



## Original Research Article

# Fecal scores and microbial metabolites in weaned piglets fed different protein sources and levels



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## ABSTRACT

This experiment studied the effects of dietary protein sources and levels on the gut health of piglets, pH value, and concentrations of microbial metabolites (ammonia-N, volatile fatty acids [VFA], and polyamines) in the distal colonic and proximal colonic digesta of piglets weaned at 21 d of age. A total of 150 early-weaned piglets were allotted randomly to 5 diets: 1) control diet (CT; 17% CP), 2) CT formulated with more soy protein concentrate (SPC19; 19% CP), 3) more fish meal (FM19; 19% CP), 4) CT formulated with more soy protein concentrate (SPC23; 23% CP), and 5) more fish meal (FM23; 23%CP). Results showed high protein level increased fecal score ( $P < 0.05$ ), but different protein sources did not ( $P > 0.05$ ). The pH value and ammonia-N concentration of digesta in the proximal and distal colon of FM23 were significantly higher ( $P < 0.05$ ) than those of CT. Acetic acid, propionic acid, butyric acid and valeric acid concentrations in the proximal colon of FM23 exceeded those of CT, SPC19, and FM19 ( $P < 0.05$ ); however, isobutyric acid and isovaleric acid were not affected ( $P > 0.05$ ). Histamine and spermidine concentrations of FM23 were higher than those of other treatments ( $P < 0.05$ ). Propionic acid and butyric acid concentrations in the distal colon were higher of FM23 than of FM19 ( $P < 0.05$ ); putrescine, histamine and spermidine were higher of FM23 than of LP and FM19 ( $P < 0.05$ ). It was concluded that high dietary CP content increased microbial metabolites (ammonia-N, histamine, putrescine) in colonic digesta and aggravated piglets' diarrhea.

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## 1. Introduction

Postweaning diarrhea is the main cause of suboptimal production of piglets in the first 2 weeks after weaning (Pluske et al.,

2003). The use of antibiotic growth promoters and high dose zinc oxide has been effective in decreasing the incidence of diarrhea (Htoo et al., 2007; Poulsen, 1995), but their routine use has negative effects and is discouraged in the industry. Some recent research had reported that feeding a low protein diet for 14 d after weaning reduced the incidence of diarrhea (Heo et al., 2008, 2009) and we have confirmed that (Wu et al., 2015). Different protein sources also affect the health and performance of piglets (Che et al., 2012). There is little understanding of how feeding a low protein content diet decreases the incidence of diarrhea. Some studies had shown that lower dietary protein level decreased the fermentation of residual protein in the large intestine (Nyachoti et al., 2006; Htoo et al., 2007). The fermentation of protein mainly occurs in the distal colon when fermentable carbohydrates have been depleted, and it results in the production of potentially

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toxic metabolites including amines, ammonia, sulfides and phenols. These metabolites are considered to be detrimental to the host's health (Windey et al., 2012).

The objectives of the present study were to determine the effects of dietary protein source and level (17%, 19% and 23.7% CP) on pH of digesta in the proximal and distal colon and concentrations of microbial-derived metabolites of piglets without feeding any antibiotic growth promoters, and to examine relationships between intestinal protein fermentation and objectively-scored diarrhea.

## 2. Materials and methods

Procedures performed in this experiment were approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences.

### 2.1. Experimental diets and feeding regimen

Piglets were allotted randomly to 5 diets: 1) control diet (CT; 17% CP), 2) CT formulated with more soy protein concentrate (SPC19; 19% CP), 3) more fish meal (FM19; 19% CP), 4) CT formulated with more soy protein concentrate (SPC23; 23% CP), and 5) more fish meal (FM23; 23% CP). The ingredients and nutrient composition of the 5 diets used in the experiment and the growth performance of piglets are given by Wu et al. (2015). All essential amino acids and minerals supplemented in amounts met or exceeded NRC (2012) nutrient standards (Wu et al., 2015). The 5 diets are therefore provided for critical comparisons of this study. First, the basal diet with low protein (CT, 17% CP) could be compared with all other diets. Second, the amount of additional protein (19% or 23.7% CP), protein source, and possible interactions could be evaluated.

### 2.2. Animals, housing, and experimental procedures

A total of 150 piglets (Duroc × Landrace × Large White; 21-d-old; BW = 5.99 ± 0.14 kg) were balanced for BW and then randomly assigned 5 treatments, each with 6 replicates, and 5 piglets per replicate (Wu et al., 2015). The consistency of the feces was evaluated daily according to 4 levels: 0, normal; 1, pasty; 2, semi-liquid; and 3, liquid (Liu et al., 2010).

### 2.3. Digesta sampling

On the last day of the experiment, one piglet with the median BW of each pen was sacrificed by an intravenous injection of sodium pentobarbital (50 mg/kg BW, Sigma, St Louis, MO, USA). Different segments of the digestive tract were located and tied off to avoid mixing of digesta. The entire intestinal tract was removed and immersed in ice-cold phosphate-buffered saline. Samples of digesta in the proximal and distal colon were snap-frozen in liquid N and stored at -80 °C until later analyses for volatile fatty acids (VFA), ammonia-N, and polyamines.

### 2.4. Volatile fatty acid analysis

The concentrations of VFA in colon digesta were determined by gas chromatography (Agilent 7890A) as described by Zijlstra et al. (1977) using crotonic acid as an internal standard. Digesta samples were thawed and 1 g samples were taken, diluted with 3 mL distilled water, mixed, and centrifuged (2,500 × g, 4 min, 4 °C). Supernatant (1 mL), 0.2 mL internal standard (42 mmol/L crotonic acid, Sigma), and 0.2 mL 10% H<sub>3</sub>PO<sub>3</sub> were mixed and re-centrifuged (20,000 × g, 10 min, 4 °C) and the supernatant was filtered through

a polyethersulphone membrane filter (0.25 μm, Whatman, UK) into a chromatographic sample vial.

### 2.5. Ammonia-N

The concentration of ammonia-N in colonic digesta was measured spectrophotometrically (Novozamsky et al., 1974). Briefly, 2 mL of phenate solution (1% phenol, 0.005% sodium nitroprusside) was added to 20 μL of diluted digesta (wt/vol = 1:5) and mixed, then 2 mL 0.7% sodium hypochlorite solution (in 0.5% sodium hydroxide, 0.4% sodium citrate) was added and mixed. Tubes were incubated in complete darkness at 40 °C for 20 min before reading absorbance at 640 nm using a Multiskan Spectrum (Molecular Devices, Sunnyvale, CA, USA). An ammonium sulfate standard solution (5 mg/L ammonia-N) was used to generate an ammonia-N standard curve, and samples were quantified using the regression equation of the standard curve.

### 2.6. Polyamine analysis

The concentrations of cadaverine, putrescine, spermidine, and spermine in digesta were determined by HPLC according to the method of Wang (2011). Briefly, the digesta (0.5 g) was diluted with 2 mL distilled water, and clarified by centrifugation (2,500 × g, 30 min, 4 °C). The supernatant layer (20 μL) was diluted with 100 μL borate buffer (pH = 8, containing 10 mg/L 1, 6-hexanediamine as an internal standard) and 20 μL of 9-fluorenylmethyl chloroformate solution (23186, Sigma–Fluka, 150 mg in 100 mL acetonitrile). Samples were mixed and incubated in the dark at 40 °C for 10 min, then mixed with 1 mL of mobile phase (acetonitrile:distilled water, 95:5) and filtered (0.22 μm, Millipore Co., Bedford, MA, USA) into chromatography vials. Samples were analyzed using a Waters 2495 instrument with a 2475 fluorescence detector, and 1 μL sample volume was injected into a 250 mm × 4 mm reversed-phase C-18 column at 40 °C, λ<sub>ex</sub> = 265 nm, λ<sub>em</sub> = 310 nm with flow rate of 0.8 mL/min.

### 2.7. Statistical analysis

The pen (replicate) of pigs was the experimental unit. The effects of diet were assessed by ANOVA using the GLM procedure of SAS 9.2 (SAS Inst., Inc., Cary, NC, USA). The control (17% CP) diet was compared with all supplemented diets. The 2 × 2 factorial of higher CP diets (source, level; SPC19, FM19, SPC23, FM23) and interaction were compared. Data are presented as least-square means with the SEM derived from the error mean square of each ANOVA for *n* = 6. *P* < 0.05 was considered to be statistically significant.

## 3. Results

### 3.1. Assessment of diarrhea in piglets

The effect of dietary protein sources and protein level on fecal scores of weaned piglets is shown in Table 1. Piglets fed 17% CP had lower fecal scores with lower incidence of diarrhea compared with piglets in other treatments (*P* < 0.05), and fecal score significantly increased with increasing CP percentage (*P* < 0.05). There were no significant differences in fecal scores of piglets between protein sources, fish or soy, at the same protein level.

### 3.2. Ammonia-N concentration and pH of proximal and distal colonic digesta

As shown in Table 2, pH of digesta in the proximal colon of pigs fed 17% CP diet was the lowest measured value (*P* < 0.05). Both level

**Table 1**  
Effects of different dietary protein sources and fecal scores of weaned piglets.<sup>1</sup>

Item	CT	SPC19	FM19	SPC23	FM23	SEM	P-value <sup>2</sup>				
							Diets	Basal	Source	Level	Source × Level
Fecal score <sup>3</sup>	0.74 <sup>b</sup>	1.52 <sup>a</sup>	1.45 <sup>a</sup>	1.77 <sup>a</sup>	1.85 <sup>a</sup>	0.09	<0.001	<0.001	0.904	0.002	0.441

CT = control treatment; SPC = soy protein concentrate; FM = fish meal.

<sup>a,b</sup> Within a row, values without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> Values are least squares means for 6 replicate pens per treatment. CT, basal diet (17% CP, low level of protein); SPC19, basal diet supplemented with soy protein concentrate (19% CP as the standard used in China); FM19, basal diet supplemented with fish meal (19% CP as the standard used in China); SPC23, basal diet supplemented with soy protein concentrate (23.7% CP as recommended by NRC); FM23, basal diet supplemented with fish meal (23.7% CP as recommended by NRC).

<sup>2</sup> Diets, all 5 diets. Basal, control compared with supplemented diets. Source, additional soy or fish protein. Level, 19% and 23.7% CP. Source × Level, the interaction of protein source and level.

<sup>3</sup> Fecal score indicates the tendency towards diarrhea: 0, normal; 1, pasty; 2, semi-liquid; and 3, liquid (Liu et al., 2010).

**Table 2**  
Effects of different dietary protein sources and pH values of proximal colonic digesta and distal colonic digesta.<sup>1</sup>

Item	CT	SPC19	FM19	SPC23	FM23	SEM	P-values <sup>2</sup>				
							Diets	Basal	Source	Level	Source × Level
Proximal colon	5.69 <sup>c</sup>	6.02 <sup>b</sup>	6.22 <sup>b</sup>	6.03 <sup>b</sup>	6.83 <sup>a</sup>	0.13	0.001	0.001	0.001	0.037	0.018
Distal colon	6.03 <sup>b</sup>	6.22 <sup>ab</sup>	6.25 <sup>ab</sup>	6.27 <sup>ab</sup>	6.63 <sup>a</sup>	0.11	0.015	0.019	0.103	0.061	0.143

CT = control treatment; SPC = soy protein concentrate; FM = fish meal.

<sup>a,b,c</sup> Within a row, values without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> Values are least squares means for 6 replicate pens per treatment. CT, basal diet with 17% CP; SPC19, 19% CP basal diet supplemented with soy protein concentrate; FM19, 19% CP basal diet supplemented with fish meal; SPC23, 23.7% CP basal diet supplemented with soy protein concentrate; FM23, 23.7% CP basal diet supplemented with fish meal.

<sup>2</sup> Diets, all 5 diets. Basal, control compared with supplemented diets. Source, additional soy or fish protein. Level, 19% and 23.7% CP. Source × Level, the interaction of protein source and level.

**Table 3**  
Effects of different dietary protein sources and ammonia-N concentrations ( $\mu\text{g/g}$ ) in proximal colonic digesta and distal colonic digesta of weaned piglets.<sup>1</sup>

Item	CT	SPC19	FM19	SPC23	FM23	SEM	P-values <sup>2</sup>				
							Diets	Basal	Source	Level	Source × Level
Proximal colon	271 <sup>b</sup>	274 <sup>b</sup>	295 <sup>b</sup>	315 <sup>ab</sup>	360 <sup>a</sup>	35.38	0.029	0.145	0.120	0.014	0.315
Distal colon	316 <sup>b</sup>	342 <sup>ab</sup>	312 <sup>b</sup>	365 <sup>ab</sup>	432 <sup>a</sup>	38.14	0.004	0.100	0.186	0.005	0.027

CT = control treatment; FM = fish meal; SPC = soy protein concentrate.

<sup>a,b</sup> Within a row, values without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> Values are least squares means for 6 replicate pens per treatment. CT, basal diet with 17% CP; SPC19, 19% CP basal diet supplemented with soy protein concentrate; FM19, 19% CP basal diet supplemented with fish meal; SPC23, 23.7% CP basal diet supplemented with soy protein concentrate; FM23, 23.7% CP basal diet supplemented with fish meal.

<sup>2</sup> Diets, all 5 diets. Basal, control compared with supplemented diets. Source, additional soy or fish protein. Level, 19% and 23.7% CP. Source × Level, the interaction of protein source and level.

and source of dietary protein had significant effects on pH in the proximal colon of piglets ( $P < 0.05$ ). At both sampling locations, the highest pH in colonic digesta occurred in FM23.

The pH and ammonia-N concentrations in digesta (Tables 2 and 3) in the proximal and distal colonic digesta of pigs fed 17% CP diet were the lowest, and those in pigs fed 23.7% fish meal supplemented diets were the highest. There were significant effects of protein level at both locations and a significant source × level interaction in the distal colon.

### 3.3. Volatile fatty acid concentrations in digesta in the proximal and distal colon

The concentrations of VFA in proximal and distal colonic digesta are summarized in Table 4. For many of the acids in proximal digesta, and some distally, protein level was more important than source. In almost all cases among the major VFA, the highest concentrations of a given VFA occurred in piglets fed the diet supplemented with the high level of fish meal. In the proximal colon, concentrations of acetic, butyric, and valeric acids in pigs fed the FM23 diet were higher than other treatments

( $P < 0.05$ ). Protein level had a significant effect on the concentration of acetic, propionic, butyric and valeric acids ( $P < 0.05$ ). The propionic acid concentration of FM23 exceeded that of FM19 ( $P < 0.05$ ). In the distal colon, increasing protein level from 19% to 23.7% raised the contents of propionic acid and butyric acid. The concentration of butyric acid of FM23 exceeded that of SPC19, FM19, SPC23 ( $P < 0.05$ ). Concentrations of isobutyric and iso-valeric acids were not significantly different among the diets ( $P > 0.05$ ).

### 3.4. Concentrations of polyamines in digesta of the proximal and distal colon

In the proximal colon (Table 5), concentrations of putrescine, histamine and spermidine in pigs fed the FM23 diet were higher than those fed diets with 17% and the 19% CP ( $P < 0.05$ ). Spermine concentration with the FM23 diet exceeded that of the FM19 diet ( $P < 0.05$ ), but there were no other differences ( $P > 0.05$ ). In the distal colon, different diets did not influence the concentration of cadaverine ( $P > 0.05$ ), but levels of putrescine, histamine and spermidine were the highest in pigs fed FM23 ( $P < 0.05$ ). Except for

**Table 4**  
Effects of different dietary protein sources and content of volatile fatty acid ( $\mu\text{mol/g}$ ) in proximal colonic digesta and distal colonic digesta of weaned piglets.<sup>1</sup>

Item	CT	SPC19	FM19	SPC23	FM23	SEM	<i>P</i> -values <sup>2</sup>					
							Diets	Basal	Source	Level	Source $\times$ Level	
Proximal colon												
Acetic acid	47.80 <sup>b</sup>	42.56 <sup>b</sup>	43.29 <sup>b</sup>	52.81 <sup>b</sup>	72.44 <sup>a</sup>	6.68	0.037	0.516	0.148	0.010	0.178	
Propionic acid	13.63 <sup>ab</sup>	14.65 <sup>ab</sup>	9.43 <sup>b</sup>	11.23 <sup>b</sup>	19.35 <sup>a</sup>	1.38	0.002	0.987	0.309	0.032	0.001	
Isobutyric acid	0.98	0.93	0.94	1.02	0.95	0.09	0.948	0.802	0.747	0.571	0.654	
Butyric acid	3.75 <sup>b</sup>	4.06 <sup>b</sup>	2.16 <sup>b</sup>	3.07 <sup>b</sup>	7.10 <sup>a</sup>	0.60	0.001	0.557	0.062	0.004	<0.001	
Isovaleric acid	0.99	0.93	0.93	1.02	0.95	0.10	0.680	0.785	0.609	0.222	0.559	
Valeric acid	1.09 <sup>b</sup>	1.29 <sup>b</sup>	0.98 <sup>b</sup>	1.15 <sup>b</sup>	2.17 <sup>a</sup>	0.20	0.006	0.194	0.104	0.023	0.005	
Acetic:propionic acid ratio	2.87	3.41	3.29	3.35	3.26	0.274	0.734	0.187	0.711	0.874	0.948	
Distal colon												
Acetic acid	62.92	56.15	49.28	52.30	78.13	8.43	0.171	0.680	0.278	0.159	0.071	
Propionic acid	24.88 <sup>ab</sup>	16.42 <sup>ab</sup>	14.86 <sup>b</sup>	16.64 <sup>ab</sup>	27.17 <sup>a</sup>	2.26	0.04	0.029	0.066	0.014	0.017	
Isobutyric acid	1.29	1.36	1.23	1.42	1.31	0.15	0.913	0.826	0.455	0.571	0.982	
Butyric acid	6.52 <sup>ab</sup>	4.35 <sup>bc</sup>	2.66 <sup>c</sup>	3.15 <sup>bc</sup>	8.65 <sup>a</sup>	0.78	<0.001	0.055	0.027	0.008	<0.001	
Isovaleric acid	1.13	1.32	1.05	1.33	1.34	0.12	0.365	0.372	0.317	0.250	0.269	
Valeric acid	2.06 <sup>a</sup>	1.39 <sup>b</sup>	1.11 <sup>b</sup>	1.31 <sup>b</sup>	2.12 <sup>a</sup>	0.24	0.026	0.046	0.280	0.070	0.038	
Acetic:propionic acid ratio	2.46	2.98	2.69	2.95	2.57	0.29	0.435	0.207	0.161	0.757	0.844	

CT = control treatment; FM = fish meal; SPC = soy protein concentrate.

<sup>a,b,c</sup> Within a row, values without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> Values are least squares means for 6 replicate pens per treatment. CT, basal diet with 17% CP; SPC19, 19% CP basal diet supplemented with soy protein concentrate; FM19, 19% CP basal diet supplemented with fish meal; SPC23, 23.7% CP basal diet supplemented with soy protein concentrate; FM23, 23.7% CP basal diet supplemented with fish meal.

<sup>2</sup> Diets, all 5 diets. Basal, control compared with supplemented diets. Source, additional soy or fish protein. Level, 19% and 23.7% CP. Source  $\times$  Level, the interaction of protein source and level.

**Table 5**  
Effects of different dietary protein sources and polyamines concentrations ( $\mu\text{g/g}$ ) in proximal colonic digesta and distal colonic digesta of weaned piglets.<sup>1</sup>

Item	CT	SPC19	FM19	SPC23	FM23	SEM	<i>P</i> -values <sup>2</sup>					
							Diets	Basal	Source	Level	Source $\times$ Level	
Proximal colon												
Putrescine	44.96 <sup>b</sup>	53.05 <sup>ab</sup>	36.52 <sup>b</sup>	60.83 <sup>ab</sup>	93.28 <sup>a</sup>	16.13	0.006	0.170	0.439	0.004	0.023	
Cadaverine	148.74	173.66	132.25	158.11	150.48	29.61	0.899	0.885	0.421	0.964	0.577	
Histamine	23.88 <sup>b</sup>	20.62 <sup>b</sup>	14.19 <sup>b</sup>	17.33 <sup>b</sup>	44.02 <sup>a</sup>	4.80	0.002	0.969	0.046	0.012	0.002	
Spermidine	13.20 <sup>b</sup>	12.14 <sup>b</sup>	9.78 <sup>b</sup>	12.88 <sup>b</sup>	19.61 <sup>a</sup>	1.41	0.003	0.805	0.142	0.002	0.006	
Spermine	2.54 <sup>ab</sup>	2.14 <sup>ab</sup>	0.93 <sup>b</sup>	1.95 <sup>ab</sup>	7.36 <sup>a</sup>	1.18	0.014	0.679	0.098	0.019	0.014	
Distal colon												
Putrescine	26.26 <sup>b</sup>	31.78 <sup>b</sup>	15.49 <sup>b</sup>	26.99 <sup>b</sup>	49.49 <sup>a</sup>	5.69	0.011	0.473	0.593	0.021	0.004	
Cadaverine	90.64	90.68	70.52	59.95	83.14	15.25	0.525	0.347	0.922	0.561	0.176	
Histamine	17.54 <sup>c</sup>	25.67 <sup>bc</sup>	18.06 <sup>c</sup>	41.00 <sup>ab</sup>	67.25 <sup>a</sup>	5.57	<0.001	0.005	0.115	<0.001	0.008	
Spermidine	12.50 <sup>b</sup>	9.25 <sup>b</sup>	7.48 <sup>b</sup>	10.40 <sup>b</sup>	21.24 <sup>a</sup>	2.59	0.016	0.838	0.127	0.008	0.020	
Spermine	3.45 <sup>ab</sup>	1.36 <sup>b</sup>	3.86 <sup>ab</sup>	1.78 <sup>b</sup>	5.24 <sup>a</sup>	0.88	0.041	0.696	0.004	0.320	0.592	

CT = control treatment; FM = fish meal; SPC = soy protein concentrate.

<sup>a,b,c</sup> Within a row, values without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> Values are least squares means for 6 replicate pens per treatment. CT, basal diet with 17% CP; SPC19, 19% CP basal diet supplemented with soy protein concentrate; FM19, 19% CP basal diet supplemented with fish meal; SPC23, 23.7% CP basal diet supplemented with soy protein concentrate; FM23, 23.7% CP basal diet supplemented with fish meal.

<sup>2</sup> Diets, all 5 diets. Basal, control compared with supplemented diets. Source, additional soy or fish protein. Level, 19% and 23.7% CP. Source  $\times$  Level, the interaction of protein source and level.

histamine, polyamines were present at higher concentrations in the proximal rather than distal colon.

#### 4. Discussion

The present study was undertaken to assess the effect of level and source of dietary protein in the diet of early-weaned piglets without in-feed antibiotic or ZnO, and to examine the relationship between intestinal fermentation of protein and incidence of diarrhea. Some previous studies (Ball and Aherne, 1988; Wellock et al., 2006, 2008) have shown such an association. In pigs, microbial digestion of dietary fermentable fiber occurs in the large intestine but, as clearly shown in the present study, the extent of intestinal fermentation is clearly influenced by dietary protein, both level and source. The differences may reflect the completeness of gastric and small intestinal digestion and hence the delivery of fermentable substrates into the intestinal environment.

##### 4.1. Influence of the protein source and level on the fecal score and pH

The fecal score reflects the digestive health of piglets. A high score indicates high incidence of diarrhea. The piglets fed 17% CP diet for a 2-wk period after weaning had a lower fecal score (firmer feces) than did piglets fed the diets with higher CP levels (19% to 23.7% CP), as per recommendations. This finding is consistent with previous studies (Nyachoti et al., 2006; Htoo et al., 2007; Yue and Qiao, 2008; Heo et al., 2010). No difference was found between piglets fed diets supplemented with fish meal or soy protein concentrate.

The pH of digesta could affect the host's health through influencing enteric pathogens. Reducing the intestinal pH can inhibit the growth of harmful bacteria in the intestine and promote the propagation of lactic acid bacteria (Smith and Jones, 1963). Decreasing dietary protein content from 23% to 17% reduced the pH of ileac digesta (Nyachoti et al., 2006). The stomach cannot produce

enough HCl, which is one cause of gastrointestinal disease in early weaned piglets (Cranwell, 1995). In contrast, the pH of ileac and cecal digesta was not affected when the pigs were fed high- or low-CP diets (Htoo et al., 2007). In the present study, the pH in the proximal colon of pigs fed the 17% CP diet was lower than that of other diets. High dietary protein levels increased the pH of colonic digesta, and diets supplemented with fish meal resulted in higher pH than those supplemented with soy concentrate protein. The reason may be that diets with more fish meal were more fermentable in the intestine, and produced more bacterial metabolites (VFA, polyamines and NH<sub>3</sub>).

#### 4.2. Influence of the protein source and level on colonic concentrations of VFA

The intestinal production of VFA reflects the fermentable fiber content of the diet and the microbial activity of intestinal contents (Williams et al., 2007). Increasing the dietary protein level raised the concentration of VFA in the intestinal tract of piglets (Nyachoti et al., 2006; Htoo et al., 2007). In contrast, some studies showed that dietary protein level did not affect the concentration of VFA (Hermes et al., 2009; Heo et al., 2010; Zhou et al., 2015). In the present study, moderate CP content (19%) of the diet supplemented with fish meal reduced the content of butyric acid in the distal colonic digesta, and high protein content (23.7%) using fish meal increased propionic acid and butyric acid at this location. The VFA are the fermented products of both proteins and carbohydrates in the digestive tract (Le et al., 2005), and concentrations were higher with the FM23 diet. There were no differences between protein sources at the lower level of supplementation (19% CP). The reason may be that the different fermentable fiber content of the diets is a more significant factor than the dietary protein level for determining the molar proportions of VFA in the colonic digesta of piglets (Hermes et al., 2009).

#### 4.3. Influence of the protein source and level on concentrations of polyamines and ammonia-N

The fermentation of protein in the large intestinal is considered to be a potential risk for the proliferation of pathogenic bacteria, and it stimulates intestinal dysbiosis (Ball and Aherne, 1988). Polyamines and ammonia-N are the main metabolites of protein fermentation, and high concentrations of these increase the incidence of diarrhea and are toxic to intestinal epithelial cells (Porter and Kenworthy, 1969; Dong et al., 1996). The polyamines are formed by decarboxylation of their corresponding amino acid precursors (Urlings et al., 1992). Increasing putrescine and cadaverine production by intestinal bacteria is associated with diarrhea of weanling pigs (Teti et al., 2002).

In the present study, the concentrations of ammonia-N, putrescine, histamine and spermidine in the proximal and distal colonic digesta of pigs fed the FM23 diet were higher than those of pigs fed the 17% CP diet, but a lesser effect was noted for diets supplemented with soy concentrate. These findings are not completely consistent with results of other similar studies. Bikker et al. (2006) reported that increasing the dietary protein level from 15.2% to 21.6% did not affect the ammonia concentration of colonic digesta, but increased the ammonia concentration of ileac digesta. Htoo et al. (2007) reported that reduced dietary CP content reduced the ammonia-N and putrescine concentrations of cecal digesta. Similarly, Schneider et al. (1989) also found that lower dietary protein level reduced the concentrations of polyamines in the large intestine of pigs. The present results suggest that diets supplemented with fish meal are more fermentable than diets containing additional soy concentrate protein.

## 5. Conclusion

The present study demonstrated that a weaning diet without in-feed antibiotics or ZnO and with a low content of CP reduced the fecal score of piglets, and the effect was not completely associated with protein fermentation in the colon. In addition, at a given level of CP, diets supplemented with fish meal rather than soy protein concentrate were more fermentable in the large intestine, yet they did not obviously associate with the fecal score.

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