

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

B. G. Bowring<sup>A,C</sup>, S. N. Jenkins<sup>B</sup> and A. M. Collins<sup>A</sup>

<sup>A</sup>Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568.

<sup>B</sup>The University of Western Australia, Crawley, WA 6009.

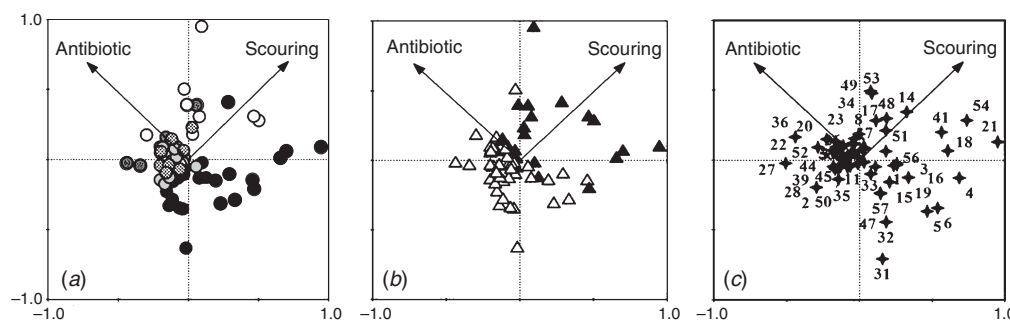
<sup>C</sup>Corresponding author. Email: bethany.bowring@dpi.nsw.gov.au

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 *Pseudobutyrvibrio* (#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 *Porphyromonadaceae* 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

R. L. Wilson<sup>A,E</sup>, R. E. Doyle<sup>A,B</sup>, G. M. Cronin<sup>C</sup> and P. K. Holyoake<sup>D</sup>

<sup>A</sup>Charles Sturt University and Graham Centre, Wagga Wagga, NSW 2678.

<sup>B</sup>Current address: The University of Melbourne, Parkville, VIC 3010.

<sup>C</sup>The University of Sydney, Camden, NSW 2570.

<sup>D</sup>Holyoake Veterinary Consulting Pty Ltd, Strathfieldsaye, VIC 3551.

<sup>E</sup>Corresponding author. Email: [rewilson@csu.edu.au](mailto:rewilson@csu.edu.au)

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<sup>®</sup> and Neovac<sup>®</sup> 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 ( $n = 6/\text{treatment}$ ). 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<sup>®</sup> (2 mL; Zoetis, Sandton, South Africa), Neovac<sup>®</sup> (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<sup>®</sup> Phase<sup>™</sup> 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, 17<sup>th</sup> Edition; UK). Tear staining data were log transformed and analysed using LMM.

The administration of turpentine, Improvac<sup>®</sup> and Neovac<sup>®</sup> 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<sup>®</sup> had lower amounts of tear staining than pigs administered Improvac<sup>®</sup> or turpentine ( $P < 0.05$ ) (Table 1).

The increases in haptoglobin and CRP concentrations indicated that the subcutaneous administration of Improvac<sup>®</sup>, Neovac<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup>, Neovac<sup>®</sup> or turpentine. Values are mean  $\pm$  SE**

Variable	Saline	Improvac <sup>®</sup>	Neovac <sup>®</sup>	Turpentine
Haptoglobin (mg/ml)	1.3 $\pm$ 0.1 <sup>a</sup>	2.2 $\pm$ 0.1 <sup>c</sup>	1.7 $\pm$ 0.1 <sup>b</sup>	2.3 $\pm$ 0.1 <sup>c</sup>
CRP (ng/ml)	802 $\pm$ 166.9 <sup>a</sup>	1805 $\pm$ 166.9 <sup>c</sup>	1235 $\pm$ 166.9 <sup>b</sup>	1705 $\pm$ 166.9 <sup>c</sup>
IET ( $^{\circ}\text{C}$ )	33.7 $\pm$ 0.22 <sup>a</sup>	33.8 $\pm$ 0.22 <sup>a</sup>	33.9 $\pm$ 0.22 <sup>a</sup>	34.4 $\pm$ 0.22 <sup>b</sup>
Tear staining (cm <sup>2</sup> )	0.06 $\pm$ 0.018 <sup>ab</sup>	0.08 $\pm$ 0.022 <sup>a</sup>	0.03 $\pm$ 0.009 <sup>b</sup>	0.08 $\pm$ 0.022 <sup>a</sup>

<sup>a,b,c</sup>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

T. La<sup>A</sup>, N. D. Phillips<sup>A</sup> and D. J. Hampson<sup>A,B</sup>

<sup>A</sup>Murdoch University, Murdoch, WA 6150.

<sup>B</sup>Corresponding author. Email: d.hampson@murdoch.edu.au

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  $\beta$ -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.* 2013a). 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.* 2013a, 2013b). 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  $\beta$ -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

S. N. Jenkins<sup>A,D</sup>, I. S. Waite<sup>A</sup>, J. Mansfield<sup>B</sup>, J. C. Kim<sup>C</sup> and J. R. Pluske<sup>B</sup>

<sup>A</sup>The University of Western Australia, Crawley, WA 6009.

<sup>B</sup>Murdoch University, Murdoch, WA 6150.

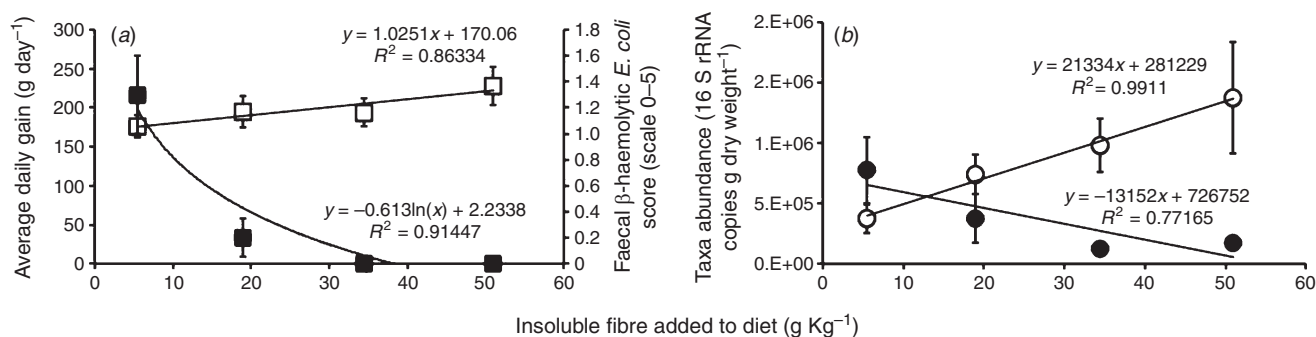
<sup>C</sup>Department of Agriculture and Food, South Perth, WA 6151.

<sup>D</sup>Corresponding author. Email: sasha.jenkins@uwa.edu.au

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<sup>®</sup> (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. 1a). It was also associated with an increased relative abundance of *Christensenellaceae* (a butyrate producer) and decreased abundance of *Lactobacillaceae* (a lactate producer) (Fig. 1b). 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<sup>®</sup>) 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

M. C. Hawley<sup>A,B</sup>, I. Svoboda<sup>A</sup> and H. J. Fallowfield<sup>A</sup>

<sup>A</sup>School of the Environment, Flinders University, Adelaide, SA 5042.

<sup>B</sup>Corresponding author. Email: [megan.hawley@flinders.edu.au](mailto:megan.hawley@flinders.edu.au)

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<sub>3</sub>), 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<sub>3</sub> to nitrate (NO<sub>3</sub>) is a potential method to alleviate the adverse effects of NH<sub>3</sub> 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<sub>3</sub> oxidation within an aerobic reactor fed AP effluent at an aeration level of 10% saturation (0.7 mg O<sub>2</sub>/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<sub>2</sub>/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<sub>4</sub>-N) concentration at the start of the experiment was 1.5 ± 0.7 g/L (mean ± SD) and that of suspended solids was 0.8 ± 0.1 g/L (Table 1). Post-aerobic treatment showed mean NH<sub>4</sub>-N and SS levels had decreased by 28.8% and 52.2% respectively. This, in conjunction with increased NO<sub>2</sub>-N and NO<sub>3</sub>-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<sub>3</sub> oxidation had occurred. Reducing NH<sub>4</sub>-N to its non-toxic form NO<sub>3</sub>-N can lead to lower disease potentials associated with NH<sub>3</sub> 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<sub>4</sub>-N to NO<sub>3</sub>-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 <sub>4</sub> -N <sup>A</sup> (g/L)	NO <sub>2</sub> -N (g/L)	NO <sub>3</sub> -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

<sup>A</sup>Chemical analysis performed: Ammonium (NH<sub>4</sub>-N); Nitrite (NO<sub>2</sub>-N); Nitrate (NO<sub>3</sub>-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

J. Ayre<sup>A,B</sup>, N. Moheimani<sup>A</sup> and M. Borowitzka<sup>A</sup>

<sup>A</sup>Murdoch University, Murdoch, WA 6150.

<sup>B</sup>Corresponding author. Email: [j.ayre@murdoch.edu.au](mailto:j.ayre@murdoch.edu.au)

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<sub>3</sub>-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<sub>3</sub>-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<sub>3</sub>-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<sub>2</sub> 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

N. N. Cheng<sup>A,B</sup> and H. J. Fallowfield<sup>A</sup>

<sup>A</sup>Flinders University, Bedford Park, SA 5042.

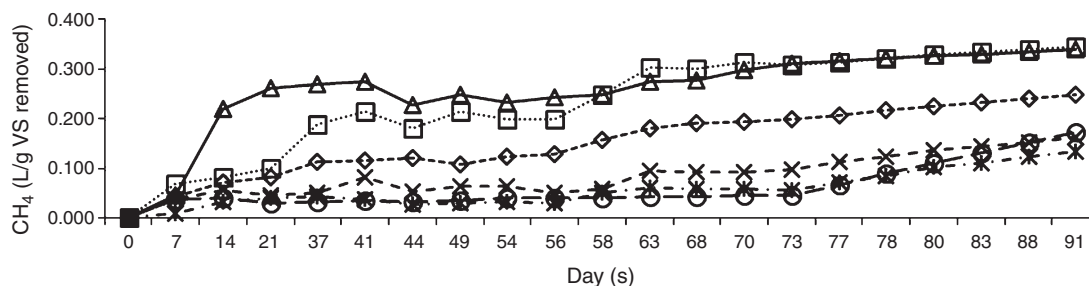
<sup>B</sup>Corresponding author. Email: ryan.cheng@flinders.edu.au

Increasingly, covered anaerobic lagoons are being considered by the Australian pork industry to manage greenhouse gas (GHG) emissions and recover methane (CH<sub>4</sub>) for energy production. Algal biomass produced in high-rate algal ponds (HRAP) treating piggery wastewater removes CO<sub>2</sub>, 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<sub>4</sub> 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<sub>2</sub> 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<sub>4</sub> 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<sub>4</sub> production compared to the 100% PS. The CH<sub>4</sub> production decreased as the ratio of ALBAZOD increased in the mixture. When the ALBAZOD ratio was beyond 7.1% A, the CH<sub>4</sub> production decreased to below 0.200 L/g VS removed. The lowest CH<sub>4</sub> (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<sub>4</sub> production with the optimum ALBAZOD mixture, the ratio is crucial in order to achieve optimum CH<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub>) 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  $\pm$  SE (n = 2).  $\triangle$ : 100% PS;  $\square$ : 96.5% PS + 3.5% A;  $\diamond$ : 92.9% PS + 7.1% A;  $\times$ : 85.4% PS + 14.6% A;  $*$ : 67.8% PS + 32.2% A;  $\circ$ : 100% A.

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

S. Tait<sup>A,E</sup>, H. Payne<sup>B</sup>, B. Cole<sup>C</sup> and R. H. Wilson<sup>D</sup>

<sup>A</sup>The University of Queensland, QLD 4072.

<sup>B</sup>Department of Agriculture and Food Western Australia, South Perth, WA 6151.

<sup>C</sup>Z-Filter Pty Ltd, Perth, WA 6155.

<sup>D</sup>Rob Wilson Consulting, Perth, WA 6012.

<sup>E</sup>Corresponding author. Email: [s.tait@uq.edu.au](mailto:s.tait@uq.edu.au)

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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- Supported by Pork CRC Limited Australia. Z-Filter Pty Ltd. provided the Z-Filter prototype and operated it during the study.

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

J. G. Froese<sup>A,B,D</sup>, J. V. Murray<sup>B</sup>, J. J. Perry<sup>C</sup> and R. D. van Klinken<sup>B</sup>

<sup>A</sup>The University of Queensland, St Lucia, QLD 4072.

<sup>B</sup>Commonwealth Scientific and Industrial Research Organization (CSIRO), Dutton Park, QLD 4102.

<sup>C</sup>CSIRO, Douglas, QLD 4811.

<sup>D</sup>Corresponding author. Email: jens.froese@uq.net.au

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: Season: Risk zone:	Below average rainfall period				Above average rainfall period			
	Summer		Winter		Summer		Winter	
	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

N. Sales<sup>A,D</sup>, D. Collins<sup>A</sup>, A. M. Collins<sup>A</sup>, T. McKenna<sup>B</sup>, M. M. Bauer<sup>B</sup>, C. R. Parke<sup>B</sup>  
 and S. Hermes<sup>C</sup>

<sup>A</sup>Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568.

<sup>B</sup>The University of Queensland, Gatton, QLD 4343.

<sup>C</sup>Animal Genetics and Breeding Unit, University of New England, Armidale, NSW 2350.

<sup>D</sup>Corresponding author. Email: narelle.sales@dpi.nsw.gov

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 ( $10.5 \pm 2.7$ : mean  $\pm$  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<sup>®</sup> 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<sup>®</sup> (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  $\mu\text{g/mL}$  (PHASE<sup>™</sup> Tridelta Development Ltd. Ireland), while the majority of pigs (54%) had Hp levels  $<1.0 \mu\text{g/mL}$  ( $0.4 \pm 0.2$ : mean  $\pm$  SD  $\mu\text{g/mL}$ ). No significant difference was observed between the Hp levels in females ( $336 \pm 38.9$ : mean  $\pm$  SD  $\mu\text{g/mL}$ ) and males ( $361 \pm 39.5$ : mean  $\pm$  SD  $\mu\text{g/mL}$ ). 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

Collection Period	Haptoglobin Concentration ( $\mu\text{g/mL}$ )								
	1st Quarter			2nd Quarter			4th Quarter		All quarters
Age group <sup>A</sup>	1	2	3	1	2	3	1	2	4
Mean $\pm$ SEM <sup>B</sup>	$162 \pm 94$	$124 \pm 30$	$45 \pm 24$	$94 \pm 56$	$110 \pm 36$	$169 \pm 150$	$716 \pm 64$	$417 \pm 77$	$348 \pm 28$
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

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

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

J. L. Black<sup>A,D</sup>, C. L. Collins<sup>B</sup>, D. J. Henman<sup>B</sup> and S. Diffey<sup>C</sup>

<sup>A</sup>John L Black Consulting, Warrimoo, NSW 2774.

<sup>B</sup>Rivalea (Australia), Corowa, NSW 2646.

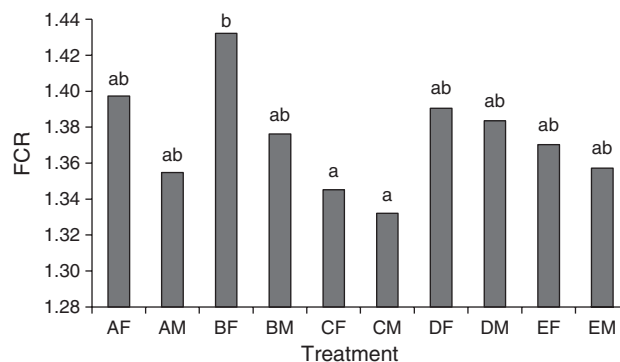
<sup>C</sup>Curtin University, Bentley, WA 6102.

<sup>D</sup>Corresponding author. Email: jblack@pnc.com.au

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<sub>2</sub> (PGE<sub>2</sub>), 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<sub>2</sub> formation; and iv) meloxicam, to inhibit COX-2 action and restrict PGE<sub>2</sub> 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. <sup>a,b</sup>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

C. Pratt<sup>A,B</sup>, M. Redding<sup>A</sup> and J. Hill<sup>A</sup>

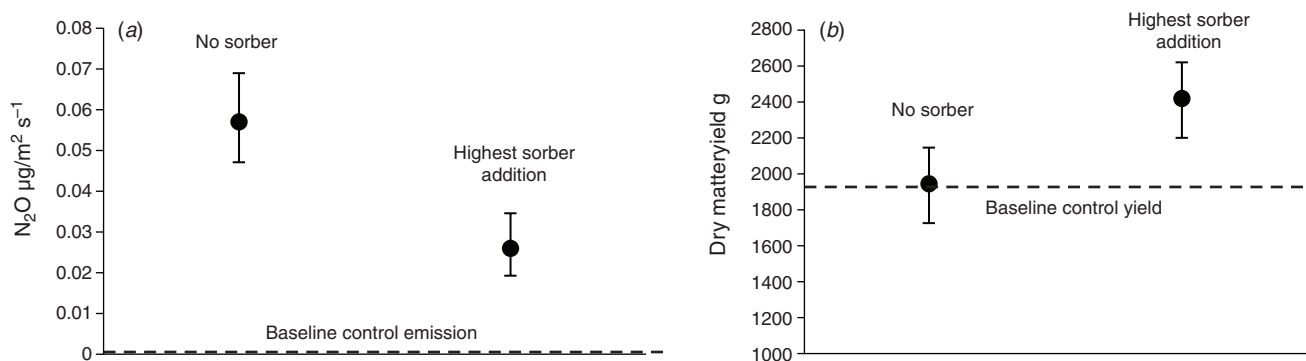
<sup>A</sup>Department of Agriculture and Fisheries, Toowoomba, 4350 QLD.

<sup>B</sup>Corresponding author. Email: Christopher.Pratt@daf.qld.gov.au

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<sub>3</sub>) volatilisation and in doing so, slowing the kinetics of nitrate formation and associated nitrous oxide (N<sub>2</sub>O) 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<sub>2</sub>O, NH<sub>3</sub>, CH<sub>4</sub>, CO<sub>2</sub>) system. In the laboratory experiments, the sorbers showed strong capacity to decrease NH<sub>3</sub> 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<sub>2</sub>O decreases (>40%) were achieved by the sorbers. As an example, mean N<sub>2</sub>O emission decreases from the field trial phase of the project are shown in Fig. 1a.

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. 1b). 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<sub>2</sub>O 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<sub>2</sub>O decreases and DMY increases.

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## Greenhouse gas emission abatement in Australian piggeries

I. R. Kruger<sup>A,D</sup>, G. W. Mills<sup>B</sup> and R. H. Wilson<sup>C</sup>

<sup>A</sup>Ian Kruger Consulting, Kootingal, NSW 2352.

<sup>B</sup>GoAhead Business Solutions, Armidale, NSW 2350.

<sup>C</sup>Rob Wilson Consulting, Perth WA 6012.

<sup>D</sup>Corresponding author. Email: [iankrugerconsulting@gmail.com](mailto:iankrugerconsulting@gmail.com)

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<sub>2</sub>-e/y with potential abatement of 54%, or 141,232 t CO<sub>2</sub>-e/y. On a whole industry basis, maximum potential abatement is 588,467 t CO<sub>2</sub>-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<sub>2</sub>-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 <sub>2</sub> -e/y) <sup>B</sup>		Average emissions intensity (kg CO <sub>2</sub> -e/kg HSCW) <sup>C</sup>	
	Baseline	Abated scenario	Baseline	Abated scenario
Farrowing only – conventional (5) <sup>A</sup>	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]

<sup>A</sup>Number in parentheses refers to number of each type of piggery studied. <sup>B</sup>CO<sub>2</sub>-e represents the global warming potential of combined nitrous oxide and methane emissions expressed as carbon dioxide equivalents. <sup>C</sup>HSCW, hot standard carcase weight.

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

A. G. Skerman<sup>A,D</sup>, S. Heubeck<sup>B</sup>, D. J. Batstone<sup>C</sup> and S. Tait<sup>C</sup>

<sup>A</sup>Department of Agriculture and Fisheries, Toowoomba, QLD 4350.

<sup>B</sup>National Institute of Water and Atmospheric Research (NIWA), Hamilton, New Zealand 3216.

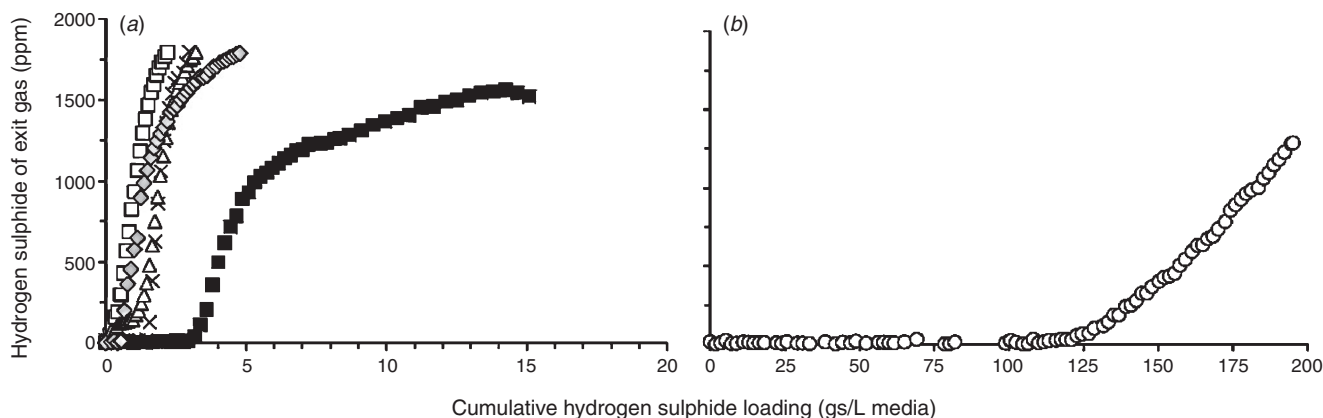
<sup>C</sup>The University of Queensland, St Lucia, QLD 4072.

<sup>D</sup>Corresponding author. Email: alan.skerman@daf.qld.gov.au

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  $H_2S$ -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  $cg_5^{\text{®}}$  commercial iron-oxide pellets (Clean-Gas, ACP Technologies Inc, USA), and the alternative media: granular steel furnace slag (<5 mm), red soil (krasnozom/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  $cg_5^{\text{®}}$  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  $cg_5^{\text{®}}$ , 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

R. Liaquat<sup>A</sup>, S. Astals<sup>B</sup>, P. D. Jensen<sup>B</sup>, D. J. Batstone<sup>B</sup> and S. Tait<sup>A,C</sup>

<sup>A</sup>Quaid-i-Azam University, Islamabad, Pakistan.

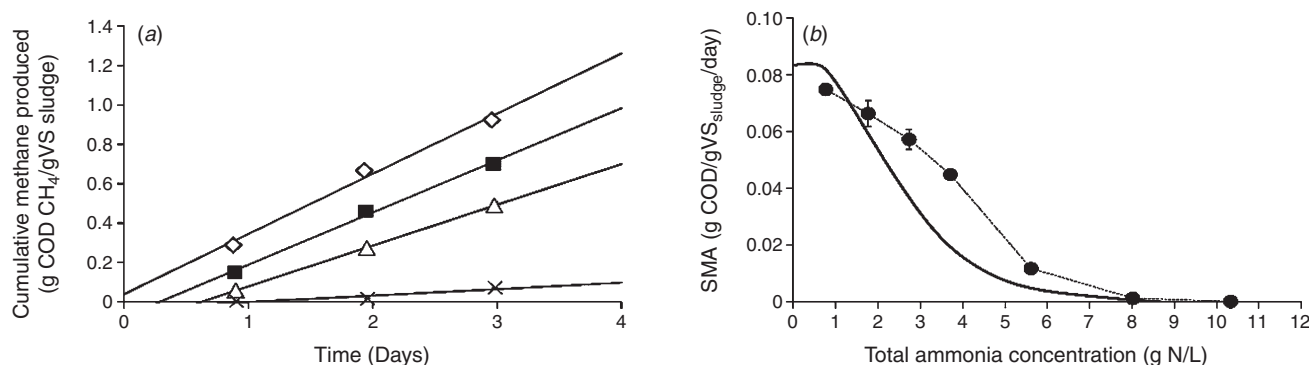
<sup>B</sup>The University of Queensland, St Lucia, QLD 4072.

<sup>C</sup>Corresponding author. Email: s.tait@uq.edu.au

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. 1b) 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. 1b), likely due to inhibition. The estimated  $KI_{50}$  of  $3.98 \pm 0.7$  g TAN/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. 1b) 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. 1b, Astals *et al.* 2015), with decrease in activity being more drastic around the  $KI_{50}$  value. These different shapes of the SMA curves (Fig. 1b) 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 ( $\diamond$ ), 2.74 g N/L ( $\blacksquare$ ), 3.71 g N/L ( $\triangle$ ), 5.61 g N/L ( $\times$ ) 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. 1a) versus  $NH_3$  content.  $KI_{50} = 3.98 \pm 0.67$  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

E. J. McGahan<sup>A</sup>, B. R. Warren<sup>A,B</sup> and R. J. Davis<sup>A</sup>

<sup>A</sup>FSA Consulting, Toowoomba, QLD 4350.

<sup>B</sup>Corresponding author. Email: [bradley.warren@fsaconsulting.net](mailto:bradley.warren@fsaconsulting.net)

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<sup>®</sup> 72-L power meter and an Envirodata<sup>®</sup> data logger. An Envirodata<sup>®</sup> 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

S. G. Wiedemann<sup>A,B</sup>

<sup>A</sup>FSA Consulting, Toowoomba, QLD 4350.

<sup>B</sup>Corresponding author. Email: [stephen.wiedemann@fsaconsulting.net](mailto:stephen.wiedemann@fsaconsulting.net)

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

S. N. Jenkins<sup>A,B</sup>, I. S. Waite<sup>A</sup>, B. Mickan<sup>A</sup> and L. K. Abbott<sup>A</sup>

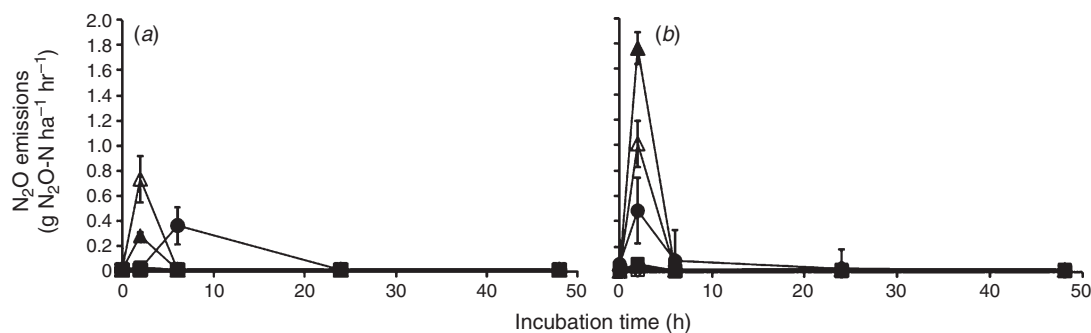
<sup>A</sup>The University of Western Australia, Crawley, WA 6009.

<sup>B</sup>Corresponding author. Email: sasha.jenkins@uwa.edu.au

Developing effective mitigation strategies for reducing nitrous oxide (N<sub>2</sub>O) 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<sub>2</sub>O emissions in Western Australian semi-arid soils and these microorganisms responded to targeted mitigation strategies for reducing N<sub>2</sub>O (Barton *et al.* 2013). However, the effect of adding pig manure to these soils on the N<sub>2</sub>O 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<sub>2</sub>O emissions following manure amendment. It was hypothesised that the amount of nitrified-N<sub>2</sub>O 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 × 5 × 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<sub>2</sub>O flux was analysed at 0, 2, 6, 24, 48, 72, 96, 120, 168 and 336 h by gas chromatography.

The N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions in semi-arid cropping systems.



**Fig. 1.** Nitrous oxide (N<sub>2</sub>O) 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

S. Hermes<sup>A,D</sup>, R. Sokolinski<sup>B</sup>, R. Johnston<sup>B</sup> and S. Newman<sup>C</sup>

<sup>A</sup>Animal Genetics and Breeding Unit, a joint venture of NSW DPI and University of New England, Armidale, NSW 2351.

<sup>B</sup>PIC Australia, Grong Grong, NSW 2652.

<sup>C</sup>Genus plc, Hendersonville, TN 37075.

<sup>D</sup>Corresponding author. Email: [Susanne.Hermesch@une.edu.au](mailto:Susanne.Hermesch@une.edu.au)

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 Hermes<sup>(2015)</sup> 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 Hermes<sup>et al.</sup> (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 Hermes<sup>(2015)</sup> 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

	N	Weekly groups			N	Monthly groups		
		SD	SE	Range		SD	SE	Range
EADG <sup>A</sup> (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 <sup>B</sup> (\$/pig)	126	5.27		25.59	28	4.38		17.41
IFCR <sup>C</sup> (\$/pig)	126	3.72		16.23	28	2.55		11.78

<sup>A</sup>Refer to text for trait abbreviations used. <sup>B</sup>IDFI, index with DFI. <sup>C</sup>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

C. G. Jose<sup>A,C</sup> and J. C. Kim<sup>B</sup>

<sup>A</sup>Murdoch University, Murdoch, WA 6150.

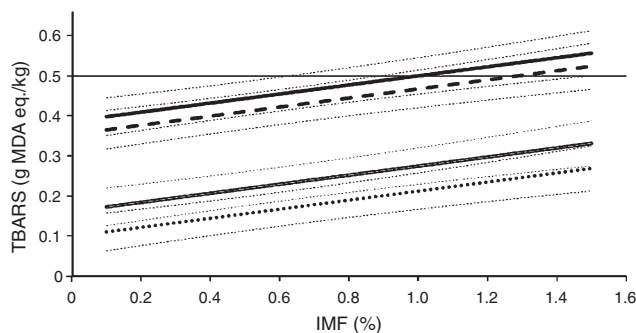
<sup>B</sup>Department of Agriculture and Food, South Perth, WA 6151.

<sup>C</sup>Corresponding author. Email: c.jose@murdoch.edu.au

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 ( $49.3 \pm 0.15$  kg, mean  $\pm$  SD) were housed in individual pens and fed a diet supplemented with either 35, 300, 500 or 700 IU of VE ( $\alpha$ -tocopherol acetate) for 6 weeks ( $n = 8$ ). The pigs were slaughtered at an average live weight of  $86.6 \pm 1.21$  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<sup>®</sup>; USA).

Loin VE content increased with increasing supplementation ( $P < 0.05$ ), with values ranging from 2.59 to 8.06 mg/kg (mean of  $5.06 \pm 0.24$  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 =  $0.61 \pm 0.19\%$ ). 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) ( $\pm$ 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

H. A. Channon<sup>A,B,C</sup>, D. N. D'Souza<sup>A</sup> and F. R. Dunshea<sup>B</sup>

<sup>A</sup>Australian Pork Limited, Barton, ACT 2600.

<sup>B</sup>The University of Melbourne, Parkville, VIC 3052.

<sup>C</sup>Corresponding author. Email: heather.channon@australianpork.com.au

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 carcasses 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.* 2015a, 2015b). 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<sup>®</sup> (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.* (2015a, 2015b). 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.* (2015a, 2015b) 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<sup>A</sup> 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 <sup>a,c</sup>	54.3 <sup>a</sup>	51.3 <sup>a,c</sup>	60.4 <sup>a</sup>	53.3 <sup>a,c</sup>	23.6
Stimulation	No	53.9 <sup>f</sup>	55.0 <sup>e</sup>	59.8 <sup>f</sup>	62.1 <sup>c</sup>	60.0 <sup>f</sup>	18.9
No stimulation	10% brine	59.0 <sup>b</sup>	63.2 <sup>b,d</sup>	62.5 <sup>b</sup>	71.7 <sup>b,d</sup>	63.5 <sup>b</sup>	10.8

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

K. L. Moore<sup>A,B,C</sup>, B. P. Mullan<sup>A</sup>, J. C. Kim<sup>A</sup>, M. Trezona<sup>A</sup> and F. R. Dunshea<sup>B</sup>

<sup>A</sup>Department of Agriculture and Food WA, South Perth, WA 6151.

<sup>B</sup>University of Melbourne, Parkville, VIC 3052.

<sup>C</sup>Corresponding author. Email: Karen.moore@agric.wa.gov.au

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<sup>®</sup>; 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 (% ≤2)	P value
	1	2	3	4	5		
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

N. A. Watson<sup>A</sup>, K. A. Dyer<sup>A</sup>, J. D. Buckley<sup>A</sup>, G. D. Brinkworth<sup>B</sup>, A. M. Coates<sup>A</sup>, G. Parfitt<sup>A</sup>,  
P. R. C Howe<sup>C</sup>, M. Noakes<sup>B</sup> and K. J. Murphy<sup>A, D</sup>

<sup>A</sup>The University of South Australia, Adelaide, SA 5001.

<sup>B</sup>Commonwealth Scientific and Industrial Research Organisation, Adelaide, SA 5000.

<sup>C</sup>The University of Newcastle, Callaghan, NSW 2308.

<sup>D</sup>Corresponding author. Email: Karen.Murphy@unisa.edu.au

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]  $34.3 \pm 0.6 \text{ kg/m}^2$  (mean  $\pm$  SEM) with moderately controlled T2DM (HbA1c  $8.1 \pm 0.2\%$ ) 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  $720 \pm 29 \text{ g/week}$  for the HPP diet and  $384 \pm 31 \text{ g/week}$  for the LPP diet ( $P < 0.001$ ). This indicates a  $90 \pm 3\%$  and  $94 \pm 3\%$  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  $-8.0 \pm 0.8 \text{ kg}$ ; LPP  $-7.6 \pm 0.8 \text{ kg}$ ) and improvements in HbA1c (HPP  $-1.5 \pm 0.2\%$ ; LPP  $-1.3 \pm 0.2\%$ ), 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

D. J. Henman<sup>A,D</sup>, S. L. Beer<sup>A</sup>, J. Lockhart<sup>B</sup> and D. D. Moore<sup>C</sup>

<sup>A</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>B</sup>BiOnyc, Orange, NSW 2800.

<sup>C</sup>Ironbark Consulting, Ironbark, QLD 4306.

<sup>D</sup>Corresponding author. Email: [dhenman@rivalea.com.au](mailto:dhenman@rivalea.com.au)

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 ( $74.7 \text{ kg} \pm 5.41 \text{ kg}$ ; mean  $\pm$  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					SEM <sup>A</sup>	P value
	SSe <sup>B</sup>	SeMet <sup>B</sup>	SeMet	SeHLan <sup>B</sup>	SeHLan		
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 <sup>a</sup>	0.70 <sup>b</sup>	1.25 <sup>a</sup>	0.88 <sup>bc</sup>	1.13 <sup>ac</sup>	0.07	0.026
Loin Se (mg/kg)	0.18 <sup>a</sup>	0.23 <sup>b</sup>	0.29 <sup>c</sup>	0.30 <sup>c</sup>	0.43 <sup>d</sup>	0.01	<0.001

<sup>A</sup>SEM, standard error of the mean. <sup>B</sup>SSe, sodium selenite; SeMet, selenomethionine; SeHLan, selenohomoalanthionine. <sup>a,b,c,d</sup>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

K. L. Moore<sup>A,B,C</sup>, B. P. Mullan<sup>A</sup>, J. C. Kim<sup>A</sup> and F. R. Dunshea<sup>B</sup>

<sup>A</sup>Department of Agriculture and Food WA, South Perth, WA 6151.

<sup>B</sup>The University of Melbourne, Parkville, VIC 3052.

<sup>C</sup>Corresponding author. Email: Karen.moore@agric.wa.gov.au

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<sup>®</sup> (Zoetis Australia, Rhodes NSW)]; and feeding regime [2.5 times maintenance (restricted  $E_m$  (kJ/d) = 444 kJ ×  $LW^{0.75}$ , 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 restrictively. 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 LW	Sex (S)	Restricted		<i>Ad libitum</i>		SED <sup>B</sup>	S	<i>P</i> -value <sup>A</sup>	
		Light	Heavy	Light	Heavy			F	W
LD <sup>E</sup> 0–14 (g/day)	E <sup>C</sup> I <sup>D</sup>	467 433	679 565	747 749	887 944	77.7	0.566	<0.001	<0.001
LD 15–28 (g/day)	E I	689 597	762 688	849 1010	972 783	71.6	0.168	<0.001	0.650
FD <sup>F</sup> 0–14 (g/day)	E I	19 32	56 132	88 31	139 112	30.0	0.937	0.035	<0.001
FD 15–28 (g/day)	E I	12 46	139 100	94 184	125 211	39.0	0.025	<0.001	0.002

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

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## Response to different pathway interventions to improve pork eating quality consistency

H. A. Channon<sup>A,B,C</sup>, D. N. D'Souza<sup>A</sup> and F. R. Dunshea<sup>B</sup>

<sup>A</sup>Australian Pork Limited, Barton, ACT 2600

<sup>B</sup>The University of Melbourne, Parkville, VIC 3052.

<sup>C</sup>Corresponding author. Email: [heather.channon@australianpork.com.au](mailto:heather.channon@australianpork.com.au)

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<sup>®</sup> (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 carcass sides and steaks from the loin only. Ageing (2 or 7 days) was allocated to muscles from each carcass 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**

Cut Cooking method	Quality grade score (Fail rate, %)				
	Silverside Roast	Silverside Stir fry	Loin Roast	Loin 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)
<i>P</i> 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

H. A. Channon<sup>A,B,C</sup>, D. N. D'Souza<sup>A</sup> and F. R. Dunshea<sup>B</sup>

<sup>A</sup>Australian Pork Limited, Barton, ACT 2600.

<sup>B</sup>The University of Melbourne, Parkville, VIC 3052.

<sup>C</sup>Corresponding author. Email: [heather.channon@australianpork.com.au](mailto:heather.channon@australianpork.com.au)

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<sup>®</sup> (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 carcass specifications (60–75 kg Trim 1; P2 8–13 mm) at 60 min after slaughter and carcasses 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 carcasses 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 carcasses (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<sup>A</sup> 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 <sup>a,c</sup>	53.0 <sup>a,c</sup>	55.4 <sup>a</sup>	57.4 <sup>a,c</sup>	54.0 <sup>a</sup>	27.1
Aitchbone	None	56.8 <sup>c,f</sup>	61.9 <sup>f</sup>	58.9 <sup>c</sup>	66.8 <sup>c,f</sup>	57.9 <sup>c</sup>	17.5
Achilles	10% brine	65.8 <sup>b,d</sup>	60.5 <sup>b</sup>	79.4 <sup>b,d</sup>	76.9 <sup>b,d</sup>	66.4 <sup>b,d</sup>	13.8

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

C. R. Ralph<sup>A,C</sup>, L. M. Staveley<sup>B</sup>, C. L. Burnard<sup>B</sup> and K. J. Plush<sup>A</sup>

<sup>A</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

<sup>B</sup>The University of Adelaide, Roseworthy, SA 5371.

<sup>C</sup>Corresponding author. Email: cameron.ralph@sa.gov.au

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  $\pm$  SEM**

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

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## Inclusion of $\text{MgSO}_4$ in the diet of sows before farrowing improves measures of piglet colostrum ingestion

K. J. Plush<sup>A,B,C</sup>, L. M. Staveley<sup>B</sup>, A. C. Weaver<sup>B</sup> and W. H. E. J. van Wetters<sup>B</sup>

<sup>A</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

<sup>B</sup>The University of Adelaide, Roseworthy, SA 5371.

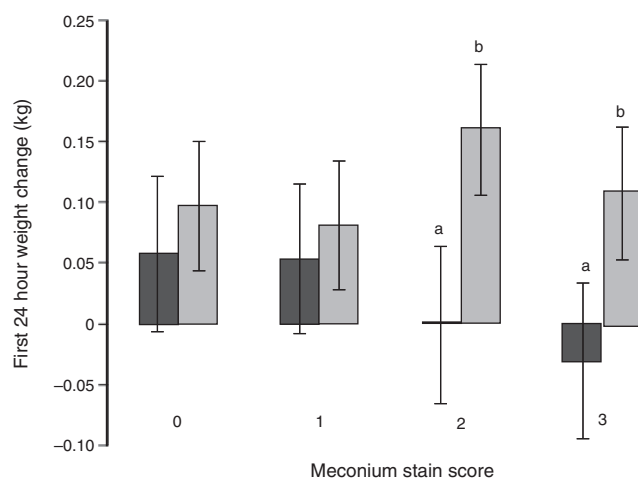
<sup>C</sup>Corresponding author. Email: kate.plush@sa.gov.au

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 ( $\text{MgSO}_4$ ) may be a suitable candidate for inclusion in a sow diet before farrowing. It was hypothesised that sows supplemented with  $\text{MgSO}_4$  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  $\text{MgSO}_4$  (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  $\pm$  SEM.

There was a tendency ( $P = 0.08$ ) for all piglets from the Mg treatment to display a higher vitality score immediately after birth ( $1.6 \pm 0.1$ ) than Control piglets ( $1.4 \pm 0.1$ ). Piglets from the Mg treatment tended ( $P = 0.07$ ) to have higher blood glucose levels ( $5.9 \pm 0.3$  mmol/L) than Control piglets ( $5.3 \pm 0.4$  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  $\text{MgSO}_4$  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  $\text{MgSO}_4$ -treated piglets. Further work should determine if these improvements result in increased piglet survival.



**Fig. 1.** Bodyweight change (kg; mean  $\pm$  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  $\text{MgSO}_4$  (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

J. S. Huser<sup>A,C,D</sup>, K. J. Plush<sup>B</sup>, W. S. Pitchford<sup>C</sup>, T. E Kennett<sup>A</sup> and D. S. Lines<sup>A</sup>

<sup>A</sup>SunPork Farms, Stirling, SA 5152.

<sup>B</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

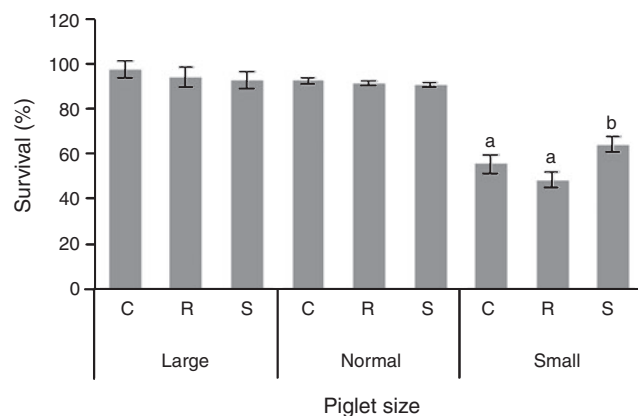
<sup>C</sup>The University of Adelaide, Roseworthy, SA 5371.

<sup>D</sup>Corresponding author. Email: sophia@austporkfarms.com.au

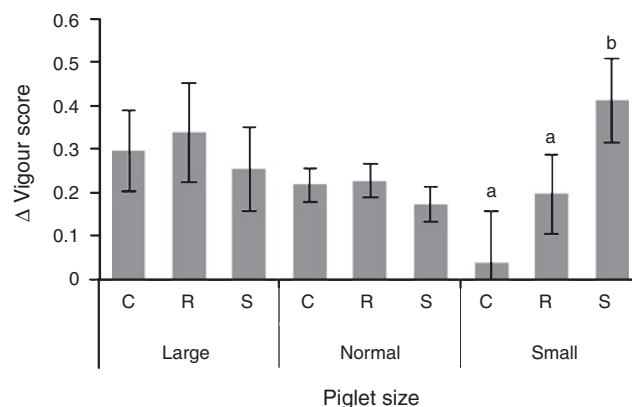
Split suckling (SS) is a management technique that could provide more sufficient colostrum to all piglets within large litters, thereby improving the chances of survival. The absorption of colostral IgG is essential for immune system development as there is no placental transfer of antibodies between 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<sup>®</sup>, 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 ( $\pm$  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)]. <sup>a,b</sup>Superscripts are significantly different ( $P < 0.05$ ).



**Fig. 2.** Mean ( $\pm$  SEM) change ( $\Delta$ ) 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)]. <sup>a,b</sup>Superscripts are significantly different ( $P < 0.05$ ).

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## A ‘two-stage’ farrowing and lactation system: piglet survival and growth performance

R. S. Morrison<sup>A,D</sup>, E. J. McDonald<sup>B</sup>, R. Z. Athorn<sup>A</sup>, E. M. Baxter<sup>C</sup> and A. J. Norval<sup>A</sup>

<sup>A</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>B</sup>The University of Sydney, Camden, NSW 2570.

<sup>C</sup>Scotland's Rural College, Edinburgh, EH9 3JG, UK.

<sup>D</sup>Corresponding author. Email: [rmorrison@rivalea.com.au](mailto:rmorrison@rivalea.com.au)

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<sup>TM</sup> 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<sub>FC</sub>: 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<sub>PS</sub>: 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 ( $25 \pm 2.7$  days; mean  $\pm$  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<sub>FC</sub> and GL<sub>PS</sub> 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 <sup>A</sup>	GL <sub>FC</sub>	PS	GL <sub>PS</sub>	SEM <sup>B</sup>	<i>P</i> value
Litters farrowed	141	36	142	36	—	—
Average number piglets weaned <sup>C,D</sup>	9.2	9.7	9.2	8.9	0.10	0.385
Live born mortality (%) <sup>D</sup>	16.6	14.6	19.9	20.3	0.823	0.094
Piglet ADG (g, birth to weaning) <sup>E</sup>	218 <sup>ab</sup>	193 <sup>c</sup>	221 <sup>a</sup>	206 <sup>bc</sup>	0.002	0.001
Piglet ADG (g, mixing to weaning) <sup>F</sup>	264 <sup>a</sup>	156 <sup>c</sup>	248 <sup>b</sup>	168 <sup>c</sup>	0.003	< 0.001

<sup>A</sup>Refer to text for treatment details. <sup>B</sup>SEM, standard error of the mean. <sup>C</sup>Included fostered piglets. <sup>D</sup>Number of piglets born alive used as a covariate. <sup>E</sup>Piglet birth weight used as a covariate. <sup>F</sup>Piglet pre-mix weight used as a covariate. <sup>a,b,c</sup>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

A. V. Strathe<sup>A</sup>, T. S. Bruun<sup>B</sup> and C. F. Hansen<sup>A,C</sup>

<sup>A</sup>University of Copenhagen, Copenhagen, Denmark.

<sup>B</sup>Danish Pig Research Centre, SEGES P/S, Copenhagen, Denmark.

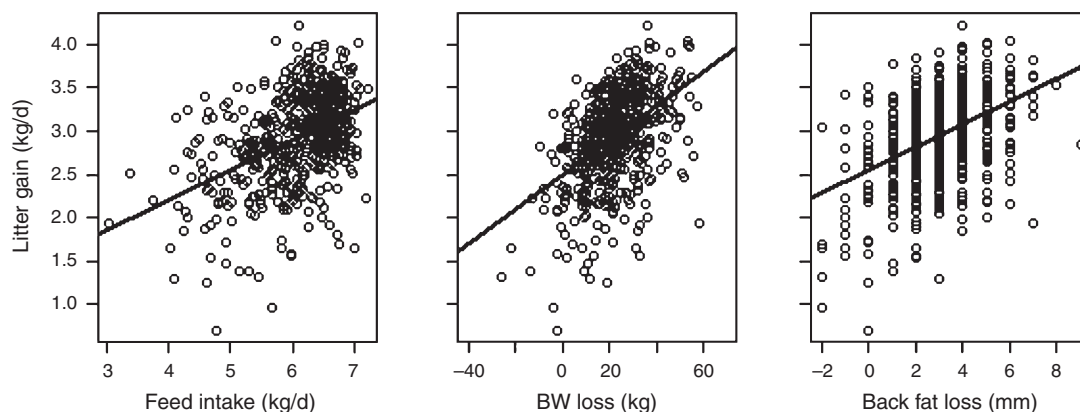
<sup>C</sup>Corresponding author. Email: cfh@sund.ku.dk

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  $2.5 \pm 1.0$ ; mean  $\pm$  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  $13.0 \pm 1.1$  piglets (mean  $\pm$  SD), had a litter gain of  $2.9 \pm 0.5$  kg/d and had an estimated milk yield of  $11.3 \pm 1.4$  kg/d. The feed intake of the sows was  $6.1 \pm 0.7$  kg/d and the sows lost  $22.5 \pm 12.7$  kg BW ( $9.1 \pm 5.1\%$  of their BW at d 2) and  $2.9 \pm 1.7$  mm BF from d 2 post-partum until weaning. The weaning-to-estrus interval (WEI) was  $5.3 \pm 6.1$  days and in the next litter, the sows gave birth to  $18.2 \pm 3.8$  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) =  $0.85 + 0.340 \times$  Feed intake (kg/d)]. A sow BW loss of 1 kg heightened daily litter gain with 20 g [Litter gain (kg/d) =  $2.48 + 0.020 \times$  BW loss (kg)], whereas a BF loss of 1 mm enhanced litter gain with 130 g/d [Litter gain (kg/d) =  $2.55 + 0.130 \times$  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

D. L. Turpin<sup>A,E</sup>, P. Langendijk<sup>B</sup>, T-Y. Chen<sup>C</sup>, D. S. Lines<sup>D</sup> and J. R. Pluske<sup>A</sup>

<sup>A</sup>Murdoch University, Murdoch, WA 6150.

<sup>B</sup>Nutreco, Boxmeer 5831, The Netherlands.

<sup>C</sup>SARDI Livestock Systems, Roseworthy, SA 5371.

<sup>D</sup>SunPork Farms, Wasleys, SA 5400.

<sup>E</sup>Corresponding author. Email: D.Turpin@murdoch.edu.au

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  $d 29 \pm 2.3$  (mean  $\pm$  SD), and IS, where piglets were separated from the sow for 8 h per day (0700 to 1500 h) starting at  $d 22 \pm 1.3$  for a week before weaning ( $d 29 \pm 1.3$ ). 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<sup>2</sup> 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 <sup>B</sup>		Cortisol (ng/mL)		N:L ratio	
Day <sup>A</sup>	–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 <sup>C</sup>	5.1	3.4			4.0	2.6		
<i>P</i> value	0.01	0.75	0.07	0.16	0.80	0.88	0.67	0.38

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

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## Teat order influences piglet performance after weaning

D. S. Lines<sup>A</sup>, E. M. de Ruyter<sup>B</sup>, W. H. E. J. van Wetters<sup>B</sup> and K. J. Plush<sup>C,D</sup>

<sup>A</sup>SunPork Farms, Wasleys, SA 5400.

<sup>B</sup>The University of Adelaide, Roseworthy, SA 5371.

<sup>C</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

<sup>D</sup>Corresponding author. Email: [kate.plush@sa.gov.au](mailto:kate.plush@sa.gov.au)

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  $28 \pm 1$  d of age (mean  $\pm$  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 nmol/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  $\pm$  SEM**

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

<sup>abc</sup>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

J. S. Huser<sup>A,C</sup>, T. E. Kennett<sup>A</sup>, K. J. Plush<sup>B</sup>, W. S. Pitchford<sup>C</sup> and D. S. Lines<sup>A,D</sup>

<sup>A</sup>SunPork Farms, Stirling, SA 5152.

<sup>B</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

<sup>C</sup>The University of Adelaide, Roseworthy, SA 5371.

<sup>D</sup>Corresponding author. Email: [dlines@austporkfarms.com.au](mailto:dlines@austporkfarms.com.au)

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<sup>®</sup>; 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 ( $\pm$  SEM)

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

<sup>A</sup>Refer to text for treatment details. <sup>B</sup>Not significant ( $P > 0.10$ ). <sup>a,b</sup>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

K. S. O'Halloran<sup>A,C</sup>, R. R. Carter<sup>B</sup>, D. J. Henman<sup>A</sup> and C. L. Collins<sup>A</sup>

<sup>A</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>B</sup>Kemin Australia, Killara, NSW 2071.

<sup>C</sup>Corresponding author. Email: ko'halloran@rivalea.com.au

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<sup>®</sup> (Kemin; Killara NSW). This study tested the hypothesis that the inclusion of Lysoforte<sup>®</sup> 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<sup>™</sup> Genetics, average parity  $2.7 \pm 0.07$  (mean  $\pm$  SE)] were 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<sup>®</sup>. 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 ( $26.6 \pm 0.15$  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<sup>®</sup> 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<sup>®</sup> inclusion (Fig. 1A). There was no difference between treatments in P2 backfat loss over all parities ( $P=0.68$ ). Sows offered the Lysoforte<sup>®</sup> 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<sup>®</sup> was higher than the controls ( $P=0.037$ ), and there was no interaction between treatment and parity ( $P=0.40$ ).

Lysoforte<sup>®</sup> 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<sup>®</sup> 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.

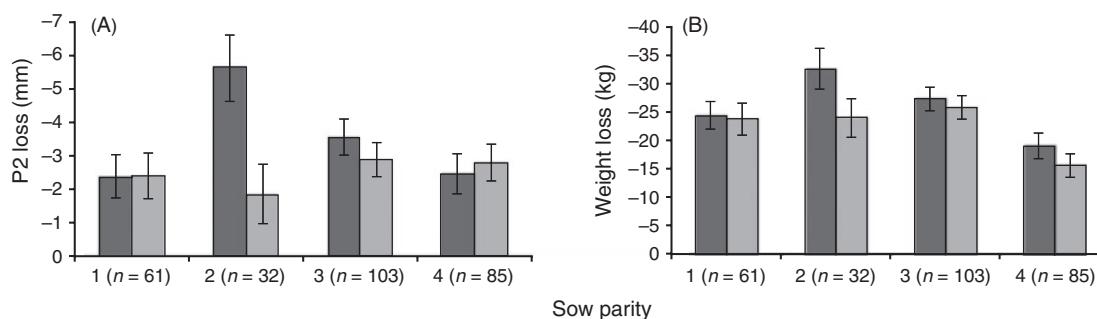


Fig. 1. The effect of Lysoforte<sup>®</sup> (□) fed during lactation vs a control diet (■) on the change in sow P2 backfat loss (A) and LW (B).

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

C. Amdi<sup>A,C</sup>, V. A. Moustsen<sup>B</sup>, G. Sørensen<sup>B</sup> and C. F. Hansen<sup>A</sup>

<sup>A</sup>University of Copenhagen, 1870 Frederiksberg, Denmark.

<sup>B</sup>SEGES, Pig Research Centre, 1609 Copenhagen V, Denmark.

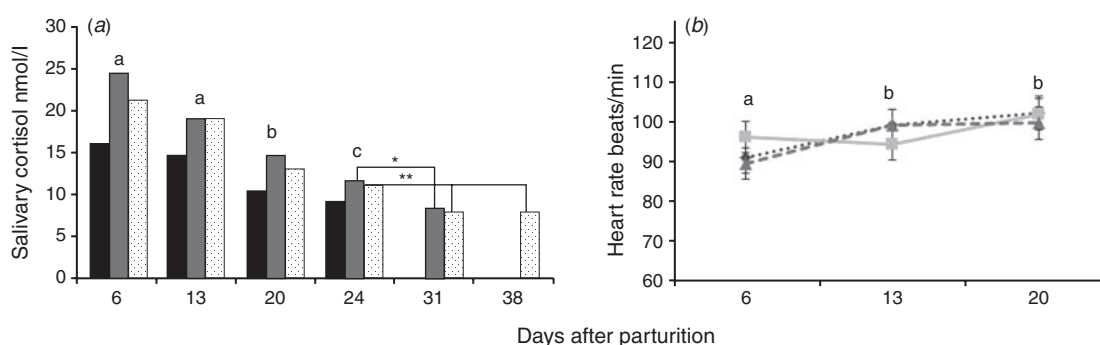
<sup>C</sup>Corresponding author. Email: ca@sund.ku.dk

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<sup>®</sup>, 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. 1a). 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. 1b).

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 ( $\pm$  pooled SE) during lactation, for control ( $\blacklozenge$ ), N1 ( $\blacksquare$ ) and N2 ( $\blacktriangle$ ) sows. Letters denote effect of time. <sup>a,b</sup>Significant effect of time ( $P < 0.05$ ).

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## Nitrous oxide for piglet gas euthanasia

J.-L. Rault<sup>A,E</sup>, N. Kells<sup>B</sup>, C. Johnson<sup>B</sup>, M. Sutherland<sup>C</sup>, R. Dennis<sup>D</sup> and D. Lay Jr.<sup>D</sup>

<sup>A</sup>The University of Melbourne, Parkville, VIC 3010.

<sup>B</sup>Massey University, Palmerston North 4410, New Zealand.

<sup>C</sup>AgResearch Ltd., Hamilton 3240, New Zealand.

<sup>D</sup>US Department of Agriculture-ARS, West Lafayette, IN 47907, USA.

<sup>E</sup>Corresponding author. Email: [raultj@unimelb.edu.au](mailto:raultj@unimelb.edu.au)

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<sub>2</sub>) gas is used but aversive to piglets (Rault *et al.* 2013). This previous research suggested that nitrous oxide (N<sub>2</sub>O; 'laughing gas') is less aversive than CO<sub>2</sub> for piglets. This research sought to: evaluate the aversiveness of inhaling N<sub>2</sub>O 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<sub>2</sub>O is less aversive to piglets than exposure to CO<sub>2</sub>.

The gas mixtures tested were: N<sub>2</sub>O and air (90%:10%; '90N'); N<sub>2</sub>O, oxygen and air (60%:30%:10%; '60N'); and, for experiments 2 and 3, CO<sub>2</sub> 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<sup>®</sup>; 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<sub>2</sub>O 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<sub>2</sub>O prefilled at 25%, 50%, or 75%; or CO<sub>2</sub> 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<sub>2</sub> ( $r = 0.40$ ,  $P = 0.06$ ), but not N<sub>2</sub>O ( $r = 0.28$ ,  $P = 0.19$ ). Overall, these data supported our hypothesis that exposure to N<sub>2</sub>O is less aversive to piglets than exposure to CO<sub>2</sub>. 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<sub>2</sub>O is less aversive than CO<sub>2</sub>, taking 13 s longer to induce full loss of consciousness in our settings. This project also demonstrated that 90% N<sub>2</sub>O 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 <sub>2</sub> O	90% CO <sub>2</sub>	90% CO <sub>2</sub> after exposure to 60% N <sub>2</sub> O	<i>P</i> value
Transitional EEG	62.10 ± 4.80 <sup>y</sup> [40–87]	45.49 ± 5.23 [39–54]	41.82 ± 5.18 <sup>x</sup> [15–51]	0.07
Isoelectric EEG	71.49 ± 7.47 [55–94]	58.66 ± 8.14 [46–68]	48.83 ± 8.07 [33–73]	0.19

<sup>x,y</sup>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

L. U. Hansen<sup>A,B</sup>

<sup>A</sup>SEGES P/S, Danish Pig Research Centre, Axeltorv 3, 1609 Copenhagen, Denmark.

<sup>B</sup>Corresponding author. Email: [luh@seges.dk](mailto:luh@seges.dk)

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 feed 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<sup>2</sup> 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<sup>®</sup>, 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
<i>P</i> 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

L. M. Hemsworth<sup>A,B</sup>, E. C. Jongman<sup>A</sup> and J. Skuse<sup>A</sup>

<sup>A</sup>The University of Melbourne, Parkville, VIC 3010.

<sup>B</sup>Corresponding author. Email: [lauren.hemsworth@unimelb.edu.au](mailto:lauren.hemsworth@unimelb.edu.au)

The Australian Pork Industry Quality Assurance Program (APIQ<sup>✓</sup>) 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 ( $\rho$ ) to examine the strength of the relationship between the assessment scores from the M and V measures, and Kappa statistics ( $\kappa$ ) 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  $\rho$  to examine the similarity between measures collected on an animal at two different time points (intra-observer), and  $\kappa$  to investigate the agreement between measures taken on an animal by multiple assessors (inter-observer).

Moderate to substantial levels of agreement ( $\kappa = 0.61$  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 ( $\rho = 0.30$  to  $0.49$ ) with slight to fair levels of agreement ( $\kappa = 0.21$  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

C. Munoz<sup>A,B</sup>, P. H. Hemsworth<sup>A</sup> and E. Jongman<sup>A</sup>

<sup>A</sup>The University of Melbourne, Parkville, VIC 3010.

<sup>B</sup>Corresponding author. Email: [cmunoz@student.unimelb.edu.au](mailto:cmunoz@student.unimelb.edu.au)

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 (parity 1 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<sup>2</sup>/sow (range 2.4–3.0 m<sup>2</sup>/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<sup>2</sup>/sow (range 2.4–3.1 m<sup>2</sup>/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<sup>®</sup>; 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

T. L. Muller<sup>A,B</sup>, R. J. E. Hewitt<sup>A</sup> and R. J. van Barneveld<sup>A</sup>

<sup>A</sup>SunPork Farms, Loganholme QLD 4129.

<sup>B</sup>Corresponding author. Email: [tracy.muller@sunporkfarms.com.au](mailto:tracy.muller@sunporkfarms.com.au)

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;  $\mu\text{mol/L}$ ), whole blood Crea ( $\mu\text{mol/L}$ ), 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
<i>Treatment</i>					
Control (9kg/d)	45.4	178.5	98.5 <sup>b</sup>	17.62 <sup>b</sup>	48.12
R1 (8kg/d)	43.8	187.1	113.8 <sup>a</sup>	21.26 <sup>a</sup>	51.11
R2 (7kg/d)	48.8	176.6	124.9 <sup>a</sup>	14.92 <sup>b</sup>	47.60
R3 (6kg/d)	45.5	170.1	138.0 <sup>a</sup>	17.12 <sup>b</sup>	48.74
SED <sup>A</sup>	4.82	17.48	13.54	1.93	2.14
<i>P</i> value	0.756	0.783	0.029	0.021	0.382

<sup>A</sup>SED, standard error of difference. <sup>a,b</sup>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

E. J. McDonald<sup>A,D</sup>, R. S. Morrison<sup>B</sup>, R. Z. Athorn<sup>B</sup>, A. J. Norval<sup>B</sup>, J. A. Downing<sup>A</sup>  
 and J. A. Lievaart<sup>C</sup>

<sup>A</sup>The University of Sydney, Camden, NSW 2570.

<sup>B</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>C</sup>Charles Sturt University, Wagga Wagga, NSW 2650.

<sup>D</sup>Corresponding author. Email: ej.mcdonald@hotmail.com

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<sup>TM</sup> 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<sub>FC</sub>: 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<sub>PS</sub>: 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  $\pm$  SD (where indicated)**

	FC <sup>A</sup>	GL <sub>FC</sub>	PS	GL <sub>PS</sub>	<i>P</i> value
WRI <sup>B</sup> (d)	5.2 $\pm$ 3.2	8.3 $\pm$ 6.6	7.4 $\pm$ 6.1	7.6 $\pm$ 6.5	0.155
Sows with WRI $\leq$ 4 d (%)	75.0 <sup>a</sup>	56.3 <sup>b</sup>	53.1 <sup>b</sup>	45.5 <sup>b</sup>	0.049
Sows successfully mated post-wean (%)	88.9	88.9	91.4	91.7	0.711
Subsequent no. piglets born alive	12.1 $\pm$ 3.1	12.0 $\pm$ 2.8	12.8 $\pm$ 2.4	11.4 $\pm$ 1.5	0.443
Counts of oestrus behaviour (counts/treatment – cumulative total)	–	45	–	42	0.961
Sows with large follicles $\geq$ 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

<sup>A</sup>Refer to text for treatment details. <sup>B</sup>WRI, weaning to re-mating interval (number of days after weaning until the sow showed signs of standing oestrus and was mated). <sup>a,b</sup>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

A. V. Strathe<sup>A</sup>, T. S. Bruun<sup>B</sup> and C. F. Hansen<sup>A,C</sup>

<sup>A</sup>University of Copenhagen, Copenhagen, Denmark.

<sup>B</sup>Danish Pig Research Centre, SEGES P/S, Copenhagen, Denmark.

<sup>C</sup>Corresponding author. Email: cfh@sund.ku.dk

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 ( $6.1 \pm 0.7$  kg, mean  $\pm$  SD;  $P = 0.23$ ) of the sows, litter size at weaning ( $13.0 \pm 1.1$ ,  $P = 0.23$ ), ADG of the litter ( $2.93 \pm 0.53$  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 ( $32.7 \pm 10.9$  kg and  $0.9 \pm 1.1$  mm), from d 2 to weaning ( $22.1 \pm 12.7$  kg and  $2.9 \pm 1.7$  mm; Table 1), from d 2 to d 17 ( $17.9 \pm 11.7$  kg and  $2.6 \pm 1.6$  mm), and from d 17 to weaning ( $8.0 \pm 7.9$  kg and  $0.7 \pm 1.5$  mm), were also all unaffected by the dietary Val:Lys ratio ( $P > 0.05$ ). Milk yield ( $11.3 \pm 1.4$  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						SE	P value
	83.9	86.4	88.0	90.5	95.3	99.1		
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

R. S. Morrison<sup>A,D</sup>, E. J. McDonald<sup>B</sup>, R. Z. Athorn<sup>A</sup>, E. M. Baxter<sup>C</sup> and A. J. Norval<sup>A</sup>

<sup>A</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>B</sup>University of Sydney, Camden, NSW 2570.

<sup>C</sup>Scotland's Rural College, Edinburgh, EH9 3JG, UK.

<sup>D</sup>Corresponding author. Email: [rmorrison@rivalea.com.au](mailto:rmorrison@rivalea.com.au)

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<sup>TM</sup> 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<sub>FC</sub>: 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<sub>PS</sub>: 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<sub>FC</sub> and GL<sub>PS</sub> 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<sub>PS</sub> and GL<sub>FC</sub> 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<sup>B</sup>**

	FC <sup>A</sup>	GL <sub>FC</sub>	PS	GL <sub>PS</sub>	SEM <sup>C</sup>	P value
13 days prior to weaning	0.50 <sup>a</sup> (–0.30)	5.11 <sup>b</sup> (0.71)	0.93 <sup>a</sup> (–0.03)	3.52 <sup>b</sup> (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

<sup>A</sup>Refer to text for treatment details. <sup>B</sup>Values are presented as back-transformed means (transformed means presented in parenthesis). <sup>C</sup>SEM standard error of the mean. <sup>a,b</sup>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

K. L. Bunter<sup>A,C</sup> and K. M. Boardman<sup>B</sup>

<sup>A</sup>AGBU, a joint venture of NSW Agriculture and the University of New England, UNE, Armidale, NSW 2351.

<sup>B</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>C</sup>Corresponding author. Email: kbunter2@une.edu.au

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  $170 \pm 3.3$  (mean  $\pm$  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  $342 \pm 15.1$  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

E. C. Greenwood<sup>A,C</sup>, J. R. Rayner<sup>B</sup>, K. J. Plush<sup>B</sup>, W. H. E. J. van Wetters<sup>A</sup> and P. E. Hughes<sup>B</sup>

<sup>A</sup>The University of Adelaide, Roseworthy, SA 5371.

<sup>B</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

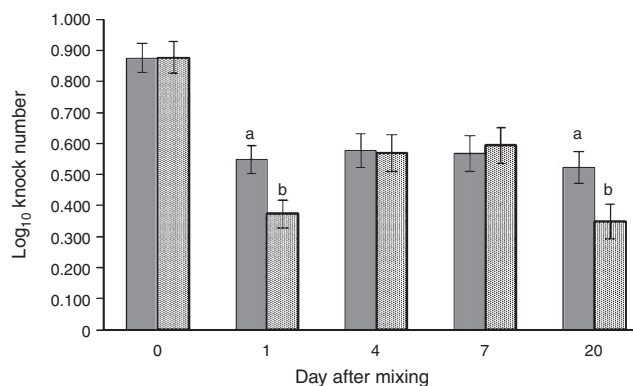
<sup>C</sup>Corresponding author. Email: emma.greenwood@adelaide.edu.au

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<sup>2</sup>/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  $\pm$  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  $\pm$  0.2 (27.6) vs 5.5  $\pm$  0.2 (35.0)] and d 20 [3.5  $\pm$  0.2 (15.0) vs 4.2  $\pm$  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<sub>10</sub>-transformed means  $\pm$  SEM. Significant differences between treatment within day are highlighted using superscripts (<sup>a,b</sup> $P < 0.01$ ).

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## Feeding behaviour, aggression and dominance in group-housed sows

J.-L. Rault<sup>A,B</sup>, H. Ho<sup>A</sup>, M. Verdon<sup>A</sup> and P. Hemsworth<sup>A</sup>

<sup>A</sup>The University of Melbourne, Parkville, VIC 3010.

<sup>B</sup>Corresponding author. Email: [raultj@unimelb.edu.au](mailto:raultj@unimelb.edu.au)

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<sup>2</sup>/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<sup>®</sup>; 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  $\pm$  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 $\pm$ 1.7	22.1 $\pm$ 1.7	26.5 $\pm$ 1.5	23.9 $\pm$ 1.8
Subdominant	26.0 $\pm$ 1.9	22.2 $\pm$ 1.8	23.9 $\pm$ 2.0	25.6 $\pm$ 2.0
Subordinate	19.2 $\pm$ 1.8	16.1 $\pm$ 1.9	21.9 $\pm$ 2.0	22.4 $\pm$ 1.6

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

T. L. Muller<sup>A,B</sup>, R. J. E. Hewitt<sup>A</sup> and R. J. van Barneveld<sup>A</sup>

<sup>A</sup>SunPork Farms, Loganholme, QLD 4129.

<sup>B</sup>Corresponding author. Email: [tracy.muller@sunporkfarms.com.au](mailto:tracy.muller@sunporkfarms.com.au)

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<sup>®</sup> (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<sup>®</sup> (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<sup>®</sup> 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<sup>®</sup>, 0.1% magnesium oxide (MgO) or 20% sugar beet pulp (SBP)

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

<sup>A</sup>SED, standard error of difference between means. <sup>a,b,c</sup>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

T. L. Muller<sup>A,B</sup>, R. J. E. Hewitt<sup>A</sup> and R. J. van Barneveld<sup>A</sup>

<sup>A</sup>SunPork Farms, Loganholme, QLD 4129.

<sup>B</sup>Corresponding author. Email: [tracy.muller@sunporkfarms.com.au](mailto:tracy.muller@sunporkfarms.com.au)

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 (Spooler *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 <sup>A</sup>	<i>P</i> 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 <sup>B</sup>	5	6	7			
Chase	0.11 <sup>b</sup>	0.22 <sup>a</sup>	0.08 <sup>b</sup>		0.06	0.031
Threat	0.08 <sup>b</sup>	0.10 <sup>b</sup>	0.35 <sup>a</sup>		0.07	<0.001
Fight time (s)	5.34	1.69	2.55		1.64	0.075

<sup>A</sup>SED, standard error of difference of means. <sup>B</sup>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. <sup>a,b</sup>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

T. L. Muller<sup>A,C</sup>, M. J. Callaghan<sup>B</sup>, R. J. E. Hewitt<sup>A</sup> and R. J. van Barneveld<sup>A</sup>

<sup>A</sup>SunPork Farms, Loganholme, QLD 4129.

<sup>B</sup>Ridley Agriproducts, Toowong, QLD 4066.

<sup>C</sup>Corresponding author. Email: [tracy.muller@sunporkfarms.com.au](mailto:tracy.muller@sunporkfarms.com.au)

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 (Spooler *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<sup>2</sup>/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	Control	Treatment Block	High feed	SED <sup>A</sup>	<i>P</i> value
Push	0.09	0.08	0.10	0.24	0.868
Chase	0.29 <sup>a</sup>	0.08 <sup>b</sup>	0.11 <sup>b</sup>	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 <sup>x</sup>	25.67 <sup>xy</sup>	25.15 <sup>y</sup>	9.76	0.084
Lying	9.13 <sup>b</sup>	13.30 <sup>a</sup>	13.66 <sup>a</sup>	11.30	0.038
Standing	50.63 <sup>a</sup>	45.91 <sup>b</sup>	45.26 <sup>b</sup>	10.85	0.006

<sup>A</sup>SED, standard error of difference between means. <sup>a,b</sup>Means in a row not having the same superscript are significantly different.

<sup>x,y</sup>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

L. J. Roberts<sup>A,B</sup> and G. J. Coleman<sup>A</sup>

<sup>A</sup>The University of Melbourne, Parkville, VIC 3010.

<sup>B</sup>Corresponding author. Email: robertsl1@student.unimelb.edu.au

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

W. H. E. J. van Wettere<sup>A,C</sup>, T. E. Kennett<sup>B</sup> and D. S. Lines<sup>B</sup>

<sup>A</sup>The University of Adelaide, Roseworthy, SA 5005.

<sup>B</sup>SunPork Farms, Stirling SA 5152.

<sup>C</sup>Corresponding author. Email: William.vanwettere@adelaide.edu.au

In the majority of lactating sows, piglet suckling 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 ( $4.4 \pm 0.23$  vs  $3.9 \pm 0.15$ ). 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:  $139.4 \pm 0.39$  vs  $147.3 \pm 0.29$  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 <sup>A</sup> (%)	Total born
	In lactation	Post-weaning	In lactation	Post-weaning	All		
0IS	0.19*	0.81*	23.0 <sup>a</sup>	30.9 <sup>b</sup>	29.4*	93	12.1
7IS	0.50*	0.50*	21.9 <sup>a</sup>	31.6 <sup>b</sup>	26.8*	100	13.1
Pooled SEM <sup>B</sup>			0.42	0.27	0.80		0.49

<sup>A</sup>FR, farrowing rate. <sup>B</sup>SEM, standard error of the mean. <sup>a,b</sup>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

G. Sørensen<sup>A,B</sup>

<sup>A</sup>SEGES P/S, Danish Pig Research Centre, 1609 Copenhagen, Denmark.

<sup>B</sup>Corresponding author. Email: GS@seges.dk

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<sup>®</sup>, 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  $\pm$  SE (per pen)

Dietary treatment (DE/day)	Low	High	<i>P</i> 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 $\pm$ 42	208 $\pm$ 42	
BW, gain to farrowing (kg)	72 $\pm$ 38	62 $\pm$ 40	0.17
Backfat P2 at mating (mm)	12.6 $\pm$ 2.03	12.5 $\pm$ 2.00	
Backfat P2 gain to farrowing (mm)	4.2 $\pm$ 1.48	3.4 $\pm$ 1.79	0.17
Total born piglets per litter	18.3 $\pm$ 1.61	17.9 $\pm$ 1.59	0.04
Farrowing rate (%)	86.8 $\pm$ 9.2	84.7 $\pm$ 10.4	0.19

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## A comparison of suckling reduction strategies along with boar exposure to induce oestrus in lactating sows

H. L. Frobose<sup>A,B</sup>, M. D. Tokach<sup>A</sup>, J. M. DeRouchey<sup>A</sup>, S. S. Dritz<sup>A</sup>, R. D. Goodband<sup>A</sup>,  
 J. L. Nelssen<sup>A</sup> and D. L. Davis<sup>A</sup>

<sup>A</sup>Kansas State University, Manhattan, KS 66506.

<sup>B</sup>Corresponding author. Email: [frobose@ksu.edu](mailto:frobose@ksu.edu)

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 ( $2.6 \pm 1.4$ ; mean  $\pm$  SD), was used in five consecutive farrowing groups (Feb to Aug). Litter size was equalised by parity ( $11.5 \pm 1.1$  piglets; mean  $\pm$  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<sup>®</sup>; 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 <sup>A</sup>	SEP	SW	24HR	SEM <sup>B</sup>	$P \leq^C$
Lactating sows inseminated <sup>D</sup> (%)	0.0	77.8	73.1	85.2	62.9	0.09	0.318
Day 18 to insemination (d)	–	5.0 <sup>ab</sup>	4.7 <sup>a</sup>	5.5 <sup>b</sup>	4.4 <sup>a</sup>	0.33	0.036
Conception rate (%)	–	80.4	62.8	87.9	59.8	0.14	0.133
Sows inseminated post-wean (%) <sup>D</sup>	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 <sup>b</sup>	78.3 <sup>ab</sup>	75.0 <sup>ab</sup>	92.0 <sup>b</sup>	66.3 <sup>a</sup>	0.08	0.094
Creep feed disappearance (g/piglet/d) <sup>E</sup>	10.8 <sup>a</sup>	13.5 <sup>a</sup>	28.6 <sup>b</sup>	11.6 <sup>a</sup>	24.4 <sup>b</sup>	1.69	0.001
Offspring ADG d 18 to 32 (g) <sup>E</sup>	222 <sup>b</sup>	215 <sup>b</sup>	164 <sup>a</sup>	217 <sup>b</sup>	165 <sup>a</sup>	9.9	0.001
Offspring ADG d 32 to 170 (g) <sup>E</sup>	894	879	890	868	879	14.6	0.715
Offspring d 170 BW (kg) <sup>E</sup>	132.5	130.2	131.0	128.8	129.6	2.09	0.734

<sup>A</sup>Refer to text for treatment details. <sup>B</sup>SEM, pooled standard error of mean. <sup>C</sup>Overall significance set at  $P < 0.05$  for individual treatment comparisons. <sup>D</sup>Controls excluded from these analyses due to lack of variance. <sup>E</sup>Piglet growth reported from two farrowing groups ( $n = 54$  litters), adjusted using d 18 body weight (BW) as a covariate. <sup>a,b</sup>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

R. Z. Athorn<sup>A,D</sup>, J. R. Craig<sup>A</sup>, E. J. McDonald<sup>B</sup>, J. A. Downing<sup>B</sup> and P. Langendijk<sup>C</sup>

<sup>A</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>B</sup>The University of Sydney, Camden, NSW 2570.

<sup>C</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

<sup>D</sup>Corresponding author. Email: rathorn@rivalea.com.au

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<sup>TM</sup> 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 <sup>A</sup>	C28 (n = 33)	IS21 (n = 58)			SS21 (n = 47)		
Lactational oestrus (%)	–	38 (22/58)			43 (20/47)		
		First heat	Skip-a-heat	NR <sup>B</sup>	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

<sup>A</sup>Refer to text for treatment details. <sup>B</sup>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

E. C. Greenwood<sup>A,C</sup>, K. J. Plush<sup>B</sup>, W. H. E. J. van Wetters<sup>A</sup> and P. E. Hughes<sup>B</sup>

<sup>A</sup>The University of Adelaide, Roseworthy, SA 5371.

<sup>B</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

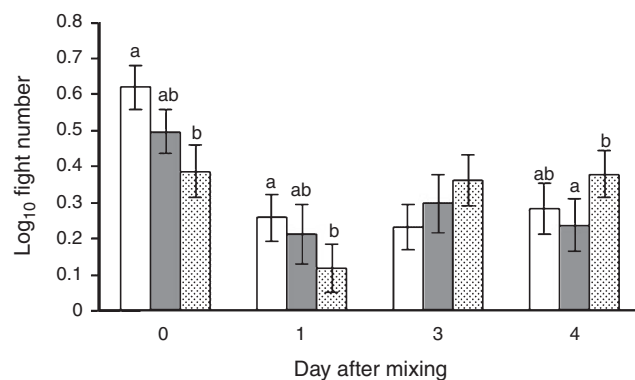
<sup>C</sup>Corresponding author. Email: emma.greenwood@adelaide.edu.au

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<sup>2</sup>/animal or greater but recent research suggests this figure is too low (Hemsworth *et al.* 2013), and so this experiment allowed 2 m<sup>2</sup>/sow (LOW), 4 m<sup>2</sup>/sow (MED) or 6 m<sup>2</sup>/sow (HIGH). The sows remained in these pens until d 4 after mixing, at which point all pens were equalised to 2 m<sup>2</sup>/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  $\pm$  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<sup>2</sup>/sow (LOW □), 4 m<sup>2</sup>/sow (MED ■) or 6 m<sup>2</sup>/sow (HIGH ▨) in group-housed sows on fight number per day/sow (on d 4 treatments were standardised to 2 m<sup>2</sup>/sow). Data are presented as log<sub>10</sub>-transformed means  $\pm$  SEM; significant differences between treatments, within day, are highlighted using superscripts (<sup>a</sup>, <sup>b</sup>  $P < 0.01$ ).

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## Maternal dietary energy rather than lysine intake during late gestation positively influences piglet birth weight

M. A. D. Goncalves<sup>A,D</sup>, K. Gourley<sup>B</sup>, S. S. Dritz<sup>A</sup>, M. D. Tokach<sup>B</sup>, N. M. Bello<sup>C</sup>, J. M. DeRouchey<sup>B</sup>,  
 J. C. Woodworth<sup>B</sup> and R. D. Goodband<sup>B</sup>

<sup>A</sup>College of Veterinary Medicine, KSU, Manhattan, KS 66506, USA.

<sup>B</sup>College of Agriculture, KSU, Manhattan, KS 66506, USA.

<sup>C</sup>College of Arts and Sciences, KSU, Manhattan, KS 66506, USA.

<sup>D</sup>Corresponding author. Email: [marcio@k-state.edu](mailto:marcio@k-state.edu)

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<sup>®</sup>; 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  $30 \pm 8.2$  g heavier (mean  $\pm$  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  $97 \pm 9.5$  g (mean  $\pm$  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.

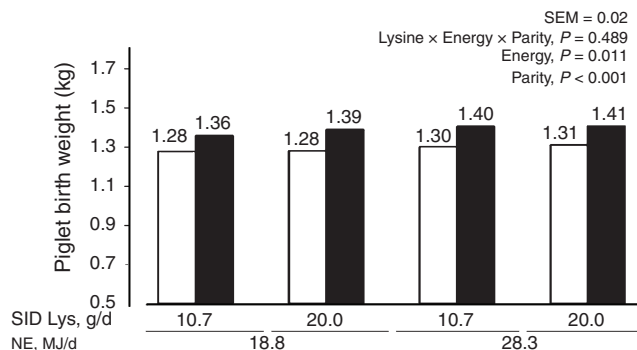


Fig. 1. Effects of lysine and energy intake during late gestation on individual piglet birth weights of gilts (□) and sows (■).

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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

A. C. Weaver<sup>A,C</sup>, K. L. Kind<sup>A</sup>, J. M. Kelly<sup>B</sup> and W. H. E. J. van Wetters<sup>A</sup>

<sup>A</sup>The University of Adelaide, Roseworthy, SA 5371.

<sup>B</sup>South Australian Research and Development Institute, Rosedale, SA 5350.

<sup>C</sup>Corresponding author. Email: [alice.weaver@adelaide.edu.au](mailto:alice.weaver@adelaide.edu.au)

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  $3.3 \pm 0.2$ , mean  $\pm$  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 ( $22.7 \pm 0.24$  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  $\pm$  SEM

	Lost more than 5% BW	Lost less than 5% BW	<i>P</i> value
N <sup>A</sup>	22	17	
Mean follicle size	$6.2 \pm 0.2$	$6.2 \pm 0.3$	NS <sup>C</sup>
% cleaved	$69.7 \pm 6.4$	$71.9 \pm 7.2$	0.066
% blastocyst/total	$31.8 \pm 3.8$	$42.2 \pm 4.3$	0.006
N <sup>B</sup>	33	14	
Ovulation rate	$22.9 \pm 0.8$	$22.0 \pm 1.3$	NS
Embryo number	$12.7 \pm 0.7$	$13.7 \pm 1.0$	NS
Embryo survival (%)	$57.0 \pm 3.4$	$64.5 \pm 5.2$	0.034

<sup>A</sup>N, number of sows slaughtered on day 21 of lactation. <sup>B</sup>N, number of sows slaughtered on d 30 after AI. <sup>C</sup>NS, not significant.

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## The influence of cumulus cells on porcine oocyte maturation in the presence of L-carnitine

A. N. Steel<sup>A,C</sup>, J. L. Lowe<sup>A</sup>, T. Somfai<sup>B</sup> and C. G. Grupen<sup>A</sup>

<sup>A</sup>The University of Sydney, Camden, NSW 2570.

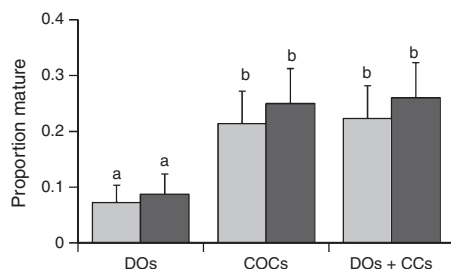
<sup>B</sup>NARO Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, Japan.

<sup>C</sup>Corresponding author. Email: alicia.steel@sydney.edu.au

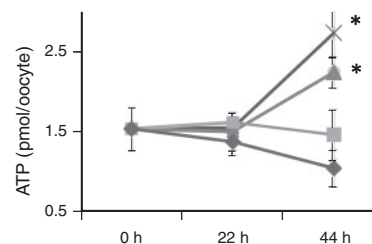
Porcine *in vitro* maturation (IVM) enables large numbers of oocytes to be salvaged from ovarian follicles and cultured *in vitro* to a stage at which they are capable of facilitating fertilisation and subsequent embryonic development. However, resultant embryos have reduced viability compared to those 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 least 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  $\pm$  SEM) at 44 hours of IVM. Oocytes were matured with  $\square$  0 mM and  $\blacksquare$  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  $\pm$  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 (X+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

J. Seyfang<sup>A,C</sup>, T. Y. Chen<sup>B</sup>, P. Langendijk<sup>B</sup> and R. N. Kirkwood<sup>A</sup>

<sup>A</sup>The University of Adelaide, Roseworthy, SA 5371.

<sup>B</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

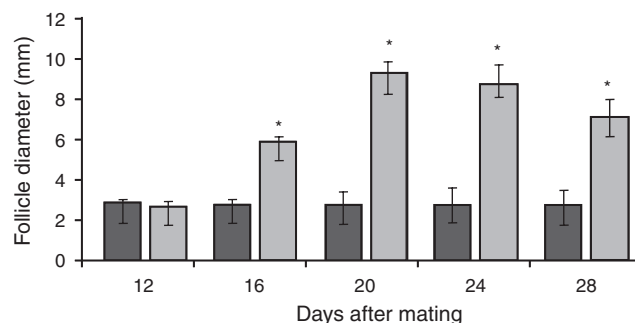
<sup>C</sup>Corresponding author. Email: [jemma.seyfang@adelaide.edu.au](mailto:jemma.seyfang@adelaide.edu.au)

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  $\times$  day interaction, and parity as fixed effects (SAS<sup>®</sup>; 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  $\pm$  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

K. L. Bunter<sup>A,B</sup>

<sup>A</sup>AGBU, a joint venture of NSW Agriculture and the University of New England, UNE, Armidale, NSW 2351.

<sup>B</sup>Corresponding author. Email: kbunter2@une.edu.au

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<sup>®</sup>; 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 (Kemmer *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

J. C. Lumby<sup>A,C</sup>, K. L. Bunter<sup>B</sup> and P. C. Wynn<sup>A</sup>

<sup>A</sup>EH Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga, NSW 2650.

<sup>B</sup>AGBU, a joint venture of NSW Agriculture and the University of New England, UNE, Armidale, NSW 2351.

<sup>C</sup>Corresponding author. Email: [jlumby@rivalea.com.au](mailto:jlumby@rivalea.com.au)

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

J. Hales<sup>A,C</sup>, V. A. Moustsen<sup>B</sup>, M. B. F. Nielsen<sup>B</sup> and C. F. Hansen<sup>A</sup>

<sup>A</sup>University of Copenhagen, Frederiksberg C, Denmark.

<sup>B</sup>SEGES, Pig Research Centre, Copenhagen, Denmark.

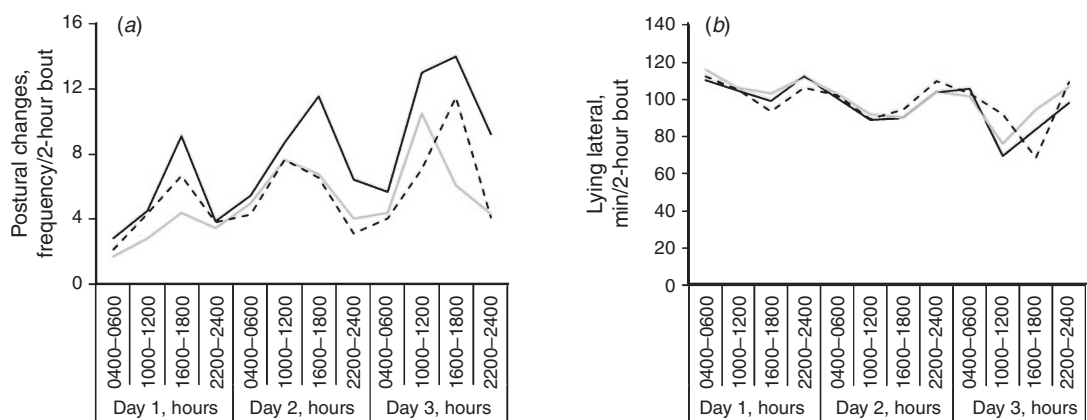
<sup>C</sup>Corresponding author. Email: [halessund@ku.dk](mailto:halessund@ku.dk)

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 sternal 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<sup>®</sup>; 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. 1a). 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. 1b). 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

M. Verdon<sup>A,C</sup>, R. S. Morrison<sup>B</sup> and P. H. Hemsworth<sup>A</sup>

<sup>A</sup>The University of Melbourne, Parkville, VIC 3010.

<sup>B</sup>Rivalea Rivalea (Australia), Corowa, NSW 2646.

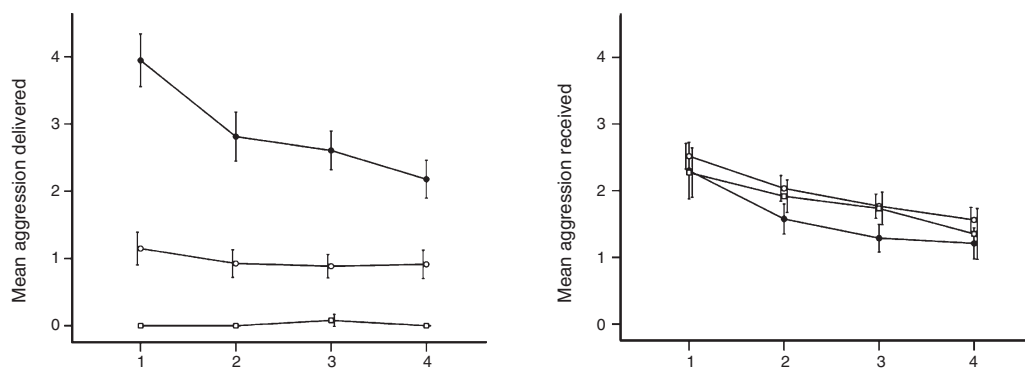
<sup>C</sup>Corresponding author. Email: [megan.verdon@unimelb.edu.au](mailto:megan.verdon@unimelb.edu.au)

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<sup>2</sup>/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 ( $\pm$  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

P. C. Condous<sup>A,C</sup>, K. J. Plush<sup>B</sup>, A. J. Tilbrook<sup>B</sup> and W. H. E. J. van Wettere<sup>A</sup>

<sup>A</sup>The University of Adelaide, Roseworthy, SA 5371.

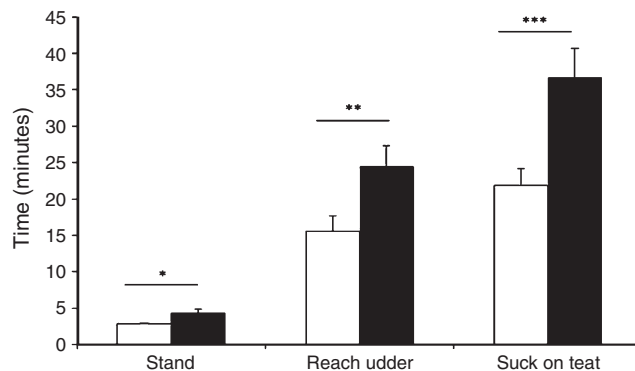
<sup>B</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

<sup>C</sup>Corresponding author. Email: [patricia.condous@adelaide.edu.au](mailto:patricia.condous@adelaide.edu.au)

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  $12.1 \pm 0.7$  and  $11.4 \pm 0.7$  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,  $3.9 \pm 0.1$  vs CLOSED,  $3.7 \pm 0.1$  mmol/L;  $P = 0.82$ ), rectal temperature at 2 hours after birth (OPEN,  $37.0 \pm 0.2$  vs CLOSED,  $37.4 \pm 0.2^\circ\text{C}$ ;  $P = 0.85$ ) or weight at 2 hours after birth (OPEN,  $1.4 \pm 0.03$  vs CLOSED,  $1.4 \pm 0.04$  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  $\pm$  SEM.

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## Pre-partum straw-directed behaviour by sows in farrowing pens is positively associated with piglet survival

G. M. Cronin<sup>A,C</sup>, G. F. Macnamara<sup>A</sup>, B. L. F. Macnamara<sup>A</sup>, M. A. Cronin<sup>A</sup>, K. E. Bøe<sup>B</sup> and I. L. Andersen<sup>B</sup>

<sup>A</sup>The University of Sydney, Camden, NSW 2570.

<sup>B</sup>Norwegian University of Life Sciences, P.O. Box 5003, 1432 Aas, Norway.

<sup>C</sup>Corresponding author. Email: greg.cronin@sydney.edu.au

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  $3.0 \pm 2.71$  (mean  $\pm$  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

W. H. E. J. van Wettere<sup>A,D</sup>, A. C. Weaver<sup>A</sup>, L. M. Staveley<sup>A</sup>, T. L. Muller<sup>B</sup>, R. J. E. Hewitt<sup>B</sup>,  
P. E. Hughes<sup>C</sup> and R. N. Kirkwood<sup>A</sup>

<sup>A</sup>The University of Adelaide, Roseworthy, SA 5005.

<sup>B</sup>Sunpork Farms, Loganholme, QLD 4129.

<sup>C</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

<sup>D</sup>Corresponding author. Email: [william.vanwettere@adelaide.edu.au](mailto:william.vanwettere@adelaide.edu.au)

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 ( $\leq 9$  vs  $\geq 12$  piglets; Small and Large, respectively) and suckled litter size (9, Small vs 12, Large). A plasma sample was collected at weaning ( $20 \pm 0.1$  days; mean  $\pm$  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  $102 \pm 0.5$  kg and  $169 \pm 1.5$  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  $\pm$  SEM

No. gilts	Small-Small 18	Litter size treatments (Gestated–Reared)		
		Small-Large 19	Large-Small 26	Large-Large 29
No. antral follicles	125.6 $\pm$ 14.96	134.2 $\pm$ 13.72	126.8 $\pm$ 12.38	104.1 $\pm$ 12.37
No. corpora lutea	11.2 $\pm$ 1.38	12.9 $\pm$ 1.19	13.1 $\pm$ 0.90	13.3 $\pm$ 0.98
Pubertal gilts (%)	39%*	47%	65%*	48%
Plasma AMH, weaning (ng/mL)	9.0 $\pm$ 0.58 <sup>b</sup>	7.3 $\pm$ 0.56 <sup>a</sup>	8.4 $\pm$ 0.46 <sup>b</sup>	7.8 $\pm$ 0.45 <sup>a</sup>
Plasma AMH, 20 weeks (ng/mL)	6.5 $\pm$ 0.43	6.2 $\pm$ 0.38	5.9 $\pm$ 0.34	6.5 $\pm$ 0.34

<sup>a,b</sup>Means in 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

T. Y. Chen<sup>A,E</sup>, C. Dickson<sup>B</sup>, D. Lines<sup>C</sup>, R. Kirkwood<sup>D</sup> and P. Langendijk<sup>A</sup>

<sup>A</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

<sup>B</sup>Lienert Australia, Roseworthy, SA 5371.

<sup>C</sup>SunPork Farms, Shea-Oak Log, SA 5400.

<sup>D</sup>The University of Adelaide, Roseworthy, SA 5371.

<sup>E</sup>Corresponding author. Email: tai.chen@sa.gov.au

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 piggyery.

The study was conducted on a commercial production unit in South Australia. Eight days before weaning, primiparous sows ( $n = 119$ ) weighing  $200 \pm 6.4$  kg (mean  $\pm$  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  $\geq 11$  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<sup>®</sup>; 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 be 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  $\pm$  SEM**

	Control ( $n = 60$ )	CHO ( $n = 59$ )
Litter size at allocation	$11.1 \pm 0.1$	$11.1 \pm 0.1$
Body weight loss (kg)	$-7.7 \pm 1.4$	$-5.8 \pm 1.2$
Energy balance (MJ ME <sup>A</sup> /d)	$-11 \pm 2^a$	$-5 \pm 2^b$
Anoestrous (%) <sup>B</sup>	15.5	13.5
Wean-mating-interval (d)	$4.8 \pm 0.1^a$	$4.3 \pm 0.2^b$
Average daily feed intake during treatment (kg)	$5.5 \pm 0.1^a$	$5.9 \pm 0.1^b$
Conception rate (%)	88	90
Total born second litter	$12.6 \pm 0.4$	$12.0 \pm 0.5$
Born alive second litter	$11.8 \pm 0.5$	$11.5 \pm 0.5$
TB <sup>C</sup>	$11.4 \pm 0.8$	$12.0 \pm 0.5$

<sup>A</sup>ME, metabolisable energy. <sup>B</sup>Sows mated  $>10$  d after weaning or not mated were considered anoestrous. <sup>C</sup>TB, total born. <sup>a,b</sup>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

T. Tsai<sup>A</sup>, S. Rajasekar<sup>A</sup> and J. C. St. John<sup>A,B</sup>

<sup>A</sup>Hudson Institute of Medical Research, Clayton, VIC 3168.

<sup>B</sup>Corresponding author. Email: [justin.stjohn@hudson.org.au](mailto:justin.stjohn@hudson.org.au)

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<sup>+</sup>) to incompetent (BCB<sup>-</sup>) COCs. Oocyte quality was also assessed by quantifying mtDNA copy number. Developmental potential of BCB<sup>+</sup> 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<sup>+</sup> to BCB<sup>-</sup> COCs per ovary was similar for each haplotype. However, mtDNA copy number for BCB<sup>+</sup> 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<sup>+</sup> 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

W. H. E. J. van Wettere<sup>A,B</sup> and L. M. Staveley<sup>A</sup>

<sup>A</sup>The University of Adelaide, Roseworthy, SA 5005.

<sup>B</sup>Corresponding author. Email: [william.vanwettere@adelaide.edu.au](mailto:william.vanwettere@adelaide.edu.au)

Piglet suckling 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  $1.2 \pm 0.09$ ; mean  $\pm$  SEM) suckling  $10.3 \pm 0.22$  piglets were used in a  $2 \times 2$  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  $18.4 \pm 0.15$  of lactation and ended at weaning on d  $27.0 \pm 0.15$  post-partum. The multi-suckling treatment consisted of three sows and their litters housed together with  $4.86 \text{ m}^2$  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  $18.4 \pm 0.15$  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  $\pm$  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	$5.4 \pm 0.42$	$5.2 \pm 0.42$	$4.3 \pm 0.47^a$	$6.1 \pm 0.41^b$
Piglet weight at weaning (kg)	$8.0 \pm 0.20$	$7.7 \pm 0.20$	$8.0 \pm 0.21$	$7.8 \pm 0.20$
Litter size at weaning	$10.1 \pm 0.25$	$10.1 \pm 0.25$	$10.3 \pm 0.26$	$9.9 \pm 0.25$
Sow LW change, day 18 to weaning (kg)	$-2.2 \pm 2.53$	$1.3 \pm 2.53$	$-0.1 \pm 2.63$	$-0.8 \pm 2.63$

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

R. Z. Athorn<sup>A,D</sup>, M. Blanes<sup>B</sup>, J. L. Patterson<sup>B</sup>, M. K. Dyck<sup>B</sup>, G. R. Foxcroft<sup>B</sup> and P. Langendijk<sup>C</sup>

<sup>A</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>B</sup>University of Alberta, AB T6G 2P5, Canada.

<sup>C</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

<sup>D</sup>Corresponding author. Email: rathorn@rivalea.com.au

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<sup>®</sup>; 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  $\pm$  SEM**

Treatment <sup>A</sup>	C28 (n = 19)	IS21 (n = 57)		
Lactational oestrus (%)	–	61 (35/57)		
		First heat	Skip-a-heat	NR <sup>B</sup>
Mating rate (%)	79 (15/19)	100 (18/18)	100 (17/17)	77 (17/22)
Pregnancy rate (%)	100 <sup>a</sup> (15/15)	83 <sup>ab</sup> (15/18)	100 <sup>a</sup> (17/17)	76 <sup>b</sup> (13/17)
Ovulation rate	23.4 $\pm$ 0.90 <sup>a</sup>	23.6 $\pm$ 0.87 <sup>a</sup>	19.6 $\pm$ 0.82 <sup>b</sup>	22.2 $\pm$ 0.94 <sup>ab</sup>
Number of live embryos	16.4 $\pm$ 1.04 (n = 14)	17.5 $\pm$ 1.15 (n = 10)	17.1 $\pm$ 1.07 (n = 12)	18.5 $\pm$ 1.09 (n = 12)
Embryonic survival (%)	70.8 $\pm$ 4.49 <sup>a</sup>	76.8 $\pm$ 4.92 <sup>ab</sup>	88.8 $\pm$ 4.63 <sup>b</sup>	85.3 $\pm$ 4.71 <sup>b</sup>
Embryonic weight (g)	1.52 $\pm$ 0.039 <sup>a</sup>	1.33 $\pm$ 0.045 <sup>b</sup>	1.46 $\pm$ 0.039 <sup>ab</sup>	1.49 $\pm$ 0.042 <sup>a</sup>
Allantochoirioic fluid volume (ml)	230.1 $\pm$ 7.78 <sup>a</sup>	187.0 $\pm$ 9.98 <sup>b</sup>	196.3 $\pm$ 8.08 <sup>b</sup>	210.1 $\pm$ 8.83 <sup>ab</sup>

<sup>A</sup>Refer to text for treatment details. <sup>B</sup>NR, non-responders. <sup>a,b</sup>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

P. M. Gurman<sup>A,B,E</sup>, G. Holds<sup>A</sup>, R. G. Jarrett<sup>C</sup>, T. Ross<sup>B</sup> and A. Kiermeier<sup>B,D</sup>

<sup>A</sup>South Australian Research and Development Institute, Urrbrae, SA 5064.

<sup>B</sup>University of Tasmania, Hobart, TAS 7001.

<sup>C</sup>Unley, SA 5061.

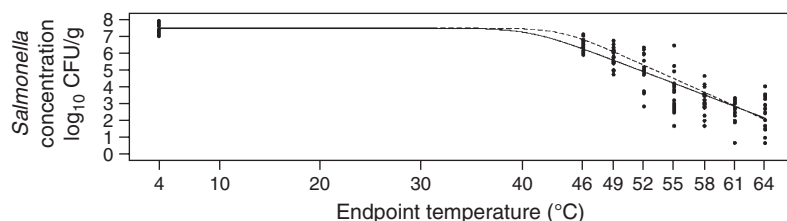
<sup>D</sup>Statistical Process Improvement Consulting and Training Pty Ltd, Gumeracha, SA 5233.

<sup>E</sup>Corresponding author. Email: Phillip.Gurman@gmail.com

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<sub>10</sub> CFU/g respectively for a 1°C increase in temperature. For both fat groups, a 5-log<sub>10</sub> 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

L. K. van Breda<sup>A,C</sup>, A. N. Ginn<sup>B</sup>, O. Dhungyel<sup>A</sup>, J. R. Iredell<sup>B</sup> and M. P. Ward<sup>A</sup>

<sup>A</sup>The University of Sydney, Camden, NSW 2570.

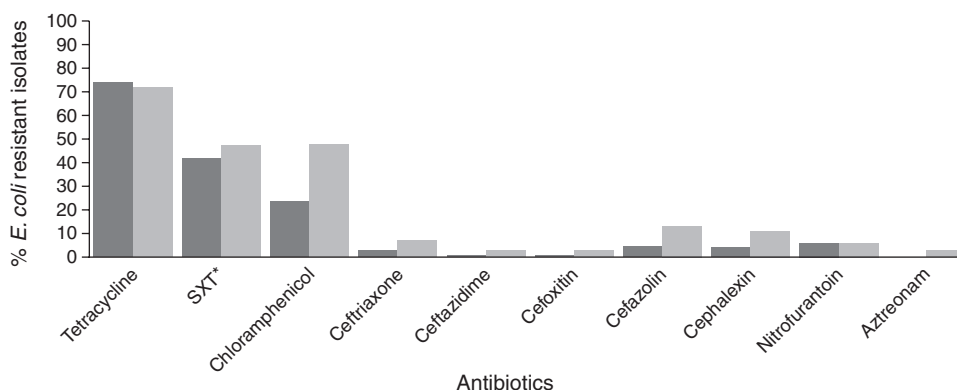
<sup>B</sup>The University of Sydney, Westmead, NSW 2145.

<sup>C</sup>Corresponding author. Email: lechelle.vanbreda@sydney.edu.au

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

D. Hamilton<sup>A,C</sup>, K. Hodgson<sup>A</sup>, A. Kiermeier<sup>A</sup> and M. McAllister<sup>B</sup>

<sup>A</sup>South Australian Research and Development Institute, Urrbrae, SA 5064.

<sup>B</sup>The University of Adelaide, Roseworthy, SA 5371.

<sup>C</sup>Corresponding author. Email: david.hamilton@sa.gov.au

*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  $2.7 \times 10^5$  *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  $2.3 \times 10^3$  and  $4 \times 10^3$  *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  $4.6 \times 10^4$  *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  $1.1 \times 10^5$  *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

M. Begum<sup>A</sup>, M. M. Hossain<sup>A</sup>, P. Y. Zhao<sup>A</sup>, J. W. Park<sup>A</sup> and I. H. Kim<sup>A,B</sup>

<sup>A</sup>Dankook University, Cheonan, Chungnam, South Korea.

<sup>B</sup>Corresponding author. Email: inhokim@dankook.ac.kr

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- $\beta$ -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  $\times$  Yorkshire  $\times$  Duroc) pigs with a bodyweight (BW) of  $20.8 \pm 3.20$  kg (mean  $\pm$  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<sup>®</sup>; 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 as suppressed *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 <sup>A</sup>
<i>Lactobacillus</i>	6.78 <sup>b</sup>	6.84 <sup>b</sup>	7.52 <sup>a</sup>	7.61 <sup>a</sup>	0.01
<i>E. coli</i>	6.65 <sup>a</sup>	6.51 <sup>ab</sup>	5.86 <sup>b</sup>	5.85 <sup>b</sup>	0.05

<sup>A</sup>SEM, standard error of the mean. <sup>a,b</sup>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

C. J. Brewster<sup>A,B</sup>, J. R. Craig<sup>A</sup>, C. L. Collins<sup>A</sup> and D. J. Henman<sup>A</sup>

<sup>A</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>B</sup>Corresponding author. Email: cbrewster@rivalea.com.au

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  $\beta$ -glucan ( $\beta$ G) 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<sup>TM</sup> Genetics, Corowa, NSW) were weaned at 26 days of age ( $8.9 \pm 0.21$  kg; mean  $\pm$  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<sup>®</sup> (Longlive Biotechnology) and 0.05% Fibosel<sup>®</sup> (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 +  $\beta$ G + SDPP also grew faster than those fed the LPS and CS XOS +  $\beta$ G 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 +  $\beta$ G + 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 <sup>b</sup>	484 <sup>ab</sup>	300 <sup>b</sup>	661 <sup>ab</sup>	3.81
CS	195 <sup>a</sup>	460 <sup>a</sup>	259 <sup>a</sup>	622 <sup>a</sup>	7.18
CS + XOS <sup>B</sup> + $\beta$ G <sup>C</sup>	262 <sup>bc</sup>	489 <sup>ab</sup>	284 <sup>ab</sup>	659 <sup>ab</sup>	1.91
CS + XOS <sup>B</sup> + $\beta$ G <sup>C</sup> + SDPP <sup>D</sup>	294 <sup>c</sup>	507 <sup>b</sup>	311 <sup>b</sup>	689 <sup>b</sup>	4.78
SED <sup>A</sup>	0.018	0.013	0.019	0.020	
<i>P</i> value	<0.01	0.01	0.04	0.02	0.07

<sup>A</sup>SED, standard error of difference of the mean. <sup>B</sup>Xylooligosaccharide. <sup>C</sup> $\beta$ -glucan. <sup>D</sup>Spray-dried porcine plasma. <sup>a,b,c</sup>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*

M. M. Capozzalo<sup>A</sup>, J. C. Kim<sup>B</sup>, J. K. Htoo<sup>C</sup>, C. F. M. de Lange<sup>D</sup>, B. P. Mullan<sup>B</sup>, J. W. Resink<sup>E</sup>,  
 C. F. Hansen<sup>F</sup> and J. R. Pluske<sup>A,G</sup>

<sup>A</sup>Murdoch University, Murdoch, WA 6150.

<sup>B</sup>Department of Agriculture and Food WA, South Perth 6151.

<sup>C</sup>Evonik Industries AG, Hanau, Germany.

<sup>D</sup>University of Guelph, Guelph, ON N1G 2W1, Canada.

<sup>E</sup>Nutreco, Boxmeer 5830, The Netherlands.

<sup>F</sup>University of Copenhagen, Copenhagen, Denmark.

<sup>G</sup>Corresponding author. Email: J.Pluske@murdoch.edu.au

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  $\times$  Large White) with an initial body weight (BW) of  $6.2 \pm 0.78$  kg (mean  $\pm$  SD) were stratified into one of four treatments according to a  $2 \times 2$  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 Sauviant *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* ( $3.44 \times 10^8$  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- $\gamma$ ). An acute phase protein index (APP Index) was calculated as follows: APP Index = (C-RP  $\times$  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- $\gamma$ , with pigs fed low Trp and high SAA having lower levels of IFN- $\gamma$ , and pigs fed either low Trp and low SAA or high Trp and high SAA having higher levels of IFN- $\gamma$  ( $P = 0.027$ ). 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- $\gamma$ ) 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 <sup>A</sup>	Trp	P value	
SID SAA:Lys	0.52	0.60	0.52	0.60			SAA	Trp $\times$ SAA
Day 0–14								
ADG <sup>B</sup> (g)	68	93	96	103	10.8	0.080	0.140	0.420
ADFI <sup>C</sup> (g)	168	175	163	175	14.1	0.872	0.492	0.852
FCR <sup>D</sup> (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- $\gamma$ (pg/mL)	6681 <sup>c</sup>	2235 <sup>a</sup>	4202 <sup>ab</sup>	4621 <sup>bc</sup>	1065.4	0.965	0.065	0.027

<sup>A</sup>SEM, standard error of the mean. <sup>B</sup>ADG, average daily gain. <sup>C</sup>ADFI, average daily feed intake. <sup>D</sup>FCR, feed conversion ratio. <sup>a,b,c</sup>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

M. Sacco<sup>A</sup> and P. L. Cakebread<sup>A,B</sup>

<sup>A</sup>The University of Melbourne, Parkville, VIC 3010.

<sup>B</sup>Corresponding author. Email: [pcake@unimelb.edu.au](mailto:pcake@unimelb.edu.au)

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<sup>®</sup>, 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  $\pm$  SE

Dental values		Feeding values	
Bite discrepancy (cm)	0.18 $\pm$ 1.29	Feeding time (min)	4.9 $\pm$ 0.39
Maxilla length (cm)	21.1 $\pm$ 2.2	Feed remaining (g)	255 $\pm$ 107
Mandible length (cm)	23.4 $\pm$ 2.5	Chews/min	175 $\pm$ 18.2
Chipped/cracked teeth (No.)	2.2 $\pm$ 1.16	Chews/min when head raised	20 $\pm$ 17.9
Displaced teeth (No.)	0.7 $\pm$ 0.83	No. of feeding events	3.1 $\pm$ 1.95
Missing/non erupted teeth (No.)	2.6 $\pm$ 3.03		
Broken teeth (No.)	0.2 $\pm$ 0.59		
Dental Wear Index	61 $\pm$ 6.6		

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## Numbers of selected bacterial species in pig faeces do not accurately represent their numbers in the ileum

B. G. Bowring<sup>A,B</sup> and A. M. Collins<sup>A</sup>

<sup>A</sup>Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568.

<sup>B</sup>Corresponding author. Email: bethany.bowring@dpi.nsw.gov.au

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<sub>10</sub> 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<sub>10</sub> numbers of *Cl. perfringens*, *E. coli*, *Enterobacteriaceae*, Lactobacilli and total bacteria (mean  $\pm$  SE), and percentages of selected bacteria in ileal mucosa and faeces

Bacterial group	Log <sub>10</sub> bacteria in ileal mucosa	Log <sub>10</sub> bacteria in faeces	Percentage bacteria in ileal mucosa relative to total	Percentage bacteria in faeces relative to total	Pearson correlation coefficient (R)
<i>Cl. perfringens</i>	7.04 $\pm$ 0.44 <sup>a</sup>	5.42 $\pm$ 0.37 <sup>b</sup>	1.35 $\pm$ 0.82 <sup>c</sup>	0.03 $\pm$ 0.02 <sup>d</sup>	0.205
<i>E. coli</i>	7.38 $\pm$ 0.67	7.54 $\pm$ 0.31	12.99 $\pm$ 6.48	1.14 $\pm$ 0.50	0.021
<i>Enterobacteriaceae</i>	8.08 $\pm$ 0.68	8.22 $\pm$ 0.35	69.87 $\pm$ 38.46	7.05 $\pm$ 3.75	0.081
Lactobacilli	5.28 $\pm$ 0.61 <sup>a</sup>	6.80 $\pm$ 0.41 <sup>b</sup>	0.27 $\pm$ 0.20	0.43 $\pm$ 0.29	0.423
Total bacteria	9.30 $\pm$ 0.21 <sup>a</sup>	10.03 $\pm$ 0.12 <sup>b</sup>	—	—	—

<sup>a,b,c,d</sup>Mean log<sub>10</sub> 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*

I. P. Oswald<sup>A,D</sup>, A. P. F. L. Bracarense<sup>B</sup> and N. Schwerin<sup>C</sup>

<sup>A</sup>INRA ToxAlim, Toulouse, France.

<sup>B</sup>University of Londrina, Brazil.

<sup>C</sup>Lallemand Pty. Ltd, Maroochydore, QLD 4558.

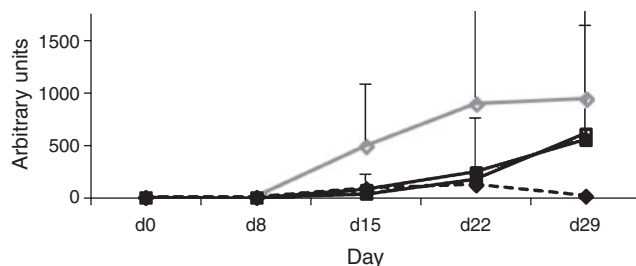
<sup>D</sup>Corresponding author. Email: [isabelle.oswald@toulouse.inra.fr](mailto:isabelle.oswald@toulouse.inra.fr)

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 ( $13.6 \pm 1.80$  kg; mean  $\pm$  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  $5 \times 10^9$  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. hyopneumoniae* 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<sup>®</sup>; 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 FB1. 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  $\mu$ m 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<sup>®</sup> as an inflammation model

R. L. Wilson<sup>A,E</sup>, R. E. Doyle<sup>A,B</sup>, G. M. Cronin<sup>C</sup> and P. K. Holyoake<sup>D</sup>

<sup>A</sup>Charles Sturt University and Graham Centre, Wagga Wagga, NSW 2678.

<sup>B</sup>Current address: The University of Melbourne, Parkville, VIC 3010.

<sup>C</sup>University of Sydney, Camden, NSW 2570.

<sup>D</sup>Holyoake Veterinary Consulting Pty Ltd, Strathfieldsaye, VIC 3551.

<sup>E</sup>Corresponding author. Email: [rewilson@csu.edu.au](mailto:rewilson@csu.edu.au)

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.* 2011a, 2011b). 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 [ $n = 8/\text{treatment}$ ; body weight  $34.5 \pm 0.51$  kg (mean  $\pm$  SE)]. Pigs were housed in pens of four (one per treatment group). Inflammation was induced using a single subcutaneous injection of Improvac<sup>®</sup> (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<sup>®</sup>; Merial, North Ryde, Australia), meloxicam (0.04 mg/kg Metacam<sup>®</sup>; Boehringer Ingelheim Vetmedica, North Ryde, Australia) or dexamethasone (1 mg/10 kg Dexason<sup>®</sup>; 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<sup>®</sup> treatment using Tridelta<sup>®</sup> Phase<sup>™</sup> 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 ( $\pm 330.8$ ) to 6612 ( $\pm 330.8$ ) and lowering to 4436 ( $\pm 330.8$ ) 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  $\pm$  SE**

Item	Saline	Ketoprofen	Meloxicam	Dexamethasone
CRP (ng/mL)	4585 $\pm$ 369	4048 $\pm$ 369	3895 $\pm$ 369	4306 $\pm$ 369
Haptoglobin (mg/mL)	1.9 $\pm$ 0.13 <sup>bc</sup>	1.4 $\pm$ 0.13 <sup>a</sup>	1.8 $\pm$ 0.13 <sup>b</sup>	2.1 $\pm$ 0.13 <sup>c</sup>
RT (°C)	39.5 $\pm$ 0.13 <sup>b</sup>	39.2 $\pm$ 0.13 <sup>a</sup>	39.2 $\pm$ 0.13 <sup>a</sup>	39.3 $\pm$ 0.13 <sup>ab</sup>

<sup>a,b,c</sup>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*

C. L. Collins<sup>A</sup>, P. McKenzie<sup>B,C</sup>, S. Beer<sup>A</sup>, K. S. O'Halloran<sup>A</sup> and A. Woeckel<sup>A</sup>

<sup>A</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>B</sup>McSwine, Seymour, VIC 3660.

<sup>C</sup>Corresponding author. Email: [peter@mcswine.com.au](mailto:peter@mcswine.com.au)

*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<sup>TM</sup> 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

P. K. Holyoake<sup>A,C</sup>, T. La<sup>B</sup>, N. D. Phillips<sup>B</sup> and D. J. Hampson<sup>B</sup>

<sup>A</sup>Holyoake Veterinary Consulting Pty Ltd, Strathfieldsaye, VIC 3551.

<sup>B</sup>Murdoch University, Murdoch, WA 6150.

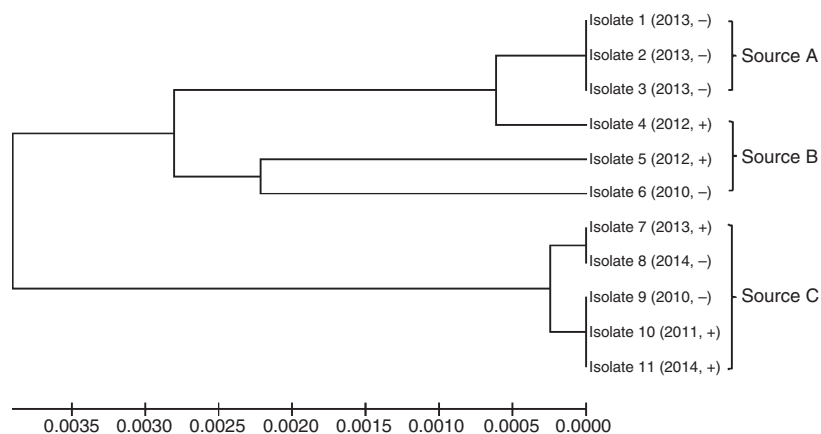
<sup>C</sup>Corresponding author. Email: trishpigvet@icloud.com

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  $\beta$ -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

S. Shafiullah<sup>A,D</sup>, M. Hernández-Jover<sup>A</sup>, D. Jordan<sup>B</sup>, M. Groves<sup>C</sup> and J. Heller<sup>A</sup>

<sup>A</sup>Charles Sturt University, Wagga Wagga, NSW 2678.

<sup>B</sup>Department of Primary Industries, Wollongbar, NSW 2478.

<sup>C</sup>The University of Queensland, Gatton, QLD 4343.

<sup>D</sup>Corresponding author. Email: [sshafiullah@csu.edu.au](mailto:sshafiullah@csu.edu.au)

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 culture 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

C. Turni<sup>A,C</sup>, R. Singh<sup>A</sup>, D. Dayao<sup>B</sup>, J. Gibson<sup>B</sup> and P. Blackall<sup>A</sup>

<sup>A</sup>The University of Queensland, St Lucia, QLD 4072.

<sup>B</sup>The University of Queensland, Gatton, QLD 4345.

<sup>C</sup>Corresponding author. Email: c.turni1@uq.edu.au

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), *fluA* (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 <sup>A</sup>					
			<i>vtaA</i>	<i>hhdA</i>	<i>hhdB</i>	<i>lsgB</i>	<i>fluA</i>	<i>capD</i>
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	+	+	+	–	–	+	–

<sup>A</sup>Potential virulence genes: *vtaA* (virulence-associated trimeric autotransporter), *hhdAB* (putative hemolysin operon), *lsgB* (lipopolysaccharide sialytransferase gene), *fluA* (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

J. Carr<sup>A,B</sup>

<sup>A</sup>Carrsconsulting, Melbourne, VIC 3000.

<sup>B</sup>Corresponding author. Email: [swineunit1@yahoo.com](mailto:swineunit1@yahoo.com)

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

B. J. Lohmar<sup>A</sup>, D. Wang<sup>B</sup>, J. Huang<sup>B</sup> and M. P. Boddington<sup>C,D</sup>

<sup>A</sup>U.S. Grains Council, Beijing Office, China World Trade Centre, Beijing, China.

<sup>B</sup>Center for Chinese Agricultural Policy, Beijing, China.

<sup>C</sup>Asian Agribusiness Consulting, Suite B209 Bai Jia Zhuang Commercial Centre, Beijing, China.

<sup>D</sup>Corresponding author. Email: michael@boddingtonconsulting.com

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 farmers 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
<i>Do you quarantine sick pigs?</i>						
Yes <sup>A</sup> (%)	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
<i>Do you mark sick pigs?</i>						
Yes (%)	74	76.8	71.4	57.4	59.2	68.4
No (%)	26	23.2	28.6	41.9	40.8	31.6
<i>Do you know withdrawal period of medication used?</i>						
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

<sup>A</sup>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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## Vitamin E but not selenium alleviates heat stress compromised metabolism in growing pigs

F. Liu<sup>A,C</sup>, J. J. Cottrell<sup>A</sup>, P. Celi<sup>B,A</sup>, B. J. Leury<sup>A</sup> and F. R. Dunshea<sup>A</sup>

<sup>A</sup>The University of Melbourne, Parkville, VIC 3010.

<sup>B</sup>The University of Sydney, Camden, NSW 2570.

<sup>C</sup>Corresponding author. Email: fanliu@student.unimelb.edu.au

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  $28.0 \pm 4.1$  kg (mean  $\pm$  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  $\alpha$ -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  $\mu$ U/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  $\mu$ M,  $P < 0.05$ ), 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<sup>A</sup>

OGTT parameters (time, min)	NRC <sup>B</sup>	20°C Se <sup>C</sup>	VE <sup>D</sup>	NRC	35°C Se	VE	SED	T <sup>E</sup>	Diet	<i>P</i> value 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 <sup>F</sup> 0 <sup>D</sup>	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					

<sup>A</sup>OGTT: oral glucose tolerance test. <sup>B</sup>Diet contains 0.24 ppm selenium, 17 IU/kg Vitamin E. <sup>C</sup>Diet contains 1.0 ppm selenium, 17 IU/kg Vitamin E. <sup>D</sup>Diet contains 0.24 ppm selenium, 200 IU/kg Vitamin E. <sup>E</sup>Temperature. <sup>F</sup>Insulin is expressed as  $\mu$ U/mL.

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## Pig feed ingredients affect enzyme diffusion coefficients

G. T. Nguyen<sup>A</sup>, W. L. Bryden<sup>A</sup>, M. J. Gidley<sup>A</sup> and P. A. Sopade<sup>A,B</sup>

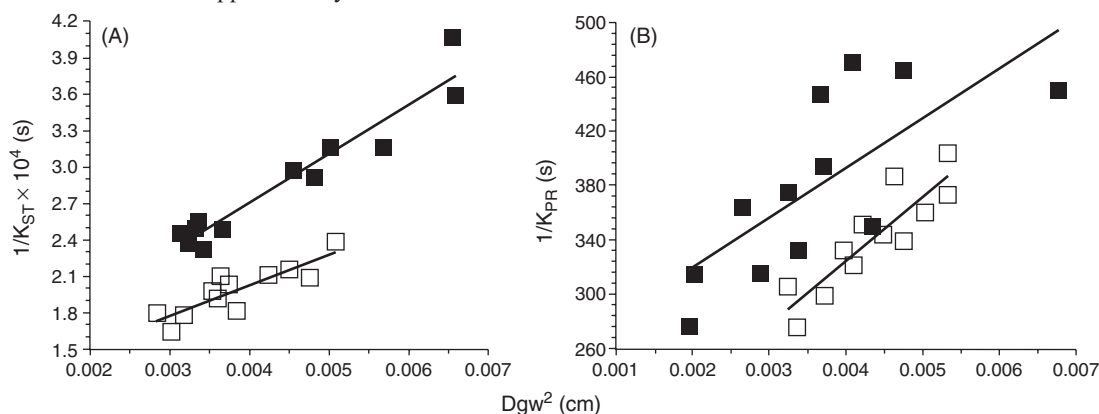
<sup>A</sup>The University of Queensland, St Lucia, QLD 4072.

<sup>B</sup>Corresponding author. Email: p.sopade@uq.edu.au

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  $1/K_{ST} \propto (D_{gw}^2/D_{IFF})$  and  $1/K_{PR} \propto (D_{gw}^2/D_{IFF})$  by regression. Minitab<sup>TM</sup> 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:  $D_{gw-grain} = 1.07 D_{gw-diet}$ ; sorghum:  $D_{gw-grain} = 0.91 D_{gw-diet}$ ,  $R^2 > 0.4$ ,  $P < 0.01$ ). However, the starch and protein contents (g/100g; mean  $\pm$  SD) were for the sorghum: grain -  $13 \pm 0.3$  protein,  $58 \pm 0.6$  starch, diet -  $23 \pm 0.5$  protein,  $33 \pm 0.7$  starch, and for the field peas: grain -  $22 \pm 0.1$  protein,  $42 \pm 0.1$  starch, diet -  $20 \pm 0.4$  protein,  $38 \pm 1.1$  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

D. J. Cadogan<sup>A,C</sup> and F. R. Dunshea<sup>B</sup>

<sup>A</sup>Feedworks, Lancefield, VIC 3435.

<sup>B</sup>The University of Melbourne, Parkville, VIC 3010.

<sup>C</sup>Corresponding author. Email: david.cadogan@feedworks.com.au

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 to rations containing high quality proteins such as soybean meal (SBM).

The study was 2 × 2 × 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<sup>®</sup> 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)	CP	CTTAD (ileum)		
						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 <sup>A</sup>			0.253	0.084	0.70	0.68	0.49	0.87
<i>P</i> 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

<sup>A</sup>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

P. Guggenbuhl<sup>A,C</sup>, E. Perez Calvo<sup>A</sup> and F. Fru<sup>B</sup>

<sup>A</sup>DSM Nutritional Products SA, Saint-Louis, France.

<sup>B</sup>DSM Nutritional Products Ltd, Basel, Switzerland.

<sup>C</sup>Corresponding author. Email: [patrick.guggenbuhl@dsm.com](mailto:patrick.guggenbuhl@dsm.com)

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* (Ronozyne 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  $7.9 \pm 0.73$  kg (mean  $\pm$  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 Ronozyne 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.* 2012a, 2012b). 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 <sup>A</sup>	P value
				H1000	H2000	H3000	Q500	Q1000	Q1500		
ADG (g)	0–14	154 <sup>a</sup>	168 <sup>a</sup>	186 <sup>ab</sup>	226 <sup>b</sup>	173 <sup>a</sup>	162 <sup>a</sup>	154 <sup>a</sup>	202 <sup>ab</sup>	6.3	0.043
	15–42	430 <sup>a</sup>	482 <sup>abc</sup>	511 <sup>bc</sup>	542 <sup>c</sup>	509 <sup>bc</sup>	442 <sup>ab</sup>	505 <sup>bc</sup>	530 <sup>c</sup>	9.5	0.024
	0–42	336 <sup>a</sup>	375 <sup>abc</sup>	400 <sup>bcd</sup>	432 <sup>d</sup>	394 <sup>bcd</sup>	347 <sup>ab</sup>	385 <sup>abcd</sup>	418 <sup>cd</sup>	7.4	0.015
ADFI (g)	0–14	256	261	279	331	280	251	250	288	7.4	NS <sup>B</sup>
	15–42	807	853	918	953	927	848	888	943	14.7	NS
	0–42	606 <sup>a</sup>	639 <sup>ab</sup>	697 <sup>bc</sup>	739 <sup>c</sup>	706 <sup>bc</sup>	644 <sup>ab</sup>	671 <sup>abc</sup>	720 <sup>bc</sup>	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

<sup>A</sup>SEM, standard error of the mean. <sup>B</sup>NS, not significant. <sup>a,b,c,d</sup>Means in 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

E. Kiarie<sup>A,B,D</sup>, M. C. Walsh<sup>A</sup>, L. Romero<sup>A</sup>, X. Yang<sup>C</sup> and S. Baidoo<sup>C</sup>

<sup>A</sup>Dupont Industrial Biosciences-Danisco Animal Nutrition, Marlborough SN8 1AA, UK.

<sup>B</sup>University of Manitoba, Winnipeg, MB R3T 2N2, Canada.

<sup>C</sup>University of Minnesota, Waseca, MN 56093, USA.

<sup>D</sup>Corresponding author. Email: [elijah.kiarie@dupont.com](mailto:elijah.kiarie@dupont.com)

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 ( $6.6 \pm 0.1$  kg; mean  $\pm$  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  $\beta$ -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 1, 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<sup>®</sup>; 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 (CTTAD) in nursery pigs

Item	Phase 1, days 0–14						Phase 2, days 15–42				
	Performance			CTTAD			Performance			ATTD	
	ADG	ADFI	G : F	GE <sup>B</sup>	CP <sup>C</sup>	BW42	ADG	ADFI	G : F	GE	CP
PC	264 <sup>a</sup>	327	0.82 <sup>a</sup>	0.80 <sup>a</sup>	0.75 <sup>a</sup>	28.9 <sup>a</sup>	664 <sup>a</sup>	989	0.67	0.73 <sup>a</sup>	0.70 <sup>a</sup>
NC	217 <sup>b</sup>	320	0.68 <sup>b</sup>	0.73 <sup>b</sup>	0.70 <sup>b</sup>	27.2 <sup>b</sup>	626 <sup>ab</sup>	996	0.64	0.70 <sup>b</sup>	0.66 <sup>b</sup>
ME	265 <sup>a</sup>	372	0.71 <sup>b</sup>	0.78 <sup>a</sup>	0.74 <sup>a</sup>	27.7 <sup>ab</sup>	618 <sup>b</sup>	983	0.63	0.74 <sup>a</sup>	0.71 <sup>a</sup>
SEM <sup>A</sup>	15.5	18.4	0.02	1.41	1.85	0.54	14.0	29.6	0.01	0.66	1.25

<sup>A</sup>SEM, standard error of means. <sup>B</sup>GE, gross energy. <sup>C</sup>CP, crude protein. <sup>a,b</sup>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

B. A. Dearlove<sup>A,B</sup>, K. E. Kind<sup>A</sup> and W. H. E. J. van Wettere<sup>A</sup>

<sup>A</sup>The University of Adelaide, Roseworthy, SA 5371.

<sup>B</sup>Corresponding author. Email: brooke.dearlove@adelaide.edu.au

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  $3.2 \pm 0.14$ ; mean  $\pm$  SE) Large White  $\times$  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  $\pm$  SE of the mean.

There were no treatment effects ( $P > 0.05$ ) (Control vs Caffeine) on total born litter size ( $11.9 \pm 0.56$  vs  $11.8 \pm 0.53$ ), 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 ( $0.29 \pm 0.09$  vs  $0.67 \pm 0.15$ ;  $P < 0.05$ ) and had more live born piglets ( $11.65 \pm 0.22$  versus  $11.01 \pm 0.23$ ;  $P < 0.05$ ). 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 Treatment	First		Middle		Last	
	Control	Caffeine	Control	Caffeine	Control	Caffeine
Time to udder (mins)	16.24 <sup>a</sup>	29.21 <sup>b</sup>	25.24	27.43	20.37 <sup>a</sup>	34.81 <sup>b</sup>
Time to suck (mins)	41.81	52.36	49.00	48.24	37.74 <sup>a</sup>	52.90 <sup>b</sup>

<sup>a,b</sup>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

J. C. Kim<sup>A,C</sup>, K. L. Moore<sup>A</sup>, M. Trezona<sup>A</sup>, M. D. Langridge<sup>A</sup>, B. P. Mullan<sup>A</sup> and J. R. Pluske<sup>B</sup>

<sup>A</sup>Department of Agriculture and Food, South Perth, WA 6151.

<sup>B</sup>Murdoch University, Murdoch, WA 6150.

<sup>C</sup>Corresponding author. Email: [jae.kim@agric.wa.gov.au](mailto:jae.kim@agric.wa.gov.au)

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*- $\alpha$ -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<sub>2</sub> (PGE<sub>2</sub>) 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<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub>, 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 <sup>A</sup>	<i>P</i> value
<i>Liver</i>							
PGE <sub>2</sub> <sup>B</sup>	26	32	22	34	25	3.8	0.170
tGSH <sup>C</sup>	3772 <sup>ab</sup>	3335 <sup>a</sup>	4138 <sup>b</sup>	3949 <sup>b</sup>	3609 <sup>ab</sup>	201.6	0.083
<i>Ileum</i>							
PGE <sub>2</sub>	108 <sup>a</sup>	126 <sup>ab</sup>	71 <sup>a</sup>	105 <sup>a</sup>	200 <sup>b</sup>	30.0	0.066
tGSH	4226	3812	4004	3704	4279	290.7	0.537
<i>mRNA expression in the colon</i>							
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

<sup>A</sup>SEM, standard error of mean. <sup>B</sup>PGE<sub>2</sub> (μg/g wet tissue), prostaglandin E<sub>2</sub>. <sup>C</sup>tGSH (μg/g wet tissue), total glutathione. <sup>a,b,c</sup>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

M. Fu<sup>A</sup>, C. L. Collins<sup>B</sup>, D. J. Henman<sup>B</sup> and E. Roura<sup>A,C</sup>

<sup>A</sup>The University of Queensland, St Lucia, QLD 4072.

<sup>B</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>C</sup>Corresponding author. Email: e.roura@uq.edu.au

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<sup>®</sup>-treated male pigs with a body weight (BW) of  $65.2 \pm 5.54$  kg (mean  $\pm$  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<sup>®</sup>; 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**

Item	Con.	Caff	Rhu	Treatments <sup>A</sup>				SEM <sup>B</sup>
				Bra	Gen	Qua	Art	
Initial BW (kg)	72.0	72.0	72.0	72.0	72.0	72.0	71.9	0.43
Final BW (kg)	114.0 <sup>ab</sup>	111.7 <sup>b</sup>	116.1 <sup>a</sup>	115.7 <sup>ab</sup>	117.5 <sup>a</sup>	117.1 <sup>a</sup>	117.7 <sup>a</sup>	0.75
Days 0 to 35								
ADG (kg)	1.20 <sup>ab</sup>	1.12 <sup>b</sup>	1.28 <sup>ab</sup>	1.25 <sup>ab</sup>	1.31 <sup>a</sup>	1.29 <sup>ab</sup>	1.31 <sup>a</sup>	0.015
ADFI (kg)	3.03 <sup>a</sup>	2.73 <sup>b</sup>	3.07 <sup>a</sup>	3.15 <sup>a</sup>	3.14 <sup>a</sup>	3.09 <sup>a</sup>	3.20 <sup>a</sup>	0.038
FCR <sup>C</sup> (kg:kg)	2.55 <sup>a</sup>	2.47 <sup>ab</sup>	2.40 <sup>b</sup>	2.55 <sup>a</sup>	2.40 <sup>b</sup>	2.40 <sup>b</sup>	2.45 <sup>ab</sup>	0.021
Carcass characteristics								
Carcass yield (%)	73.8	73.9	73.2	73.6	73.9	74.1	74.0	0.15
HSCW <sup>D</sup> (kg)	84.2 <sup>ab</sup>	82.6 <sup>b</sup>	85.5 <sup>ab</sup>	85.6 <sup>ab</sup>	86.9 <sup>a</sup>	86.8 <sup>a</sup>	87.1 <sup>a</sup>	0.58
Backfat depth (mm) <sup>E, F</sup>	13.5 <sup>a</sup>	11.5 <sup>b</sup>	12.8 <sup>ab</sup>	13.8 <sup>a</sup>	14.3 <sup>a</sup>	13.4 <sup>ab</sup>	13.7 <sup>a</sup>	0.24
Loin depth (mm) <sup>F</sup>	52.9 <sup>ab</sup>	51.9 <sup>b</sup>	54.0 <sup>ab</sup>	54.2 <sup>ab</sup>	53.4 <sup>ab</sup>	56.5 <sup>a</sup>	56.2 <sup>a</sup>	0.53

<sup>A</sup>Con., Control; Caff, caffeine; Rhu, rhubarb; Bra, brassica; Gen, gentian; Qua, quassia; Art, artemisia. <sup>B</sup>SEM, standard error of the mean. <sup>C</sup>FCR, feed conversion ratio. <sup>D</sup>HSCW, hot standard carcass weight. <sup>E</sup>P2 position. <sup>F</sup>Analysed using HSCW as a covariate. <sup>a,b</sup>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

J. J. Cottrell<sup>A</sup>, A. J. Gabler<sup>A</sup>, S. C. Grimshaw<sup>A</sup>, D. J. Cadogan<sup>B</sup> and F. R. Dunshea<sup>A,C</sup>

<sup>A</sup>The University of Melbourne, Parkville, VIC 3010.

<sup>B</sup>Feedworks, Lancefield, VIC 3435.

<sup>C</sup>Corresponding author. Email: fdunshea@unimelb.edu.au

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  $7.96 \pm 0.047$  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 <sup>A</sup>	Diet	P value	
		Bovine	Ovine	Porcine			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
<i>Days 1–7</i>								
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
<i>Days 1–21</i>								
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

<sup>A</sup>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

R. J. E. Hewitt<sup>A,D</sup>, D. Isaac<sup>B</sup>, J. Vande Ginste<sup>C</sup> and R. J. van Barneveld<sup>A</sup>

<sup>A</sup>SunPork Farms, Loganholme, QLD 4129.

<sup>B</sup>BEC Feed Solutions Pty Ltd, Carole Park, QLD 4300.

<sup>C</sup>Nuscience, Drongen, Belgium 9031.

<sup>D</sup>Corresponding author. Email: robert.hewitt@sunporkfarms.com.au

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<sup>®</sup> 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<sup>®</sup> Pig, with or without the presence of medication, will enhance the performance of weaner pigs.

Newly-weaned pigs aged 23 days and weighing  $5.1 \pm 0.10$  kg (mean  $\pm$  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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> Pig in the presence (T1) or absence (T2) of medication compared with a medicated Control diet**

	Control	T1	T2	SED <sup>A</sup>	P value
Entry weight (kg)	5.1	5.1	5.1	0.27	0.963
Exit weight (kg)	11.9 <sup>a</sup>	12.9 <sup>b</sup>	11.7 <sup>a</sup>	0.18	<0.001
ADG <sup>B</sup> (kg)	0.242 <sup>a</sup>	0.279 <sup>b</sup>	0.237 <sup>a</sup>	0.007	<0.001
ADFI <sup>C</sup> (kg)	0.38 <sup>a</sup>	0.39 <sup>a</sup>	0.34 <sup>b</sup>	0.011	0.003
FCR <sup>D</sup> (kg:kg)	1.56 <sup>a</sup>	1.40 <sup>b</sup>	1.45 <sup>b</sup>	0.032	<0.001
Week 1 ADG (kg)	0.090 <sup>a</sup>	0.125 <sup>b</sup>	0.094 <sup>a</sup>	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 <sup>a</sup>	1.14 <sup>b</sup>	1.29 <sup>b</sup>	0.082	0.002
Deaths	10	0	1	$\chi^2$ (2, n = 140) = 16.99, $P < 0.001$	

<sup>A</sup>SED, standard error of difference between means. <sup>B</sup>ADG, average daily gain. <sup>C</sup>ADFI, average daily feed intake. <sup>D</sup>FCR, feed conversion ratio. <sup>a,b</sup>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

J. R. Pluske<sup>A,E</sup>, J. L. Black<sup>B</sup>, J. C. Kim<sup>C</sup> and F. R. Dunshea<sup>D</sup>

<sup>A</sup>Murdoch University, Murdoch, WA 6150.

<sup>B</sup>John L. Black Consulting, Warrimoo, NSW 2774.

<sup>C</sup>Department of Agriculture and Food, South Perth, WA 6151.

<sup>D</sup>The University of Melbourne, Parkville, VIC 3010.

<sup>E</sup>Corresponding author. Email: J.Pluske@murdoch.edu.au

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  $66.9 \pm 0.19$  kg (mean  $\pm$  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%  $\text{CaCl}_2 + 2.2\%$   $\text{Na}_5\text{P}_3\text{O}_{10}$ ; 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%  $\text{CaCl}_2 + 2.2\%$   $\text{Na}_5\text{P}_3\text{O}_{10}$  in the first week, so subsequently the inclusion rate was halved for each additive (to 2%  $\text{CaCl}_2 + 1.1\%$   $\text{Na}_5\text{P}_3\text{O}_{10}$ ). Overall, ADFI was lowest ( $P = 0.007$ ) in pigs fed  $\text{CaCl}_2 + \text{Na}_5\text{P}_3\text{O}_{10}$  (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  $\text{CaCl}_2 + \text{Na}_5\text{P}_3\text{O}_{10}$  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  $\text{CaCl}_2 + \text{Na}_5\text{P}_3\text{O}_{10}$  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  $\text{CaCl}_2 + \text{Na}_5\text{P}_3\text{O}_{10}$  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	$\text{CaCl}_2 + \text{Na}_5\text{P}_3\text{O}_{10}$	CDCA	LA	LSD	<i>P</i> value
<i>BW (kg)</i>						
d 7 <sup>A</sup>	76.0	72.9	76.8	77.5	2.18	0.002
d 14 <sup>A</sup>	86.9	81.8	86.7	86.8	3.54	0.018
d 21 <sup>A</sup>	93.8	89.8	94.9	93.4	3.53	0.039
<i>ADFI<sup>B</sup> (kg)</i>						
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
<i>Overall performance</i>						
ADG <sup>C</sup> (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 <sup>D</sup> (kg:kg)	2.81	2.81	2.52	2.55	0.235	0.023

<sup>A</sup>Day 0 BW used as a covariate. <sup>B</sup>ADFI, average daily feed intake. <sup>C</sup>ADG, average daily gain. <sup>D</sup>FCR, feed conversion ratio.

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## Use of trace mineral analysis to quantify the efficacy of mineral supplementation

T. L. Muller<sup>A,B</sup>, R. J. E. Hewitt<sup>A</sup> and R. J. van Barneveld<sup>A</sup>

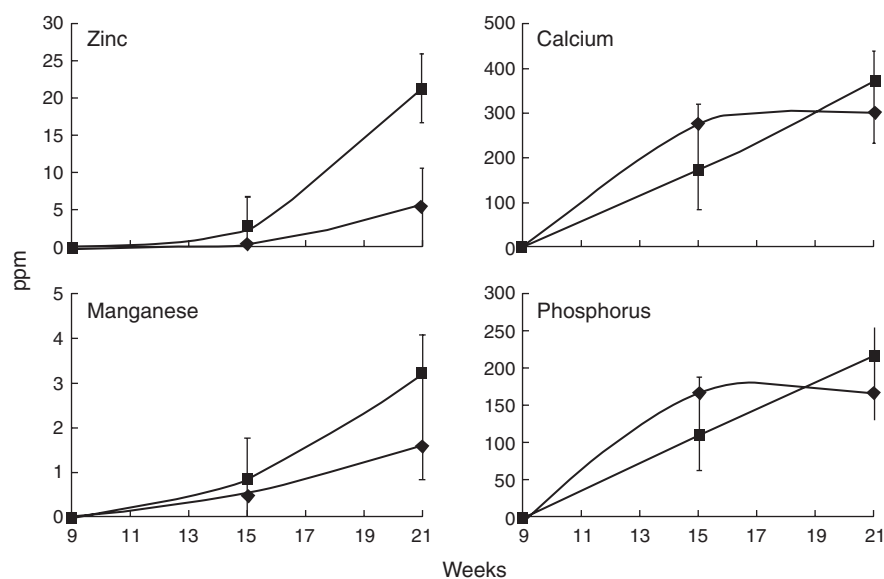
<sup>A</sup>SunPork Farms, Loganholme, QLD 4129.

<sup>B</sup>Corresponding author. Email: [tracy.muller@sunporkfarms.com.au](mailto:tracy.muller@sunporkfarms.com.au)

Trace minerals, in particular those that are complexed to organic molecules, have been associated with decreased foot lesions and lameness (Anil *et al.* 2010a) and have led to increased numbers of pigs born alive and litter birth weights (Anil *et al.* 2010b). 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 (Avalia<sup>®</sup>; 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

E. Kiarie<sup>A,B,D</sup> and R. Petracek<sup>C</sup>

<sup>A</sup>Dupont Industrial Biosciences-Danisco Animal Nutrition, Marlborough SN8 1AA, UK.

<sup>B</sup>University of Manitoba, Winnipeg, MB R3T 2N2, Canada.

<sup>C</sup>Prairie Swine Centre Inc., Saskatoon, SK S7H 5N9, Canada.

<sup>D</sup>Corresponding author. Email: [elijah.kiarie@dupont.com](mailto:elijah.kiarie@dupont.com)

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 ( $9.2 \pm 0.16$  kg BW; mean  $\pm$  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<sup>®</sup>; 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 <sup>A</sup>	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

<sup>A</sup>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

P. Y. Zhao<sup>A</sup>, J. W. Park<sup>A</sup>, J. M. Heo<sup>B</sup>, J. H. Yoo<sup>B</sup>, S. D. Upadhaya<sup>A</sup> and I. H. Kim<sup>A,C</sup>

<sup>A</sup>Dankook University, Cheonan, Chungnam, South Korea.

<sup>B</sup>Chungnam National University, Daejeon, South Korea.

<sup>C</sup>Corresponding author. Email: inhokim@dankook.ac.kr

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) ( $205 \pm 1.6$  kg; mean  $\pm$  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<sup>®</sup>; 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

Y. Lei<sup>A,B</sup>, H. L. Li<sup>A</sup>, P. Y. Zhao<sup>A</sup>, J. W. Park<sup>A</sup> and I. H. Kim<sup>A,C</sup>

<sup>A</sup>Dankook University, Cheonan, Chungnam, South Korea.

<sup>B</sup>DadHank Biotechnology Corporation, Chengdu, Sichuan, China.

<sup>C</sup>Corresponding author. Email: inhokim@dankook.ac.kr

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  $237 \pm 1.9$  kg (mean  $\pm$  SE; BW measured at 7 days before farrowing) was allotted into one of four treatments using a  $2 \times 2$  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<sup>®</sup>; 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 <sup>A</sup>	<i>P</i> 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 <sup>B</sup>	W21	SE	<i>P</i> 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

<sup>A</sup>SE, standard error. <sup>B</sup>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

P. Y. Zhao<sup>A</sup>, J. W. Park<sup>A</sup>, J. M. Heo<sup>B</sup>, J. H. Yoo<sup>B</sup>, J. S. Jeong<sup>A</sup> and I. H. Kim<sup>A,C</sup>

<sup>A</sup>Dankook University, Cheonan, Chungnam, South Korea.

<sup>B</sup>Chungnam National University, Daejeon, South Korea.

<sup>C</sup>Corresponding author. Email: inhokim@dankook.ac.kr

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  $6.6 \pm 0.29$  kg (mean  $\pm$  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

P. Y. Zhao<sup>A</sup>, J. W. Park<sup>A</sup>, S. Mohana Devi<sup>A</sup>, K. Y. Lee<sup>B</sup> and I. H. Kim<sup>A,C</sup>

<sup>A</sup>Dankook University, Cheonan, Chungnam, South Korea.

<sup>B</sup>Morningbio Co., Ltd, Cheonan, Chungnam, South Korea.

<sup>C</sup>Corresponding author. Email: inhokim@dankook.ac.kr

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  $252 \pm 11.7$  kg (mean  $\pm$  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 $\times$ 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<sub>2</sub>S 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

J. W. Park<sup>A</sup>, P. Y. Zhao<sup>A</sup>, M. Begum<sup>A</sup>, M. M. Hossain<sup>A</sup> and I. H. Kim<sup>A,B</sup>

<sup>A</sup>Dankook University, Cheonan, Chungnam, South Korea.

<sup>B</sup>Corresponding author. Email: inhokim@dankook.ac.kr

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  $25.1 \pm 2.01$  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<sup>®</sup>; 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 (Irekhorre *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 <sup>A</sup>	<i>P</i> value	
					Linear	Quadratic
<i>Growth performance (Overall)</i>						
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
<i>Haematological profiles (d 4)</i>						
RBC (×10 <sup>6</sup> /uL)	6.95	6.86	7.21	0.167	0.246	0.271
WBC (×10 <sup>3</sup> /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

<sup>A</sup>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

S. J. Wilkinson<sup>A,D</sup>, B. P. Mullan<sup>B</sup>, J. C. Kim<sup>B</sup> and J. A. Downing<sup>C</sup>

<sup>A</sup>Feedworks, Lancefield, VIC 3435.

<sup>B</sup>Department of Agriculture and Food Western Australia, South Perth, WA 6151.

<sup>C</sup>The University of Sydney, Camden, NSW 2570.

<sup>D</sup>Corresponding author. Email: [stuart.wilkinson@feedworks.com.au](mailto:stuart.wilkinson@feedworks.com.au)

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  $40 \pm 0.2$  kg (mean  $\pm$  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**

<i>n</i> -6: <i>n</i> -3 PUFA ratio	4:1	8:1	12:1	SEM <sup>A</sup>	<i>P</i> value
<i>BW (kg)</i>					
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
<i>ADG (kg)</i>					
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
<i>FCR (kg : kg)</i>					
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
<i>Carcass measurements</i>					
HSCW <sup>B</sup> (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

<sup>A</sup>SEM, standard error of the mean. <sup>B</sup>HSCW, hot standard carcass weight.

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## Increasing zinc via an inorganic source (ZnO) in high calcium finisher diets improves growth performance

J. R. Craig<sup>A,B</sup>, T. McDonald<sup>A</sup>, C. J. Brewster<sup>A</sup> and D. J. Henman<sup>A</sup>

<sup>A</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>B</sup>Corresponding author. Email: [jcraig@rivalea.com.au](mailto:jcraig@rivalea.com.au)

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<sub>3</sub>) 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  $\pm$  0.43; mean  $\pm$  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<sub>3</sub>) (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<sup>®</sup> 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  $\pm$  SEM

Treatment	Control	Control + 550 ppm ZnO	Control + 550 ppm Bioplex Zn
ADG <sup>A</sup> (kg)			
Days 21–35	1.2 $\pm$ 0.04 <sup>a</sup>	1.4 $\pm$ 0.06 <sup>b</sup>	1.2 $\pm$ 0.03 <sup>a</sup>
Days 0–35	1.1 $\pm$ 0.03 <sup>ab</sup>	1.2 $\pm$ 0.03 <sup>b</sup>	1.0 $\pm$ 0.03 <sup>a</sup>
FCR <sup>B</sup> (kg:kg)			
Days 21–35	2.6 $\pm$ 0.05 <sup>a</sup>	2.3 $\pm$ 0.07 <sup>b</sup>	2.7 $\pm$ 0.07 <sup>a</sup>
Days 0–35	2.4 $\pm$ 0.05 <sup>a</sup>	2.3 $\pm$ 0.04 <sup>b</sup>	2.5 $\pm$ 0.07 <sup>a</sup>
HSCW (kg)	67.9 $\pm$ 1.29 <sup>ab</sup>	69.2 $\pm$ 1.33 <sup>a</sup>	65.4 $\pm$ 1.29 <sup>b</sup>
Carcass P2 (mm)	11.4 $\pm$ 0.51 <sup>a</sup>	12.0 $\pm$ 0.55 <sup>a</sup>	11.6 $\pm$ 0.61 <sup>a</sup>

<sup>A</sup>ADG, average daily gain. <sup>B</sup>FCR, feed conversion ratio. <sup>a,b</sup>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

W. H. E. J. van Wettere<sup>A,C</sup>, L. M. Staveley<sup>A</sup>, A. C. Weaver<sup>A</sup> and K. J. Plush<sup>B</sup>

<sup>A</sup>The University of Adelaide, Roseworthy, SA 5005.

<sup>B</sup>South Australian Research and Development Institute, Roseworthy, SA 5371.

<sup>C</sup>Corresponding author. Email: [William.vanwettere@adelaide.edu.au](mailto:William.vanwettere@adelaide.edu.au)

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  $3.9 \pm 0.19$ ; mean  $\pm$  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 ( $13.0 \pm 0.3$ ), born alive ( $12.2 \pm 0.3$ ) or stillborn ( $0.8 \pm 0.1$ ) (all mean  $\pm$  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 SEM <sup>A</sup>
	0.0%	2.5%	5.0%	First	Middle	Last	
Time to udder (sec)	49.1 <sup>b</sup>	22.5 <sup>a</sup>	28.0 <sup>a</sup>	30.3	35.4	32.1	3.90
Time to suckle (sec)	51.0 <sup>b</sup>	32.0 <sup>a</sup>	42.5 <sup>b</sup>	47.9 <sup>b</sup>	44.0 <sup>ab</sup>	33.5 <sup>a</sup>	2.39
24 h piglet LW gain, kg	0.05 <sup>a</sup>	0.08 <sup>ab</sup>	0.10 <sup>b</sup>	0.08	0.07	0.08	0.07
24 h glucose (mmol/l)	5.90 <sup>a</sup>	6.77 <sup>b</sup>	6.12 <sup>a</sup>	6.43	6.18	6.19	0.31
24 h immunocrit	0.124	0.127	0.132	0.137 <sup>b</sup>	0.127 <sup>ab</sup>	0.120 <sup>a</sup>	0.01

<sup>A</sup>SEM, standard error of the mean. <sup>a,b</sup>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*

S. L. Beer<sup>A,C</sup>, C. L. Collins<sup>A</sup>, D. J. Henman<sup>A</sup> and A. Naylor<sup>B</sup>

<sup>A</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>B</sup>Alltech Inc., Roseworthy, SA 5371.

<sup>C</sup>Corresponding author. Email: [sbeer@rivalea.com.au](mailto:sbeer@rivalea.com.au)

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<sub>4</sub>) 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<sub>4</sub> 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<sub>4</sub>) 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 carcass 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 <sup>A</sup>	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

<sup>A</sup>SED, standard error of difference between means.

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## Composition of enzyme mixtures influences the faecal digestion of a weaner pig feed

D. W. Zheng<sup>A</sup>, L. Kang<sup>A</sup>, N. Dong<sup>A,B</sup> and B. J. Hosking<sup>A</sup>

<sup>A</sup>AsiaPac (Dongguan) Biotechnology, Dongguan City, Guangdong PRC 523808.

<sup>B</sup>Corresponding author. Email: ningdong@asiapac.cn

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  $12.8 \pm 0.59$  kg (mean  $\pm$  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 (EC3.2.1.8, 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<sup>®</sup>, 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 <sup>A</sup>	P value <sup>B</sup>
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 <sup>a</sup>	0.88 <sup>a</sup>	0.89 <sup>ab</sup>	0.91 <sup>b</sup>	0.90 <sup>ab</sup>	0.005	0.024
Acid detergent fibre	0.34 <sup>a</sup>	0.47 <sup>ab</sup>	0.45 <sup>ab</sup>	0.47 <sup>ab</sup>	0.50 <sup>b</sup>	0.035	0.022
Neutral detergent fibre	0.61	0.63	0.62	0.65	0.65	0.023	NS

<sup>A</sup>SE, standard error. <sup>B</sup>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

D. W. Zheng<sup>A</sup>, L. Kang<sup>A</sup>, A. Wang<sup>A</sup>, N. Dong<sup>A,B</sup> and B. J. Hosking<sup>A</sup>

<sup>A</sup>AsiaPac (Dongguan) Biotechnology Co., Ltd, Dongguan City, Guangdong, PRC 523808.

<sup>B</sup>Corresponding author. Email: ningdong@asiapac.cn

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)  $31.8 \pm 0.32$  kg mean  $\pm$  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<sup>®</sup>, 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  $41.8 \pm 0.83$  kg and  $0.955 \pm 0.039$  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 <sup>A</sup>	P value <sup>B</sup>
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 <sup>ab</sup>	13.8 <sup>ab</sup>	13.7 <sup>a</sup>	13.7 <sup>a</sup>	13.9 <sup>b</sup>	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 <sup>a</sup>	0.48 <sup>ab</sup>	0.45 <sup>ab</sup>	0.50 <sup>ab</sup>	0.52 <sup>b</sup>	0.011	0.015
NDF	0.60 <sup>b</sup>	0.62 <sup>bc</sup>	0.58 <sup>ab</sup>	0.63 <sup>c</sup>	0.54 <sup>a</sup>	0.009	0.001
ADF	0.30 <sup>b</sup>	0.35 <sup>b</sup>	0.41 <sup>b</sup>	0.35 <sup>b</sup>	0.23 <sup>a</sup>	0.0208	0.001

<sup>A</sup>SEM, standard error mean. <sup>B</sup>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

P. Guggenbuhl<sup>A,C</sup>, E. Perez Calvo<sup>A</sup> and F. Fru<sup>B</sup>

<sup>A</sup>DSM Nutritional Products SA, Saint-Louis, France.

<sup>B</sup>DSM Nutritional Products Ltd., Basel, Switzerland.

<sup>C</sup>Corresponding author. Email: [patrick.guggenbuhl@dsm.com](mailto:patrick.guggenbuhl@dsm.com)

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*-(Ronozyne 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  $23.5 \pm 1.96$  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 Ronozyne 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.* 2012a). 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.* 2012a, 2012b). 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 <sup>A</sup>	P value
			H1000	H2000	H3000	Q500	Q1000	Q1500		
CTTAD P	0.35 <sup>a</sup>	0.35 <sup>a</sup>	0.52 <sup>bc</sup>	0.52 <sup>bc</sup>	0.54 <sup>bc</sup>	0.47 <sup>b</sup>	0.51 <sup>bc</sup>	0.55 <sup>c</sup>	0.010	<0.0001
P excretion (%)	0.25 <sup>c</sup>	0.31 <sup>d</sup>	0.18 <sup>ab</sup>	0.18 <sup>ab</sup>	0.17 <sup>a</sup>	0.20 <sup>b</sup>	0.19 <sup>ab</sup>	0.17 <sup>ab</sup>	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 <sup>B</sup>
Ca excretion (%)	0.49 <sup>a</sup>	0.62 <sup>b</sup>	0.41 <sup>a</sup>	0.46 <sup>a</sup>	0.48 <sup>a</sup>	0.42 <sup>a</sup>	0.40 <sup>a</sup>	0.43 <sup>a</sup>	0.010	<0.0001
Plasma P (mg/dL)	4.0 <sup>a</sup>	4.7 <sup>a</sup>	5.9 <sup>b</sup>	6.3 <sup>b</sup>	6.3 <sup>b</sup>	6.3 <sup>b</sup>	6.0 <sup>b</sup>	6.6 <sup>b</sup>	0.14	<0.0001
Plasma Ca (mg/dL)	12.9 <sup>a</sup>	15.0 <sup>b</sup>	12.2 <sup>a</sup>	12.4 <sup>a</sup>	12.3 <sup>a</sup>	11.9 <sup>a</sup>	12.0 <sup>a</sup>	11.5 <sup>a</sup>	0.17	<0.0001
Plasma INO (mg/L)	7.01 <sup>ab</sup>	5.70 <sup>a</sup>	8.67 <sup>bcd</sup>	9.88 <sup>cd</sup>	11.1 <sup>d</sup>	7.68 <sup>abc</sup>	9.01 <sup>bcd</sup>	10.90 <sup>d</sup>	0.324	<0.0001
Bone ash (%)	48.2 <sup>a</sup>	53.0 <sup>b</sup>	55.5 <sup>bc</sup>	58.1 <sup>c</sup>	57.7 <sup>c</sup>	56.8 <sup>bc</sup>	57.7 <sup>c</sup>	58.0 <sup>c</sup>	0.55	<0.0001
Break force (N) <sup>C</sup>	146 <sup>a</sup>	198 <sup>ab</sup>	271 <sup>bc</sup>	369 <sup>c</sup>	347 <sup>c</sup>	273 <sup>bc</sup>	293 <sup>bc</sup>	293 <sup>bc</sup>	12.3	<0.0001

<sup>A</sup>SEM, standard error of the mean. <sup>B</sup>NS, not significant. <sup>C</sup>N, newtons. <sup>a,b,c,d,e</sup>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

P. Guggenbuhl<sup>A,C</sup>, E. Perez Calvo<sup>A</sup> and F. Fru<sup>B</sup>

<sup>A</sup>DSM Nutritional Products SA, Saint-Louis, France.

<sup>B</sup>DSM Nutritional Products Ltd., Basel, Switzerland.

<sup>C</sup>Corresponding author. Email: [patrick.guggenbuhl@dsm.com](mailto:patrick.guggenbuhl@dsm.com)

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  $7.91 \pm 0.73$  kg (mean  $\pm$  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 <sup>A</sup>	P value
			H1000	H2000	H3000	Q500	Q1000	Q1500		
CTTAD P	0.28 <sup>a</sup>	0.30 <sup>a</sup>	0.50 <sup>c</sup>	0.55 <sup>cd</sup>	0.54 <sup>cd</sup>	0.43 <sup>b</sup>	0.51 <sup>c</sup>	0.59 <sup>d</sup>	0.001	<0.0001
P excretion (%)	0.40 <sup>d</sup>	0.47 <sup>c</sup>	0.28 <sup>b</sup>	0.25 <sup>b</sup>	0.26 <sup>b</sup>	0.31 <sup>c</sup>	0.27 <sup>b</sup>	0.23 <sup>a</sup>	0.009	<0.0001
CTTAD Ca	0.52 <sup>b</sup>	0.45 <sup>a</sup>	0.59 <sup>bcd</sup>	0.65 <sup>d</sup>	0.56 <sup>bc</sup>	0.52 <sup>b</sup>	0.61 <sup>cd</sup>	0.64 <sup>d</sup>	0.009	<0.0001
Ca excretion (%)	0.47 <sup>c</sup>	0.64 <sup>d</sup>	0.41 <sup>abc</sup>	0.35 <sup>a</sup>	0.44 <sup>bc</sup>	0.47 <sup>c</sup>	0.39 <sup>ab</sup>	0.36 <sup>a</sup>	0.011	<0.0001
Plasma P (mg/dL)	4.2 <sup>a</sup>	6.3 <sup>b</sup>	7.8 <sup>c</sup>	8.2 <sup>c</sup>	8.1 <sup>c</sup>	6.7 <sup>b</sup>	7.7 <sup>c</sup>	8.7 <sup>c</sup>	0.16	<0.0001
Plasma Ca (mg/dL)	12.3 <sup>b</sup>	13.2 <sup>c</sup>	12.3 <sup>b</sup>	11.2 <sup>a</sup>	11.7 <sup>ab</sup>	11.6 <sup>ab</sup>	12.2 <sup>b</sup>	11.4 <sup>ab</sup>	0.10	<0.0001
Plasma INO (mg/L)	7.40 <sup>a</sup>	7.53 <sup>a</sup>	12.10 <sup>abc</sup>	14.60 <sup>c</sup>	13.90 <sup>bc</sup>	9.12 <sup>ab</sup>	13.00 <sup>bc</sup>	16.10 <sup>c</sup>	0.533	<0.0001
Bone ash (%)	61.2 <sup>a</sup>	63.2 <sup>b</sup>	65.2 <sup>bc</sup>	65.4 <sup>bc</sup>	64.3 <sup>bc</sup>	63.7 <sup>bc</sup>	64.7 <sup>bc</sup>	65.9 <sup>c</sup>	0.27	<0.0001
Breaking force (N) <sup>B</sup>	141 <sup>a</sup>	324 <sup>ab</sup>	519 <sup>bc</sup>	714 <sup>c</sup>	623 <sup>bc</sup>	375 <sup>ab</sup>	610 <sup>bc</sup>	744 <sup>c</sup>	37.8	<0.0001

<sup>A</sup>SEM, standard error of the mean. <sup>B</sup>N, newtons. <sup>a,b,c,d,e</sup>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

R. S. Toledo<sup>A,B</sup>, A. G. Rocha<sup>A</sup> and C. Schaefer<sup>A</sup>

<sup>A</sup>Cooperativa Central Aurora, Chapeco, Santa Catarina, Brazil.

<sup>B</sup>Corresponding author. Email: rodrigo-toledo@auroraalimentos.com.br

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<sup>®</sup>; 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 <sup>A</sup>	<i>P</i> 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 <sup>B</sup> (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

<sup>A</sup>SEM, standard error of the mean. <sup>B</sup>Dig Lys intake: [(ADFI 23–36 kg\*1.18% SID Lys) + (ADFI 36–58 kg\*1.12% SID Lys) + (ADFI 58–88 kg\*1.02% SID Lys) + (ADFI 88–100 kg\*0.85% SID Lys) + (ADFI 100–120 kg\*1.03% SID Lys)]/Number of trial days.

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## Lysine requirements of modern genotype finisher pigs

K. L. Moore<sup>A,B</sup>, B. P. Mullan<sup>A</sup> and J. C. Kim<sup>A</sup>

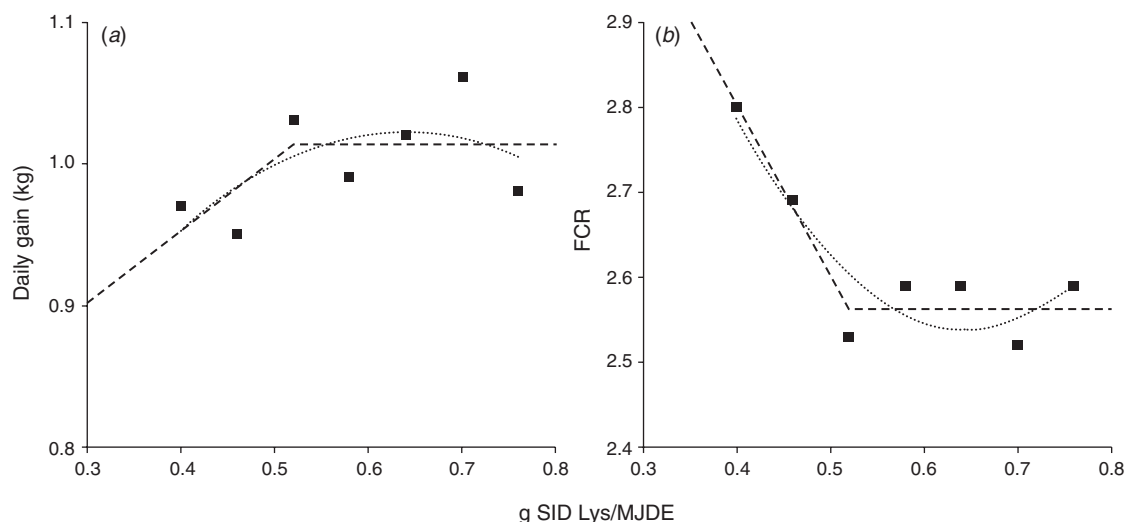
<sup>A</sup>Department of Agriculture and Food WA, South Perth, WA 6151.

<sup>B</sup>Corresponding author. Email: karen.moore@agric.wa.gov.au

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  $\times$  Landrace  $\times$  Duroc) was used in a  $2 \times 7$  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  $63.6 \pm 0.44$  to  $103 \pm 0.55$  (mean  $\pm$  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  $\pm$  SE). Data has been fitted with linear plateau (---) and quadratic (···) models.

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## Sulphur amino acid requirements of commercially-grown finisher pigs

J. C. Kim<sup>A,E</sup>, C. L. Collins<sup>B</sup>, J. L. Black<sup>C</sup>, K. L. Moore<sup>A</sup>, M. Trezona<sup>A</sup>, B. P. Mullan<sup>A</sup> and J. R. Pluske<sup>D</sup>

<sup>A</sup>Department of Agriculture and Food, South Perth, WA 6151.

<sup>B</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>C</sup>John L Black Consulting, Warrimoo, NSW 2774.

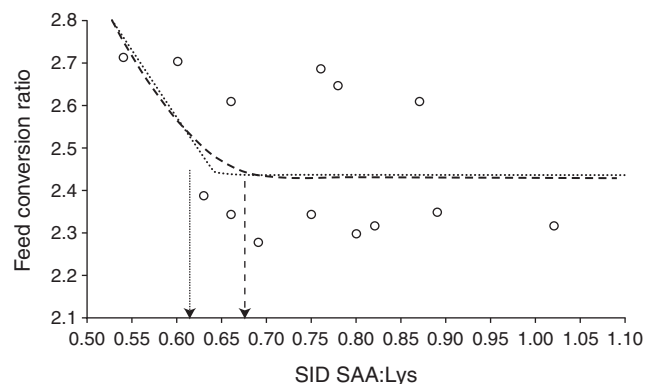
<sup>D</sup>Murdoch University, Murdoch, WA 6150.

<sup>E</sup>Corresponding author. Email: [jae.kim@agric.wa.gov.au](mailto:jae.kim@agric.wa.gov.au)

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  $50.1 \pm 0.46$  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 =  $0.64 \pm 0.29$ ) or a quadratic-plateau model (broken line,  $R^2 = 0.25$ ,  $P < 0.001$ , SID SAA:Lys =  $0.71 \pm 0.30$ ) 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

G. T. Nguyen<sup>A</sup>, W. L. Bryden<sup>A</sup>, M. J. Gidley<sup>A</sup> and P. A. Sopade<sup>A,B</sup>

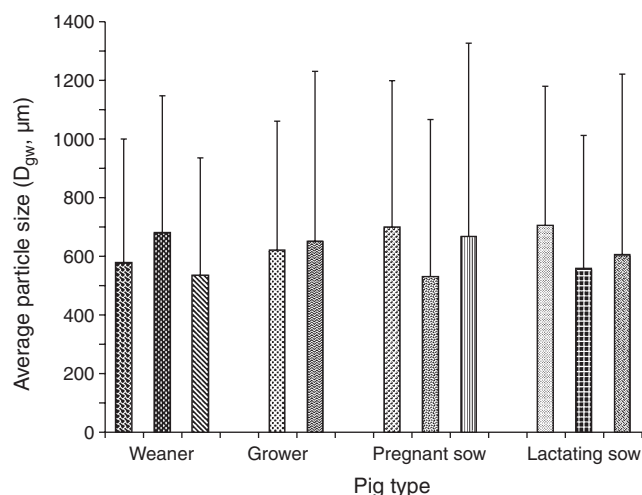
<sup>A</sup>The University of Queensland, St Lucia, QLD 4072.

<sup>B</sup>Corresponding author. Email: p.sopade@uq.edu.au

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  $\mu\text{m}$ ), and  $S_{gw}$  was 400–700  $\mu\text{m}$  (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  $\mu\text{m}$ , 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

G. T. Nguyen<sup>A</sup>, C. Collins<sup>B</sup>, D. Henman<sup>B</sup>, S. Diffey<sup>C</sup>, A. M. Tredrea<sup>D</sup>, J. L. Black<sup>E</sup>, W. L. Bryden<sup>A</sup>,  
 M. J. Gidley<sup>A</sup> and P. A. Sopade<sup>A,F</sup>

<sup>A</sup>The University of Queensland, St Lucia, QLD 4072.

<sup>B</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>C</sup>Curtin University, Bentley, WA 6102.

<sup>D</sup>The University of Sydney, NSW 2390.

<sup>E</sup>John L Black Consulting, Warrimoo, NSW 2774.

<sup>F</sup>Corresponding author. Email: p.sopade@uq.edu.au

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 (HMF-DMF, HMF-DMVC, HMVC-DMF, and HMVC-DMVC). 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  $6.8 \pm 0.1$  kg (mean  $\pm$  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  $\mu$ m, with up to 50% of the particles being higher than 1000  $\mu$ m 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  $\mu$ m) 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  $\mu$ m as the optimum particle size range for sorghum fed to weaner pigs. Feed mills, therefore, need not grind sorghum below 400  $\mu$ m for milling economy, and particle size above 800  $\mu$ m 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 <sup>A</sup>
$D_{gw}$ -row ( $\mu$ m)	442	581	659	823	539	570	609	640	450	515	605	689	
$S_{gw}$ -row ( $\mu$ m)	294	453	558	704	317	421	414	482	279	397	487	630	
Particles >1000 $\mu$ m (%)	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

<sup>A</sup>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

G. T. Nguyen<sup>A</sup>, C. Collins<sup>B</sup>, D. Henman<sup>B</sup>, S. Diffey<sup>C</sup>, A. M. Tredrea<sup>D</sup>, J. L. Black<sup>E</sup>, W. L. Bryden<sup>A</sup>,  
 M. J. Gidley<sup>A</sup> and P. A. Sopade<sup>A,F</sup>

<sup>A</sup>The University of Queensland, St Lucia, QLD 4072.

<sup>B</sup>Rivalea (Australia), Corowa, NSW 2646.

<sup>C</sup>Curtin University, Bentley, WA 6102.

<sup>D</sup>The University of Sydney, NSW 2390.

<sup>E</sup>John L Black Consulting, Warrimoo, NSW 2774.

<sup>F</sup>Corresponding author. Email: p.sopade@uq.edu.au

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  $7.3 \pm 0.10$  kg (mean  $\pm$  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  $\mu$ m, and had up to 45% of the particles greater than 1000  $\mu$ m. The disc-milled pea had a wider  $D_{gw}$  range (200  $\mu$ m) 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  $\mu$ m, 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  $\mu$ m) 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 <sup>A</sup>
$D_{gw}$ -row ( $\mu$ m)	576	709	745	811	569	580	604	675	561	585	694	809	
$S_{gw}$ -row ( $\mu$ m)	443	589	657	737	447	451	479	571	436	478	597	703	
Particles >1000 $\mu$ m (%)	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

<sup>A</sup>SEM, standard error of the mean.

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## Rapid changes occur in feed efficiency after infection with *Mycoplasma hyopneumoniae* and *Pasteurella multocida*

G. L. Wyburn<sup>A</sup>, G. J. Eamens<sup>B</sup>, D. Collins<sup>A</sup> and A. M. Collins<sup>A,C</sup>

<sup>A</sup>Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568.

<sup>B</sup>5 Wirraway Street, Raby, NSW 2566.

<sup>C</sup>Corresponding author. Email: [alison.collins@dpi.nsw.gov.au](mailto:alison.collins@dpi.nsw.gov.au)

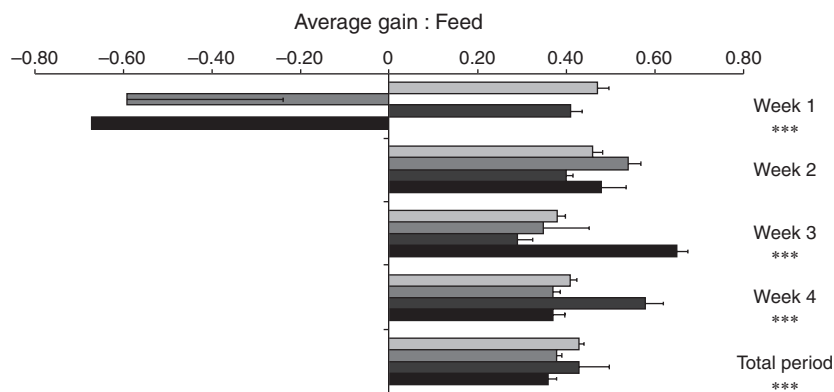
*Mycoplasma hyopneumoniae* (*Mhp*) and *Pasteurella multocida* (*Pm*) are the agents most frequently isolated from lungs affected with enzootic pneumonia. Primary infection with *Mhp* predisposes pigs to opportunistic infection with *Pm*, causing increased lung damage and reduced weight gains and feed 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  $24.0 \pm 0.53$  kg (mean  $\pm$  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 in G : 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

R. Pieper<sup>A,C</sup>, W. Vahjen<sup>A</sup>, M. Taciak<sup>B</sup>, E. Świąch<sup>B</sup>, M. Barszcz<sup>B</sup>, J. Skomiał<sup>B</sup> and J. Zentek<sup>A</sup>

<sup>A</sup>Freie Universität Berlin, Berlin, Germany.

<sup>B</sup>The Kielanowski Institute of Physiology and Nutrition, Jablonna, Poland.

<sup>C</sup>Corresponding author. Email: robert.pieper@fu-berlin.de

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<sub>3</sub>). 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<sub>3</sub> 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

J. W. Park<sup>A</sup>, P. Y. Zhao<sup>A</sup>, R. A. Valientes<sup>B</sup> and I. H. Kim<sup>A,C</sup>

<sup>A</sup>Dankook University, Cheonan, Chungnam, South Korea.

<sup>B</sup>DSM Nutritional Products Philippines, Inc. Unit 1803, One Global Place, 1634 Taguig City, Philippines.

<sup>C</sup>Corresponding author. Email: [inhokim@dankook.ac.kr](mailto:inhokim@dankook.ac.kr)

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  $7.2 \pm 0.33$  kg (mean  $\pm$  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  $3000 \times g$  at  $4^{\circ}\text{C}$  for 15 min to prepare plasma for determination of cortisol, TNF- $\alpha$ , 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  $3000 \times g$  at  $4^{\circ}\text{C}$  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<sup>®</sup>; 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- $\alpha$ , 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

I. Stensland<sup>A</sup>, J. C. Kim<sup>B</sup>, J. Mansfield<sup>A</sup> and J. R. Pluske<sup>A,C</sup>

<sup>A</sup>Murdoch University, Murdoch, WA 6150.

<sup>B</sup>Department of Agriculture and Food, South Perth, WA 6151.

<sup>C</sup>Corresponding author. Email: J.Pluske@murdoch.edu.au

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  $\beta$ -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  $\times$  Landrace) weighing  $7.2 \pm 1.02$  kg (mean  $\pm$  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<sup>®</sup>, 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  $1.03 \times 10^9$  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 <sup>A</sup>			SEM <sup>C</sup>	D	P value <sup>B</sup>	
	Control	ZnO	OACP			T	D $\times$ T
% pigs with PWD	25 <sup>a</sup>	4 <sup>b</sup>	29 <sup>a</sup>				
DI (%) <sup>E</sup>	5.06 <sup>a</sup>	0.62 <sup>b</sup>	6.25 <sup>a</sup>	1.526		0.026	
<i>E. coli</i> score <sup>F</sup>							
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				

<sup>A</sup>Refer to text for treatment details. <sup>B</sup>D, day; T, treatment. <sup>C</sup>SEM, standard error of the mean. <sup>D</sup>PWD was defined as pigs having FC score of 4. <sup>E</sup>Mean proportion of days pigs had diarrhoea (FC = 4) in the 14 d after weaning; <sup>F</sup>Agar plates scored from 0–5 according to numbers of streaked sections containing  $\beta$ -haemolytic *E. coli*, where 0 = no growth and 5 = growth in fifth section of the plate. <sup>a,b</sup>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

K. L. Moore<sup>A,B</sup>, J. C. Kim<sup>A</sup> and B. P. Mullan<sup>A</sup>

<sup>A</sup>Department of Agriculture and Food WA, South Perth, WA 6151.

<sup>B</sup>Corresponding author. Email: karen.moore@agric.wa.gov.au

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 <sup>A</sup>	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) <sup>B</sup>	2.32	2.34	2.28	0.048	0.501
g SID Lys intake/kg LW gain	22.2 <sup>a</sup>	20.6 <sup>b</sup>	20.5 <sup>b</sup>	0.297	0.002
DE intake (MJ DE/kg LW gain)	39.3 <sup>b</sup>	36.0 <sup>a</sup>	38.1 <sup>ab</sup>	0.856	0.007
Carcase weight (kg) <sup>C</sup>	68.7	67.1	68.6	1.02	0.204
Dressing percentage	70.6 <sup>a</sup>	70.0 <sup>ab</sup>	69.8 <sup>b</sup>	0.328	0.050
P <sub>2</sub> 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

<sup>A</sup>SED, standard error of difference between means. <sup>B</sup>FCR, feed conversion ratio. <sup>C</sup>Hot carcase weight: AUSMEAT trim 13-head off, flare off, fore trotters off, hind trotters on. <sup>a,b</sup>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

R. J. E. Hewitt<sup>A,B</sup>, A. Corso<sup>A</sup> and R. J. van Barneveld<sup>A</sup>

<sup>A</sup>SunPork Farms, Loganholme, QLD 4129.

<sup>B</sup>Corresponding author. Email: robert.hewitt@sunporkfarms.com.au

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.* 2014a). 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  $4.5 \pm 0.67$  kg (mean  $\pm$  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<sup>TM</sup> 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.* (2014a) in that the Low group were ‘too late to catch up’, however, the results of Douglas *et al.* (2014b) 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 <sup>A</sup>	P value
Entry weight (kg)	4.4 <sup>a</sup>	3.8 <sup>b</sup>	5.2 <sup>c</sup>	0.11	<0.001
Exit weight (kg)	80.2 <sup>a</sup>	75.9 <sup>b</sup>	84.8 <sup>c</sup>	1.10	<0.001
ADG <sup>B</sup> (kg)	0.647 <sup>a</sup>	0.617 <sup>b</sup>	0.680 <sup>c</sup>	0.010	<0.001
ADFI <sup>C</sup> (kg)	1.27 <sup>a</sup>	1.20 <sup>b</sup>	1.36 <sup>c</sup>	0.031	<0.001
FCR <sup>D</sup> (kg : kg)	1.96	1.95	2.00	0.044	0.499
Age at slaughter (days)	163 <sup>a</sup>	170 <sup>b</sup>	156 <sup>c</sup>	2.1	<0.001
Entry weight CV (%)	13.9 <sup>a</sup>	9.7 <sup>b</sup>	6.9 <sup>b</sup>	1.36	<0.001
Exit weight CV (%)	11.8	10.4	11.3	1.20	0.381

<sup>A</sup>SED, standard error of difference between means. <sup>B</sup>ADG, average daily gain. <sup>C</sup>ADFI, average daily feed intake. <sup>D</sup>FCR, feed conversion ratio.  
<sup>a,b,c</sup>Means in a row not having the same superscript are significantly different.

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