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Short communication: Effect of oral polyamine supplementation pre-weaning on piglet growth and intestinal characteristics

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*A high proportion of piglets fail to adapt to the changing composition of their diet at weaning, resulting in weight loss and increased susceptibility to pathogens. Polyamines are present in sow milk and promote neonatal maturation of the gut. We hypothesised that oral spermine and spermidine supplementation before weaning would increase piglet growth and promote gastrointestinal development at weaning. In Experiment One, one pair of liveweight (LW)-matched piglets per litter from first and third lactation sows received 2 ml of a 0 (Control) or 463 nmol/ml spermine solution at 14, 16, 18, 20 and 22 days of age (*n *= 6 piglets/treatment per parity). Villus height and crypt depth in the duodenum and jejunum were measured at weaning (day 23* postpartum*). In Experiment Two, piglets suckling 18 first and 18 third lactation sows were used. Within each litter, piglets received 2 ml of either water (Control), 463 nmol/ml spermine solution or 2013 nmol/ml spermidine solution at 14, 16, 18, 22 and 24 days of age (*n *= 54 piglets/treatment per sow parity). Piglets were weighed individually at 14, 18, 24 (weaning) and 61 days of age. In Experiment One, oral spermine supplementation resulted in a 41% increase in villus height, a 21% decrease in crypt depth and 79% decrease in the villus height : crypt depth ratio compared with control piglets (*$P < 0.01$*). In Experiment Two, spermine and spermidine-supplemented piglets suckling first lactation sows grew faster (*$P < 0.05$*) between days 14 and 18* postpartum *than control piglets: 0.230 ± 0.011 and 0.227 ± 0.012* v. *0.183 ± 0.012 kg/day, respectively. Spermine supplementation tended (*$P < 0.1$*) to increase piglet LW gain from weaning to day 37 post-weaning compared with control piglets (0.373 ± 0.009* v. *0.341 ± 0.010 kg/day). In conclusion, spermine supplementation increased villus height at weaning, and appears to have the potential to improve the pre- and post-weaning growth of conventionally weaned piglets.*

Keywords: piglets, polyamines, gastrointestinal tract, weaning, growth

Implications

In piglets, oral supplementation with polyamines before weaning increased the absorptive surface area of the gastrointestinal tract at weaning, and positively affected piglet growth pre- and post-weaning. Spermine and spermidine-supplemented piglets suckling first lactation sows gained significantly more weight during the first 4 days of supplementation. Spermine supplementation pre-weaning tended to increase piglet growth after weaning. These positive effects suggest that oral supplementation with polyamines, at low doses, can increase the capacity of piglets to cope with weaning, and thus reduce the negative impacts of weaning on piglet performance within commercial pig production systems.

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Introduction

Piglet growth during the immediate post-weaning period is often compromised due to their failure to adapt to the abrupt change from milk to solid cereal-dominated diets (Lalles *et al*., 2007). This period of reduced growth is associated with reduced voluntary feed intake, as well as structural and functional changes in the small intestine, which cause a decrease in the digestive and absorptive capacity of the weaned piglet (Lalles *et al*., 2007). As a result, piglet welfare, performance and survival, as well as the efficiency of pig production, are all limited (Pluske *et al*., 1997).

During the suckling period and weaning process the gastrointestinal tract of the neonate matures and undergoes substantial changes that facilitate the shift in diet composition experienced at weaning (Xu *et al*., 2000). The triggers for many of these changes are delivered via the maternal milk. In particular, milk-borne polyamines (putrescine, spermidine and

spermine) play an important role in neonatal gut maturation, increasing in concentration in both maternal milk and the intestinal mucosa of rats during weaning (Peulen *et al.*, 2000). In conventional production systems, piglets are normally weaned at 21 to 28 days of age, ~56 days earlier than would occur under natural conditions. It is, therefore, suggested that the milk consumed by piglets before commercial weaning lacks the required bioactives to facilitate the dietary transition that occurs at weaning. Spermidine concentrations in sow's milk increase as lactation progresses peaking on day 49 of lactation (Kelly *et al.*, 1991). Oral supplementation with spermine advanced intestinal maturation in piglets dosed from 2 to 15 days of age (Sabater-Molina *et al.*, 2009) and 11 to 14 days of age (Cheng *et al.*, 2006). However, animal age appears to be a critical determinant of the intestinal response to spermine supplementation (Shimizu *et al.*, 1993). It is, therefore, important to determine the impact of oral supplementation with spermine or spermidine before weaning on the intestinal development and performance of conventionally weaned 21 to 28-day-old piglets before and after weaning.

In light of this, two studies were conducted. Experiment One investigated the effect of oral spermine supplementation before weaning on the intestinal villus height and crypt depth of piglets weaned at 24 days of age. Experiment Two investigated whether oral supplementation with either spermine or spermidine before weaning would increase piglet growth before and after weaning at 24 days of age. In both studies, a comparison between the response of piglets suckling first and third lactation sows with the polyamine supplementation was included, based on evidence that suckled dam parity affects piglet performance pre- and post-weaning (Miller *et al.*, 2013).

Material and methods

All animal procedures were approved by the University of Adelaide's Animal Ethics Committee and conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes, 7th edition (2004). Both experiments were conducted in South Australia, with Experiment One conducted at the University of Adelaide's 300 sow herd research piggery, and Experiment Two conducted on a 7500 sow commercial facility. Spermine and spermidine were obtained from Sigma-Aldrich (Sigma Aldrich, Castle Hill, New South Wales, Australia). The doses of spermine and spermidine used were calculated to provide 20% more than normally received in sow's milk. This figure was based on an estimated average intake of 950 ml/piglet per day, and sows milk containing 4.87 nmol/ml of spermine and 21.19 nmol/ml of spermidine (Sabater-Molina *et al.*, 2009).

Experiment One: effect of oral spermine supplementation pre-weaning on gastrointestinal morphology

The experimental design was a 2×2 factorial, incorporating two maternal parities (first *v.* third lactation sows; $n = 6$ sows and litters/parity) and two levels of spermine supplementation (0 *v.* 926 nmol spermine). Within 24 h of birth, litter size was standardised to, and maintained throughout lactation at, 10 piglets. On day 14, male piglets were weighed, ranked according to liveweight (LW) and pair-matched according to LW. For each litter, the pair of piglets with the mean LW for the litter were selected, with only piglets born to that sow used. Within litter, one piglet from each pair was allocated to receive either 2 ml water (Control) or 463 nmol/ml spermine (Sp) solution. Doses of spermine and water were delivered in 2 ml of water using an oral drench gun, with piglets drenched every 2nd day from days 14 to 22 *postpartum*.

Individual piglet LW was recorded on days 14, 18 and 22 postnatal age. On day 23 *postpartum*, piglets were euthanised, dissected and the entire gastrointestinal tract removed via tubal ligation. The small intestine was divided into equal thirds, with the proximal third assumed to be the duodenum, followed by the jejunum. Segments (2 cm) were dissected from the beginning of the duodenum and the middle of jejunum, flushed with cold sterile saline solution, opened longitudinally and fixed in buffered formalin for 24 h. Fixed samples were dehydrated, cleared and embedded in paraffin wax. The embedded samples were then sectioned at 7 μm for staining. Sections were stained with haematoxylin–eosin for histological assessment of gastrointestinal morphology. Image analysis was used to measure villus height (distance from the opening of the crypt to the villus tip) and crypt depth (distance from the base to the opening of the crypt) of 10 villi in each section (VideoPro®, version 6.210; Leading Edge Pty Ltd, Australia).

Statistical analysis. Data are expressed as mean ± SEM, unless otherwise stated. A general ANOVA was used to determine the effects of spermine supplementation and maternal parity on piglet weight and weight gain. Intestinal region was included in ANOVA to determine the effect of spermine supplementation and maternal parity on intestinal morphology. Between-treatment differences were examined using LSD. Significance was determined as $P < 0.05$. Analysis was conducted using Genstat, 10th edition (Rothamsted Experimental Station, Harpenden, UK).

Experiment Two: effect of oral spermine or spermidine supplementation on piglet growth before and after weaning

Experimental design and measurements. The experimental design was a 2×3 factorial, incorporating two maternal parities (first *v.* third lactation sows; $n = 18$ sows and litters/parity) and three polyamine supplements (0 *v.* 925 nmol of spermine solution *v.* 4026 nmol spermidine solution). Within 24 h of parturition (day 1), piglets were weighed and litter size was standardised to, and maintained throughout lactation at, 10 piglets. On day 14, piglets were weighed. Within litters, nine piglets were allocated to receive either 2 ml of water (Control), 463 nmol/ml spermine (Sp) or 2013 nmol/ml spermidine (Spd) solution. Piglet allocation was conducted to ensure that average LW for each treatment per litter was equal. Doses of spermine, spermidine and water were delivered using an oral drench gun, with piglets drenched every 2nd day from

days 14 to 24 *postpartum*. Piglets were weighed individually on days 1, 14, 18 and 24 (weaning) of lactation and again on day 61 *postpartum* (day 37 post-weaning).

Statistical analysis. Data are expressed as mean ± SEM, unless otherwise stated. A general ANOVA was used to determine the effects of spermine and spermidine supplementation and maternal parity on piglet growth. Between-treatment differences were examined using LSD. Significance was determined as $P < 0.05$. Analysis was conducted using Genstat 10th Edition (Rothamsted Experimental Station, Harpenden, UK).

Results

Experiment One: effect of oral spermine supplementation pre-weaning on gastrointestinal morphology

There was no effect of spermine supplementation on piglet LW or LW gain during the experimental period (Table 1). Piglets suckling third as compared with first lactation sows gained more weight between days 14 and 18 of lactation ($P < 0.05$; Table 1). Oral spermine supplementation increased villus height, reduced crypt depth and increased the villus : crypt depth ratio compared with control piglets ($P < 0.05$; Table 1). Villus height and villus height : crypt depth were greater for piglets suckling third compared with first lactation sows ($P < 0.05$; Table 1).

Experiment Two: effect of oral spermine or spermidine supplementation on piglet growth before and after weaning

There were no main effects of spermine or spermidine treatment on piglet LW. Spermine and spermidine tended ($P < 0.1$) to increase piglet LW gain between days 14 and 18. There was a significant interaction between treatment and parity for piglet LW gain from days 14 to 18. For piglets suckling first lactation sows, spermine and spermidine supplementation increased LW gain from days 14 to 18 ($P < 0.05$; Table 2). Piglets that received spermine tended to gain more LW between weaning (day 24) and day 61 *postpartum* compared with control piglets ($P = 0.075$; Table 2).

Discussion

Taken together, the current data indicated a positive effect of polyamine supplementation on piglet growth and intestinal development. Spermine supplementation increased the surface area available for nutrient absorption at weaning. Both spermine and spermidine supplementation stimulated a positive, albeit, transient increase in the pre-weaning growth of piglets suckling first lactation sows. These data extend previous evidence that supplementary polyamines can alter intestinal development in early-weaned piglets, by demonstrating the positive effects of spermine and spermidine supplementation on conventional weaning age piglets. Importantly, these data provide preliminary evidence that spermine supplementation pre-weaning has the potential to increase piglet growth post-weaning.

Our finding that spermine supplementation altered morphology of the gastrointestinal tract is consistent with previous outcomes in 14-day-old suckling (Cheng *et al*., 2006; Sabater-Molina *et al*., 2009) and 24-day-old weaned (Kang *et al*., 2012) piglets. However, the currently observed increase in villus height and decrease in crypt depth contrasts with the reduced villus height and increased crypt depth observed in piglets receiving a spermine supplement before 2 weeks of age (Cheng *et al*., 2006; Sabater-Molina *et al*., 2009). It is plausible that these differences reflect the ages of the piglets used, and animal age has been identified as a critical determinant of the intestinal response to spermine

Table 1 *Effect of oral spermine supplementation from days 14 to 22* postpartum *and maternal parity on piglet liveweight (LW) and LW gain before weaning, as well as intestinal characteristics on day 23* postpartum *(Experiment One)*

	Spermine treatment			Maternal parity			Measurement site		
	C	Sp	SEM	First lactation	Second lactation	SEM	Duodenum	Jejunum	SEM
Piglet LW (kg)									
Day 14 *postpartum*	4.73	4.55	0.113	4.81	4.47	0.113	–	–	–
Day 18 *postpartum*	5.77	5.64	0.124	5.75	5.65	0.124	–	–	–
Day 22 *postpartum*	6.97	6.83	0.139	6.98	6.82	0.139	–	–	–
Piglet LW gain (kg/day)									
Days 14 to 18 *postpartum*	0.260	0.272	0.011	0.235[a]	0.297[b]	0.011	–	–	–
Days 18 to 22 *postpartum*	0.301	0.297	0.014	0.306	0.291	0.014	–	–	–
Days 14 to 22 *postpartum*	0.281	0.284	0.009	0.271	0.293	0.009	–	–	–
Intestinal measures									
Villus height (μm)	372[c]	523[d]	13.8	407[c]	489[d]	19.2	440	455	20.9
Crypt depth (μm)	280[d]	220[c]	9.2	261	239	10.9	239	261	10.9
Villus height : crypt depth	1.4[c]	2.5[d]	0.10	1.6[c]	2.2[d]	0.13	2.0	1.9	0.15

C = control; Sp = Spermine.
[a,b]Values within a row, and main effect, with different superscript letters differ significantly at $P < 0.05$.
[c,d]Values within a row, and main effect, with different superscript letters differ significantly at $P < 0.01$.

Table 2 *Effect of three oral supplementation treatments (Control (C)* v. *spermine (Sp)* v. *spermidine (Spd)) from days 14 to 24* postpartum *and maternal parity (first lactation* v. *second lactation) on piglet liveweight (LW) and LW gain (LWG) (Experiment Two)*

							Treatment interactions						
	Polyamine treatment				Maternal parity			First lactation			Second lactation			
	C	Sp	Spd	SEM	First lactation	Second lactation	SEM	C	Sp	Spd	C	Sp	Spd	SEM
Piglet LW (kg)														
Day 1	1.58	1.57	1.59	0.032	1.51[a]	1.65[b]	0.027	1.50	1.51	1.52	1.67	1.63	1.66	0.046
Day 14	4.30	4.32	4.36	0.092	4.18[a]	4.49[b]	0.076	4.17	4.18	4.18	4.44	4.46	4.56	0.131
Day 18	5.22	5.25	5.33	0.031	5.03[a]	5.53[b]	0.026	4.93	5.08	5.07	5.54	5.44	5.62	0.044
Day 24	6.65	6.63	6.77	0.065	6.29[a]	7.11[b]	0.055	6.19	6.32	6.36	7.16	6.97	7.21	0.094
Day 61	19.83	20.25	19.65	0.287	19.57[a]	20.28[b]	0.234	19.50	19.69	19.50	19.19	20.83	19.80	0.394
Piglet LWG (kg/day)														
Days 1 to 14	0.221	0.196	0.197	0.015	0.209	0.200	0.013	0.242	0.193	0.192	0.197	0.200	0.202	0.020
Days 14 to 18	0.219*	0.239*	0.243*	0.008	0.214[a]	0.256[b]	0.007	0.183[c]	0.230[d]	0.227[d]	0.259[d]	0.248[d]	0.261[d]	0.012
Days 18 to 24	0.224	0.228	0.244	0.012	0.208[a]	0.259[b]	0.010	0.207	0.201	0.218	0.244	0.259	0.273	0.017
Days 14 to 24	0.227	0.232	0.242	0.008	0.213[a]	0.257[b]	0.007	0.206	0.214	0.219	0.251	0.251	0.268	0.012
Days 24 to 61	0.341*	0.373*	0.355*	0.007	0.340[a]	0.375[b]	0.006	0.313	0.358	0.348	0.371	0.390	0.364	0.011

[a,b]Values within a row, and main effect, with different superscript letters differ significantly at $P < 0.01$.
[c,d]Values within a row, and between individual treatments, with different superscript letters differ significantly at $P < 0.05$.
*Values within a row, and main effect, with different superscript letters differ significantly at $P < 0.1$.

supplementation in rodent pups (Shimizu *et al*., 1993). In partial support of an age-dependent effect, Kang *et al*. (2012) reported increased villi height and decreased crypt depth in piglets receiving varying doses of spermine for 3 days following weaning at 21 days of age. The differing intestinal response to spermine supplementation in the present compared with previous studies may also be attributed to differences in the concentrations provided. Based on the mean spermine levels present in milk (Sabater-Molina *et al*., 2009), the dose provided in the present study was designed to represent an approximate increase of 20% above the daily level consumed via the milk. The concentrations of spermine used in the previous studies were all substantially higher than those used in the present study: 10 times (Sabater-Molina *et al*., 2009), 1891 times (Cheng *et al*., 2006) and between 324 and 1971 times (Kang *et al*., 2012) the current dose. It is, therefore, suggested that timing of supplementation, rather than the dose provided, is responsible for the differences in intestinal alterations observed in the present compared with the previous studies of suckling piglets.

As supported by previous studies (Cheng *et al*., 2006; Kang *et al*., 2012), spermine supplementation increased piglet LW gain in the present study. A positive effect of polyamines on intestinal glucose absorption has been proposed (Larque *et al*., 2007), with enhanced maturation of the intestinal immune system also reported in spermine-supplemented neonatal mice (ter Steege *et al*., 1997). It is plausible that alterations in intestinal function in combination with the observed increase in the surface area available for nutrient absorption in spermine-supplemented piglets were responsible for their improved growth before weaning. The current data provided preliminary evidence that spermine supplementation pre-weaning can promote piglet growth following weaning. The impaired growth which occurs post-weaning is attributed to villus atrophy and crypt hyperplasia, and a resultant decrease in nutrient absorption. Villus atrophy and crypt hyperplasia occur as a result of increased villus cell loss and increased crypt cell production (Pluske *et al*., 1997). It is suggested that polyamine supplementation may decrease the severity of post-weaning villus atrophy and crypt depth hyperplasia.

In summary, it is evident from the current data that spermine supplementation, at relatively low doses, can increase intestinal absorptive area at weaning and promote piglet growth following weaning. It also appears that piglets suckling first lactation sows benefit more from the provision of supplementary polyamines, suggestive of deficiencies in either maternal milk composition or deficits in intestinal development. Based on the current data, it is, therefore, suggested that supplementary spermine, even at low doses, is a potential strategy to alleviate the negative impacts of weaning on piglet performance.

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