

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

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

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

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

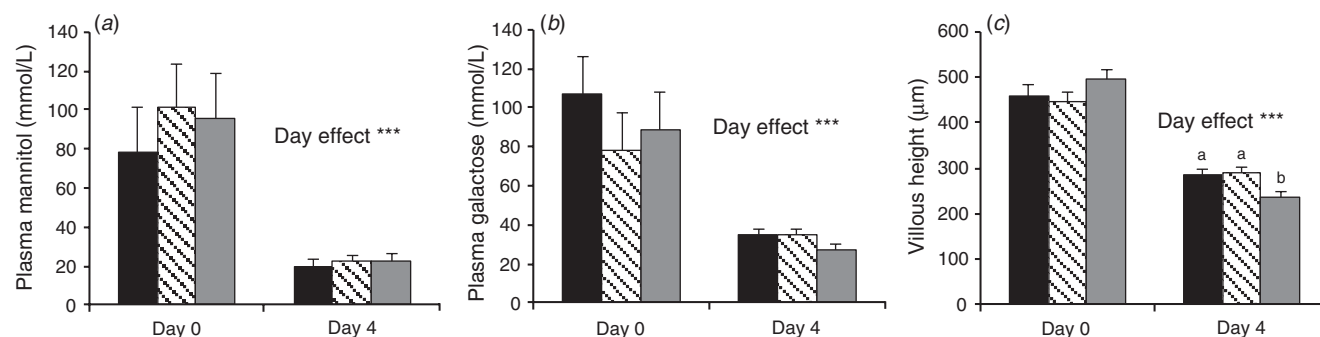


Fig. 1. (a) Plasma MAN concentration, (b) plasma GAL concentration, and (c) jejunum villous height in pigs killed either at weaning or 4 days later. CW = black ($n = 5$), IS16 = black stripe ($n = 5$) and IS8 = grey ($n = 5$). ^{a,b}Indicates differences between treatments within each day.

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

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

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

There were interactions between sanitary conditions and dietary treatments in crypt depth and villous-crypt ratio (V : C) on d 7 ($P < 0.01$) and 14 ($P < 0.001$) (Table 1). Pigs that were housed in poor sanitary conditions had lower ($P < 0.05$) AID of dry matter than pigs that were housed in sanitary conditions on d 14. A diet supplemented with 2% cellulose decreased ($P < 0.05$) AID of crude protein compared to pigs fed a diet with 0 or 1% cellulose. Our results indicated that a diet with 1% added cellulose increased V : C ratio, but feeding a diet containing cellulose impaired the AID of crude protein and energy on d 14 in both sanitary and poor sanitary conditions.

Table 1. Effects of environmental conditions and dietary treatments of cellulose on ileal morphology and apparent ileal digestibility (AID; %) in weaned pigs over 14 days

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

^APooled standard error of the mean. ^BSignificance level: NS, not significant; †, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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

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

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

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

Table 1. Diet description and analysed standardised ileal digestible Trp:LNAA composition

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

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

Parameter	Dietary (D)				Treatment (T)		s.e.m.	P-value		
	1	2	3	4	Control	Infected		D	T	D × T
ADG (g)	162 ^b	184 ^{a,b}	163 ^b	219 ^a	179	185	13.6	0.010	0.681	0.729
ADFI (g)	259 ^b	284 ^{a,b}	261 ^{a,b}	315 ^a	279	281	16.2	0.050	0.936	0.753
FCR	1.51	1.41	1.71	1.21	1.56	1.36	0.152	0.126	0.158	0.657

^{a,b}Mean values within a row that have different superscript are significantly different ($P < 0.05$).

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

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

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

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

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

Treatment Trait ^B	Least square mean \pm s.e.				Parity (P)		P -value ^A	
	CON	CLA	MCFA	BOTH	GILT	SOW	D	P
IgG _{d0}	82.8 \pm 12.7	81.0 \pm 10.4	85.5 \pm 10.7	78.8 \pm 14.2	93.1 \pm 9.5	70.9 \pm 7.5	NS	*
IgG _{d21}	0.34 \pm 0.06	0.30 \pm 0.05	0.37 \pm 0.05	0.33 \pm 0.06	0.36 \pm 0.04	0.31 \pm 0.04	NS	NS
IgG _S	16.3 \pm 1.7 ^{ab}	17.4 \pm 1.5 ^a	12.3 \pm 1.4 ^b	14.0 \pm 2.0 ^{ab}	13.1 \pm 1.3	16.9 \pm 1.1	*	**
β HBA	0.85 \pm 0.07	0.84 \pm 0.06	0.82 \pm 0.06	0.84 \pm 0.08	0.90 \pm 0.05	0.78 \pm 0.05	NS	*
Mortality	14.4 ^{ab}	11.2 ^a	16.0 ^b	15.0 ^b	14.0	14.3	*	NS

^ANS, not significant, $P \geq 0.10$; * $P < 0.10$; ** $P < 0.05$. ^BIgG concentration expressed as mg/mL, β HBA expressed as mM, mortality expressed as %. ^{a,b}Different superscripts within rows denote significant pairwise differences between diets ($P < 0.05$).

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

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

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

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

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

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

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

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

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

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

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

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

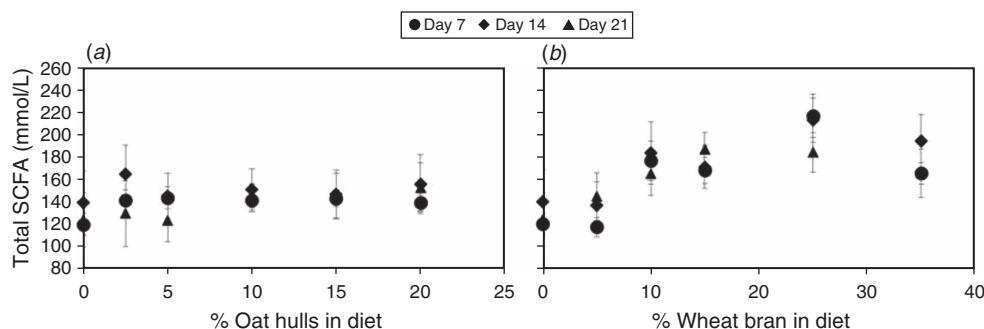


Fig. 1. Total SCFA (mmol/L of faecal water) in faeces from (a) OH diets and (b) WB diets.

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

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

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

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

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

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

^ART, rectal temperature on d 19 of lactation. ^BRR, respiration rate on d 19 of lactation.

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

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

Cinnamon did not ameliorate RR, RcT and ST or blood acid-base balance. Fasted glucose concentrations were lower in cinnamon supplemented pigs (6.55 v. 5.65 for Control v. Cinnamon, $P = 0.050$), but no interaction with HS was observed and no other influence of cinnamon on glucose and insulin kinetics were observed. Therefore the hypothesis that cinnamon would improve insulin sensitivity in HS pigs was not supported. The effects of HS on pig thermoregulation, blood biochemistry and insulin sensitivity were marked. Heat stress increased RR ~8-fold, from 22 to 172 breaths/min. The increase in respiration resulted in reduced blood CO₂ concentrations (pCO₂ 53.2 v. 47.3 mmHg for TN v. HS $P < 0.001$). As blood pH regulates the rate of biochemical reactions on a global basis it is very tightly regulated; however, HS pigs tended to have more alkaline blood than TN pigs (7.419 v. 7.432, $P = 0.087$). Blood HCO₃⁻ concentrations were reduced in HS pigs (34.4 v. 31.6 mmol/L, $P = 0.002$), indicating increased buffering by excretion of excess HCO₃⁻. Compared to TN, urinary pH was lower in HS pigs (6.38 v. 5.41, $P < 0.001$), indicating that urinary bicarbonate excretion occurred before 7 days during HS. Heat stress reduced haematocrit (31.3 v. 26.1%, $P < 0.001$), indicating an expansion in plasma volume possibly due to reduced H₂CO₃ formation from CO₂ and H₂O. Heat stress reduced the maximal glucose (9.62 v. 8.18 mmol, $P = 0.014$) and insulin concentrations (1.06 v. 0.67 ng/mL) following the intravenous (IV) glucose bolus. Glucose area under the curve (AUC)_{0–60} was not influenced by HS; however, the insulin AUC_{0–60} was significantly lower in HS pigs (0.242 v. 0.169 ng/mL.h, $P = 0.005$). Collectively these results show that HS pigs had a lower insulin response to an IVGTT. This may be in part due to reduced glucose concentrations after the IV bolus due to an expansion in blood volume or an increase in insulin sensitivity. In summary HS alters blood acid-base balance, leading to a loss of minerals such as bicarbonate and an apparent expansion in blood volume. It is expected that the energetic cost of panting, buffering and loss of nutrients such as bicarbonate contribute to compromised production efficiency in HS pigs.

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

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

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

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

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

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

^AN, Newton. ^BNS, not significant. ^{a–e}Means in a column not having the same superscript are significantly different.

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

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

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

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

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

	BL \pm s.e.	Fitting model CLP \pm s.e.	Q, R^2	Mean/n metab. ^A	Previous dose–response studies Reference	Value
Ile:Lys	0.53 ± 0.09	0.54 ± 0.18	0.54, 0.59	0.53	Soumeh <i>et al.</i> (2014)	0.52
Leu:Lys	0.96 ± 0.14	1.06 ± 0.24	1.10, 0.89	1.04	Soumeh <i>et al.</i> (2015a)	0.93
Val:Lys	0.66 ± 0.14	0.68 ± 0.22	0.69	0.68	Soumeh <i>et al.</i> (2015b)	0.70

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

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Factors influencing the measure of creatinine in non-reproductive pigs

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

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

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

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

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

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

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

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

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

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

Table 1. Effects of Ca and P concentrations on growth performance of nursery pigs from d 0 to 28

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

^As.e.m., standard error of the mean. ^{a–d}Means with different superscripts within a row differ ($P < 0.05$).

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

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

Six Large-White male piglets with 10.2 ± 0.53 kg bodyweight were used to perform the intestinal gene expression analysis. Five porcine bitter taste receptors T2R1, T2R4, T2R7, T2R10, T2R20 and T2R39 were selected based on their high gene sequence homology with the human orthologues and their affinity to several compounds (i.e. caffeine, quinine, amarogentin and saccharin) known to be bitter to humans and pigs. Epithelium layers and smooth muscle layers in the stomach, duodenum and oesophagus were separated and analysed for the expression of the bitter taste receptor genes (Tas2rs). Total RNA was extracted from the tissue samples using the TRIZOL-chloroform method. RNA purification, cDNA synthesis and real-time qPCR assays using SYBR green were performed. The relative gene expression levels were estimated using the Pfaffl method (Pfaffl 2001) taking into account the cycle threshold values of both the candidate genes and of the two reference genes ACTB₃ and GAPDH. GraphPad Prism 7 software (GraphPad Software Inc., La Jolla, CA, USA) was used to analyse the gene expression data using a one-way ANOVA and Fischer's least significant difference (l.s.d.) analysis for multiple comparisons.

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

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

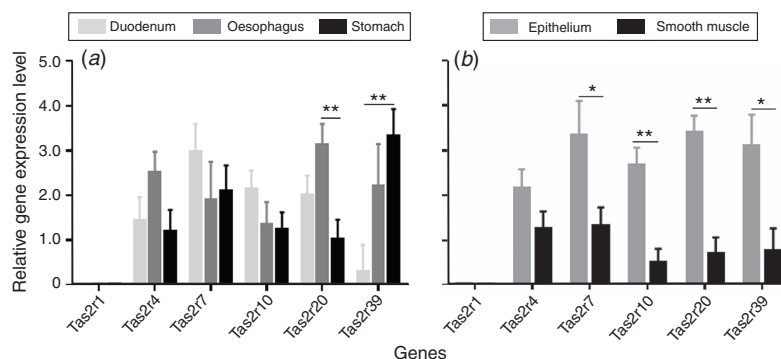


Fig. 1. Expression of porcine Tas2r1, 4, 7, 10, 20 and 39 in the gastrointestinal tract of pigs (mean \pm s.e.m., $n = 6$) (a) Normalised expression of bitter genes of interest in the pig duodenum, oesophagus and stomach. (b) Normalised expression of the bitter genes of interest in the epithelium and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

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Importance of connectivity grains for AusScan NIR prediction accuracy

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

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

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

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

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Black JL, Hughes RJ, Diffey S, Tredrea AM, Flinn PC, Spragg JC, Kim JC (2014) *Proceedings of the Australian Poultry Science Symposium* **25**, 23–30.

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

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

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

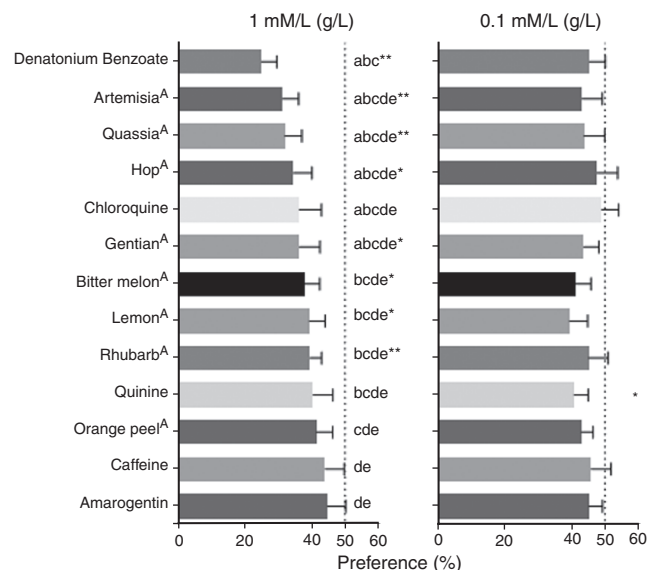


Fig. 1. Mean (\pm s.e.) preference rate (as % of the total double choice solution intake) between a 50 mM sugar solution and the same solution added with one of thirteen bitter compounds or plant extracts. [^]Treatments with the superindex are plant extracts. * or ** indicates that the preference values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

Reference

Fu M, Collins C, Henman D, Roura E (2015) *Chemical Senses* **40**, 363.

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Preference thresholds for four limiting essential amino acids in piglets

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

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

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

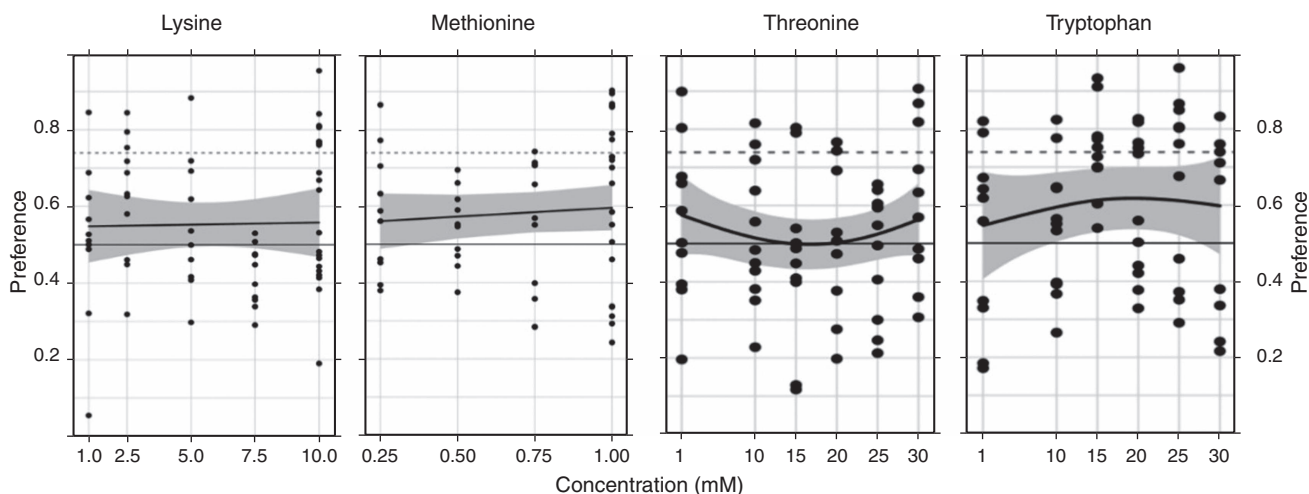


Fig. 1. Dose-dependent preference values for Lysine, Methionine, Tryptophan and Threonine solutions in pigs. Preference is shown as a fraction of 1 (equivalent to 100%). Shaded regions in the figures represent approximate 95% confidence bands ($P < 0.05$). The dashed lines depict the preference value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

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

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

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

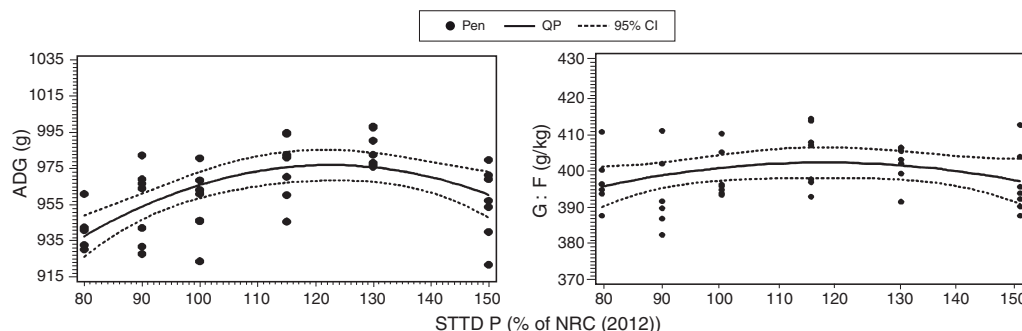


Fig. 1. Fitted QP regression model for ADG and G : F as a function of increasing STTD P in 24–130 kg pigs.

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

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

Eight hundred and forty Improvac vaccinated male pigs (PrimeGroTM Genetics, 57.8 kg \pm 0.66 kg) were randomly allocated to four dietary treatments in a 2 \times 2 factorial design (high and low DE level and LSB addition at 0 or 10⁹ CFU/kg), with Pen as the experimental unit. Pigs were housed in commercial pens (14 pigs/pen and 15 pens/treatment) with feed and water available *ad libitum*. The experiment commenced in February 2016 and over the 42 day experimental period there were 25 days with maximum pen temperatures exceeding 30°C and 12 days over 35°C. Diets contained 14.0 or 12.8 MJ DE/kg and 0.62 g standardised ileal digestible lysine/MJ DE. Pen weights and feed use was measured at 0, 21 and 42 d. Carcass fat depth (P2) was measured after slaughter. Statistical analysis was conducted using ANOVA (SPSS v21.0, IBM, Armonk, NY, USA).

As expected, pigs fed diets with higher DE grew faster and more efficiently and had fatter carcasses than those fed a low DE diet (Table 1). The addition of LSB at 10⁹ CFU/kg increased feed intake of pigs fed low-energy, but not high-energy, diets. Pigs fed LSB also had improved feed efficiency, particularly in higher DE diets. There was an interaction between DE and LSB for carcass fat at the P2 site. Pigs fed high DE diets with LSB were leaner than those without LSB, whereas in low DE diets LSB addition produced a fatter carcass. The results support the hypothesis that in hot weather, LSB increased feed intake in pigs fed a low energy diet, most likely due to reduced heat increment during digestion. However, in high DE (high starch) diets feed efficiency is improved. The use of the live yeast Levucell SB is a useful tool for maintaining pig growth performance over hot weather.

Table 1. Growth performance of finisher pigs fed two digestible energy (DE) levels with and without Levucell SB (LSB)

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

^AADG, average daily gain. ^BFCR, feed conversion ratio. ^CADI, average daily intake. ^DP2, fat depth. NS, not significant. ^{a-d}Means in a row not having the same superscript are significantly different (*P* < 0.05).

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Cooling innovations for loose farrowing pens in summer

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

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

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

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

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

^ANumber of piglets born alive used as covariate in analysis. Liveborn mortality figures are calculated for each litter (taking into account fostering adjustment).

^BPiglet birthweight used as covariate in analysis.

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

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

A total of 108 male pigs (Duroc × (Yorkshire × Landrace)) weaned at 21 days of age with initial bodyweight (BW) of 6.2 ± 0.4 kg (mean \pm s.e.m.) were randomly allocated to one of three dietary treatments (0, 1, 2% cellulose; Accent Microcell Pvt. Ltd, India). The BW was measured individually on d 0, 7, and 14. Feed consumption was recorded weekly on a pen basis. The concentrations of interleukin 1 β (IL-1 β ; R&D Systems, Minneapolis, MN, USA), tumour necrosis factor α (TNF- α ; R&D Systems), prostaglandin E₂ (PGE₂; MyBioSource, San Diego, CA, USA), leukotriene B₄ (LTB₄; MyBioSource), and cyclooxygenase-2 (COX-2; MyBioSource) in plasma were quantified using commercially available ELISA kits according to the manufacturers' instructions described by Piñeiro *et al.* (2009) on d 0, 7 and 14. Data were analysed as completely randomised block design, using general linear model procedure of ANOVA (SPSS v22.0, IBM, Armonk, NY, USA).

Pigs reared under sanitary environmental conditions had higher ADG ($P < 0.05$) and improved feed efficiency for 14 days after weaning compared to their counterparts (Table 1). Pigs housed in poor sanitary conditions reduced the cytokines TNF- α , COX-2, and PGE₂ ($P < 0.05$) for 14 days after weaning compared to those in sanitary environmental conditions. Feeding a diet with dietary cellulose (i.e. up to 2%) lowered COX-2 concentration ($P < 0.05$) without compromising growth performance for 14 days after weaning independent of environmental conditions. Our results indicated that pigs fed a diet supplemented with cellulose (i.e. up to 2%) did not impair growth performance of weaned pigs, and could maintain and/or reduce plasma cytokine concentrations (i.e. COX-2) for 14 days after weaning regardless of environmental conditions.

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

Item	Environmental conditions		s.e.m.	Dietary treatments			s.e.m.	P-value	
	Sanitary	Poor sanitary		Cellulose 0%	Cellulose 1%	Cellulose 2%		Environmental conditions	Dietary treatments
ADG (g)	339.7	167.6	18.8	280.1	254.4	226.5	27.51	0.003	0.617
ADFI (g)	392.8	317.8	24.44	382.1	372.0	312.0	25.7	0.229	0.566
FCR (g/g)	1.2	2.1	0.17	1.5	1.9	1.6	0.2	0.036	0.666
Diarrhoea index (%)	12.8	24.0	2.58	24.8	17.9	12.5	2.66	0.041	0.196
IL-1 β (pg/mL)	0.0	13.3	4.80	0.0	19.9	0.0	4.84	0.179	0.144
TNF- α (pg/mL)	5.1	17.6	1.90	16.9	10.8	6.4	2.21	0.002	0.063
COX-2 (pg/mL)	102.8	123.7	2.67	125.3	112.4	102.1	3.29	<0.001	0.023
PGE ₂ (pg/mL)	17.5	60.3	4.10	38.5	40.8	37.4	5.57	0.002	0.947
LTB ₄ (pg/mL)	258.0	413.7	49.45	323.6	436.3	247.6	53.57	0.168	0.297

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

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

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

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

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

Table 1. Growth performance in weaned pigs fed different levels of Ca, Phy and R

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

^ANS, not significant. ^{a–c}Means in a column not having the same superscript are significantly different.

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

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

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

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

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

Table 1. *Myo*-inositol concentrations in the small intestine segments and plasma in weaned pigs

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

^ANS, not significant. ^{a–c}Means in a column not having the same superscript are significantly different.

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

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The presence of phytate (inositol-6-phosphate, IP₆) and its lower degree phosphorylation (IP_x) esters have been reported to significantly reduce digestion and absorption of minerals and proteins in monogastric animals, even at low concentrations (Wilcock and Walk 2016). *In vivo* reduction of IP₆ and IP_x ester content through superdosing of enhanced *Escherichia coli* phytase consistently improved nutrient utilisation efficiency and growth performance of monogastric animals (Wilcock and Walk 2016). Moreover, as the pharmaceutical dose of zinc oxide in piglet diets interacted with phytate and other divalent minerals, supplementation of 2500 FTU/kg phytase improved performance in weaned pigs (Walk *et al.* 2013). However, there still is a perception in the pork industry that piglets will not respond to phytase superdosing when offered an animal protein-based diet with low phytate content. This study tested the hypothesis that enhanced *E. coli* phytase at 2500 FTU/kg will improve performance of piglets offered both animal protein (AP) and plant protein (PP)-based diets, containing low and moderate amounts of phytate, respectively.

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

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

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

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

NS, not significant; SE, standard error.

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Alternative milk derivatives do not reduce weaner pig performance

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

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

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

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

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

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

^{a,b}Means in a row with different superscripts differ significantly ($P < 0.05$). ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SED, standard error of difference of means; Treat, treatment effects; Week, entry week effects; T x W, interaction effects.

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

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

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

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

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

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

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

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Using hydroxyl selenomethionine as an antioxidant for weaned piglets

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

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

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

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

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

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

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

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In vivo digestion of encapsulated essential oils in weaned pigs

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

A 200 g mixture including thyme, nerolina and peppermint gum EO were added to a solution of 20 g/L of alginate and 0.5 g of Tween 80 (Croda International, Snaith, DN14 9AA, UK) and blended for 1 min using an Ultraturrax homogeniser (IKA Works, Staufen im Breisgau, Germany). A low flow peristaltic pump with a 21 G needle dropped the emulsion into a 20 g/L CaCl solution. The droplets were left to harden for 30 min. Microcapsules were washed, drained, and stored in a sealed container at 4°C. A catheter was surgically implanted in the external jugular vein of 12 5-week-old male pigs. Treatments consisted of a single oral dose of free EO (FEO) mixed with the morning meal (0.45 mg/kg BW of the principal compounds of each EO (PCEO)), encapsulated EO (EEO, 76 mg/kg BW of microencapsulation) and control (C, feed without EO). Blood samples were collected at 5, 10, 15, 30, 45, 60, 120, 180, 240 and 300 min. Animals were euthanised ~10 h after receiving the treatment and gastrointestinal content samples were collected. Samples were analysed by gas-chromatography/mass spectrometry. Data were analysed by ANOVA using Minitab 16 (Minitab Inc., State College, PA, USA).

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

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

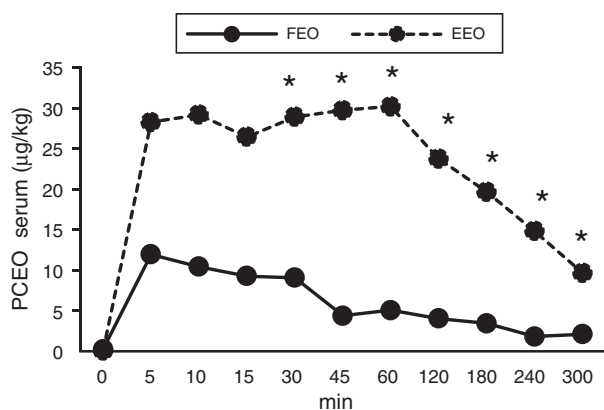


Fig. 1. Concentration of principal components of EO (PCEO) found in serum of pigs after oral treatment with free (FEO) or encapsulated (EEO) essential oils. Asterisks show significant differences between treatments at $P < 0.05$.

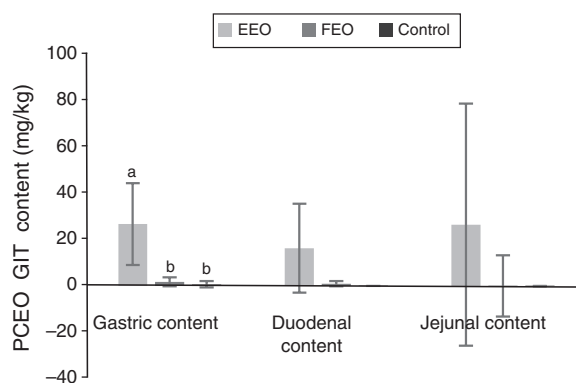


Fig. 2. Concentration of principal components of EO (PCEO) found in the GIT of pigs around 10 h after oral treatment of free (FEO) or encapsulated (EEO) essential oils. Superscripts show significant differences between treatments at $P < 0.05$.

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

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

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

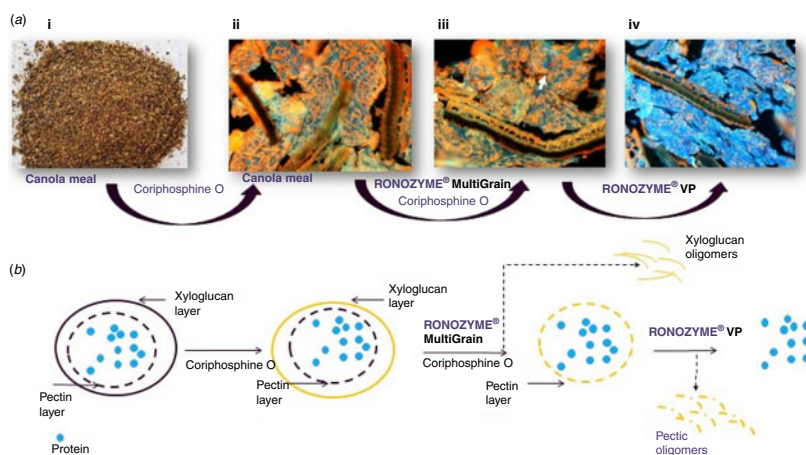


Fig. 1. (a) Canola meal (i) is stained with Coriphosphine O, which stains cell walls orange, and proteins show blue autofluorescence (ii). On incubation at RT for 3 h with Ronozyme[®] MultiGrain a xyloglucanase containing product the outer intact cell walls disappear, revealing a more diffused cell wall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

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

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

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

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

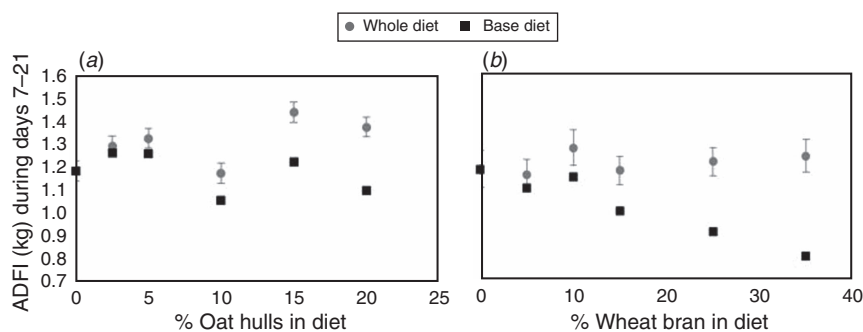


Fig. 1. ADFI (kg) from 7 to 21 days for whole diets containing OH (a) or WB (b). Intake of the base diet was calculated by subtracting the proportion of WB or OH from whole diet.

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

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

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

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

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

Table 1. Effect of *Curcuma aromatica* (CA) and inositol monophosphate (IP) supplementation in lactating Parity 3 sows and piglets

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

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

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

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

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

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

Table 1. Effect of the NSP-enzyme + phytase on growth performance and carcass traits

	Positive control	Negative control	NC + (NSPase + Phytase)	<i>P</i> -value
Initial weight (kg)	23.9	23.9	23.6	0.020
Final weight (kg)	98.9 ^a	94.3 ^c	96.9 ^b	<0.001
Weight gain (g/d)	823 ^a	773 ^c	805 ^b	<0.001
Feed intake (kg/d)	1.91	1.87	1.86	0.35
FCR ^A	2.33	2.41	2.32	0.054
Ash in bone (g/kg)	556 ^a	533 ^b	558 ^a	<0.001
Carcass weight (%)	89.0 ^a	86.0 ^b	88.0 ^a	<0.001
Lean meat (%)	58.3	58.5	58.4	0.35
Backfat (mm)	14.2	13.6	13.8	0.096
Muscle thickness (mm)	60.2	59.1	59.2	0.29

^AFCR, feed conversion ratio. ^{a-c}Means with different superscripts within a row differ ($P < 0.05$).

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The reproductive value of enrichment to sows at farrowing

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

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

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

Table 1. The effects of the provision of lucerne before and at parturition on sow reproductive performance

	Control		Lucerne		P-value
	Mean	s.e.m. ^A	Mean	s.e.m.	
Log ₁₀ farrowing duration (min) ^B	2.22 (166.0)	0.05	2.31 (204.2)	0.04	0.174
Log ₁₀ piglet interval (min) ^B	1.18 (15.1)	0.05	1.24 (17.4)	0.05	0.364
Total piglets born	10.9	0.5	11.0	0.5	0.834
Piglets born alive	10.4	0.5	10.9	0.5	0.450
Piglets born dead	0.4	0.1	0.1	0.1	0.027
Piglet deaths	0.8	0.2	0.6	0.1	0.328
Piglets weaned	10.5	0.3	10.6	0.3	0.865

^As.e.m., standard error of the mean. ^BBack-transformed means are presented in brackets.

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

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

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

The average duration of sham chewing bouts was 54 s on D3 (s.d. 47) and 78 s on D8 (s.d. 173), and the difference in average bout duration between the 2 days was not significant ($Z = -1.11$, $P = 0.27$). The average frequency of sham chewing bouts was 8 bouts/sow/d on D3 and 5 bouts/sow/d on D8, and the difference in average frequency of bouts between the 2 days was significant ($Z = -2.16$, $P = 0.03$). 85% of sows were identified as performing sham chewing behaviour on D3 and 70% of sows on D8; however, the difference between the days was not significant ($Z = -1.34$, $P = 0.18$). Sham chewing was observed across the day, both pre- and post-feed drops. The proportion of sows identified as performing sham chewing behaviour with continuous sampling was significantly different to 5 min IPS on both D3 ($Z = -2.45$, $P = 0.01$) and D8 ($Z = -2.65$, $P = 0.01$), and 2 min IPS on D3 ($Z = -2.00$, $P = 0.05$) but not D8 ($Z = -1.73$, $P = 0.08$). When comparing the two instantaneous sampling methods, the proportion of sows identified as performing sham chewing behaviour was significantly different on D8 ($Z = -2.00$, $P = 0.04$) but not D3 ($Z = -0.82$, $P = 0.41$). Using 5 min IPS, 65% of sows were identified as performing sham chewing behaviour on D3 and 35% of sows on D8. While for 2 min IPS, 55% of sows were identified as performing sham chewing behaviour on D3 and 55% of sows on D8. Thus, while 2 min IPS was more accurate in identifying sows sham chewing than 5 min IPS, both instantaneous point sampling methods failed to identify some sows that continuous sampling identified.

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

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

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

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

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

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

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

	Herd 1 Free access stalls		Herd 2 Floor feeding	
Group size	35		14	
Area (m ² /sow)	3.5		3.0	
	Straw	Maize	Straw	Maize
Number of sows included in the study	554	1,068	608	758
Number of pens	16	16	47	25
Sham chewing, % sows 5 h after feeding	62 ^a	48 ^b	41 ^a	16 ^b
Number of sows examined	62	90	47	77
Stomach ulcer, % of sows with score over 6	48	45	32	46

^{a,b}Significantly different ($P < 0.05$).

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Agonistic interactions in stable group housed swine using a Gestal[®] free access stall over two gestation periods

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

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

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

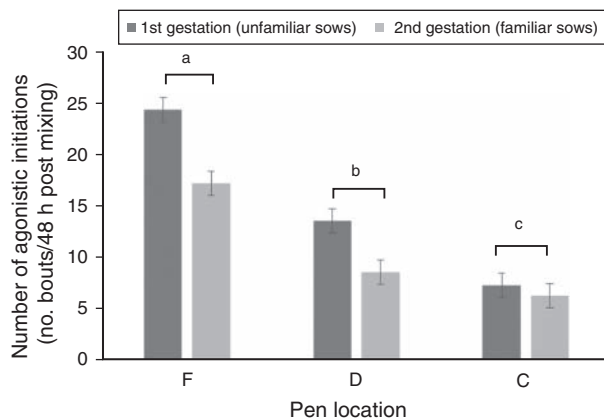


Fig. 1. Mean (\pm s.e.) agonistic initiations performed (bouts/48 h, $n = 38$) in group housed sows across two gestation periods (first gestation ■; second gestation ■) and area within in pen (F, feeding area; D, drinker area; and C, concrete flooring). Data are presented as back-transformed values.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

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

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

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

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

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

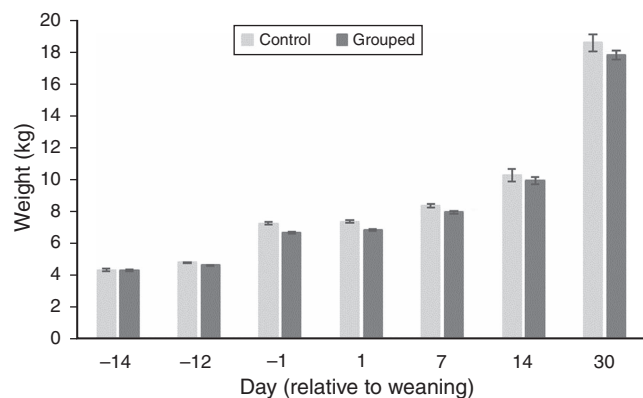


Fig. 1. Pre- and post-weaning piglet growth from control piglets and piglets grouped for the last 14 days of lactation. Day 0 represents weaning.

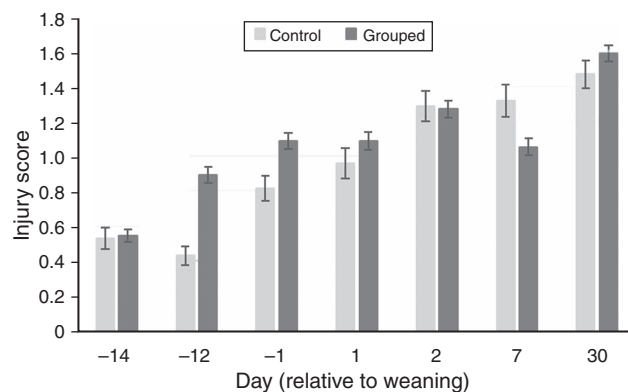


Fig. 2. Pre- and post-weaning injury scores from control and piglets grouped for the last 14 days of lactation. Day 0 represents weaning.

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

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

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

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

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

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

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

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Nurse sows display altered reproduction in the next gestation

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

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

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

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

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

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

^A95% confidence intervals rather than s.e.m. are presented for binary data. NS, not significant.

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

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

Sows ($n = 811$, parity 3.2 ± 0.1) were randomly allocated to treatment; CON fed lactation mash, MgSO₄ fed lactation mash with added magnesium sulphate (2.85 kg/tonne), SUPP fed lactation mash mixed with an algae supplement high in magnesium and calcium (5.5% and 30% respectively; 5 kg/tonne). Sows were fed 2.5 kg of the treatment diets from 5 days prior, and *ad libitum* to 3 days after farrowing. From 4 days to weaning, all sows were fed the CON diet *ad libitum*. Sows that farrowed between 0700 and 1600 h were checked every 40 min and if no progression was noted, were manually assisted. Sows were fostered to 11.52 ± 0.03 piglets 12 to 24 h after farrowing, and where possible this was conducted within treatment. All data analyses were carried out in SPSS v24.0 (IBM, Armonk, NY, USA) with the number of times a sow was assisted, the number of piglets pulled manually and all piglet mortality using a generalised linear model with Poisson distribution, percentage of sows requiring assistance using a generalised linear model with binary distribution and total pigs born, pigs born alive and number of piglets weaned using a general linear model.

The percentage of sows requiring assistance was unchanged by treatment, but the number of sow assists, and number of piglets pulled manually were reduced in the SUPP treatment compared with CON and MgSO₄ ($P < 0.05$; Table 1). Total piglets born, and piglets born alive did not differ, but number of piglets born dead increased in MgSO₄ sows compared to CON ($P = 0.01$). More piglets died from d 1 to 3 in the SUPP treatment than CON ($P = 0.05$), but number of piglets weaned was similar for all treatments.

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

Table 1. Mean \pm s.e.m performance traits for sows fed a standard lactation diet (CON) and those supplemented with magnesium sulphate (MgSO₄) or an algae supplement high in magnesium (SUPP) from 5 days before to 3 days after farrowing

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

^{a,b}Means in a row with different superscripts differ significantly ($P < 0.05$). ^A95% confidence intervals are presented for binary traits rather than s.e.m. NS, not significant.

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

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Play behaviour in early life may have an important role in the cognitive and social development of piglets. Research also supports play as a potential positive welfare indicator (Brown *et al.* 2015). Environmental enrichment can provide opportunities to express exploratory behaviours that could lead to increased play behaviour. Our aim was to determine the effects of access to lucerne on piglet play. The hypothesis was that the provision of lucerne throughout lactation would increase play behaviour in piglets. Approximately 1 kg of lucerne hay was added to the farrowing crate daily for the first week of lactation, then every second day until weaning. Piglets from nine control litters ($n = 98$) and 13 enriched litters ($n = 141$), born across several days, were recorded for 24 h on a set day, 2 weeks after the farrowing period begun. Behavioural analysis was carried out for 2 h of video footage (11:00 to 13:00) for all litters by instantaneously sampling every 15 s. Behaviours expressed were grouped into; play, active, rest or nursing, and were mutually exclusive to one another. Piglets were not individually identified, but each piglet was nominated to one of the behaviour groups at each sample point. Binominal generalized linear mixed models with treatment and piglet age, and their interaction, fitted as factors and sow as a random factor were used to analyse incidents of play, nursing and active behaviours (Bates *et al.* 2014). Analyses were performed in R (R v3.3.2; R Core Team 2017). Treatment did not statistically influence any of the behaviours ($P > 0.05$). Age influenced nursing ($P = 0.016$) and play ($P = 0.04$), but the relationships were not linear. Behaviour varied widely between litters (Fig. 1). The occurrences of play were low and occurred sporadically, but, observationally, they appeared more common after a nursing event.

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

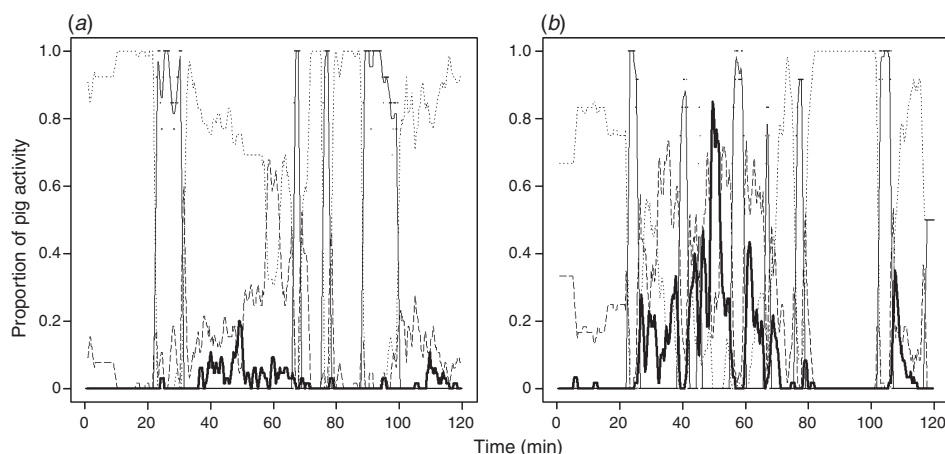


Fig. 1. Behavioural profiles of two litters (Control (a) and Lucerne (b)), showing the proportion of nursing (thin black), active (broken), play (thick black) and resting (dots) over the 2 h observation period.

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Strategies to reduce the pain of tail docking in piglets

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

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

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

Table 1. Mean total plasma cortisol (ng/mL) concentrations at 15 and 30 min post-treatment

	No handling	Sham	Clipper	Cauteriser	Meloxicam + Clipper	Meloxicam + Cauteriser	s.e.m.	P-value
Cortisol 15 min	88.6 ^a	138.4 ^b	186.7 ^c	169.5 ^{cd}	163.2 ^{bed}	144.3 ^{bd}	3.97	< 0.001
Cortisol 30 min	212.6 ^a	276.2 ^b	317.7 ^b	267.5 ^c	261.8 ^c	238.7 ^{ac}	6.73	0.001

^{a-d}Within rows values with different superscripts are significantly different ($P < 0.05$). s.e.m., standard error of mean.

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

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

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

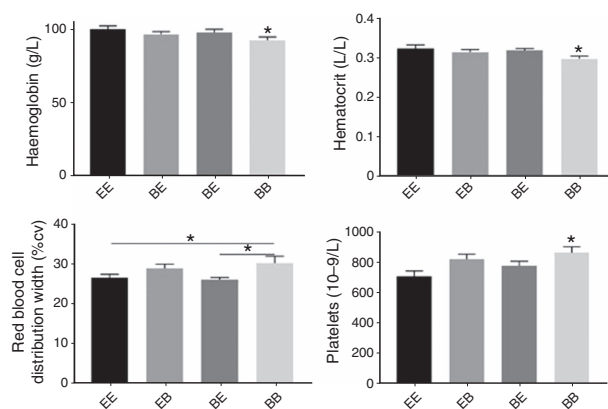


Fig. 1. The effect of enrichment on four red blood cell parameters. *indicates significance ($P < 0.05$).

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

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

The litters ($n = 1551$ piglets) of 112 sows (Large White \times Landrace, PrimeGroTM Genetics, Corowa, NSW, Australia) were allocated to one of three treatments over four time replicates: 1) Group lactation (GL) from 7 days post partum (GL₇, $n = 48$ litters), 2) GL from 14 days post partum (GL₁₄, $n = 48$ litters), or (3) Farrowing crate (FC; $n = 16$ litters). All dams farrowed in standard farrowing crates, where FC litters remained (with their dams) until weaning. GL₇ and GL₁₄ litters were transferred (with their dams) from farrowing crates to GL pens (one pen of five sows at 8.4 m²/sow and one pen of seven sows at 8.1 m²/sow, per treatment and replicate) at 7 and 14 days post partum, respectively. All treatments were weaned at 28 days post partum. Treatments were balanced for sow parity, weight and litter size, and there were no treatment differences in litter weight and sex ratio, or variation in these variables. Four focal piglets (one average-sized male and one average-sized female from a high and a low parity dam) per GL pen, and two focal piglets per FC (selected as per GL piglets), were video recorded from 0700 to 1700 h on the day after mixing (D2) and 2 days before weaning (pre-weaning, PW). Of the four FC litters per replicate, two were video recorded on the same days as GL₇ (FC₇) and the other two recorded on the same days as GL₁₄ (FC₁₄). Piglet time-budgets were observed using point sampling with 5 min intervals. Behaviours were analysed with LMM and GLMM models (SPSS v23.0, IBM, Armonk, NY, USA), with the main and interactive effects of housing (GL v. FC), litter age at mixing (7 v. 14 days) and observation day (D2, PW), as repeated-measures and controlling for pen and replicate as random factors (Table 1). Effects of GL₁₄, GL₇ and FC treatments on aggression following mixing with unfamiliar (ratio 50 : 50) pigs post-weaning was also analysed for one replicate (five pens/treatment, 12 pigs/pen).

Time spent suckling declined over lactation for all housing treatments. GL piglets suckled mostly from their dam at d 2 post-mixing, but mostly from other dams at d 26. The higher incidence of potentially harmful manipulations (manipulating ears, belly/tail) in FC compared to GL treatments at d 26 requires further investigation. No other piglet behaviours were affected by lactation housing. Post-weaning, FC pigs delivered 57 to 72% more single bites/knocks, and engaged in 43% more fights post weaning than GL pigs. The means for GL₇, GL₁₄ and FC piglets were, respectively, 17.0, 11.2 and 39.8 bites/knocks per pig ($P < 0.001$) and 1.29, 1.48 and 2.45 fights per pig ($P = 0.006$). This suggests that group-lactation systems can reduce piglet's aggression post-weaning.

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

	GL ₇		GL ₁₄		FC ₇ ^A		FC ₁₄ ^B		Housing	<i>P</i> -value Day	Age
	D2	PW	D2	PW	D2	PW	D2	PW			
Suckling (total) ^C	17.7	16.6	15.8	12.4	16.0	13.7	14.0	12.4	0.16	0.11	0.02
Suckling other dam ^C	3.8	10.6	3.9	9.1	N/A	N/A	N/A	N/A	N/A	<0.01	0.66
Nosing sow ^C	1.4	1.5	0.9	1.4	0.6	1.0	1.1	0.9	0.18	0.05	0.37
Manipulating	0.1 ^a	0.2 ^a	0.0 ^a	0.2 ^a	0.5 ^b	1.6 ^b	0.8 ^b	3.1 ^b	0.06 ^D	<0.01 ^D	0.48
Aggression ^E	0.7	0.7	0.7	0.2	0.8	0.8	1.2	0.7	0.13	0.14	0.08
Play	0.4	0.2	0.3	0.4	0.6	0.3	0.6	0.2	0.47	0.05	0.88

^{A,B}Observed on the same days at GL₇ and GL₁₄, respectively. ^C $y = \sin^{-1}(\sqrt{X})$ back-transformed means. ^DHousing \times day interaction, $P < 0.01$. ^EBites, knocks, fights. ^{a-c}When there was an interaction, superscripts indicate where means differ.

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

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

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

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

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

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

	Control	Glutamine	<i>P</i> -value
Total pigs born per litter	13.02 \pm 0.21	13.03 \pm 0.22	0.975
Total pigs born alive per litter	11.72 \pm 0.19	11.74 \pm 0.20	0.945
Average birthweight of all piglets born alive (kg)	1.53 \pm 0.02	1.51 \pm 0.02	0.318
Variation in live born weight ^A (%)	18.97 \pm 0.43	19.10 \pm 0.48	0.843
Average piglet weaning weight ^B (kg)	7.45 \pm 0.08	7.25 \pm 0.08	0.070
Variation in piglet weaning weights ^{A,B} (%)	21.28 \pm 5.50	22.05 \pm 5.27	0.316

^ACoefficient of variation = (s.d./mean) \times 100%. ^BAge at weaning included in the analysis as a covariate.

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

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

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

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

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

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

Treatment Trait	Least square mean \pm s.e.				Parity (P)		P -value ^A	
	Diet (D)				GILT	SOW	D	P
	CON	CLA	MCFA	BOTH				
Δ SW (kg)	-22.7 ± 1.8	-18.0 ± 1.8	-19.4 ± 1.7	-17.9 ± 1.8	-13.6 ± 1.3	-25.4 ± 1.3	NS	*
Δ P2 (mm)	-3.6 ± 0.4^a	-2.0 ± 0.4^b	-2.6 ± 0.4^{ab}	-2.5 ± 0.4^{ab}	-1.3 ± 0.3	-4.0 ± 0.3	*	*
NBA	12.6 ± 0.4^a	11.6 ± 0.4^b	12.3 ± 0.4^{ab}	12.1 ± 0.4^{ab}	11.2 ± 0.3	13.1 ± 0.3	**	**
Piglet d 0 to 21 ADG (g/d)	207 ± 4	195 ± 4	197 ± 4	199 ± 4	176 ± 3	223 ± 3	NS	**

^ANS, not significant, $P \geq 0.10$; * $P < 0.10$; ** $P < 0.05$. ^{a,b}Different superscripts within rows denote significant pairwise differences between diets ($P < 0.05$).

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

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

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

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

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

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

** $P < 0.01$.

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

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

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

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

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

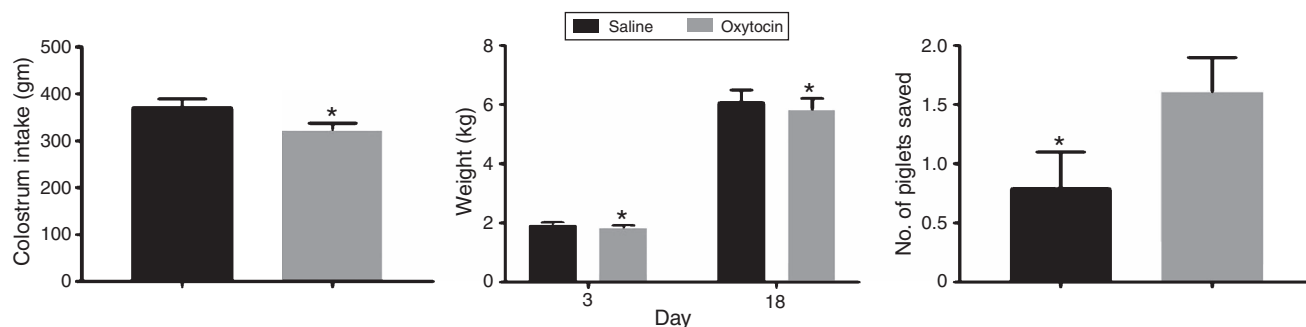


Fig. 1. The effect of 25 µg of oxytocin delivered intranasally to gilts, on colostrum intake, piglet weight at d 3 and 18 and the number of piglets saved. *, $P < 0.05$.

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

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

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

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

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

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

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

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

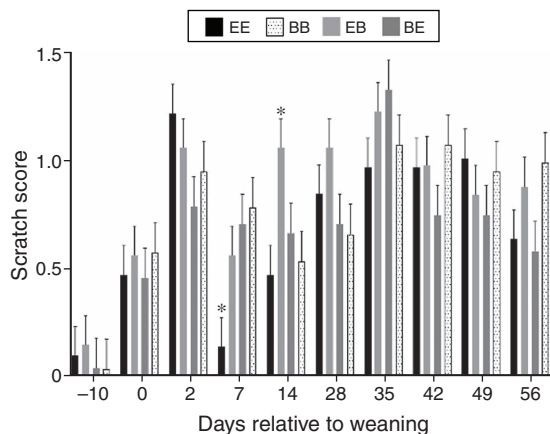


Fig. 1. The effects of enrichment on scratch score in piglets measured weekly around the weaning period. *Indicates significant differences between treatments post weaning.

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

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

Fifty female pigs were selected at weaning and randomly allocated (21 days, mean weight 5.4 ± 0.5 kg (mean \pm s.d.)) into three dietary treatment groups ($n = 25$) and maintained through to slaughter (~21 weeks of age). Isoenergetic and isonitrogenous diets were fed to treatment groups throughout the experiment containing a base vitamin/mineral premix (120 ppm Zn for ZnSO₄, 50 ppm Mn from MnO, 15 ppm Cu from CuSO₄) with the inorganic treatment group receiving an additional 750 g/t of the premix. Amino acid complex (AAC) treatment received 750 g/t of Availa[®] Sow (Zinpro Corp., Eden Prairie, MN, USA) delivering an additional 50 ppm Zn, 20 ppm Mn and 10 ppm Cu from amino acid complexed minerals. Pigs were held in a group housing system with feed and water offered *ad libitum*. The left and right rump was shaved of all hair at the start of the experiment (Muller *et al.* 2015) and a pooled hair sample was taken before slaughter at 21 weeks. Hoof samples, from each of the rear and front limb, were also collected at 21 weeks, with both hair and hoof samples analysed for their trace mineral content. Data were analysed using the Univariate GLM and correlation procedures (GENSTAT 15, VSN International, Hemel Hempstead, UK).

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

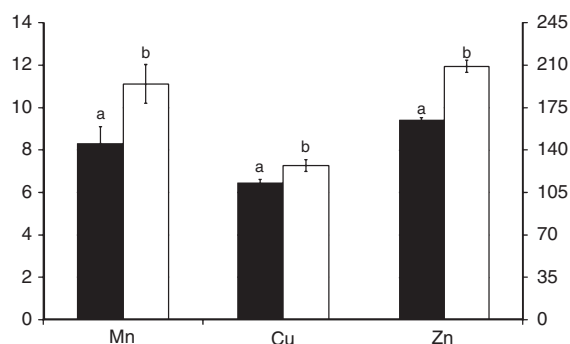


Fig. 1. Concentration (ppm) of Mn, Cu, (primary-axis) and Zn (secondary-axis) in the hoof of growing gilts fed control diets (■) or diets supplemented with amino acid complexed minerals (□; Mn, Cu, and Zn) from weaning to 21 weeks of age. ^{a,b}Means with different superscripts differ significantly ($P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

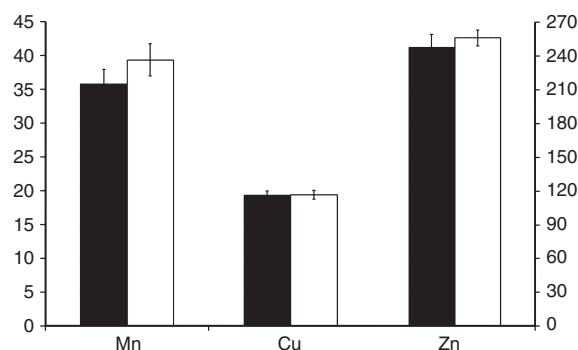


Fig. 2. Concentration (ppm) of Mn, Cu, (primary-axis) and Zn (secondary-axis) in the hair of growing gilts fed control diets (■) or diets supplemented with amino acid complexed minerals (□; Mn, Cu, and Zn) from weaning to 21 weeks of age.

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A poured block reduces feeding associated aggression in sows during gestation

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

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

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

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

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

^ASED, standard error of difference of the means. ^{a,b}Means in a row with different superscripts differ significantly ($P < 0.05$).

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

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

The experiment was conducted at the May Farm pig unit, Camden, NSW, with 19 Large White × Landrace litters containing 197 piglets from 10 days to 9 weeks old. Litters remained together throughout the experiment, with weaning occurring at about d 26. Litters were allocated at random to one of three block-shape treatments: (1) Cube; (2) Brick; or (3) Wedge. Pigs and enrichment blocks (one block per pen, fixed on 10 mm thick steel rod) were weighed weekly, and blocks were replaced weekly with the same treatment shape. Within litters, the number of pigs observed to interact oro-nasally with the enrichment block was recorded from video (AHD1 Mega Pixel Cameras and AHD 1080P Digital Recorder, CCTV Central, Mount Waverley, Vic., Australia) using a point sample technique. Four focal pigs per litter were also marked enabling quantification from the video of bout duration of interactions with the blocks. Interactions were recorded on each minute over the first 30 min after block replacement, on each minute over the first 5 min/h for the next 23 h after block replacement, and on each minute over the first 5 min/h on the fourth day after block replacement. Behaviour data were analysed using Generalised Linear Mixed Models while weight data were analysed with Linear Mixed Models in REML (GENSTAT 17, VSN International, Hemel Hempstead, UK).

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

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

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

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

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

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

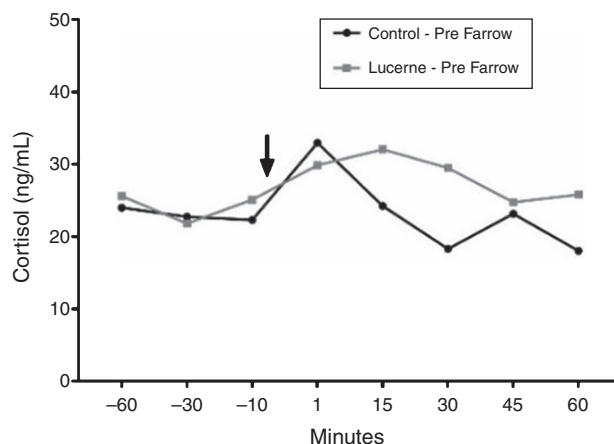


Fig. 1. The mean plasma concentration of cortisol (ng/ml) for sows for 60 min before, and 60 min after, the introduction of a feed cart. Arrow indicates the introduction of the feed cart.

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

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Group-lactation housing may improve sow welfare by increasing the opportunity for the sow to move about and express social and maternal behaviours (van Nieuwamerongen *et al.* 2014). This study tested the hypothesis that group-housed lactating sows would (1) engage in less aggression and more positive social interactions when mixed at 7 rather than 14 days post partum; and (2) show better maternal behaviour (nursing behaviour and sow-piglet interactions) than sows housed in farrowing crates. One hundred and twelve sows (Large White × Landrace, PrimeGroTM Genetics, Corowa, NSW; Parity 1 to 7) and their litters were allocated to one of three treatments over four time replicates: (1) group lactation (GL) from 7 days post partum (GL₇, $n = 48$ sows); (2) GL from 14 days post partum (GL₁₄, $n = 48$ sows); or (3) farrowing crate (FC; $n = 16$ sows). All sows farrowed in standard farrowing crates, where FC sows remained until weaning. However, GL₇ and GL₁₄ sows and their litters were transferred from farrowing crates to GL pens (one pen of five sows at 8.4 m²/sow and one pen of seven sows at 8.1 m²/sow, per treatment and replicate) at 7 and 14 days post partum, respectively. All treatments were weaned at 28 days post partum. Treatments were balanced for sow parity, weight and litter size, and there were no treatment differences in litter weight and sex ratio, or within pen/crate variation in these variables. For GL pens, two focal sows per pen (one high and one low parity) were video recorded from 0700 to 1700 on the day after mixing (D2) and 2 days before weaning (pre-weaning, PW). Of the four FC sows per replicate, two were video recorded on the same days as GL₇ (FC₇) and two on the same days as GL₁₄ (FC₁₄). Data gathered continuously from video records included sow aggressive and nursing behaviours while sow time-budgets were observed using point sampling at 5-min intervals. Behaviours were analysed with LMM and GLMM models (SPSS v23.0, IBM, Armonk, NY, USA), with the main effects of housing (GL v. FC), litter age at mixing (7 v. 14 days) and observation day (D2, PW), as repeated-measures and controlling for pen and replicate as random factors (Table 1). There were no significant interactions between main effects.

Aggression (bites/knocks) between GL sows increased by nearly 40% from d 2 to 26; however, there was no effect of age at mixing on aggression. GL₁₄ sows engaged in more positive interactions with conspecifics (i.e. nosing sow) than GL₇ sows. Sows spent less time lying in GL pens compared to FC and interacted with piglets more frequently. Whilst there was no difference in number of nursings between GL and FC treatments, GL housing disrupted nursing behaviour, as evidenced by reduced proportion of successful nursing bouts, a tendency for increased sow terminated bouts and a longer inter-nursing interval, compared to FC sows. Sow aggression and disrupted nursing behaviour in GL may result in compromised welfare and growth of sows and piglets, and is being investigated.

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

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

^{A,B}Observed on the same days at GL₇ and GL₁₄, respectively. ^CFrequencies. ^DProportion of observations or nursing bouts. ^E $y = 1/x$ back-transformed means presented (min).

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

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

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

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

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

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

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

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

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

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

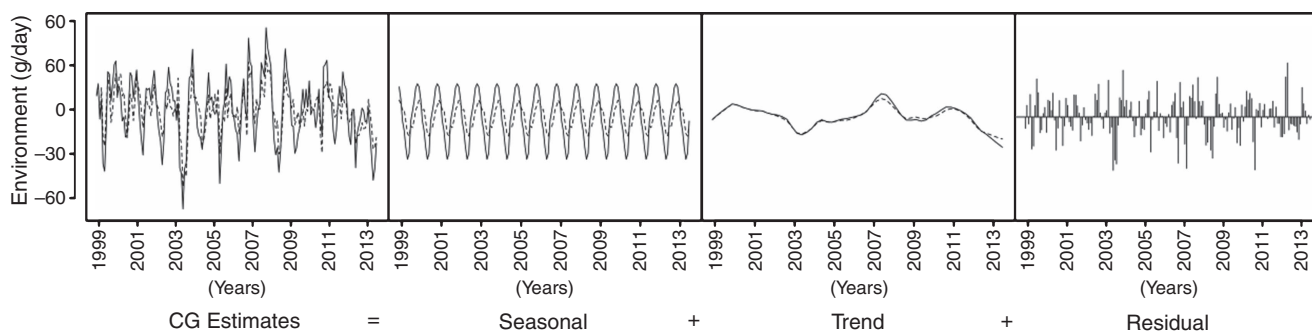


Fig. 1. Contemporary group (CG) estimates (— unadjusted and --- adjusted for minimum monthly temperatures), decomposed into seasonal, trend and residual components using time series analysis.

separately for genetic improvement of traits such as disease resilience.

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Modifying procedures to assess immune competence in mature boars

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

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

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

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Breeder dam parity does not affect lifetime reproductive performance

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

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

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

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

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

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

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

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Early lifetime performance parameters affecting selection and reproductive success in gilts

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The sow replacement rate in Australia is 56.1%, with the average parity at which a sow is culled, currently sitting at 4.1 (Australian Pork Limited 2013). There are several key reasons for premature sow turnover, with failure to express pubertal oestrus and poor reproductive performance during the early parities considered a major cause for removal. Most on farm selection criteria for replacement gilts focus on gilt attributes at selection into the breeding herd. However, including criteria from early lifetime performance parameters such as birthweight, weaning weight, and pre-weaning growth may aid in selecting gilts with a higher probability of reproductive success (Knauer 2016). The hypothesis of this study was gilts that are born heavier and which do not have any growth deficits during the pre-weaning or post-weaning period will have a higher probability of (1) being selected into the breeding herd, and (2) displaying pubertal oestrus, resulting in at least one successful mating.

From January 2014 until March 2015, individual weights at birth and d 21 were recorded on 10 480 multiplier gilts (Large White × Landrace, PrimeGro™ Genetics, Corowa, NSW, Australia) born at the genetic supply unit of a large commercial pig producer located in southern New South Wales, Australia. As a matter of routine recording, the date of birth, parity and gestation length of the dam, as well the number of total piglets born in the litter, were known for each individual gilt. Post weaning weights were recorded 2 weeks after weaning on a subset of 3288 gilts. Of the 10 480 gilts included in the project, 8852 (84.5%) gilts were selected to enter the breeding herd and were sent to five different sites on the one farm. A subset of selected gilts had data for weight ($n = 7446$) and P2 backfat ($n = 3399$) at selection. Of the gilts selected, 7612 (72.6%) were mated and 6870 (65.5%) farrowed at least once. The significance of early weights and development for the probabilities (0/1) of a gilt being selected (SEL), mated (MATE) and successfully farrowing at least one litter (FARR), was investigated using stepwise logistic regression (PROC LOGISTIC, SAS v9.4, SAS Institute Inc., Cary, NC, USA). Apart from year-quarter of birth (season) and dam parity group (four levels: Parities 1, 2, 3–5, 5+), early in life explanatory variables submitted to the procedure included deciles (allocated within birth year-quarter) and were all treated as class effects. Within each explanatory variable, gilts with missing records were allocated to a separate class. Site (five levels) was also submitted for MATE and FARR. Only factors significant at $P < 0.05$ were included in the final models for each trait. The significance of the difference between each factor level and the reference decile was assessed via the odds-ratio. The sixth decile was the reference level for each explanatory variable. The corresponding probability of gilts being selected, mated and farrowing for deciles significantly different to Decile 6 were back-calculated using the corresponding odds and the odds-ratios.

Season of birth was the most significant factor contributing to SEL ($P < 0.0001$). After season, gilts from the lowest decile for birthweight were 5% less likely to be selected than gilts in Decile 6 ($P < 0.001$). Gilts in the lowest 10% for 21 days weight and post-weaning gain had a 14% lower probability for SEL compared to Decile 6 ($P < 0.0001$). The probability of SEL was also reduced by 6% for gilts in the lowest 30% for weaning age ($P < 0.05$). Season and site were the most significant factors affecting both MATE and FARR ($P < 0.0001$). Relative to the reference level, gilts in the lowest 10% for pre-weaning gain, the lowest 20% for weight at selection, and the lowest 30% for P2 backfat at selection also had a reduced MATE, with corresponding reductions of 9%, 7% and 10%, respectively ($P < 0.01$). Gilts in the four lowest deciles for pre-weaning gain had a 10% reduction in the probability of FARR, whereas gilts in the highest 20% for post-weaning gain, or the top 30% for P2 backfat at selection, had an increased probability of FARR (+10% and +8%) relative to the reference level. The association between P2 backfat and FARR was generally linear. Being in the low deciles for birthweight, 21 days weight and post-wean gain were detrimental for surviving to selection or meeting the minimum weight requirement at selection age. Once selected, being in the lower deciles for pre-weaning gain, selection weight and P2 backfat at selection decreased the probability of being mated and successfully farrowing a litter whereas, being in the higher deciles for post-wean gain and P2 backfat at selection significantly increased a gilts' probability of success.

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Caffeine increases gestation length on a commercial farm

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In recent years, pressure for sow prolificacy has been applied to increase the number of pigs sold/sow/year. However, selection pressure on sow prolificacy and the resultant increase in litter sizes has adverse consequences, most notably increased stillbirths, lower birthweights, lower viability at birth and hence poor survival (Quiniou *et al.* 2002). Small scale studies have demonstrated that maternal caffeine supplementation on the day before parturition decreased stillbirths and increased piglet temperature at birth (Superchi *et al.* 2013, 2016). Recent data (B. A. Dearlove and W. H. E. J. van Wettere, unpubl. obs.) indicated that 3 days of caffeine supplementation (6 g/d) before parturition extended gestation length and increased piglet temperature shortly after birth. The aim of the current study was to determine the impact of caffeine supplementation of sow diets before parturition on gestation length and the incidence of stillbirths under commercial conditions. We hypothesised that caffeine would extend gestation length and reduce stillbirths.

Large White, Landrace, and Duroc sows ($n = 348$, parity 2.85 ± 0.10) were allocated to one of three treatment groups, Control (CTL, $n = 122$), 3 g/d caffeine (CAF3, $n = 111$) and 6 g/d caffeine (CAF6, $n = 115$). Treatment began at d 112 of gestation and continued until farrowing (CAF3: mean 3.74 ± 0.14 days treatment; CAF6: 3.91 ± 0.14 days treatment). Gestation length, total born, born alive and born dead, and piglet survival to processing (processing occurred within the first 24 h of life), d 4 and d 21 post partum were recorded. Statistical analysis was performed using a mixed general linear model (SPSS v24.0, IBM, Armonk, NY, USA) with treatment, breed, parity and whether the piglets were purebred or crossbred with total born as a covariate. Sow was treated as a random effect. Data from individual piglets were treated as repeated-measures on the sow in similar mixed linear models including the sow as a random factor. Data are presented as estimated means \pm s.e. from mixed models. Total born (12.04 ± 0.39), born alive (10.92 ± 0.19) and stillbirths (0.93 ± 0.15) were unaffected by treatment. Treatment also did not affect piglet survival to processing ($96.76 \pm 0.86\%$), d 4 ($91.50 \pm 1.34\%$) and d 21 post partum ($89.54 \pm 1.88\%$). Gestation length was increased in the CAF6 group compared to the CTL group (CTL: 115.22 ± 0.21 ; CAF3: 115.76 ± 0.22 ; CAF6: 116.11 ± 0.23 ; $P < 0.01$) and tended to be increased in the CAF3 compared to the CTL group ($P < 0.08$). There was also a breed difference in gestation length with Landrace and Duroc sows having an extended gestation on the CAF6 treatment (Table 1).

The failure of caffeine to reduce stillbirths in this study may have been due to the intense supervision received under experimental conditions compared to commercial conditions (Superchi *et al.* 2013, 2016), suggesting that further studies should be conducted to confirm if caffeine supplementation does represent a commercial strategy to improve piglet survival. However, consistent with our previous data, gestation length was increased in response to caffeine supplementation. This is an important finding, as sows which farrow prematurely (< 115 days) are likely to produce more stillborn and low viability piglets, with fewer of their piglets surviving to weaning (Vanderhaeghe *et al.* 2011). Maternal caffeine supplementation may, therefore, be a useful strategy for herds in which premature farrowing is a problem.

Table 1. Gestation length (d) for Large White, Landrace and Duroc sows receiving either no (CTL), 3 (CAF3) or 6 (CAF6) caffeine a day from d 112 of gestation until farrowing

	CTL	3 g	6 g
Large White ($n = 111$)	114.67 ± 0.33	115.47 ± 0.29	114.77 ± 0.34
Landrace ($n = 203$)	115.30 ± 0.19^a	115.59 ± 0.27^{ab}	116.35 ± 0.27^c
Duroc ($n = 34$)	114.42 ± 0.44^a	115.13 ± 0.47^{ab}	115.99 ± 0.43^b

Data are represented as means \pm s.e. Significant differences within sow breed are highlighted using superscripts (^{a-c} $P < 0.05$).

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Sow dimensions increase with increasing parity but not with increasing litter size

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In most of indoor farrowing accommodations, sows are housed in crates. During mating and gestation many sows are in stalls, or are being fed in feeding stalls or in Electronic Sow Feeders. Increasing consumer awareness of sow welfare makes it important to ensure that sows do not risk injuries associated with crates or stalls that are too small (Pedersen 2015). Therefore, it is important that crates, stalls and feeding stations meet the space requirements of sows. Dimensions of Danish crossbred-sows were measured several years ago (Moustsen *et al.* 2011). Based on those measurements, recommendations for dimensions of crates, stalls, and pens were decided on. Body dimensions of sows increase with increasing parity but whether or not increased litter size leads to increases in body dimensions, or whether larger sows give birth to larger litters, has not been investigated. The objective of the present study was to determine whether body dimensions of Danish crossbred sows had increased (Moustsen *et al.* 2011) and if larger sows gave birth to larger litters. The average parity of the measured sows was 3.2, ranging from Parity 1 to 10. Sows farrowed, on average, 8.8 days before measuring. Length, width, height and depth of ~40 hyper-prolific crossbred (Large White × Landrace) sows, in 10 production herds, were measured (Table 1). The sows were in a standing position on a level surface when measured. Length was measured with a carpenter's rule as a straight line from snout to behind hind legs. Three measurements were taken and the average used in analysis. Depth, and width at the shoulders was measured using a specially developed calliper. Depth was measured in the middle section of the sow between the front and hind legs, from the dorsal to the ventral surface, and can be used as an estimate of the width of the sow's body when lying. The height was measured using the carpenter's rule. Number of liveborn and stillborn piglets were recorded. Data were analysed using generalized mixed models (SAS EG 7.1, SAS Institute Inc., Cary, NC, USA) with length, width, height and depth in turn as response variables, and parity group (1, 2 to 3, 4+) and total born (< or > than the median of the parity group) as explanatory variables. Herd was included as a random effect. In the statistical analysis, means were compared by Type 3-test. Length, width, height and depth increased significantly with increasing parity ($P < 0.001$), however, within parity group there was no difference in body dimensions between sows having litter size less or higher than the median of the group.

It is important that housing facilities allow the sows to stand up and lie down unhindered and enables all piglets easy access to the udder. Therefore, knowledge of sow dimensions is important. The average litter size in Danish production herds has increased over the years. However, this study concluded that there was no significant correlation between the measured sow dimensions and litter size. In addition, sow dimensions were similar to a previous study (Moustsen *et al.* 2011). It is expected that recommendations based on Moustsen *et al.* (2011) will continue to ensure sows' ability to stand up and lie down unhindered.

Table 1. Litter size and body dimensions of 405 Danish crossbred sows in 2017. Mean \pm s.e. and 5th to 95th percentiles (*in italics*)

	Parity 1 ($n = 114$)		Parity 2 to 3 ($n = 130$)		Parity 4+ ($n = 161$)		<i>P</i> -value
Total born (n)	16.2 \pm 0.3 ^a	<i>11–22</i>	18.3 \pm 0.4 ^b	<i>9–25</i>	19.1 \pm 0.3 ^b	<i>13–25</i>	<0.001
Length (cm)	169 \pm 0.8 ^a	<i>155–183</i>	181 \pm 0.6 ^b	<i>170–190</i>	192 ^c \pm 0.5 ^c	<i>180–203</i>	<0.001
Height (cm)	83 \pm 0.4 ^a	<i>76–89</i>	87 \pm 0.3 ^b	<i>81–92</i>	90 \pm 0.3 ^c	<i>84–96</i>	<0.001
Width (cm)	38 \pm 0.2 ^a	<i>34–42</i>	40 \pm 0.2 ^b	<i>36–45</i>	42 \pm 0.3 ^c	<i>38–47</i>	<0.001
Depth (cm)	57 \pm 0.3 ^a	<i>51–63</i>	61 \pm 0.3 ^b	<i>56–66</i>	65 \pm 0.1 ^c	<i>60–71</i>	<0.001

^{a–c}Significant differences between parity groups within rows.

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A single intravenous injection of Kisspeptin evokes an increase in luteinising hormone in 15- and 18-week-old gilts

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Kisspeptin is a neuropeptide essential to the regulation of the gonadotrophin releasing hormone (GnRH) neuroendocrine system (Lehman *et al.* 2010). Sheep, rodents and humans deficient in the gene that codes for kisspeptin, KISS1, never reach sexual maturity and kisspeptin is a key regulator of seasonality in sheep (Goodman *et al.* 2007). Expression of KISS1 mRNA is attenuated in the non-breeding season of sheep and injection of kisspeptin during the nonbreeding season can stimulate oestrus in ewes (Smith 2012). While the role of kisspeptin in the neuroendocrine control of reproduction in humans, sheep and rodents is well established, little is understood about its role in pigs. We hypothesised that a single intravenous injection of kisspeptin would evoke an increase in plasma concentrations of luteinising hormone (LH) in 15- and 18-week-old gilts and that this increase would be of similar magnitude in each age group.

We conducted the experiment over two replicates at the Roseworthy Piggery, Roseworthy, SA. Gilts were identified and tagged at birth. One week before commencement of the experiment, gilts were transferred to individual pens to acclimatise to the experimental environment. Gilts were then fitted with indwelling ear-vein catheters. In Replicate 1, 18-week-old gilts were allocated to three treatments ($n = 6$ per treatment). They were injected with either saline, 5 mg of Kisspeptin 10 (Pheonix Pharmaceuticals Inc., Burlingame, CA, USA) or 10 mg Kisspeptin 10. In Replicate 2, 15-week-old gilts were allocated to two treatments ($n = 6$ per treatment). They were injected with either saline or 10 mg of Kisspeptin 10. On the day of the experiment blood was collected every 15 min for 1 h before injection and then every 15 min for 6 h after injection. Data were analysed using a repeated-measures analysis of variance in SPSS v24.0 (IBM, Armonk, NY, USA).

The concentration of LH after kisspeptin injection was greater than before kisspeptin injection for 15- and 18-week-old gilts ($P < 0.05$) (Fig. 1). Panel A (Fig. 1) shows that the increase in LH in 18-week-old gilts after a 5 mg injection of kisspeptin was not significantly different from the increase in LH after a 10 mg injection of kisspeptin. There was no significant difference in the increase in LH between 15-week-old gilts given 10 mg of kisspeptin or 18-week-old gilts given 5 mg or 10 mg of kisspeptin.

Our data indicate that the neuroendocrine production of LH in pigs is stimulated by kisspeptin. This effect is evident in pigs that are 15 weeks old. Further research into the role of kisspeptin in the control of reproduction and seasonality in pigs is warranted.

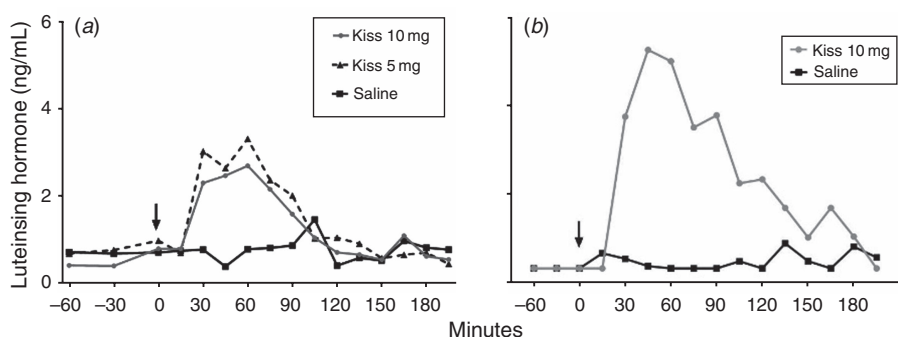


Fig. 1. Mean luteinising hormone concentration in response to a single intravenous kisspeptin (Kiss) injection for 18- (a) and 15-week-old (b) gilts. A 5 mg or 10 mg dose was given to 18-week-old gilts and a 10 mg dose was given to 15-week-old gilts. Injections were given at time 0 as indicated by the arrow. Blood samples were collected every 15 min commencing 60 min before injection and concluding 6 h after injection.

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Lifetime sow productivity is influenced by both body protein and body fat reserves after first-litter weaning

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Sow longevity and lifetime productivity is a key factor in determining herd productivity and lowering production costs. Sow replacement rates are high in Australia, with an average of 59% sow turnover (Benchmarking Report 2015, R. Campbell, pers. comm.) and the trends do not indicate any improvement. The main cause of sow turnover includes low litter size and reproductive failure, with many sows being culled prematurely. Hughes *et al.* (2010) suggested management practices that reduce sows entering the herd with excessive body reserves and are too heavy, would promote sow longevity. This study tested the hypothesis that sow longevity and lifetime performance is affected by body reserves in young sows.

In a study herd of 1637 weaned, Parity 1, first-cross sows (Large White × Landrace, PrimeGro GeneticsTM, Corowa, NSW, Australia), body reserves of protein and fat at mating following first litter weaning (Parity 1) were determined based on predictive equations (Smits *et al.* 2017). Sows were ranked independently for body protein and fat at Parity 1 mating as the lowest 25%, (LOW, $n = 409$), median 50% (MED, $n = 819$), and the highest 25% (HIGH, $n = 409$) cohort. The effects (mean ± s.e.) of body protein or fat cohort at mating on lifetime performance were determined by one-way GLM univariate ANOVA (SPSS v21.0, IBM, Armonk, NY, USA) using body fat or body protein mass, respectively, as a linear covariate. Data excludes any sow culled or removed before mating as a Parity 1 sow. Sows ranked with a HIGH body protein mass, adjusted to a constant body fatness, produced fewer litters and less piglets ($P < 0.05$) in their lifetime compared to sows with LOW or MED body protein reserves (Table 1). There was a minimal level of body fat reserves associated with lifetime performance, with MED or HIGH fat reserves, adjusted to a constant body protein, producing more piglets and lasting longer in the herd (LOW fat v. HIGH fat; $P = 0.053$). These results differ to other publications (Clowes *et al.* 2003), and this could be due to different genetics and feeding regimens resulting in sows with different protein and fat masses between studies.

In conclusion, our data provides evidence that sows with body protein not exceeding 26 kg, and body fat mass no less than 41 kg, last longer and are more productive than large lean sows. Furthermore, we suggest that breeding sows need to be individually fed throughout life so that body reserves, particularly fat levels, can be maintained as suggested by Bunter *et al.* (2010).

Table 1. The effect of sow body protein and fat mass at Parity 1 mating on lifetime sow performance

	Sow body protein mass at Parity 1 mating ^A			<i>P</i> value Main effect protein
	LOW (avg 26.0 kg)	MED (avg 28.8 kg)	HIGH (avg 31.5 kg)	
Lifetime litters	4.4 ± 0.1 ^y	4.2 ± 0.1 ^y	3.6 ± 0.1 ^x	<0.001
Lifetime live born	49.0 ± 1.3 ^y	47.3 ± 0.9 ^y	40.1 ± 1.3 ^x	<0.001
Lifetime total born	54.5 ± 1.5 ^y	52.8 ± 1.0 ^y	45.5 ± 1.5 ^x	<0.001
	Sow body fat mass at Parity 1 mating ^B			Main effect fat
	LOW (avg 31.4 kg)	MED (avg 40.6 kg)	HIGH (avg 51.0 kg)	
Lifetime litters	3.8 ± 0.1 ^x	4.2 ± 0.1 ^y	4.1 ± 0.1 ^{x,y}	0.013
Lifetime live born	42.5 ± 1.3 ^x	47.0 ± 0.9 ^y	46.4 ± 1.3 ^y	0.016
Lifetime total born	47.7 ± 1.5 ^x	52.9 ± 1.0 ^y	52.0 ± 1.5 ^y	0.014

^AMain effect body protein analysed with body fat (mean BF: 41.1 ± 0.2 kg) at Parity 1 mating included as a linear covariate. ^BMain effect body fat analysed with body protein (mean BP: 28.8 ± 0.1 kg) at Parity 1 mating included as a linear covariate. ^{x,y}Within rows, mean values differ $P < 0.05$.

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Effect of parity and stage of gestation on maternal growth and feed efficiency of gestating sows

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Previous research in regards to gestating sow nutrient requirements (Noblet *et al.* 1990) has been used to develop models based on the sow's body condition, parity and stage of gestation. However, data are limited pertaining to the application of these models in current commercial sow herds to determine maternal growth and efficiency of feed usage of modern sows. Therefore, the objective of this study was to evaluate the effect of parity and stage of gestation on maternal weight gain and efficiency of feed use in gestating sows from a commercial sow farm. A total of 712 females were group-housed from d 5 to 112 of gestation and individually fed with electronic sow feeders (ESF). Feed intake and bodyweight (BW) were recorded daily throughout gestation. Gilts (Parity 1) and sows, received 27.2 and 30.5 MJ ME per d based on set feeding strategies. Gilts and sows received 2.0 and 2.3 kg per day throughout the course of gestation. Maternal weight gain, not including products of conceptus, and feed efficiency was predicted using a series of equations to model nutrient utilisation in gestation (Dourmad *et al.* 2008). Data were divided into three parity groups and gestation was divided into three periods. Averages for each period were reported for all predictions with the exception of G : F, where the median for each period was reported. Data were analysed using PROC MIXED procedure in SAS (v9.4, SAS Institute Inc., Cary, NC, USA).

Parity 2 sows had the greatest ($P < 0.05$) energy use for maternal protein and fat deposition in all stages of gestation while Parity 1 sows had a negative energy balance during the final stage of gestation (Table 1). At every stage of gestation, maternal gain decreased with parity ($P < 0.05$). Regardless of parity, maternal average daily gain (ADG) decreased ($P < 0.05$) from d 39 to 74 before increasing ($P < 0.05$) during the final stage of gestation. Parity 1 sows had the greatest ($P < 0.05$) maternal ADG in all gestation periods. Parity 1 sow maternal G : F decreased ($P < 0.05$) in each sequential period of gestation. Parity 1 sow G : F was greater ($P < 0.05$) than Parity 2 and 3+ sows in most gestation periods.

Overall, this study and subsequent prediction models show how stage of gestation and parity affect growth of different tissue pools, sow maternal BW, and feed usage throughout the course of gestation. Further research is needed to investigate these differences and if there is an impact on subsequent performance.

Table 1. Maternal growth and feed efficiency of sows as influenced by parity and stage of gestation

	5–39	Day of gestation 40–74	75–109	Probability, <i>P</i>
Energy available for maternal protein and lipid deposition, KJ				
Parity 1	5035 ^{ax} ± 90.7	2895 ^{bx} ± 90.7	–145 ^{cx} ± 90.7	<0.001
Parity 2	7395 ^{ay} ± 104.4	5775 ^{by} ± 104.4	3016 ^{cy} ± 104.4	<0.001
Parity 3+	5431 ^{az} ± 86.3	4251 ^{bz} ± 86.3	1397 ^{cz} ± 86.3	<0.001
ADG (kg)				
Parity 1	0.47 ^{ax} ± 0.011	0.27 ^{bx} ± 0.011	0.41 ^{cx} ± 0.011	<0.001
Parity 2	0.32 ^{ay} ± 0.013	0.04 ^{by} ± 0.013	0.15 ^{cy} ± 0.013	<0.001
Parity 3+	0.23 ^{az} ± 0.011	–0.04 ^{bz} ± 0.011	0.34 ^{cz} ± 0.011	<0.001
G : F ^A				
Parity 1	1.29 ^{ax} ± 0.110	0.67 ^{bx} ± 0.110	–1.24 ^{cx} ± 0.110	<0.001
Parity 2	0.67 ^{ay} ± 0.127	–0.04 ^{by} ± 0.127	1.13 ^{cy} ± 0.127	<0.001
Parity 3+	0.88 ^{ay} ± 0.105	–0.34 ^{by} ± 0.105	0.17 ^{cz} ± 0.105	<0.001

^AMaternal feed efficiency is reported as G : F and was determined using the following equation: G : F = Maternal ADG (kg)/energy available for maternal deposition (kg).

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Development of non-invasive methods to monitor the transfer of dietary volatile compounds in pigs

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The transfer of volatile compounds from the maternal diet to amniotic fluid and milk has been previously evaluated using gas chromatography–mass spectrometry (GC/MS) (Palou *et al.* 2015). Both of these maternal fluids have disadvantages associated with the sampling procedures (i.e. sow monitoring at farrowing and oxytocin injection). Blood sampling is another method used to evaluate the transfer of dietary volatile compounds; however, this procedure is invasive, which causes stress and discomfort in the animals. The aim of this study was to develop a non-invasive user friendly methodology to assess the transfer of essential oil (EO) volatile compounds from feed to saliva and carpal gland secretion in pigs. We hypothesised that the quantification of volatile dietary compounds to saliva and carpal gland secretions will be indicative of the amount consumed and the transfer rate in the pre-absorptive and post-absorptive stages, respectively.

This trial was conducted at the Herston Medical Research Centre. Twelve post-weaning piglets were individually penned and allocated into one of three treatments: (1) single dose of a mix of EO in feed (EOF, 0.45 mg/kg BW of the principal compound of each EO (EOPC)); (2) single dose of a mix of EO intravenously (IV) injected (EOIV, 32 µg/kg bodyweight (BW) of each EOPC); and (3) a control consisting of standard feed without EO. Treatments were provided with the morning meal. The EO comprised lemon ironbark, peppermint gum, nerolina, clove, thyme, cinnamon, oregano, geraniol and anethole. Saliva, carpal gland secretion and serum were collected at different time points: 5, 15, 30, 45, 60, 120 and 180 min after administration of the treatments. Saliva was collected by approaching the mouth of the pig with a sea sponge attached to tweezers. While piglets were distracted chewing on the sponge, carpal gland secretion was collected by a procedure consisting of a gauze sponge impregnated in distilled water to gently wipe the skin in two directions (top to bottom and left to right) repeated three times ('skin washing'). In order to collect multiple blood samples a catheter was surgically implanted in the external jugular vein of all piglets. Data was statistically analysed using ANOVA in Minitab 16 (Minitab Inc., State College, PA, USA), to evaluate the transfer of EOPC to saliva, skin washing and serum between the three treatments at each time point. Paired *t*-test was performed to evaluate differences in the concentration of EOPC between serum with saliva and skin washing in treated pigs. The EOPC were analysed by headspace–solid phase micro extraction–CG/MS.

Overall there were significantly higher ($P < 0.05$) concentrations of EOPC in saliva compared to serum in EOF piglets while significantly lower ($P < 0.05$) concentrations were found in saliva compared to serum in EOIV piglets. Additionally, the concentrations of volatile compounds in skin washings were significantly lower ($P < 0.05$) than in serum in both EOF and EOIV treatment groups. These results indicated that the high levels of dietary EOPC found in saliva of piglets in EOF treatment are explained by direct contact of saliva with the volatile compounds present in feed. The result suggests that the levels of dietary volatile compounds found in saliva after oral consumption of EO are indicative primarily of aerial transfer from feed contents in the gastrointestinal tract, which, in turn, may be related to recent intake. On the other hand, the EOPC levels found in skin washings were absorbed from the gastrointestinal tract first, transferred to blood (or lymph) and then secreted through the carpal glands. Thus, EOPC in carpal gland secretions would indicate post-absorptive transfer efficiency and stability in biological tissues. To the best of our knowledge, this is the first report that monitors the transfer of dietary volatile compounds to saliva and carpal gland secretions.

It was concluded that the data offered two new approaches of monitoring the transfer of dietary volatile compounds to maternal fluids: one related to pre-absorptive (saliva) and the other to post-absorptive (carpal gland secretion) events. These results are relevant to the understanding of maternal-offspring imprinting through maternal diets in mammalian species including humans. Further investigation is required to evaluate the correlation between levels of dietary volatile compounds found in saliva and skin washing with those found in milk and amniotic fluid in pigs.

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Predicting oestrus and ovulation in sows using the vulva, cervical mucus and body temperature

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The most common method of oestrus detection in sows relies on observing behavioural changes indicative of sexual receptivity. This technique is time consuming, labour intensive and due to its subjective nature, often provides inconsistent results between observers (Soede *et al.* 2011). Consequently, it would be beneficial to identify alternative markers for oestrus, and more importantly for the onset of ovulation, by validating more quantifiable and objective measures. The hypothesis follows that fluctuations in vulva size, mucus composition and body temperature will occur within 24 h of ovulation and would be suitable measures to assist with the determination of the onset of oestrus.

In this pilot study, a range of biological markers were measured on 46 multiparous (mean parity \pm s.e.m. 3.22 ± 0.15 , range = 2–5) Large White \times Landrace sows over the anticipated period of oestrus, based on time post-weaning. Vulva size, vulva and ear temperature and cervical mucus pH, viscosity and crystallisation patterns, were recorded. Measurements were recorded at 12 h intervals from 3 days post-weaning to 2 days after the last observed oestrus behaviour. Vulva size was measured with a ruler and calculated as length \times width. Cervical mucus was extracted using a Rocket cervical mucus syringe and split into three aliquots. One aliquot was air-dried on a microscope slide, observed at $\times 100$ magnification and classified into six patterns based on the predominant shapes present (Abusineina 1962). The remaining aliquots were used to detect pH using test strips and viscosity length by measuring the stretch of mucus with a ruler (Rijnders *et al.* 2007). Internal vulva and ear canal temperatures were obtained using an infrared gun at a distance of 10 cm from the body surface and corrected for ambient temperature. The time of predicted ovulation was defined as 30 h before an increase in faecal progesterone (P4) as determined by ELISA, accounting for the 24 h hormone passage rate (Shaw and Foxcroft 1985).

The measurements were mapped to three time points; the onset of behavioural oestrus determined by the first instance of standing heat in response to back pressure, 24 h before predicted ovulation and the point of predicted ovulation (Table 1). Each marker was analysed using an ANOVA in GENSTAT 16 (VSN International, Hemel Hempstead, UK). Mucus pH ($P < 0.001$) decreased at the point of predicted ovulation to facilitate sperm survival during conception. The predominant mucus crystallisation pattern changed from large irregular fern shapes at the onset of behavioural oestrus to shortened, linear patterns 24 h before predicted ovulation ($P = 0.013$) potentially indicating a decrease in ionic compounds resulting from elevated oestrogen levels. There were no significant differences in vulva size, mucus viscosity, vulvar temperature or ear temperature detected between the time points.

These results indicated that cervical mucus properties have potential as an alternative oestrus detection tool in addition to existing monitoring programs. Further investigation is required to determine if using these markers can reduce the time for oestrus detection and to predict insemination timing results in satisfactory fertility.

Table 1. Mean values for biological markers measured at the first instance of standing heat in response to back pressure and 24 h before and at the time of predicted ovulation

Biological marker	Onset of behavioural oestrus	24 h before predicted ovulation	Predicted ovulation (30 h before P4 peak)
Vulva size (cm ²)	7.1 \pm 0.9	6.7 \pm 0.8	4.9 \pm 0.8
Mucus pH	7.7 \pm 0.1 ^a	7.7 \pm 0.1 ^a	7.2 \pm 0.1 ^b
Mucus viscosity (cm)	0.5 \pm 0.1	0.4 \pm 0.1	0.6 \pm 0.1
Mucus crystallisation	Large, irregular shapes	Short linear streaks	Short linear streaks
Corrected vulvar temperature (°C)	30.9 \pm 0.5	30.7 \pm 0.4	30.5 \pm 0.5
Corrected ear temperature (°C)	32.6 \pm 0.5	31.6 \pm 0.5	30.5 \pm 0.4

Data presented as mean \pm s.e.m. ^{a,b}Within the same row, values with different superscripts differ significantly.

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The pre-ovulatory luteinising hormone surge is affected by the sex ratio of a gilt's birth litter

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Female reproduction can be affected by exposure to excessive concentrations of androgens *in utero*, resulting in masculinisation (Veiga-Lopez *et al.* 2009). Gilts may be exposed to excessive concentrations of androgens *in utero* from developing male littermates, as occurs in rodents (vom Saal and Bronson 1978). Gilts from male-biased litters may be masculinised and present with decreased reproductive potential due to differences in the functioning of their hypothalamo-pituitary-gonadal axis (HPG) (Veiga-Lopez *et al.* 2009). We hypothesised that the preovulatory surge of luteinising hormone (LH) of gilts from male-biased litters would be delayed in onset and would have an attenuated amplitude compared to gilts from female-biased litters.

Large White x Landrace gilts were selected from male-biased (>60% males, $n = 10$) or female-biased (>60% females, $n = 9$) litters. From 19 weeks of age gilts were rehoused into groups of four and began boar exposure in a detection mating area for 1 h daily for detection of puberty. To synchronise second oestrus, gilts received 5 mL/d of an orally active progestogen, altrenogest, commencing 12 days after the detection of puberty. Once all gilts had expressed puberty the altrenogest was withdrawn. Four days after withdrawal of altrenogest blood samples were collected via indwelling jugular vein catheters every 4 h until the end of subsequent oestrus. Plasma was stored at -20°C until required for LH assay using a double antibody radioimmunoassay. The assay sensitivity was 0.4 ng/mL and the intra and inter assay coefficients of variation were, 11.9% and 20.3%, respectively. A surge was defined as described by Barb *et al.* (1982). Data were analysed using a one-way ANOVA (SPSS v22.0, IBM, Armonk, NY, USA).

The onset of the LH surge was significantly delayed (56 ± 3.3 v. 43 ± 3.8 h, $P < 0.05$) for gilts from male-biased litters compared to gilts from female-biased litters (Fig. 1). The duration of the LH and the time from the onset of the LH surge to peak amplitude was significantly less (30 ± 2.2 v. 38 ± 1.2 h, and 6 ± 0.9 v. 12 ± 1.4 , respectively, $P < 0.05$) for gilts from male-biased litters than for gilts from female-biased litters. There was no difference between groups in the amplitude of the LH peak or the time from beginning sampling to reach the LH peak.

These data partially support our hypothesis in that the LH surge was delayed in gilts from male-biased litters but there was no difference in amplitude of the LH surge. Nonetheless, we present evidence that gilts from male-biased litters display a delayed LH surge, an attenuated duration of the LH surge and a reduced time from the onset of LH surge to the peak. Combined, these data suggest that the response of the HPG axis during oestrus is different between gilts from male-biased litters and gilts from female-biased litters. These differences may affect reproductive performance and therefore, with further research, the sex ratio could be used as a selection tool to improve the breeding herd.

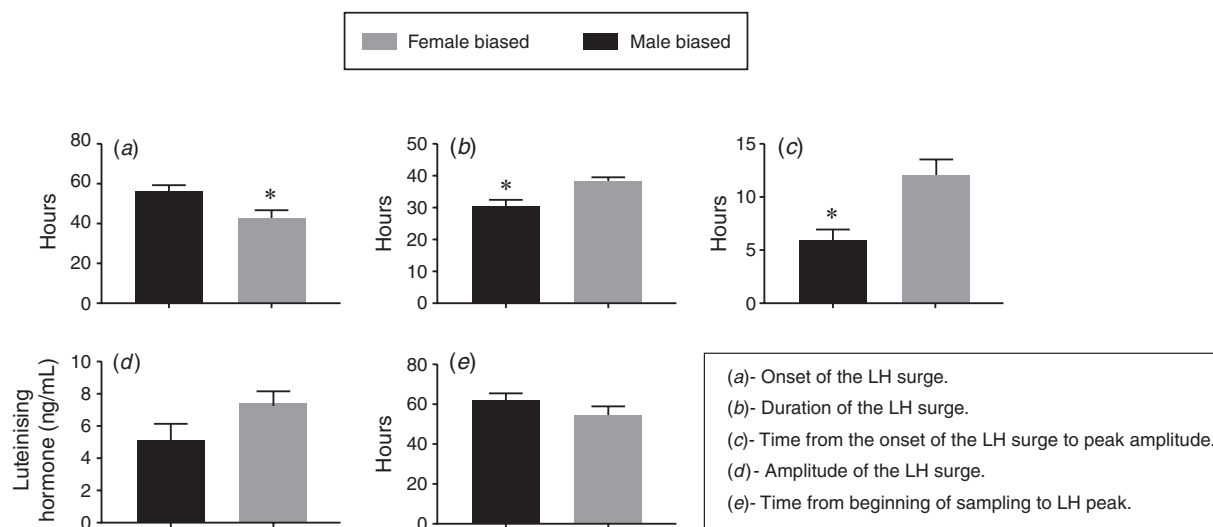


Fig. 1. Characteristics of the pre-ovulatory luteinising hormone surge for gilts from male biased and female biased litters (* = $P < 0.05$).

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The effects of the *in utero* environment on gilt performance

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Traditionally, the pork industry has focused most of its attention on the management of replacement gilts in the post-selection (16–21 weeks) phase. However, in many species it is now understood that the prenatal environment of an animal plays an equally important role as the pre-pubertal environment. In dairy cows, the age of the dam at first calving, the dam's milk yield and body condition score during gestation accounted for a significant proportion of the total phenotypic variance of calving interval and non-return rate of the daughter cows (Banos *et al.* 2007). There is growing evidence to suggest more research is needed on the long-term effects of the maternal environment. The aim of this study was to determine the efficacy of easily identifiable, early predictors of a gilt's lifetime reproductive performance. We hypothesised that the proportion of female to male fetuses would significantly affect the *in utero* environment in which a gilt develops, thereby affecting ovarian development and overall performance.

This study used 166 Large White × Landrace gilts, which were identified and weighed at birth, weaning, week 18 and week 21. Reproductive tracts were collected post slaughter at 21 weeks of age and antral follicles on both ovaries were counted and classified as either small (1–3.99 mm) or large (>4 mm). An analysis of variance (ANOVA), unbalanced design (GENSTAT 15, VSN International, Hemel Hempstead, UK) was used to determine the effect of proportion of females in the gestated litter (low, <40%; normal, 41–59%; high, >60%) on surface antral follicle counts, birthweight and growth rates.

The ovaries of gilts from female-biased litters contained a higher total number of surface antral follicles ($P < 0.05$) (Table 1) than those of gilts from litters with low proportions of females. The total small follicle counts were higher ($P < 0.05$) in gilts from female-biased litters. Large antral follicle counts did not differ between females from male-biased, normal or female-biased litters. Female birthweight was reduced in female-biased litters in comparison to litters with low proportions of females (1.16 ± 0.02 kg v. 2.09 ± 0.02 kg; $P < 0.05$). Female piglets born into litters with low proportions of females showed an increased average daily weight gain to weaning ($P < 0.05$) and remained significantly heavier until slaughter ($P < 0.05$).

The results of this study support the hypothesis that the *in utero* environment in which a gilt develops significantly affects the birthweight, and subsequent growth performance of an individual animal. Sexual maturity appears to be occurring independently of the *in utero* sex ratios, as there was no significant difference observed in large antral follicle numbers. However, the significantly higher number of small antral follicles observed in gilts from female-biased litters, indicates that these animals may have a higher ovarian reserve in comparison to gilts from normally distributed or male-biased litters.

Table 1. Piglet d 1 weight, average daily gains from birth to weaning, number of small (1–3.99 mm) and large (>4 mm) antral follicles present on the ovaries of 21-week-old gilts from gestational litters with low (<40%), normal (41–59%) and high (60%) proportions of females

	Low <40% 39	Proportion of females Normal 41–59% 81	High >60% 46
No. of gilts			
No. of follicles			
1–3.99 mm	116.7 ± 11.3 ^a	138.1 ± 7.9 ^a	165.6 ± 10.6 ^b
>4 mm	23.1 ± 2	18.5 ± 1.4	20.7 ± 1.9
Total follicle number	136.4 ± 11 ^a	155.5 ± 7.8 ^a	184.7 ± 10.4 ^b
Piglet weight			
D 1 weight (kg)	2.09 ± 0.02 ^a	1.62 ± 0.01 ^b	1.16 ± 0.02 ^c
Average daily gain (g)	210.9 ± 13.5 ^a	165.9 ± 9.5 ^b	168.1 ± 12.8 ^b

^{a–c}Means in a row with different superscripts differ significantly ($P < 0.05$).

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Can serum levels of anti-Müllerian hormone and oestradiol in juvenile gilts be used to predict future reproductive performance?

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The current process for selecting breeding gilts results in significant economic loss for the pig industry. In Australia, it is estimated that around 40% of selected gilts are culled before Parity 3 (Plush *et al.* 2016). Thus, there is a need for effective markers of future reproductive performance in young gilts.

We previously tested whether serum levels of anti-Müllerian hormone (AMH) and oestradiol (E2) in 80-day-old juvenile gilts would be useful markers of future ovarian and uterine properties and found that AMH was indicative of uterine capacity while E2 marked puberty attainment at 160 days of age (Steel *et al.* 2016). We also examined whether AMH and E2 in juvenile gilts aged 60, 80 and 100 days were predictive of mating and litter parameters over three parities. It was found that basal levels of AMH were not associated with mating and litter parameters and while E2 was associated with the proportion of piglets born alive and proportion stillborn at Parity 1 (Steel *et al.* 2017). The present study was conducted to determine the repeatability of our previous experimental results across different herds. It was hypothesised that, similar to our previous studies, E2 levels in juvenile gilts could be used to predict Parity 1 reproductive performance and, in contrast to other species, AMH could not.

Blood samples were obtained from 80-day-old Landrace x Large White gilts from two Australian commercial piggeries located in South-Eastern QLD (Farm A: PIC AustraliaTM Genetics, Grong Grong, NSW, Australia; $n = 101$) and in Southern NSW (Farm B: PrimeGroTM Genetics, Corowa, NSW, Australia; $n = 187$). Sera AMH and E2 were measured via competitive inhibition ELISA kits, CEA228Po and CEA461Ge, respectively (Cloud-Clone Corp., Katy, TX, USA). The mean mating age of gilts was 227.8 ± 12.5 (s.d.) days of age at Farm A and 208.6 ± 19.6 days of age at Farm B. Age at first heat (only at Farm A), first mating outcomes, gestation length, number of mummified, stillborn, and live piglets from the first litter and any culling information were recorded. Data was analysed using regression, restricted maximum likelihood and generalized linear model methods, with Farm as a factor, via the statistical package R (v3.3.3, R Foundation, Vienna, Austria).

Our two previously mentioned studies (Steel *et al.* 2016, 2017), were conducted only at Farm B. Similar to previous results, a single sample of AMH at 80 days of age was not predictive of Parity 1 mating, litter or culling parameters at both farms ($P > 0.05$). In contrast to our previous findings at Farm B, serum E2 levels were negatively associated with age at first heat at Farm A ($b = -0.021$, $P = 0.011$, $R^2 = 0.10$), positively associated with proportion of piglets born alive at both farms ($OR = 1.005$, $P < 0.001$) and were negatively associated with the proportion stillborn at Farm A ($OR = 0.991$, $P = 0.003$), but not at Farm B ($P > 0.05$). The inconsistent results between farms could be due to genetic variation studies, but reasons for disparities between experiments on the same farm are currently unclear. However, it is evident that at a time in juvenile gilts when ovarian cells first become responsive to gonadotrophins, serum E2 levels, which likely influence the development of the female reproductive tract, are indicative of first parity outcomes. It should be noted that the sample size of the present study ($n = 288$) was much greater than that of the earlier studies (Steel *et al.* 2016: $n = 48$; Steel *et al.* 2017: $n = 72$).

The findings of the present study validate those obtained previously showing that serum AMH levels in 80-day-old gilts are not predictive of first parity outcomes. The relationship between serum E2 levels at 80 days of age and the proportion of stillborns and piglets born alive at Parity 1 warrants further investigation.

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Commercial evaluation of a mating in lactation protocol

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Alternative management of the sow and her litter in conventional farrowing accommodation to uncouple mating from the weaning process has been investigated over recent years in Australia. The main rationale behind this research was to enhance the weaning process for the piglet without compromising sow productivity. Extending weaning age or gradual weaning through intermittent suckling can help piglets adapt to the weaning process, resulting in a decrease in the incidence and/or severity of the post-wean growth check (Kuller *et al.* 2004). However, increasing weaning age will decrease the number of litters per sow per year and gradual weaning will result in some sows cycling whilst they are still lactating, leading to an increase in sow non-productive days (Downing 2015). In order to improve the piglet weaning experience in combination with maintaining sow performance, mating sows whilst they are still lactating may be a viable option. The aim of this study was to compare subsequent reproductive outcomes between sows that were mated during lactation and those that were mated after weaning under commercial production conditions.

This study was conducted over a 12 month period between August 2015 and July 2016. The lactation oestrus (LO) induction protocol consisted of sow and piglet separation (placement of a solid board within the farrowing crate that separated the piglets from the sow) along with fence line boar exposure during the last week of lactation. All sows were monitored for signs of oestrus and were mated in the farrowing crate by artificial insemination if they displayed standing oestrus. Those sows that did not display oestrus during lactation were mated at their first standing oestrus after weaning. Subsequent reproductive outcome data was analysed using GLM analysis or Chi-squared (χ^2) (SPSS v24.0, IBM, Armonk, NY, USA). The percentage of sows (Parity 3.1 \pm 0.10) that had a lactation oestrus was 40%. Sows mated during lactation (responders, $n = 166$) had a lactation length (LL) of 29.8 \pm 0.37 days and a wean to re-mating interval (WRI) of -1.3 ± 0.34 days compared to a LL of 28.8 \pm 0.28 days and WRI of 6.5 \pm 0.27 days for those sows mated after weaning (non-responders, $n = 253$) ($P < 0.05$). The farrowing rate for responders was 78% compared to 88% for non-responders ($\chi^2 = 7.99$; $P < 0.05$) and piglets born alive was 10.7 \pm 0.46 for responders and 11.2 \pm 0.41 for non-responders ($P > 0.05$). The number of sows that displayed oestrus during lactation dropped during summer and those that were mated had a lower farrowing rate and piglets born alive compared to both responders mated during winter, spring and autumn and non-responders mated in summer and winter, spring and autumn (Fig. 1).

In conclusion, lactation length and season had a significant effect on the number of sows that responded to the LO induction protocol. Additionally, subsequent reproductive outcomes were negatively affected for sows mated during lactation in summer compared to those mated during lactation in other seasons. However, there was no difference in subsequent reproductive outcomes between sows mated in lactation outside of summer and those mated after weaning.

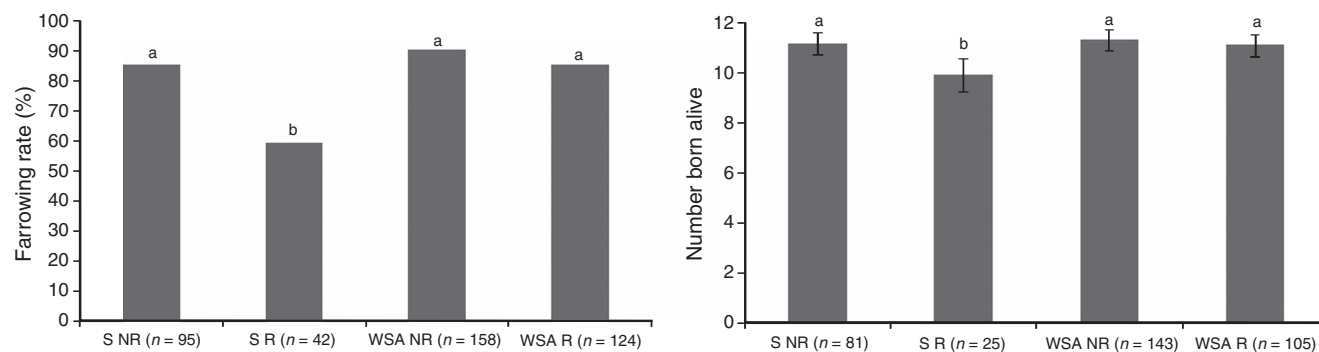


Fig. 1. Farrowing rate and born alive for non-responders (NR) and responders (R) according to season. S NR (Summer – Non-Responders); S R (Summer – Responders); WSA NR (Winter, Summer and Autumn – Non-Responders); WSA R (Winter, Summer and Autumn – Responders). ^{a,b}are significantly different from each other ($P > 0.05$).

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Melatonin fed in early gestation increases fetal weight

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Uterine blood flow and placenta size are determinants of the amount of nutrients reaching the attached fetuses within the uterus. Inevitably, selection for prolificacy increases the number of fetuses *in utero* and, therefore, already limits nutrient exchange between the maternal and fetal circulations. Uterine capacity is considered to be limiting when the number of developing conceptuses exceeds 14 (Dziuk 1968). Consequently, the excessive intrauterine crowding occurring in a proportion of mature prolific sows between d 25 and 40 of gestation leads to limited placental development by d 30, and subsequently to intrauterine growth retardation (IUGR) and reduced birthweight of entire litters (Foxcroft 2012). Melatonin has been used in sheep models to induce umbilical vasodilation and increase the amount of blood flow and nutrients reaching the fetus (Thakor *et al.* 2010), providing a valuable treatment for pregnancies experiencing IUGR. The aim of this project was to determine whether supplementing diets with melatonin between d 25 and d 50 of pregnancy in gilts would increase fetal growth to d 50 of gestation.

Pre-pubertal gilts ($n = 29$) were induced to ovulate at 22 weeks of age using a combination of PG600 (Intervet America, Inc., Millsboro, DE, USA) and daily boar exposure. At their induced oestrus, gilts were inseminated twice (24 h apart) using commercial pooled semen doses (80 mL doses of 3×10^{-9} sperm per dose, ≤ 4 days old: SABOR Pty Ltd, Clare, SA, Australia). Days 25 to 50 of gestation, gilts were treated with either 5 mL canola oil (CTL), 18 mg melatonin in 5 mL canola oil (MEL18) or 36 mg melatonin in 5 mL canola oil (MEL36). Day 50 of pregnancy, gilts were slaughtered at a commercial abattoir. Reproductive tracts were dissected, and the number of corpora lutea (CL; ovulation rate), number and weight of fetuses, fetal crown–rump length, as well as placental weights were recorded. Statistical analysis was performed using a mixed general linear model (SPSS 24.0, IBM, Armonk, NY, USA) with treatment, replicate, fetal sex and gilt as fixed effects and litter size as a covariate. Data are presented as estimated means \pm s.e. from mixed models.

The number of fetuses recovered was not different among treatments (9.82 ± 1.70 , 8.34 ± 1.30 and 8.93 ± 1.60 , for CTL, MEL18 and MEL36 respectively). Melatonin treatment did not affect CL, fetal and placenta weights combined, placental weights and crown–rump lengths ($P > 0.05$). Fetuses from the MEL36 group were heavier ($P < 0.05$) compared to the CTL group (CTL, 65.77 ± 4.78 g; MEL18, 73.42 ± 5.08 , MEL36, 81.85 ± 3.80).

The current limited data from an experimental gilt model provides preliminary evidence that oral supplementation with melatonin from early to mid-gestation can increase fetal weight at d 50. Studies with larger numbers, and in situations of more extreme intrauterine crowding and limited placental development, are needed to confirm the benefits of using melatonin to increase litter weights when intrauterine crowding in early gestation is linked to subsequent litter-wide IUGR and low birthweight. Studies that follow the pregnancy to farrowing also need to be conducted to ensure the effects of melatonin on fetal weight are continuing through to birth after treatment ceases at d 50 of gestation.

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Aerobic and algal treatment for piggery effluent and water reuse: design of an integrated wastewater treatment plant

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Anaerobically digested piggery effluent (ADPE) has high ammonia (NH_3) and suspended solids concentration, which precludes using it as a substrate for the growth of most microalgae for effluent treatment, disinfection and sustainable biomass energy production. Concentrations of NH_3 of 3630 ± 1250 mg $\text{NH}_3\text{-N/L}$, chemical oxygen demand 8933 mg/L (Hu *et al.* 2013), and phosphate levels of 620 mg/L (Olguín *et al.* 2003) have been reported in ADPE. In the absence of oxidation of ammonium-N ($\text{NH}_4^+\text{-N}$) to nitrate (NO_3^-), some dilution will always be needed if used for algae culture. A recent review (Buchanan *et al.* 2013) recommended using an integrated wastewater treatment approach that incorporates a nitrifying aerobic treatment step to reduce the NH_4^+ concentration in the effluent without dilution thereby promoting algal growth for biomass energy production, to likely reduce greenhouse gas (GHG) emissions. The proposed integrated system also has the potential to increase pig productivity and profitability via improving the quality of effluent reused in piggery operations. The objective of this project is to construct an integrated on-farm aerobic-algal treatment system and evaluate the performance in terms of biomass energy production, reduced GHG emissions and higher quality effluent for reuse. This research has the potential to improve profitability via increased on-farm bioenergy production, availability of high quality reuse water, improved pig health and reduction in operating costs.

The Roseworthy Piggery in South Australia was chosen for the pilot study and preliminary effluent analyses were conducted to enable design of the integrated wastewater treatment plant (Fig. 1). Anaerobically digested piggery effluent from an existing lagoon is transferred to a reception pit and followed by aerobic reactor at programmed intervals. NH_3 is converted to NO_3^- under aerobic conditions using naturally occurring populations of aerobic nitrifying bacteria. The resulting treated slurry with greatly reduced NH_3 load is then pumped into a sedimentation tank where the supernatant liquid phase is delivered to a makeup tank. Finally, effluent from the makeup tank is transferred into high rate algal pond (HRAP) for nutrient removal via growth of the algal biomass. The HRAP is mixed by an eight bladed paddlewheel and its hydraulic residence time is determined by the rate of slurry addition and/or change in HRAP depth (maximum operating depth 0.5 m). Treated HRAP effluent is returned to sump and then to anaerobic pond before land spreading. Through this direct demonstration, farmers can form an opinion about the feasibility of implementing an integrated waste system to their own operations. An integrated wastewater treatment system offers the potential to treat waste safely and provide additional on farm energy, while reducing GHG emissions and producing high quality water for reuse in piggery operations.

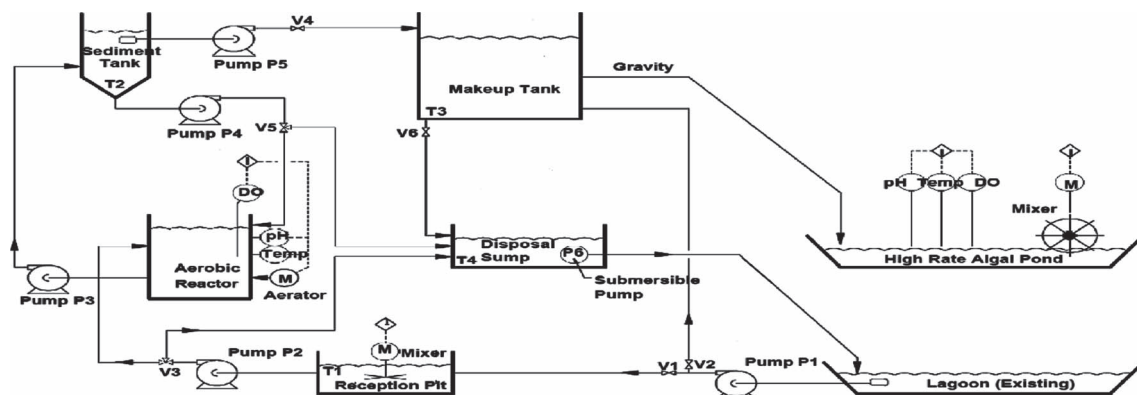


Fig. 1. Flinders University Roseworthy integrated aerobic-algal wastewater treatment research facility.

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Assessing the suitability of microalgae biomass produced from piggery waste as a fertiliser

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The use of microalgae for the recapture of nutrients, water and bioenergy from the treatment of piggery effluent is a promising technology (Astals *et al.* 2015; Nwoba *et al.* 2016). The harvested microalgae biomass has the potential to be developed into a high quality, concentrated and balanced fertiliser due to its high nitrogen (N) and phosphorous (P) content that is easy to handle and transport (Mulbry *et al.* 2005). However, there is still uncertainty regarding the re-use of microalgae in terms of its impact on plant N uptake, dry matter production, N mineralisation, and soil microbial activity. The aims of this study were 2-fold: (1) to investigate the effectiveness of microalgae biomass to supply N to wheat in comparison with a synthetic N fertiliser, ammonium nitrate (NH₄NO₃), and (2) to assess the release of inorganic N (potential N mineralisation rate) following the application of microalgae biomass to soil. Microalgae biomass grown on undiluted, untreated piggery anaerobic digestion effluent was obtained from the Algae R&D Centre, Murdoch University. First, a pot experiment having a 2 × 5 factorial arrangement of treatments was undertaken to test the feasibility of using microalgae as a fertiliser with factors being two N sources (microalgae biomass and NH₄NO₃) applied at five N equivalent levels (0, 10, 20, 40, 80 kg N/ha) for 8 weeks. The trial was planted with wheat (*Triticum aestivum* L.) and arranged in randomised block design with four replicates per treatment. Second, a laboratory incubation experiment was conducted to quantify the potential N mineralisation of the microalgae using the same experimental design for the pot trial but without the plant. Total dry biomass, root and shoot production were determined using standard methods. Soil mineral N was analysed colourimetrically using a modified hydrazine reduction method. The carbon dioxide (CO₂) production (microbial activity) was measured using an infrared gas analyser.

Utilisation of the microalgae biomass as an alternative fertiliser significantly improved the overall wheat yield and shoot production (Fig. 1), particularly at higher application rates. However, shoot production was slightly lower in the plants receiving the microalgae when compared to the mineral fertiliser NH₄NO₃. The soil N mineralisation rate was positively correlated with the amount of microalgae biomass applied implying that the microalgae provides an available nitrogen source for plants but not to the same extent as NH₄NO₃. This is because a large proportion of the N in microalgae is in an organic form and needs to be mineralised by soil microorganisms before it is plant available. Consequently, an increase in microbial activities (CO₂ production) was observed in the soil amended with the microalgae biomass. Further studies to determine the agronomic and economic value of microalgae as a fertiliser are needed.

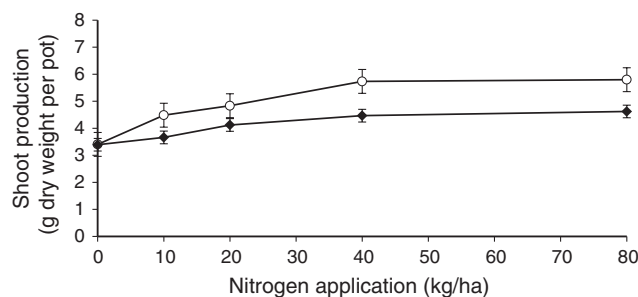


Fig. 1. Effect of mineral fertiliser NH₄NO₃ (○) and microalgae biomass (●) on shoot production (mean ± s.e.m., where *n* = 3) for wheat when applied at different levels (equivalent to 0 to 80 kg N/ha).

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Effect of feed wastage on piggery effluent characteristics

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Across the Australian pork industry, a 5% change in feed wastage is 82 000 tonnes feed/year, with a current value of approximately \$38m. While its importance is widely recognised, there are currently no practical robust methods to quantify feed wastage. Feed wastage influences feed efficiency, shed (manure), effluent management practices and effluent characteristics. Accordingly, the present study hypothesised that feed wastage could be estimated from effluent characteristics.

To relate feed wastage to effluent characteristics, the study used an innovative modelling and experimental approach to simulate different rates of feed wastage and assess its effects on piggery effluent. Quantities of pig feed (wheat and barley based grower diet), faeces, urine, flush water (clean bore water) and shed effluent (collected over 24 h in an agitated sump) were sampled from a commercial batch grower shed, housing 535 pigs (average 45 kg live weight at 13 weeks of age). Pre-determined proportions of these samples were mixed to simulate shed effluent having four different rates of feed wastage (Treatments A–D). Treatment A was composed of faeces, urine and flush water only, to simulate zero feed wastage. Treatment B was raw effluent discharged from the shed. Treatments C and D were composed of raw shed effluent with added feed, to simulate higher rates of feed wastage. The resulting samples were analysed to evaluate the total solids (TS), volatile solids (VS) and biochemical methane potential (BMP). Analyses of variance (ANOVA) followed by protected least significant difference (l.s.d.) testing were performed on the analysis results, at the 5% level, using GENSTAT 16.1 (VSN International, Hemel Hempstead, UK). The AUSPIG growth and production simulation model (Davies *et al.* 1998) was used to simulate the age, live weight, P2 back-fat and feed intake of the pigs in the trial shed over their entire growth cycle (wean-to-finish). The genotype settings and feed intake factors in the model were adjusted so that measured and predicted performance parameters (growth rate, P2 backfat and feed intake) were similar for the batch of pigs. For each of the four treatments, the extent of feed wastage was then estimated using the AUSPIG model (as reference for comparison) and separately by total solids mass balance.

Estimated feed wastage in the trial shed on the sampling day (Treatment B) was 4.2% from the mass balance calculations and 6.9% from the AUSPIG model. The difference between these two estimates would likely be indistinguishable with normal production data variability. This extent of wastage represents current industry best practice, supported by inspection of the shed indicating virtually no visible spilt feed. The analysis further confirmed that increasing levels of feed wastage resulted in an increased BMP due to the energy content of the waste feed, and also increased concentrations of TS and VS (Table 1). However, the increased feed costs associated with higher levels of feed wastage outweigh potential cost savings from increased methane recovery (higher BMP) for on-farm energy use because energy currently is a relatively minor contributor to total production costs (~4%) compared to feed (~60%).

Overall, the results support the stated hypothesis, showing that feed wastage consistently affects and can be estimated from effluent characteristics. A logical next step is to adapt industry-standard effluent characteristic models such as PigBal (Skerman *et al.* 2013) to also estimate feed wastage.

Table 1. Feed wastage and mean analysis results for treatments A, B, C and D

Parameter	Units	A	B	C	D	s.e.m.
Feed wastage (mass balance)	%	0.0	4.2	9.4	15.2	
Feed wastage (AUSPIG)	%	0.0	6.9	12.1	17.8	
TS	%	1.51 ^a	1.81 ^b	2.06 ^c	2.29 ^d	0.04
VS	%	1.24 ^a	1.46 ^b	1.70 ^c	1.93 ^d	0.03
BMP (B ₀)	L CH ₄ /kg VS	284.6 ^a	326.6 ^b	360.9 ^c	383.3 ^d	5.4

s.e.m., pooled standard error of mean. ^{a–d}Means in a row with different subscripts are significantly different ($P < 0.05$).

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The effect of dam parity on growth, white blood cell count, haemoglobin and immunoglobulin levels of weaner pigs

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Dam parity affects the growth rate of finisher pigs and gilt progeny have lower growth rates than progeny from multiparous sows, although the magnitude of this effect varies between herds (Hermesch and Li 2013). Gilt progeny had lower levels of immunoglobulin (Ig) G and IgA at birth (Klobasa *et al.* 1986). However, differences in IgG and IgA were the reverse between piglets from gilts v. multiparous sows at 2 (IgA) and 3 (IgG) weeks of age, due to higher *de-novo* synthesis of Ig in piglets from gilt litters (Klobasa *et al.* 1986). Similarly, Miller *et al.* (2013) found no dam-parity effect on multiple immune parameters measured in piglets. This study hypothesised that gilt progeny have reduced growth and similar haematological and immunoglobulin levels in weaner pigs in comparison to progeny from multiparous sows.

All traits were recorded from January 2013 until October 2014 in 799 Large White pigs that originated from 217 litters and 209 dams. Serum samples were taken at an age of 37.7 ± 3.4 days and a weight of 11.3 ± 2.17 kg after being weaned at 27.1 ± 2.4 days with a mean weight of 8.9 ± 1.30 kg. Capture ELISA were performed using purified Ig (Sigma Aldrich, Castle Hill, NSW, Australia) or known reference serum as standards and polyclonal antibody sets (Bethyl Laboratories, Montgomery, TX, USA). Quantitative analysis of the samples was executed using four parameter logistic fit (4PL) software. Dam parity and weekly batch were fitted as fixed effects for all traits using the SAS software (v9.4, SAS Institute Inc., Cary, NC, USA). Sex had significant effects on growth rate until weaning (GRW), white blood cell count (WBC) and haemoglobin (HGB). Age was fitted as a linear covariable for IgA. Dam parities above the sixth parity were defined as one level in the analyses.

The statistical power to detect differences between parities was large due to the large number of records and dam parity was a statistically significant effect for most traits in Table 1. Dam parity affected growth rate most, which was lowest for gilt progeny. For example, pre-weaning growth of gilt progeny was 20 g/d and 28 g/d lower than growth of progeny of second- and fourth-parity (P2, P4) sows. Similarly, growth from weaning to 5 weeks (GR5) was 25 g/d and 34 g/d lower in gilt progeny in comparison to progeny from P2 and P4 sows. These differences can be expressed relative to the mean (or standard deviation, s.d.) of each trait to make a comparison between traits possible. The growth gap of gilt v. P4 progeny was 9% of the mean (63% of the s.d.) for GRW and 16% of the mean (29% of s.d.) for GR5. Gilt progeny had superior WBC and HGB than progeny from multiparous sows. Further, IgA levels were not significantly different in gilt progeny compared to progeny from multiparous sows. The levels of IgG in progeny tended to increase with parity number and gilt progeny had significantly lower IgG levels than progeny from P3 or P6 sows; however, dam parity was not a significant effect overall.

These results illustrate the reduced capacity of younger sows to support the growth potential of their progeny. Haematological and immunoglobulin levels of weaner pigs were not inferior in gilt progeny.

Table 1. Least square means (standard error) for dam parity (P1 to P6) of growth rate until weaning (GRW) and from weaning to 5 weeks (GR5) as well as white blood cell count (WBC), haemoglobin (HGB) and immunoglobulin (IgA, IgG) levels in weaner pigs

Trait	P1	P2	P3	P4	P5	P6	P-value
GRW	310 (3.75) ^a	330 (3.25) ^b	328 (3.65) ^b	337 (4.56) ^b	333 (5.67) ^b	330 (3.27) ^b	<0.0001
GR5	186 (8.24) ^a	211 (7.14) ^b	205 (8.010) ^{bc}	220 (10.01) ^c	206 (12.45) ^{bc}	224 (7.17) ^c	<0.023
WBC	15.5 (0.40) ^a	16.6 (0.35) ^{bc}	16.6 (0.39) ^{ab}	18.1 (0.49) ^c	17.0 (0.60) ^{bc}	17.0 (0.61) ^b	0.0059
HGB	117 (0.78) ^a	112 (0.67) ^b	114 (0.75) ^{bc}	113 (0.94) ^b	116 (1.18) ^{ac}	113 (0.68) ^b	<0.0001
IgA	0.51 (0.025) ^a	0.44 (0.021) ^b	0.52 (0.030) ^a	0.48 (0.037) ^{ac}	0.48 (0.037) ^{abc}	0.48 (0.021) ^{abc}	0.07
IgG	8.38 (0.46) ^a	8.77 (0.40) ^{ab}	9.66 (0.44) ^{bc}	9.73 (0.55) ^{abc}	9.48 (0.69) ^{abc}	9.91 (0.40) ^c	0.10

^{a-c}Means in a row with a different superscripts differ significantly, $P < 0.05$.

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The effect of parity on haemoglobin levels in sows prior to farrowing and in 1-day-old piglets

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A decreased haemoglobin level in piglets is associated with a decreased survival rate (Rootwelt *et al.* 2012). While haemoglobin levels have been studied in piglets, few studies have focused on sows. In sows, low iron levels were associated with reduced reproductive performance (Büffler *et al.* 2017). Haemoglobin levels in sows decrease with increasing parities (Gannon *et al.* 2011a). It has been suggested that the body haemoglobin reserves cannot be restored between parities (Auvigne *et al.* 2010). In this study it was hypothesised that with an increasing parity, both the sow haemoglobin and the piglet haemoglobin decrease.

In 2012 and 2013, haemoglobin samples were collected from 171 Landrace and 216 Large White sows with 581 litters on a breeding farm in the region of Adelaide, South Australia. The sow parity ranged from 1 to 5+, there were between 75 and 162 observations per parity. A droplet of blood was collected from sows 5.0 ± 2.7 days before farrowing via the ear vein. The haemoglobin levels were estimated using the HemoCue Hb²⁰¹⁺ analyser (HemoVue AB, Angelholm, Sweden), which was validated by Gannon *et al.* (2011b). Some sows had observations in multiple parities, resulting in 568 observations on haemoglobin level. The haemoglobin levels of three piglets in each litter (selected visually as a light, medium and heavy piglet) were measured on 1-day-old piglets before iron supplementation based on the same procedure used in sows. The average haemoglobin level of the three piglets was used for analysis ($n = 503$). A linear model was fitted in R with haemoglobin measurement date (66 levels) and parity (five levels) added as fixed effects (R v3.3.2, R Foundation, Vienna, Austria). Breed was also tested as a fixed effect and was found not to be significant. The R function TukeyHSD was used to determine which means were significantly different from each other.

Sow haemoglobin ranged between 74 and 146 g/L, with a mean of 111.6 ± 12.8 g/L. Average piglet haemoglobin ranged between 58 and 149.5 g/L, with a mean of 103.3 ± 15.1 g/L. The linear model showed a decreasing trend of sow haemoglobin levels with increasing parity ($R^2 = 0.27$), while the average piglet haemoglobin stayed relatively constant with increasing parity ($R^2 = 0.12$) (Table 1).

This study showed that with increasing parity, sow haemoglobin level decreased, while piglet haemoglobin levels stayed constant. The haemoglobin levels found in this study were lower than found in the study of Gannon *et al.* (2011a). A haemoglobin level of 100 g/L has been suggested as the normal-range threshold for sows (Gannon *et al.* 2011a). In this study, the mean sow haemoglobin level was above this threshold for all parities. However, 6.8% of the sows in Parity 1 and 36% of the sows in Parity 5+ were below this threshold. Awareness of the increasing danger of anaemia in later parities could help identify problem cases earlier, thereby ensuring that sow health and welfare is not compromised.

Table 1. The least-squares mean (SE) level of sow haemoglobin and the average litter haemoglobin across parities

	Parity 1	Parity 2	Parity 3	Parity 4	Parity 5+
Sow haemoglobin (g/L)	118.2 ^a (1.0)	111.1 ^b (1.1)	109.6 ^b (1.2)	107.1 ^b (1.3)	106.7 ^b (1.4)
Average piglet haemoglobin (g/L)	101.5 ^a (1.4)	106.8 ^{ab} (1.5)	103.3 ^a (1.6)	100.8 ^{ac} (1.7)	106.3 ^a (1.9)

^{a-c}Means in a row with a different superscripts differ significantly, $P < 0.05$.

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The effects of selecting growing pigs for a high growth rate and low backfat on sow characteristics

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The effects of selecting for finisher traits on the development of breeding sows is not well understood (Lewis and Bunter 2013). It has been estimated by Hermesch *et al.* (2010) that for a 1 g increase in estimated breeding value (EBV) for average daily gain (ADG) of the growing pig, the sow weight (SWT) before farrowing increased on average by 0.32 kg. For a 1 mm decrease in backfat (BF) EBV of the growing pig, sow backfat (SBF) decreased on average by 1.56 mm. The aim of this study was to evaluate the effects of historical selection for a high ADG and low BF in growing pigs on the sows' weight, fatness and haemoglobin levels (Hb). It was hypothesised that sows with a higher genetic potential for lean meat growth as growing pigs are both heavier and leaner in each parity.

In 2012 and 2013 data were collected on 171 Landrace and 216 Large White sows with 581 litters. Sow weight, SBF, and Hb were measured ~5 days before farrowing, upon transfer to the farrowing facilities in Parity 1 to 5+. There were between 164 (Parity 1) and 75 (Parity 5+) observations per parity. PIGBLUP was used to obtain EBVs for ADG and BF on the growing pig, using own and relatives' records. Pedigree was available for 50 431 animals from 260 sires and 2055 dams born between 2004 and 2013. There were 563, 573 and 568 observations for SWT, SBF and Hb, with a mean (standard deviation; s.d.) of 266 (35.2) kg, 18.6 (3.6) mm, and 112 (12.8) g/L. The EBV for ADG ranged from -47.06 to 82.81 and the EBV for BF ranged from -2.63 to 0.19. The average SWT was 230, 253, 280, 297 and 308 kg from Parity 1 to Parity 5+. Observations that deviated more than 3 s.d. from the mean were excluded. Measurement date (65 levels), parity (five levels) and breed (only for SBF, two levels) were fitted as fixed effects in linear models for SWT, SBF and Hb using R (R v3.3.2, R Foundation, Vienna, Austria). In addition, litter weight and ADG EBV were fitted as linear covariables for SWT, ADG EBV was fitted as linear covariable for Hb, and BF EBV was fitted as linear covariable for SBF. Regressions within parity group were also estimated to evaluate the effect of EBV for ADG or BF on sow characteristics within parity.

Across parities, regression coefficients were only significant for BF EBV (Table 1). With a 1 mm decrease in BF EBV, sow fatness reduced by 0.6 mm. With a 1 g increase in ADG EBV in the growing pig, SWT increased by 0.32 kg and Hb decreased by 0.15 g/L in Parity 1. The effect of ADG EBV on SWT and Hb was not significantly different from zero in later parities. Lewis and Bunter (2013) found genetic correlations between weight at selection and weight across parities ranging from 0.54 in Parity 1 to 0.32 in Parity 5.

This study found that the EBV for ADG and BF affected SWT, SBF and Hb, although the magnitude of effects changed over parities. Until the first farrowing, gilts with higher genetic merit for growth had a higher SWT and lower Hb. Due to feed restrictions and other management strategies, sows might not be able to express their genetic potential for growth. The EBV for BF had a positive effect on SBF, but was lower than found by Hermesch *et al.* (2010). Feeding strategies might affect the observed relationship between growing animals and the mature sow herd. This relationship should be explored further for the development of selection strategies to improve growth of growing pigs while limiting mature size of sows.

Table 1. The regression coefficients (SE) of EBV for ADG or BF on sow characteristics across (additive model) and within (interaction model) parity, significant effects in bold ($P < 0.05$)

	Across	Parity 1	Parity 2	Parity 3	Parity 4	Parity 5+
ADG EBV on sow weight	0.02 (0.06)	0.32 (0.12)	-0.07 (0.18)	0.07 (0.17)	0.00 (0.17)	-0.21 (0.17)
BF EBV on sow fatness	0.59 (0.26)	0.15 (0.52)	0.33 (0.80)	0.79 (0.80)	0.56 (0.82)	1.32 (0.82)
ADG EBV on sow Hb	-0.04 (0.03)	-0.15 (0.06)	0.00 (0.09)	0.03 (0.08)	-0.04 (0.09)	0.01 (0.09)

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Predicting body protein and body fat for breeding sows of a modern commercial genotype

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Modern sow genotypes have changed considerably since body composition studies were conducted over 25 years ago on Australian genotypes. Earlier predictive equations were developed on datasets of slaughtered gilts and first-litter sows (e.g. Mullan and Williams 1990) or used genotypes from the United Kingdom (Large White × Landrace crossed with Landrace × Meishan; Gill 2006). As part of a larger project to evaluate sow lifetime performance and longevity over several parities (Smits *et al.* 2017), we established predictive equations based on measured live animal data for body protein and fat of sows before mating for Australian maternal genotypes. The hypothesis was that predicted and actual tissues reserves were similar.

Over six replicates, six unmated gilts (40 weeks of age) and 52 mixed parity (1 to 9) sows at weaning were selected for the study (Large White × Landrace F1 cross, PrimeGro GeneticsTM). Prior to slaughter, animals were measured for several traits including ultrasound (PieMedical, linear array probe 5 MHz) fat depth thickness measured at 65 mm from midline at last rib (P2) and 20 mm from midline at junction of the tail (LEG); loin muscle depth at P2; shoulder height; girth circumference at foreleg and last rib; live weight (LWT); and parity. After 24 h, carcasses were split into primals and measured for protein and fat content using dual X-ray absorptiometry (Suster *et al.* 2003). Viscera were collected from the abattoir and weighed full and empty, frozen and then ground, freeze-dried and analysed for protein and fat using chemical analysis methods. Live empty bodyweight was calculated from the empty cold carcass weight plus the estimated blood volume (7% of live weight). Regression was used to develop the predictive equations with the highest regression (r^2) for protein and fat content in the empty live weight. The least significant effect was removed from the model containing all factors in a step-wise fashion to develop a parsimonious model.

The predictive equations described with the highest regression in the model were as follows:

$$\text{Body Protein (kg)} = 6.13 + \alpha + 0.14 \pm 0.01 \times \text{LWT} - 0.18 \pm 0.05 \times \text{P2} - 0.13 \pm 0.04 \times \text{LEG} \quad (r^2 : 0.96, P < 0.05) \quad (\text{Eqn 1})$$

where $\alpha = -2.16$ for parity 0; -0.83 for parities 1–2; 0.33 for parities 3–5; 0 for parities > 5

$$\text{Body Fat (kg)} = \alpha + 0.24 \pm 0.05 \times \text{LWT} + 1.07 \pm 0.24 \times \text{P2} + 0.50 \pm 0.21 \times \text{LEG} - 37.3 \quad (r^2 : 0.79, P < 0.05) \quad (\text{Eqn 2})$$

where $\alpha = 12.7$ for parity 0; 4.51 for parities 1–2; 1.69 for parities 3–5; 0 for parities > 5 .

There was a high consistency between actual protein and fat contents in the empty bodyweight and predicted values (Table 1). Adding additional parameters such as loin muscle diameter, girth dimensions and shoulder height did not increase the accuracy of the equation for predicting body tissue mass when live weight was recorded.

These equations provide a valuable tool for predicting changes in body protein and fat reserves in this commercial genotype across a range of parities, weights and backfat measures.

Table 1. Comparison of actual tissue values (mean \pm s.e.) in the empty bodyweight of unmated gilts and sows with predictive body protein (BPROT) and fat (BFAT) tissue reserves

Parity	LWT (kg)	P2 (mm)	LEG (mm)	Actual BPROT (kg)	Actual BFAT (kg)	Predicted BPROT (kg) ^A	Predicted BFAT (kg) ^B
0 (6)	193.0 \pm 5.8 ^C	17.8 \pm 1.0 ^C	21.0 \pm 1.0 ^C	24.8 \pm 1.0 ^C	48.4 \pm 3.1 ^C	25.9	50.8
1 (14)	212.0 \pm 4.4	15.9 \pm 1.0	21.9 \pm 1.5	28.5 \pm 0.5	44.7 \pm 2.3	30.3	45.4
2 (13)	222.7 \pm 5.7	12.8 \pm 0.9	20.4 \pm 1.1	31.6 \pm 0.7	40.6 \pm 3.1	32.6	43.8
4–5 (16)	246.9 \pm 5.6	15.0 \pm 0.8	21.2 \pm 1.1	35.1 \pm 0.8	47.3 \pm 2.2	36.8	49.5
7 (9)	262.4 \pm 6.6	16.9 \pm 1.6	23.7 \pm 1.5	36.3 \pm 0.9	52.5 \pm 2.9	38.0	54.7

^ABody protein (kg) and ^Bbody fat (kg) in empty bodyweight, calculated from Eqns 1 and 2 above.

^CUnmated at 40 weeks of age. (), Number of sows sampled in dataset.

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The effect of pre-slaughter factors on meat quality varies between muscle cuts

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The Australian pork industry has focused on developing an eating quality pathway (Channon *et al.* 2016) to improve quality and consistency of pork; however, this has focused on conventional housed pigs with an average HCW of 74.9 kg. This study investigated the effect of housing type (H), carcass weight (W) and sex (S) on objective pork quality. The hypothesis tested was that housing type, carcass weight and sex, as individual and additive factors, does not affect objective quality of several pork cuts. A total of 384 Large White × Landrace commercial pigs (PrimeGro Genetics™, Corowa, NSW, Australia) were selected over eight replicates in a 2 × 2 × 3 factorial experiment with the main treatments being: (1) Housing: Conventional (CON) and deep-litter (DL); (2) Sex: Female or castrated pigs (IM); and (3) Carcass weight: Light 60–70 kg (L), Medium 70.1–80 kg (M) or Heavy 80.1–91 kg (HV). Methods are previously described by Lealiifano *et al.* (2017) with objective meat quality measurements (shearforce, pH, drip loss, L*, a* and b*) conducted 24 h post-slaughter on *Musculus longissimus thoracis et lumborum* (loin), *M. biceps femoris* (silverside) and *M. gluteus medius* (rump) from the carcass's left side. Data was analysed using ANOVA (GENSTAT 16, VSN International, Hemel Hempstead, UK).

There was a significant main effect of H on the shear force values, such that overall meat cuts from DL were lower than meat cuts from CON pigs (Table 1). There was no effect of carcass weight on the objective meat quality measures of the loin. Shear force in the rump decreased as carcass weight increased (4.22 kg v. 4.02 kg v. 3.97 kg, SE 0.09 for L, M and HV, respectively, $P = 0.012$). The increase in carcass P2 with increasing weight could have affected the increased tenderness associated with heavier carcasses. The silverside drip loss increased with an increase in slaughter weight (3.19%, 3.41%, 3.64%, SE 0.002, $P = 0.024$ for L, M and HV carcass weights, respectively). The increased drip loss in the silverside could be due to increased muscle size and a slower chilling rate for the heavier carcasses. Loin objective quality was not significantly affected by S differences. IM had lower drip loss, higher pH in the rump and silverside (SS) compared to females (Rump Drip loss: 3.46% v. 3.96%, SE 0.01, $P = 0.025$; SS Drip loss: 3.12% v. 3.70%, SE 0.01, $P = 0.005$; Rump pH: 5.47 v. 5.44, SE 0.01, $P = 0.005$; SS pH: 5.53 v. 5.49, SE 0.01, $P = 0.01$ respectively). There were no significant interactions of shear force by cut type due to S, W, or interactions with H. Deep litter carcasses had an increased drip loss level in the silverside over the three carcass weights (L: 3.26% v. 3.12%, SE 0.27; M: 3.63% v. 3.18%, SE 0.27; HV: 4.10% v. 3.18%, SE 0.27 DL v. CON housed respectively $P = 0.049$). There were also significant H × W interactions (L: 44.99 v. 47.05, SE 0.42; M: 45.08 v. 45.83, SE 0.42; HV: 44.81 v. 46.32, SE 0.42 DL v. CON housed respectively $P = 0.049$) and S × H × W interactions (L IM: 45.62 v. 46.95, SE 0.59 respectively; L Female: 44.37 v. 47.15, SE 0.59 respectively; M IM: 45.30 v. 45.92, SE 0.59 respectively; M Female: 44.86 v. 45.74, SE 0.59 respectively; HV IM: 44.45 v. 46.84, SE 0.59 respectively; HV Female: 45.18 v. 45.80, SE 0.59 respectively $P = 0.010$) for L* value in the silverside.

The hypothesis was rejected as these results show that housing had a significant effect on key objective meat quality measures, with carcass weight and to a lesser extent sex impacting meat quality. The effects however were varied between meat cuts.

Table 1. Means and standard error of the difference (SED) for the effect of housing on pH, Minolta L*, drip loss (%) and shearforce (kg) in the loin, rump and silverside (SS)

Housing Objective measurement	Conventional			Deep Litter			SED	P-value
	Loin	Rump	SS	Loin	Rump	SS		
pH	5.45	5.49	5.53	5.44	5.42	5.49	0.01	<0.001
L*	49.45	47.75	46.40	49.06	49.06	44.96	0.20	<0.001
Drip loss (%)	5.97%	3.67%	3.16%	6.70%	3.74%	3.67%	0.15	0.010
Shear force (kg)	5.08	4.21	4.78	4.69	3.94	4.65	0.07	0.002

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The incidence of pale soft exudative pork in entire male pigs from an Australian herd

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Pale, soft, exudative (PSE) pork is caused by protein denaturation resulting from high temperature and low pH conditions occurring in the early post-mortem period. This changes the light reflectance and water binding characteristics of meat. However, the description of PSE varies within the literature and the Australian Pork industry has no definition for PSE pork. Since the removal of the halothane gene from the Australian herd (Channon and Warner 2011), the incidence of PSE pork may have been underestimated, with no studies conducted to determine the prevalence of PSE pork as it is no longer seen as an issue. However, factors other than the halothane gene are known to lead to the temperature and pH conditions required to cause PSE (Scheffler and Gerrard 2007). The objective of this study was to present data for the incidence of PSE, based on published PSE classifications, in an audit of carcasses from an Australian pork farm.

Data from 198 randomly selected entire male, Large White × Landrace carcasses, sourced from one farm, were collected over 6 days in an Australian abattoir. The pH of the *Musculus longissimus dorsi* was measured at 0.75, 3 and 72 h post-mortem. A 50 g sample of the *M. longissimus dorsi* was collected at 72 h post-mortem to measure colour values for lightness (L^*) and was then subsequently used to measure drip loss over a 24 h period. Five published PSE definitions of varying parameters were selected to test the incidence of PSE pork in the samples collected (Table 1). The carcasses that fell outside these thresholds were considered PSE and are presented as a proportion of the consignment (%). The prevalence of PSE was highly variable depending on which thresholds were used. Only six carcasses in total (3%) had an L^* value of greater than 60, to be considered PSE in Category 1 (Warriss and Brown 1995). Although paleness is an attribute of PSE, the colour of fresh meat is not associated with eating quality and is a consumer preference, thus colour alone should not be used to determine PSE. Of the 198 carcasses sampled, 63.6% would be considered PSE based on Category 2 (Warner *et al.* 1997) for L^* and drip loss percentage. The fact that almost two-thirds of all carcasses had a drip loss over 5% highlights that water holding capacity can increase without L^* increasing to high levels. Determination of PSE using an ultimate pH threshold of pH 5.5 (Category 3; Gajana *et al.* 2013) resulted in 68% of carcasses being classified as PSE. However, ultimate pH is independent of the rate of pH decline, so can be low without causing protein denaturation (Scheffler and Gerrard 2007), therefore ultimate pH alone is a poor indicator of PSE. Categories 4 (Bee *et al.* 2007) and 5 (Offer 1991) take into account rate of pH decline hence are a more accurate presentation of circumstances in post-mortem muscle that cause PSE. Although the rates described are lower than Categories 2 and 3, high rates still existed with 28.3 and 39.4% respectively, highlighting a likely issue with PSE in Australian pork when 4 of the 5 published thresholds are applied.

The Australian pork industry must consider the development of an industry standard for describing PSE pork. The current data presents a high incidence of PSE across most definitions; thus, PSE could explain some eating quality variation in Australian pork.

Table 1. The incidence (%) of PSE in 198 carcasses based on five published definitions for PSE

Category	Source	Definition	Incidence of PSE (%)
1	Warriss and Brown (1995)	$L^* > 60$	3.00%
2	Warner <i>et al.</i> (1997)	$L^* > 50$; Drip loss $> 5\%$	63.60%
3	Gajana <i>et al.</i> (2013)	pHu < 5.5	68.20%
4	Bee <i>et al.</i> (2007)	pH @ 3 h < 5.7	28.30%
5	Offer (1991)	Rate pH decline ≥ 0.02 units/min ^A at 45 min	39.40%

^AThe rate of pH decline after 45 min post mortem, assuming starting pH was 7.

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Prevalence survey of *Toxoplasma gondii* in hearts from Western Australian sows

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Toxoplasma gondii (*T. gondii*) is a protozoan parasite that infects warm-blooded animals, including humans, with an estimated 30% of the world's population seropositive for *T. gondii* (FAO/WHO 2014). Infection in humans can result from the consumption of uncooked or undercooked meat from infected animals. The World Health Organization has classified *T. gondii* as high priority in a report on the global burden of foodborne hazards based on the potential for serious ongoing pathological conditions (FAO/WHO 2014). *Toxoplasma gondii* from the meat of small ruminants, pork, beef and game ranked fourth in a global ranking tool of foodborne parasites by 'importance' and their primary food vehicle. Congenital toxoplasmosis may cause abortion, fetal death or central nervous system abnormalities, chorioretinitis and encephalitis in neonates.

The aim of this study was to estimate the prevalence of *T. gondii* in sow meat from Western Australia (WA). The sampling strategy was based on the numbers of sows required for a national baseline survey (a minimum of 300 samples would be required to give 95% confidence in a national prevalence estimate) using a randomised sampling framework and pig numbers proportional to annual production. Western Australia has 12% of the Australian sow population resulting in a total sample of 40 sow hearts from six free range and 14 intensive farms in WA. The sow hearts were collected at slaughter by abattoir employees, frozen to -20°C and transported to the SARDI Food Safety and Innovation laboratory. Heart tissue underwent acid/pepsin digestion (Dubey 1998) followed by DNA extraction using the Wizard Genomic DNA Extraction Kit (Promega Corp., Madison, WI, USA). All DNA extracts were analysed using polymerase chain reaction (PCR) for a mammalian house-keeping gene (Frericks and Esser 2008) and the *T. gondii* 529 base pair fragment (Opsteegh *et al.* 2010).

Toxoplasma gondii DNA was detected in two samples from different intensive indoor production herds, resulting in an estimated prevalence of *T. gondii* in sow hearts from WA of 5% (s.d. $\pm 1.4\%$). An earlier pilot study using the same methodology (APL project 2014/506) estimated the prevalence in sow hearts ($n=92$ from 62 herds) from south-eastern Australia at 9.8% (s.d. $\pm 3.1\%$). Combined, the prevalence of *T. gondii* in sow hearts is 8.3% (s.d. $\pm 3.2\%$) with no statistical significant difference between the prevalence estimates for WA and south-eastern Australia ($P=0.57$). It should be noted that this data does not represent the national prevalence of *T. gondii* in Australian pork due to limited geographic range and numbers sampled. Despite this, the data does indicate that further investigation is warranted to determine the actual prevalence in the Australian herd so that strategies are appropriately implemented to minimise public health risks associated with the consumption of uncooked comminuted fermented meats.

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Deep litter housed pigs have a faster pH decline compared to conventional housed pigs

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The Australian pork industry has focused on developing an eating quality pathway (Channon *et al.* 2016) to improve the quality and consistency of pork. However, studies have generally focused on conventional housed pigs with an average HCW of 74.9 kg. This study was conducted to identify the effect of deep litter compared to conventional housing systems, over three carcass weights and two sexes of pigs. The hypothesis tested was that housing (H), carcass weight (W) and sex (S), does not affect carcass objective quality.

A total of 384 Large White \times Landrace commercial pigs (PrimeGro GeneticsTM, Corowa, NSW, Australia) selected over eight replicates were used in a $2 \times 2 \times 3$ factorial experiment with the main factors being: (1) housing: conventional partially-slatted birth to bacon housing system (CON) v. deep-litter grow-out system (DL); (2) sex: female (F) or castrated male pigs (IM) (Improvac[®], Zoetis, Rhodes, NSW, Australia); (3) carcass weight specification (Trim 1): Light 60 to 70 kg, 8 to 16 mm P2 (L); Medium 70.1 to 80 kg, 8 to 16 mm P2 (M); Heavy 80.1 to 91 kg, 8 to 16 mm P2 (HV). Pigs of different sex were kept separately on the farms, during transport (~4 h) and in lairage. On arrival at the abattoir a sub-sample per housing type and sex were held in lairage with access to water before slaughter the next day. For each replicate, 48 pigs were selected within housing type, sex, and carcass weight specifications. Carcasses were chilled 24 h at 2°C. Carcass pH and temperature were measured (loin) at 45 min, 90 min, 3, 6 and 24 h post-slaughter using a pH meter (MPI, Topeka, KS, USA). Data were analysed using ANOVA (GENSTAT 16, VSN International, Hemel Hempstead, UK).

The effect of housing, carcass weight and sex on objective quality measures is reported separately (Lealiifano *et al.* 2017). DL carcasses were 1 mm fatter compared to CON (SE 0.26, $P=0.002$), and were fatter at target carcass weights (L: 10.3 v. 9.8, SE 0.36; M: 11.8 v. 11.1, SE 0.36; HV: 13.5 v. 11.9, SE 0.36 DL v. CON respectively $P=0.023$). This could be due to ambient temperature variability in the deep litter housed pigs. DL carcasses had a faster pH decline (Fig. 1a) perhaps indicative of increased glycolytic rate post-slaughter as a consequence of increased pre-slaughter stress or higher glycogen stores. Carcass temperature was unaffected by housing ($P>0.05$). Rate of chilling was slower in HV carcasses compared to L; however, there was no difference for 24 h carcass temperature between the weight categories (Fig. 1b). Carcass P2 increased by 1 mm with every increase in 10 kg carcass weight (SE 0.22, $P=0.001$). Carcass pH was unaffected by weight differences ($P>0.05$). The combination of the increased muscle size and carcass P2 caused greater heat inertia and a slower chilling in the heavier carcass. There was an H \times S interaction ($P<0.05$) at 45 min post-slaughter only (IM: 6.35 v. 6.53 SE 0.04; F: 6.43 v. 6.47, SE 0.04, $P=0.031$ for DL and CON, respectively). The hypothesis was rejected as these results show that H, W and S, significantly influence objective carcass quality but with a large variability between factors.

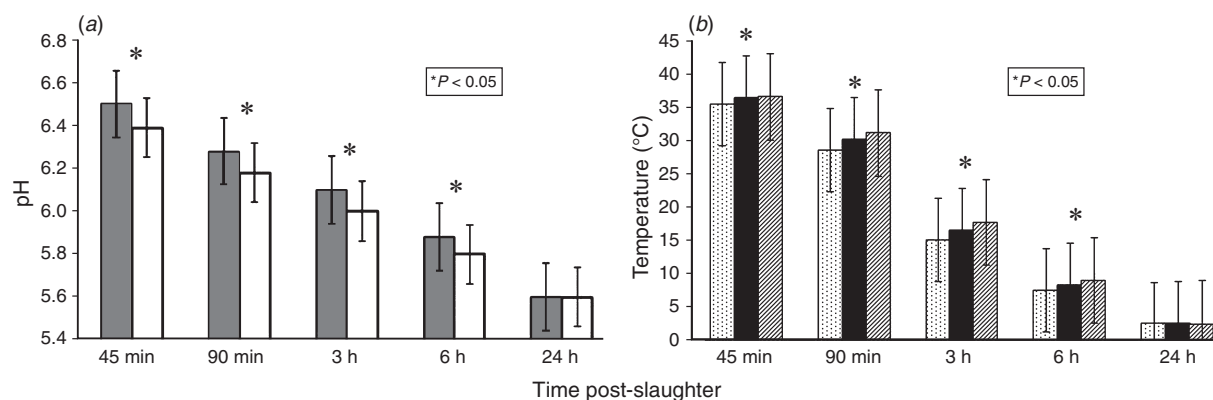


Fig. 1. (a) Carcass pH of DL (white) and CON (grey) pigs, and (b) Carcass temperature of L (dots), M (solid black), and HV (grey stripes) pigs from 45 min to 24 h post-slaughter.

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Development of an algorithm to correlate Physi-Trace pig liver data with pork meat data

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Physi-Trace was designed to facilitate the provenance of pork back to a point of origin. Throughout Physi-Trace, it has been shown that it is possible to validate whether a pork product is Australian or not and to establish the origin (farm/tattoo) of Australian pork. This has been possible through continued sampling of known origin pork meat and the subsequent trace elemental analysis and statistical interpretation of the resulting data. A system is now in place that will facilitate the traceability of fresh Australian pork. Associated with the traceability of fresh pork is the traceability of pork offal. Preliminary research by Kreitals (2013) indicated that it was possible to link the elemental profile of offal varieties to the elemental profiles of fresh pig meat using several algorithms thus allowing the origin determination of pig offal to be conducted using the Physi-Trace framework. The algorithms developed by Kreitals (2013) were limited to a single processor and required further research to verify their robustness. The objective of this research was to undertake a detailed study to develop a universal algorithm that could be used to trace analytical data for liver back to their equivalent pork meat data and establish region of origin.

The experiment required the collection of samples (livers and raw meat) from pigs at participating Physi-Trace export processors, and represented two large, two medium and one small grower from each processor. Seven samples were taken from each grower totaling 203 raw meat and 203 liver samples. The samples were digested in a mixed acid solution at 90°C overnight before dilution with high purity (18 MΩ) water and were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) to determine trace element profiles. Due to the accumulation of some elements in the livers, a reduced set of analytes (Table 1) was developed for accuracy.

This investigation produced an algorithm that could be used to transform Physi-Trace elemental data for pork liver into 'equivalent' data that can be compared with pork meat data to determine property of origin. Fig. 1 is a linear discriminant analysis plot of the raw meat elemental data from all five growers from a specific processor with the addition of the transformed liver data from Grower 3.

From Fig. 1, all five growers can be easily discriminated and the transformed liver data for Grower 3 groups well with the raw meat data from Grower 3. Similar results were obtained for 86% of all the liver samples tested showing that the developed algorithm could be used to transform pork liver elemental data into data that could be used to compare with the elemental data of raw meat in the Physi-Trace database to assess the origin of the liver sample. To trace back to a processor of origin, the success rate of the algorithm was 97%.

Table 1. Reduced set of analytes used for all 'conversion' of pork liver data to equivalent pork meat data

7 Li	9 Be	11 B	45 Sc	49 Ti
51 V	53 Cr	55 Mn	59 Co	66 Zn
69 Ga	74 Ge	75 As	77 Se	85 Rb
88 Sr	89 Y	98 Mo	111 Cd	115 In
121 Sb	126 Te	133 Cs	138 Ba	208 Pb
Na 589.592 nm	Mg 285.213 nm	Al 167.019 nm	P 178.222 nm	S 181.972 nm
K 766.491 nm	Ca 422.673 nm			

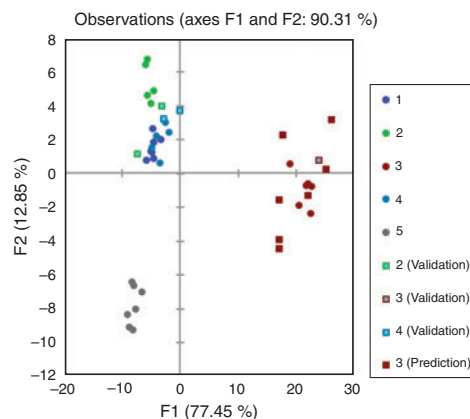


Fig. 1. Scatter plot showing discrimination between five groups from a single region. Modified liver data for Liver Group 3 agrees with corresponding Meat Group 3.

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Reduced antimicrobial resistance in weaner pigs treated with Detach[®] following natural challenge with F4 *Escherichia coli*

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Aminoglycosides and zinc oxide (ZO) are used to prevent post-weaning diarrhoea (PWD) caused by Enterotoxigenic *Escherichia coli* (ETEC), but their use can lead to antibiotic resistance. Detach (Anatara Lifesciences Ltd, Brisbane, Qld, Australia) protects pigs from PWD but is not bactericidal. Detach[®] acts by (1) inactivating host receptors, preventing ETEC colonisation and (2) inhibiting intestinal fluid secretion caused by ETEC toxins (Mynott *et al.* 1997; Chandler and Mynott 1998). This study tested the hypothesis that Detach[®] could control PWD in weaner pigs without increasing the incidence of antimicrobial resistance (AMR) in *E. coli*.

Seventy-two weaner pigs (mixed gender) from nine gilt litters were randomly allocated into four treatment groups with two piglets from each litter per treatment. Each group, six pens of three pigs ($n = 18$ per group), were housed in separate rooms to avoid faecal contamination. Detach[®] (4 mL) was administered orally (DT) the day before weaning (d -1) and on d 7. All pigs were acclimatised to non-medicated feed between d 0 and 7. Zinc oxide (2500 ppm) or neomycin sulphate (8 mg/kg, NS) was administered in feed between d 7 and 20. Non-medicated feed was supplied to Control (CT) and DT pigs from d 0 to 40, and to Zn and NS pigs from d 20 to 40. Pen feed intake and individual weight gains were recorded weekly and faeces were collected from each pig on d 6, 19 and 39 and faecal consistency scores recorded daily. Pathogenic F4 ETEC were quantified in d 6 faeces to compare infection levels between groups. Four *E. coli* isolates from each faecal sample were tested for AMR to seven commonly used antimicrobials. Differences in proportions of resistant *E. coli* between groups were analysed with a Kruskal–Wallis One-Way ANOVA (GENSTAT 18, VSN International, Hemel Hempstead, UK). Two NS pigs died on d 19 and 39 due to a *Streptococcus suis* infection and chronic pericarditis, respectively. Low numbers of F4 ETEC were detected at d 6 in all pigs (median = 280 ETEC), with a significantly higher proportion in CT relative to NS pigs ($P = 0.042$). During the treatment period, diarrhoea was numerically higher in CT piglets between d 7 and 19 (44% to 11% in other groups), but mean faecal consistency scores did not differ between treatments, and CT pigs recovered after d 20. Immediately following removal of ZO and NS, 25% of NS and ZO pigs had diarrhoea requiring electrolytes. Weight gains over the trial period (d 7 to 39) were NS, 23.13^a kg; ZO, 22.02^c kg; DT, 21.07^b kg and CT, 22.77^c kg (different superscripts indicate $P < 0.05$). Feed intake was NS, 25.77^a kg; ZO, 24.45^b kg; DT, 23.66^c kg and CT, 27.00^d kg. At d 6, *E. coli* AMR was not significantly different between groups, but by d 19 *E. coli* from DT pigs had reduced AMR to neomycin (1.4% compared to 12.7%), tetracycline, sulphamethoxazole/trimethoprim and lincospectin relative to NS pigs (Fig. 1). At Day 39, ZO pigs had significantly increased resistance to tetracycline.

Detach[®] effectively controlled diarrhoea following natural challenge without inducing resistance to antimicrobials in *E. coli*, but did not lead to the growth advantages observed in NS treated pigs.

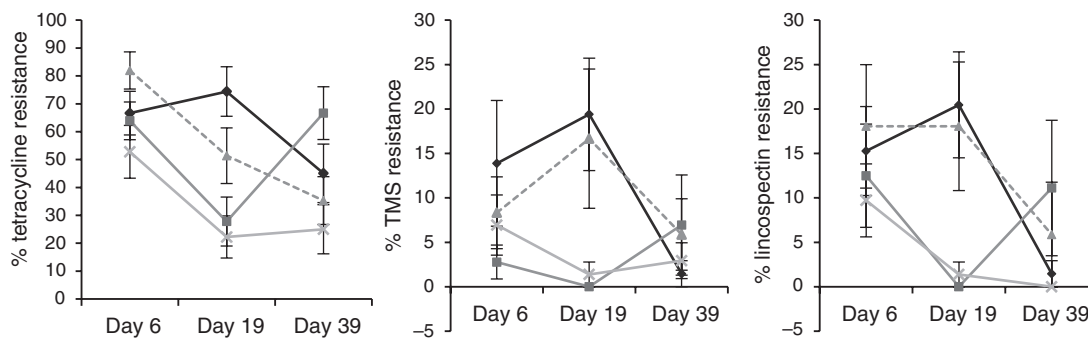


Fig. 1. Percentage of *E. coli* with resistance to tetracycline, sulphamethoxazole/trimethoprim (TMS) and lincospectin in —●— NS, —■— ZO, —×— DT and —▲— CT pigs.

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The emergence of community associated MRSA (ST93) in piggery workers and associated risk factors

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In Europe, livestock associated (LA) MRSA (ST398) carriage in pig enterprises has emerged as an occupational challenge where a high prevalence has been reported in pig farmers (Cuny *et al.* 2009) and pigs (Morcillo *et al.* 2015). Recently, a high prevalence of MRSA carriage has been found in pigs and people working in a pig farm in Australia, where 84% of total MRSA positive workers ($n = 31$) were carrying community associated (CA) MRSA (ST93) and 16% LA-MRSA (ST398) (Sahibzada *et al.* 2017). The objective of this study was to determine the potential risk factors associated with different MRSA clone carriage with regard to occupational pig exposure on this farm.

Information was collected from the participants via questionnaires. Associations between MRSA carriage and the presence of potential risk factors were investigated using the statistical package R v3.3.3 (R foundation, Vienna, Austria), fitting univariable generalised linear models (GLM) with binomial distribution logit link function. Significance was set at 0.05. Clone-specific carriage trend was compared separately for each clone with the overall MRSA non-carrier by excluding the counter-clone from the baseline model.

A total of 52 piggery workers participated in the study, 77% male and 23% female. No significant association was found for MRSA carriage on this farm with age, gender, ethnicity, number of years working with pigs, chronic disease, or history of hospitalisation. Pig contact and contact intensity (number of hours working in direct pig contact), the role of pig workers, and level of education were noted to be significantly ($P < 0.01$) associated with MRSA and MRSA clone carriages. The prevalence of MRSA carriage was 85.2% for persons working in pig sheds, 60% for those in maintenance roles and 50% amongst feedmill workers. No MRSA carriage was found in those with administrative or pastoral roles. The odds ratio (OR) for MRSA carriage in workers who had high school or lower levels of qualifications was 3.96 (CI = 1.25–13.84, $P = 0.02$) compared with those with tertiary education. Univariable logistic regression analysis showed that the odds of ST93 carriage increased by 7% (OR 1.07, CI = 1.02–1.14) for each hour increase in pig contact in a week. A similar higher odds ratio was noted for ST398 (OR 1.08, CI = 1.02–1.21).

ST93 has been frequently isolated from communities in Australia. However, this strain has never been reported as an occupational risk, unlike ST398 which has been studied thoroughly and linked with pig contact and contact intensity.

We describe for the first time the CA-MRSA clone carriage of ST93 as an occupational risk for piggery workers which is strongly associated with intensity of contact between workers and pigs. Given that it has been reported on a single farm, it is important to investigate MRSA carriage in humans and pigs on other pig farms.

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Disinfectant susceptibility in south east Australian pig herds

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Escherichia coli (ETEC) causes severe diarrhoeal diseases in piglets resulting in large production losses to the Australian pig industry. Antibiotics are often used to manage this problem, but resistance can develop over time. Disinfectants can be used as an alternative (or additional) control option for *E. coli*, by reducing *E. coli* environmental contamination. However, disinfectant effectiveness has been poorly investigated in Australia. To assess disinfectant resistance, a cross-sectional survey of 22 commercial pig herds located in south-east Australia was conducted between September 2013 and May 2014. Fifty faecal samples were collected from each herd, 10 from pre- and 40 from post-weaned piglets. Faecal samples were collected off the floor of piglet pens (approximately five samples per pen). Fifteen presumptive *E. coli* isolates were randomly selected from diarrhoeal and non-diarrhoeal samples (including β - and non- β -haemolytic isolates) and confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) (Microflex LT MALDI BioTyper; Bruker Biosciences, Preston, VIC, Australia). Isolates ($n = 325$) were screened for susceptibility to six veterinary disinfectants using MIC broth microdilution and breakpoints according to CLSI guidelines (CLSI 2012). *Escherichia coli* isolated from pre- and post-weaned piglet pens showed high susceptibility ($\geq 95\%$) to five of the six disinfectants screened (Table 1).

Disinfectant use in combination with strict cleaning and hygiene protocols could effectively manage *E. coli* disease and limit other enteric bacteria commonly found in the piglet pen environment. This study highlights the effectiveness of disinfectant use as a management tool for *E. coli* associated with diarrhoea in piglets. Further work could monitor the effectiveness of these disinfectants on-farm to reduce other species of endemic bacteria.

Table 1. Disinfectant phenotypic resistance in 325 *E. coli* isolates from Australian domestic piglet pens

Disinfectant	Manufacturer	Concentration	<i>E. coli</i> resistance (%)
Virkon [®]	DuPont (Wilmington, DE, USA)	1:100	Nil
		1:200	Nil
Farm Fluid S [®]	Antec International Ltd (Sudbury, UK)	1:100	Nil
		1:200	Nil
Nu-quat [®]	Bunzl Distribution Midcentral Inc (St Louis, MO, USA)	1:50	1.8
		1:100	4.0
Microtech 7000	Artech Technologies Pty Ltd (Breakwater, Vic., Australia)	1:500	Nil
		1:1000	3.4
F10 [™]	Health and Hygiene Pty Ltd (Roodepoort, South Africa)	1:100	2.2
		1:200	4.6
Iodophore	Not commercially available	1:85	100.0
		1:170	100.0

Reference

CLSI (2012) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Testing for Bacteria Isolated From Animals; Secondary Informational Supplement. VET01–S2. **33**, 1–188. (Clinical and Laboratory Standards Institute: Wayne, PA)

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Investigation of a novel porcine bacterium by whole genome sequencing and mouse inoculation

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A bacterial isolate collected from a lung lesion of a pig at slaughter was identified as a member of the family *Pasteurellaceae* using standard biochemical testing. Antibiotic sensitivity was tested using the CDS method and the isolate was found to be susceptible to ampicillin, cephalixin and enrofloxacin and resistant to tetracycline. Further classification was inconclusive, and the isolate failed to group within any existing genus or species. The lung lesions were typical of those seen after infection with *Actinobacillus pleuropneumoniae*, but preliminary 16S rRNA gene sequencing indicated that the closest relative was *Haemophilus parasuis*. The work described here aimed to further investigate this novel bacterium by biochemical testing, full genome sequence analysis and assessment of its pathogenicity in a mouse model of infection.

Full genome analysis was completed using Illumina (Illumina Inc., San Diego, CA, USA) and Oxford (Oxford Nanopore Technologies, Oxford, UK) nanopore sequencing, with comparisons of conserved genes suggesting that the novel bacterium was most closely related to *H. parasuis*. Concatenation and phylogenetic assessment of the housekeeping genes, *atpD*, *infB* and *rpoB* showed that the organism lay in a distinct monophyletic position within the *Pasteurellaceae* phylogenetic tree (Fig. 1). Assessment of the pathogenicity of the organism was performed by inoculation of 6 week old BALB/c mice with the novel bacterium at different concentrations via the intranasal and intraperitoneal routes. The lesions caused after infection were compared to those seen in a positive control group infected with *A. pleuropneumoniae*. The lungs, liver and spleen were assessed for the severity and type of lesions using histopathological examination. Intranasal inoculation resulted in interstitial pneumonia and bronchitis. The findings suggested that the lesions caused by this yet unclassified member of the *Pasteurellaceae* differed significantly from those described in previously published studies in which mice were inoculated with *G. parasuis* (De la Fuente *et al.* 2007).

The results suggested that further research is needed to assess the prevalence of this bacterium in pig populations, as well as to examine its pathogenicity for its natural host, as mouse models only provide an indication of the potential of the organism to cause acute disease. Its potential to cause chronic disease will need to be assessed to fully understand the type and severity of impact this bacterium may have on commercial pig farms.

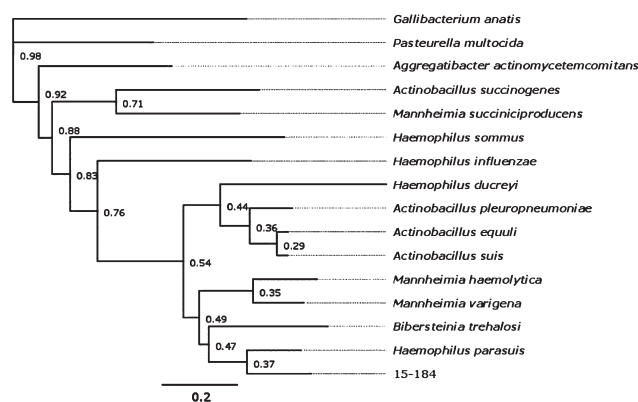


Fig. 1. Phylogenetic tree based on concatenation of the *atpD*, *infB* and *rpoB* genes from selected members of the family *Pasteurellaceae*. The randomised accelerated maximum likelihood (RAxML) tree was built in Geneious v.7.1 using the rapid hill-climbing algorithm. Bootstrap values from 100 trees are indicated as percent confidence values for the branching.

Reference

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Haptoglobin in oral fluid samples from pens of pigs can potentially be used to estimate herd inflammatory status

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Until recently, plasma haptoglobin (Hp) content has been used as a biomarker for immune system activation in pigs (Parra *et al.* 2006). The major limitation of this is the need to collect representative blood samples, which can be problematic and create errors in terms of collecting representative samples of a herd. Alternatively, measuring Hp in oral fluid samples to discriminate an unhealthy herd has been proposed (Soler *et al.* 2013). Therefore, the hypotheses of the present study were that (1) immune system activation of grower/finisher pigs can be discriminated by measuring Hp content in oral fluid (OF) samples, and (2) the Hp contents in the OF samples collected from pigs in a pen (the group) are significantly correlated with that in the OF samples collected from individual pigs in that group.

To test the hypotheses, seven commercial farms of varying health status (healthy *v.* unhealthy, as assessed by veterinarians) were selected. To test the first hypothesis, five pens per farm were randomly selected for OF and plasma sample collection from individual pigs. A total of 340 grower pigs were sampled for plasma and OF. To test the second hypothesis, two additional pens were randomly selected from each farm and an individual OF sample and a single-point OF sample were collected by hanging a cotton rope for 40 min in a pen. A total of 110 individual OF samples and 12 group pens OF samples were collected in this process. One group pen OF sample was removed from the dataset due to dehydration, as the water supply was interrupted on the sampling day. The samples were analysed for Hp content using a commercially available ELISA kit (Aviva Systems Biology, San Diego, CA, USA). Correlation and a simple linear regression analyses were conducted using GENSTAT 15 (VSN International, Hemel Hempstead, UK).

The mean (\pm s.e.) plasma and OF Hp contents determined from 340 pigs were 0.73 (0.03) mg/mL and 0.51 (0.04) μ g/mL, respectively. The Hp contents in the OF samples were significantly correlated with that in the plasma samples ($r = 0.44$, $P < 0.001$). The cut-off point of the OF Hp content for discrimination between healthy and unhealthy pigs was 1.5 μ g/mL (Fig. 1a). The single-point OF sample collected by hanging a cotton rope in a pen linearly correlated with the mean Hp content determined by collection of OF samples from individual pigs in the same group ($r = 0.967$, $P < 0.001$, Fig. 1b). This suggests that OF is a potentially useful sample for measuring the degree of immune system activation. However, and as the slope indicates in the regression equation, the Hp content in the group OF sample was higher by 2.56 μ g/mL per unit of Hp than the average of individual OF samples. This would indicate an adjustment for the cut-off point may be required when single-point OF sample is used to evaluate inflammatory status of a herd.

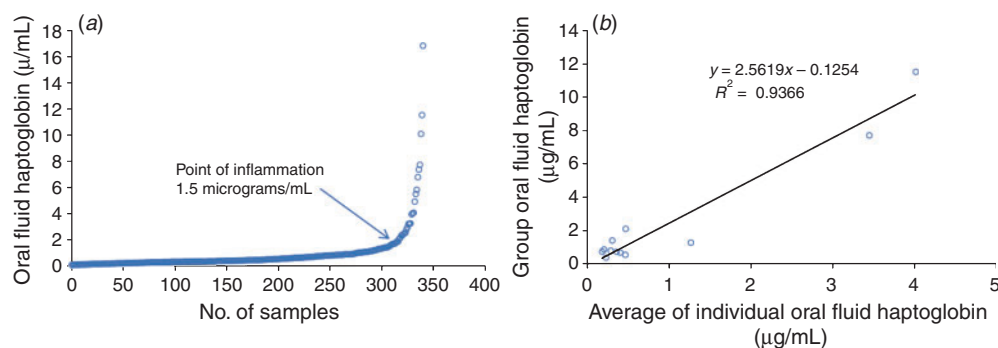


Fig. 1. Oral fluid haptoglobin concentration in individual pigs (a), and the relationship between mean haptoglobin content in individual *v.* group oral fluid samples (b).

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Novel feed additives controlling *Salmonella typhimurium* in pigs

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Non-typhoidal salmonellosis is estimated to cause 93.8 million cases of acute gastroenteritis and 155 000 deaths globally each year, ~85% of which are estimated to be foodborne. Pork products are among the top food-borne sources of salmonellosis globally (FAO/WHO 2016). Reduction of contamination in the feed-to-food chain is necessary to reduce the number of human cases. Since antimicrobial resistance is on the rise, there is an urgent need for new antibacterial strategies to reduce salmonellosis. This paper investigates antibacterial properties of a mixture of organic acids (formic and lactic acid) with two novel feed additives: mannanase-hydrolysed copra meal (CM) and rye overgrown with mycelium of *Agaricus subrufescens* (ROM) using a porcine *Salmonella typhimurium* infection model. Formic and lactic acid were included because of their antibacterial properties (van Immerseel *et al.* 2006), while the other components were supposed to reach the intestinal tract where they may competitively bind to *S. typhimurium*. Furthermore, mannanase-hydrolysed copra meal contains β 1–4 mannobiose, which may increase IgA-response and reduce *S. typhimurium* shedding after infection (Agunos *et al.* 2007).

In vivo activity of the feed additive blend was evaluated in a *S. typhimurium* challenge study with 24 piglets individually housed directly after weaning. Piglets were fed a control diet or the same diet supplemented with the combination of organic acids, CM and ROM. To provide the disease challenge, piglets received feed containing 10^9 CFU *S. typhimurium* from 5 days after weaning for seven consecutive days. The pigs were monitored for *S. typhimurium* shedding and serum immunoglobulin A (IgA) (3 days post infection). Data were analysed using the GLM procedure (SAS v9.4, SAS Institute Inc., Cary, NC, USA).

In all treatment groups *S. typhimurium* infection resulted in a mild increase in body temperature ($<0.5^\circ\text{C}$), mild diarrhoea. The combination of feed additives significantly decreased *S. typhimurium* peak shedding during the first week after infection (4.0 ± 0.27 v. 5.1 ± 0.27 log CFU/gram faeces; $P < 0.01$; Fig. 1). Immunoglobulin A serum levels, 3 days post infection, were negatively correlated with the level of shedding 3 days post infection in both the control group ($r^2 = 0.66$; $P = 0.03$) and the treatment group ($r^2 = 0.62$; $P = 0.01$); however, supplementation of the combined feed additives had no effect on IgA levels (Fig. 2).

In conclusion, the feed additive combination did not influence serum IgA levels in pigs. However, the combination of organic acids with CM and ROM showed inhibiting effects towards the disease and therefore may be useful to control *S. typhimurium* in pigs.

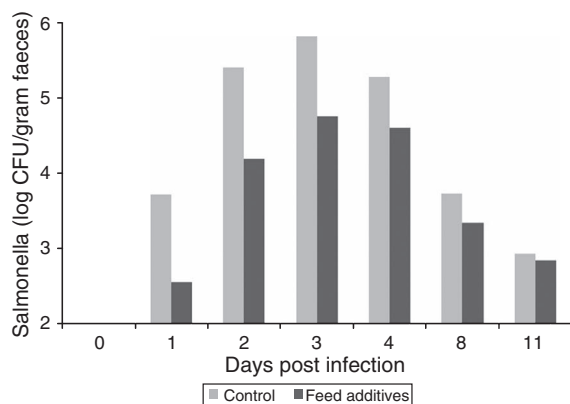


Fig. 1. *Salmonella* shedding after challenge.

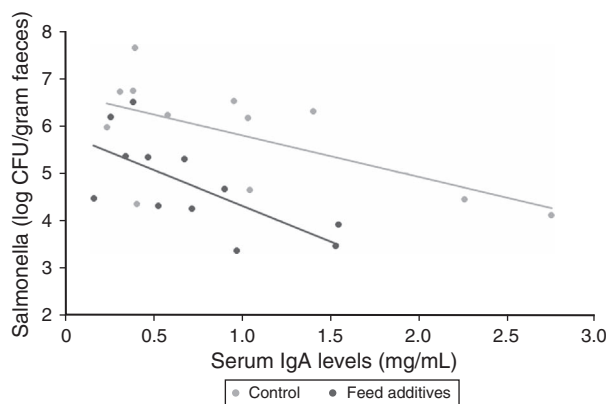


Fig. 2. Correlation of *Salmonella* shedding with IgA levels.

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***Lactobacillus acidophilus* fermentation product as an alternative to therapeutic zinc oxide in weaned pig diets on performance and response to *Escherichia coli* challenge**

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Previous research in pigs with chronic K88+ (F4) *Escherichia coli* has demonstrated that feeding *Lactobacillus acidophilus* fermentation product (LAFP; SynGenX[®], Diamond V, Cedar Rapids, IA, USA) at 1 kg/MT, in combination with carbadox and zinc oxide (ZnO) improves health and reduces the frequency of injectable treatments and mortalities (Probst Miller *et al.* 2016). Additionally, the combination of 1 kg/MT LAFP and oxytetracycline, or 2 kg/MT LAFP alone, has been shown to reduce *E. coli* in weaned pigs (An *et al.* 2015). Zinc oxide, fed at therapeutic levels, has been used to alleviate diarrhoea associated with weaning stress. However, concerns about the environmental impact of high inclusion levels of zinc, as well as the potential of zinc to drive antibiotic resistance in bacteria, may limit its use in the future. Therefore, we hypothesise that LAFP can aid in reducing the impact of *E. coli* on pig health and performance, demonstrating similar benefits as therapeutic levels of ZnO.

In the first of two studies, 288 piglets were weaned at 28 days (7.41 kg bodyweight (BW)) and randomly assigned to one of four dietary treatments (12 pens per treatment; three boars:three gilts per pen): Negative Control devoid of antibiotics (NC); Positive Control – NC + 3000 ppm ZnO (PC); NC + 1 kg/MT LAFP in pre-starter and 0.5 kg/MT LAFP in starter (LA1); and NC + 2 kg/MT LAFP in pre-starter and 1 kg/MT LAFP in starter (LA2). Diets were provided for 35 days (Pre-starter from weaning to 12 kg BW; Starter from 12 kg BW to the end of the study). Pigs and feeders were weighed on d 0, 7, 14, and 35 to determine BW, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). Data was analysed using GENSTAT 17 (VSN International, Hemel Hempstead, UK). In the first week post-weaning, a trend was observed in ADG and FCR in which PC had higher ADG and lower FCR than NC, with both LA1 and LA2 being intermediate. Significant improvements in ADG ($P = 0.024$) and FCR ($P = 0.013$) were observed using contrast comparisons between NC and the combination of LA1 and LA2. In the starter period (d 14 to d 34) and in the overall nursery period PC had higher ADG and final BW ($P < 0.01$) than NC and LA1, with LA2 being intermediate. Feed-to-gain was higher in NC compared to LA1 and LA2 combined (1.51 v. 1.44 g/g; $P = 0.03$) in starter period and in the overall nursery period (1.44 v. 1.36 g/g; $P = 0.022$). There were no differences in performance between PC and LA2 throughout the study.

In the second study, 48 piglets, 24 boars:24 gilts (Topigs Tempo x (Great Yorkshire x Finnish Landrace)), weaned at 26 days of age (7.1 kg BW) were allocated to four treatments: Negative Control devoid of antibiotics (NC2); Positive Control – NC2 + 2500 ppm ZnO (PC2); NC2 + 1 kg/MT LAFP (SG1); and NC2 + 2 kg/MT LAFP (SG2). Diets were offered to the piglets for 22 days. On d 10, piglets were orally challenged with 5 mL of 8.9 Log of nalidixine resistant *E. coli*. During d 11 to 15, 18, 20 and 22, faecal scores were measured (Gerritsen *et al.* 2012) and samples were collected to quantify nalidixine resistant *E. coli* and data was analysed using GENSTAT (17th edition). On d 14 ($P = 0.064$), 15 ($P = 0.064$) and 18 ($P = 0.041$), faecal *E. coli* excretion was lowest in boars fed SG2, with no differences between gilts and boars fed other diets. On d 20, pigs fed SG2 showed lower faecal excretion of *E. coli* than pigs fed with NC2, PC2 and SG1 (3.4 v. 4.3, 4.3 and 4.4 Log CFU/g faeces, respectively; $P = 0.031$). Overall faecal score was lowest for SG2 ($P < 0.05$). Pigs provided PC2 tended to have higher ADG ($P = 0.053$) compared with NC2 and SG1 (466 v. 359 and 385 g/d, respectively), with SG2 intermediate (406 g/d).

In summary, inclusion of ZnO at therapeutic levels improved performance compared to negative control fed animals. Feeding LAFP at 2 kg/MT resulted in performance comparable to therapeutic ZnO and additionally reduced faecal score and shedding of *E. coli* following challenge in diets that did not contain antibiotics or other additives. In conclusion, LAFP at 2 kg/MT may be a potential alternative for therapeutic ZnO in piglet diets post-weaning.

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Effect of the combination of three yeast strains on post weaning piglets after an experimental *E. coli* infection

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Weaning imposes stress on the piglets, which may affect their general health, therefore, it is necessary to keep a well balanced microflora to protect against colonisation of pathogenic microorganisms in the gastrointestinal tract (Jensen 1998). Yeast fractions have been shown to have a positive effect on gut health in weaning piglets. A blend of three inactivated yeasts strains (YANG[®]; Lallemand SAS, comprising two complementary species of *Saccharomyces cerevisiae* and one strain of *Cyberlindnera jadinii*) has shown positive antibacterial effects *in vitro* (Dunière *et al.* 2016). The objective of this trial was to evaluate the effect of the yeast blend on *E. coli* faecal counts and IgG blood concentration of weaned piglets with an *E. coli* challenge. It was hypothesised that both the *E. coli* excretion and the immunoglobulin G (IgG) concentration will be lower, as an effect of counteracting the effects of the challenge given by *E. coli* inoculation.

The trial was run at Schothorst Feed Research (SFR), and 36 male piglets (Topigs Tempo × (Great Yorkshire × Finnish Landrace)) aged 25 ± 0.91 days and weighing 6.83 ± 0.51 kg at weaning, were housed in pairs in 1 m² pens. Pens were allocated to one of the experimental treatments: negative control (NC), positive control (PC), and YANG (Y, 800 g/ton). The test product was offered in feed for the entire experiment. In PC treatment, piglets were daily treated with colistin (100 000 IU Coliplus, Bimeda, Llangefni, UK[®]) via drinking water at a dose of 2 000 000 IU/mL per kg of bodyweight. During the first 3 days, all piglets received colistin to reduce the presence of *E. coli* in the gut. On d 10, all the piglets were challenged orally with 5 mL of 8.80 ± 0.07 log CFU *E. coli*/mL. The *E. coli* strain was isolated from an SFR herd and made resistant to nalidixin to facilitate its identification. Faecal samples were taken on d 8, 11, 15, and 22 and blood samples on d 8, 15 and 22. The experimental data were analysed using the mixed model (repeated-measurements) using GENSTAT 18 (VSN International, Hemel Hempstead, UK), with dietary treatment and day as fixed effects. Significance was set at $P < 0.05$, with pen being the experimental unit. Before challenge, faecal excretion of *E. coli* in all the piglets was lower than detectable levels. Piglets fed diet Y had lower faecal *E. coli* excretion than piglets fed NC ($P < 0.001$). Piglets treated with colistin showed the lowest *E. coli* excretion in the faeces. Piglets fed diet Y tended to have lower IgG blood concentration ($P < 0.10$) than piglets on the PC diet. The study design did not allow detection of growth performance between dietary treatments. Results are shown in Table 1.

It was concluded that the inclusion of YANG[®] at a dose of 800 g/ton decreased faecal *E. coli* excretion by ~0.5 log CFU compared with piglets in NC treatment. An effect on the humoral immune response was suggested, but needs further investigation.

Table 1. *E. coli* excretion (log CFU/g faeces) in faeces and IgG concentration in blood (mg/mL) before (d 8) and after (d 11 to 22) *E. coli* challenge

Day	<i>E. coli</i> excretion			d	P^A	d × T	IgG concentration			d	P^A
	NC	PC	Y				NC	PC	Y		
8 ^A	1	1	1	–	–	–	3.31	3.10	2.96	–	0.15
11 ^A	7.63 ^b	3.26 ^a	6.44 ^b	–	<0.01	–	–	–	–	–	–
15 ^A	6.67 ^b	1.44 ^a	6.12 ^b	–	<0.01	–	2.82	2.84	2.73	–	0.93
22 ^A	4.09 ^b	1 ^a	4.30 ^b	–	<0.01	–	2.93	3.19	2.73	–	0.16
Overall	6.26 ^c	1.48 ^a	5.66 ^b	<0.01	<0.01	<0.01	3.02 ^{xy}	3.04 ^y	2.80 ^x	0.03	0.08

^A P -value from one way ANOVA analysis. d, day; T, treatment; d × T, interaction day × treatment. ^{a–c}Means with different superscript are significantly different.

^{x,y}Different superscript indicates a trend for a significant difference ($P < 0.10$).

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Pen hygiene and piglet mortality in farrowing pens with partly solid floor, changes through lactation

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Commercial viability of free farrowing pens relies on good performance and functionality of the pens. Design recommendations suggest a partly solid floor to provide a comfortable lying area and to accommodate sow nesting behaviour (Baxter *et al.* 2011). However, dunging behaviour can be difficult to control when sows are loose and maintaining good hygiene on solid areas can require more effort than fully slatted floors. Improving piglet survival can also be labour intensive, but experiences from practice show both performance and hygiene improves as experience with the system grows. The objective of this study was to investigate how hygiene and piglet mortality developed with increasing experience with the system.

The study was conducted over 3 years in two commercial Danish herds, each with 1200 sows, and SWAP (Sow Welfare And Piglet protection) farrowing pens. The pens had 60% and 40% solid concrete floor in Herd A and B, respectively, and cast iron slats. The solid floor was divided into different areas: 'creep', 'sow area' and 'area under sloping wall'. Areas were scored for cleanliness ('clean and dry', 'partly dirty' or 'soiled') once a week, and sow cleanliness was assessed ('clean and dry', 'partly dirty' or 'dirty'). Cause of death of piglets within herds was recorded ('weak', 'crushed', 'starvation/run', 'leg problems', 'other') and Herd A also recorded weekly performance figures. Data on hygiene and performance was analysed for effects of herd, season, time and week in lactation using linear models (SAS v9.4, SAS Institute Inc., Cary, NC, USA). Results showed a decrease in total piglet mortality (stillborn and liveborn deaths of total born) from 26.8% in the first quarter to 22% in the last quarter ($P < 0.01$) 2 years later. In this period, there was an increase in the number of liveborn piglets/litter (16.5 ± 0.15 v. 17.9 ± 0.15 , $P < 0.01$) and a higher weaned piglets/litter (13.3 ± 0.24 v. 14.2 ± 0.18 , $P < 0.05$), indicating that performance improved over time. Crushing was the main cause of death ($P < 0.001$) with 58%, 64% and 59% of mortality attributed to crushing in lactation week 1, 2 and 3 + 4, respectively. Cleanliness of the sow area differed between herds ($P < 0.001$) and seasons ($P < 0.001$). The proportion of clean sow areas increased after farrowing ($P < 0.001$) whereas cleanliness of the area under the sloping wall decreased during lactation ($P < 0.001$) (Fig. 1). Only piglets can defecate under the sloped wall and the reduced cleanliness indicates that piglets developed unwanted dunging behaviour. Sows were cleaner in Herd B compared to Herd A ($P < 0.001$) but in both herds sow cleanliness decreased through lactation ($P < 0.001$). In Herd A, 70% of sows were clean before farrowing, decreasing to 55% in lactation week 3 + 4. In Herd B, 96% of sows were clean before farrowing and 87% were clean in week 3 + 4. These results are consistent with the reduced cleanliness of pens and also indicate an effect of the different floor profiles on sow cleanliness.

This study indicates that experience with a system improves performance and that the effect of interventions might be improved by aiming specific interventions at specific time points.

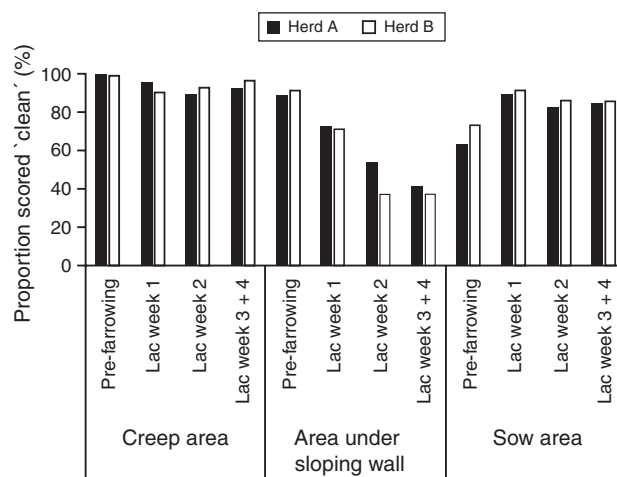


Fig. 1. Cleanliness of pen areas through lactation in Herds A and B. Effect of lactation week: creep area, $P < 0.001$; area under sloping wall, $P < 0.001$; sow area, $P < 0.001$.

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Topical zinc oxide improves shoulder lesion healing compared with other treatments

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A shoulder lesion is formed when the scapular spinal process is placed under prolonged pressure reducing blood supply and eventually causing tissue necrosis (Rolandsdotter *et al.* 2009). Shoulder lesions are developed primarily in the farrowing unit, and are more typical of sows with poor body condition, lameness, and scars from previous incidence (Kaiser *et al.* 2013). These lesions negatively impact sow welfare, and adversely affect consumer opinion of current conventional farrowing systems. The aim of this study was to assess the effectiveness of four treatments on the healing of shoulder lesions. We hypothesised that topical zinc oxide administration would improve the healing of lesions compared with cetrimide, benzalkonium and aluminium sprays.

A total of 399 lesions were graded (BPEX 2011) and measured for lesion diameter pre-treatment (~d 12 lactation), d 7 post-treatment and again at weaning (~d 25 lactation). Each shoulder lesion was randomly allocated using the Rand function in excel to one of the following treatments: 0.8% cetrimide solution ($n = 103$), 0.2% benzalkonium solution ($n = 104$), 4% aluminium powder ointment ($n = 90$) and, 15.25% zinc oxide ($n = 102$). All groups were topically treated daily until weaning. Lesion grade and diameter were analysed using a general linear model, shed was fitted as a random term, with farrowing month, parity, body condition score, and treatment as fixed effects (SPSS v24.0, IBM, Armonk, NY, USA). Percentage of sows with sores at weaning was analysed using binary logistic regression with the same model.

There was no significant difference in shoulder lesion grading or diameter before treatment. At both the post treatment measurements (d 7 post-treatment and at weaning), zinc oxide had significantly reduced the diameter of the lesion compared with cetrimide, with the other two treatments being intermediate (Table 1). A similar pattern was observed for lesion grade. Fewer sows from the zinc oxide treatment presented with lesions at weaning compared with cetrimide and benzalkonium, with aluminium being intermediate.

Using a 15.25% zinc oxide ointment to treat shoulder lesions improved healing compared with other available treatment options such as antiseptic sprays and aerosol band aids.

Table 1. Lesion diameter (cm) and grade (0 to 5) pre-treatment, d 7, and weaning and lesion presence at weaning for sows treated with cetrimide, benzalkonium, aluminium and zinc oxide daily

	Cetrimide		Benzalkonium		Aluminium		Zinc Oxide		<i>P</i> -value
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	
Lesion diameter (cm)									
Pre-treatment	3.5	0.6	3.5	0.6	3.4	0.6	3.6	0.6	NS
7 days post-treatment	3.6 ^a	0.2	3.3 ^{ab}	0.2	3.1 ^{ab}	0.2	2.8 ^b	0.2	0.03
Weaning	3.0 ^a	0.2	2.7 ^{ab}	0.2	2.5 ^{ab}	0.2	2.3 ^b	0.2	0.007
Lesion grade (score 0 to 5)									
Pre-treatment	1.7	0.2	1.7	0.2	1.7	0.2	1.7	0.2	NS
7 days post-treatment	2.0 ^a	0.1	1.9 ^a	0.1	1.8 ^{ab}	0.1	1.6 ^b	0.1	0.004
Weaning	1.2 ^a	0.3	1.2 ^a	0.3	0.9 ^b	0.3	0.7 ^b	0.3	0.001
Weaning presence (%) ^A	94 ^a (86–97)		97 ^a (91–99)		87 ^{ab} (76–93)		77 ^b (66–85)		0.001

^{a,b}Means in a row with different superscripts differ significantly ($P < 0.05$). ^AConfidence intervals are presented in brackets.

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Microencapsulated feed additives allow improved production efficiency in weaner pigs

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Zinc oxide (ZnO) is widely used at high levels (3000 ppm) in order to control enteric disease in weaner pigs (Poulsen 1995), nevertheless, regulatory issues and the need to reduce environmental loads of zinc has resulted in lower allowable inclusion rate of zinc (Case and Carlson 2002). Industry must look at alternative methods that allow for lower inclusion levels without reducing efficacy. This study aimed to assess performance benefits offered by the inclusion in weaner diets of microencapsulated blends of ZnO, essential oils and organic acids as an alternative to high levels of ZnO, with the hypothesis that microencapsulated blends would show equivalent performance to the 'standard' ZnO treatment.

One hundred and forty pigs (C29 × 400, PIC, Grong Grong NSW) entered the experiment weekly (CHM PP 75/15) at weaning (21 days) and were on experiment for 28 days. Five weeks' worth of entries were utilised in this experiment with 10 pens of pigs ($n = 14$) entered per week, resulting in 10 replicates per treatment. A base starter diet (14.9 MJ digestible energy (DE)/kg, 0.9 g standardised ileal digestible lysine/MJ DE) was formulated, which included 3000 ppm of bentonite. Treatments differed by part or total substitution of the bentonite with the additive, such that diets remained isoenergetic and isonitrogenous, and were Control (Ctrl) with no additive, (ZnO) containing 3000 ppm ZnO, P(ZnO) containing 1000 ppm of protected ZnO (Zinco-Plus™, Jefo Nutrition Inc., Canada), P(OA+EO) containing 1000 ppm of a protected blend of organic acids and essential oils (Porcinat+™, Jefo Nutrition Inc.) and P(ZnO)+P(OA+EO) containing combination of 1000 ppm of protected organic acids and essential oils and 1000 ppm of protected ZnO. Microencapsulated additives were based on a fat matrix protection for controlled release of active ingredients in the gastrointestinal tract. Data were analysed by ANOVA using GENSTAT 18 (VSN International, Hemel Hempstead, UK) with treatment as main effect, entry week as blocking factor and entry weight as a covariate, differences between treatments were determined by l.s.d. ($P < 0.05$). There was a significant blocking effect related to entry week, however, there were no interactions, so only main effects are shown in Table 1.

The supplementation of a combination of P(ZnO)+P(OA+EO) had a significant positive impact on growth performance of weaner pigs by comparison to the control treatment, P(ZnO)+P(OA+EO) pigs had a higher exit weight ($P < 0.008$) and a lower feed conversion ratio ($P < 0.032$). The combination of P(ZnO)+P(OA+EO) did not differ in performance to the ZnO treatment and thus appears to be a viable alternative to high levels of free ZnO.

Table 1. Weight and growth performance of Ctrl weaner pigs compared to weaner pigs receiving ZnO, P(ZnO), P(OA+EO) or a combination of both microencapsulated products for 4 weeks

	Ctrl	ZnO	P(ZnO)	P(OA+EO)	P(ZnO)+ P(OA+EO)	SED	P-value
d 0 weight (kg)	5.8	5.7	5.9	5.9	5.8	0.28	0.921
d 28 weight (kg)	13.2 ^a	14.3 ^c	13.6 ^{ab}	13.6 ^{ab}	13.9 ^{bc}	0.27	0.008
d 28 ADFI (kg/d)	0.38 ^{ab}	0.42 ^c	0.39 ^{ab}	0.38 ^a	0.40 ^{bc}	0.012	0.005
d 28 ADG (kg/d)	0.266 ^a	0.302 ^c	0.277 ^{ab}	0.279 ^{ab}	0.291 ^{bc}	0.010	0.008
d 28 FCR (kg/kg)	1.44 ^c	1.40 ^{abc}	1.42 ^{bc}	1.35 ^a	1.39 ^{ab}	0.027	0.022

^{a-c}Means in a row with different superscripts differ significantly ($P < 0.05$). AGD, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SED, standard error of difference of the means.

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Zinc oxide presentation can influence weaner performance

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Weaning is associated with a period of feed interruption and a transition to solid feed that can result in transient changes in gut morphology leading to impaired performance. Zinc oxide (ZnO) has been found to benefit gut health with reported effects such as increased expression of antimicrobial peptides, gut flora stabilisation and bactericidal function (Pluske *et al.* 2007). Zinc oxide is included in weaner diets at rates well above nutrient requirements. Environmental concerns due to low bioavailability and retention of Zn (Case and Carlson 2002) and implication in the development of antimicrobial resistance in enteric bacteria (Yazdankhah *et al.* 2014) have led calls to restrict the use of ZnO. This study investigated weaner performance when using modified organic versions of ZnO, with the null hypothesis that there would be no effect on performance between ZnO treatments.

Five hundred and sixty male pigs (20 days, 5.86 ± 0.17 kg) entered the experiment over a 4-week period and were sorted by size and assigned to pens ($n = 14$). Pens were weighed and allocated to one of four treatments using a randomised block design, resulting in 10 replicates per treatment. Treatments consisted of isoenergetic and isonitrogenous weaner diets (14.85 MJ DE/kg, 0.87 g standardised ileal digestible lysine/MJ DE) including no ZnO (control), 3000 ppm ZnO, 1000 ppm of a protected ZnO (PZnO) or 300 ppm of an enhanced surface ZnO (EZnO). Diets and water were offered *ad libitum* throughout the 28 days of the experimental period. Pens of pigs and feed refusal were weighed weekly and water usage was also measured weekly. Data were analysed by ANOVA with treatment as a fixed factor, entry week as blocking factor and entry weight as a covariate. Removals were tested for significance via Chi-squared analysis. Significant differences between treatments were determined by l.s.d. ($P < 0.05$, GENSTAT 18, VSN International, Hemel Hempstead, UK).

There was no significant difference between diets containing no ZnO (control), 3000 ppm of ZnO or 1000 ppm of the PZnO; however, the 300 ppm EZnO treatment pigs consumed less feed, grew slower and ended the experiment significantly lighter than all other treatments ($P < 0.05$; Table 1). There was no significant difference in morbidity and mortality between treatments ($\chi^2(3) = 5.76$, $P = 0.124$). The lower feed intake of the EZnO treatment suggests that this product may have affected the palatability of the diet, which is supported by a consistent, but not significant, increase in water usage compared to the other treatments.

The similar performance of the pigs fed PZnO compared to those fed ZnO confirms our null hypothesis and affords us a viable alternative if restrictions on the inclusion rate of ZnO are imposed in the future.

Table 1. Performance of weaner pigs fed diets containing no zinc oxide (–ve Control), 3000 ppm of zinc oxide (ZnO), 1000 ppm of a protected zinc oxide (Pro ZnO) or 300 ppm of an enhanced surface zinc oxide (ES ZnO)

	Control	ZnO	PZnO	EZnO	SED	Treat	P-value Week	T × W
Entry weight (kg)	5.8	5.8	5.8	5.8	0.6	1.00	0.62	0.85
Exit weight (kg)	13.4 ^b	13.7 ^b	13.3 ^b	12.4 ^a	0.4	0.010	<0.001	0.45
ADG (kg/d)	0.269 ^b	0.279 ^b	0.264 ^b	0.234 ^a	0.012	0.010	0.002	0.45
ADFI (kg/d)	0.39 ^b	0.40 ^b	0.38 ^{ab}	0.35 ^a	0.02	0.033	0.010	0.68
FCR (kg/kg)	1.45	1.44	1.42	1.50	0.03	0.07	0.029	0.06

^{a,b}Means in a row with different superscripts differ significantly ($P < 0.05$). ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SED, standard error of difference of means; Treat, treatment effects; Week, entry week effects; T × W, interaction effects.

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A comparative study of the efficacy of Detach[®] versus zinc oxide to control post-weaning diarrhoea in pigs

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Diarrhoea is a major cause of ill-thrift and mortality in piglets post-weaning. Control strategies include improved hygiene, antibiotics and high concentrations of in-feed zinc oxide (ZnO). There are increasing concerns regarding the use of antibiotics and ZnO because of the potential for developing bacterial resistance, hence the need for non-antimicrobial alternatives to control diarrhoea. Detach[®] (Antara Lifesciences Ltd, Brisbane, Qld, Australia), comprised of bromelain, a proteolytic extract from pineapple stems, is one alternative as it has been shown to reduce diarrhoea in piglets through its anti-attachment and anti-secretory effects in the intestine (Chandler and Mynott 1998; Mynott *et al.* 1997). The objective of this study was to compare the efficacy of Detach[®] to ZnO in-feed treatment in a Spanish herd with a history of post-weaning diarrhoea due to *Escherichia coli*. Healthy, 25-day-old pigs were ear tagged and randomly assigned at weaning (d 0) to one of four groups (72 piglets per group): (1) Detach[®]; (2) Detach[®]+ZnO; (3) ZnO; or (4) nil treatment. Pigs in Groups 1 and 2 received a single 4 mL drench of Detach[®] on d 0, whilst 2500 ppm ZnO (Apsamix Zinc, Andres Pintaluba S.A., Reus, Spain) was included in the diet of pigs in Groups 2 and 3 from d 0 to 14. All pigs received a diet containing 2500 ppm ZnO from d 15 to 42, as per the standard practice on this farm. Pigs were housed in 48 pens (six piglets per pen) in two identical nursery rooms. Each pig was examined from d 2 to 16 and on d 19, 26, 33 and 42 and scored for faecal consistency (normal (0), pasty/semi-liquid (1) or liquid/watery (2) and clinical condition (0 = normal, 1 = depressed). The combined faecal and clinical scores were used to categorise pigs as morbid/sick (Score 2/3) or healthy (Score 0/1). The number of sick days per pen was analysed using a linear mixed model with room/gender as random effects and treatment as the fixed effect (GENSTAT for Windows, 2007). The number of pigs with diarrhoea per pen was analysed pairwise as binomial data using Binomial Logistic Regression (LogXact v8.0, Cytel, Cambridge, MA, USA).

Over the d 0 to 14 and 0 to 42 post-weaning period, Detach[®] and ZnO were equally effective at reducing diarrhoea compared to controls ($P < 0.05$) (Fig. 1). Detach[®] and ZnO-treatment groups had fewer sick days ($P < 0.05$) and less antibiotic treatments than controls ($P < 0.05$), with 15, 0, 2 and 33 treatments administered to the Detach[®], Detach[®]+ZnO, ZnO and control groups, respectively. Weight gains from d 0 to 14 were Detach[®] 0.7 kg, Detach[®]+ZnO 1.14 kg, ZnO 1.03 kg and control 0.57 kg, with ZnO-treated pigs gaining more weight than the other groups ($P < 0.05$). Neither treatment had a significant effect on weight gain between d 0 to 42; Detach[®] 12.4 kg, Detach[®]+ZnO 12.5 kg, ZnO 12.6 kg and control 12.2 kg.

Under the conditions of this study, a single dose of Detach[®] at weaning was as effective as in-feed ZnO in reducing the prevalence of diarrhoea and antibiotic treatments post-weaning compared to untreated pigs.

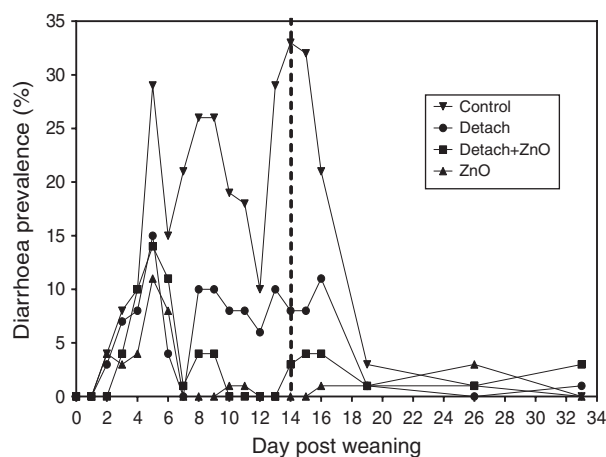


Fig. 1. Prevalence of diarrhoea in piglets treated with a single dose of Detach[®] at weaning, compared with ZnO added in feed for 2 weeks and nil treatment (control). Dashed line indicates d 14 when ZnO was included in the feed of all groups.

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Effects of microencapsulated organic acids and essential oils supplementation on performance and rectal temperature in challenged weaning pigs

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Lipopolysaccharide (LPS) is a complex of lipids and sugars and is an essential component of the cell walls of gram negative bacteria (e.g. *Escherichia coli* and *Salmonella spp.*). Injection of LPS induces pathologic phenomena, which can lead to immune and inflammatory responses (Wu *et al.* 2015). Addition of organic acids in feed can improve the growth of piglets (Costa *et al.* 2013), and essential oils are considered anti-inflammatory (Yoon *et al.* 2010). The objective of the study is to determine the effect of microencapsulated blends of organic acids and essential oils (MOE) supplementation on rectal temperature and growth in weaning pigs subjected to a LPS challenge.

A total of 20 weaning (21 days) pigs ((Landrace × Yorkshire) × Duroc) with an initial bodyweight of 4.43 ± 0.6 kg (mean \pm s.d.) were randomly allocated to four treatments with five replicates per treatment per pen for a 28 days experimental period. The average temperature of the pig accommodation area was $30 \pm 1^\circ\text{C}$. Treatments were: (1) basal diets + 0.2% MOE+ LPS injection; (2) basal diets + 0.2% MOE + saline injection; (3) basal diets + LPS injection; and (4) basal diets + saline injection. The basal diet contained 14.9 MJ/kg digestible energy and 1.65% lysine. On d 28, LPS (SIGMA, from *Escherichia coli* O111:B4, L2630) was injected at 100 $\mu\text{g}/\text{kg} \times \text{bodyweight}$. Injections were injected intramuscularly into the thigh, and rectal temperature of the pigs was recorded after injection of LPS or saline 0, 4, 8, 12 and 16 h. All data were analysed by ANOVA using the General Linear Models (GLM) procedure of SAS (v9.2, SAS Institute Inc., Cary, NC, USA). Results are presented in Table 1.

Supplement with 2% MOE significantly improved the final bodyweight, average daily gain (ADG) and average daily feed intake (ADFI) ($P < 0.05$). However, feed efficiency (G:F) was not significantly different ($P > 0.05$). At 4, 8, 12, and 16 h after challenge, LPS (+MOE+LPS, –MOE+LPS) gave significantly higher rectal temperature than the saline treatment ($P < 0.01$). In conclusion, LPS injection can increase rectal temperature and MOE supplementation increased growth performance in weaning pigs, however, MOE could not inhibit the LPS-induced hyperthermia.

Table 1. Effects of microencapsulated blends of organic acids and essential oils on growth performance and rectal temperature in weaning pigs challenged by LPS

	+MOE	−MOE	s.e.m. ^A	P-value		
Initial BW (kg)	4.41	4.45	0.13	0.816		
Final BW (kg)	7.40	8.47	0.31	0.023		
ADG (g)	0.21	0.29	0.02	0.035		
ADFI (g)	0.27	0.32	0.01	0.0001		
G: F	0.79	0.89	0.08	0.358		
	+MOE	−MOE	s.e.m. ^A	P-value		
	+LPS	−LPS	+LPS	−LPS	MOE	LPS
0 h	39.8	39.4	39.5	40.1	0.144	0.891
4 h	40.5	39.4	40.3	39.2	0.177	0.300
8 h	40.6	39.4	40.4	39.1	0.158	0.171
12 h	40.3	39.2	40.3	39.3	0.134	0.647
16 h	40.4	39.3	40.1	38.9	0.225	0.110

^As.e.m., standard error of the mean.

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Effect of probiotic and xylanase on growth performance, nutrient digestibility, blood profiles, and faecal microflora in growing pigs

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There is evidence that probiotics, also referred to as direct-fed microbials (DFM), can improve gut microbial balance and intestinal health (Dersjant-Li *et al.* 2015). In addition, an interaction between multi-enzymes and DFM has been reported (Romero *et al.* 2013). The hypothesis of this study was that there might be a beneficial interaction when a probiotic and xylanase are fed to growing pigs.

A total of 120 growing pigs (Yorkshire × Landrace) × Duroc with an average bodyweight (BW) of 25.22 ± 1.88 kg were randomly allotted to four experimental diets based on initial BW and sex (six replicate pens per treatment; two gilts and three barrows/pen). The experiment lasted for 6 weeks and dietary treatments included: CON, basal diet, CON + 0.002% *Enterococcus faecium* (EF1), and CON + 0.005% *E. faecium* (EF2) and CON + 0.002% *E. faecium* + 0.01% *Endo-1,4-β-xylanase* (9000 U/g) (EX). Feed intake and BW were recorded initially at week 3 and 6 of the experimental period to calculate average daily gain (ADG), feed intake (ADFI), and gain to feed ration (G : F). Chromium oxide was added to the diet as an indigestible marker at 0.20% of the diet for 7 days before faecal collection at d 42, when samples were collected by rectal massage from at least two pigs per treatment to determine the digestibility (ATTD) of dry matter (DM), nitrogen (N) and energy. At week 6, blood samples (3 mL) were collected (four pigs/treatment) into non-heparinised tubes to obtain serum to determine the total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL), creatinine concentrations, blood urea nitrogen (BUN), and glucose concentrations. Faecal samples were collected directly via massaging the rectum of two pigs (one gilt and one barrow) in each pen and then pooled and placed on ice for transportation to the laboratory where analysis was immediately performed to determine faecal *E. coli* and *Lactobacillus* counts. Data were analysed in accordance as a completely randomised design using the GLM procedure (SAS v9, SAS Institute Inc., Cary, NC, USA). Pen was used as the experimental unit. Differences among the treatment means were determined by using the Tukey's test with $P < 0.05$ indicating significance.

During the whole period, pigs fed the EX diet had increased ADG compared to pigs fed the CON and EF1 diets (718 g v. 643 and 681 g, respectively, $P < 0.05$). The ADG of pigs fed the EF2 diet increased compared to pigs fed the CON diet (703 g v. 643 g, respectively, $P < 0.05$). The G : F of pigs fed the EF2 and EX diets increased compared to pigs fed the CON and EF1 diets (0.431 and 0.436 v. 0.389 and 0.407, respectively, $P < 0.05$). At week 6, pigs fed the EF2 and EX diets had better G : F compared to pigs fed the CON diet (75.5 and 75.6% v. 73.2%, respectively, $P < 0.05$). Pigs fed the EF2 diet had decreased creatinine concentrations in the blood compared to pigs fed the CON diet (0.95 mg/dL v. 1.25 mg/dL, respectively, $P < 0.05$) at the sixth week. Pigs fed the EF2 and EX diets had higher faecal *Lactobacillus spp.* concentrations compared to the CON diet (7.55 and 7.61 v. 7.42 log₁₀ CFU/g, respectively, $P < 0.05$). In addition, pigs fed the EX diet had increased *Lactobacillus spp.* counts compared to pigs fed the EF1 diet (7.61 v. 7.48 log₁₀ CFU/g, respectively, $P < 0.05$). Pigs fed the EX diet had decreased *E. coli* counts compared to pig fed the CON diet (6.23 v. 6.46 log₁₀ CFU/g, respectively, $P < 0.05$) at week 6. However, no significant difference was observed for ATTD of DM and N, Glucose, BUN, HDL, LDL of blood at week 6, or ADFI during the overall study among treatments ($P < 0.05$).

In conclusion, supplementation of growing pig diets with a combination of *E. faecium* and endo-1,4-β-xylanase is capable of improving growth performance, nutrient digestibility, increasing faecal *Lactobacillus*, reducing faecal *E. coli* counts and decreasing creatinine concentrations in the blood in growing pigs compared to pigs fed CON, and probiotic only supplementation. The data suggested that the combination of *E. faecium* and endo-1,4-β-xylanase could offer more benefits than when used alone.

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***In vitro* antimicrobial activity of essential oils against enterotoxigenic *Escherichia coli* found in a nation-wide commercial farm survey**

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The antibacterial activity of some natural essential oils (EO) have the potential to reduce reliance on antibiotics to prevent post-weaning diarrhoea (PWD) caused by enterotoxigenic *Escherichia coli* (ETEC). However, a systematic screening of EO against the most common ETEC strains in Australia has never been established and is the main objective of the current research. We hypothesised that the sensitivity to the EO (alone or combined) by the selected ETEC strains prevalent in Australian piggeries was serotype-dependent or pathotype-dependent.

Ninety-one different *E. coli* isolates were collected from Victoria, New South Wales, Queensland, Western Australia and Southern Australia pig nurseries and characterised by the principal ETEC virulence genes (LT, Sta, Stb, stx₁, stx₂, eaeA, ehxA, F4, F5, F6 and F18) using multiplex PCR. An initial screening of 18 EO using a broth microdilution method against the two isolates with the most common ETEC serotypes (O:141ab and O:149) was done to find the minimum inhibitory concentrations (MIC). The EO with a MIC ≤0.1% for non-natives and ≤0.65% for native EO were selected and subjected to a checkerboard method to study potential synergies between EO combinations. A fractional inhibitory concentration index (FICI; defined as (MIC_{EO1} in the combination/MIC_{EO1}) + (MIC_{EO2} in the combination/MIC_{EO2}) of ≤0.5 was set as an indicator of synergistic activity in the EO combinations tested. Finally, the 91 isolates were tested against the most successful combinations of oils (Do *et al.* 2015).

Our results showed that the most common virulence gene was Stb present in 42 isolates followed by LT (33) and Sta (31). Twelve of the *E. coli* strains isolates had no ETEC virulence genes. Oregano, clove, thyme and cinnamon for non-natives and lemon myrtle, lemon ironbark, peppermint gum and nerolina for natives presented average MIC of 0.02%, 0.08%, 0.08%, 0.07% and 0.1%, 0.4%, 0.55%, 0.65%, respectively. The synergies between different EO combinations are shown in Table 1. Based on the MIC we observed resistance, low, medium, high, and very high sensitivities for 38.3%, 28.3%, 13.3%, 6.6% and 13.3% respectively. No relationship between sensitivity with serotypes and pathotypes was found.

In conclusion, our results confirmed that the virulence gene profile of ETEC found in Australian pig nurseries is similar to previous publications from elsewhere. Different ETEC serotypes showed different sensitivities to EO (Gutierrez *et al.* 2008). The non-native EO presented a lower MIC compared with native EO; however, the native EO had a better synergistic potential hypothetically due to a differential mode of action. Additional studies are warranted.

Table 1. Synergistic antimicrobial activity of essential oils in combinations against *E. coli* serotypes O:141 and O:149

EO mix	Concentration (%)	Serotype	FIC
Oregano/clove	0.005/0.005	O:141	0.32
Thyme/nerolina	0.02/0.04	O:141	0.33
Clove/nerolina	0.02/0.04	O:141	0.33
Cinnamon/lemon ironbark	0.017/0.025	O:141	0.42
Lemon ironbark/nerolina	0.05/0.04	O:141	0.23
Peppermint gum/nerolina	0.068/0.081	O:141	0.25
Cinnamon/nerolina	0.0175/0.081	O:149	0.37
Peppermint gum/nerolina	0.137/0.081	O:149	0.37
Thyme/nerolina/ peppermint gum	0.0025/0.02/0.068	O:141	0.18
Clove/lemon ironbark/nerolina	0.005/0.025/0.08	O:141	0.27
Thyme/nerolina/peppermint gum	0.0025/0.04/0.068	O:149	0.21
Clove/lemon ironbark/nerolina	0.0025/0.05/0.08	O:149	0.27

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Cereal and protein sources fed to pigs after weaning influence faecal shedding of β -haemolytic *Escherichia coli*

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Feeding different cereal and (or) protein sources after weaning influences production, the structure and function of the gastrointestinal tract, and the incidence of post-weaning colibacillosis (PWC; Heo *et al.* 2013). Several studies show that white rice can replace other cereals in nursery diets, and with animal protein sources, can reduce the incidence of PWC (Montagne *et al.* 2004). However, an imbalance between amounts and (or) types of carbohydrates and proteins entering the large intestine in pigs fed rice-based diets may induce PWC (Pluske *et al.* 2007). This experiment examined whether feeding a different cereal base (rice or wheat), in combination with sources of animal proteins or vegetable proteins, would influence the excretion of β -haemolytic *Escherichia coli* and antibiotic treatments given to pigs after weaning.

A total of 84 newly weaned pigs (Large White x Landrace), aged 24 days and weighing 6.7 ± 0.13 kg (mean \pm s.e.m.), were used in a 3×2 factorial arrangement of treatments with the respective factors being (1) three cereal types (extruded medium-grain rice, extruded long-grain rice, or hammer-milled wheat); and (2) two dietary protein sources (animal v. vegetable). Diets were formulated to contain adequate levels of energy and nutrients for pigs of this genotype and age, and had a crude protein content of 200 g/kg. Pigs were offered their respective diets in groups of four for the first 7 days after weaning, and for the final 2 weeks were housed individually (0.42 m²/pig). Each pen was equipped with a nipple water drinker and a stainless steel feed trough. Pigs were swabbed for the presence or absence of β -haemolytic *E. coli* upon arrival and then on d 2, 5, 6 and 8 after weaning, and the number of pigs injected with antibiotics for clinical PWC (as assessed by the stockperson) was recorded. Treatment effects were assessed by two-way ANOVA for a factorial design using StatView 5.0 for Windows (AddSoft Pty Ltd, Australia). The results are shown in Table 1.

The number of antibiotic treatments given for clinical PWC after weaning was similar for all treatments ($P > 0.05$). Feeding vegetable proteins showed a strong tendency to reduce ($P = 0.057$) faecal shedding of *E. coli* after weaning compared to pigs fed animal protein sources. This difference was caused predominately by the greater swab score in pigs fed diets with long-grain rice plus animal protein diet ($P = 0.069$). This suggests feeding this form of rice, which has the most amylose and least amylopectin, in the presence of vegetable proteins reduced ETEC colonisation and subsequent shedding of β -haemolytic *E. coli*.

Table 1. Interaction means for antibiotic treatments and faecal swab score recorded in pigs after weaning

Cereal source	Protein source	Antibiotic treatments (per pig)	Faecal swab score ^A
Medium-grain rice	Animal	1.4	0.5
	Vegetable	0.7	0.7
Long-grain rice	Animal	1.0	0.6
	Vegetable	0.6	0.3
Wheat	Animal	1.0	0.8
	Vegetable	1.0	0.8
Standard error of mean		0.07	0.03
		<i>Probability</i>	
Cereal source		0.792	0.461
Protein source		0.230	0.057
Cereal * Protein		0.638	0.069

^AScored from 0 to 5: 0 = no growth of β -haemolytic *E. coli*; 5 = heavy colonisation of β -haemolytic *E. coli*.

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A potential new species of porcine *Actinobacillus* defined by multi-locus sequence analysis

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In recent years the submission of *Actinobacillus*-like strains sourced from the respiratory tract of pigs to our laboratory has increased. Our usual diagnostic method for identification of non-routine organisms is 16S rDNA sequencing. However, this method does not have the discriminatory power to separate very closely related species in the *Actinobacillus* complex. This has prompted a project to improve the identification of *Actinobacillus*-like strains by using multi-locus sequencing analysis (MLSA). The *recN*, *rpoA* and *thdF* housekeeping genes were chosen for this analysis due to their proven ability to predict whole-genome DNA-DNA similarity.

For a total of 36 field isolates, identified by the 16S rDNA sequencing as unusual species – *A. porcitosillarum*, *A. minor*, *A. porcitosillarum/minor* complex, *A. porcinus*, *A. indolicus* and *A. rossi* – three genes, *recN*, *rpoA* and *thdF*, were sequenced and aligned to publicly available data of type strains within the genus *Actinobacillus* to make multiple alignments of DNA sequences constructed by Clustal in Geneious version 8.0.5. The genome similarity index was calculated according to Kuhnert and Korczak (2006). Two species specific PCR for *Haemophilus parasuis* were used to exclude this species from the study.

None of the strains were positive in the species specific *H. parasuis* PCR. A potential new species, consisting of 17 isolates, was identified with a genome similarity index of 0.56 to the closest related type strain – *A. indolicus* (similarity index > 0.4 and > 0.9 indicating the same genus and species, respectively; Table 1). The type strains of *H. parasuis* and *A. indolicus* formed a group with nine isolates. A further seven isolates did not fit into a group due to lack of congruence of the *thdF* gene phylogeny with *recN* and *rpoA* and their identity remains uncertain. The analysis also pointed to the inadequacy of the 16S rDNA identification as none of the strains identified as *A. porcitosillarum/minor* were confirmed as such. It also highlighted that no single gene by itself can be used for identification.

Further work is in progress to look at the pathology associated with the 17 strains identified as a potential new species, to determine the significance of these organisms for the pork industry.

Table 1. Sequence of isolates and type strains were aligned with CLUSTAL. Values of greater than 96% belonged to the same species, values between 84 to 96% were uncertain and values below 84% had no similarity

No. of isolates	16S identification	<i>recN</i>	%	<i>rpoA</i>	%	<i>thdF</i>	%	Monophyletic group with
8	<i>A. indolicus</i> / <i>H. parasuis/A. rossi</i>	<i>H. parasuis</i> / <i>A. indolicus</i>	>96.05	<i>A. indolicus</i> / <i>H. parasuis</i>	>97.44	Uncertain	>92.20	<i>H. parasuis</i> / <i>A. indolicus</i>
1	<i>A. porcitosillarum</i> / <i>minor</i>	Uncertain	84.96	<i>A. indolicus</i> / <i>H. parasuis</i>	98.57/97.70	Uncertain	94.44	
3	<i>A. minor</i>	<i>A. minor</i>	100	<i>A. minor</i>	99.62	Uncertain	94.64	None
4	<i>A. porcitosillarum</i> / <i>minor</i>	No similarity	<84	no similarity/ <i>minor</i>	<84	<i>A. minor</i> / uncertainty	<97.92	
2	<i>A. porcinus</i>	<i>A. porcinus</i>	<99.2	<i>A. porcinus</i>	99.87	<i>A. porcinus</i>	<99.71	<i>A. porcinus</i>
1	<i>A. porcinus</i>	Uncertain	84.96	<i>A. porcinus</i>	99.87	Uncertain	87.29	
7	<i>A. indolicus/H. parasuis</i>	No similarity	<84	<i>A. indolicus/H. parasuis</i>	97.18/<97.50	Uncertain	85.22	New species
10	<i>A. porcitosillarum</i> / <i>minor</i>	No similarity	<84	<i>A. indolicus/H. parasuis</i>	97.18/97.44	Uncertain	>85.14	

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Effects of a multi-strain probiotic on growth performance and faecal microflora in weaner pigs

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Weaning stress leads to pigs being susceptible to gastrointestinal disorders and digestive disturbances, thus resulting in a high incidence of diarrhoea and depressed growth rate (Lallès *et al.* 2007). It has been suggested that probiotics could promote intestinal health and alleviate weaning stress in young pigs (Lan *et al.* 2016). Therefore, the objective of the present study was to evaluate the effect of a multi-strain probiotic (*Bacillus subtilis* 1.0×10^9 cfu/g and *Bacillus licheniformis* 1.0×10^9 cfu/g) on growth performance and faecal microflora in weaner pigs.

A total of 120 weaner pigs ((Landrace \times Yorkshire) \times Duroc) with an average bodyweight (BW) of 7.84 ± 1.75 kg (28 days of age) were randomly allocated to four experimental diets: (1) CON, basal diet; (2) CON + 0.1% multi-strain probiotics; (3) CON + 0.2% multi-strain probiotics; and (4) CON + 0.3% multi-strain probiotics. All data were statistically analysed using the GLM procedure of the SAS v9 (SAS Institute Inc., Cary, NC, USA). Orthogonal comparisons were conducted using polynomial regression to determine linear, quadratic and cubic effects. Feed consumption was recorded on a pen basis during the experiment, and individual pig BW was recorded at initial, d 7, 14 and 42 of the experimental period to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain : feed (G : F). *Lactobacillus*, *E. coli* were determined by serial dilution (10^{-1} to 10^{-7}) on selective media. The results are shown in Table 1.

In this study, during d 1 to 7, dietary supplementation of three concentrations of a multi-strain probiotic increased (linear effect, $P < 0.05$) the G : F. During d 14 to 42 and d 1 to 42, increasing the level of the dietary multi-strain probiotic improved (linear effect, $P < 0.05$) the ADG and G : F. At the end of experiment, dietary supplementation with the multi-strain probiotic decreased (linear effect, $P < 0.05$) the faecal *E. coli* counts.

In conclusion, dietary supplementation of a multi-strain probiotic (*B. subtilis* and *B. licheniformis*) exerted a positive influence on growth rate and faecal microflora in weaner pigs.

Table 1. Effect of probiotics on growth and faecal microflora in weaner pigs

Items	CON ^A	TRT1 ^B	TRT2 ^C	TRT3 ^D	Linear	<i>P</i> -value Quadratic	Cubic	SEM ^E
d 1 to 7								
G : F	0.813 ^b	0.826 ^{ab}	0.852 ^{ab}	0.860 ^a	0.0118	0.8630	0.6096	0.013
d 14 to 42								
ADG (g)	497 ^b	515 ^{ab}	525 ^{ab}	544 ^a	0.0147	0.9895	0.7547	12
G : F	0.601 ^b	0.627 ^{ab}	0.639 ^a	0.640 ^a	0.0250	0.2989	0.9447	0.012
d 1 to 42								
ADG (g)	417 ^b	434 ^{ab}	441 ^{ab}	453 ^a	0.0141	0.7490	0.7147	9
G : F	0.653 ^b	0.672 ^{ab}	0.688 ^a	0.689 ^a	0.0028	0.2983	0.7395	0.008
<i>E. coli</i> log ₁₀ cfu/g	5.83 ^a	5.78 ^b	5.75 ^b	5.67 ^b	0.0135	0.5337	0.1790	0.02

^ACON, basal diets. ^BTRT1, CON + 0.1% probiotics. ^CTRT2, CON + 0.2% probiotics. ^DTRT3, CON + 0.3% probiotics. ^ESEM, standard error of the mean.

^{a,b}Means with the same superscript are not statistically different.

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Does dietary supplementation of gamma-aminobutyric acid improve performance in weaner pigs experimentally infected with *Escherichia coli* and given adrenocorticotrophic hormone?

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Gamma-aminobutyric acid (GABA) is a non-protein amino acid and a major inhibitory neurotransmitter in the central nervous system. Previous research completed on rats and humans has shown that GABA stimulates voluntary feed intake and regulates stress hormone secretion in humans (Kruk and Pycocock 1983). This neurotransmitter also plays important roles in motor function, emotions, appetite regulation and nutrient utilisation efficiency (Lelevich *et al.* 2009; Zhang *et al.* 2012). The aim of this study was to use both an enterotoxigenic *Escherichia coli* (ETEC) infection model and an adrenocorticotrophic hormone (ACTH) injection to simulate a pathogenic and endocrine stressful weaning event. We hypothesised that an increased supplementation of GABA in diets of weaned pigs will improve growth performance and reduce cortisol production.

A total of 96 newly weaned male pigs (Large White × Landrace) with the Mucin 4+ allele were stratified into eight treatments based on weaning weight, sow parity and location in the building (eight treatments × 12 pigs = 96 pigs). The study was designed as a 2 × 4 factorial arrangement with respective factors being (1) without/with ETEC infection plus ACTH and (2) four dietary GABA (Sigma-Aldrich; MO, USA) levels (0, 60, 80, 100 mg/kg). All pigs were fed the same base diet (20% protein, 5% fat, 1.2% lysine, 5% crude fibre, 0.85% calcium and 0.4% salt). On d 8 and 9 after weaning, all piglets were orally inoculated with ETEC (0.8 mL via two gelatinised capsules; serotype O149:K88) as well as being given 5 IU ACTH (Synacthen; Juno Pharmaceuticals, Vic., Australia) intramuscularly (IM), which occurred an hour beforehand. Pigs in the non-infected, non-ACTH group were given IM 0.2 mL of sterile saline and sham infected with two gelatinised capsules of phosphate-buffered saline. Faecal consistency score, diarrhoea index, and the number of therapeutic antibiotic treatments were recorded. Faecal β-haemolytic *E. coli* shedding was measured on d 0, 7, 10, 11 and 14 after weaning. Blood samples were collected on d 6, 9 (1 h after infection) and 14 from eight pigs per treatment. Plasma cortisol was assessed using ELISA from Enzo Life Sciences (Cat. No. ADI-900-071, NY, USA). Data were analysed by two-way analysis of variance using SPSS (v21.0, IBM, Armonk, NY, USA).

There were no differences ($P > 0.05$) between the four GABA diets for ADG, ADFI or FCR over the 21 days duration of the study (Table 1). Pigs in the non-infected, non-ACTH group gained 35 g/d more than pigs in the ETEC infection plus ACTH group during week 3 ($P = 0.098$; data not shown). In total, 73.2% of pigs in the infection plus ACTH group developed diarrhoea between d 8 to 14 compared to 26.8% in the non-infected, non-ACTH group of pigs ($P = 0.001$). These data indicate that eliciting both an ETEC infection challenge and an acute stress response after weaning caused no overall detrimental effects on production or diarrhoea; however, further investigation will establish blood measurements more indicative of the stress response during this period.

Table 1. Effects of dietary treatments, ETEC+ACTH infection or sham-infection on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) from d 0 to 21 after weaning

Item	GABA (mg/kg) (D)				Treatment (T)		s.e.m.	D	P-value	
	0	60	80	100	Sham	Infection			T	D × T
ADG (g)	186	191	187	168	189	176	13.1	0.63	0.33	0.95
ADFI (g)	290	295	279	260	291	272	17.4	0.50	0.26	0.95
FCR (g:g)	1.62	1.58	1.53	1.60	1.57	1.59	0.02	0.76	0.72	0.67

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