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Effects of Oral Administration of Spermine on the Development of Small Intestine and Growth Performance of Weaned Pigs

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Abstract: The effects of spermine on the development of small intestine and growth performance of newly weaned pigs were investigated. Thirty pigs were used and the treatment of spermine was through oral administration for the 1st 3 days after weaning with the dose of 0, 300, 600, 900 or 1200 μmol per pig daily. The administration of spermine improved Average Daily Feed Intake (ADFI) and Average Daily Gain (ADG) in the periods 0-14 ($p<0.05$) but had no significant effects on the ratio of Feed to Gain (F/G) and protein/DNA contents of the duodenal or ileal mucosa. The protein/DNA content of the jejunum was significantly increased at the doses of 300 and 600 μmol day^{-1} spermine but was declined at the higher doses. The villus height in the duodenum ($p<0.01$) and ileum ($p<0.05$) increased with an increase in spermine doses. The crypt depth was reduced by spermine in the jejunum and the ratio of villus height/crypt depth was increased by spermine in the duodenum, jejunum and ileum ($p<0.05$). Additionally, the treatment of spermine at tested doses showed no effect on D-xylose absorption by the small intestine. These results indicate that oral administration of spermine for 3 days after weaning increased ADG and ADFI, enhanced the development of the small intestinal mucosa and improved intestinal morphology.

Key words: Piglets, spermine, growth performance, intestinal morphology, intestinal protein/DNA, D-xylose

INTRODUCTION

The polyamines such as spermidine, spermine and putrescine are vital for the functions and renewal of the intestinal epithelium cells (Greco *et al*., 2001). They have been shown to stimulate cell growth and differentiation (Bardocz *et al*., 1995; Greco *et al*., 2001), improve glucose transport (Johnson *et al*., 1995), increase intestinal motility (Fioramonti *et al*., 1994) and regulate disaccharidase activities (Deloyer *et al*., 1996). Previous studies have also demonstrated that exogenous sources of polyamines seemed to be essential for the growth and development of small intestinal and colonic mucosa in addition to the endogenous synthesis of polyamines inside mucosal cells (Loser *et al*., 1999). Oral administration of polyamines to suckling rodents has been reported to induce intestinal maturation at weaning (Dufour *et al*., 1988; Wild *et al*., 1993; Capano *et al*., 1994; Kaouass *et al*., 1994; Ter Steege *et al*., 1997; Peulen *et al*., 1998). However, dietary supplementation of putrescine did not affect tissue levels of polyamines in nursing rats (Greco *et al*., 2001). In contrast, diets with spermidine and spermine appeared to be necessary for many important biological processes in rodents and humans (Deignan *et al*., 2007).

Spermine is a low molecular weight polyamine which might induce the maturation of the small intestinal mucosa when orally ingested by suckling rats (Dufour *et al*., 1988). Peulen *et al.* (1998) suggested that this spermine-induced maturation seemed to be identical to the natural process which occurred following weaning.Weaned pigs are highly susceptible to various stressors such as bacterial diseases and gut development is important for them, not only to protect them from enteric pathogens but for digestion and absorption of nutrients for growth (Kang *et al*., 2008; Liu *et al*., 2008). However, little is known about the effects of spermine on the development of intestine for the weaning pigs. The

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objective of this study was to investigate the effect of spermine on the performance and small intestinal development for the weaning pig.

MATERIALS AND METHODS

Pig trial: A total of 30 barrows (Yorkshire x Landrace, 5.08±0.61 kg) were weaned at 21±2 days of age and placed in individual pens with free access to feed and water (Deng *et al*., 2009). The basal diet which mainly contained 57.4% maize, 23.4% soybean meal and 5% wheat midding were formulated to meet or exceed NRC in 1998 recommendations for all nutrients (Table 1). The pigs were randomly assigned to five groups and received 0, 300, 600, 900 and 1200 μmol spermine (Sigma Chemical Inc., St. Louis, MO, USA) daily by oral administration, via intragastric gavage for the 1st 3 days after weaning. Each treatment had six replicates in which each individual pig was treated as a replicate. Feed consumption was measured and pigs were weighed on day 0, 7 and 14 to calculate the Averaged Daily Gain (ADG), Average Daily Feed Intake (ADFI) and the ratio of Feed to Gain (F/G). On day 7 and 14, pigs were orally administrated 10% D-xylose solution (1 mL kg^{-1} BW) and blood samples were collected after 1 h. On day 14, all the pigs were killed under anesthesia with an intravenous injection of pentobarbital sodium (50 mg kg^{-1} BW) and intestinal samples were collected (Yin *et al*., 2001). The animal-use protocol for this research was approved by the Animal Care and Use Committee of Hubei province.

Table 1: Composition and calculated nutrient content of the experimental diet [a]

Items	Values
Ingredients (g kg^{-1})	
Maize	574.00
Soybean meal (CP 43%)	234.00
Wheat middlings	50.00
Fish meal (CP 63%)	36.00
Rice protein powder	14.00
Fat powder	20.00
Dry whey	30.00
Acidifier[b]	2.00
Premix[c]	40.00
Total	1000.00
Calculated analysis[d]	
Digestible energy (MJ kg^{-1})	13.86
Crude protein (%)	19.50
Calcium (%)	0.80
Phosphorus (%)	0.70
Lysine (%)	1.28
Methionine + Cystine (%)	0.65

[a]Expressed on air dry weight basis; [b]A blend of phosphoric acid, citric acid and lactic acid to be used as a feed additive; [c]Premix provided for 1 kg of complete feed: Vitamin A, 18000 IU; Vitamin D_3, 4000 IU; Vitamin E, 40 I U; Vitamin K_3, 4 mg; Vitamin B_1, 6 mg; Vitamin B_2, 12 mg; Vitamin B_6, 6 mg; Vitamin B_{12}, 0.05 mg; Biotin, 0.2 mg; Folic acid, 2 mg; Niacin, 50 mg; Calcium pantotheniate, 25 mg; Fe, 100 mg; Cu, 150 mg; Mn, 40 mg; Zn, 100 mg; I, 0.5 mg; Se, 0.3 mg; [d]Calculated values

Sample collection: Blood samples were collected from anterior vena cava into heparinized vacuum tubes and then centrifuged at 3,000×g for 10 min at 4°C to obtain plasma. Plasma was stored at -80°C until analysis (Tang *et al*., 2005). The small intestine was dissected free of its mesentery and kept on ice immediately. The segment was divided into three regions, duodenum (from the pylorus to the ligament of trietz), jejunum (the proximal segment of the rest of the small intestine) and ileum (a distal segment 15 cm proximal to the ileocecal junction) (Jiang *et al*., 2009). The three segments (duodenum, jejunum and ileum) were rinsed thoroughly with ice-cold physiological saline solution to remove digesta. At duodenum and approximately mid-jejunum and mid-ileum, two adjacent segments (approximately 3 and 10 cm in length) were removed. The 10 cm intestinal segments were opened longitudinally and the mucosa was collected by scraping with a sterile glass microscope slide and frozen immediately in liquid nitrogen. Samples of mucosa were stored at -80°C until use.

Measurement of mucosal protein and DNA: Protein concentrations of mucosal homogenates were determined using the method of Bradford (1976) with bovine serum albumin (Biosharp, USA) as the standard. Mucosal DNA was estimated according to the method of Labarca and Paigen, 1980) using the bisbenzimidazole fluorescent dye (Hoechest 33258, Sigma Chemical Inc., St. Louis, MO, USA) in fluorescent buffer (2 mol NaCl; 50 mmol Na_3PO_4, pH 7.4) with calf-thymus DNA (Sigma Chemical Inc., St. Louis, MO, USA) as the standard. The fluorescence was measured at excitation (365 nm) and emission (458 nm) wavelengths in a fluorometer (RFLP-5301PC, Shimadzu Corporation, Japan). The ratio of mucosal protein: DNA was expressed as micrograms of protein per nanogram DNA.

Histology evaluation: The 3 cm intestinal segments were processed, embedded and stained according to procedures described by Luna (1968). Briefly, the segment was flushed and submerged in cold Phosphate-buffered Saline (PBS, 3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH = 7.4) for approximately 5 min before being transferred to fresh chilled fixative solution (10% formalin). After approximately 5 min in FEA (formalin, 95% ethanol and glacial acetic acid solution), each segment was placed into a 30 mL scintillation vial containing fresh fixative solution and stored at 4°C for 24 h then the solution was replaced with 70% ethanol and samples were then stored at room temperature. Segments were dehydrated over a 2 days period using increasing concentrations of ethanol (80% to

absolute) and chloroform. Segments were embedded in paraffin, refrigerated (4°C) and sliced into 4 μm crosscut sections by a microtome (American Optical Co., Scientific Instrument Div., Buffalo, NY, USA) and stained with hematoxylin and eosin. Morphometric measurements were performed by person who was blind to the treatment groups using light microscopy with a computer assisted morphometric system (Motic Images Advanced 3.2 software, Motic China Group Co., Ltd., China). The identities of all tissue slides were disguised, thus the examination was conducted in a blinded manner. The height, width and crypt depth (micrometers) of ten well-oriented villi per sample were measured.

Measurement of plasma D-xylose: The D-xylose absorption test was carried out according to the method described by Mansoori *et al.* (2009). Briefly, 50 μL of the collected plasma was added to 5 mL of phloroglucinol (Sigma Chemical Inc., St. Louis, MO, USA) colour reagent solution and heated at 100°C for 4 min. The samples were allowed to cool to room temperature in a water bath. D-xylose (Sigma Chemical Inc., St. Louis, MO, USA) standard solutions were prepared by dissolving D-xylose in saturated benzoic acid (prepared in deionized water) to obtain 0, 0.7, 1.3, 2.6 mmol L^{-1}. They were added to colour reagent solution alongside the samples. The absorbance of all samples and standard solutions were measured using a spectrophotometer (Model 6100, Jenway LTD., Felsted, Dunmow, CM6 3LB, Essex, England, UK) set at 554 nm.

Statistical analysis: Data were subjected to analysis of General Linear Model and Regression using SPSS 13.0 software (SPSS Inc. Chicago, IL, USA). Means were compared using Duncan's multiple comparisons test. The experimental unit for all statistical procedures was individual pigs and the significance level for all tests was set at $p<0.05$.

RESULTS AND DISCUSSION

Growth performance: The effects of spermine on pig growth performance are shown in Table 2. The high doses of spermine (900 and 1,200 μmol day^{-1}) significantly improved the ADFI and ADG in the periods of 0-14 days ($p<0.05$) but not the F/G ($p>0.05$). All the treatments with low doses of spermine (≤300 μmol day^{-1}) had no effect on the average daily feed intake, ADG and F/G.

Mucosal protein/DNA levels and ratioes: The effects of spermine on the contents of mucosal protein and DNA and their ratios in the small intestine are shown in Table 3. There were no differences in the levels of protein, DNA and the ratio of protein to DNA in all the samples of small intestine ($p>0.05$).

Histological observation: The data on the villus height, crypt depth and the ratios of villus height and crypt depth of small intestine are shown in Table 4. In general, the increasing level of spermine increased the villus height of duodenum and ileum ($p<0.05$). The treatment had no effect on the crypt depth except for the samples of jejunum. The ratios of villus height and crypt depth were nearly all increased by the treatment of spermine ($p<0.05$).

D-xylose absorption: The concentrations of D-xylose in plasma are shown in Table 5. The treatment of spermine had no effect on the absorption of D-xylose into the plasma.

Exogenous dietary polyamines not only are necessary for normal body metabolism but could contribute to growth and health significantly (Bardocz *et al.*, 1993). In sow's milk, the level of spermine was higher than spermidine throughout the lactation in sow's milk (Motyl *et al.*, 1995). Sabater-Molina *et al.* (2009) reported that milk formulas supplemented with polyamines at maternal milk physiologic doses (including

Table 2: The effect of spermine on the performance of pigs for 14 days post weaning[c,d]

		Spermine (μmol day^{-1})					p-value	
Items	Control	300	600	900	1200	SEM	Linear	Quadratic
Average daily feed intake (g day^{-1})								
0~7 day	175.35[a]	176.52[a]	188.53[ab]	193.86[b]	192.11[b]	2.727	0.003	0.012
7~14 day	220.61[a]	224.39[ab]	226.83[ab]	254.16[bc]	259.20[c]	5.809	0.004	0.014
0~14 day	197.98[a]	200.46[a]	207.68[ab]	224.01[b]	225.66[b]	4.044	0.002	0.009
Average daily gain (g day^{-1})								
0~7 day	69.61	79.30	78.15	85.77	85.33	8.303	0.073	0.190
7~14 day	104.56[a]	114.68[a]	129.81[b]	132.61[b]	139.12[b]	12.174	<0.001	<0.001
0~14 day	87.10[a]	97.04[ab]	103.95[b]	109.20[b]	112.18[b]	6.472	0.001	0.002
Feed/gain (kg DM of feed consumed per kg of body weight gain)								
0~7 day	2.52	2.39	2.42	2.27	2.25	0.878	0.300	0.597
7~14 day	2.11	1.96	1.77	1.92	1.86	0.050	0.134	0.141
0~14 day	2.27	2.09	2.02	2.05	2.01	0.052	0.126	0.213

[a,b]Means in the same row with no superscripts or that have a common superscri; pt letter do not differ significantly ($p>0.05$); [c]A total of 30 piglets were used, 1 pig/pen and 6 replicate pens per treatment; [d]The spermine was administered orally for the first 3 days after weaning

Table 3: The effect of spermine on the protein and DNA levels and the protein/DNA ratio of the small intestine measured in pigs 14 days after weaning

		Spermine (μmol day^{-1})					p-value	
Sites	Control	300	600	900	1200	SEM	Linear	Quadratic
Duodenum								
Protein (mg g^{-1})	56.25	61.87	60.44	58.17	55.54	1.609	0.660	0.408
DNA (mg g^{-1})	5.31	5.81	5.89	5.50	5.25	0.153	0.455	0.591
Protein/DNA	9.51	10.84	10.32	11.75	10.66	0.354	0.704	0.864
Jejunum								
Protein (mg g^{-1})	50.66	58.07	56.64	53.40	50.24	1.367	0.578	0.111
DNA (mg g^{-1})	5.52	5.44	5.69	5.59	5.74	0.148	0.657	0.574
Protein/DNA	8.49	10.73	10.01	9.59	9.01	0.240	0.414	0.319
Ileum								
Protein (mg g^{-1})	46.38	54.31	49.20	49.11	53.91	1.536	0.373	0.674
DNA (mg g^{-1})	5.53	5.96	5.66	5.69	6.62	0.169	0.122	0.129
Protein/DNA	8.54	9.22	9.36	8.65	8.19	0.281	0.527	0.347

Means in the same row with no superscripts or that have a common superscript letter do not differ significantly (p>0.05)

Table 4: The effect of spermine on the histology of pig small intestines measured 14 days after weaning

		Spermine (μmol day^{-1})					p-value	
Sites	Control	300	600	900	1200	SEM	Linear	Quadratic
Duodenum								
VH (μm)	281.72^{b}	317.76^{c}	307.30^{b}	359.79^{a}	348.31^{ac}	7.665	<0.010	<0.010
CD (μm)	230.17	203.16	231.16	208.70	226.99	5.736	0.984	0.719
VH/CD	1.15^{b}	1.62^{a}	1.29^{ab}	1.79^{a}	1.63^{a}	0.334	<0.010	<0.010
Jejunum								
VH (μm)	278.84	331.21	327.88	330.33	356.25	10.190	0.029	0.081
CD (μm)	260.48^{a}	204.03^{b}	233.94^{ab}	202.41^{b}	202.29^{b}	6.401	<0.010	0.015
VH/CD	1.07^{b}	1.49^{ab}	1.46^{ab}	1.64^{a}	1.72^{a}	0.377	<0.010	<0.010
Ileum								
VH (μm)	272.32^{b}	323.44^{a}	323.21^{a}	325.56^{a}	350.11^{a}	8.541	<0.010	0.200
CD (μm)	234.16	223.31	208.16	216.20	209.21	4.303	0.060	0.118
VH/CD	1.19^{b}	1.55^{ab}	1.56^{ab}	1.72^{ab}	1.75^{a}	0.462	<0.010	<0.010

VH = Villus Height, CD = Crypt Depth, VH/CD = Villus Height/Crypt Depth; $^{a-c}$Means in the same row with no superscripts or that have a common superscript letter do not differ significantly (p>0.05)

Table 5: The effects of spermine on D-xylose absorption into the plasma*

		Spermine (μmol day^{-1})					p-value	
D-xylose	Control	300	600	900	1200	SEM	Linear	Quadratic
Day 7	0.19	0.23	0.27	0.25	0.24	0.101	0.137	0.067
Day 14	0.21	0.29	0.32	0.22	0.39	0.026	0.100	0.263

*The absorption is expressed by the level of D-xylose in the plasma samples (unit: mmol L^{-1}). Blood samples were collected 1 h after administration of the D-xylose solution on day 7 and 14 after weaning, respectively

5 nmol mL^{-1} of spermine) slightly enhanced gut growth and maturation in neonatal piglets. Excess oral ingestion of spermine might have widespread health effects, including the induction of food allergies due to the contribution of spermine to maturation of both the immune system and the small intestinal mucosa (Sugita *et al*., 2011). But Sousadias and Smith (1995) had shown that the level of oral spermine was >0.25 g per chick daily, chick growth would be decrease. In the present study, the maxmium of spermine intake for the piglets was 0.17 and 0.08-0.17 g per pig daily could increase the ADG for the piglets during the entire experimental time which was also in consistent with the histology results. Thus according to the current study, weaned pigs might have a requirement for an appropriate amount of exogenous spermine.

Small intestinal morphology has typically been used as an estimate of intestinal health in pigs (Argenzio *et al*., 1990; Zijlstra *et al*., 1996). In general, measurements of villus height and crypt depth give an indication of the likely maturity and functional capacity of entercoytes (Hampson, 1986). Longer villi height and shorter crypt depth suggest that rates of enterocyte proliferation and exfoliation are higher (Shirkey *et al*., 2006).

Abrams (1977) have demonstrated that shorter villi and deeper crypts were caused by resident bacterial flora when compared with germ-free animals. In the present study, the spermine treatment increased the villus height and villus height: crypt depth ratios suggesting a positive effect on intestinal development. The enhanced intestinal maturity may have contributed to the higher feed intake by spermine treated pigs.

Polyamines can be absorbed like food components. Their absorption occurs mainly in the upper part of the gut including duodenum, jejunum and ileum where they are involved in enterocyte maturation and metabolism (Bardocz *et al*., 1998). In the present study, the spermine effects on the development of intestine were evident in the jejunum and ileum suggesting that the effects of spermine on intestinal development were likely associated

with the absorptive site of spermine. It is also known that polyamines are essential for protein synthesis in addition to their positive effects on intestinal maturation and functions (Pegg, 1986; Johnson, 1988). They also affect the growth of intestine by interacting with DNA and protein (Feuerstein *et al*., 1986). The protein: DNA ratio has thus been used as indices of cell hypertrophy (increased cell size) (Jin *et al*., 1994). In the present study, researchers did not observe the effect of spermine that changed the protein/DNA ratio of the small intestine.

D-xylose (a pentose sugar) is absorbed from the upper small intestinal tract. It is poorly metabolized by the body and has been widely applied for the investigation of small intestinal absorption (Gyr *et al*., 1974). When D-xylose absorption is decreased, it is likely that there is a problem not only with nutrient uptake but also with water which is not being absorbed at a rate necessary for maintenance of life processes (Doerfler *et al*., 2000). In the present study, D-xylose was used to examine the absorptive function of weaning piglets. Researchers did not detect concentration changes of D-xylose in the plasma with the increasing doses of spermine.

CONCLUSION

The present study has demonstrated that oral administration of spermine for 3 days after weaning could stimulate the development of the small intestinal mucosa and improve intestinal morphology in addition to its effect to increase the average daily feed intake of piglets. Given that the enhancement of intestinal development can help the reduction of growth retardation and diarrhea incidence commonly associated with weaning of piglets, the observations may have a practical implication and further studies are thus warranted.

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