



Nutrient digestibility, blood profiles and fecal microbiota are influenced by chitooligosaccharide supplementation of growing pigs

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ABSTRACT

This study was conducted to evaluate the effects of dietary chitooligosaccharide (COS) supplementation on growth performance, nutrient digestibility, blood profiles and fecal microbiota in growing pigs. A total of 144 [(Landrace × Yorkshire) × Duroc] pigs with an initial body weight of 23.6 ± 1.1 kg were allotted to one of the following dietary treatments: 1) basal diet; 2) basal diet with 44 mg/kg of tylosin (100 mg/kg tylosin); 3) basal diet with 5 g/kg of COS and 4) basal diet with 5 g/kg COS and 44 mg/kg tylosin. There were nine replications per treatment with four pigs per pen. Throughout the experiment, pigs that were treated with a combination of COS and tylosin had a lower ADFI ($P=0.02$) and higher gain/feed ratio ($P<0.05$) than the other treatments. In addition, administration of either COS or tylosin alone significantly increased the digestibility of dry matter, nitrogen and gross energy ($P<0.05$). The red blood cell (RBC) and white blood cell (WBC) counts, as well as the serum albumin concentrations were not affected by COS or tylosin supplementation. However, the lymphocyte proportion and serum total protein concentration were increased in pigs fed tylosin supplemented diets compared with those pigs fed diets not supplemented with tylosin ($P<0.05$). Administration of tylosin significantly increased serum IgG concentration ($P=0.02$); however, treatment with COS or tylosin supplementation had no effect on the total cholesterol or triglyceride concentrations. The serum HDL cholesterol concentration was significantly increased in pigs treated with COS ($P=0.02$) compared to the pigs fed diets without COS. The COS administration also decreased the number of fecal *Escherichia coli* ($P<0.01$), whereas the number of fecal *Lactobacilli* was not influenced by either COS or tylosin administration. Results of the current study indicate that dietary supplementation of COS can improve nutrient digestibility and haematological profiles, as well as decrease of fecal *E. coli* populations in growing pigs.

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1. Introduction

Antibiotic growth promoters (AGP) have been widely used in the livestock industry for several decades due to their excellent health and growth promotion properties. However, the use of AGP is associated with potential antibiotic residues and the generation of drug resistance. Therefore, the AGP has been discontinued in many countries. To date, the European Union has completely banned the use of AGP, and research for alternatives to the use of AGP in the livestock industry is developing. In 1986, discontinuation of the use of AGP in

Sweden led to serious piglet production problems, followed by an increased use of therapeutic antibiotics (Wierup, 2001). Such experiences have demonstrated that alternatives to AGP should be considered carefully before any widespread changes in production are implemented.

Chitosan, which is one of the richest natural macromolecular compounds on earth, is a polyglucosamide that is primarily extracted from the shells of crustaceans (such as shrimp and crabs), as well as the cell walls of fungi. Recently, it has been suggested that chitooligosaccharide (COS), derived by chemical and enzymatic hydrolysis of chitosan, has a higher activity and more physiological functions than chitosan due to its lower molecular weight. The COS has been shown to have antibacterial activity (Jeon et al., 2000; Liu et al., 2008). Liu et al. (2008)

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investigated that effect of COS supplementation on intestinal structure and fecal shedding of total *Escherichia coli* and Lactobacilli in weaning pigs and found that Lactobacilli were increased while *E. coli* were decreased when COS was used at a level of 200 mg/kg. In addition, it has been reported that administration of COS to livestock improves gut health and immunity (Sekiguchi et al., 1994; Okamoto et al., 2003), regulates blood lipid content (Hayashi and Ito, 2002), and increases the concentration of serum growth hormone and insulin-like growth factor-1 (Tang et al., 2005). However, the results of studies evaluating the effects of COS have been inconsistent. Therefore, this study was conducted to 1) determine the effects of dietary COS supplementation on growth performance, nutrient digestibility, blood profiles and fecal microtiota in growing pigs and 2) evaluate whether COS can be an alternative substance to antibiotics.

2. Materials and methods

2.1. Experimental design, animals and diets

The experimental protocol of the current study was approved by the Animal Care and Use Committee of Dankook University. A total of 144 [(Landrace × Yorkshire) × Duroc] pigs with an initial body weight (BW) of 23.6 ± 1.1 (mean $\pm$ SD) kg were assigned by BW, sex, and litter according to a randomized complete block design. The experiment consisted of four dietary treatments with nine replications per treatment and four pigs per pen (two females and two males). Pigs were housed in an environmentally controlled facility and room temperature was maintained at approximately 24 °C. Each pen (1.2 × 1.6 m) was equipped with a self-feeder and nipple waterer to allow *ad libitum* access to feed and water throughout the experimental period. The experimental treatments included: 1) basal diet; 2) basal diet with 44 mg/kg of tylosin (100 mg/kg tylosin); 3) basal diet with 5 g/kg of COS and 4) basal diet with 5 g/kg COS and 44 mg/kg tylosin (100 mg/kg tylosin). The diets were based on maize and rice bran as the cereal source and soybean meal as the main protein source (Table 1). All diets were provided in mash form and formulated to meet or exceed the NRC (1998) recommendations for all nutrients, regardless of treatment. Treatment additives were included in the diet by replacing the same amount of maize, and all diets had the same lysine and digestible energy content by manipulation of soybean meal and fat source. The COS used in the current study was provided by EASY BIO System Inc. (Korea) and produced by microbial fermentation of the shells of crustaceans by *Aspergillus niger*, *Aspergillus oryzae*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Lactobacillus acidophilus*. The preparation contained 8.6% crude protein, 14.5% crude fat, 22.6% crude ash, 10.7% crude fiber, 9.0% moisture, 3.0% chitooligosaccharide.

2.2. Sampling and measurements

The experimental diets were provided for a 42-day period during which the individual BW and feed consumption of each pen were monitored in order to calculate the ADG, ADFI and gain/feed ratio. The coefficient of total tract apparent digestibility (CTTAD) for dry matter (DM) and nitrogen (N), and gross energy was also determined at the end of experiment.

Table 1

Formula and chemical compositions of diets (as-fed basis).

Ingredients (g/kg)	
Ground maize	599.3
Soybean meal	239.0
Rice bran	50.0
Molasses	40.0
Animal fat	26.1
Rapeseed meal	20.0
Defluorinated phosphate	11.6
Calcium carbonate	4.4
L-lysine	3.4
Salt	1.5
Vitamin premix ^a	1.0
Mineral premix ^b	1.0
dl-methionine	1.0
Choline chloride	0.8
L-threonine	0.9
Analyzed composition	
Digestible energy (MJ/kg)	14.5
Crude protein (g/kg)	181.2
Lysine (g/kg)	10.8
Calcium (g/kg)	7.3
Total phosphorus (g/kg)	5.8

^a Provided per kg of complete diet: 6500 IU of vitamin A; 950 IU of vitamin D₃; 27 IU of vitamin E; 2 mg of vitamin K; 3.6 mg of vitamin B₂; 1.3 mg of vitamin B₆; 23 µg of vitamin B₁₂; 15 mg of pantothenic acid; 26 mg of niacin and 0.03 mg of biotin.

^b Provided per kg of complete diet: 54 mg of Cu; 70 mg of Fe; 70 mg of Zn; 50 mg of Mn; 0.5 mg of I; 0.5 mg of Co and 0.25 mg of Se.

Chromic oxide (Cr₂O₃) was used as an indigestible marker. Pigs were fed diets containing Cr₂O₃ (2 g/kg) for 5 days prior to the collection day, and fresh fecal grab samples were obtained once daily from at least two pigs in each pen on the last two days of the experiment. All fecal samples, as well as feed samples, were stored in a freezer at −20 °C until they were analyzed. Prior to chemical analysis, fecal samples were thawed and dried at 70 °C for 72 h, after which they were ground to pass through a 1-mm screen. All feed and fecal samples were analyzed for DM and N according to the procedures described by the AOAC (1995). Chromium was analyzed by UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) and nitrogen was measured using a Kjeltac 2300 analyzer (Foss Tecator AB, Hoeganaes, Sweden). Gross energy was determined using a Compensated Jacket Calorimeter 6100 (Parr Instrument Co., Moline, IL USA).

At the beginning of the experiment, two pigs (one female and one male) were randomly selected from each pen ($n = 72$) and bled via jugular venipuncture using a sterile needle and either a 5-mL vacuum or a K₃EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). The same pigs were bled again at the end of the experiment. The serum was then separated by centrifugation, and an aliquot was stored at −4 °C until it was analyzed for total protein, albumin and IgG using an automatic biochemistry blood analyzer (HITACHI 747, Japan). Serum total and HDL cholesterol concentrations were determined enzymatically (Cholesterol Kit No. 352, Sigma Chemical, St. Louis, MO, USA). In addition, the serum triglyceride concentration was also analyzed enzymatically (triglyceride Kit No. 339, Sigma Chemical). The whole blood samples were analyzed for red blood cell (RBC), white blood cell (WBC) and lymphocyte counts using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA).

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