The effects of magnolol supplementation on growth performance, meat quality, oxidative capacity, and intestinal microbiota in broilers

Qian Xie, Kaihuan Xie, Jinhui Yi, Zehe Song, Haihan Zhang, and Xi He[1]

College of Animal Science and Technology, Hunan Agricultural University, Ministry of Education Engineering Research Center of Feed Safety and Efficient Use, Hunan Engineering Research Center of Poultry Production Safety, Hunan Co-Innovation Center of Animal Production Safety, Changsha, 410128, China

ABSTRACT The aim of the present study was to evaluate the effect of magnolol (**MAG**) on growth performance, meat quality, oxidative capacity, and intestinal microbiota in the yellow-feather broilers. A total of 360 one-day-old male yellow-feather broiler chicks were allocated into 5 groups of 6 replicates and 12 chickens per replicate, were fed a basal diet supplemented with 0 (Control group, **CON**), 100, 200, 300, or 400 mg/kg MAG for 51 d. The results showed that dietary supplementation with 200 and 300 mg/kg MAG increased the average daily gain (**ADG**) but decreased feed to gain ratio (**F**/**G**) during the overall periods ($P < 0.05$). Dietary MAG increased significantly the redness value (a^*) of the breast muscle ($P < 0.05$), and decreased the water loss rate and shear force of the breast meat ($P < 0.05$). MAG supplement reduced the malondialdehyde (**MDA**) levels, and increased the glutathione (**GSH**), superoxide dismutase (**SOD**), and total antioxidant capacity (**T-AOC**) levels in breast muscle and jejunum. PCR analysis showed that MAG increased the levels of NF-E2-related factor 2 (**Nrf2**), NAD(P)H/quinone oxidoreductase 1 (**NQO1**), heme oxygenase-1 (**HO-1**), glutathione-S transferase (**GST**), glutamate-cysteine ligase catalytic subunit (**GCLC**), glutamate-cysteine ligase modifier subunit (**GCLM**), and SOD expressions ($P < 0.05$). Analysis of differential enrichment of gut microbiota found that *Faecalibacterium* in the cecum of MAG supplemented broilers increased, and *Coprobacillus* has decreased ($P < 0.05$). In conclusion, MAG improved growth performance, meat quality of the broilers and antioxidant capacity, and modulated gut microbiota homeostasis.

Key words: magnolol, meat quality, antioxidant capacity, Nrf2, gut microbiota

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INTRODUCTION

The intensive artificial selection and high energy input in the diet for the last decade significantly increase the daily gain of body weight and shorten the raising period for commercial broilers, but unexpectedly exert the heavy metabolic burdens and oxidative stress (Mishra and Jha, 2019). Oxidative stress characterized as elevated reactive oxygen species (**ROS**) level could result in the deterioration of meat quality and leads to great economic loss for the industry every year (Xing et al., 2019). Therefore, the supplementation of antioxidants in the diets may have a dual effect for the commercial broilers on improving meat quality and maintaining growth performance.

Previous studies suggested that natural plant extracts such as resveratrol, oregano essential oil, and aloe vera had profound impacts on ameliorating the oxidative stress for animals (Avila-Ramos et al., 2012; Zhang et al., 2018; Amber et al., 2021). MAG is a neolignan, which is highly enriched in the bark of *Magnolia officinalis* (Liu et al., 2019). It has been reported that MAG showed great potentials to attenuate oxidative stress and alleviate cellular inflammation through scavenging the mitochondrial dysfunction and enhancing the phagocytosis in vitro (Dong et al., 2013; Parray et al., 2018; Chen et al., 2019b). MAG is also able to ameliorate oxidative stress and protect neurons from lipid oxidation for animals in the hippocampus of senescence-accelerated mice (Akagi et al., 2004). A previous study demonstrated that MAG could relieve the heatstroke-induced cerebral ischemic injury through inhibiting free radical formation in vivo (Chang et al., 2003). However, rarely studies reported the effect of MAG on commercial broiler as a feed additive and its underlying mechanism also need to be explored.

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[1]Corresponding author: hexi111@126.com

Oxidative stress is induced by the excessive cellular accumulation of ROS and serves as an important factor causing the gut microbiota perturbation, woody breast, and white striping myopathy (Rehman et al., 2018; Salles et al., 2019). NF-E2-related factor 2-Kelch-like ECH-associated protein 1 (**Keap1**) signaling pathway plays an essential role in preventing the cellular oxidative stress by the transcriptional activity of Nrf2 to induce the gene expression of antioxidants, such as HO-1, NQO1, SOD, and GST. The mechanism of bioactive compounds such as phenols, carotenoids, alkaloids, or sulfur to activate the Nrf2- Keap1 pathway was either through the direct induction of Nrf2 expression or through inhibition of the dimerization of Nrf2 and Keap1 or enhancement of the transcriptional activity of Nrf2 (Fakhri et al., 2020). MAG, known as an antioxidant, has been shown to stimulate the expression of Nrf2 and HO-1 in hepatocytes and hepatic steatosis mice (Kuo et al., 2020).

Magnolia bark now is extensively cultivated in China and Japan, the bioactive compound, MAG is also used in Chinese medical ingredients, food supplements, and cosmetics (Sarrica et al., 2018). However, the application of MAG for poultry production is still unknown. In this study, we investigated the effects of MAG supplementation on the growth performance, meat quality, oxidative capacity, and intestinal microbiota in yellow-feathered broilers.

MATERIALS AND METHODS

Animals and Experimental Design

The protocols used in the animal experiments were approved by the Institutional Animal Care and Use Committee at Hunan Agricultural University. A total of 360 male yellow-feathered broiler chicks with similar hatching weights (40.14 ± 0.77 g) were allocated into a completely randomized design with 5 treatments for a 51 d (Broilers reach slaughter weight at this time.) feeding trial. Each treatment consisted of 6 replicates (cages) with 12 birds each. The dietary treatments were 1) CON, basal diet; 2) MAG100, basal diet supplemented with 100 mg/kg MAG; 3) MAG200, basal diet supplemented with 200 mg/kg MAG; 4) MAG300, basal diet supplemented with 300 mg/kg MAG; 5) MAG400, basal diet supplemented with 400 mg/kg MAG. The dosage of magnolol reference Lin et al. (2017). The MAG used in this study with a purity of approximately 98.0% from J Microlta biological resources Co., Ltd. (Hunan, China.). The ingredient composition of the basal diet and its nutrient levels are presented in Table 1. Room temperature maintained at 34°C ± 1°C during the first week of age and gradually decreased to 25°C ± 1°C toward the end of the third week and thereafter until the 56 d. Broilers were reared in a temperature-controlled house under a light schedule of 24 h of light and were provided with mash feed and water ad libitum.

Table 1. Composition and nutrient level of the basal diet (g/kg, as-fed basis unless otherwise stated).

Items	1 to 28 d	29 to 51 d
Ingredients, %		
Corn	65.80	66.30
Wheat bran	0.60	1.00
Soybean meal	25.90	26.90
Fish meal	2.00	0
Soybean oil	1.70	1.80
Premix[1]	4.00	4.00
Total[2]	100.00	100.00
Nutrient levels		
Metabolizable energy (MJ/kg)	12.88	12.32
Crude protein, %	23.12	20.54
Lysine, %	0.99	0.88
Methionine, %	0.27	0.26
Calcium, %	0.91	0.90
Total phosphorus, %	0.42	0.36

[1]Premix provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3 2,500 IU; vitamin E 20 mg; vitamin K_3 3 mg; vitamin B_1 3.0 mg; vitamin B_2 8.0 mg; vitamin B_6 7.0 mg; vitamin B_{12} 0.03 mg; pantothenic acid 20.0 mg; nicotinic acid 50.0 mg; biotin 0.1 mg; folic acid 1.5 mg; Fe 96 mg; Cu 25 mg; I 0.9 mg; Zn 98 mg; Mn 105.4 mg; Se 0.3 mg.
[2]Calculated according to NRC (1994).

Growth Performance Measurement

All birds in each replicate were weighed individually after a 12-h feed deprivation on 28 and 51 d of age, and the feed consumption was recorded per replicate to calculate average daily feed intake (**ADFI**), average daily gain (**ADG**), and feed to gain ratio (**F/G**) during the 0 to 28 d, 29 to 51 d, and 0 to 51 d.

Sample Collection

On 28 and 51 d of age, one bird from each replicate of groups (6 birds from each treatment in total) was randomly selected for sampling after a 12-h feed withdrawal period. Birds were sacrificed by cervical dislocation. The breast muscle, intestinal samples. and cecal chyme were collected and stored at −80°C for further analysis.

Meat Quality

Meat quality was determined within 45 min after being sacrificed. The pH value of the breast muscle was measured using a pH meter (Beijing Bulader Technology Development Co., Ltd. Beijing, China.). The luminance (L*), redness (a*), and yellowness (b*) of the breast muscle were measured using a colorimeter (Threenh Technology Co., Ltd. Shenzhen, China.). Shear force of each sample was measured using a Digital Meat Tenderness Meter (Engineering College of the Northeast Agricultural University, Harbin, China.). The water loss rate was measured using a pressure gravimetric method (Chen et al., 2019a) (Nanjing Soil Instrument Factory Co., Ltd. Nanjing, China.). The water loss rate (%) was calculated as follows:

$$\text{The water loss rate (\%)} = \text{initial weight} - \text{final weight}/\text{initial weight} \times 100\%.$$

Determination of Antioxidants in the Breast Muscle and Jejunum

Under cold conditions in an ice water bath, the tissue homogenate was prepared using 0.9% NaCl at a weight (g)-to-volume (mL) ratio of 1:9. The homogenate supernatant was obtained by centrifugation (3,500 rpm) for 10 min. The levels of MDA, GSH, SOD, and T-AOC were assayed in the homogenate supernatant of the breast muscle and jejunum using the commercial assay kits purchased from Jiancheng Bioengineering Research Institute (Nanjing, China).

Gene Expression

Total RNA of the breast muscle was isolated using the SteadyPure Universal RNA Extraction Kit (Accurate Bioengineering Co., Ltd., Hunan, China). The mRNA expression levels of Nrf2, HO-1, NQO1, GST, GCLC, GCLM, SOD, and β-actin were measured by quantitative real-time PCR (**RT-qPCR**) technique with the primers shown in Supplementary Table 1. The RT-qPCR was performed using the TB Green Premix Ex Taq (TaKaRa, Biotechnology, Dalian, China). The mRNA levels were calculated using the $2^{-\Delta\Delta CT}$ method.

Characterization of Gut Microbiota by 16S rRNA Gene Sequencing

Cecal chyme samples of broilers were collected for analyzing the composition of gut bacterial communities, at the end of experiment. Briefly, the cecal chyme of broilers were collected separately into a 1.5-mL tube, and freeze-dried to achieve constant weight. An equal amount of chyme from each group was washed by DNase-free water to clean the surface, and then chyme DNA was extracted using a QIAamp PowerFecal DNA Kit (QIAGEN, Venlo, Netherlands), following the manufacturer's manual. The V3−V4 region of the 16S rRNA gene was amplified using forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR cycling conditions consisted of an initial denaturation of 2 min at 94°C, and 25 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. The quality of the amplified 16S rRNA gene was checked on 0.8% agarose gels. Amplicon sequencing was performed on the Illumina MiSeq System at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

Statistics Analysis

All data were analyzed with one-way ANOVA followed by Duncan's multiple range test using SPSS 26.0 software (SPSS Inc., Chicago, IL). Data were expressed as means and pooled SEM. The significance was declared at $P < 0.05$.

RESULTS

Growth Performance

The effects of MAG supplementation on the growth performance of yellow-feathered broilers were presented in Table 2. The results showed that dietary supplementation with MAG had a significant improvement on the chicken final body weight, ADG, and F/G compared with the CON group during da 1 to 51 ($P < 0.05$). At the 1 to 28 d, dietary treatments did not affect growth performance. But supplementing with 200 and 300 mg/kg MAG showed higher ADG, and lower F/G than the CON group at the 28 to 51 d ($P < 0.05$).

Meat Quality

The effect of MAG addition in the diet on the meat quality of breast muscle was presented in Table 3. Supplementation of 300 mg/kg MAG in the diet significantly increased the redness (a^*) of the breast muscle relative to other groups ($P < 0.05$). Compared with the CON group, supplementing with 100, 300, and 400 mg/kg MAG decreased the water loss rate ($P < 0.05$). Besides, MAG addition decreased shear force compared to the CON group ($P < 0.05$) and the dosage effect

Table 2. Effects of magnolol supplementation on growth performance of broilers.

Items	CON	MAG100	MAG200	MAG300	MAG400	SEM	*P*-value
Initial body weight (g)	40.21	39.42	40.38	40.27	40.18	0.160	0.301
Final body weight (kg)	2.05^{bc}	2.03^{c}	2.15^{a}	2.19^{a}	2.13^{ab}	0.018	0.006
1 to 28 d							
ADFI (g)	52.32	50.90	51.74	51.86	52.84	0.373	0.576
ADG (g)	28.50	27.69	28.36	29.19	29.13	0.239	0.248
F/G	1.84	1.84	1.83	1.78	1.82	0.012	0.444
29 to 51 d							
ADFI (g)	129.06^{bc}	125.75^{c}	132.52^{ab}	136.93^{a}	134.67^{ab}	1.155	0.004
ADG (g)	52.71^{b}	52.84^{b}	57.22^{a}	57.84^{a}	55.25^{ab}	0.613	0.003
F/G	2.45	2.38	2.32	2.37	2.44	0.016	0.052
1 to 51 d							
ADFI (g)	89.54^{ab}	87.25^{b}	90.75^{ab}	92.88^{a}	92.39^{a}	0.652	0.020
ADG (g)	39.42^{bc}	39.03^{c}	41.37^{a}	42.11^{a}	40.91^{ab}	0.346	0.006
F/G	2.27^{a}	2.23^{abc}	2.20^{c}	2.21^{bc}	2.26^{ab}	0.009	0.027

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; F/G, feed to gain ratio.

$^{a-c}$Means within a column not sharing a common superscript differ significantly ($P < 0.05$). Each mean represents 6 replicates per treatment, with 12 layers per replicate.

Table 3. Effects of magnolol supplementation on meat quality of broilers.

Items	CON	MAG100	MAG200	MAG300	MAG400	SEM	*P*-value
pH (24 h)	4.74	4.54	4.65	4.67	4.60	0.032	0.334
L*	51.44	52.15	52.37	51.47	51.50	0.461	0.957
a*	0.88[ab]	0.53[b]	0.60[b]	1.31[a]	0.54[b]	0.091	0.021
b*	5.43	6.64	6.35	6.10	6.54	0.249	0.544
Water loss rate, %	31.86[a]	26.17[c]	29.54[ab]	26.23[c]	27.36[bc]	0.562	0.000
Shear force (kg)	1.96[a]	1.72[b]	1.65[b]	1.64[b]	1.67[b]	0.027	0.000

Abbreviations: L*, lightness; a*, redness; b*, yellowness.
[a-c]Means within a column not sharing a common superscript differ significantly ($P < 0.05$). Each mean represents 6 replicates per treatment, with 12 layers per replicate.

was not significant ($P > 0.05$). However, MAG had no effect on the pH, lightness (L*), and yellowness (b*) of the breast muscle ($P > 0.05$).

Antioxidant Capacity in Breast Muscle and Intestine

The effects of MAG on the antioxidant capacity of the breast muscle were shown in Table 4. On 28 d of age, compared with the CON group, dietary supplementation of 100 and 200 mg/kg MAG increased GSH activity in the breast muscle ($P < 0.05$). The SOD activity of the MAG100 group was greater than that of the CON group ($P < 0.05$), whereas there were no significant effects of MAG supplementation on MDA and T-AOC ($P > 0.05$). On 51 d of age, the administration of MAG reduced breast muscle MDA levels and SOD activity ($P < 0.05$).

As shown in Table 5, on d 28, compared with the CON group, supplementing with 100 mg/kg MAG increased T-AOC levels and SOD activity ($P < 0.05$), and all MAG supplemented groups have a lower MDA level in the jejunum ($P < 0.05$). On d 51, broiler in the MAG200, MAG300, and MAG400 groups tended to have greater GSH activity than the CON group ($P < 0.05$).

Expression of Nrf2 Signaling Pathway-Related Genes in Breast Muscle

In order to explore the effect of MAG on Nrf2 signaling, we assessed the impact of MAG on the gene expression associated with Nrf2 signaling pathway in breast muscle. The results were listed in Figure 1. The gene expressions of Nrf2, NQO1, HO-1, GCLC, and SOD in the MAG200 and MAG400 groups were significantly

Table 4. Effects of magnolol on antioxidant capacity of the breast muscle in broilers.

Items	CON	MAG100	MAG200	MAG300	MAG400	SEM	*P*-value
28 d							
MDA (nmol/mg protein)	0.08	0.06	0.07	0.07	0.06	0.005	0.530
T-AOC (U/mg protein)	0.07	0.10	0.09	0.11	0.07	0.005	0.064
GSH (mg/g protein)	1.62[b]	2.59[a]	2.25[a]	1.38[b]	1.70[b]	0.117	0.001
SOD (U/mg protein)	15.23[bc]	18.12[a]	17.02[abc]	17.47[ab]	14.54[c]	0.439	0.030
51d							
MDA (nmol/mg protein)	0.38[a]	0.21[b]	0.19[b]	0.18[b]	0.24[b]	0.020	0.004
T-AOC (U/mg protein)	0.33[ab]	0.44[a]	0.39[a]	0.29[ab]	0.19[b]	0.028	0.019
GSH (mg/g protein)	2.62	4.62	3.66	3.56	3.87	0.224	0.132
SOD (U/mg protein)	64.51[a]	49.24[b]	41.17[bc]	35.00[c]	43.24[bc]	2.307	0.000

Abbreviations: GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase, T-AOC, total antioxidant capacity.
[a-c]Means within a column not sharing a common superscript differ significantly ($P < 0.05$), n = 6. Each mean represents 6 replicates per treatment, with 1 layer per replicate.

Table 5. Effects of magnolol on antioxidant capacity of the jejunum in broilers.

Items	CON	MAG100	MAG200	MAG300	MAG400	SEM	*P*-value
28 d							
MDA (nmol/mg protein)	0.34[a]	0.24[b]	0.23[b]	0.19[b]	0.18[b]	0.016	0.012
T-AOC (U/mg protein)	2.79[b]	3.58[a]	2.99[ab]	2.30[b]	2.42[b]	0.135	0.006
GSH (mg/g protein)	32.36	40.31	33.75	34.48	41.34	1.462	0.172
SOD (U/mg protein)	129.78[b]	181.63[a]	143.70[b]	135.28[b]	159.28[ab]	6.042	0.021
51 d							
MDA (nmol/mg protein)	0.55	0.34	0.42	0.46	0.44	0.038	0.615
T-AOC (U/mg protein)	2.67	2.52	3.51	2.72	2.05	0.165	0.083
GSH (mg/g protein)	29.10[d]	36.35[cd]	62.53[a]	47.12[bc]	52.07[ab]	2.904	0.001
SOD (U/mg protein)	181.32	187.46	199.89	163.60	185.57	5.173	0.309

Abbreviations: GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase, T-AOC, total antioxidant capacity.
[a-d]Means within a column not sharing a common superscript differ significantly ($P < 0.05$). Each mean represents 6 replicates per treatment, with 1 layer per replicate.

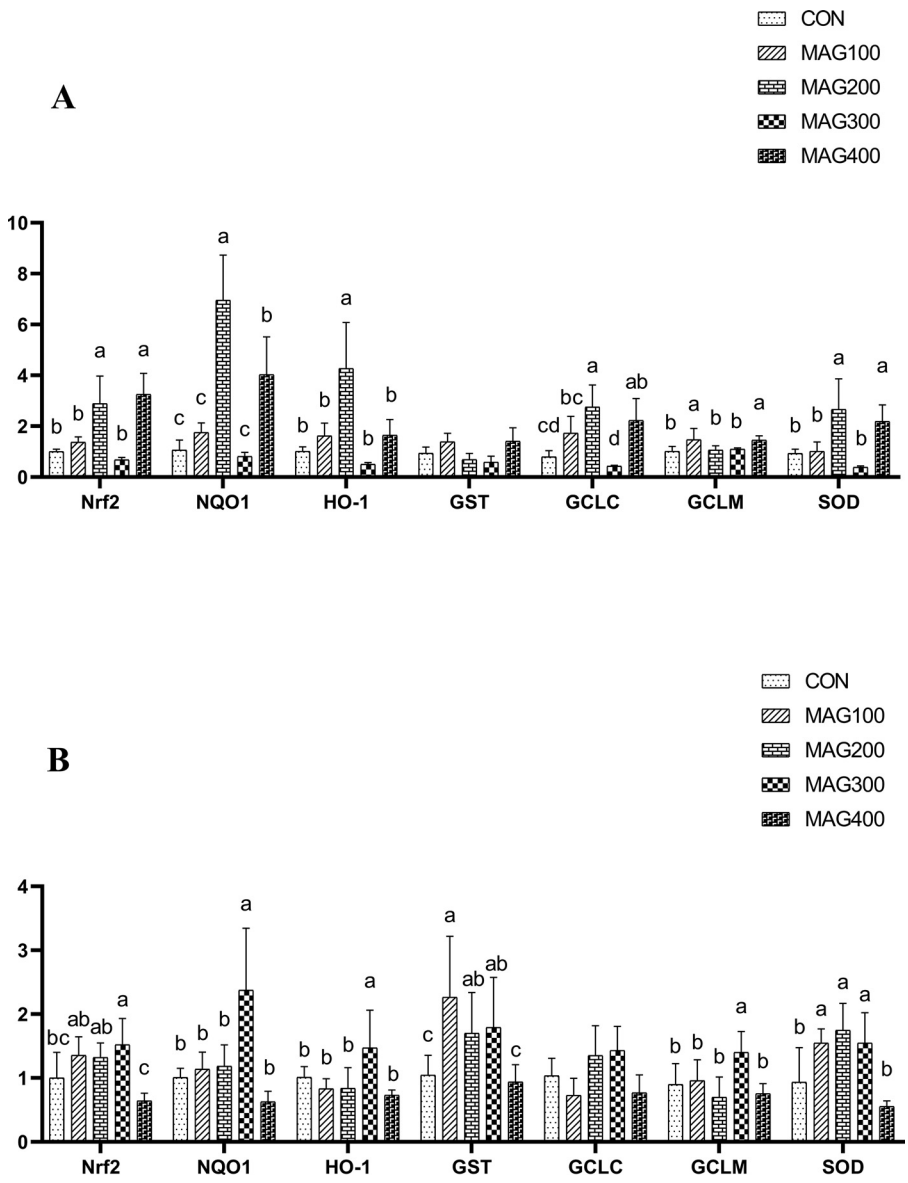

Figure 1. Effects of magnolol on mRNA levels of Nrf2 signaling pathway-related genes at d 28 (A) and d 51 (B) in breast muscle. Abbreviations: HO-1, heme oxygenase-1; GST, glutathione-S transferase; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase modifier subunit; Nrf2, NF-E2-related factor 2; NQO1, NAD(P)H/quinone oxidoreductase 1; SOD, superoxide dismutase.

higher than the CON group on d 28 ($P < 0.05$). Besides, the MAG100 and MAG400 group showed a significant upregulation of GCLC mRNA level in breast muscle compared to CON group ($P < 0.05$). On 51 d of age, compared with the CON group, dietary supplementation of 100 mg/kg and 200 mg/kg MAG increased GST and SOD mRNA expression ($P < 0.05$). In addition, the MAG200 group showed a significant increase of SOD mRNA level in breast muscle compared to the CON group ($P < 0.05$). Moreover, the Nrf2, NQO1, HO-1, GST, GCLM, and SOD mRNA expression of MAG300 group was significantly higher than the CON group ($P < 0.05$).

Alpha Diversity of Cecal Microbiota

The diversity of cecal microbiota is shown in Table 6. Compared with the CON group, MAG supplementation decreased the observed species richness (Chao1) of cecal microbiota at 28 d of age ($P < 0.05$). However, species diversity (Shannon and Simpson) of cecal microbiota did not change in broilers fed with MAG at d 28 or 51.

Taxonomic Composition of Cecal Microbiota

The phylum level taxonomic composition analysis (Figure 2, Supplementary Table 2) showed that *Firmicutes, Bacteroidetes, Proteobacteria, Tenericutes,* and *Actinobacteria* were the dominant bacteria in chicken cecum both at early and late stages. Additionally, the changes of microbial composition between different treatment groups were not apparent either at the early stage or late stage

At the genus level (Figure 2, Supplementary Table 3), all MAG supplemented groups have a lower *Coprobacillus* (0.07−0.16%) abundance at d 28 ($P < 0.05$), and the MAG100 group showed a significant increase of *Faecalibacterium* (19.45%) abundance at d 51 ($P < 0.05$).

Table 6. Effect of magnolol on richness and diversity of gut microbiota in broilers.

Items	CON	MAG100	MAG200	MAG300	MAG400	SEM	*P*-value
28 d							
Chao1	3,571.13[a]	2,803.54[b]	2,629.24[b]	2,651.62[b]	2,245.08[b]	130.346	0.011
Observed_species	2,543.56	2,150.42	2,078.02	2,093.68	1,885.16	83.871	0.148
Shannon	6.69	6.44	6.31	6.80	6.48	0.165	0.906
Simpson	0.90	0.88	0.88	0.92	0.90	0.011	0.763
51d							
Chao1	3,114.39	2,404.74	2,370.59	2,695.21	2,305.75	112.112	0.116
Observed_species	2,258.58	1,845.72	1,813.10	2,135.86	1,861.96	79.061	0.278
Shannon	7.04	6.29	6.02	7.04	6.53	0.195	0.378
Simpson	0.94	0.91	0.88	0.94	0.90	0.012	0.361

[a-b]Means within a column not sharing a common superscript differ significantly ($P < 0.05$). Each mean represents 6 replicates per treatment, with 1 layer per replicate.

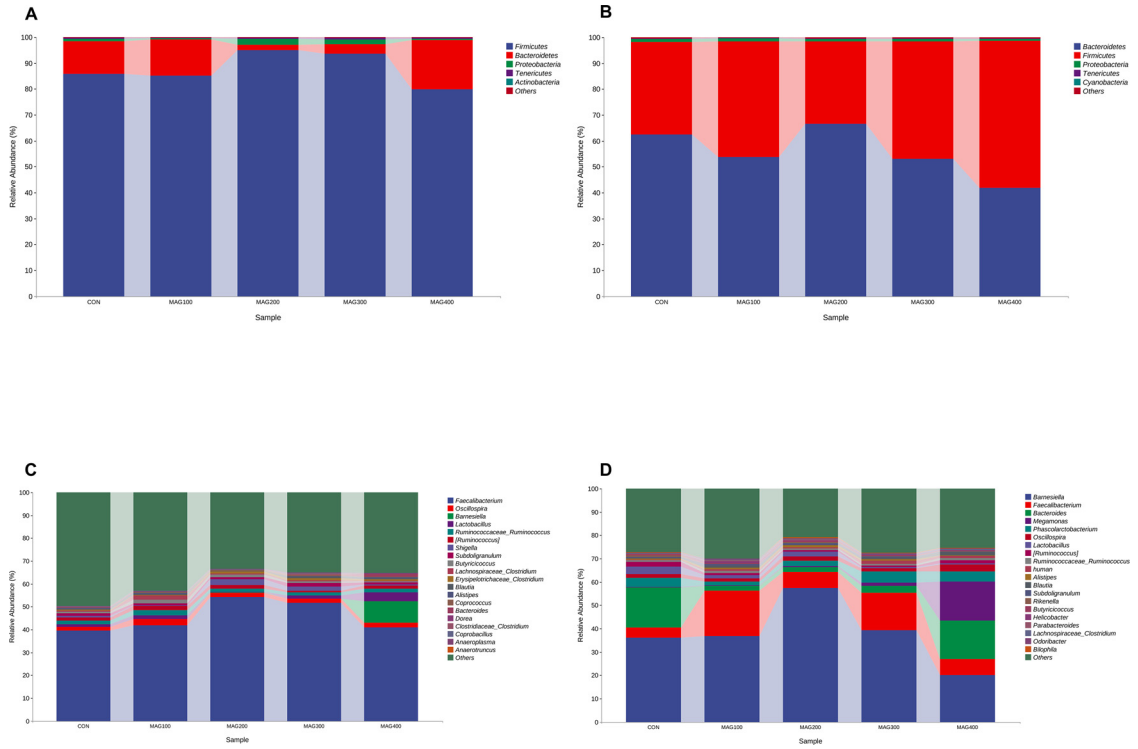

Figure 2. Phylum-level and genus-level taxonomic composition of the cecal microbiota in different groups. (A) 28 d Phylum-level, (B) 51 d Phylum-level, (C) 28 d Genus-level, (D) 51 d Genus-level.

DISCUSSION

MAG was known as a natural polyphenolic compound and was found to have antioxidant activity (Amorati et al., 2015). Several studies have suggested dietary polyphenols can improve the growth performance of livestock and poultry via their antioxidant activity (Eid et al., 2003; He et al., 2019). Starčević et al. (2015) reported that phenolic compounds used in small doses can act as health promoters. Polyphenol-rich grape seed could be supplemented in the diet of broiler chickens to improve performance, enhance antioxidant capacity, and decrease detrimental bacteria in the ileum (Abu Hafsa and Ibrahim, 2018). In the present study, we found that MAG supplementation could increase ADG, but decrease F/G in yellow-feather broilers. It is worth mentioning that during the whole rearing period, the 200 mg/kg and 300 mg/kg MAG group showed improved ADG by 4.9 and 6.8%, and F/G was significantly decreased by 3.2 and 2.6%, as compared with the control group. Similar to our findings, MAG improved the antioxidant capacity, intestinal mucosal status, and growth performance of *Linwu* ducks (Lin et al., 2017). These data suggest that MAG may be a potential candidate to improve the growth performance of animals.

Physiological characteristics including appearance and texture directly affect the willingness of consumers to purchase meat products. The pH value, color, water loss rate, and shear force were widely used to evaluate the sensory characteristics of meat quality (Castellini et al., 2002). Myoglobin content difference and its oxidation states at the muscle surfaces is the primary determinant for the meat color. When oxidation of oxymyoglobin to metmyoglobin and the color of the muscle changes from bright red to reddish-brown (Suman and Joseph, 2013). Several investigators have reported that the lipid oxidation process promotes

oxymyoglobin oxidation, but the preservation of fresh meat color following the inclusion of antioxidant ingredients (O'Grady et al., 2001; Faustman et al., 2010). Studies have reported that MAG can trap radicals and are active to reduce $ONOO^-$ and $^1O_2$ (Zhao and Liu, 2011). Simultaneously, MAG radicals do not react with molecular oxygen and produce no superoxide radical (Amorati et al., 2015). Interestingly, we also found that dietary MAG supplementation decreased the MDA level of the breast muscle in yellow-feather broilers on 51 d of age, which indicated that MAG supplementation improved oxidative stability of breast muscle. In the current study, MAG significantly increased a* of the breast muscle, which is related to the antioxidant activity of MAG. In this study, MAG supplementation improved physical characteristics of muscle (i.e., color, water loss rate, or shear force), and a dosage of 300 mg/kg MAG supplementation exhibits a better effect. The water loss rate is used to indicate the water-holding capacity of muscle (Huang et al., 2021). Shear force is a valuable and intuitive parameter for evaluating muscle tenderness (Bowker et al., 2011). Myofibrillar proteins exert a vital role in meat quality particularly with water-holding capacity (Chen et al., 2017). ROS will attack the muscle proteins and result in oxidative modification, thus affecting meat water-holding capacity (Zhang et al., 2013). Yi et al. (2021) reported that due to the strong hydrophobic interaction and covalent bond the beef myofibrillar proteins aggregation under excessive oxidative modification induced by high linoleic acid concentration resulted in decreased gel water-holding capacity. In addition, meat with a higher water-holding capacity may show accelerated tenderization, yielding improved meat quality (Qiao et al., 2001). Lin et al. (2020) indicated that MAG additive improves the meat quality of *Linwu* ducks by modulating antioxidative status. MAG administration remarkably increased the a* of leg muscle and decreased water loss rate of leg and breast muscle, which is similar to the result of our study. Therefore, these results collectively imply that the improvement in meat quality might have resulted from the antioxidant properties of MAG.

Several studies have shown that MAG can improve oxidation stability (Lu et al., 2014; Nishiyama et al., 2019). Chen et al. (2001) reported that the inhibitory effects of intimal hyperplasia and MCP-1 expression in balloon injured aorta of cholesterol-fed rabbits might be attributed to the antioxidant capacity of MAG. In laying hens, MAG significantly improves SOD activity (Chen et al., 2021), which outcome was similar to our study. Our study confirmed that MAG could exert antioxidative activities by improving breast muscle and jejunum SOD, GSH, and T-AOC levels and decreasing MDA levels. This means that MAG may be an effective antioxidant, which could improve the oxidative stability of broilers. Nrf2 is a redox-sensitive nuclear transcription factor that augments cellular defense against oxidative damage. Nuclear factor erythroid 2−related factor 2 is present in the cytosol under normal conditions, and when oxidative stress occurs, Nrf2 is translocated into the nucleus and binds to the antioxidant response element to augment the expression of antioxidant genes (Chen et al., 2019c). Broiler chickens fed MAG in the present study exhibited significantly increased mRNA levels of Nrf2 in breast muscle, suggesting that the antioxidant capacity of breast muscle has favorable changes under MAG supplementation, thereby improving the meat quality (Huang et al., 2021; Rajgopal et al., 2016). Interestingly, the expression of Nrf2 downstream related mRNA genes in breast muscles was also upregulated, including NQO1, HO-1, GCLC, and GCLM. Studies have shown that magnolia cortex extract can significantly suppress cytotoxicity induced by H_2O_2 or 6-hydroxydopamine (**6-OHDA**) by inducing NQO1 and catalase in PC12 cells (Nishiyama et al., 2019). HO-1 is the rate-limiting enzyme in heme catabolism and catalyzes the formation of equimolar carbon monoxide, biliverdin, and ferrous iron, which provide an inducible defense system against oxidant stress (Salerno et al., 2017). Kuo et al. (2020) observed that MG administration increased the expression of HO-1 and SOD2 by promoting the translocation of Nrf2 into the nucleus, and the induction of HO-1 and SOD2 led to the reduction of oxidative stress in rats. GCLC and GCLM form a heterodimer, which is the rate-limiting step in the synthesis of GSH (Lu, 2013; Jin et al., 2019). We observed that MAG increased the expression of NQO1, HO-1, GCLC, and GCLM levels of broilers. Therefore, it is reasonable to believe that MAG strengthens oxidative stability by activating NQO1, HO-1, and GSH synthesis-related gene expression in the Nrf2 signaling pathway.

Gut microbiota contributes to the maintenance of the normal physiological function of the intestine, providing a series of beneficial effects on their hosts, such as nutrient digestion and protection from invasive pathogens (Chang et al., 2020; Sliżewska et al., 2020). The data on the intestinal microbiota of birds has shown that *Firmicutes, Bacteroidetes, Proteobacteria, Tenericutes*, and *Actinobacteria* are the major intestinal phyla, which suggests their importance in host metabolism and physiology (Chen et al., 2020; Das et al., 2020). Similar to these reports, this study indicated that MAG did not change the major bacterial phylum in the cecum of yellow-feather broilers. However, we found that MAG induced differentially enriched bacterial species at genus taxonomic levels in broilers. In our study, the abundance of *Faecalibacterium* in the cecum of MAG-supplemented broilers increased, and *Coprobacillus* have decreased. *Faecalibacterium* has been associated with a healthy gut status which fermentation produces mainly short-chain fatty acids such as butyrate (Minamoto et al., 2015; Li et al., 2019; Ye et al., 2019). As the preferred energy source for Intestine epithelial cells, butyrate is a microbial metabolite that is exhibiting a wide spectrum of positive effects, such as improve growth performance and gut function (So. et al., 2018; Wang et al., 2019). Indoleproprionic acid (**IPA**) is an antioxidant metabolite produced by gut microbes from dietary tryptophan. Menni et al. (2019) found that *Faecalibacterium* prasunitzii, which has been shown to not produce IPA, is

positively correlated with IPA levels. *Faecalibacterium* may thrive in a similar environment to that of IPA producers and hence they may be signatures for IPA production even if they lack this function. In our study, we observed that MAG improves growth performance, breast muscle and intestinal antioxidant capacity, which may be related to the up-regulation of *Faecalibacterium* abundance. *Coprobacillus* has been proven to be a pathogen, but its associations with the health of the broilers are still unknown (Heo et al., 2020). This means that MAG may improve of broilers gut health by reducing pathogenic bacteria. It can be speculated that supplemented with MAG probably improves growth performance, antioxidant capacity, and gut health of broiler, which should be associated with its multiple effects on the differential enrichment of gut microbiota. However, further studies are required on the effect of these variably abundant microbial species.

CONCLUSIONS

In this study, we demonstrated that MAG can improve the growth performance, meat quality, oxidative stability, and altered the function features of gut microbiota of yellow-feather broilers.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.101722.

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