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Effects of chito-oligosaccharide on intestinal mucosal amino acid profiles and alkaline phosphatase activities, and serum biochemical variables in weaned piglets

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ABSTRACT

The present experiment was conducted to determine the effects of chito-oligosaccharide (COS) on intestinal mucosal amino acid profiles and alkaline phosphatase (ALP) activities, and serum biochemical variables in weaned piglets. A total of 24 piglets (BW = 7.82 ± 0.21 kg) were weaned at 25 d of age and were blocked by body weight, sex, and litter and randomly assigned to one of two treatments consisting of a basal diet (CON) or the basal diet supplemented with 30 mg/kg COS for a 14-d period. Each treatment was assigned to 6 pens (2 piglets/pen) and 6 piglets (3 males and 3 females) were randomly selected from each treatment (1 pigs/pen) for blood and tissue sampling. Dietary supplementation with COS increased ($P < 0.05$) serum IgG and urea nitrogen contents, and tended to increase ($P < 0.10$) serum calcium. The ileal mucosal ALP activity in piglets fed with COS diet were greater ($P < 0.05$) than that in CON piglets. Dietary supplementation with COS increased ($P < 0.05$) the contents of Asn and Cys, and tended to increase ($P < 0.10$) the contents of Asp and Orn in the small intestinal mucosa of weaned piglets. Moreover, the contents of short chain fatty acid (SCFA) in caecal and colonic digesta of weaned piglets were affected ($P < 0.05$) by dietary COS supplementation. There were interactions ($P < 0.05$) between dietary COS and intestinal section for mucosal AA contents and digesta SCFA contents in weaned piglets. In conclusion, the results of the present experiment suggest that dietary supplementation with COS affects intestine and immune functions of weaned piglets.

1. Introduction

Weaned piglets often face post-weaning challenges, including low feed intake, intestinal dysfunction, and growth retardation (Blecha and Charley, 1990; Pluske et al., 1997). Antibiotic growth promoters (AGP) have been applied to piglet diets to solve post-weaning problems for several decades (Gong et al., 2014; Wang et al., 2015). However, the use of AGP was reported with potential antibiotic residues and bacterial resistance, and many countries (e.g. European) have already banned antibiotics used as growth promoters (Van den Bogaard and Stobberingh, 2000). Chito-oligosaccharide (COS), which can be obtained by chemical and enzymatic hydrolysis of poly-chitosan, was reported to have a role in improving growth performance of weaned piglets and was considered as a potential alternatives to AGP (Singla and Chawla, 2001; Tang et al., 2005; Yin et al., 2008; Sun et al., 2009; Huang et al., 2005, 2007, 2016; Xiao et al., 2013a, 2014; Xiong et al., 2015d). The COS has been shown to have beneficial effects on intestinal microbial population, with increasing Lactobacilli and

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decreasing Escherichia coli, of weaned piglets (Kong et al., 2014; Han et al., 2007). The intestinal morphology, barrier function, antioxidant capacity, and short chain fatty acid (SCFA) contents of weaned piglets were affected by dietary supplementation with COS (Yang et al., 2012; Zhou et al., 2012; Kong et al., 2014; Xiong et al., 2015a). Moreover, the growth performance, apparent digestibility, diarrhea incidence of weaning piglets were also improved by dietary supplementation of COS (Han et al., 2007; Liu et al., 2008; Chen et al., 2009; Yang et al., 2012). Tang et al. (2005) showed that serum contents of growth hormone and insulin-like growth factor-I were increased when weaned piglets were given a diet with 250 mg/kg COS. These results indicated that COS benefited intestinal microecology, morphology and functions, and growth performance of weaned piglets. The intestinal mucosa of piglets has high rate of amino acid (AA) catabolism and alkaline phosphatase (ALP) is regarded as a key marker enzyme when considering intestinal primary digestive and absorptive functions changes (Hodin et al., 1995; Stoll et al., 1998). In the present experiment, we hypothesized that dietary supplementation with COS would affect intestine mucosal AA profiles and ALP activities, and serum biochemical variables of weaned piglets. Therefore, the objective of the present study was to determine intestinal mucosal AA profiles and ALP activities, and serum biochemical variables in weaned piglets fed a control or COS-supplemented diet.

2. Materials and methods

The experimental design and procedures in the present study were reviewed and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Science (Yin et al., 2011).

2.1. Animals and experimental treatments

A total of 24 piglets (Duroc × Landrace × Yorkshire, BW = 7.82 ± 0.21 kg) were weaned at 25 d of age. The piglets were blocked by body weight (BW), sex, and litter and randomly assigned to one of two treatments consisting of a basal diet (CON) or the basal diet supplemented with 30 mg/kg COS for a 14-d period (Li et al., 2009). The basal diet met the National Research Council (2012) nutrient specifications for 5–10 kg BW pigs as described by Xiong et al. (2015b) (Table 1). Each diet had 6 pens with 2 piglets/pen (1 male and 1 female). Piglets had free access to feed and drinking water at all times throughout the experimental period (Fang et al., 2009). The COS used in the present experiment was produced by the enzymolysis of crab shell using chitinase, cellulase, and chitosanase after the crab shell was powered and treated by acetic acid. The COS composed of several chitosan oligomer with molecular weigh about 800–2500 Da. The water solubility and the sugar content of the COS products was more than 99% and 85%, respectively.

Table 1
Ingredient and chemical composition of the basal diet (as-fed basis).

Ingredient	Content, %
Wheat	24.5
Extruded corn	28.5
Spray-dried whey	10
Extruded soybean	13.8
Soybean meal	10.3
Fish meal	4.5
Limestone	0.6
Monocalcium phosphate	0.6
Salt	0.30
L-Lys · HCl	0.4
Soy oil	3
Vitamin and mineral premix[a]	3.5
Calculated analysis	
NE, kcal/kg	2500
CP, %	20
Total Lys, %	1.40
Total Met, %	0.35
Total Thr, %	0.87
Ca, %	0.70
Available P, %	0.43

[a] The vitamin-mineral premix supplied per kilogram of feed: 10,000 IU of vitamin A (trans-retinyl acetate), 1,000 IU of vitamin D3 (cholecalciferol), 80 IU of vitamin E (all-rac-tocopherol acetate), 2.0 mg of vitamin K3 (menadione dimethylpyrimidinol bisulfite), 0.03 mg of vitamin B_{12}, 12 mg of riboflavin, 40 mg of niacin, 25 mg of D-pantothenic acid (D-Ca pantothenate), 0.25 mg of biotin, 1.6 mg of folic acid, 3.0 mg of thiamine (thiamine mononitrate), 2.25 mg of pyridoxine (pyridoxine HCl), 300 mg of choline (choline chloride), 150 mg of Fe (ferrous sulfate), 100 mg of Zn (zinc oxide), 30 mg of Mn (manganese sulfate), 25 mg of Cu (copper sulfate), 0.5 mg of I (potassium iodate), 0.3 mg of Co (cobalt sulfate), 0.3 mg of Se (sodium selenite), and 4.0 mg of ethoxyquin.

2.2. Sample collection and plasma metabolite analysis

At 39 d of age, six piglets (3 males and 3 females) were randomly selected from each treatment (1 pigs/pen) for blood and tissue sampling. Blood samples were collected into 10 mL tubes via jugular vein puncture and centrifuged at 3000 × *g*, 4 °C for 10 min to recover serum (Tan et al., 2012). Serum samples were immediately stored at −80 °C until required for measuring biochemical variables (Xiao et al., 2013b). Piglets were held under general anaesthesia (C3H2ClF5O) and euthanized by an intravenous (jugular vein) injection of 4% sodium pentobarbital solution (40 mg/kg BW). The gastrointestinal tract was removed immediately, dissected, and divided into 6 segments: stomach, duodenum, jejunum, ileum, caecum, and colon. The contents of caecum and colon were collected, and immediately frozen in liquid nitrogen and stored at –80 °C until required for measuring SCFA contents. Intestinal tissue from the middle sections of the duodenum, jejunum, and ileum (approximately 20 cm of each tissue) were aseptically isolated, flushed with phosphate-buffered saline, and then the mucosal sampling were collected (Xiong et al., 2015a,b). Mucosal tissue samples were immediately frozen in liquid nitrogen and stored at –80 °C until required for the analysis of intestine mucosal AA profiles and ALP activities (Xiong et al., 2016; Kong et al., 2009a,b). Serum contents of total protein, IgG, IgM, urea nitrogen, ammonia, glucose, phosphorus, calcium, cholesterol, and triglycerides were measured using Biochemical Analytical Instrument (Beckman CX4) and commercial kits (Sino-German Beijing Leadman Biotech Ltd, Beijing, China; Yang et al., 2013).

2.3. Amino acids contents in jejunal and ileal mucosa

To measure the AA contents in jejunal and ileal mucosa, about 0.2 g of liquid nitrogen pulverized samples were homogenized with 1 mL of an ice-cold PBS on ice for 3 min, and then centrifuged at 3000 × *g* and 4 °C for 10 min. The supernatants (10 μL) were used for analyzing protein content using the Bicinchoninic Acid assay (Beyotime biotechnology, China). The remaining supernatant was deproteinized using 7.5% trichroacetic acid and then centrifuged at 12,000 × *g*, 4 °C for 15 min. The supernatant was also