]	0.680[c]	0.010	<0.001
ADFI[C] (kg)	1.27[a]	1.20[b]	1.36[c]	0.031	<0.001
FCR[D] (kg : kg)	1.96	1.95	2.00	0.044	0.499
Age at slaughter (days)	163[a]	170[b]	156[c]	2.1	<0.001
Entry weight CV (%)	13.9[a]	9.7[b]	6.9[b]	1.36	<0.001
Exit weight CV (%)	11.8	10.4	11.3	1.20	0.381

[A]SED, standard error of difference between means. [B]ADG, average daily gain. [C]ADFI, average daily feed intake. [D]FCR, feed conversion ratio.
[a,b,c]Means in a row not having the same superscript are significantly different.

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Chapter 11: Index of Authors

Manipulating Pig Production XV

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

Melbourne, Australia

22–25 November 2015

Edited by Professor John Pluske and Dr Jo Pluske
AUSTRALASIAN PIG SCIENCE ASSOCIATION (INC.)
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Preface

The global pork industry has and will continue to face many factors that contribute to the volatility surrounding pork supply and demand. Over recent years these factors have included feed costs, disease outbreaks, animal welfare, product quality and consumer preference. The Australian industry has not been sheltered from these issues and can take many lessons from experiences internationally. The imminent phase out of piglet castration in the EU by 1 January 2018 is an example of how key stakeholders can influence production methods. With the 2018 deadline approaching, the A. C. Dunkin Memorial lecture at this year's conference will provide participants with an overview as to how the European pork industry has responded to the phase out and how the consumer is influencing the adoption of alternative technologies. While the issues may be different, the impact of consumer expectation on current and future production methods is as relevant locally as it is to pork producers in other parts of the world.

The Australian industry has had the fortune of considerable investment in research and development over the past decade with support through the Cooperative Research Centre (CRC) for an Internationally Competitive Pork Industry and the CRC for High Integrity Australian Pork in addition to the ongoing investment from Australian Pork Limited. This support into local R&D provides the industry with innovation that is integral in addressing drivers of profitable pork production in a global market. The 2015 conference presents recent developments across a diverse range of disciplines impacting on production efficiency, sustainability and profitability of pork production. I encourage all delegates to fully value the contributions across such a diverse range of topics. I particularly thank the contributions of all our invited speakers in stimulating discussion and new ideas for our industry.

From its inception, APSA was established to provide a forum for excellence in science related to all aspects of the pig industry and to promote interaction between scientists from different disciplines and from different countries. APSA continues to uphold these aims, with a particular emphasis on fostering the development of young scientists into our industry. It is once again very pleasing to see such a large group of students and early career scientists attend and contribute to APSA.

It has been a pleasure to preside over APSA for the past two years and to have contributed to facilitating the networking of those involved in pork production. Thank you for your support of the conference and I hope you will continue to be part of the future success of the association.

Dr Cherie Collins
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.

Previous recipients:

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Dr David Hennessy (2007)
Dr Michael Taverner (2009)
Dr Ian Williams (2011)
Professor Frank Dunshea (2013)
Dr Bruce Mullan (2013)

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 focussed 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:

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Kaye Coates (1999)
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The Australasian Pig Science Association (APSA) biennial conference continues to go from strength to strength and is now widely recognised as one of the premier international pig science forums. The hard work and dedication of many people over the years has seen APSA grow from humble beginnings in Albury in 1987 to the international conference that it is today attracting delegates from around the world. The growth and success of APSA has come about from the hard work and dedication of many people over almost 30 years and is set to continue for many more.

The 2015 conference would not have been possible without the considerable time and effort contributed by many individuals. The continued support of members and colleagues within the pig science community in the submission of high quality papers is gratefully acknowledged. The APSA committee would also like to thank all those that have attended the 2015 conference, particularly those that have travelled considerable distances to participate.

The APSA conference proceedings are internationally recognised for their scientific and editorial standard. There are very few conferences now held where such a high quality proceedings are produced prior to the conference. The effort and dedication of Professor John Pluske and Dr Jo Pluske as editors for the 2015 proceedings, Manipulating Pig Production XV is gratefully acknowledged. The 2015 proceedings was a milestone for APSA, with the proceedings 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 all that have made this transition a success, in particular the assistance of Dr Chris Anderson, CSIRO Publishing for his willingness to take on the APSA proceedings and his guidance and support during this process. The committee would also like to thank all of the referees that reviewed the scientific content of the papers, your contribution is gratefully acknowledged.

The contribution of sponsors is critical for the success of the APSA biennial conference. I would like to acknowledge Australian Pork Limited and the Pork CRC for High Integrity Australian Pork for their generous support of APSA once again as Principle Sponsors. I would also like to thank all of our sponsors of the 2015 conference (listed on the Sponsors page), we truly appreciate your support.

A conference of this size does not come together without the hard work and dedication of a number of people. I would like to sincerely thank the 2015 committee for all of their efforts, it was a wonderful experience to be part of the team. Accordingly, thank you to Dr Alison Collins (Vice President), Dr Dave Cadogan (Immediate Past President), Amy Lealiifano (Secretary), Emalyn Loudon (Treasurer), Professor John Pluske, Professor Frank Dunshea, Dr Jae Cheol Kim and Dr Pat Mitchell. My personal gratitude in particular goes to Amy Lealiifano for her efforts above and beyond the role of secretary in keeping the committee on track. I would also like to extend my sincere thanks to the team at YRD for their assistance in the 2015 conference as secretariat. It was a pleasure to work alongside Kate Murphy, Phoebe White and the YRD team.

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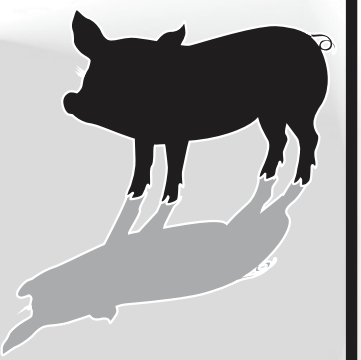

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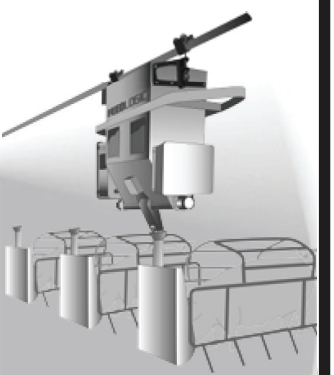

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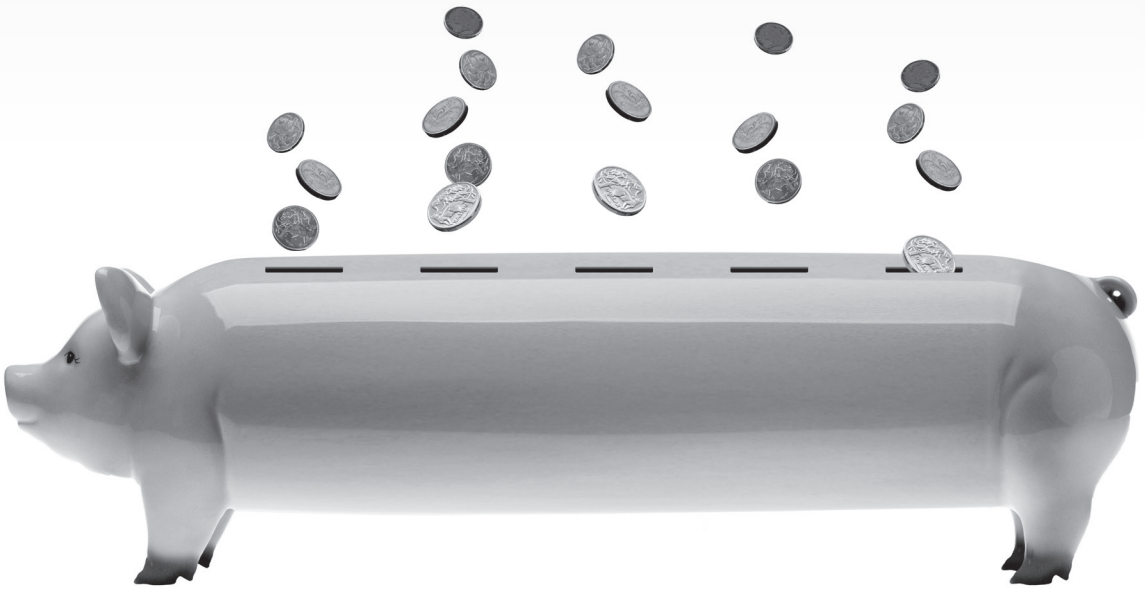

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Jemma Lumby, 2015 Agricultural Graduate

"*Moving from South West Queensland to the town of Corowa was a big move. However the region has a lot to offer. It is situated on the border of New South Wales and Victoria with the Murray River being the state line. There is a great sporting and outdoors culture and is only a short drive to the cities of Albury and Wodonga. It is also only a few hours drive to Melbourne and the ski slopes. I chose the Rivalea Graduate Program as it offered a great career pathway. Being an integrated company it provides opportunities in a diverse array of interlinking businesses and sectors of the company. The program is a combination of hands on work along with trial based project work. There are opportunities to work alongside a range of different people and professionals. The company has fantastic training and educational programs to enabling a smooth path into a great career.*"

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