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# Effects of supplementation of chito-oligosaccharide on the growth performance, nutrient digestibility, blood characteristics and appearance of diarrhea in weanling pigs

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ABSTRACT

This study was conducted to investigate effects of supplementation with a chito-oligosaccharide (COS) on growth performance, nutrient digestibility, blood characteristics and diarrhea incidence in weanling pigs. A total of 120 Landrace × Yorkshire–Duroc pigs (21 ± 1 d of age) with an average initial BW of 7.10 ± 0.48 kg were randomly allotted to four dietary treatments, with six pens per treatment and five pigs per pen. A maize–soybean meal-based diet was formulated as a basal diet. Experimental treatments included: 1) CON, basal diet; 2) ANT: basal diet + antibiotics (phase 1, 40 mg/kg avilamycin and 100 mg/kg oxytetracycline; phase 2 and phase 3, 40 mg/kg chlortetracyclinean and 100 mg/kg neomycin); 3) COS1, basal diet + 1 g/kg COS; and 4) COS2, basal diet + 2 g/kg COS. The experiment consisted of three phases (d 0 to d 7, d 8 to d 21 and d 22 to d 42) and lasted 42 d. Improved growth performance and total tract apparent digestibility (TTAD) of dry matter (DM) and nitrogen (N) only applied to the high inclusion level of COS (2 g/kg) ($P<0.05$) and both performance and digestibility were lower ($P<0.05$) than for the group supplemented with antibiotics. However, lymphocyte concentration and appearance of the diarrhea decreased ($P<0.05$) in response to supplementation of COS. The results of the current study indicated that dietary supplementation with 2 g/kg COS enhanced growth performance and improve TTAD of DM and N.



## 1. Introduction

In response to a decrease in the therapeutic effectiveness of antibiotics for treatment of a wide array of bacterial infections in humans, several European countries have banned the use of dietary antibiotics (Simon et al., 2003). Also, antibiotics supplementation in the animal diet will be banned in South Korea in July, 2011. Therefore, there is increasing demand to find effective, stable and safe alternatives of feed-grade antibiotics to promote growth and prevent disease in livestock. Functional oligosaccharide has been shown to improve growth performance and enhance the host health status (Gibson and Roberfroid, 1995). Chitosan is a polymer of glucoamines which can be made by deacetylating the chitin that is located in the cell wall of fungi and exoskeletal area of insects and Crustacea (Muwwarelli, 1997). Chitin and its derivatives (chitosan, chitin, oligosaccharides, chitooligosaccharide) have unique characteristics that are different from oligosaccharide (Kim et al., 1998). Chito-oligosaccharide (COS) is an oligosaccharide that can be easily obtained by chemical and enzymatic hydrolysis of polychitosan (Knaul et al., 1999). However, due to the insolubility, the application of polychitosan in animals is limited. In contrast, COS has a low molecular weight, good solubility, and low viscosity (Chae et al., 2005). In addition, several recent studies have demonstrated the health benefits of COS (Chen et al., 2009; Meng et al., 2010; Yan et al., 2009; Yan and Kim, 2011; Zhou et al., 2009). For example, COS has been shown to decrease

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the establishment of pathogens in the intestine (Vishu Kumar et al., 2005) and to enhance immune function (Okamoto et al., 2003). A previous study showed that dietary supplementation with 250 mg/kg COS increased serum growth hormone (GH), the insulin-like growth factor-I (IGF-I) and protein synthesis which in turn might have caused improved growth performance in early-weaned pigs (Tang et al., 2005).

However, there is little knowledge of the possible benefits of COS, and effects of dietary supplementation of COS is not consistent due to the COS supplementation levels in the diet. We hypothesized that high COS supplementation level would bring benefits on the growth performance in post weanling pigs and COS could be an apposite alternative of antibiotics. Therefore, this study was conducted to test the effects of dietary supplementation with COS on the growth performance, nutrients digestibility, blood characteristics and appearance of diarrhea in post weanling pigs.

## 2. Materials and methods

### 2.1. Preparation of chito-oligosaccharide (COS)

The COS used in this study was the natural products of chitin, chitosan and chitosan oligosaccharides that were produced by fermentation using live probiotics, including *Aspergillus oryzac*, *Bacillus subtillus*, *Saccharomyces cerevisiae* and *Lactobacillus acidophilus*. The fermentation product was comprised of: 18.6% crude protein, 14.5% crude fat, 10.7% crude fiber, 22.6% crude ash, 9.0% moisture, 4.3% calcium, 1.7% phosphorus, 4.0% chitin chitosan, and 3.0% chitosan oligosaccharide.

The COS was composed of several chitosan oligomers with molecular weight of about 1500 Da. The water solubility of the COS supplement was greater than 99%. To separate and quantify the different oligomers contained in the COS supplement, a standard sample was prepared by mixing 6 chitosan oligomers (Sigma, St. Louis, MO) using identical concentrations of each. The separation and quantification of oligomers was done by HPLC using an evaporative light-scattering detector (Thermo Finnigan, San Jose, CA, USA) and Asahipak NH2P-50-4E (4.6× 250 mm; Shodex, Tokyo, Japan). Generally, a concentration of 20 mg/ml COS was used for HPLC analysis. The mobile phase consisted of acetonitrile and a 0.3% ammonia solution at pH 10. Each run lasted for 60 min. The concentration of acetonitrile in mobile phase was 75% from 0 to 5 min and then reduced to 50% in 40 min, followed by a further reduction to 10 in 50 min and then a return to 75% in 60 min. The elution flow rate was maintained at 1.0 ml/min (typical operating pressure ~ 1200 psi). The composition of experimental COS was shown in Table 1.

**Table 1**
Composition of experimental chito-oligosaccharide.[a]

| Oligomers | Number of β-(1,4)-linked *N*-acetylglucosamine | Concentration, mg/ml |
|---|---|---|
| Chitobiose | Consisting of 2 β-(1,4)-linked *N*-acetylglucosamine | 0.65 |
| Chitotriose | Consisting of 3 β-(1,4)-linked *N*-acetylglucosamine | 1.98 |
| Chitotetrose | Consisting of 4 β-(1,4)-linked *N*-acetylglucosamine | 4.99 |
| Chitopentose | Consisting of 5 β-(1,4)-linked *N*-acetylglucosamine | 6.21 |
| Chitohexose | Consisting of 6 β-(1,4)-linked *N*-acetylglucosamine | 3.21 |

[a] The concentration of COS used for HPLC analysis was 20 mg/ml.

### 2.2. Animals, diets and experimental design

A total of 120 crossbreed Landrace × Yorkshire–Duroc pigs with an initial body weight (BW) of 7.10 ± 0.48 kg were used in a 42-d experiment. The pigs were weaned at 21 d of age and then selected by weight and were allotted to one of the four treatments using a completely randomized block design. The pigs in each group were fed experimental diets that consisted of un-supplemented control diet based on corn, soybean meal or similar diets. Experimental treatments included: 1) CON, basal diet; 2) ANT: basal diet + antibiotics (phase 1, 40 mg/kg avilamycin and 100 mg/kg oxytetracycline; phase 2 and phase 3, 40 mg/kg chlortetracyclinean and 100 mg/kg neomycin); 3) COS1, basal diet + 1 g/kg COS; and 4) COS2, basal diet + 2 g/kg COS (Table 2). Supplementation of antibiotics and COS was replaced by maize. There were 6 replicate pens per treatment with 5 pigs per pen. The stainless steel pens were 0.5 × 0.6 × 2.0 m and had a slatted plastic floor. Each pen was provided with a stainless steel feeder and a nipple waterer that allowed for ad libitum access to feed and water throughout the experiment. Ventilation was provided by a mechanical system, and lighting was automatically regulated to provide 12 h of artificial light per day. The ambient temperature within the room was approximately 30 °C and was decreased by 1 °C each week. Pigs were fed the phase 1 experimental diets for one week, the phase 2 experimental diets for two weeks and the phase 3 experimental diets for three weeks. The animal care and use protocol was approved by the Animal Care and Use Committee of Dankook University.

### 2.3. Experimental procedures and sampling

Individual pig BW and feed intake by pen were determined at the termination of each dietary phase and then used to calculate ADG (average daily gain), ADFI (average daily feed intake) and G:F (gain:feed).

During each dietary phase, 2 g/kg $Cr_2O_3$ was incorporated per kg of diet as indigestible marker. On days 7, 21 and 42, fecal samples were collected from at least three pigs per pen by rectal massage. All fresh fecal and feed samples were stored in a refrigerator at −20 °C until analysis. Determination of total tract apparent digestibility of the dry matter (DM) and nitrogen (N) was performed according to the AOAC (1995) procedures. The chromium was analyzed by UV absorption spectrophotometry (Shimadzu, UV-1201, Kyoto, Japan). Nitrogen was measured using a Kjeltec 2300 analyzer (Foss Tecator AB, Hoeganaes, Sweden).

At the beginning of the experiment, two healthy pigs were selected at random from each pen (n = 48) and blood samples were collected via jugular venipuncture. The same pigs were bled again on d 42. At each collection time, the