Poultry Science®

Effects of garcinol supplementation on the performance, egg quality, and intestinal health of laying hens in the late laying period

Weilei Yao,[*,†] Enling Wang,[*,†] Yan Zhou,[‡] Yanxu Han,[*,†] Shimin Li,[*,†] Xinyi Yin,[*,†] Xinlei Huang,[*,†] and Feiruo Huang[*,†,1]

[*]*Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, China;* [†]*Frontiers Science Center for Animal Breeding and Sustainable Production, Wuhan 430070, China; and* [‡]*Wuhan Academy of Agricultural Sciences, Wuhan 430072, China*

ABSTRACT The problem of rapid decline in egg production performance and poor egg quality is a key obstacle to improving the economic benefits of laying hens. Garcinol is an antioxidant polyphenol plant extract that has multiple physiological functions. Diets with the appropriate amount of garcinol might be able to improve the performance traits and health of late laying hens. Therefore, this study was conducted to evaluate the utilization of garcinol in late laying hens. A total of 400 healthy 59-wk-old Tingfen No. 6 hens were randomly allocated into 4 dietary treatment groups and fed a basal diet supplemented with 0, 100, 300, and 500 mg/kg garcinol for 12 wk, denoted the Con, LG, MG, and HG groups, respectively. The results showed that the addition of garcinol in the diet tended to increase the egg production rate compared with that of the control group ($P = 0.080$), while the average egg weight was significantly lower ($P < 0.05$) during the whole period of the experiment. The results showed that MG group hens had higher egg quality and strengthened antioxidant capacity in their serum ($P < 0.05$). Moreover, the laying hens in the MG group had significantly decreased crypt depth (**CD**) and increased villus height (**VH**) in the jejunum and ileum ($P < 0.05$), as well as an increased ratio of VH to CD ($P < 0.05$) and increased expression levels of *Occludin* ($P < 0.05$) and *Claudin-2* ($P < 0.05$) in the jejunum to improve intestinal barrier function. In addition, dietary supplementation with garcinol influenced the cecal microbiota of laying hens, which was characterized by changes in the microbial community composition, including increased abundances of Firmicutes, *Romboutsia*, and *Ruminococcus torques*. In conclusion, dietary 300 mg/kg garcinol supplementation could increase the egg production and egg quality of late laying hens, which may be attributed to the antioxidant effects of garcinol and the improvement of intestinal morphology and epithelial barrier function as well as the regulation of mucosal immune status by altering microbial composition.

Key words: garcinol, laying hens, production performance, egg quality, cecal microbiota

2023 Poultry Science 102:102939
https://doi.org/10.1016/j.psj.2023.102939

INTRODUCTION

In the poultry industry, the laying performance and egg quality of laying hens in the late phase usually decline, with increased mortality on occasion. The decrease in laying performance is mainly manifested in a decrease in the laying rate; a decline in egg quality, such as eggshell quality or Haugh unit, and an increase in the egg-feed ratio (Lv et al., 2019). During the aging process and under the influence of various stress factors, late laying hens are often in a subhealthy state, with changes in metabolic homeostasis, an increase in reactive oxygen species (**ROS**) and a decrease in the body's ability to resist oxidative stress, while at the same time, the damage to immunocompetent cells is reduced, the homeostatic balance of the immune response is disrupted, and immune function is reduced (Mishra and Jha, 2019; Shini et al., 2019). In addition, the gut microbial composition of laying hens changes significantly with age, and the balance of the gut flora is easily imbalanced in the late laying period, such that the amount of beneficial bacteria decrease and that of harmful bacteria increase, the digestive function of laying hens is weakened and inflammatory responses are easily induced, which ultimately leads to a decrease in production performance (Xiao et al., 2021). Studies have shown that compromised intestinal functions, immune imbalance and

Received June 14, 2023.
Accepted July 15, 2023.

[1]Corresponding author: huangfeiruo@mail.hzau.edu.cn

intestinal flora disturbance due to high-intensity production are ascribed to the poor egg performance of hens in the late laying period (Sugiharto, 2016; Yang et al., 2020). Therefore, producers need to deal with this change by improving antioxidant function and intestinal health of laying hens in order to deal with the performance loss of late-phase egg production.

At present, many studies have shown that plant extracts play an important role in improving the health and production performance of laying hens and have been widely used and promoted in the laying hen breeding industry (Akyildiz and Denli, 2016; Mahfuz et al., 2021). Among them, plant polyphenol extract is a kind of additive that has been proven to be effective in a large number of alternative studies due to its biological effects and has great potential for development (Abdel-Moneim et al., 2020). Garcinol (molecular formula, $C_{38}H_{50}O_6$) is a polyphenol isolated from the fruit rind of *Garcinia indica* that contains active groups similar to those of curcumin. In addition, garcinol has been reported to demonstrate a range of physiological processes in cells and mice in vitro, including anti-inflammatory, antioxidant, and antibacterial activities (Schobert and Biersack, 2019). Our previous study reported that supplementation of garcinol in the diet of pregnant rats can improve the antioxidant function of the liver (Yao et al., 2020). Moreover, it has been reported that garcinol supplementation increases the levels of intestinal symbiotic bacteria in mice to prevent intestinal disorders induced by a high-fat diet (Lee et al., 2019). In previous study, dietary supplementation with garcinol was found to inhibit the growth of harmful bacteria in the gut and ameliorate intestinal barrier dysfunction and inflammation in weaned piglets (Wang et al., 2020). Therefore, it is reasonable to speculate that garcinol can promote the performance of laying hens by improving the antioxidant function and intestinal microbial homeostasis in the late laying period.

Previous studies reveal that garcinol has been used to prevent or treat intestinal disorders. However, there are few reports about the effects of dietary supplementation with garcinol in late laying hens. Thus, the present study was first conducted to investigate the effects of garcinol on the production performance, egg quality, and intestinal health of laying hens in the late laying period and to explore the appropriate dosage of garcinol to estimate its potency as a plant feed additive and to provide a theoretical and experimental basis for the biological effects of garcinol and its application.

MATERIALS AND METHODS

Birds and Experimental Design

This study was conducted in Shendan in Hubei Province from April to July 2021. This experiment was approved by the Animal Care and Use Committee of the College of Animal Sciences and Technology, Huazhong Agricultural University and was conducted in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The animal handling protocol permit number is HZAUSW-2022-0006. A total of 400 Tingfen No. 6 laying hens (59-wk old) were randomly assigned to 4 dietary treatments with 10 replicates per treatment. The control group (**Con**) received a standard maize and soybean meal basal diet (Table 1), formulated according to the composition and nutrient levels in line with the Agricultural Trade Standardization of China (MAPRC, 2004). The 3 treatment groups (LG, MG, and HG) received a basal diet supplemented with 100, 300, and 500 mg/kg garcinol, respectively. Before the start of the experiment, all hens were fed a basal diet for 2 wk and were similar in body size and had similar egg production. The formal experimental period lasted 14 wk (from 61 to 73 wk of age). Birds were housed in stainless steel cages (38.1 cm width × 50 cm length × 40 cm height). The room environment was controlled at 22°C, and a daily lighting schedule of 16 h light and 8 h dark was adopted throughout the whole trial. Hens were allowed free access to experimental diets and water.

Table 1. Composition and nutrient levels of basic diets.

Ingredient	Content (%)	Nutrient levels[2]	Content
Corn	62.30	ME (kcal/kg)	2662
Soybean meal	25.00	CP (%)	15.73
Soybean oil	0.70	CF (%)	2.47
Limestone	9.00	Ca (%)	4.02
premix[1]	3.00	TP (%)	0.35
Total	100.00	Lysine (%)	0.80
		Methionine + cysteine (%)	0.64

Abbreviations: CF, crude fiber; CP, crude protein; ME, metabolizable energy.

[1]The premix is DSM L33 premix. Provided per kilogram of diet: vitamin A 9,900 IU, vitamin D_3 4,000 IU, vitamin E 25 IU, vitamin K 32.5 IU, vitamin B_1 2 mg, vitamin B_2 6 mg, vitamin B_6 4 mg, vitamin B_{12} 0.024 mg, biotin 0.2 mg, pantothenic acid 10 mg, nicotinamide 35 mg, folacin 1 mg, choline 360 mg, Fe 80 mg, Cu 10 mg, Mn 100 mg, Zn 100 mg, I 1.1 mg, Se 0.3 mg, and methionine 1.5 g.

[2]Nutrient levels were calculated values.

Performance and Egg Quality Parameters

Daily egg production was recorded and expressed as the output rate per hen per day. Eggs were weighed twice a week according to the replicate basis. The provided and residual feed amount of each replicate were recorded every week. The egg production rate, average egg weight, average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**) were calculated each week. At wk 4, 8, and 12 of the trial, 20 eggs from each treatment group were randomly selected to determine the egg quality. Eggshell color [L*(lightness), a*(redness), and b*(yellowness)] values were measured once for each egg on the equatorial region by a color meter. Eggshell strength was evaluated using an eggshell force gauge (EFR-01, ORKA Food Technology Ltd., Israel). The albumen height, egg yolk color, and Haugh unit were evaluated using an egg multitester (DEF-6000, Tokyo, Japan). Additionally, the eggshell thickness of each egg was determined using a Vernier caliper.

Sample Collection

At 73 wk of age, 10 hens per group with body weights close to the average were randomly selected after 10 h of feed deprivation. Blood samples were collected from the wing vein using vacuum blood collection tubes. The serum was separated by centrifugation at 3,000 × *g* for 15 min and stored at −20°C until it was used for the biochemical analysis. Then, the hen from each group was killed by jugular bloodletting. Tissue samples of the duodenum, jejunum, and ileum were immediately collected and rinsed with cold saline (NaCl 9 g/L, 4°C) and fixed in paraformaldehyde. Other segments of the jejunum were opened and thoroughly rinsed with sterile normal saline, and then the mucosa was collected by scraping with glass slides, immediately frozen in liquid nitrogen, and kept in a freezer (−80°C) for measurements of gene expression and biochemical analysis.

Serum Antioxidant Capacity and Immune-Related Indicator Analysis

The levels of glutathione peroxidase (**GSH-Px**), catalase (**CAT**), total superoxide dismutase (**T-SOD**), total antioxidant capacity (**T-AOC**), and malondialdehyde (**MDA**) in serum were determined using commercial kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Company, Jiangsu, China). Similarly, the kit provided by Nanjing Jiancheng Biotechnology company (Nanjing Jiancheng Biotechnology, Nanjing, China) was used to detect 9 indices, including interleukin-2 (**IL-2**), interleukin-6 (**IL-6**), tumor necrosis factor (**TNF-α**), immunoglobulin A (**IgA**), immunoglobulin G (**IgG**), immunoglobulin M (**IgM**), lipopolysaccharides (**LPS**), cortisol (**CORT**), and D-lactate (**LD**) acid, by enzyme-linked immunosorbent assay.

Intestinal Morphology Analysis

Paraformaldehyde-fixed duodenum, jejunum, and ileum were sliced to observe intestinal morphology. The intestinal sample tissue was removed from the fixative, embedded in paraffin after being trimmed, dehydrated, transparentized, and waxed. After the wax block was made, the samples were sectioned and then stained with eosin. Finally, the sections were selected according to the sectioning and staining effects, and neutral resin was used for mounting. The sections of each tissue were observed using an optical microscope and photographed. The villus height (**VH**) and corresponding crypt depth (**CD**) were measured by microscope image processing software (Image-Pro Plus 6.0), and the ratio of VH to CD (VH/CD) was calculated. Five values were taken from each slice, and the average value was calculated for data processing and analysis.

Gene Expression of the Jejunum

Total RNA was extracted from the ileum using TRIzol Reagent, and reverse transcription was performed using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Dalian, China). Quantitative real-time PCR was performed using TB Green Premix Ex Taq II (Takara) by a Light Cycler 96 PCR System to examine the mRNA levels. Relative RT-PCR was performed to measure gene expression of Claudin-2, Occludin, and Zo-1mRNAs. The primers used were shown in Table 2. Relative quantification of gene expression was determined by $2^{-\Delta\Delta Ct}$.

Table 2. Primer sequences of target and reference genes.

Gene name	Primer sequence (5′−3′)
Claudin-2	F: CATACTCCTGGGTCTGGTTGGT
	R: GACAGCCATCCGCATCTTCT
Occludin	F: ACGGCACCTACCTCAA
	R: GGGCGAAGAAGCAGATGAG
Zo-1	F: CAACGTAGTTCTGGCATTATTCG
	R: GGAGGATGCTGTTGTCTCGG
β-actin	F: AGTGTCTTTTTGTATCTTCCGCC
	R: CCACATACTGGCACTTTACTCCTA

Cecal Microbiota Analysis

Bacterial genomic DNA was extracted from the cecal digesta according to the manufacturer's instructions. The extracted genomic DNA samples were detected by 1% agarose gel electrophoresis. All sequence data were processed using the QIIME software package (V1.9.1). The sequences were compared on the Greengenes database (http://greengenes.secondgenome.com). The Mothur package (V1.30.2) was used to identify and remove chimeric sequences. Using the Greengene database, the columns were divided into different OTUs according to the 97% similarity threshold, and each OTU was usually regarded as a microbial species. A Venn diagram was obtained by comparing OTUs between samples or groups. Finally, Venn diagrams with common and unique OTUs were used to identify similarities between samples from different treatment groups. Microbial *α*-diversity was analyzed using the MOTHUR v1.30.2 program. Microbial *β*-diversity uses nonmetric multidimensional scaling (**NMDS**) to evaluate the pairing distance among samples. Linear discriminant analysis (**LDA**) combined effect size measurements (**LEfSe**) were applied to identify the relative richness (LDA > 2) of bacteria between groups.

Statistical Analysis

All the results from experiment were analyzed by using the 1-way ANOVA, performed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Replicate ($n = 10$) served as the experimental unit. Data were analyzed using the general linear model (**GLM**) with SPSS 16.0 (SPSS Inc., Chicago, IL). The results in the tables are shown with the means ± SEM, and other figure results are presented with means ± SEM. The $P < 0.01$,

$P < 0.05$, and $P \leq 0.10$ were deemed the statistical high significance, significance, and tendency, respectively.

RESULTS

Effects of Dietary Garcinol on Egg Production Performance

As shown in Table 3, garcinol had no significant effect on the ADFI ($P > 0.10$). Additionally, dietary garcinol supplementation did not affect the FCR ($P > 0.10$). At the fourth week of the experiment, LG and MG group hens had increased egg production rates compared with those in the control group, with increases of 4.38 and 4.05%, respectively. In addition, the average egg weight was significantly reduced due to garcinol supplementation, but there was no significant effect on feed intake and FCR ($P > 0.05$). During wk 5 to 8 of the experiment, the average egg weight of the LG group was significantly lower than that of the control group ($P < 0.05$), while the egg production rate of the MG group increased by 3.56% compared to that of the control group. The average egg weight of the LG and MG groups was significantly lower than that of the control group from wk 9 to wk 12 ($P < 0.05$). During wk 8 to 12 of the experiment, the addition of garcinol had no significant effect on the laying rate, but the average egg weight of the LG and MG groups was significantly lower than that of the control group ($P < 0.05$). Additionally, throughout the trial period, the addition of garcinol in the diet tended to increase the egg production rate compared with that of the control group ($P = 0.080$). Specifically, the egg production rate in the LG and MG groups increased by 1.81 and 2.84%, respectively, while the average egg weight was significantly lower ($P < 0.05$).

Effects of Dietary Garcinol on Egg Quality

The effects of garcinol on the egg quality-related characteristics of fresh eggs are listed in Table 4. According to the data in the table, up to wk 4, the LG group had a significantly improved albumen height ($P < 0.05$) and Haugh unit. The shape index decreased as the level of garcinol added to the diet increased ($P = 0.083$). Up to wk 8, compared with the LG and HG groups, the MG group protein height and Haugh unit were significantly increased ($P < 0.05$), and LG group significantly increased the egg shape index ($P < 0.05$). However, other characteristics were not significantly different among the groups ($P > 0.10$). Up to wk 12, protein height and Haugh units significantly increased with the addition of garcinol ($P < 0.01$), while the shape index exhibited an increasing trend. Nevertheless, compared with the control group, the LG and MG groups showed significantly decreased eggshell brightness (L*) ($P < 0.01$) and a significant decrease in eggshell yellowness (b*) ($P < 0.05$). The eggshell redness (a*) in the LG and MG groups tended to increase compared to that of the control group ($P = 0.074$). In addition, dietary garcinol supplementation had no effect on eggshell strength, yolk color, eggshell thickness, or eggshell color ($P > 0.05$).

Effects of Dietary Garcinol on Serum Antioxidant Capacity

The effects of garcinol on the antioxidant capacity are shown in Figure 1. The results showed no significant effect on the activity of GSH-PX activity concentrations among the different treatment groups ($P > 0.05$). As shown in the figure, compared with the control group, the MG group had significantly increased levels of T-SOD and CAT in serum ($P < 0.05$). Furthermore, the

Table 3. Effect of garcinol on egg production performance of late laying hens.

Item	The level of garcinol mg/kg				*P* value
	0 (Con)	100 (LG)	300 (MG)	500 (HG)	
Wk 1−4					
Laying rate (%)	90.10 ± 1.96	94.05 ± 1.17	93.75 ± 0.82	89.25 ± 1.67	0.051
Average egg weight (g)	60.52 ± 0.84[a]	57.59 ± 0.40[b]	58.40 ± 0.44[b]	58.34 ± 0.84[b]	0.021
ADFI (g/d/hen)	107.28 ± 1.87	105.60 ± 2.70	111.84 ± 3.99	109.56 ± 3.7	0.553
Feed conversion ratio (g/g)	1.98 ± 0.06	1.95 ± 0.02	2.04 ± 0.03	2.11 ± 0.05	0.056
Wk 5−8					
Laying rate (%)	89.95 ± 1.58	91.96 ± 2.06	93.15 ± 0.97	88.99 ± 1.39	0.231
Average egg weight (g)	59.85 ± 0.77[a]	57.52 ± 0.56[b]	58.54 ± 0.43[ab]	57.90 ± 0.54[ab]	0.040
ADFI (g/d/hen)	110.57 ± 2.83	113.04 ± 4.91	110.18 ± 4.06	113.31 ± 1.92	0.903
Feed conversion ratio (g/g)	2.06 ± 0.03	2.15 ± 0.08	2.02 ± 0.04	2.21 ± 0.04	0.066
Wk 9−12					
Laying rate (%)	88.83 ± 1.46	87.02 ± 1.63	89.48 ± 1.40	87.85 ± 2.10	0.223
Average egg weight (g)	59.67 ± 0.78[a]	57.36 ± 0.49[b]	57.85 ± 0.38[ab]	58.06 ± 0.66[ab]	0.049
ADFI (g/d/hen)	107.78 ± 4.10	104.07 ± 3.20	110.153 ± 1.13	110.10 ± 1.01	0.382
Feed conversion ratio (g/g)	2.04 ± 0.06	2.09 ± 0.05	2.13 ± 0.05	2.15 ± 0.06	0.060
Wk 1−12					
Laying rate (%)	89.57 ± 1.35[ab]	91.19 ± 1.26[ab]	92.11 ± 0.97[a]	88.70 ± 1.28[b]	0.038
Average egg weight (g)	59.99 ± 0.71[a]	57.35 ± 0.43[b]	58.33 ± 0.40[b]	58.10 ± 0.64[b]	0.014
ADFI (g/d/hen)	108.57 ± 2.54	106.23 ± 2.22	110.95 ± 2.27	110.93 ± 1.65	0.402
Feed conversion ratio (g/g)	2.08 ± 0.02[b]	2.04 ± 0.04[b]	2.07 ± 0.03[b]	2.15 ± 0.03[a]	0.014

Abbreviation: ADFI, average daily feed intake.
[a,b]Within a row, values with no common superscripts indicate a significant difference ($P < 0.05$).

Table 4. Effect of garcinol on the egg quality of laying hens during the late laying period.

Item	The level of garcinol (mg/kg)				*P* value
	0 (Con)	100 (LG)	300 (MG)	500 (HG)	
Wk 4					
Shell strength (kg/m^2)	3.235 ± 0.087	3.279 ± 0.103	3.444 ± 0.094	3.307 ± 0.096	0.443
Shell thickness (mm)	0.334 ± 0.005	0.336 ± 0.006	0.338 ± 0.004	0.327 ± 0.005	0.364
Shape index	1.340 ± 0.007	1.341 ± 0.007	1.330 ± 0.009	1.317 ± 0.007	0.083
Albumen height (mm)	6.47 ± 0.18^{ab}	6.87 ± 0.33^{a}	6.20 ± 0.17^{ab}	6.00 ± 0.19^{b}	0.048
Haugh unit	79.81 ± 1.15	82.21 ± 2.01	78.48 ± 1.25	76.86 ± 1.32	0.078
Yolk color	12.65 ± 0.27	12.70 ± 0.29	12.55 ± 0.26	12.85 ± 0.29	0.895
L*	84.10 ± 0.47	84.14 ± 0.47	83.81 ± 0.76	84.87 ± 0.54	0.600
a*	3.57 ± 0.31	3.63 ± 0.24	4.01 ± 0.38	3.41 ± 0.37	0.618
b*	15.86 ± 0.67	16.14 ± 0.57	16.67 ± 0.74	15.81 ± 0.65	0.787
Eggshell color	26.61 ± 1.92	27.03 ± 1.73	28.74 ± 2.54	25.32 ± 1.99	0.708
Wk 8					
Shell strength (kg/m^2)	3.406 ± 0.074	3.229 ± 0.084	3.363 ± 0.079	3.306 ± 0.083	0.444
Shell thickness (mm)	0.332 ± 0.005	0.326 ± 0.005	0.332 ± 0.004	0.325 ± 0.007	0.639
Shape index	1.331 ± 0.009^{b}	1.355 ± 0.010^{a}	1.324 ± 0.005^{b}	1.335 ± 0.008^{ab}	0.043
Albumen height (mm)	6.31 ± 0.22^{ab}	6.01 ± 0.29^{b}	6.91 ± 0.26^{a}	5.79 ± 0.30^{b}	0.023
Haugh unit	78.52 ± 1.53^{ab}	75.96 ± 2.17^{b}	82.99 ± 1.73^{a}	74.73 ± 2.56^{b}	0.025
Yolk color	7.85 ± 0.20	7.75 ± 0.30	8.19 ± 0.25	7.80 ± 0.25	0.582
L*	82.68 ± 1.22	81.77 ± 1.01	80.34 ± 0.99	83.27 ± 1.13	0.254
a*	4.92 ± 0.41	5.12 ± 0.41	4.97 ± 0.35	4.35 ± 0.49	0.583
b*	17.33 ± 0.85	17.00 ± 0.86	17.09 ± 0.48	17.04 ± 0.96	0.992
Eggshell color	32.70 ± 3.05	33.63 ± 2.61	35.49 ± 2.34	31.05 ± 3.10	0.726
Wk 12					
Shell strength (kg/m^2)	3.402 ± 0.097	3.439 ± 0.069	3.399 ± 0.095	3.486 ± 0.115	0.910
Shell thickness (mm)	0.341 ± 0.006	0.330 ± 0.005	0.323 ± 0.005	0.336 ± 0.006	0.132
Shape index	1.334 ± 0.008	1.348 ± 0.008	1.328 ± 0.008	1.320 ± 0.007	0.092
Albumen height (mm)	4.81 ± 0.22^{B}	7.97 ± 0.36^{A}	7.97 ± 0.37^{A}	8.34 ± 0.38^{A}	<0.01
Haugh unit	67.46 ± 1.85^{B}	89.20 ± 2.36^{A}	90.07 ± 1.83^{A}	92.01 ± 1.82^{A}	<0.01
Yolk color	7.82 ± 0.15	8.08 ± 0.15	7.74 ± 0.12	7.93 ± 0.13	0.351
L*	90.91 ± 1.09^{A}	82.41 ± 0.79^{B}	81.67 ± 0.86^{B}	90.94 ± 0.77^{A}	<0.01
a*	4.11 ± 0.44	4.58 ± 0.43	4.95 ± 0.36	3.48 ± 0.40	0.074
b*	20.57 ± 0.90^{a}	17.69 ± 0.86^{b}	17.91 ± 0.51^{b}	19.02 ± 0.40^{ab}	0.032
Eggshell color	29.76 ± 2.91	33.20 ± 2.58	34.75 ± 2.25	27.43 ± 2.58	0.189

L* = lightness; a* = redness; b* = yellowness.
^{a,b}Within a row, values with no common superscripts indicate a significant difference ($P < 0.05$).
^{A,B}Within a row, values with no common superscripts indicate a high significant difference ($P < 0.01$).

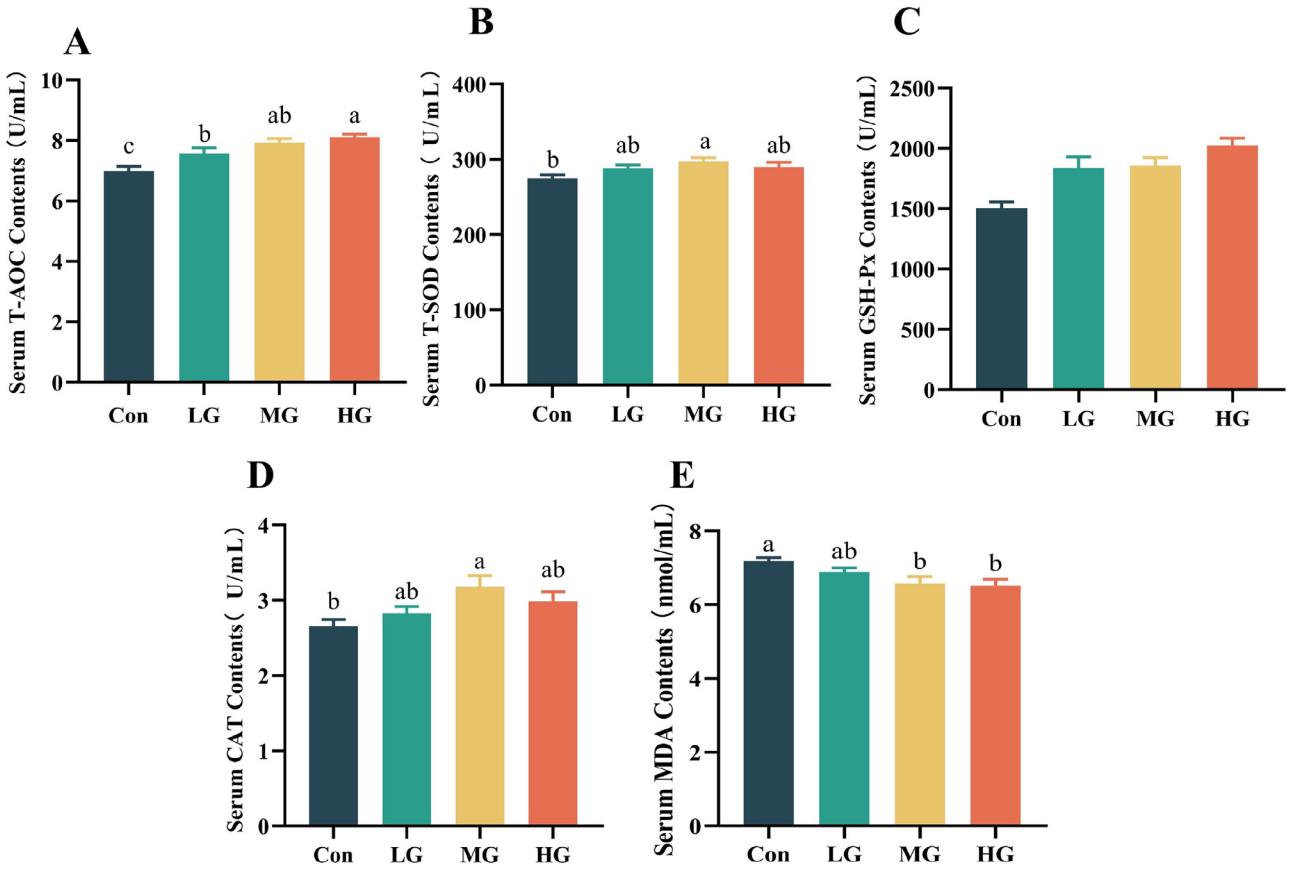

Figure 1. Effects of garcinol on the serum antioxidant index of late laying hens. (A) The contents of T-AOC in serum, (B) The contents of T-SOD in serum, (C) The contents of GSH-Px in serum, (D) The contents of CAT in serum, (E) The contents of MDA in serum. $^{a-c}$ Values in a row with no common letters indicate a significant difference ($P < 0.05$).

MDA levels decreased significantly ($P < 0.05$), and the levels of T-AOC in serum increased significantly ($P < 0.05$) with increasing levels of garcinol added to the diets.

Effects of Dietary Garcinol on Immune Indices

To evaluate the effects of garcinol on immune responses in the intestine of laying hens during the late laying period, the serum levels of immunoglobulin, cytokines IL-2, IL-6, TNF-α and CORT, D-lactate, LPS, and immune-related indicators of intestinal mucosa were determined in this experiment (Figure 2). There was no effect of dietary garcinol on the serum levels of IgG, IgM, TNF-α, and CORT ($P > 0.05$). In addition, compared with the other groups, the MG group had significantly increased serum IgA content ($P < 0.05$). The laying hens of the MG group had lower concentrations of IL-2 and IL-6 ($P < 0.05$) and a higher level of IgA ($P < 0.01$) in serum than that of the control laying hens. Moreover, the MG and HG groups had significantly decreased serum levels of LPS and D-lactate compared to that of the control group ($P < 0.05$).

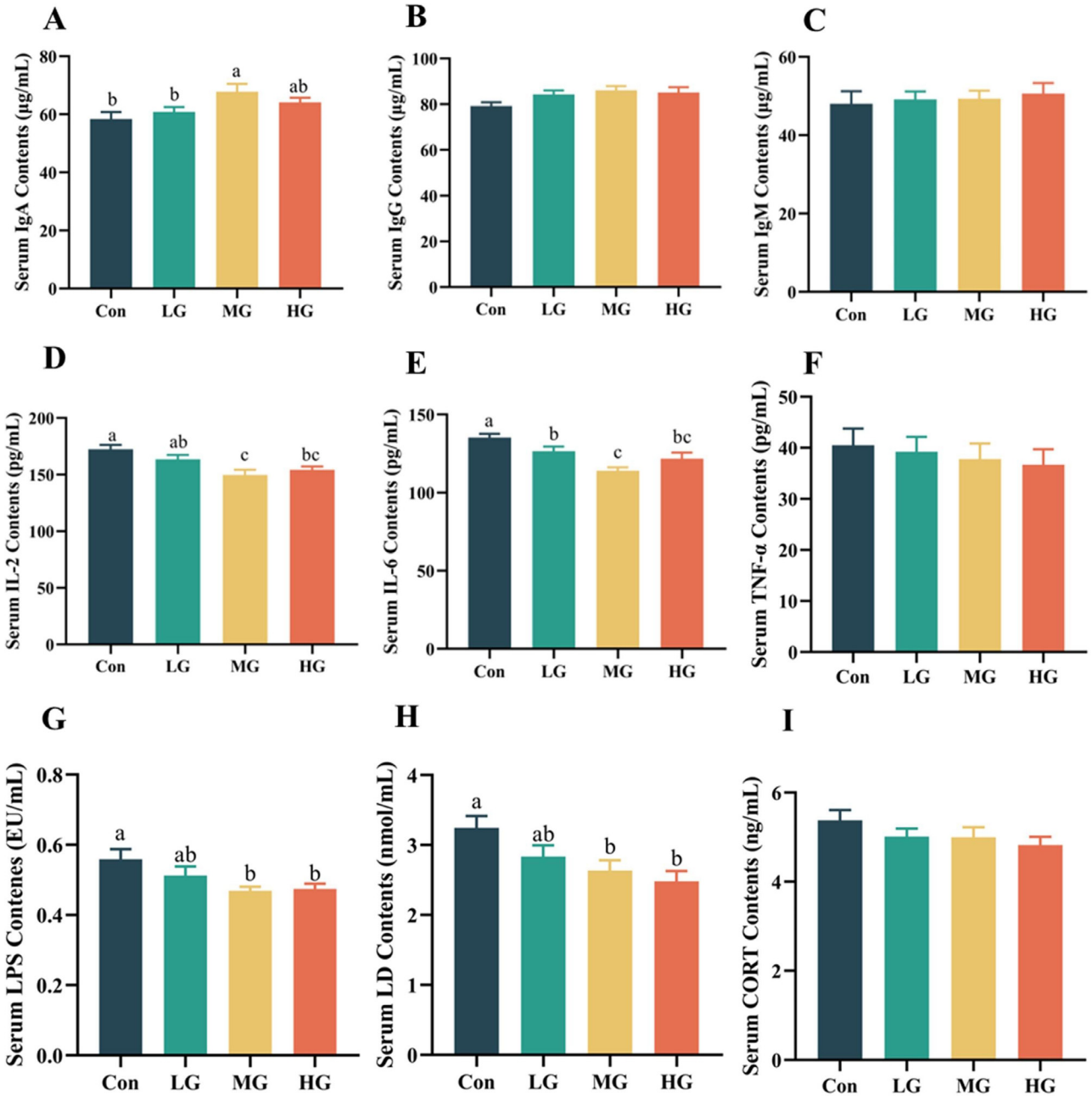

Figure 2. The effect of garcinol on immune indices in the serum of late laying hens. (A) The contents of IgA in serum, (B) The contents of IgG in serum, (C) The contents of IgM in serum, (D) The contents of IL-2 in serum, (E) The contents of IL-6 in serum, (F) The contents of TNF-α in serum, (G) The contents of LPS in serum, (H) The contents LD of in serum, (I) The contents of CORT in serum. $^{a-c}$Values in a row with no common letters indicate a significant difference ($P < 0.05$).

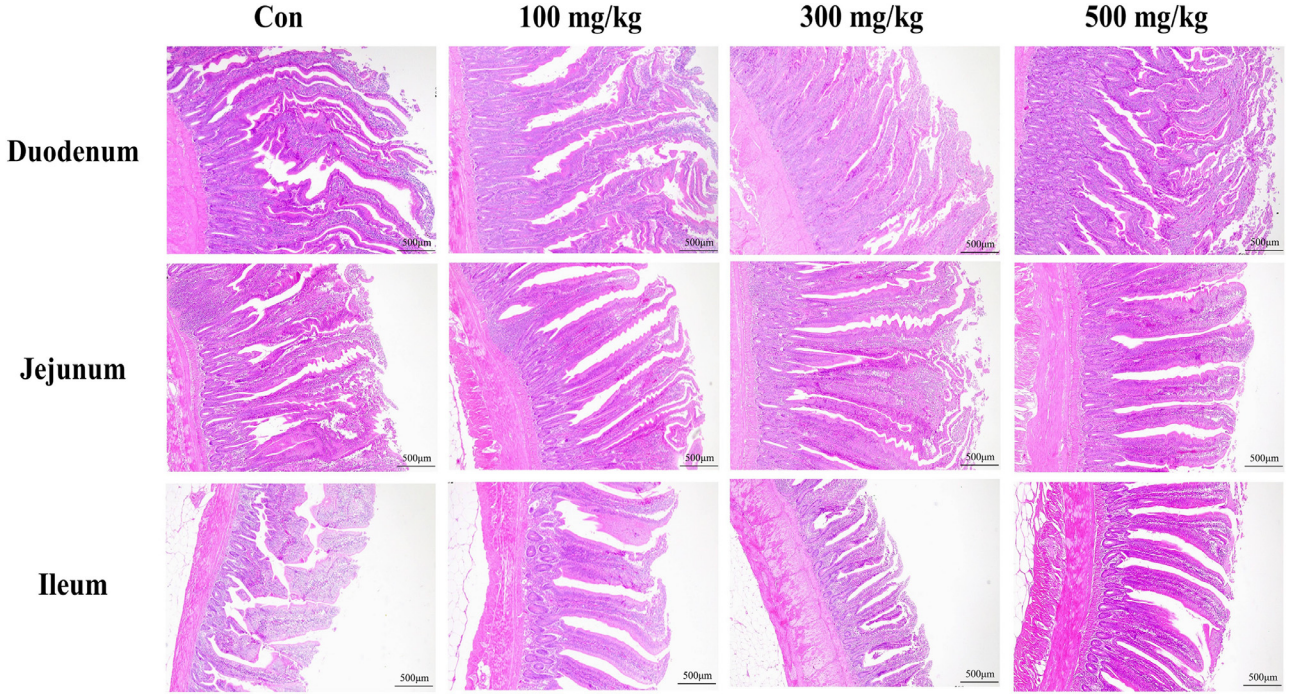

Figure 3. Effects of garcinol on histopathological changes of duodenum, jejunum, and ileum with H&E staining (original magnification of 40×).

Effects of Dietary Garcinol on Intestinal Histomorphology

The histomorphology of the intestinal segment is shown in Figure 3. It can be observed from the figure that the intestinal villi in the control group showed a general breakage, the whole intestinal villi were not straight and the intestinal villi were sparse between each other; when the diet was supplemented with garcinol, the number of breaks was reduced, and there was a significant recovery (the intestinal villi were more relatively intact and healthier) compared with that in the control group. The data in Table 5 show that compared to the control group, dietary supplementation with garcinol increased the villus height of the jejunum and ileum ($P < 0.05$) and decreased the crypt depth of the jejunum and ileum ($P < 0.05$). Moreover, the effect in the MG group was significantly better than that in the other treatment groups. Then, the VH/CD values were calculated, and the results showed that the VH/CD ratio of the duodenum, jejunum, and ileum in the dietary garcinol supplementation group was increased, among which the values in the MG and HG groups significantly increased ($P < 0.05$).

Effects of Dietary Garcinol on the Gene Expression of Tight Junctions

Gene expression related to the intestinal mucosa barrier function of the jejunum is shown in Figure 4. Dietary garcinol significantly increased *Occludin* expression compared to that in the control group ($P < 0.05$); the mRNA expression of *Claudin-2* was significantly upregulated in the LG and MG groups compared to the control

Table 5. Effects of garcinol on the morphological parameters of the intestinal mucosa of laying hens during the late laying period.

	The level of garcinol (mg/kg)				
Item	0 (Con)	100 (LG)	300 (MG)	500 (HG)	*P* value
VH (μm)					
Duodenum	1396.407 ± 61.366	1546.802 ± 84.061	1478.926 ± 35.157	1539.789 ± 77.082	0.380
Jejunum	1224.645 ± 35.452^{b}	1266.562 ± 67.383^{b}	1444.078 ± 63.839^{a}	1363.048 ± 48.421^{ab}	0.046
Ileum	776.377 ± 29.406^{c}	958.330 ± 52.733^{a}	821.446 ± 31.303^{bc}	935.953 ± 41.232^{ab}	0.010
CD (μm)					
Duodenum	435.666 ± 23.780	332.919 ± 46.099	369.949 ± 30.843	381.366 ± 22.689	0.191
Jejunum	300.093 ± 19.025^{a}	232.446 ± 8.951^{b}	246.568 ± 16.552^{b}	239.835 ± 8.286^{b}	0.011
Ileum	178.488 ± 11.22^{a}	185.982 ± 8.730^{a}	145.003 ± 8.813^{b}	159.807 ± 10.029^{ab}	0.031
V/C					
Duodenum	3.302 ± 0.281^{b}	5.112 ± 0.557^{a}	4.150 ± 0.294^{ab}	4.127 ± 0.298^{ab}	0.024
Jejunum	4.213 ± 0.188^{b}	5.711 ± 0.430^{a}	6.148 ± 0.351^{a}	5.829 ± 0.405^{a}	<0.01
Ileum	4.498 ± 0.241^{c}	5.245 ± 0.222^{bc}	5.805 ± 0.324^{ab}	6.087 ± 0.256^{a}	<0.01

Abbreviations: CD, crypt depth; V/C, villus height to crypt depth ratio; VH, villus height.
$^{a-c}$Within a row, values with no common superscripts indicate a significant difference ($P < 0.05$).

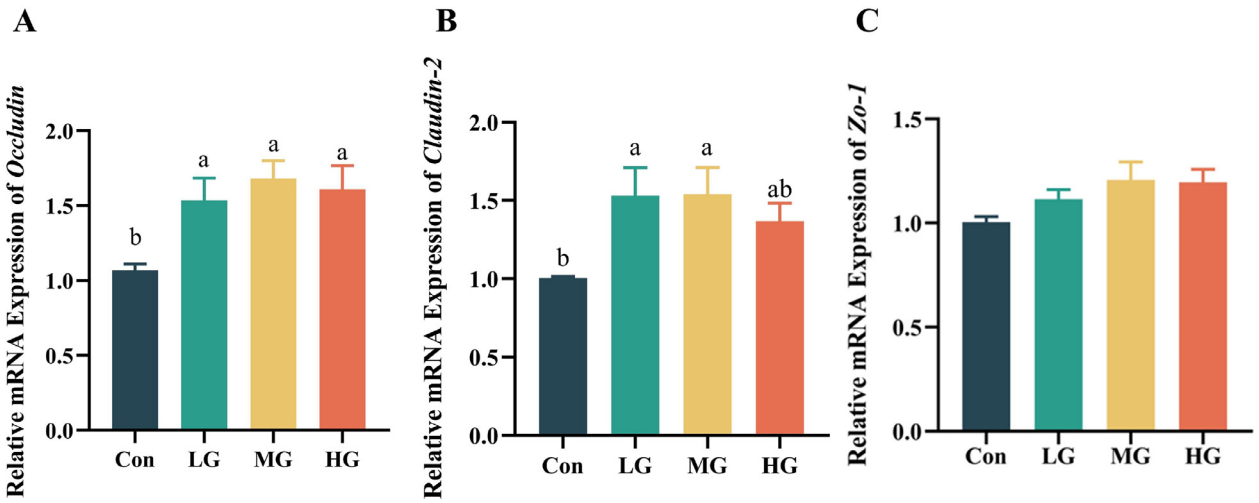

Figure 4. Effects of garcinol on tight junctions of late laying hens. (A) Relative mRNA expression of *Occludin*; (B) Relative mRNA expression of *Claudin-2*; (C) Relative mRNA expression of *ZO-1*. [a, b]Values in a row with no common letters indicate a significant difference ($P < 0.05$).

group ($P < 0.05$). In contrast, the mRNA expression of *Zo-1* was not significantly different among the 4 groups.

Effects of Dietary Garcinol on Gut Microbiota

As shown in Figure 5, the dietary addition of garcinol markedly increased the number of OTUs of cecum bacteria compared to that of the control group. Figure 5A to D demonstrates the effect of different concentrations of garcinol on the alpha diversity and beta diversity of cecum bacteria. The alpha diversity values (Shannon, Simpson, Ace, and Chao1) were analyzed based on 16S rRNA gene sequencing data. The alpha diversity results showed that richness index (Ace and Chao) in the 3 garcinol groups were higher than those in the control group, but there was no significant difference among the 3 treatment groups. The results of the multidimensional scaling (NMDS) (Figure 5F) based on Bray–Curtis at the genus level revealed distinct differences in microbiota structure among the groups. Furthermore, the bacterial relative abundance of each group was appraised at the phylum and genus levels. Figure 5G and H displays the top 10 phyla and top 10 genera found in the cecal contents of the laying hens. Firmicutes, Bacteroidetes, Actinobacteria, WPS-2, and Spirochaetota were the most prevalent phyla. Further analysis at the genus level revealed that the dominant flora in cecal contents were *Bacteroides, Lactobacillus, Romboutsia*, unclassified Lachnospiraceae and *Ruminococcus torques*. The LEfSe results at the phylum to genus levels shown in Figure 5J indicated that the number of superior bacteria in the MG group was significantly higher than that in the other 2 groups.

DISCUSSION

Improving the production performance and egg quality of late laying hens is key to improving their economic efficiency (Dai et al., 2020). Previous research has confirmed that garcinol possesses antioxidant, anti-inflammatory, anticancer, and antibacterial properties (Hemshekhar et al., 2011), all of which promote its potential application. The results gathered in this study show that the treatment for the MG group was effective in increasing the egg production performance of hens at the end of the laying cycle during the first 4 wk; however, no significant effects were observed on the ADFI and FCR. Previous studies have shown that plant polyphenols have beneficial effects on the performance of laying hens in the late laying period (Akyildiz and Denli, 2016). Supplementation of 250 mg/kg thymol in a basic diet resulted in improved productive performance of laying hens (Abd El-Hack et al., 2021). Similarly, Ding et al. found that adding tea polyphenols to the diet of hens during the late laying phase improved their production performance (Ding et al., 2020). However, a previous study showed that polyphenols had no effect on egg weight (Yang et al., 2018). This discrepancy might be due to the differences in the age of the laying hens, which is the most important factor affecting the egg weight of freshly laid eggs. The reason for the lower effect of high levels of garcinol compared to other levels in this trial may be due to the lower adaptability of hens to high levels.

Egg quality declines significantly after peak production, for example, enlarged egg size, increased broken egg rate, decreased eggshell quality, and reduced albumen height (Liu et al., 2018; Saleh et al., 2019). Studies have shown that dietary administration of magnolol could significantly improve albumen height and Haugh unit, but adding more than 300 mg/kg negatively affected egg quality (Chen et al., 2021). Another study found that albumen height and Haugh units increased in all supplemented groups after adding curcumin to the diet of laying hens (Galli et al., 2018), which is consistent with the results of this study. In the present study, the significant increases in the albumen height and Haugh unit suggested that dietary garcinol supplementation improves the internal egg quality during the late laying period. This might be attributed to the antioxidant property of

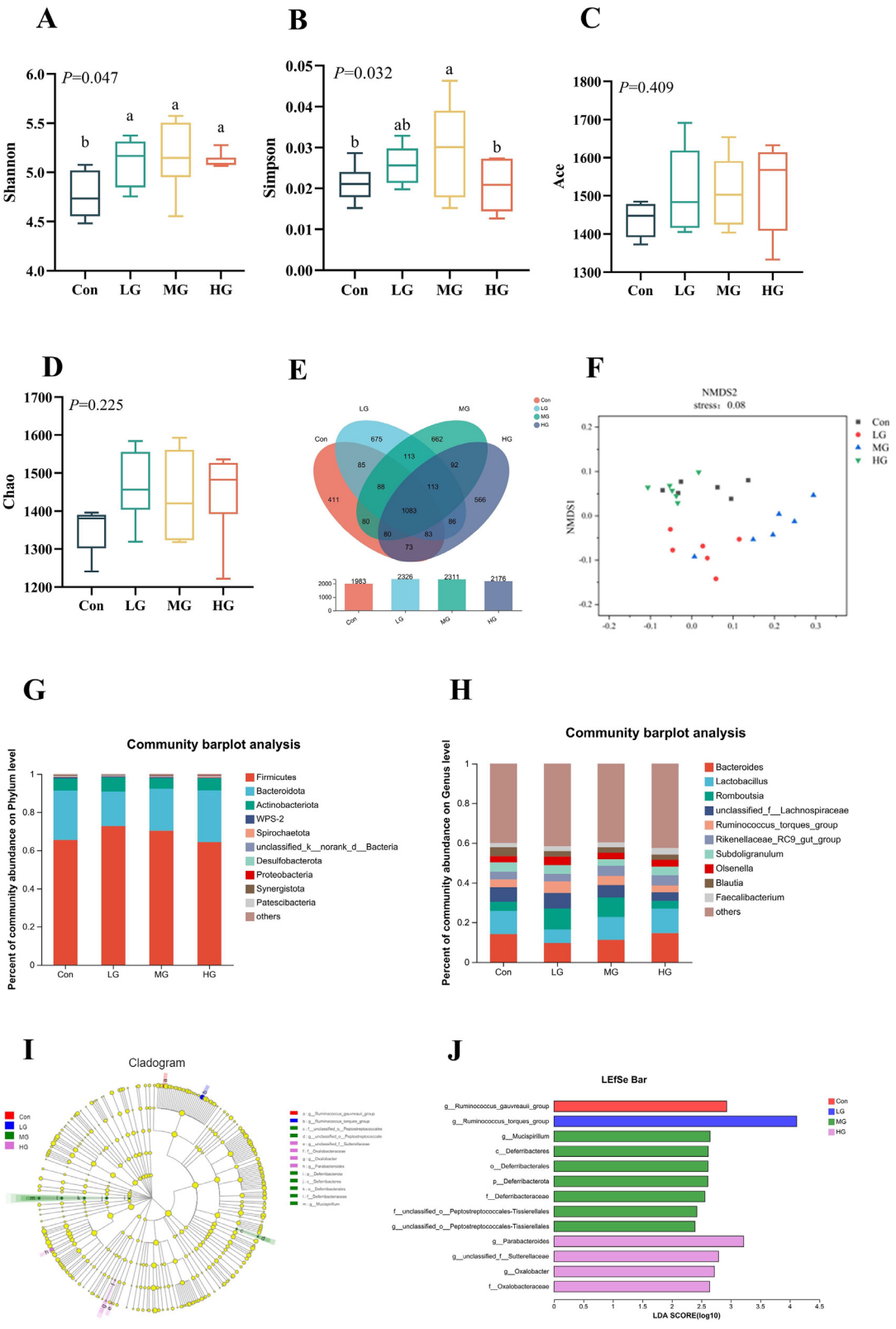

Figure 5. Effect of garcinol on cecal microbiota. (A) Shannon index, (B) Simpson index, (C) Ace index, (D) Chao index, (E) Venn diagram of cecal microflora OTUs, (F) NMDS analysis at the genus level, (G) Relative abundance of cecal microbiota at the phylum level, (H) Relative abundance of cecal microbiota at the genus level, (I) Evolutionary branch diagram of the intestinal flora at all levels, (J) Histogram of LDA value distribution. $^{a-c}$Values in a row with no common letters indicate a significant difference ($P < 0.05$).

garcinol, which probably increased albumen quality through lower lipid and protein oxidation. Additionally, adding garcinol to the diet significantly decreased L* and b* only at wk 12, implying the effect of garcinol may be delayed. Moreover, decreasing the shape index within a certain range is beneficial for reducing the damage and loss rate of eggs during transportation (Zhan et al., 2019). In this study, the medium dose (300 mg/kg) garcinol was the most effective in terms of production performance and egg quality of late laying hens.

The resistance of late laying hens to oxidative stress is reduced (Subramanian and James, 2010), and the addition of antioxidative substances to the diet helps improve the ability of the body to scavenge free radicals and maintain the redox balance. According to the results of the experiment, the laying hens fed garcinol had higher T-SOD and CAT activity and lower MDA content in serum, suggesting that garcinol was effective in improving the antioxidant status of laying hens, which might contribute to increased albumin height and Haugh unit. Many studies have demonstrated that dietary supplementation with plant polyphenols such as curcumin, tea polyphenols, and quercetin improved the antioxidant status of laying hens (Yuan et al., 2016; Galli et al., 2018; Yang et al., 2018). Our results are similar to these studies. This might be explained by the radical-scavenging activity of antioxidant compounds. Polyphenols can prevent fatty acid oxidation and, as a result, affect the fatty acid profile by protecting unsaturated fatty acids, which are prone to free radical damage. It is plausible that a similar mechanism may have occurred in the present experiment. IgG, IgM, and IgA are 3 major immunoglobulin classes in poultry (Kong et al., 2007). In the present study, we observed that dietary garcinol feed supplementation improved the humoral immune status of late laying hens by increasing their serum levels of IgA. Furthermore, dietary supplementation with garcinol decreased the content of proinflammatory factors such as IL-2, IL-6, and TNF-α in serum, which indicated that the addition of garcinol feed to the diet significantly improved the immunity of late laying hens. Our results also demonstrated the anti-inflammatory effect of garcinol in the serum of laying hens. Similarly, nutritional supplementation with antioxidants from plant spices such as epigallocatechin gallate (**EGCG**) and garlic reduced the level inflammatory cytokines in laying hens (Cheng et al., 2017; Ogbuewu et al., 2021). Hence, these positive effects of using garcinol indicate that garcinol has a certain regulatory effect on the immunity of late laying hens and can enhance the body's immunity, which might be due to its anti-inflammatory and antioxidant mechanisms.

Damage to the intestinal barrier is an important cause of redox imbalance and reduced immune function in late laying hens. In the present study, it was found that the addition of 300 or 500 mg/kg garcinol to the basal diet of late laying hens significantly reduced serum concentrations of both LPS and D-lactate. The levels of LPS, D-lactate, and CORT in the serum can reflect the level of inflammation and the integrity of the intestinal barrier (Collins et al., 2013; Zafar and Saier, 2018). The reduction in LPS and D-lactate might be due to the antibacterial activity of garcinol and its modulatory effects on intestinal microbial composition, resulting in less pathogen-induced damage to enterocytes. It may also further diminish the risk of pathogen invasion to intestinal epithelial cells and promote their ability to regenerate villi. The results of the intestinal morphology showed that the MG group had a significantly increased VH and VH/CD ratio in the jejunum and ileum compared with that of the control group. Disruption of tight junctions and microbiota dysbiosis due to long-term egg production would enable the translocation of luminal pathogens and toxins. It would subsequently lead to inflammation and tissue damage, which may be partially responsible for the lower nutrient absorption and the compromised laying performance of laying hens in the late production period (Feng et al., 2021). The results of this study showed that the expression levels of the tight junction proteins *Occludin, Zo-1*, and *Claudin-2* increased with garcinol supplementation, which may enhance intestinal barrier function to some extent. These results revealed that garcinol can directly promote intestinal health by enhancing intestinal barrier function and decreasing intestinal inflammation in laying hens. Therefore, the strengthened epithelial barrier in response to garcinol treatment would be beneficial for the maintenance of intestinal health and production performance of laying hens.

To better understand the favorable effects of garcinol, further analysis was conducted on the gut microbiota since its interactions with the gut play a crucial role in the maintenance of the physiological function of host health (Lynch and Hsiao, 2019; Abd El-Hack et al., 2023). Emerging evidence demonstrates that the diversity of the intestinal microbiota of chickens is largely influenced by age and diet (Videnska et al., 2014). The results of the gut microbiota analysis showed that there was no difference in the alpha richness index between groups, whereas the results of the alpha diversity index and beta diversity analysis showed significant clustering according to dietary treatments, indicating that the gut microbiota community structure was altered by the addition of garcinol. Interestingly, our study showed that there was a certain difference in the relative abundance of the gut microbiota. The MG group had reduced levels of *Bacteroides* while increasing the relative abundance of Firmicutes, *Romboutsia*, and *Ruminococcus*. Firmicutes facilitate cellulose digestion in the gut, so their higher abundance aids in meeting the nutritional and energetic needs of animals and helps to balance the gut microbiota and protect against pathogens (Garneau et al., 2008; Sun et al., 2016; Murugesan et al., 2018). Both *Romboutsia* and *Ruminococcus* are beneficial intestinal bacteria (Wang et al., 2019). The production of butyric acid is usually significantly positively correlated with *Ruminococcus*. In addition, *Ruminococcus* was also reported to be related to the degradation and utilization of polysaccharides in chickens (Nguyen et al., 2021). Additionally, there were 7 potential biomarkers with significantly different abundances in the MG group, and further research is needed to explore the functions of these biomarkers in Frontiers in regulating the gut health of laying hens. The MG group had an increased relative abundance of Deferribacterota, *Romboutsia, Mucispirillum*, and Peptostreptococcales-Tissierellales. The phylum Deferribacterota can obtain energy through specialized or parthenogenetic anaerobic metabolism, which is beneficial for the regulation of the immune system. Bacteria of the genus *Romboutsia* are an important

probiotic that have a positive effect on the maintenance of intestinal and body health, and the genus *Mucispirillum* is also a type of beneficial intestinal bacteria that is positively correlated with immune function. Combined with the overall results, supplementation with 300 mg/kg garcinol in the diet can improve the structure of the cecum microbiota of late laying hens and has the ability to promote the growth of beneficial bacteria, thereby increasing their production performance.

CONCLUSIONS

In summary, dietary supplementation with 300 mg/kg garcinol could improve production performance and egg quality by ameliorating antioxidant status and immunity, improving intestinal barrier function and altering intestinal microflora composition, which could lead to the development of a new green feed additive in late laying hens. These findings may provide insights into the underlying rationalization of garcinol utilization in laying hens.

ACKNOWLEDGMENTS

This study was supported by the National Key Research and Development Program (grant no. 2021YFD1300300), the National Natural Science Foundation of China (grant no. 32072742).

DISCLOSURES

The authors have no conflicts of interest to declare regarding this research.

REFERENCES

Abd El-Hack, M. E., B. A. Alaidaroos, R. M. Farsi, D. E. Abou-Kassem, M. T. El-Saadony, A. M. Saad, M. E. Shafi, N. M. Albaqami, A. E. Taha, and E. A. Ashour. 2021. Impacts of supplementing broiler diets with biological curcumin, zinc nanoparticles and bacillus licheniformis on growth, carcass traits, blood indices, meat quality and cecal microbial load. Animals (Basel) 11:1878.

Abd El-Hack, M. E., H. M. Salem, A. F. Khafaga, S. M. Soliman, and M. T. El-Saadony. 2023. Impacts of polyphenols on laying hens' productivity and egg quality: a review. J. Anim. Physiol. Anim. Nutr. (Berl.) 107:928–947.

Abdel-Moneim, A. E., A. M. Shehata, S. O. Alzahrani, M. E. Shafi, N. M. Mesalam, A. E. Taha, A. A. Swelum, M. Arif, M. Fayyaz, and M. E. Abd El-Hack. 2020. The role of polyphenols in poultry nutrition. J. Anim. Physiol. Anim. Nutr. (Berl.) 104:1851–1866.

Akyildiz, S., and M. Denli. 2016. Application of plant extracts as feed additives in poultry nutrition. Anim. Sci. 59:2260.

Chen, F., H. Zhang, E. Du, F. Jin, C. Zheng, Q. Fan, N. Zhao, W. Guo, W. Zhang, S. Huang, and J. Wei. 2021. Effects of magnolol on egg production, egg quality, antioxidant capacity, and intestinal health of laying hens in the late phase of the laying cycle. Poult. Sci. 100:835–843.

Cheng, M., X. Zhang, Y. Miao, J. Cao, Z. Wu, and P. Weng. 2017. The modulatory effect of (-)-epigallocatechin 3-o-(3-o-methyl) gallate (egcg3 ″me) on intestinal microbiota of high fat diet-induced obesity mice model. Food Res. Int. 92:9–16.

Collins, H. M., M. K. Abdelghany, M. Messmer, B. Yue, S. E. Deeves, K. B. Kindle, K. Mantelingu, A. Aslam, G. S. Winkler, and T. K. Kundu. 2013. Differential effects of garcinol and curcumin on histone and p53 modifications in tumour cells. BMC Cancer 13:1–11.

Dai, D., S. G. Wu, H. J. Zhang, G. H. Qi, and J. Wang. 2020. Dynamic alterations in early intestinal development, microbiota and metabolome induced by in ovo feeding of l-arginine in a layer chick model. J. Anim. Sci. Biotechnol. 11:19.

Ding, X., J. Du, K. Zhang, S. Bai, Q. Zeng, H. Peng, Y. Xuan, Z. Su, and J. Wang. 2020. Tandem mass tag-based quantitative proteomics analysis and gelling properties in egg albumen of laying hens feeding tea polyphenols. Poult. Sci. 99:430–440.

Feng, J., M. Lu, J. Wang, H. Zhang, K. Qiu, G. Qi, and S. Wu. 2021. Dietary oregano essential oil supplementation improves intestinal functions and alters gut microbiota in late-phase laying hens. J. Anim. Sci. Biotechnol. 12:72.

Galli, G. M., A. S. Da Silva, A. H. Biazus, J. H. Reis, M. M. Boiago, J. P. Topazio, M. J. Migliorini, N. S. Guarda, R. N. Moresco, and A. F. Ourique. 2018. Feed addition of curcumin to laying hens showed anticoccidial effect, and improved egg quality and animal health. Res. Vet. Sci. 118:101–106.

Garneau, J. E., D. M. Tremblay, and S. Moineau. 2008. Characterization of 1706, a virulent phage from Lactococcus lactis with similarities to prophages from other Firmicutes. Virology 373:298–309.

Hemshekhar, M., K. Sunitha, M. S. Santhosh, S. Devaraja, K. Kemparaju, B. Vishwanath, S. Niranjana, and K. Girish. 2011. An overview on genus garcinia: phytochemical and therapeutical aspects. Phytochem. Rev. 10:325–351.

Kong, X., G. Wu, Y. Liao, Z. Hou, H. Liu, F. Yin, T. Li, R. Huang, Y. Zhang, and D. Deng. 2007. Dietary supplementation with Chinese herbal ultra-fine powder enhances cellular and humoral immunity in early-weaned piglets. Livest. Sci. 108:94–98.

Lee, P. S., C. Y. Teng, N. Kalyanam, C. T. Ho, and M. H. Pan. 2019. Garcinol reduces obesity in high-fat-diet-fed mice by modulating gut microbiota composition. Mol. Nutr. Food Res. 63:1800390.

Liu, X. T., X. Lin, Y. L. Mi, W. D. Zeng, and C. Q. Zhang. 2018. Age-related changes of yolk precursor formation in the liver of laying hens. J. Zhejiang Univ. Sci. B 19:390–399.

Lv, Z. P., S. J. Yan, G. Li, D. Liu, and Y. M. Guo. 2019. Genistein improves the reproductive performance and bone status of breeder hens during the late egg-laying period. Poult. Sci. 98:7022–7029.

Lynch, J., and E. Hsiao. 2019. Microbiomes as sources of emergent host phenotypes. Science 365:1405–1409.

Mahfuz, S., Q. Shang, and X. Piao. 2021. Phenolic compounds as natural feed additives in poultry and swine diets: a review. J. Anim. Sci. Biotechnol. 12:1–18.

Ministry of Agriculture of the People's Republic of China (MAPRC). 2004. Chicken Feeding Standard (NY/T33-2004). China Standard Press, Beijing&China.

Mishra, B., and R. Jha. 2019. Oxidative stress in the poultry gut: potential challenges and interventions. Front. Vet. Sci. 6:60.

Murugesan, S., K. Nirmalkar, C. Hoyo-Vadillo, M. Garcia-Espitia, D. Ramirez-Sanchez, and J. Garcia-Mena. 2018. Gut microbiome production of short-chain fatty acids and obesity in children. Eur. J. Clin. Microbiol. Infect. Dis. 37:621–625.

Nguyen, H. T., M. R. Bedford, S. B. Wu, and N. K. Morgan. 2021. Soluble non-starch polysaccharide modulates broiler gastrointestinal tract environment. Poult. Sci. 100:101183.

Ogbuewu, I., V. Okoro, and C. Mbajiorgu. 2021. Meta-analysis of the responses of laying hens to garlic (Allium sativum) supplementation. Anim. Feed Sci. Technol. 275:114866.

Saleh, A. A., EaM. Ahmed, and T. A. Ebeid. 2019. The impact of phytoestrogen source supplementation on reproductive performance, plasma profile, yolk fatty acids and antioxidative status in aged laying hens. Reprod. Domest. Anim. 54:846–854.

Schobert, R., and B. Biersack. 2019. Chemical and biological aspects of garcinol and isogarcinol: recent developments. Chem. Biodivers. 16:e1900366.

Shini, A., S. Shini, and W. L. Bryden. 2019. Fatty liver haemorrhagic syndrome occurrence in laying hens: impact of production system. Avian Pathol. 48:25–34.

Subramanian, M. V., and T. J. James. 2010. Age-related protective effect of deprenyl on changes in the levels of diagnostic marker enzymes and antioxidant defense enzymes activities in cerebellar tissue in wistar rats. Cell Stress Chaperones 15:743–751.

Sugiharto, S. 2016. Role of nutraceuticals in gut health and growth performance of poultry. J. Saudi Soc. Agric. Sci. 15:99–111.

Sun, B. H., X. Wang, S. Bernstein, M. A. Huffman, D. P. Xia, Z. Y. Gu, R. Chen, L. K. Sheeran, R. S. Wagner, and J. H. Li. 2016. Marked variation between winter and spring gut microbiota in free-ranging Tibetan macaques (Macaca thibetana). Sci. Rep. 6:26035.

Videnska, P., K. Sedlar, M. Lukac, M. Faldynova, L. Gerzova, D. Cejkova, F. Sisak, and I. Rychlik. 2014. Succession and replacement of bacterial populations in the caecum of egg laying hens over their whole life. PLoS One 9:e115142.

Wang, W. W., J. Wang, H. J. Zhang, S. G. Wu, and G. H. Qi. 2019. Transcriptome analysis reveals mechanism underlying the differential intestinal functionality of laying hens in the late phase and peak phase of production. BMC Genom. 20:970.

Wang, T., W. Yao, J. Li, Y. Shao, Q. He, J. Xia, and F. Huang. 2020. Dietary garcinol supplementation improves diarrhea and intestinal barrier function associated with its modulation of gut microbiota in weaned piglets. J. Anim. Sci. Biotechnol. 11:12.

Xiao, S. S., J. D. Mi, L. Mei, J. Liang, K. X. Feng, Y. B. Wu, X. D. Liao, and Y. Wang. 2021. Microbial diversity and community variation in the intestines of layer chickens. Anim. (Basel) 11:840.

Yang, J. X., M. T. Chaudhry, J. Y. Yao, S. N. Wang, B. Zhou, M. Wang, C. Y. Han, Y. You, and Y. Li. 2018. Effects of phytoestrogen quercetin on productive performance, hormones, reproductive organs and apoptotic genes in laying hens. J. Anim. Physiol. Anim. Nutr. (Berl.) 102:505–513.

Yang, J., K. Zhan, and M. Zhang. 2020. Effects of the use of a combination of two bacillus species on performance, egg quality, small intestinal mucosal morphology, and cecal microbiota profile in aging laying hens. Probiotics Antimicrob. Proteins 12:204–213.

Yao, W. L., T. X. Wang, J. Xia, J. Li, X. H. Yu, and F. R. Huang. 2020. Dietary garcinol attenuates hepatic pyruvate and triglyceride accumulation by inhibiting P300/CBP-associated factor in mid-to-late pregnant rats. J. Nutr. 150:231–239.

Yuan, Z. H., K. Y. Zhang, X. M. Ding, Y. H. Luo, S. P. Bai, Q. F. Zeng, and J. P. Wang. 2016. Effect of tea polyphenols on production performance, egg quality, and hepatic antioxidant status of laying hens in vanadium-containing diets. Poult. Sci. 95:1709–1717.

Zafar, H., and M. H. Saier Jr. 2018. Comparative genomics of transport proteins in seven bacteroides species. PLoS One 13:e0208151.

Zhan, H. Q., X. Y. Dong, L. L. Li, Y. X. Zheng, Y. J. Gong, and X. T. Zou. 2019. Effects of dietary supplementation with Clostridium butyricum on laying performance, egg quality, serum parameters, and cecal microflora of laying hens in the late phase of production. Poult. Sci. 98:896–903.