Animal Feed Science and Technology 169 (2011) 275–280

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Short communication

Evaluation of dietary supplementation of delta-aminolevulinic acid and chitooligosaccharide on growth performance, nutrient digestibility, blood characteristics, and fecal microbial shedding in weaned pigs

L. Yan, I.H. Kim*

Department of Animal Resource and Science, Dankook University, Cheonan, Choognam 330-714, Republic of Korea

ARTICLE INFO

Article history:
Received 17 February 2011
Received in revised form 27 June 2011
Accepted 27 June 2011

Keywords:
Chitooligosaccharide
Delta-aminolevulinic acid
Growth performance
Weaned pig

ABSTRACT

Eighty commercial cross-bred pigs [(Duroc×Yorkshire)×Landrace, weaned at 21 d, body-weight (BW) = 5.97 ± 0.46 kg] were allocated to 1 of 4 treatments [4 replicates with 5 pigs per pen (3 barrows and 2 gilts)] to determine the effects of delta-aminolevulinic acid (ALA) and chitooligosaccharide (COS) in weaned pigs using a 2×2 factorial design, with 2 levels of COS (0 or 3 mg/kg) and ALA (0 or 3 mg/kg). In this study, pigs fed diets supplemented with ALA did not affect the overall growth performance, although dietary ALA supplemented diets increased ($P < 0.05$) average daily gain (ADG) during 2–5 wk. Inclusion of ALA did not affect the ($P > 0.05$) the nutrient digestibility. Dietary ALA supplementation increased ($P < 0.05$) red blood cells (RBC), serum iron, total iron and total iron binding capacity (TIBC) in pigs. A synergistic effect ($P < 0.05$) between ALA and COS was observed on serum immunoglobulin G (IgG) concentrations. Moreover, inclusion of COS decreased the *Escherichia coli* numbers throughout the experiment. No difference ($P > 0.05$) was observed on the nutrient digestibility and blood characteristics with COS supplementation. A synergistic interaction ($P < 0.05$) was also observed between ALA and COS on fecal shedding of *E. coli* at d 13. In conclusion, pigs fed ALA (3 mg/kg) had no effect on overall growth performance but increased the iron status in weaned pigs. Inclusion of COS (3 g/kg) increased the lymphocyte count in blood and reduce the fecal *E. coli* in weaned pigs. A synergistic effect between ALA and COS was observed on the total IgG concentration and fecal *E. coli* in weaned pigs.

1. Introduction

Delta-aminolevulinic acid (ALA), which is the precursor in the heme biosynthetic pathway, has been used in clinical photodynamic therapy for a long time (Nishikawa and Murooka, 2001). Hallberg and Hulthen (2000) had reported that ALA can be converted to protoporphyrin IX through several biochemical reactions, which in turn incorporate iron via the enzyme ferrochelatase to form heme. Chen et al. (2008) also suggested that ALA supplementation in livestock could affect the synthesis of heme and positively influence the iron status and immunity of animals. Therefore, the inclusion of ALA may provide a new strategy for optimizing growth and health of pigs.

Abbreviations: ALA, delta-aminolevulinic acid; COS, chitooligosaccharide; ADG, average daily gain; G:F, gain:feed; DM, dry matter; WBC, white blood cell; RBC, red blood cell; Hb, haemoglobin; TIBC, total iron binding capacity.

* Corresponding author. Tel.: +82 41 550 3652; fax: +82 41 565 2949.
E-mail address: inhokim@dankook.ac.kr (I.H. Kim).

0377-8401/$ – see front matter.
doi:10.1016/j.anifeedsci.2011.06.017

Chitooligosaccharide (COS), which is easily obtained by chemical and enzymatic hydrolysis of polychitosan, has shown high antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella cholera* (Jung et al., 2006). Tang et al. (2005) had demonstrated that dietary COS increased the concentration of serum growth hormone and insulin-like growth factor-I (IGF-I) mRNA level. Several studies also suggested COS could be used as a good alternative to dietary antibiotics (Liu et al., 2008; Wang et al., 2009; Zhou et al., 2009; Yan et al., 2010). Moreover, it was previously suggested that chitosan or chitosan derivatives could accelerate the absorption of iron by chelating iron at low pH increasing the solubility of iron in the intestines (Xia et al., 2011; Liao et al., 2007). A clinical study reported that ingestion of chitosan can effectively improve serum hemoglobin levels of patients with chronic renal failure (Jing et al., 1997). Therefore, the objective of this study was to determine if single or combined supplementation of ALA and COS may exert positive effects on growth performance, coefficient of apparent total tract digestibility (CATTD), immunological blood characteristics, and fecal microbial shedding in weaned pigs.

2. Materials and methods

The protocols used for the current experiment were approved by the Animal Care and use Committee of Dankook University.

2.1. Preparation of delta-aminolevulinic acid and chitooligosaccharide

Both the ALA and COS used in the current study were produced by EASY BIO System Inc. (Seoul, South Korea). Briefly, ALA was produced by recombinant *E. coli* containing the *R. capsulatus* heme A gene under the control of the constitutive promoter to a maximum concentration of 21 mM in the absence of levulinic acid, an inhibitor of ALA dehydratase. The COS was produced by fermentation of the shells of crustaceans by *Aspergillus oryzae*, *Bacillus subtillus*, *Saccharomyces cerevisiae*, and *Lactobacillus acidophilus*. The preparation contained 186 g/kg CP, 145 g/kg crude fat, 226 g/kg crude ash, 107 g/kg crude fibre, 90 g/kg moisture, and 246 g/kg nitrogen free extract. The preparation contained at least 40 g/kg chitin chitosan and 30 g/kg chitooligosaccharide (EASY BIO System Inc., Seoul, South Korea). The average molecular weight of ALA and chitooligosaccharide was about 131.13 g/mol and 2000 Da, respectively. The degree of the acetylation for the chitooligosaccharide is 75%.

2.2. Experimental design, animals, and facilities

Eighty cross-bred [(Duroc×Yorkshire)×Landrace] piglets, weaned at 21 d of age with an average initial bodyweight (BW) of 5.97 ± 0.46 kg were allocated to one of four treatments with 4 replicates each consisting of 5 pigs (3 barrows and 2 gilts) per pen in a 2×2 factorial design, with 2 levels of ALA (0 or 3 mg/kg) and COS (0 or 3 mg/kg). The dosages of COS and ALA used in the current study is based on our previous study (Chen et al., 2008, 2009; Wang et al., 2009). A two-phase feeding program composed of phases 1 (d 0–14) and 2 (d 14–35) was employed (Table 1). Pigs received the same phase 1 control diet for 2 d of acclimation prior to the experiment. Diets used in the present study were formulated to meet or exceed the nutrient recommendations of National Research Council (NRC, 1998). Diets were freeze-dried and ground through a 1-mm screen in a Wiley mill to determine dry matter (DM), crude protein (CP), phosphorous (P), and calcium (Ca) (AOAC, 2000). Gross energy (GE) was determined using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, IL, USA). The amino acid (AA) composition of the experimental diets was determined following acid hydrolysis with 6 N HCl at 110 °C for 24 h using an AA analyzer (Biochrom 20, Pharmacia Biotech, Cambridge, England). Sulfur-containing amino acids were analysed after cold performic acid oxidation overnight and subsequent hydrolysis. All pigs were housed in an environmentally controlled nursery room. The stainless steel pens were 0.5 m×0.6 m×2.0 m with a slatted plastic floor. Each pen was provided with a stainless steel feeder and a nipple waterer that allowed *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 at the start of the experiment and decreased by 1 °C each wk.

2.3. Sampling and measurements

Individual pig BW and feed consumption of each pen was monitored weekly to calculate average daily gain (ADG), average feed intake (ADFI), and gain:feed (G:F). Chromium oxide (Cr_2O_3, 2 g/kg) was added to the diets as an indigestible marker at the beginning of 5 wk (29 d) to measure digestibility. Fecal grab samples were obtained via massaging the rectum from at least 2 pigs (1 barrow and 1 gilt) in each pen at the end of 5 wk (34 d) to determine the coefficient of apparent total tract digestibility (CATTD) of DM and nitrogen (N). All the feed and fecal samples were freeze-dried and finely ground to be able to pass through a 1-mm screen, and stored in a refrigerator at −20 °C until analysis. The DM and N concentrations were determined according to the AOAC (2000). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) following the method described by Williams et al. (1962). The CATTD of DM and N were calculated using the following formula: CATTD = 1 − [(Nf×Cd)/(Nd×Cf)], where Nf = nutrient concentration in feces (% DM),