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Original Research Article

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

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# 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 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).

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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

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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.

years to prevent pathogen infection and to improve performance in

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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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  $\times$  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  $\alpha 3$  (GST $\alpha 3$ ), 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  $\beta$ -actin from *Anas platyrhynchos*, primer pairs were designed with Primer 5.0 for quantitative real-time PCR (Table 2). The  $\beta$ -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  $^\circ C$  for 10 s and 55  $^\circ C$  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  $\mu$ m 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

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#### Table 1

Composition	and	nutrient	levels	of basal	diets	(air-drv	basis)
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Item	Ingredients, %	Item	Nutrient levels, <sup>1</sup> %
Corn	58.50	Metabolizable energy, MJ/kg	11.72
Soybean meal	10.15	Crude protein	15.60
Times powder	10.00	Calcium	0.82
Wheat bran	10.00	Total phosphorus	0.61
Cottonseed meal	3.20	Available phosphorus	0.32
Rapeseed meal	3.20	Salt	0.35
Soybean oil	0.86	Lysine	0.70
Limestone	1.38	Methionine	0.39
Dicalcium phosphate	1.15	Methionine + Cysteine	0.68
NaCl	0.30	Threonine	0.55
98.5% DL-Methionine	0.17	Tryptophan	0.17
78% L-Lysine	0.09	Dry matter	86.97
1% Premix <sup>2</sup>	1.00	Crude fiber	3.20
Total	100.00		

<sup>1</sup> Nutrient levels are calculated values.

<sup>2</sup> The premix provided the following (per kilogram of complete diet) micronutrients: vitamin A 12,000 IU, vitamin D<sub>3</sub> 2,500 IU, vitamin E 20 mg, vitamin K<sub>3</sub> 3 mg, vitamin B<sub>1</sub> 3 mg, vitamin B<sub>2</sub> 8 mg, vitamin B<sub>6</sub> 7 mg, vitamin B<sub>12</sub> 0.03 mg, D-pantothenic acid 20 mg, nicotinic acid 50 mg, biotin 0.1 mg, folic acid 1.5 mg, Cu (as copper sulfate) 9 mg, Zn (as zinc sulfate) 110 mg, Fe (as ferrous sulfate) 100 mg, Mn (as manganese sulfate) 100 mg, Se (as sodium selenite) 0.16 mg, I (as potassium iodide) 0.6 mg.

#### Table 2

Primer sequences used for real-time quantitative PCR.

Sequences of the primer pair	GenBank accession No.	Fragment length, bp
5'-AGTACCCCATTGAACACGGT-3'	EF667345	197
5'-ATACATGGCTGGGGTGTTGA-3'		
5'-TTCGAGAAGTGCGAGGTGAA-3'	KU048803	156
5'-GTTCCAGGAGATGTCGTTGC-3'		
5'-TTTGCTGAGAACTACGGGGT-3'	KU048804	192
5'-GGGGCTGTATCTCTTCACCA-3'		
5'-AGAGAGCCCTGATCGACATG-3'	KU048805	177
5'-AGTCTTGGCCGTGTTGTTTC-3'		
5'-TGCCTACACTCGCTATCTGG-3'	KU048806	183
5'-AGGTCCATCTCAAGGGCATT-3'		
5'-AATGTGCGTGACTGACAACC-3'	KU048802	196
5'-ACGTTCATCCTCCTTCAGCA-3'		
5'-CAGTCCTTGGGCTACTTGGA-3'	KU048807	197
5'-CGGTTGGTCATGGGGTTGTA-3'		
5'-GACCTGCCCTACGACTATGG-3'	KU048809	167
5'-TGAAGTGACACCTGAGCTGT-3'		
5'-TGGACCAAAGGATGCAGAGA-3'	KU048808	200
5'-CATTCCCAGTTAGCGTGCTC-3'		
5'-CGCCTTGAAGCTCATCTCAC-3'	KM109969	176
5'-TTCTTGCCTCTCCTGCGTAT-3'		
	Sequences of the primer pair 5'-AGTACCCCATTGAACACGGT-3' 5'-ATACATGGCTGGGGGTGTTGA-3' 5'-TTCCAGGAAGTGCGAGGTGAA-3' 5'-GTTCCAGGAGATGTCGTTGC-3' 5'-GGGCGTGATCTCTTCACCA-3' 5'-AGGACGCCTGATCGCGTGTTGTTC-3' 5'-AGGTCTTGGCCGTGTTGTTC-3' 5'-AGGTCCATCCTCAACGGCATT-3' 5'-AGGTCCATCCTCCAGGCATT-3' 5'-AGGTCCATCCTCCAGGCATT-3' 5'-AGGTCCATCCTCCTCAGCA-3' 5'-CGGTTGGTCATGCGCTACTTGGA-3' 5'-CGGTTGGTCATGGGCTTGTA-3' 5'-GACCTGCCCTACGGCATTGGA-3' 5'-GACCTGCCCTACGGCATGGA-3' 5'-GACCTGCCCTACGGCATGTG-3' 5'-TGGACCAAGGATGCAGCGA-3' 5'-TGGACCAAGGATGCAGCGA-3' 5'-CGATTCCCGCTACGCGCTC-3' 5'-CGCTTGAAGCTCATCTCAC-3' 5'-TCTTGCCCTCCTCCGCGTAT-3'	Sequences of the primer pairGenBank accession No.5'-AGTACCCCATTGAACACGGT-3'EF6673455'-ATACATGGCTGGGGGTGTTGA-3'KU0488035'-TTCCAGGAAGTGCGAGGTGAA-3'KU0488035'-GTTCCAGGAGATGTCGTTGC-3''KU0488045'-GGGGCTGATCTCTTCACCA-3''KU0488055'-AGAGAGCCCTGATCGACATG-3'KU0488055'-AGCTTGGCCGTGTTGTTC-3''KU0488065'-AGCTCACCGCTATCTGGC-3'KU0488065'-AGGTCCATCTCACAGGGCATT-3''KU0488065'-AGGTCCATCTCACAGGGCATT-3''KU0488025'-AGGTCCATCTCACACGCA''KU0488025'-AGTCCTTGGCTACTTGGACAACC-3'KU0488075'-CGGTTGGTCATGGACAACC-3'KU0488075'-CGGTTGGTCATGGACTATGG-3'KU0488095'-TGAACTGACACCTGAGCTGT-3''KU0488085'-CACTCCACCTACGACGAGCA-3'KU0488085'-CGCTTGAACACCAGGGCTGT-3''KU0488085'-CGCTTGAACACCAGGATGCAGAGA-3'KU0488085'-CATTCCCAGTTAGGGTCCT-3''KU0488085'-CATTCCCAGTTAGCGTGCTC-3''KU0488085'-CATTCCCAGTTAGCGTGCTC-3''KU0488085'-CATTCCCAGTTAGCGTGCTC-3''KU0488085'-TTCTAGCCTCTCCAGCATAT-3''KU0488085'-CATTCCCAGTTAGCGTGCTC-3''KU0488085'-TTCTAGCCTCTCCTGCGTAT-3''KU048808

GPX1 = glutathione peroxidase-1; GPX4 = glutathione peroxidase-4; GSTa3 = glutathione S-transferase  $\alpha3$ ; HO-1 = heme oxygenase-1; CAT = catalase; Keap-1 = kelch-like ECH-associated protein 1; MnSOD = manganese superoxide dismutase; SOD1 = superoxide dismutase-1; Nrf2/ECH = nuclear factor erythroid-2-related factor 2/erythroid-derived CNC homology factor.

villus to the villus crypt junction) and crypt depth (from villus crypt junction to the base of the crypt) from each slide were determined, using an image analysis system (Image-Pro, Media Cybernetics, Inc., Silver Springs, MD) (Jiang et al., 2012; Shen et al., 2009). The lengths of 10 intact villi and their associated crypts were measured in each slide.

# 2.6. Statistical analysis

Statistical analysis of data was done with Statistical Package for Social Sciences (SPSS) 19.0 (IBM, Armonk, New York). One-way ANOVA model was performed to test all data. Replicate was used as the experimental unit. Results were presented as means and pooled standard errors of the means (SEM). When the main effects were significant, the differences among means were further determined using the Duncan's multiple range. Differences between means of all groups were considered significant at P < 0.05, and *P*-values between 0.05 and 0.10 were considered as a trend. Orthogonal polynomial contrasts were used to determine linear and quadratic responses of defined characters to different MA levels.

# 3. Results

# 3.1. Growth performance

Compared with BD, MA200 and MA300 increased ADG (P < 0.05) (Table 3). A positive linear MA dose—response relationship (P = 0.003) for ADG was observed (Table 3). In addition, there were no significant differences (P > 0.05) on average final weight, ADFI and F/G among each group (Table 3). These data reveal that MA can increase ADG of *Linwu* ducks, relative to BD.

# 3.2. The mRNA expression levels of hepatic antioxidant-related genes

Compared with BD, MA200 and MA300 increased (P < 0.05) the SOD1 and CAT mRNA expression levels (Fig. 1B and D). The MnSOD mRNA expression level was significantly higher (P < 0.05) in

Item	Groups <sup>1</sup>					SEM	P-value	P-value	P-value	
	BD	MA100	MA200	MA300	CS300			Linear	Quadratic	
Average initial weight, g	968.74	965.12	963.67	960.24	971.08	2.104	0.545	0.227	0.984	
Average final weight, g	1,494.03	1,499.76	1,511.04	1,505.75	1,509.66	3.730	0.608	0.254	0.539	
ADG, g	25.01 <sup>b</sup>	25.46 <sup>ab</sup>	26.07 <sup>a</sup>	25.98 <sup>a</sup>	25.65 <sup>ab</sup>	0.119	0.021	0.003	0.249	
ADFI, g	181.68	182.24	184.01	183.05	181.42	1.268	0.974	0.654	0.795	
F/G	7.26	7.16	7.06	7.04	7.07	0.041	0.413	0.055	0.582	

Effects of dietar	y magnolol levels on gro	owth performance in	Linwu ducks (49 to 70 days)	).

ADG = average daily body weight gain; ADFI = average daily feed intake; F/G = feed/gain ratio.

<sup>a,b</sup> Within a row, values with different superscripts differ significantly (P < 0.05).

Table 3

<sup>1</sup> BD = basal diet; MA100, MA200, MA300 = BD supplemented with 100, 200 or 300 mg magnolol additive per kg of diet, respectively; CS300 = BD supplemented with 300 mg/kg of an antibiotic additive (10% colistin sulfate).

MA200, MA300 and CS300 than that in BD (Fig. 1C). Moreover, MA200 and MA300 tended to increase (0.05 < P < 0.10) HO-1, GPX4 and Nrf2/ECH mRNA expression levels (Fig. 1A, G and H). The positive linear MA-dose response relationships were observed for CAT, SOD1, MnSOD, HO-1 and Nrf2/ECH mRNA expression levels (P < 0.05). In addition, there was no significant difference (P > 0.05) on expression levels of GST $\alpha$ 3, GPX1 and Keap-1 among each group (Fig. 1E, F and I). These data demonstrated that MA might improve the antioxidant status by regulating the expression of hepatic antioxidant-related genes.

# 3.3. The change in intestinal mucosal morphology

The intestinal mucosal morphologies including the duodenum, jejunum and ileum were observed by HE staining. Ducks fed diets with MA had more intact intestinal mucosa than that with BD and CS300 at the duodenum, jejunum and ileum (Fig. 2). MA300 and CS300 increased (P < 0.01) duodenal villus height/crypt depth ratio (V/C) (Table 4), compared with BD. Ileal villus height and V/C were significantly higher (P < 0.05) in MA200 and MA300 than that in BD

(Table 4). Moreover, MA200 also elevated ileal V/C (P < 0.05), compared with CS300. The positive linear MA-dose response relationships were observed for ileal villus height, ileal V/C and duodenal V/C (P < 0.01). In addition, there were no significant differences on crypt depth in the duodenum, jejunum and ileum among each group (P > 0.05) (Table 4). These data revealed that the ducks fed MA showed the intact status of intestinal mucosal morphology.

# 4. Discussion

Several lines of studies have suggested dietary polyphenols can improve the growth performance of livestock and poultry through the mechanisms involving the antioxidant activity and protective effect for the organs (Hashemi and Davoodi, 2011; Liu et al., 2014; Wallace et al., 2010; Windisch et al., 2008). Magnolol was known as a natural polyphenolic compound and was found to have antioxidant activity (Shen et al., 2010). The antioxidant activity of magnolol was observed to protect the structure and functions of organs (Kim et al., 2013; Mei et al., 2016; Shen et al., 2010; Zhao and



**Fig. 1.** Effects of dietary magnolol levels on the mRNA expression levels of hepatic antioxidant related genes in 70 days *Linwu* ducks. *HO-1* = heme oxygenase-1;  $GST\alpha 3$  = glutathione S-transferase  $\alpha 3$ ; SOD1 = superoxide dismutase-1; MnSOD = manganese superoxide dismutase; CAT = catalase; GPX1 = glutathione peroxidase-4; Nrf2/ECH = nuclear factor erythroid-2-related factor 2/erythroid-derived CNC homology factor; *Keap-1* = kelch-like ECH-associated protein 1. BD = basal diet; MA100, MA200, MA300 = BD supplemented with 100, 200 or 300 mg magnolol additive per kg of diet, respectively; CS300 = BD supplemented with 300 mg/kg of an antibiotic additive (10% colistin sulfate). <sup>a,b</sup> Within each figure, bars with different superscripts differ significantly (P < 0.05).



**Fig. 2.** Effects of dietary magnolol levels on intestinal mucosal morphology (A = Duodenum; B = Jejunum; C = Ileum) in 70 days *Linwu* duck (40  $\times$ ). BD = basal diet; MA100, MA200, MA300 = BD supplemented with 100, 200 or 300 mg magnolol additive per kg of diet, respectively; CS300 = BD supplemented with 300 mg/kg of an antibiotic additive (10% colistin sulfate).

# Table 4

Effects of dietary magnolol levels on intestinal mucosal morphology in 70 days Linwu ducks.

Item	Groups <sup>1</sup>					SEM	P-value	P-value	P-value	
	BD	MA100	MA200	MA300	MA300			Linear	Quadratic	
Duodenum										
Villus height, µm	746.01	767.45	772.54	785.94	777.12	5.538	0.213	0.034	0.742	
Crypt depth, μm	200.08	192.43	187.25	174.91	180.73	3.509	0.175	0.027	0.756	
V/C	3.74 <sup>c</sup>	4.01 <sup>bc</sup>	4.15 <sup>abc</sup>	4.51 <sup>a</sup>	4.33 <sup>ab</sup>	0.078	0.009	< 0.001	0.724	
Jejunum										
Villus height, µm	636.93	649.58	666.36	681.77	662.99	5.735	0.120	0.011	0.908	
Crypt depth, µm	165.84	163.26	159.54	151.62	156.76	3.493	0.762	0.237	0.756	
V/C	3.88	4.04	4.22	4.53	4.26	0.096	0.278	0.041	0.733	
Ileum										
Villus height, µm	634.92 <sup>b</sup>	667.33 <sup>a</sup>	686.17 <sup>a</sup>	678.99 <sup>a</sup>	660.94 <sup>ab</sup>	5.529	0.020	0.004	0.067	
Crypt depth, μm	150.75	149.12	136.12	143.70	144.41	1.835	0.085	0.054	0.228	
V/C	4.22 <sup>c</sup>	4.48 <sup>bc</sup>	5.07 <sup>a</sup>	4.73 <sup>ab</sup>	4.59 <sup>bc</sup>	0.080	0.005	0.004	0.054	

V/C = villus height/crypt depth.

 $^{a-c}$  Within a row, values with different superscripts differ significantly (P < 0.05).

<sup>1</sup> BD = basal diet; MA100, MA200, MA300 = BD supplemented with 100, 200 or 300 mg magnolol additive per kg of diet, respectively; CS300 = BD supplemented with 300 mg/kg of an antibiotic additive (10% colistin sulfate).

Liu, 2011). These data suggest that magnolol may be a potential candidate to improve growth performance of animals. In the present study, we found that magnolol supplementation could increase ADG and modulate antioxidant status of *Linwu* ducks.

Although many studies on the antioxidant action of polyphenols were carried out under artificial heat stress, the aim of this study was to examine the antioxidant action of magnolol under practical duck production settings in cages, and to explore the possibility as a replacer for antibiotics. Thus, we investigated the antioxidant status at the end of the trial by examining the expression level of Nrf2-mediated antioxidant enzyme genes. The data revealed that MA, compared with BD, significantly enhanced the mRNA levels of hepatic *SOD1*, *MnSOD2* and *CAT*, and also increased the mRNA levels of *HO-1*, *Nrf2* and *GPX4*. Superoxide dismutase is an enzyme which alternately catalyzes the dismutation of superoxide (O<sub>2</sub>) radical into either O<sub>2</sub> or H<sub>2</sub>O<sub>2</sub> (Loboda et al., 2016). Glutathione peroxidase is an enzyme

that catalyzes the degradation of heme and generates antioxidant molecules, biliverdin and carbon monoxide (Kikuchi et al., 2005; Ryter et al., 2006). Catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide to  $H_2O$  and  $O_2$  (Chelikani et al., 2004). Thus, MA might up-regulate the mRNA level of these antioxidant-related genes to modulate the antioxidant status and performance of *Linwu* ducks.

The intact morphology of the mucosa in the duodenum, jejunum and ileum is one of the most important indication of intestinal health as well as digestive and absorptive capacity in poultry (Jiang et al., 2012; Tossou et al., 2016). The morphology can be expressed as villus height, crypt depth and their ratio. Crypt depth is associated with the level of cell turnover. The lengthening of the villus (villus height) increases the surface area for nutrient absorption (Yason et al., 1987). The villus are also continually renewed as they are vulnerable to ordinary sloughing as well as pathogenic assault and pathogen-initiated inflammation (Li et al., 2006). Therefore, longer villi and a higher V/C ratio indicate

better digestive and absorptive capabilities. In our study, we found that MA significantly enhanced ileal villus height and duodenal V/C ratio. Mei et al. (2016) also observed that magnolol pretreatment attenuates heat stress-induced injury in intestinal epithelial cell-6 (IEC-6) and then maintained the intact structures and functions of the small intestine. Based on the improvement in the intestinal mucosal morphology by supplementing with magnolol, we considered that magnolol might keep the intact structures and functions of the intestinal mucosa of the intestinal mucosa by its antioxidant activity, which further enhanced the digestive, absorptive and metabolic capabilities of the gut, leading to increased growth performance in *Linwu* ducks.

## 5. Conclusions

The MA enhanced the growth performance of *Linwu* ducks by modulating the antioxidant status, and improving intestinal mucosal morphology. The effects obtained by MA were similar to that elicited by CS although the mechanisms may have differed. Thus, MA will be a potential candidate as a replacer for in-feed antibiotics to improve the growth performance of *Linwu* ducks in the cage-rearing production system.

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