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Effects of long-term dietary glycerol monolaurate supplementation on productivity, egg quality, intestinal mucosal morphology and serum parameters of laying hens

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Research

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ABSTRACT

Objective: This study evaluated the long-term effects of supplementation with a medium-chain monoglyceride, glycerol monolaurate (GML), on the productive performance, egg quality, intestinal mucosa structure and serum parameters of laying hens.

Methods: A total of 120 17-week-old Hy-Line brown laying hens were randomly distributed into 4 groups with 5 replicates and 6 hens per replicate, that were fed corn-soybean meal-based diets supplemented with 0, 0.15, 0.30 or 0.45 g/kg GML for 52 weeks.

Results: The results showed that, compared with the control group, the laying rate and average egg weight in the 0.30 g/kg GML group were significantly increased ($p<0.05$), and the feed conversion ratio in the 0.30 g/kg GML group was significantly decreased ($p<0.05$) from 58 to 69 weeks. At 58 weeks of age, eggshell thickness was significantly increased ($p<0.05$) in the 0.15 and 0.30 g/kg GML groups. At 69 weeks of age, eggshell thickness and eggshell strength were significantly increased ($p<0.05$) in all the GML groups. In the serum, the activities of aspartate transaminase and alkaline phosphatase were significantly decreased ($p<0.05$) in all the GML groups. The activity of glutamic-pyruvic transaminase and the total cholesterol and low-density lipoprotein-cholesterol concentrations were significantly decreased ($p<0.05$) while the high-density lipoprotein-cholesterol concentration was significantly increased ($p<0.05$) in the 0.30 and 0.45 g/kg GML groups. Regarding intestinal morphology, supplementation with 0.30 g/kg GML significantly increased ($p<0.05$) the villus height and crypt depth of the duodenum, the villus height of the jejunum and the villus length to crypt depth ratio of the jejunum.

Conclusion: These results suggest that GML supplementation during weeks 58-69 could improve laying performance and egg quality in laying hens, possibly because of the favorable effects of GML on the intestinal mucosa structure, lipid profiles and liver function.

Keywords: Laying Hens; Glycerol Monolaurate; Egg Quality; Intestinal Morphology; Serum Property

1. INTRODUCTION

Antibiotics have been extensively applied in the poultry industries to prevent disease and infections, improve growth and productivity performance, and increase efficiency of feed utilization [1]. However, an increasing number of countries have gradually restricted or banned the use of antibiotics as feed additives due to growing concerns regarding outbreaks of resistant bacteria [2], and potential damage to gut microbiota [3]. Given these concerns, alternatives to antibiotics as feed additives has been developed by feed additive researchers and manufacturers of animal nutrition, including feed enzymes, fatty acids, probiotics, prebiotics and plant extracts [4]. Medium chain fatty acids (MCFAs) have been reported to be effective as feed additives in improving serum lipid profiles, decreasing fatty deposition and regulating gut structure in laying hens and broilers [5]. Zeitz et al. [6] showed that dietary fats rich in lauric and myristic acids increased the feed conversion efficiency and breast meat percentage of broilers and attributed this to the improvements of gut health.

Glycerol monolaurate (GML), a monoglyceride of lauric acid, naturally exists in breast milk, coconut oil and palm oil [7]. The antimicrobial property of GML suggests GML has potential as an alternative to antibiotics. GML has broad antimicrobial activity, and is especially effective against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus*, possibly through the disruption of the plasma membranes of the bacteria [8]. In addition, GML is effective in killing bacterial spores and some *Staphylococcus aureus* strains with antibiotic-resistant biofilms [9]. Furthermore, GML has been reported to prevent simian immunodeficiency virus (SIV) transmission and postoperative infection due to its strong antiviral properties [10]. With digestion and absorption features similar to those of MCFAs, GML has also been used to regulate animal physiological function and gut microbiota [11]. Zhang et al. reported that GML can modulate human immune system by regulating lipid dynamics in T cells and preventing cytokine production and exotoxin stimulation [12]. For the above reasons, GML has been investigated for its effects as a feed additive on the health, productivity and egg properties of chickens [13]. In addition, Yuniwarti et al. [14] found that virgin coconut oil containing lauric acid as a dietary supplement could be converted into GML in the body of broilers and increase chicken body weight.

In our preliminary study, short-term GML supplementation was found to significantly improve the body weight and physiological and biochemical parameters of broilers and laying hens (data not shown). The present work aimed to evaluate, for the first time, the long-term effects of basal diets supplemented with GML on the productive performance, egg quality, intestinal mucosa structure and serum parameters of Hy-Line Brown laying hens. The results of this study may further contribute to the broader application of GML and provide insight into its potential as an alternative of antibiotics.

2. MATERIALS AND METHODS

2.1. Husbandry and experimental diets

The protocol was approved by the Institutional Animal Care and Use Committee of Zhejiang University (Protocol No. ZJU-BEFS-2016004), and the experiments were conducted according to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

One hundred twenty Hy-Line Brown hens of 17 weeks of age were randomly assigned to 4 groups. Each treatment consisted of 5 replicates with 6 hens per replicate and was randomly distributed in the facility. Three adjacent cages (38 × 35 × 28 cm; length × width × height) with 2 birds per cage were considered as an experimental replicate. The hens were housed in cages with a light regime of 16L:8D with free water and food intake. A phase feeding program (phase I: 17 to 58 weeks of age; phase II: 59 to 69 weeks of age) was used with different diets, which were corn-soybean meal-based and formulated to meet the nutrient requirements of the National Research Council (NRC, 2012). The compositions of the diets for phase I and II are shown in Table 1. Hens in the control group were fed with the basal diet, whereas hens in other 3 experimental treatments received the basal diets supplemented with 0.15, 0.30 and 0.45 g/kg GML, respectively. GML was purchased from Hangzhou Kangyuan Food Science and Technology Co., Ltd. (Hangzhou, China).

Table 1. Ingredient composition and nutrient levels of basal diets (g/kg).

Item	Value	
	Phase I	Phase II
Ingredients		
Corn	62.9	64.9
Soybean meal	23.85	21.78
Pebble	4.51	4.51
Limestone	3.42	3.42
Vitamin-mineral premix[1]	4.7	4.7
Cod-liver oil	0.03	0.03
Rapeseed oil	0.59	0.66
Calculated analysis		
ME (MJ kg^{-1})	11.85	11.98
Crude protein	16.1	15.8
Crude fat	28.6	29.1
Lys	0.81	0.75
Met + Cys	0.65	0.60
Met	0.35	0.32
Ca	3.73	3.78
P	0.60	0.57

[1] Providing, per kg diet: vitamin A (from vitamin A acetate), 9940 IU; vitamin D_3, 4950 IU; vitamin E (from DL-α-tocopheryl acetate), 24 mg; vitamin B_1, 2 mg; vitamin B_2, 5.8 mg; vitamin B_6, 3 mg; vitaminB_{12}, 0.020 mg; biotin, 0.15 mg; Cu, 25 mg; Fe, 746 mg; Mn, 149 mg; Zn, 65 mg; Se, 0.30 mg.

Phase I: 17 - 58 weeks of age, Phase II: 59 - 69 weeks of age. ME: metabolizable energy; Ca: calcium; P: phosphorus.

2.2. Production performance

Egg production, egg weight, and mortality were recorded daily during the trial. The laying rate was defined as the ratio of the total number of laid eggs to the number of laying hens. Feed intake each week was determined by subtracting the weight of the remaining feed from the weight of the feed provided. The feed conversion ratio (FCR) was calculated as grams of total feed intake per gram of egg weight.

2.3.Egg quality

A total of 75 eggs per treatment (15 eggs from each replicate) laid on the last week of the 58^{th} and 69^{th} weeks, were randomly collected to determine the egg quality. The eggs were broken and separated into shell, albumen and yolk for weighing and their proportions were calculated. The egg quality parameters, including albumen height, Haugh unit, yolk color, eggshell strength and eggshell thickness, were measured with a digital egg tester (DET-6000, Nabel Co., Ltd., Japan).

2.4. Serum parameters

At the end of the trial, blood samples of 3 hens per replicate were collected via axillary vein after a 12 h fast. Each sample was immediately centrifuged at 3000 r min^{-1} for 15 min. The serum was decanted into a 1.5 mL polypropylene tube and stored at -80 ºC for the subsequent detection of serum parameters. The activities of aspartate transaminase (AST), alkaline phosphatase (ALP), glutamic-pyruvic transaminase (GPT), and gamma glutamyl transferase (GGT) and the concentrations of triglyceride, total cholesterol, high-density lipoprotein-cholesterol (HDLC), low density lipoprotein-cholesterol (LDLC), blood urea nitrogen, total protein, albumin, globulin, total bilirubin, creatinine, calcium (Ca), and phosphorus (P) in the serum were determined using commercially available kits (Jiancheng Co., Ltd., Nanjing, China) and automatic analyzers (COBASC 311, Roche Group, Switzerland).

2.5. Intestinal mucosa structure

At the end of the trial, 1 cm sections of the duodenum, jejunum and ileum from the medial portion were collected from laying hens, washed with physiological saline solution and fixed in 4% buffered paraformaldehyde. The tissue samples were embedded in paraffin, serially sectioned to 20 μm thickness, and then stained with hematoxylin and eosin. The villus height and crypt depth (4 hens from each replicate) were observed and measured with an optical microscope (DM4000B, LEICA Co. Ltd., Germany). The villus height to crypt depth ratio (VCR) of the duodenum, jejunum and ileum was calculated as the villus length divided by the crypt depth.

2.6. Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). The results are expressed as the means ± standard deviations (SD). Differences among treatments were separated by the Tukey test for multiple comparisons, and probability values less than 0.05 were considered as significant.

Table 2. Effects of different levels of GML supplementation on productive performance of hens

Item	Control	GML (g/kg diet)		
		0.15	0.30	0.45
Laying rate (%)				
Phase I	85.67 ± 0.45^{bc}	86.53 ± 0.31^{ab}	86.93 ± 0.35^{a}	85.47 ± 0.32^{c}
Phase II	80.50 ± 0.26^{b}	80.95 ± 0.14^{b}	86.20 ± 0.36^{a}	77.77 ± 0.81^{c}
Whole trial	84.60 ± 0.30^{bc}	85.40 ± 0.30^{b}	86.77 ± 0.25^{a}	83.90 ± 0.69^{c}
Average egg weight (g)				
Phase I	61.81 ± 0.43^{ab}	62.37 ± 0.40^{a}	62.33 ± 0.15^{a}	61.30 ± 0.36^{b}
Phase II	64.39 ± 0.29^{bc}	65.23 ± 0.32^{ab}	65.36 ± 0.47^{a}	64.03 ± 0.25^{c}
Whole trial	62.28 ± 0.30^{ab}	62.97 ± 0.35^{a}	63.04 ± 0.42^{a}	61.89 ± 0.26^{b}
Feed intake (g hen^{-1} per day)				
Phase I	106.33 ± 1.53	105.67 ± 2.08	105.69 ± 2.52	105.67 ± 1.53
Phase II	109.33 ± 1.53	108.33 ± 2.08	109.00 ± 2.00	109.06 ± 1.04
Whole trial	106.96 ± 1.30	106.22 ± 1.72	106.36 ± 1.58	106.36 ± 1.29
FCR (g:g)				
Phase I	2.01 ± 0.03	1.96 ± 0.04	1.95 ± 0.04	2.02 ± 0.04
Phase II	2.11 ± 0.02^{ab}	2.05 ± 0.04^{b}	1.93 ± 0.04^{c}	2.19 ± 0.03^{a}
Whole trial	2.03 ± 0.03^{ab}	1.98 ± 0.04^{ab}	1.94 ± 0.04^{b}	2.05 ± 0.04^{a}

Different letters in the same row indicate values significantly different (*P<0.05*) among the groups. Phase I: 18 - 58 weeks of age, Phase II: 59 - 69 weeks of age, the whole trial: 18 - 69 weeks of age. FCR: feed conversion ratio.

3. RESULTS AND DISCUSSION

3.1. Productive performance

The effects of GML supplementation on the productive performance of laying hens are shown in Table 2. During phase I, phase II and the whole trial, no significant difference in the body weight or mortality rate was observed among the treatments. Relative to that in the control group, the laying rate in the 0.30 g/kg GML group was significantly increased (*P<0.05*) by 1.47%, 7.08% and 2.57% during phase I, phase II and the whole trial, respectively. The laying rate in the 0.15 g/kg GML group was increased slightly relative to that in the control group, but the difference was not significant. However, relative to that in the control group, the laying rate in the 0.45 g/kg GML group was significantly decreased (*P<0.05*) by 3.39% during phase II and slightly but not significantly decreased during phase I, indicating that the effect of GML on laying rate was dose-dependent. The highest average egg weight was observed in the laying hen group receiving 0.30 g/kg GML (65.36 g) and was significantly higher than that of the control group (*P<0.05*). In the other GML groups, dietary GML supplementation had no significant effect on average egg weight during phase I or phase II. Feed intake was slightly decreased following 0.15, 0.30 and 0.45 g/kg dietary GML supplementation in both phase I and phase II, but did not significantly differ from that of the control group. In addition, relative to the control treatment, 0.15 and 0.30 g/kg dietary GML supplementation decreased the FCR, whereas 0.45 g/kg GML slightly increased the FCR; however, a significant difference from the control value was observed only for the 0.30 g/kg GML group during phase II. These results confirmed that 0.30 g/kg dietary GML supplementation was optimal in this trial. In addition, the trends across the different levels of GML supplementation with respect to the effects of GML on the production performances were very similar between the two phases.

Our results showed that long-term dietary GML supplementation significantly influenced the productive performance of laying hens, which is consistent with the reported effects of MCFAs as feed additives [15]. Van et al. [15] found that replacing part of the soybean oil and animal fat in the broiler diet with 0.3% capric acid and 2.7% lauric acid could improve the FCR. Moreover, MCFAs have been reported to improve productive performance and egg quality in poultry feeding, possibly through beneficial effects on digestion, adsorption, physiological regulation and gut health [16]. As one of the monoglycerides of MCFAs, GML might improve productive performance and egg quality in laying hens via similar mechanisms.

3.2. Egg quality

The effects of the different diets on the egg quality of laying hens were evaluated and are shown in Table 3. Albumen height and Haugh unit were increased by 0.30 g/kg dietary GML supplementation at the 58th and 69th weeks, but the differences were not significant. No effect of 0.15 or 0.45 g/kg GML supplementation was observed, and compared with the control group, the 0.45 g/kg GML group had higher values of albumen height and Haugh units at the 58th week but lower values of these variables at the 69th week. These results implied that the optimal concentration of GML might differ among different phases; therefore, in the present study, we divided the trial period into two feeding phases (phase I and II) for analysis. Different effects of feed additives among different feeding phases have also been observed in another research [17]. Compared with the control group, the yolk color was not significantly affected by dietary GML supplementation at the 58th week but was significantly ($P<0.05$) improved by 11.11% and 14.60% in the 0.30 and 0.45 g/kg GML groups, respectively, at the 69th week. In addition, relative to that in the control group, eggshell thickness was significantly ($P<0.05$) increased by 5.56% and 8.33% in the 0.15 and 0.30 g/kg GML groups, respectively, at the 58th week and by 8.33% in all three groups at the 69th week. Much greater eggshell strengths were observed at the 69th week in all the groups compared with the control group, with eggshell strength significantly ($P<0.05$) increasing by 5.71%, 6.49% and 6.75% in the 0.15, 0.30 and 0.45 g/kg GML groups, respectively. Regarding the egg components, compared with the control group, at the 58th week, the albumen proportion was significantly ($P<0.05$) increased by 4.53% in the 0.30 g/kg GML group, and the yolk proportion was significantly increased by 3.62% in 0.45 g/kg GML group. In addition, no significant change in albumen and yolk content was observed in all the groups at the 69th week. Relative to that in the control group, the eggshell proportion was significantly increased in the 0.30 and 0.45 g/kg GML groups at the 58th week and in the 0.15 g/kg GML group at the 69th week.

Table 3. Effects of different levels of GML supplementation on egg quality of laying hens

Item	Control	GML (g/kg diet)		
		0.15	0.30	0.45
Albumen height (mm)				
58 weeks of age	6.93 ± 0.5	6.54 ± 0.8	7.10 ± 0.7	7.21 ± 0.7
69 weeks of age	7.91 ± 0.5	7.63 ± 0.8	8.02 ± 0.9	7.84 ± 0.6
Haugh units				
58 weeks of age	79.47 ± 3.83	81.41 ± 5.76	82.88 ± 4.66	83.73 ± 4.53
69 weeks of age	85.26 ± 2.21	85.54 ± 2.32	85.34 ± 3.34	85.17 ± 2.03
Yolk color				
58 weeks of age	7.02 ± 0.48	7.00 ± 0.32	7.04 ± 0.47	7.08 ± 0.45
69 weeks of age	6.30 ± 0.48^{c}	6.56 ± 0.52^{bc}	7.00 ± 0.47^{ab}	7.22 ± 0.67^{a}
Eggshell thickness (mm)				
58 weeks of age	0.36 ± 0.01^{b}	0.38 ± 0.02^{a}	0.39 ± 0.02^{a}	0.37 ± 0.02^{ab}
69 weeks of age	0.36 ± 0.01^{b}	0.39 ± 0.02^{a}	0.39 ± 0.01^{a}	0.39 ± 0.02^{a}
Eggshell strength (kgf m^{-2})				
58 weeks of age	3.42 ± 0.43^{b}	4.00 ± 0.46^{a}	3.76 ± 0.42^{ab}	3.70 ± 0.44^{ab}
69 weeks of age	3.85 ± 0.13^{b}	4.07 ± 0.16^{a}	4.10 ± 0.23^{a}	4.11 ± 0.16^{a}
Albumen proportion (%)				
58 weeks of age	56.79 ± 1.62^{b}	56.04 ± 0.83^{b}	59.36 ± 0.60^{a}	56.87 ± 1.35^{b}
69 weeks of age	58.72 ± 3.32	59.60 ± 2.48	58.58 ± 1.77	58.19 ± 2.17
Yolk proportion (%)				
58 weeks of age	26.78 ± 1.09^{b}	27.02 ± 0.79^{ab}	27.21 ± 0.79^{ab}	27.75 ± 0.76^{a}
69 weeks of age	26.69 ± 1.50	26.65 ± 1.63	26.46 ± 1.32	26.62 ± 1.62
Eggshell proportion (%)				
58 weeks of age	9.23 ± 0.22^{b}	9.15 ± 0.24^{b}	9.51 ± 0.30^{a}	9.62 ± 0.27^{a}
69 weeks of age	8.86 ± 0.50^{b}	9.48 ± 0.40^{a}	9.06 ± 0.59^{ab}	9.16 ± 0.48^{ab}

Different letters in the same row indicate values significantly different ($P<0.05$) among the groups. Measured as a proportion (weight on weight) of the whole egg, expressed in percentage (%). kgf: kilogram force.

Adequate eggshell strength and thickness are essential for protection against the penetration of and contamination with pathogens. Eggshell quality is influenced by many factors, such as genetics, environment, nutrition, and the health status of layer hens [18]. Our study showed that the dietary GML supplementation increased eggshell thickness, eggshell strength and eggshell proportion. Lee et al. [16] reported that a microencapsulated organic acid blend (17% fumaric acid, 13% citric acid and 10% malic acid) containing 1.2% MCFAs (capric and caprylic acids) improved egg production, eggshell strength, Haugh unit and serum Ca concentration in laying hens, indicating that the positive effects of MCFAs and GML treatment on the eggshell parameters were possibly related to improved calcium absorption. Moreover, the improvements in the egg quality of the yolk proportion and color might be attributable to changes in lipid compositions because GML was reported to promote the absorption of lipid and lipid soluble pigments, such as zeaxanthin in the diet as an effective emulsifier [19].

3.3. Serum parameters

The influences of different level of GML supplementation on the serum biochemical indices of laying hens were analyzed (Table 4). In the 0.15, 0.30 and 0.45 g/kg GML groups, AST activity was significantly decreased relative to that in the control group by 14.86%, 17.20% and 7.11%, respectively, and ALP activity was significantly decreased by and 16.60%, 49.35% and 28.38%, respectively. Furthermore, the GPT activity was significantly decreased compared with the control level by 34.36% and 21.00% in the 0.30 and 0.45 g/kg GML groups, respectively. In the other GML groups, the GGT activity was lower than that in the control group, but the differences were not significant. Relative to that in the control group, the total cholesterol concentration was significantly decreased by 22.70%, 44.99% and 42.74% in the 0.15, 0.30 and 0.45 g/kg GML groups, respectively. In the 0.30 and 0.45 g/kg GML groups, the LDLC concentration was significantly decreased relative to the control concentration by 30.16% and 44.44%, respectively, whereas the HDLC concentration was significantly increased by 27.39% and 35.22%, respectively. No significant effect of treatment was observed on the concentration of serum triglyceride, urea nitrogen, total protein, albumin, globulin, total bilirubin or creatinine. Ca concentration was significantly increased by 24.87% in the 0.30 g/kg GML group relative to the control group, which indicated that the positive effect of GML on Ca absorption might have accounted for the enhanced eggshell strength in laying hens. However, no significant effect of treatment was observed on P concentration.

Serum plasma parameters could be used as supplementary indicators in the estimation of toxic effects in birds [20]. Elevated activities of serum plasma enzymes are typically indicative of organ damage [21]. In our study, serum ALP, AST, GPT and GGT activities in the GML groups were decreased relative to the levels in the control group, indicating improvements in liver function. These improvements might be attributable to increased antioxidant capacity under lipid oxidative stress after GML supplementation, as GML supplementation has been reported to increase the activities of superoxidase dismutase (SOD) and glutathione peroxidase (GSH-Px) [21].

The cholesterol profile was significantly improved in laying hens receiving GML supplementation in this study, which is consistent with the effects of MCFAs [22]. MCFAs and medium-chain triglycerides have been reported to enhance the capacity to transport excess cholesterol and to lower serum cholesterol levels [23]. Shokrollahi et al. [22] showed that dietary MCFAs (caproic acid < 3%, caprylic acid = 30%, capric acid = 56%, lauric acid = 10%, other fatty acids < 0.03%) significantly decreased LDLC and total cholesterol level, and increased the level of HDLC. Additionally, Zeng et al. [24] reported that dietary MCTs significantly prevented abdominal fat accumulation in high fat diet-fed mice without affecting serum total triglyceride concentration. These reports suggest that GML might play important roles in the modulation of lipid metabolism by regulating cholesterol metabolism, a possibility that requires further investigation.

Table 4. Effects of different levels of GML supplementation on serum parameters of laying hens (69 weeks of age)

Item	Control	GML (g/kg diet)		
		0.15	0.30	0.45
AST (IU/L)	223.32 ± 8.88[a]	190.14 ± 6.40[c]	184.90 ± 8.12[c]	207.44 ± 10.75[b]
ALP (IU/L)	672.40 ± 36.15[a]	560.80 ± 48.87[b]	340.60 ± 59.90[c]	481.60 ± 79.72[b]
GPT (IU/L)	13.62 ± 0.93[a]	11.84 ± 1.39[ab]	8.94 ± 0.91[c]	10.76 ± 0.73[bc]
GGT (IU/L)	23.00 ± 1.58	22.60 ± 2.41	22.40 ± 1.14	21.60 ± 2.30
Triglyceride (mmol/L)	12.61 ± 0.69	13.09 ± 0.59	13.20 ± 0.30	12.84 ± 0.61
Total Cholesterol (mmol/L)	4.89 ± 0.57[a]	3.78 ± 0.38[b]	2.69 ± 0.40[c]	2.80 ± 0.33[c]
LDLC (mmol/L)	0.63 ± 0.08[a]	0.75 ± 0.14[a]	0.44 ± 0.07[b]	0.35 ± 0.07[b]
HDLC (mmol/L)	2.30 ± 0.12[b]	2.60 ± 0.45[ab]	2.93 ± 0.40[a]	3.11 ± 0.20[a]
Blood urea nitrogen (mmol/L)	0.44 ± 0.11	0.42 ± 0.04	0.36 ± 0.09	0.32 ± 0.13
Total protein (g/L)	55.98 ± 3.28	55.28 ± 2.83	54.40 ± 1.28	53.10 ± 2.10
Albumin (g/L)	18.30 ± 1.16	17.04 ± 1.30	18.28 ± 1.85	18.36 ± 0.58
Globulin (g/L)	37.68 ± 3.01	38.24 ± 3.01	36.12 ± 1.59	34.74 ± 2.35
Total bilirubin (μmol/L)	0.84 ± 0.05	0.88 ± 0.08	0.92 ± 0.08	0.88 ± 0.08
Creatinine (μmol/L)	9.20 ± 0.84	9.24 ± 0.87	10.03 ± 1.02	9.80 ± 1.30
Ca (mmol/L)	5.59 ± 0.36[b]	6.09 ± 0.44[b]	6.98 ± 0.28[a]	6.15 ± 0.21[b]
P (mmol/L)	1.68 ± 0.14	1.79 ± 0.22	1.88 ± 0.31	1.63 ± 0.17

Different letters in the same row indicate values significantly different ($P<0.05$) among the groups. AST: aspartate transaminase; ALP: alkaline phosphatase; GPT: glutamic-pyruvic transaminase; GGT: gamma glutamyl transferase; LDLC: low-density lipoprotein cholesterol; HDLC: high density lipoprotein cholesterol.

3.4. Intestinal mucosa structure

The effects of the different levels of GML supplementation on the intestinal mucosa structure of laying hens are shown in Table 5 and Figure 1. Relative to the values in the control group, the villus height and crypt depth of the duodenum were significantly increased by 17.74% and 10.55%, respectively, in the 0.15 g/kg GML group and by 17.63% and 11.27%, respectively, in the 0.30 g/kg GML group. The villus height of the jejunum was significantly increased by 3.06% in 0.15 the g/kg GML group, and the VCR of the jejunum was significantly increased by 21.08%, 9.48% and 7.52% in the 0.15, 0.30 and 0.45 g/kg GML groups, respectively.

Villus height and crypt depth are important indices of intestinal digestion and absorption capacity, which are mainly determined by the rates of enterocyte regeneration and apoptosis [25]. Hanczakowska et al. [26] reported that dietary MCFA (2 g/kg capric acid) significantly increased the villus height and crypt depth of piglets by 31.33% and 20.71%, respectively. The improvement to the intestinal epithelial cell structure of laying hens due to GML supplementation in this study is consistent with a previous study of dietary MCFAs supplementation [26], which con

firms that GML and MCFAs improve productive performance by influencing digestion and adsorption via a similar mechanism. As medium-chain glycerides can be directly consumed as energy sources for enterocytes [5], dietary GML supplementation could stimulate enterocyte renewal and differentiation, thereby increasing the villus height and crypt depth. Moreover, the *in vivo* antibacterial effects of GML could contribute to improving intestinal health of animals by regulating the gut microbiota and inhibiting bacterial toxin production and inflammation reactions [11]. The potent anti-inflammatory action of GML could reduce the release of macrophages and the excess production of pro-inflammatory cytokines, which cause tissue damage and increase energy expenditure by interfering with adenosine triphosphate (ATP) synthesis and producing intensive stress proteins nonessential for growth [27]. Moreover, unlike the parameters in the upper digestive tract (duodenum and jejunum), the villus height, crypt depth and VCR of ileum were not significantly influenced by GML supplementation, suggesting selective effects of GML on metabolite and gut microbiota modulation, which is consistent with previous study [28].

Table 5. Effects of different levels of GML supplementation on intestinal mucosa structure of laying hens (69 weeks of age)

Intestine	Item	Control	GML (g/kg diet)		
			0.15	0.30	0.45
Duodenum	Villus length (μm)	166.37 ± 4.34^{c}	195.89 ± 5.78^{a}	183.93 ± 9.09^{b}	159.78 ± 4.55^{c}
	Crypt depth (μm)	28.93 ± 1.06^{b}	34.03 ± 1.36^{a}	32.19 ± 1.64^{a}	28.68 ± 0.74^{b}
	VCR (μm:μm)	5.75 ± 0.16	5.76 ± 0.13	5.72 ± 0.12	5.57 ± 0.09
Jejunum	Villus length (μm)	139.42 ± 7.30^{b}	169.96 ± 6.68^{a}	143.68 ± 4.18^{b}	150.25 ± 8.94^{b}
	Crypt depth (μm)	22.80 ± 1.39	22.95 ± 0.56	21.45 ± 0.79	22.82 ± 1.14
	VCR (μm:μm)	6.12 ± 0.06^{c}	7.41 ± 0.34^{a}	6.70 ± 0.24^{b}	6.58 ± 0.15^{b}
Ileum	Villus length (μm)	127.85 ± 5.38	120.11 ± 13.66	123.84 ± 9.22	123.59 ± 5.83
	Crypt depth (μm)	28.42 ± 0.72	28.35 ± 0.62	27.25 ± 0.70	28.22 ± 0.82
	VCR (μm:μm)	4.50 ± 0.27	4.23 ± 0.41	4.54 ± 0.31	4.38 ± 0.28

Different letters in the same row indicate values significantly different ($P<0.05$) among the groups.

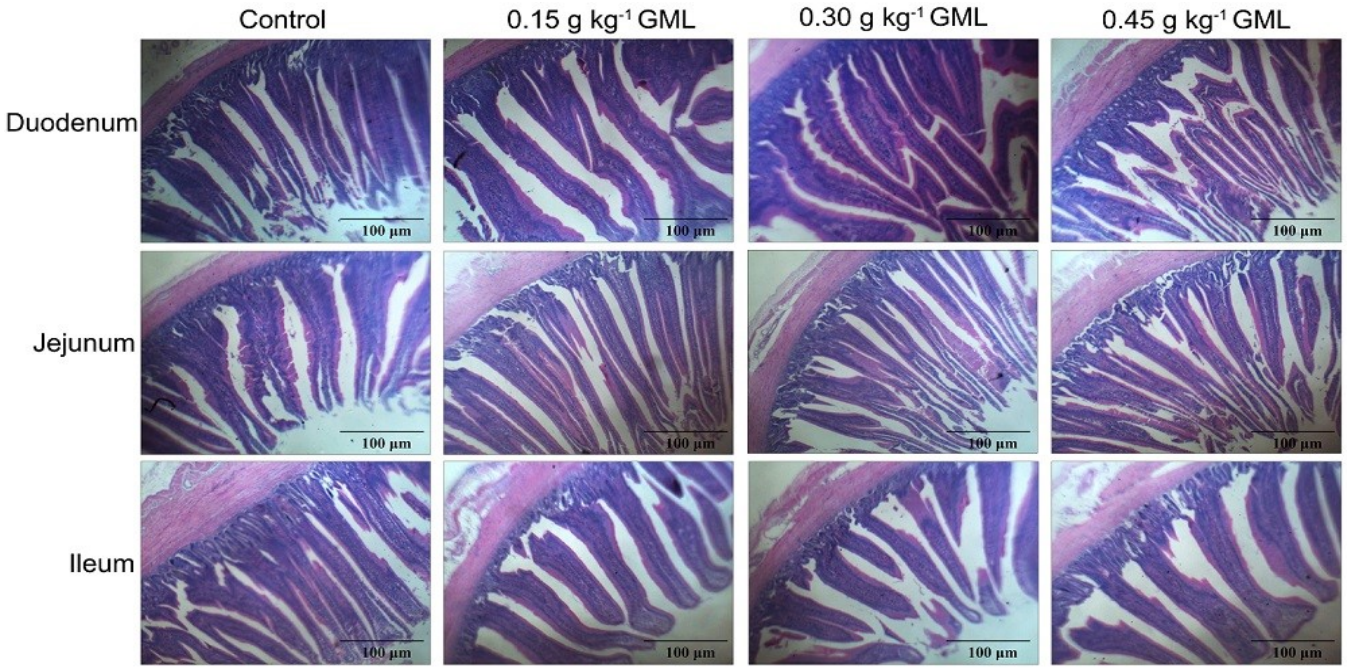

Figure 1. Intestinal morphology (duodenum, jejunum and ileum) of laying hens fed diet with 0, 0.15, 0.30 or 0.45 g/kg GML supplementation.

4. CONCLUSION

The abuse of antibiotics in poultry feed has caused a series of problems, including antibiotic residues in meat and egg products, disorders of human gut microbiota, contamination of the environment, and the development and spread of antibiotic-resistant bacteria, which calls for research and development on natural alternatives to antibiotics. GML is generally recognized as safe by the USA Food and Drug Administration and has been widely used as a food additive for its emulsifying and antimicrobial properties. Analysis of serum plasma parameters and intestinal mucosa morphology in this study supported the view that GML is a safe and advantageous substitute for traditional antimicrobial drugs in the poultry industry. In addition, dietary GML supplementation was proven to be useful for improving productive performance and egg quality in laying hens. These improvements are possibly the results of the improved intestinal mucosa structure and serum physiological and biochemical parameters, such as AST, ALP, and GPT activities and total cholesterol, LDLC, HDLC and Ca levels. Collectively, these results suggested that GML has promise as an alternative to conventional antimicrobials in laying hens to improve weight gain and feed conversion. Furthermore, these results suggest that GML has potential as a functional feed additive to increase laying performance and egg quality, possibly through its beneficial effects on the intestinal structure, lipid profiles and liver function.

Competing interests

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Author's contributions

Conception and design: H. Cai, M. Zhao, F. Feng. Acquisition of data: H. Cai, M. Liu, J. Li, M. Zhao. Analysis and interpretation of data: H. Cai, C. Cai, M. Zhao. Manuscript drafting and revising: H. Cai, C. Cai, M. Zhao.

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