Animal Nutrition 3 (2017) 132–138

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Magnolol additive as a replacer of antibiotic enhances the growth performance of *Linwu* ducks

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ARTICLE INFO

Article history:
Received 20 January 2017
Received in revised form
25 March 2017
Accepted 28 March 2017
Available online 4 April 2017

Keywords:
Magnolol
Linwu duck
Growth performance
Antioxidant-related gene expression
Intestinal mucosal status

ABSTRACT

Magnolol rich in *Magnolia officinalis* is a bioactive polyphenolic compound. The aim of this study was to examine the effects of magnolol additive (MA) on growth performance, expression levels of antioxidant-related genes, and intestinal mucosal morphology of *Linwu* ducks aged from 49 to 70 days, comparing with that of an antibiotic additive (colistin sulfate [CS]). A total of 275, 49-day-old ducks were assigned to 5 groups with 5 cages of 11 ducks each and fed diets supplemented with 0, 100, 200 and 300 mg of MA/kg and 300 mg of CS/kg for 3 weeks, respectively. The results showed that the average daily body weight gain (ADG) was increased significantly in MA-fed groups (200 and 300 mg/kg), compared with the basal diet (BD) group ($P < 0.05$). The mRNA levels of superoxide dismutase-1 (*SOD1*), manganese superoxide dismutase-2 (*MnSOD2*) and catalase (*CAT*) were also increased significantly in MA groups ($P < 0.05$). In addition, hematoxylin and eosin staining revealed that *Linwu* ducks fed the diets with MA had more intact intestinal mucosa than those fed the BD and CS diets. In addition, ileal villus height, ileal villus height/crypt depth ratio (V/C) and duodenal V/C were also improved significantly ($P < 0.05$). Taken together, these data demonstrated that MA is an effective feed additive to enhance the growth performance of the *Linwu* ducks by improving the antioxidant and intestinal mucosal status, suggesting that MA will be a potential additive to replace antibiotic (CS).

1. Introduction

The negative effects of stress on poultry production have received considerable concern. Stress can be caused by mechanical, thermal, infectious and chemical stimuli in modern intensive rearing mode. Stress can induce an imbalance between the production and elimination of reactive oxygen species (ROS). On the other hand, antibiotics have been used worldwide for more than 50 years to prevent pathogen infection and to improve performance in the poultry industry (Choi et al., 2014). However, the sustained use of antibiotics as growth promoting feed additives has increased the risk of drug resistance (Castanon, 2007; Dibner and Richards, 2005). Moreover, there is growing evidence that consumers demand poultry products to be safe, healthy and high quality. These situations require society to develop a healthy and sustainable poultry industry by reducing or replacing antibiotics used in feed. Recent studies have suggested that many phytochemicals have profound impacts on the growth performance and antioxidant status of animals (Wallace et al., 2010). For example, resveratrol (Liu et al., 2014), oregano essential oil (Horosava et al., 2006) and aloe vera (Shokri et al., 2016) have been reported to improve the growth performance of farm animals.

Magnolol is a 4-allyl-2-(5-allyl-2-hydroxy-phenyl) phenol, and is present in considerable quantities in the bark of the Houpu magnolia (*Magnolia officinalis*) (Lin et al., 2016a). Previous data

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2017.03.004

2405-6545/

revealed that magnolol had anti-inflammatory (Wang et al., 2015), antineoplastic (Wu et al., 2014), anti-stress (Chang et al., 2003) and antidiarrheic effects (Guerra-Araiza et al., 2013; Xia et al., 2013). Especially, a strong antioxidant effect has been observed both in *in vitro* and *in vivo* assays for magnolol (Shen et al., 2010). *In vitro*, magnolol exhibited effective antioxidant abilities detected with the methods of 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging, 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Ferric ions (Fe^{3+}) reducing power, superoxide anion and hydroxyl radical-scavenging assays (Amorati et al., 2015; Li and Chen, 2012; Ogata et al., 1997; Zhao and Liu, 2011). *In vivo*, magnolol can protect against organs and tissues injury by scavenging free radicals and activating the antioxidant or detoxifying enzymes in rats (Chen et al., 2009; Chang et al., 2003; Loong et al., 2002) and mice (Kim et al., 2013; Lu et al., 2015a, 2015b). Moreover, the intestinal mucosal status has also been reported to affect growth performance of poultry (An et al., 2016; Feng et al., 2010; Shen et al., 2015; Yang et al., 2012).

Based on the bioactive properties of magnolol, we investigated the effects of magnolol additive (MA) on growth performance, expression levels of antioxidant-related genes and intestinal mucosal status in *Linwu* ducks, a major indigenous dual purpose type breed of ducks in China (Lin et al., 2016b). The potential for magnolol to replace antibiotics was discussed in antibiotic additive.

2. Materials and methods

2.1. Birds, diets, and experimental design

Two hundred and seventy-five female *Linwu* ducks, 42 days of age, free of infectious disease, were obtained from Hunan Shunhua Duck Industrial Development Company, China, and transferred to the laboratory of the Department of Animal Nutrition and Feeding Technology, Hunan Institute of Animal Science and Veterinary Medicine. Magnolol additive was extracted from *Magnolia officinalis* at the National Research Center of Engineering Technology for Utilization of Functional Ingredients from Botanicals by the method described previously (Long, 2009). Briefly, magnolol was extracted with methanol as solvent, and separated with silica gel column chromatography. Then purity of magnolol was identified as 98.1% by high performance liquid chromatography (HPLC). The ducks were supplied *ad libitum* access to feed and water throughout the trial period. After a 1-week adaptation period, *Linwu* ducks were individually weighed and divided into 5 groups without significant difference on average initial weight among groups; each group (55 *Linwu* ducks) was further subdivided into 5 cages (11 ducks/cage), and the dimension of each cage was 120 cm × 120 cm. Group 1 received a basal diet (BD). Group 2 received BD supplemented with 300 mg/kg of an antibiotic additive (10% colistin sulfate [CS] manufactured by Guangzhou Xingda Animal's Pharmaceutical Company, China) (CS300). The remaining 3 groups received, respectively, the BD supplemented with 100, 200 or 300 mg MA/kg of diet (MA100, MA200, and MA300). The BD was formulated in accordance with the Nutrient Requirements of Meat-type Duck (China, NY/T 2122-2012) and the Nutrient Requirements of Ducks (NRC, 1994) (Table 1). The feeding period was 21 days. The mean daily temperature during the trial was 28.3 °C. On day 70, liver and intestinal tract were taken from birds for further analysis. All the experimental procedures were approved by the Institutional Animal Care and Use Committee of Hunan Agricultural University.

2.2. Growth performance

Body weight of *Linwu* ducks was individually measured at the beginning (day 49) and the end of the trial (day 70). Feed intake per cage was recorded daily. The average daily feed intake (ADFI), average daily body weight gain (ADG) and feed/gain ratio (F/G) were calculated according to the data from each cage.

2.3. Data and sample collection

On day 70, after 12 h fasting, 5 *Linwu* ducks in each group (1 duck in each cage) with live weights close to the mean were immediately slaughtered by cervical dislocation, as described by Murawska (2012). The liver was immediately removed from the carcass, frozen in liquid nitrogen, and stored at −80 °C until analysis. The small intestine was promptly moved out and divided into 3 parts: duodenum, jejunum and ileum. A 2-cm segment of the intestine was cut from the midpoint of the duodenum, jejunum, and ileum. These intestinal tissue samples were lightly flushed with physiological saline (154 mmol/L), blotted dry with filter paper and fixed into 10% neutral buffered formalin for further analysis of intestinal mucosal morphology (Applegate et al., 2005; Watkins et al., 2004).

2.4. Quantification of mRNA expression by real-time PCR

Total RNA from the liver was isolated using Trizol reagent (TaKaRa, Tokyo, Japan), and then treated with DNase I (Thermo Fisher Scientific Inc., USA). The cDNA was synthesized from 1 μg of RNA with a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., USA) according to the manufacturer's instructions. Based on the cloned complete sequences (https://www.ncbi.nlm.nih.gov/genbank/) of heme oxygenase-1 (*HO-1*), glutathione S-transferase α3 (*GSTα3*), superoxide dismutase-1 (*SOD1*), manganese superoxide dismutase-2 (*MnSOD*), catalase (*CAT*), glutathione peroxidase-1 (*GPX1*), glutathione peroxidase-4 (*GPX4*), nuclear factor erythroid-2-related factor 2/erythroid-derived CNC homology factor (*Nrf2/ECH*), kelch-like ECH-associated protein 1 (*Keap-1*) and β-actin from *Anas platyrhynchos*, primer pairs were designed with Primer 5.0 for quantitative real-time PCR (Table 2). The β-actin gene was used as the housekeeping gene. All primers were synthesized and purified by Sangon Biotech Co. Ltd (Shanghai, China). Reaction volume of 20 μL mixture contained 10 μL Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 1 μL cDNA template, 1 μL of each of the upstream and downstream primers, and 7 μL sterilized deionized water. The amplification parameters for all the genes of the thermocycler (CFX Connect, Bio-Rad, Inc., USA) were a preheat period of 3 min at 95 °C followed by 45 cycles of 95 °C for 10 s and 55 °C for 20 s, and a melting curve ramping from 65 to 95 °C with an increasing temperature of 0.5 °C. All samples analyses were carried out in triplicate and the average values were indexed. The target gene expression was normalized to that of the selected reference gene, and the relative gene expression was calculated using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The mRNA levels were expressed as the fold change relative to the mean value of the BD group, which was arbitrarily defined as 1.0.

2.5. Measurement of intestinal mucosal morphology

Measurement of intestinal mucosal morphology was described previously (Jiang et al., 2012). Briefly, 2 cm-intestinal tissue samples of the duodenum, jejunum and ileum were embedded in paraffin. A microtome (RM-2235, Leica microsystems AG., Hessen, Germany) was used to make 5 or 6 μm slices that were mounted in glass slides and subsequently stained with hematoxylin and eosin (HE staining). Finished slides were observed under an Olympus Van-Ox S microscope (Opelco, Washington, DC) and the typical microscopic fields were selected to take photos. Villus height (from the tip of the