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Effects of dietary levels of chito-oligosaccharide on ileal digestibility of nutrients, small intestinal morphology and crypt cell proliferation in weaned pigs

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ABSTRACT

There is continued need for novel agents to improve intestinal function in weaned pigs. Some evidence suggests that chito-oligosaccharide (COS) supplements may enhance pigs' intestinal function after weaning. The present study was designed to examine the effect of COS supplementation on growth performance, nutrient digestibility and small intestinal functions in weaned pigs as an effective alternative to antibiotic addition in post-weaning diets. For the experiment, weanling pigs were divided into 5 groups (13–14 animals per group) and received either a basal diet, a supplemented diet with 75, 150 or 225 mg/kg COS, or a supplemented diet with 110 mg/kg lincomycin for 56 days. Growth, feed efficiency, hematological and biochemical profiles, nutrient's ileal digestibility, small intestinal morphology and crypt cell proliferation were measured at 28 and 56 days of the experiment. Pigs supplemented with 150 mg/kg COS or lincomycin showed: (i) consistently more digestible ileal contents (e.g. crude protein, crude fat, ash, calcium, and phosphorus), (ii) increased absorption capacity (e.g. increased villus height and the villus height/crypt depth ratio for three intestinal segments) on day 28 of the experiment and (iii) more active cell division (as indicated by Ki-67 marker of duodenal and jejunal crypt cells) on day 56 of the experiment ($P < 0.05$, respectively). These data suggest that 150 mg/kg COS might be a useful dietary supplement to promote nutrient absorption and digestibility efficiency.

1. Introduction

After weaning, piglets are exposed to nutritional, social and environmental stresses that lead to morphological and functional adaptations in the gastrointestinal tract (Pluske et al., 1997). Post-weaning stressors can affect feed intake, growth performance, and predisposition to diseases (Lalles et al., 2007). In many countries, antibiotics are added to pig starter diets as growth promoters and to prevent infections. However, antibiotic addition to animal feed has been prohibited or limited in many nations due to antibiotic-resistance which threatens human health (Smith et al., 2002). Therefore, alternative strategies to improve weaned pigs' health and performance while satisfying consumer safety and farm's profitability must be sought (Gong et al., 2014).

Chito-oligosaccharide (COS) is a D-glucosamine oligomer produced via hydrolysis of polymeric chitosan. In weanling pigs, COS appears to be effective in enhancing intestinal health and immune response by modulating the balance of intestinal microbiota (Huang et al., 2007; Liu et al., 2010; Tang et al., 2005; Walsh et al., 2012; Yin et al., 2004). Some studies suggested that dietary COS supplementation could be affecting metabolic response, small intestinal morphology, nutrient digestibility and growth performance in weaners (Chen et al., 2009; Han et al., 2007; Huang et al., 2016; Liu et al., 2008; Xiao et al., 2014; Xie et al., 2015; Xiong et al., 2015; Yang et al., 2012; Zhou et al., 2012). Moreover, findings in some studies suggest that the effect of COS might be dependent to the supplementation level in starter diets. Due to its low molecular weight (MW), COS might be mediating changes in the activity of some digestive enzymes thus improving apparent digestion of most nutrients and intestinal health based on results from a previous study in poultry (Wang et al., 2015). However, COS mechanism of action is not fully understood and COS-mediated changes in cellular function and cellular markers remain to be studied.

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The hypothesis of the present study was that COS supplementation in starter diets could be used as alternatives to antibiotics in feed for swine production by modifying gut function leading to improve nutrient bioavailability in the small intestine, thus improving piglets' health and performance. Therefore, the objective of the present study was to test COS supplementation as a growth promoter alternative to antibiotic addition in post-weaning diets and its effect in pigs' gut function.

2. Materials and methods

All procedures described in this experiment were approved by the Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University.

2.1. Preparation of COS

The COS supplement used in this experiment was produced by the Department of Biochemistry, Faculty of Science, Chulalongkorn University, Thailand. The COS was obtained by enzymatic hydrolysis of the chitosan present in shrimp shell. A mixture of chitinase and chitosanase were used. Briefly, shrimp shell was deproteinized by soaking in 1 L of 1 *N* NaOH solution for 24 h, and then demineralized by soaking in 1 *N* HCl for a further 24 h. Pigments and other lipid soluble substances were removed by extraction with 95% ethanol at 75 °C. The resultant product was deacetylated in 50% (w/w) NaOH solution until more than 90% degree of deacetylation (DD) was achieved. The chitosan product was then solubilized in 1% acetic acid solution and subjected to enzymatic hydrolysis. The COS was purified by precipitation with NaOH and washed with distilled water until the pH was neutral. In this study, the lyophilized COS was solubilized in an appropriate volume of 1% acetic acid solution. Its average MW determined by gel permeation chromatography was about 7.5 kDa.

2.2. Animals and experimental design

Sixty-eight female piglets (Large WhitexLandracexDuroc) weaned at 18 days of age, were purchased from a local commercial swine farm and housed in individual cages at the Animal Nursery Facility, Faculty of Veterinary Science, Chulalongkorn University. The average initial body weight of animals was 5.68 ± 0.07 kg. Individually housed pigs constituted the experimental unit. All weaners received a basal experimental diet for 2 days, during the acclimatization period prior to the start of the trial. The pigs were randomly allocated into one of the five dietary treatment groups with 13 or 14 animals per treatment and fed for 56 days. All animals received the same basal diet during the experiment (Table 1). Treatment groups differed only in their COS or antibiotic supplementation level: (1) Control diet, no COS or lincomycin supplementation; (2) 75 mg/kg of COS supplementation; (3) 150 mg/kg of COS supplementation; (4) 225 mg/kg of COS supplementation; and (5) 110 mg/kg of lincomycin supplementation. Ileal nutrient digestibility was estimated by adding 0.25% chromic oxide (Cr_2O_3) to all the experimental diets for 5 days prior to collection day (days 28 and 56). Pigs had ad libitum access to water and feed. All experimental diets were provided as a meal formulated to contain similar nutrient contents and to meet or exceed the NRC (2012) recommendations for post-weaning pigs. The proximate analysis of diets and ileal digesta for dry matter, crude protein, crude fiber, ether extract, ash, calcium and phosphorus contents were performed according to the AOAC (2006) procedures. Gross energy (GE) content of the diet was measured by a bomb calorimeter (Leco corporation, USA). During the 56 days of the study, the pigs were weighed weekly, and feed intake was recorded daily by weighing feed at 0800 h and again at 1800 h. Body weight gain (BWG), average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G: F) were computed on a weekly basis to monitor pigs' growth and feed efficiency. Mortality was monitored daily throughout the experiment.

Table 1
Ingredients, calculated and measured compositions of experimental pig diets (*as*-fed basis).

Indices	Amount
Ingredient, %	
Broken rice	40.00
Full-fat soybean	25.00
Ground corn	18.00
Fish meal, 58% crude protein	5.00
Soybean meal, 44% crude protein	3.60
Skim milk	6.00
Dicalcium phosphate	1.70
Vitamin mineral premix[a]	0.35
Sodium chloride	0.35
Calculated composition[b]	
Crude protein, %	22.00
Crude fat%	4.70
Crude fiber, %	3.43
Calcium, %	0.85
Available phosphorus, %	0.82
Tryptophan, %	0.28
Methionine + Cystine, %	0.66
Lysine, %	1.30
Metabolizable energy, kcal/kg	3323.23
Measured composition	
Dry matter, %	90.66
Crude protein, %	22.58
Ether extract, %	5.43
Crude fiber, %	4.01
Calcium, %	0.82
Phosphorus, %	0.77
Ash, %	6.59
Gross energy, kcal/kg	4222.80

[a] Provided the following per kilogram of diet: vitamin A, 8400 IU; vitamin D3, 945 IU; vitamin E, 0.0126 g; vitamin K, 0.0021 g; vitamin B1, 0.0011 g; vitamin B2, 0.0022 g; vitamin B6, 0.0016 g; vitamin B12, 0.02 mg; nicotinic acid,0.0126 g; pantothenic acid 0.063 g; folic acid, 0.0053 g; biotin, 0.0315 mg; choline, 0.1750 g; Cu, 0.1260 g; Fe, 0.1050 g; Mn, 0.0210 g; Co, 0.0007 g; I, 0.0007 g; Zn, 0.07 g; Se, 0.00007 g.
[b] Calculated values according to NRC (2012).

2.3. Hematology, total protein and blood urea nitrogen sample collection and analysis

On days 28 and 56 of the experiment, two blood samples were randomly collected from four to five pigs per treatment group via puncture of anterior vena cava into a vacutainer tube coated with or without anticoagulant (heparin) as appropriate. Blood sampling was performed from 0900 to 1000 h. The blood samples were immediately placed on ice and centrifuged at 2000*g* for 10 min at 4 °C to obtain plasma or serum and immediately stored at −20 °C until analysis. The white blood cells and red blood cells in whole blood were counted using an automatic blood analyzer (Coulter 1890, Diamond Diagnostic Inc. Holliston, USA). Concentrations of total plasma protein (TP) and serum blood urea nitrogen (BUN) were determined by an automatic biochemistry analyzer (Lysis ID B0567, Italy).

2.4. Ileal digesta sample collection and analysis

After blood samples were collected at 28 and 56 days of the experiment, three pigs from each group were sedated (i.m. azaperone, 6 mg/kg BW) and euthanized (i.v. pentobarbital, 50 mg/kg BW) to collect stomach digesta (for pH measurement), ileal digesta (for apparent nutrient digestibility) and small intestinal tissue (for histomorphology and immunohistochemistry determinations). The pH of the digesta samples from the stomach were determined immediately postmortem using a laboratory pH meter (Mettler Toledo Instrument Co., Ltd., USA). The ileal digesta from each pig was obtained immediately postmortem and stored at −20 °C until the analysis was