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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.

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*, 40 min, 4 °C). Supernatant (1 mL), 0.2 mL internal standard (42 mmol/L crotonic acid, Sigma), and 0.2 mL 10% H_3PO_3 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, λex = 265 nm, λem = 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

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