IMPROVING PIGLET PERFORMANCE THROUGH INCREASED POLYAMINE LEVELS IN SOW MILK

CONFIDENTIAL

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Co-operative Research Centre for an Internationally Competitive Pork Industry

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Executive Summary

Piglet growth during the immediate post-weaning period is often compromised as a result of their failure to adapt to the changing composition of their diet, specifically the switch from a milk dominated to a cereal dominated diet. Commonly referred to as post-weaning growth check, this period of adaptation and resultant decline in growth exerts a significant impact on overall herd feed conversion efficiency, impacting on the time taken to attain market weights, and in severe cases resulting in piglet mortality. Structural and functional changes in the small intestine that cause a decrease in digestive and absorptive capacity in the weaned pig are key contributors to the growth check in weaned piglets. Under natural conditions, certain strategies are present in both the lactating mother and her suckling offspring that are designed to assist with the challenges presented to the young animal at the time of weaning. During the suckling period and weaning process the gastrointestinal tract of the young animal matures and undergoes substantial changes in order to cope with the shift in diet experienced at weaning. The triggers for many of these changes are delivered to the young via the milk. Polyamines (putrescine, spermidine, and spermine) are components of colostrum and milk, and play an important role in regulating cell growth and proliferation, the stabilisation of DNA, RNA transcription, protein synthesis, apoptosis and the regulation of immune responses. In particular, milk-borne polyamines play an important role in neonatal gut maturation, increasing in concentration in both milk and intestinal mucosa of suckling offspring during weaning.

The objective of the two studies conducted as part of this project were to determine the effect of orally dosing suckling piglets with polyamines on small intestinal structure and function, as well as rate of liveweight gain. In study one, piglets suckling either parity one or parity three sows received an oral dose of either spermine (20% of estimated daily intake via sows milk) or water every second day from day 14 to 24 of lactation (n = 60 piglets per treatment). In study two, piglets suckling either parity one or parity three sows received an oral dose of either spermine (20% supplement level), spermidine (20% supplement level) or water every second day from day 14 to 24 of lactation (n = 54 piglets / treatment). In both studies, piglet liveweight (LW) was recorded on days 3, 14 and 24 post-partum, as well as day 61 post-partum (study two only). In study one only, a subset of 6 piglets per treatment were sacrificed at 24 days of age, and intestinal samples collected to determine treatment effects on intestinal structures and disaccharidase activity.

Taken together, our date demonstrate that polyamine supplementation (either spermine or spermidine) prior to weaning can significantly increase piglet liveweight gain pre-weaning. Further, the data obtained from study two suggest a greater response to polyamine supplementation in piglets suckling parity one sows. It is also evident that spermine induced increases in piglet liveweight gain are accompanied by increased villous height and decreased crypt depth in the duodenum and jejunum, structural changes which effectively increase the surface area available for nutrient absorption. Such structural changes may reduce the severity of post-weaning villous atrophy and crypt-cell hyperplasia, essentially reducing the extent to which post-weaning growth is impaired.

In conclusion, the findings of this study are extremely promising and have identified a strategy to potentially improve piglet growth post-weaning, or at least reduce the severity of post-weaning growth disorders. However, in order to maximise the efficacy of this strategy, and thus the commercial viability, further research is required in the following areas: determination of whether polyamine supplementation during the peri- or post-weaning period alters intestinal development and improves piglet growth; development of more commercially relevant methods of delivering additional polyamines to piglets, for example in feed, water, or by increasing levels in sows milk.

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1. Introduction

Piglet growth during the immediate post-weaning period is often compromised as a result of their failure to adapt to the changing composition of their diet, specifically the switch from a milk dominated to a cereal dominated diet. Commonly referred to as post-weaning growth check, this period of adaptation and resultant decline in growth exerts a significant impact on overall herd feed conversion efficiency, impacting on the time taken to attain market weights, and in severe cases resulting in piglet mortality.

Early-weaned piglets are at a disadvantage because their digestive systems are not adequately developed to handle the digestion and absorption of a typical weaning diet based on grains and vegetable proteins. The gastrointestinal tract and the digestive enzymes that break down sugars, starch and protein take time to mature and can be extremely variable between piglets. As a result, it is common for piglets at this stage to experience a growth lag. This 'post-wean growth check' results in growth performance of the weaned piglet that is significantly below genetic potential. A significant amount of research has investigated the various factors responsible for this drop in performance and interventions that alleviate the post-weaning piglet growth gap (reviewed by Pluske et al., 1997); however, it continues to be a major problem in just about every type of pig production system in the Asia Pacific region.

Structural and functional changes in the small intestine that cause a decrease in digestive and absorptive capacity in the weaned pig are key contributors to the growth check in weaned piglets. Mediators of intestinal structure and function post-weaning have been widely reviewed and have been shown to reduce the impact of the post-wean growth gap (Pluske et al., 1997). However, little attention has been given to modulators of intestinal structure and function pre-weaning. Under natural conditions, certain strategies are present in both the lactating mother and her suckling offspring that are designed to assist with the challenges presented to the young animal at the time of weaning. During the suckling period and weaning process the gastrointestinal tract of the young animal matures and undergoes substantial changes in order to cope with the shift in diet experienced at weaning. The triggers for many of these changes are delivered to the young via the milk.

Polyamines (putrescine, spermidine, and spermine) are components of colostrum and milk, and play an important role in regulating cell growth and proliferation, the stabilisation of DNA, RNA transcription, protein synthesis, apoptosis and the regulation of immune responses. In particular, milk-borne polyamines play an important role in neonatal gut maturation, increasing in concentration in both milk and intestinal mucosa of suckling offspring during weaning (Luk et al., 1988; Luk 1990).

Maturation of the intestine is known to occur in the first few postnatal weeks in many vertebrates. Natural weaning by a sow can take place at anywhere up to 6 months of age and the spermidine/spermine peak in sows milk doesn't occur until approximately day 49 of lactation (Kelly et al.,1991). The function of milk-borne polyamines is to trigger a series of changes in the gastrointestinal tract of the suckling offspring that help it adapt to the progressive transition from a milk dietary regime to a solid dietary regime. Early weaning programs designed to increase sow productivity have driven the average weaning age below 21 days. Weaning piglets at 21 days, or earlier, may mean that they have insufficiently

matured gastrointestinal tracts, which may result in poor adaptation to post-weaning diets and therefore reduced post-weaning performance.

Studies in rats demonstrate that oral administration of spermidine and spermine, at appropriate doses, induces all the modifications in the digestive tract that occur at weaning, namely, (a) in the intestine: variations in the specific activities of disaccharidases and peptidases, gene expression, the level of receptors to polymeric immunoglobulins (RPIs), tissue histology, and the intestinal permeability to macromolecules; (b) maturation of the intestinal immunological system; (c) an increase in the specific activity of enzymes contained in the pancreas; (d) a change in the growth rate and biochemical properties of the liver. Thus increasing administering polyamines to piglets earlier in a sow's lactation (ie, creating a peak prior to day 21) may accelerate the maturation of the piglet gut making it better adapted to early-weaning.

The objective of the current project was to manipulate maturation of the piglet gut during the suckling period with the aim of improving pre-weaning survivability and dietary adaptation, leading to a subsequent increase in post-weaning performance. This project tested the hypothesis that administration of polyamines to piglets from 14 days of age until weaning would promote maturation of the gastrointestinal tract and increase piglet growth pre- and post- weaning.

2. Methodology

Two distinct experiments were undertaken. Experiment one was conducted as an honours project for Pork CRC funded student Tahlia Sobko. Experiment two was conducted in part by two summer scholarship students, with the stipend of Luisa Panetta kindly provided by the Pork CRC.

Experiment One: Effect of oral spermine supplementation pre-weaning on piglet growth and intestinal development

This experiment was conducted at the University of Adelaide's piggery at Roseworthy, South Australia. The experimental design was a 2 x 2 factorial, incorporating two maternal parities (parity 1 versus parity 3 sows) and two levels of polyamine supplementation (0 versus 925.3 nmol/ml spermine supplementation). Within 24 hours of parturition, litter size was standardised to, and maintained throughout lactation at, 10 piglets. Following standardisation, piglets were weighed. Within each litter, piglets were ranked according to liveweight (LW) and pair matched according to liveweight, with one piglet from each pair allocated to receive either 2 ml of water (**W**) or 2 ml of 462.7 nmol/ml spermine solution (**S**). The dose of spermine provided was calculated to provide 20% more spermine than normally received in sows milk, this figure was based on an average intake of 950 ml milk per day, and sows milk containing 4.87 nmol/ml (Sabater-Molina et al., 2009). Twelve parity one (**Par1**) and 12 parity three (**Par3**) sows, and their litters were used. The four experimental treatments were as follows (n = 60 piglets / treatment); **SPar1**, piglets suckling parity one sows and receiving an oral dose of spermine solution; **SPar3**, piglets suckling parity three sows and receiving an oral dose of spermine solution; **WPar1**, piglets suckling parity one sows and receiving an oral dose of water; **WPar3**, piglets suckling parity three sows and receiving an oral dose of water.

Individual piglet LW was recorded on day 3, 14 and 24 post-parturition, with weaning occurring on day 24. Doses of spermine and water were delivered using an oral drench gun, with piglets drenched every second day from day 14 to 22 post-partum. A sub-set of 6 piglets / treatment were sacrificed on day 24 post-parturition, with gastrointestinal samples collected to measure villus height and crypt depth, as well as sucrase and maltase activity. Milk samples were collected from sows on days 1, 3, 14 and 24 post-parturition, and analysed for protein, fat, lactose, non-fat solids and somatic cell count.

Treatment effects on all measures were determined using a general analysis of variance, with piglet LW on day 1 and 14 included in the model as covariates The effects of maternal parity on milk composition were determined using a general analysis of variance. All statistical analysis was conducted using Genstat, 10th edition (Committee of the Statistics Department, Rothamsted Experimental Station, Herpenden).

Experiment Two: Effect of oral spermine or spermidine supplementation on piglet growth pre-and post-weaning (a commercial study).

This experiment was conducted on a 7,500 sow facility, situated approximately 70 km north of Adelaide, South Australia. The experimental design was a 2 x 3 factorial, incorporating two maternal parities (parity 1 versus parity 3 sows) and three polyamine supplements (0 versus 925.3 nmol/ml spermine versus 4026.1 nmol/ml spermidine supplementation). Within 24 hours of parturition, litter size was standardised to 10 piglets. Following standardisation, piglets were weighed. Within each litter, piglets were ranked according to liveweight (LW) with piglets allocated based on liveweight to receive either 2 ml of water (**W**), 2 ml of 462.7 nmol/ml or spermine solution (**S**) or 2 ml of 2013 nmol/ml of spermidine (**SP**D) solution. Only nine piglets per litter were included in the experiment, with the additional piglet receiving no treatment at all. The dose of spermine and spermidine provided was calculated to provide a supplementation level of 20% above what would normally be received in sows milk, this figure was based on an average intake of 950 ml milk per day, and sows milk containing 4.87 nmol/ml of spermine and 21.2 nmol/ml of spermidine (Sabater-Molina et al., 2009). Eighteen parity one (**Par1**) and 18 parity three (**Par3**) sows, and their litters were used. The six experimental treatments were as follows (n = 54 piglets / treatment); **SPar1**, piglets suckling parity one sows and receiving an oral dose of spermine solution; **SPDPar1**, piglets suckling parity one sows and receiving an oral dose of spermidine solution; **SPar3**, piglets suckling parity three sows and receiving an oral dose of spermine solution; **SPDPar3**, piglets suckling parity three sows and receiving an oral dose of spermidine solution **WPar1**, piglets suckling parity one sows and receiving an oral dose of water; **WPar3**, piglets suckling parity three sows and receiving an oral dose of water.

Individual piglet LW was recorded on day 3, 14, 18, 24 and 61 post-parturition, with weaning occurring on day 24. Doses of spermine, spermidine and water were delivered using an oral drench gun, with piglets drenched every second day from day 14 to 22 post-partum. Sows were weighed and P2 backfat measured on days 1 and 24 post-partum, with milk samples collected on days 3, 14 and 24 post-parturition, and analysed for protein, fat, lactose, non-fat solids and somatic cell count. The concentrations of fat and protein in collected milk samples were measured by infrared spectroscopy using a Bentley 2500 Combi instrument (Bentley Instruments, Chaska, Minnesota, USA). A standardised milk solution was used prior to each assay run to confirm the calibration stability of the instrument readings.

Treatment effects on all measures were determined using a general analysis of variance, with piglet LW on day 1 included in the model as a covariate. In addition, a comparison between polyamine supplementation (spermine and spermidine treatment data combined) and no supplementation (water) was also conducted using a general analysis of variance model, with piglet LW on day 1 included in the model as a covariate. The effects of maternal parity on body composition as well as milk composition were determined using a general analysis of variance. All statistical analysis was conducted using Genstat, 10th edition (Committee of the Statistics Department, Rothamsted Experimental Station, Herpenden).

3. Outcomes

Experiment One: Effect of oral spermine supplementation pre-weaning on piglet growth and intestinal development

Spermine supplemented piglets were significantly heavier on days 14 and 24 post-parturition (Figure 1). Prior to start of treatment (day 1 – 14), spermine piglets gained 190 more grams than water piglets (Table 1; $P < 0.05$). From day 14 to 24 of lacation, spermine supplementation increased LW gain by 320 g (Table 1; $P < 0.01$). Liveweight gain between days 14 and 24 was significantly higher ($P < 0.01$) for piglets suckling parity three compared parity one sows (Table 1). Lactose and Protein concentrations were significantly higher in milk collected from Sows compared to Gilts (Figure 2). Compared to piglets suckling parity 1 sows, piglets suckling parity three sows were significantly ($P < 0.01$) heavier on day 1 (1.58 ± 0.03 versus 1.44 ± 0.03 kg) and tended ($P = 0.09$) to be heavier on day 24 post-partum (7.18 ± 0.13 versus 6.85 ± 0.13 kg). Piglet LW gain was unaffected by maternal parity between days 1 and 14 of lactation (Table 1), but was significantly ($P < 0.05$) higher between days 14 and 24 of lactation for piglet suckling parity three compared to parity one sows (Table 1). There was a significant, positive, correlation between piglet weight on day 1 and day 14 post-partum ($P < 0.001$; $R^2 = 28.3$) and day 1 and day 24 post-partum ($P < 0.001$; $R^2 = 18.1$).

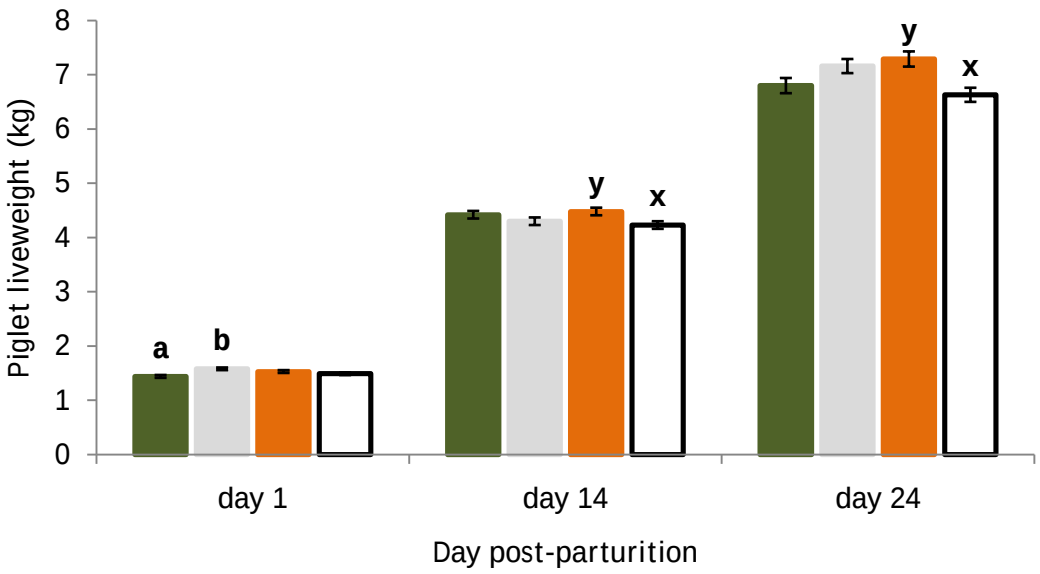

Figure 1 Liveweight of piglets suckling either parity 1 (■) or parity 3 (■) sows and orally dosed with either spermine (■) or water (□). [a,b] Means with different superscripts within each parity differ significantly (P < 0.05); [x,y] Means with different superscripts between treatment differ significantly (P < 0.05)

Table 1 Differences in liveweight gain (kg) for piglets suckling parity 1 or parity 3 sows, and piglets receiving an oral dose of spermine or water

	Piglet liveweight gain (kg)	
	Day 1 - 14	Day 14 - 24
Maternal parity		
Parity 1	2.85 ± 0.08	2.43 ± 0.08^{b}
Parity 3	2.89 ± 0.08	2.87 ± 0.08^{a}
Oral supplement		
Spermine	2.97 ± 0.08^{y}	2.81 ± 0.08^{b}
Water	2.76 ± 0.08^{x}	2.49 ± 0.08^{a}

Within main effects, means with different superscripts differ significantly; ^{ab} P<0.05;^{xy} P < 0.01

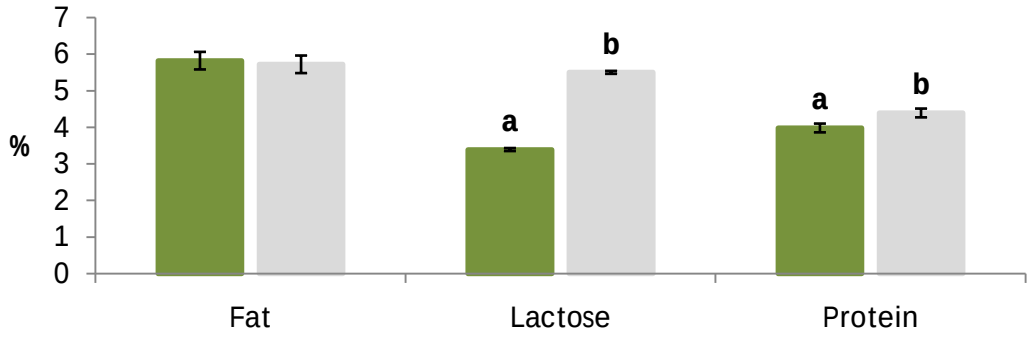

Figure 2 Concentration of fat, lactose and protein in milk samples collected from parity 1 (■) and parity 3 (■) sows. [ab] within compound, means with different superscripts differ significantly (P < 0.01).

Spermine supplementation significantly increased villi height and decreased crypt depth in the duodenum and jejunum (Table 2), resulting in a significantly lowered villus height to crypt depth ratio (Figure 4). There was a significant effect of parity (one versus three) on piglet villi height in the duodenum (399.6 ± 14.7 versus 480.1 ± 14.7) and jejunum (412.8 ± 16.7 versus 496.9 ± 16.7); villus height: crypt depth in the duodenum (1.7 ± 0.1 versus 2.23 ± 0.1) and jejunum (1.57 ± 0.1 versus 2.2 ± 0.1). However, crypt depth in the duodenum (428.0 ± 13.9 and 230.4 ± 13.9) and jejunum (274.2 ± 11.1 and 247.0 ± 11.1) was similar for piglet suckling parity 1 and parity 3 sows, respectively.

Table 2 Differences in villus morphology in the duodenum and jejunum of piglets suckling Parity One or Parity Three sows and supplemented with spermine solution or water

	Parity One sows		Parity Three sows	
	Spermine	Water	Spermine	Water
Duodenum				
Villi Height (μm)	487.1± 15.3^{ax}	312.1±19.3^{bx}	537.6±29.6^{ay}	422.6±15.5^{by}
Crypt Depth (μm)	232.1±8.5^{a}	264.0±23.8^{b}	194.7± 7.1^{a}	266.1±29.4^{b}
VH:CD$_1$	2.1±0.1^{ax}	1.2±0.1^{bx}	2.8±0.2^{ay}	1.7±0.2^{by}
Jejunum				
Villi Height (μm)	503.8±22.8^{ax}	321.7±11.7^{bx}	563.2±28.8^{ay}	430.7±27.2^{by}
Crypt Depth (μm)	244.7± 7.1^{a}	303.6±13.2^{b}	206.1±20.6^{a}	288.0±18.5^{b}
VH:CD$_1$	2.1±0.1^{ax}	1.1±0.1^{bx}	2.8±0.3^{ay}	1.5±0.2^{by}

[ab] Means with different superscripts within each parity differ significantly; *P*<0.001
[xy] Means with different superscripts between parities differ significantly; *P*<0.001.
[1] VH:CD; villus height to crypt depth ratio

Table 3 Effects of orally administering spermine on brush border disaccharidase activities in the duodenum and jejunum of 24 day old piglets suckling parity 1 or parity 3 sows.

	Specific activities of disaccharidase, (U/g protein)		
	Sucrase	Maltase	Lactase
Maternal Parity			
Parity 1	38.5[b]	231.6[b]	68.5
Parity 2	24.6[a]	94.6[a]	52.8
Oral supplement			
Spermine	34.2	192.3[b]	61.5
Water	28.8	134.0[a]	59.9
Intestinal Site			
Duodenum	38.2[b]	154.2	52.2*
Jejunum	24.9[a]	172.1	69.1*
Pooled SEM	*4.05*	*16.53*	*7.15*

Means with different superscripts within main effects differ; [ab] *P*<0.0f; *P = 0.098

Experiment Two: Effect of oral spermine or spermidine supplementation on piglet growth pre-and post-weaning (a commercial study).

Overall, piglets suckling parity three sows were significantly heavier on days 1, 14, 18, 24 and 61 post-partum, and gained significantly more LW between days 14 and 24 and day 24 and 61 post-partum compared to those suckling parity one sows (Table 4). However, piglet LW gain between days 1 and 14 post-partum was unaffected by maternal parity. Piglet LW on day 1, 14, 24 and 61 was unaffected by polyamine supplementation (1.58 ± 0.04; 4.32 ± 0.12; 6.67 ± 0.52; 19.91 ± 0.48 kg, respectively). However, there was a significant interaction between maternal parity and polyamine supplementation with regards to piglet LW on day 18 and piglet LWG between day 14 and 18. Specifically, piglets suckling parity one sows and receiving either spermine or spermidine tending (P=0.09) to be heavier on day 18 (5.25 ± 0.04 and 5.23 ± 0.04 versus 5.11 ± 0.04 kg), and grew significantly (P < 0.05) faster between 14 and 18 post-partum compared to unsupplemented piglets (0.24 ± 0.01 and 0.22 ± 0.01 versus 0.19 ± 0.01 kg / day). There was no effect of spermine or spermidine supplementation on the growth of piglets suckling parity 3 sows.

Table 4 Effect of maternal parity (three (Par3) versus one (Par1)) and oral piglet treatment (Water (W) versus Polyamine (Poly)) on piglet liveweight (LW).

		Piglet LW (kg)				
		D1	D14	D18	D24	D61
Sow parity	Par3	1.65[b]	4.35	5.32[d]	6.88[d]	20.57[d]
	Par1	1.51[a]	4.29	5.20[c]	6.46[c]	19.30[c]
Treatment	Water	1.58	4.29	5.22	6.64	19.85
	Polyamine	1.57	4.33	5.27	6.67	19.96
Interactions	WPar3	1.67	4.28	5.34[b]	6.92	20.43
	PolyPar3	1.64	4.39	5.31[b]	6.85	20.63
	Wpar1	1.50	4.31	5.11[a]	6.39	19.29
	PolyPar1	1.51	4.29	5.24[b]	6.50	19.31
Pooled SEM		*0.05*	*0.12*	*0.11*	*0.20*	*0.57*

[cd] within column, and main effect, indicate significant difference; P < 0.05
[ab] within column indicate significant interaction between parity and treatment; P = 0.05

As there was no significant difference between spermine and spermidine in terms of piglet LW and rate of LWG, further analysis was conducted comparing the effects of polyamine supplementation with no (water) supplementation on piglet performance (Tables 4 and 5). Overall, polyamine supplementation tended (P = 0.07) to increase piglet LWG between day 14 and 18 post-partum. However, there was a significant interaction between maternal parity and piglet response to polyamine supplementation, with polyamine supplementation significantly (P < 0.05) increasing piglet LW on day 18 post-partum and the rate of LWG between days 14 and 18 (Table 4 and 5). There was no effect on LW or LWG of piglets suckling parity 3 sows. There was a significant (P < 0.001) correlation between piglet LW at 1 and 24 days of age (Figure 3) and 24 and 61 days of age (Figure 4).

Table 5 Effect of maternal parity (three (Par3) versus one (Par1)) and oral piglet treatment (Water (W) versus Polyamine (Poly)) on piglet liveweight (LW) gain

		Piglet LW gain (kg/d)				
		d1-14	d14-18	d18-24	d14-24	Wean - D 61
Sow parity	Par3	0.199	0.249[d]	0.234	0.241[b]	0.376[b]
	Par1	0.194	0.216[c]	0.208	0.213[a]	0.330[a]
Treatment	Water	0.195	0.219[*]	0.223	0.227	0.344
	Polyamine	0.197	0.238[*]	0.220	0.227	0.355
Interactions	WPar3	0.195	0.251[b]	0.249	0.247	0.376
	PolyPar3	0.201	0.248[b]	0.233	0.239	0.375
	Wpar1	0.195	0.190[a]	0.210	0.209	0.315
	PolyPar1	0.193	0.229[b]	0.208	0.215	0.337
Pooled SEM		*0.01*	*0.01*	*0.03*	*0.02*	*0.02*

Differences within column, and main effect, indicate significant difference; [cd]P<0.05, *P=0.07
[cd]within column indicate significant interaction between parity and treatment; P<0.05

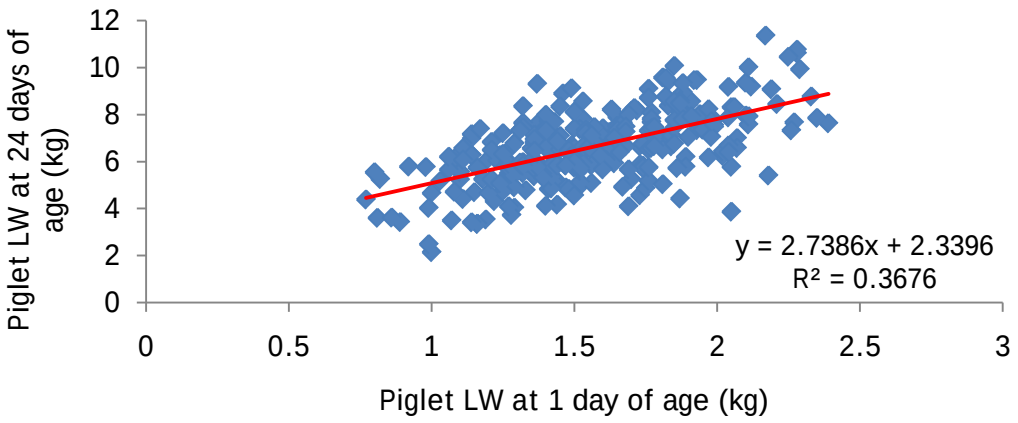

Figure 3 Correlation between piglet liveweight (LW; kg) at 1 and 24 days of age

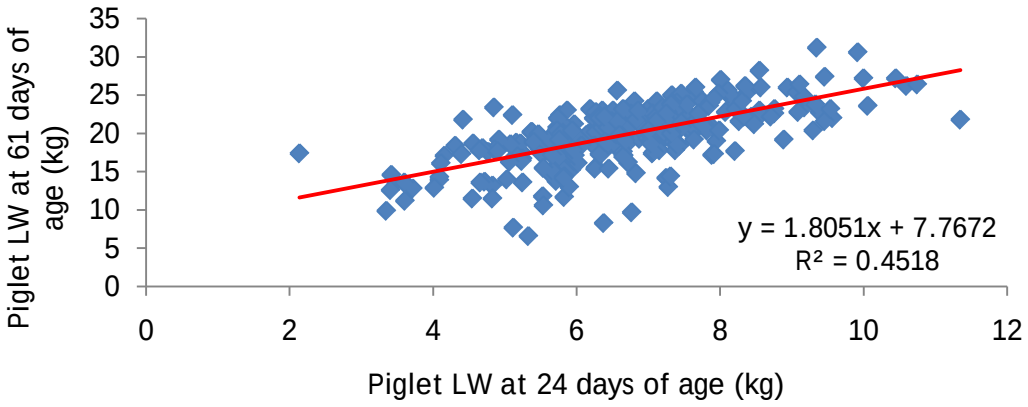

Figure 4 Correlation between piglet liveweight (LW; kg) at 24 and 61 days of age

Parity 3 sows were significantly heavier on day 1 of lactation and at weaning compared to parity 1 sows (Table 6). Although the amount of LW and P2 backfat loss was similar for parity 3 and parity 1 sows, parity 1 sows had significantly lower P2 backfat at weaning compared to parity 3 sows (Table 6). Parity 1 sows lost a significantly higher proportion of P2 backfat during lactation than parity 3 sows (19.6 ± 3.28 versus 10.2 ± 3.70%). Similarly, parity one sows lost significantly more eye muscle during lactation, and therefore had a smaller eye muscle at weaning than parity 3 sows (Table 6). The effect of sow parity and day of lactation on milk composition are presented in Figure 5. Overall, compared to milk collected from parity one sows, parity three sow milk tended ($P = 0.06$) to contain more fat (6.8 ± 0.21 versus 6.3 ± 0.19%), contained significantly ($P < 0.03$) more protein (4.8 ± 0.06 versus 4.6 ± 0.06%) and had a significantly (< 0.05) higher somatic cell count (719.200 ± 108,000 versus 314,500 ± 99,300 cells). More specifically, milk collected from parity three sows on day 3 of lactation had significantly ($P < 0.05$) higher concentrations of fat and protein, but lower concentrations of lactose compared to parity one sows. However, nutrient composition of the milk was similar for both parity groups on days 14 and 24 of lactation. Interestingly, somatic cell count was significantly ($P < 0.05$) higher on

days 3 and 14 of lactation in milk collected from parity 3 compared to parity 1 sows (Figure 5).

Table 6 Effect of sow parity (three (Par 3) versus one (Par1) on changes in sow liveweight (LW), P2 backfat (P2) and Maximum eye muscle depth (MMD) during lactation

	Sow parity		
	$Par3_1$	$Par1_2$	*Pooled SEM*
LW D1	275^b	206^a	*8.53*
LD Wean	257^b	192^a	*8.55*
LW Loss	-19.2	-13.9	*3.56*
P2 D1	19.7	18.7	*1.69*
P2 Wean	18.5^b	15.6^a	*1.46*
P2 Loss	-1.8	-3.0	*0.8*
MMD D1	49.9	47.4	*1.69*
MMD Wean	50.9^b	44.8^a	*1.6*
MMD Loss	1.13^b	-2.56^a	*1.54*

^{ab} within row indicate significant interaction between parity; P < 0.05

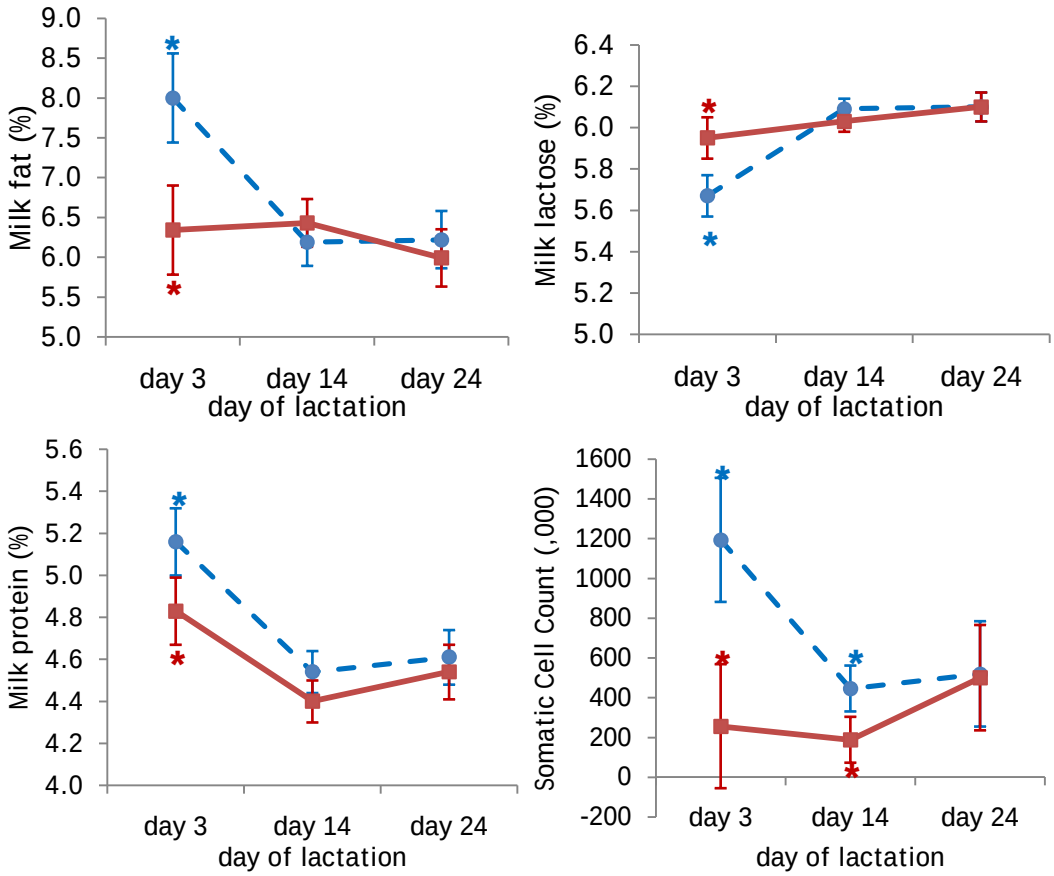

Figure 5 Fat, lactose, protein and somatic cell count of milk collected from parity three (●) and parity one (■) sows on days 3, 14, and 24 of lactation. *indicates significant difference; P<0.05

4. Application of Research

Taken together, the results of the current studies demonstrate that orally dosing suckling piglets with the polyamines spermine or spermidine can improve liveweight gain. An increase in the surface area available for nutrient absorption in the duodenum and jejunum following polyamine supplementation is likely responsible for the observed increase in growth rate. Interestingly, in the commercial study (study 2), the beneficial effect of polyamine supplementation was only evident in piglets suckling parity one sows, with liveweight gain increased for the first four days of the supplementation period. However, whether this is due to deficient levels of polyamines in the milk of parity one sows, or decreased polyamine synthesis in the progeny of parity one sows is not known.

It is evident from our data that spermine supplementation increases villous height and decreases crypt depth, a change in intestinal morphology that increases the surface area for nutrient absorption. An increase in nutrient absorptive area, as well as improved glucose transport (Larque et al., 2007), may explain the increased rate of liveweight gain observed in polyamine supplemented piglets. The reduction in piglet growth which commonly occurs in the five days following weaning can be attributed to decreased villous height (villous atrophy) and increased crypt depth (crypt hyperplasia), and a resultant decrease in nutrient absorption. Villous atrophy and crypt hyperplasia occur as a result of increased villous cell loss and increased crypt-cell production (Pluske et al., 1997). Absorbed rapidly in the duodenum and jejunum, polyamines play an important role in cellular growth and proliferation, and also appear to increase glucose absorption (Larque et al., 2007). Previous reports (i.e. Ewtushik et al., 2000; Cheng et al., 2006; Sabater-Molina et al., 2009) support our finding that polyamine supplementation alters intestinal morphology. It is, therefore, suggested polyamine supplementation may decrease the severity of post-weaning villous atrophy and crypt-depth hyperplasia. In support of this, Wu et al. (2000) observed increased polyamine synthesis and villus height in piglets treated with cortisol, and suggested this is a potential mechanism for the beneficial effects of glucocorticoids on gut maturation in neonates as well as villous recovery following viral enteritis. Interestingly, injecting suckling piglets with glucocorticoids has also been shown to increase sucrase and maltase activity (Chapple et al., 1989), and we have demonstrated increased maltase activity in polyamine supplemented piglets. Further, incidences of post weaning mortality were reduced and growth rate increased when piglets weaned at 14 days of age received an injection of glucocorticoids (Chapple et al., 1989).

It is unclear why, in the second study, only piglets suckling parity one sows exhibiting an increase in liveweight gain in response to oral polyamines. The three weeks prior to parturition are characterized by rapid growth and increased functional capacity of the piglet's gastrointestinal tract (Sangild et al., 2002). During this period, fetal development depends entirely on nutrients obtained from the dam. There is convincing evidence (i.e. Rehfeldt et al., 2011) that imbalanced and / or inadequate maternal nutrient intake during gestation impairs fetal

development and alters post-natal growth (Rehfeldt et al., 2011). Furthermore, intra-uterine growth retarded (IUGR) pigs display altered development of the gastrointestinal tract during the early post-natal period (D-Inca et al., 2011). It could, therefore, be suggested that pre- and post-natal development of the gastrointestinal tract is impaired in lighter birthweight piglets, albeit to a lesser degree than observed in IUGR piglets. Consistent with previous studies (i.e. Pork CRC 2D-101), our data demonstrate that progeny of parity one sows were on average 140 g lighter at birth than those of parity three sows. The increased intestinal surface area for nutrient absorption in response to polyamine supplementation represents a plausible mechanism responsible for the improved growth of polyamine supplemented piglets. The capacity of polyamines to increase the growth rate of piglets suckling parity one but not parity three sows could suggest that either the surface area for nutrient absorption is lower in these animals, or the quantity of nutrients ingested is lower. With regards to the latter, it is logical to suggest that a polyamine induced increase in absorptive surface area would increase the absorption of nutrients without the need to increase the quantity ingested.

Consistent with previous studies (CRC Final Report: 2D-101), the present findings demonstrate reduced growth rate in piglets suckling parity one compared to parity three sows. Interestingly, fostering parity one progeny onto multiparous sows resulted in growth rates comparable to multiparous sow progeny suckling multiparous sows. Conversely, fostering multiparous sow progeny onto parity one sows decreased piglet growth rate to equal that of non-fostered parity one progeny (CRC Final Report 2D-101). Based on this data it would appear that reduced milk output, either volume or nutrient content, from parity one sows, rather than differences in progeny growth potential or intestinal development, are responsible for their reduced growth rates. Although milk output was not measured in this study, significant differences in the composition of milk collected from parity one and parity three sows were observed, with a higher protein concentrations in parity three sow milk consistent across both studies, with increase fat concentration only evident in study two. Interestingly, the greatest differences in milk composition were evident earlier in lactation, before differences the growth rates of the two parity groups began to diverge. It is, therefore, suggested that differences in milk volume rather than nutrient content are more likely responsible for the observed differences in growth rate. However, regardless of the cause, it is apparent from our data that it is not until day 14 of lactation that parity related differences in nutrient supply begin to limit piglet growth potential.

It is interesting that milk collected from parity three sows had a significantly higher somatic cell count compared to parity one sows. Somatic cell counts are routinely used throughout the dairy industry to identify cows with mammary gland infections (mastitis) caused by bacterial infection. Somatic cells are essentially defense cells which migrate to the mammary gland in order to combat bacterial infections, with levels in milk positively correlated with the degree of infection. High somatic cells counts are associated with reduced milk yield in cattle, sheep and goats. The relationship between somatic cell counts and incidences of mastitis, metritis and agalactia syndrome (MMA), and piglet performance and

possibly subsequent reproduction performance may be an area worthy of future investigation.

5. Conclusion

In conclusion, it is evident from the current data that orally dosing piglets with polyamines (either spermine or spermidine) results in an immediate increase in liveweight gain accompanied by an increase in the surface area for nutrient absorption in the gastrointestinal tract. It is, therefore, suggested that increasing the levels of polyamines ingested by piglets, either prior to, or during, the weaning process may decrease the severity of post-weaning villous atrophy and crypt cell hyperplasia, and thus improving post-weaning growth. Interestingly, based on the second study, it is apparent that piglets suckling parity one sows are more likely to benefit from receiving additional polyamines.

In order to maximize the commercial benefits of our findings, further research is required, including;

- Determining the effects of polyamine supplementation post-weaning on piglet growth and survival
- Identifying whether deficiencies in polyamine concentrations are responsible for the reduces growth of piglets suckling parity one sows, and based on this information developing strategies to increase the polyamine content of sows milk
- Developing alternative methods of delivering polyamines directly to piglets, either via water supply or in creep diets
- Identifying whether the beneficial effects of polyamines on intestinal absorptive surface area and growth are restricted to parity one sow progeny.
- Conducting a large, commercial trial to determine the effects of polyamine supplementation on piglet survival and growth through to slaughter

In addition, there is recent evidence to support a beneficial effect of polyamine supplementation on the systemic immune system of suckling rat pups (Perez-Cano et al., 2010), suggesting that polyamine supplementation during the early postnatal period may be beneficial, and requires investigation.

6. Limitations/Risks

The major limitation to the outcomes of this study is the small sample size used, with additional studies required to identify alternative (more practical) methods of providing piglets with additional polyamines. Equally, it is possible based on the current data that only piglets with sub-optimal nutrient intake (i.e. parity one progeny) or impaired intestinal development will receive benefit from polyamine supplementation.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

- That additional studies are conducted to determine the following:
    - The optimal timing of polyamine supplementation relative to weaning to improve piglet growth and survival
    - Whether the use of polyamine supplementation should be targeted to 'at-risk' piglets or those receiving sub-optimal nutrient supply
    - Whether it is possible to increase the polyamine concentration of sows milk by natural methods
    - Whether increasing the polyamine content of creep diets and/or water supply is beneficial for piglet performance
    - The effects of polyamine supplementation during the first week of life on immune status
    - Whether the high somatic cell count of older parity sows is negatively affecting the growth of suckling piglets and in severe cases subsequent reproductive performance.

8. References

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9. Appendix 1 - Notes

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10. Appendices

Appendix 1: