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Original research article

Effects of protein sources and levels in antibiotic-free diets on diarrhea, intestinal morphology, and expression of tight junctions in weaned piglets

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ABSTRACT

This study examined effects of dietary protein sources and levels on intestinal health of 21 to 35 d-old weaned piglets fed antibiotics-free diets. A total of 150 weaned piglets (21 d of age) were allotted to 5 dietary treatment groups. Diets were formulated, based on corn-soybean meal, with different protein sources (fish meal and soy protein concentrate) to provide different dietary CP levels. Piglets within 5 dietary treatments were fed diets as follows, respectively: 1) control diet of 17% CP (control); 2) 19% CP diets formulated with more soy protein concentrate (SPC19); 3) fish meal (FM19); 4) 23.7% CP diets formulated with more soy protein concentrate (SPC23); 5) fish meal (FM23). The results showed that piglets from control group had higher ADG and lower incidence of diarrhea compared with those of other groups ($P < 0.05$). The incidence of diarrhea of piglets in FM19 group was lower than those from SPC23 group and FM23 group ($P < 0.05$). With the higher CP levels, villous height and villous height to crypt depth ratio of piglets in the duodenum and jejunum were decreased ($P < 0.05$), but crypt depth was increased ($P < 0.05$). Comparing control group and other groups, we found the expression of inflammatory cytokines interleukin-1β (IL-1β) and interferon-γ (IFN-γ) were increased ($P < 0.05$) in the jejunum and colon of piglets, as did cystic fibrosis transmembrane conductance regulators (CFTR) in the distal colon. The relative transcript abundance of Zonula occludens-1 (ZO-1) in the jejunum, and occludin in the jejunum and ileum of piglets fed 23.7% CP diets were reduced compared with those fed control diet ($P < 0.05$). In conclusion, the 17% CP diet without in-feed antibiotics helped improve growth performance and relief of diarrhea of 21 to 35 d-old weaned piglets. Dietary CP level, rather than its source (either fish meal or soy protein concentrate), has more significant impacts on the growth performance and intestinal health of 21 to 35 d-old weaned piglets when fed antibiotics-free diets.

1. Introduction

Early weaning of piglets is often associated with gut disorders such as mucosal inflammation (Pié et al., 2004), intestinal barrier dysfunction (Wijtten et al., 2011) and diarrhea (Caspary, 1992; van Beers Schreurs et al., 1992). In-feed antibiotics in weaning diets have been used as preventative measures to alleviate these problems for decades (Cromwell, 2002). Increased concerns about negative effects of antibiotics (Chen et al., 2005; Jensen, 2006), such as antibiotic-resistant bacteria have led to a partial and then a total ban on the preventive use of antibiotics in feed (Wierup, 2001; Gallois et al., 2009). Accordingly, the control of post-weaning

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diarrhea requires alternative strategies other than dietary antibiotics.

Both dietary protein levels and sources are important causes of diarrhea in weaned piglets (Pluske et al., 2002). Evidence shows that feeding a low-protein diet (< 18% CP) in the post-weaning period improves fecal consistency and intestinal health (Ball and Aherne, 1982; Wellock et al., 2006; Kong et al., 2007), However, these results are based on antibiotic diets. Despite the protective effects of lower-protein diet, lower-protein diet-fed pigs compromised growth performance compared with pigs fed a higher-protein diet (Nyachoti et al., 2006; Wellock et al., 2006; Yue and Qiao, 2008). Previous studies showed that feeding diets containing 19 to 23% CP could help meet the growth needs of weaning piglets (Htoo et al., 2007; Opapeju et al., 2009). Therefore, it is important to determine whether performance and intestinal health would be affected in piglets if dietary CP is increased from 17.3% without in-feed antibiotics. In addition, weaned piglets fed animal protein sources appear to have a superior feeding value than plant protein sources, which partly due to the plant proteins are less digestible than animal proteins (Yu et al., 2002; Yue and Qiao, 2008). For that reason, this study investigated the effects of dietary CP levels (17, 19, and 23.7%) and protein sources on the growth performance and intestinal health in weaned piglets without feeding any antibiotics growth promoters.

2. Materials and methods

2.1. Animals, diets, and housing

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

A total of 150 male weaned piglets (Duroc × Landrace × Large White, 21 d of age, initial BW 5.99 ± 0.14 kg) were randomly assigned in the balance of BW to 1 of 5 treatments, each with 6 replicates (pens) of 5 piglets.

Diets (Table 1) were formulated to provide 17% CP (control), 19% CP (Chinese recommended level Ministry of Agriculture of the People's Republic of China, 2004), and 23.7% CP (NRC recommended level NRC, 2012), respectively, with increasing dietary percentages of soy protein concentrate (SPC19 and SPC23) or fish meal (FM19 and FM23) for the latter two higher CP diets. Control diet contained 17.5% soybean meal and 3% fish meal. All diets were pelleted without any in-feed antibiotics growth promoters and the levels of all essential amino acids met or exceeded the standard of NRC (2012).

The experiment lasted for 2 weeks. The experiment was conducted during summer with an average room temperature of 31 ± 2°C. Water and feed were provided ad libitum throughout the 14-d study. Piglets were weighed at the beginning and the end of the experiment and daily feed intake was recorded with each replicate. Appearance and behaviour of the animals and occurrence of diarrhea were checked daily to evaluate the health status of piglets. Fecal consistency was assessed visually and classified at 4 levels as described previously (Liu et al., 2010): 0, normal; 1, pasty; 2, semi-liquid; and 3, liquid. The piglets were considered to have diarrhea when the fecal consistency was at level 2 or 3, and the incidence of diarrhea was calculated using the number of pig days with diarrhea in each pen as percentage of total pig days during that time interval.

2.2. Slaughter procedure and sampling

On the final day of the experiment, one piglet with BW close to the average weight was chosen from each pen, fasted overnight, blood sampled from the anterior vena cava into heparinised vacutainers, then killed by an intravenous injection of sodium pentobarbital (50 mg/kg BW, Sigma). Blood was centrifuged (800 × g, 10 min, 4°C) and plasma samples were held at −80°C until analysis.

Following killing and a midline abdominal incision, the pyloric valve, ileocaecal junction and distal colon were tied to prevent mixing of digesta, and digesta was collected from the distal colon.

The separation of intestinal tract was according to the methods described by Yang et al. (2014) with slight modifications. Briefly, the entire intestinal tract was removed and divided into 4 segments: duodenum, to about 10 cm distal to the pylorus; jejunum, the middle portion; ileum, about 5 cm proximal to the ileocaecal junction; colon, the distal section. The segments were cut longitudinally to expose mucosa and washed three times with ice-cold phosphate buffered saline (PBS). Mucosa from the jejunum, ileum and colon was scraped with glass slides, snap-frozen in liquid nitrogen and stored at −80°C until for further use. Finally, three 2 × 2 cm sections from consistent locations in the duodenum, jejunum, and ileum were fixed in 10% neutral formalin for morphometric analysis.

Table 1
Composition and nutrient level of the experimental diets (as dry-matter basis).[1]

Item	Control	SPC19	FM19	SPC23	FM23
Ingredients, %					
Corn	62.09	59.67	60.22	53.33	53.71
Extruded soybean meal	17.50	17.50	17.50	17.50	17.50
Fish meal	3.00	3.00	7.37	3.00	16.20
Soy protein concentrate		4.36		13.41	
Whey powder	10.00	10.00	10.00	10.00	10.00
Soybean oil	2.85	1.75	2.00		1.35
L-Lys·HCl	0.70	0.48	0.41	0.04	
DL-Met	0.22	0.15	0.12		
L-Thr	0.23	0.13	0.11		
L-Trp	0.06	0.02	0.03		
L-Ile	0.16	0.03	0.04		
L-Val	0.20	0.08	0.06		
L-His	0.03				
L-Phe	0.05				
Premix[2]	0.16	0.16	0.16	0.16	0.16
Dicalcium phosphate	0.79	0.73	0.06	0.61	
Limestone	0.96	0.94	0.92	0.90	0.08
Acidifier	0.30	0.30	0.30	0.30	0.30
TiO_2	0.40	0.40	0.40	0.40	0.40
NaCl	0.15	0.15	0.15	0.15	0.15
$NaHCO_3$	0.15	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrient levels, %					
Crude protein (analyzed)	17.00	19.00	19.00	23.70	23.70
DE, MJ/kg	3.40	3.40	3.40	3.40	3.40
Ca	0.80	0.80	0.80	0.80	0.80
Available P	0.40	0.40	0.40	0.40	0.63
Lys	1.36	1.35	1.35	1.36	1.48
Met + Cys	0.77	0.77	0.76	0.76	0.82
Thr	0.86	0.87	0.86	0.95	0.99
Trp	0.25	0.24	0.25	0.30	0.28
Arg	0.94	1.08	1.10	1.37	1.42
Val	0.92	0.93	0.92	1.09	1.12
Leu	1.40	1.59	1.60	1.98	1.98
Ile	0.74	0.73	0.73	0.95	0.91
His	0.43	0.47	0.47	0.61	0.61
Phe	0.81	0.89	0.87	1.15	1.08

SPC = soy protein concentrate; FM = fish meal.

[1] Control = control diet of 17% CP; SPC19 = 19% CP diets formulated with more soy protein concentrate; FM19 = 19% CP diets formulated with more fish meal; SPC23 = 23.7% CP diets formulated with more soy protein concentrate; FM23 = 23.7% CP diets formulated with more fish meal.

[2] Provided per kilogram of diet: vitamin A, 11,000 IU; vitamin D_3, 1,100 IU; vitamin E, 250 IU; vitamin K, 2.5 mg; vitamin B_{12}, 87.5 μg; vitamin B_1, 5 mg; vitamin B_2, 16.5 mg; nicotinamide, 75 mg; pantothenate, 50 mg; folic acid, 1.5 mg; vitamin B_6, 50 mg; biotin, 250 μg; choline chloride, 2.5 mg; Fe ($C_4H_2FeO_4$), 100 mg; Cu ($CuSO_4 \cdot 5H_2O$), 6 mg; Mn ($MnSO_4 \cdot H_2O$), 4 mg; Zn ($ZnSO_4 \cdot H_2O$), 100 mg; I [Ca $(IO_3)_2$], 0.14 mg; Se (Na_2SeO_3), 0.30 mg; Co ($CoSO_4 \cdot 7H_2O$), 6 mg.