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Effects of dietary glycerol monolaurate on productive performance, egg quality, serum biochemical indices, and intestinal morphology of laying hens*

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Abstract: Glycerol monolaurate (GML) has been widely used as an effective antibacterial emulsifier in the food industry. A total of 360 44-week-old Hy-Line brown laying hens were randomly distributed into four groups each with six replicates of 15 birds, and fed with corn-soybean-meal-based diets supplemented with 0, 0.15, 0.30, and 0.45 g/kg GML, respectively. Our results showed that 0.15, 0.30, and 0.45 g/kg GML treatments significantly decreased feed conversion ratios (FCRs) by 2.65%, 7.08%, and 3.54%, respectively, and significantly increased the laying rates and average egg weights. For egg quality, GML drastically increased albumen height and Haugh units, and enhanced yolk color. Notably, GML increased the concentrations of polyunsaturated and monounsaturated fatty acids and reduced the concentration of total saturated fatty acids in the yolk. The albumen composition was also significantly modified, with an increase of 1.02% in total protein content, and increased contents of His (4.55%) and Glu (2.02%) under the 0.30 g/kg GML treatment. Additionally, GML treatments had positive effects on the lipid metabolism of laying hens, including lowering the serum triglyceride and total cholesterol levels and reducing fat deposition in abdominal adipose tissue. Intestinal morphology was also improved by GML treatment, with increased villus length and villus height to crypt depth ratio. Our data demonstrated that GML supplementation of laying hens could have beneficial effects on both their productivity and physiological properties, which indicates the potential application of GML as a functional feed additive and gives us a new insight into this traditional food additive.

Key words: Glycerol monolaurate (GML); Laying hen; Productive performance; Egg quality; Lipid metabolism
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1 Introduction

The improvement of poultry growth performance and production quality and quantity are the major concerns for the poultry feed industry. Antibiotics have been used as growth promoters for a long time, but in recent decades, an increase in antibiotics abuse in poultry farming has been threatening the safety of related food products and public health. Since restrictions on the use of antibiotics were

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introduced by the European Union in 2006 (Thacker, 2013), natural functional feed additives, such as vitamin E, turmeric, and fish oil, have been considered as alternatives (Basmacioglu et al., 2003; Skřivan et al., 2010; Bonilla et al., 2017). Medium-chain fatty acids (MCFAs; C8–C12) have recently been reported to have positive effects on growth performance, production quality, and gut health as a natural feed additive, and have proven to be good alternatives to antibiotics in the poultry industry (Shokrollahi et al., 2014; van der Aar et al., 2017). MCFAs are absorbed efficiently and transported directly to the liver via the portal venous system, rather than being incorporated into chylomicrons for transport through the lymphatic system or peripheral circulation (Odle, 1997). van der Hoeven-Hangoor et al. (2013) showed that adding 0.3% capric acid (C10:0) and 2.7% lauric acid (C12:0) to the broiler diet to partly replace soybean oil and animal fat could improve feed conversion efficiency. Zeitz et al. (2015) found that dietary fats rich in C12:0 and myristic acid (C14:0) could increase feed conversion efficiency, breast meat percentage, and gut health in broilers. Furthermore, both free and esterified MCFAs could improve poultry health, production performance, and intestinal health (Zentek et al., 2011; Zeitz et al., 2015). Chiang et al. (1990) found that supplementation with 3%–5% medium-chain triglycerides (MCTs) in the feed of broilers improved feed utilization efficiency. Furuse et al. (1992) reported that MCTs also influenced protein utilization and fat deposition in growing chicks.

Glycerol monolaurate (GML, $C_{15}H_{30}O_4$), a natural monoglyceride of C12:0, occurs naturally in breast milk, coconut oil, and palmetto, which are available in the feed industry (Witcher et al., 1996; Zentek et al., 2011). GML has been used as an antimicrobial and antiviral emulsifier, and was determined to be “generally recognized as safe” (GRAS) by the US Food and Drug Administration. Broilers treated with a mixture of GML and butyric acid (C4:0) glycerides showed an 8.8% higher average end body weight and a 3.3% increase in feed conversion efficiency, and those positive effects were also observed during field trials at a commercial broiler farm (Meirhaeghe et al., 2015). Wieland et al. (2015) reported that feeding 0.006% GML based on body weight had no negative impact on liver function in

newborn calves. Moreover, it has been reported that dietary virgin coconut oil, containing C12:0, could be turned into GML in the body and increase the body weight of broilers (Yuniwanti et al., 2012). Supplementation of coconut oil in the feed of laying hens also significantly affected the lipid composition of their eggs, with increased C14:0 and C12:0 levels (Thomsen, 1966, 1967). However, the effects of GML on the production performance and egg quality of laying hens are still unknown.

In the present study, we hypothesized that GML has the potential to improve the production performance, egg quality, and metabolic status of laying hens. We evaluated and compared the effects of different concentrations of dietary GML supplementations on the laying performance, egg quality, yolk nutrient composition, albumen nutrient composition, intestinal morphology, and serum biochemical parameters of laying hens. We also explored the potential relationship between production performance and the metabolic benefits of GML treatment in laying hens. Our results provide evidence for extending the application of GML in the poultry industry.

2 Materials and methods

2.1 Husbandry and experimental treatment

A total of 360 Hy-Line brown laying hens (44-week-old) with uniform body weight and laying rate were randomly divided into four equal treatments, each with six replications ($n=15$). Three hens were housed per cage (38 cm×35 cm×28 cm), with a manual feeder and nipple drinker, in a controlled environment (12 h daylight cycle, lights off at 18:00, 22–26 °C). The hens in five adjacent cages on one tier were considered an experimental replicate. The study protocol was approved by the Institutional Animal Care and Use Committee of Zhejiang University, Hangzhou, China (Protocol Number ZJU-BEFS-2016004), and the study was performed according to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, USA. The feeding trial lasted for 8 weeks, and 120 birds (5 hens from each replicate, 30 hens per group) were sacrificed after 12-h fasting. Serum samples were collected from the jugular vein into vacuum tubes (5 mL) containing coagulant gel, and were

centrifuged for 15 min at 3000 r/min. Jejunum samples (about 1 cm) and abdominal fat pad samples were collected and washed in 0.01 mol/L phosphate-buffered saline (PBS), then fixed in 4% paraformaldehyde for subsequent histopathological analysis.

2.2 Diets and glycerol monolaurate

The experimental diets were based on corn and soybean meal according to recommendations of the National Research Council (Pesti, 1995). Hens in the control group were fed the basal diet, whereas hens in the three experimental treatments received the basal diets supplemented with 0.15, 0.30, or 0.45 g/kg GML. Feed and water were provided for ad libitum consumption. GML was provided by the Hangzhou Kangyuan Food Science and Technology Co., Ltd. (Hangzhou, China). The experimental diets were stored in a dry and well-ventilated storeroom. The composition of the basal diet is presented in Table 1.

2.3 Production performance

Egg number, egg weight, and feed consumption were recorded on a daily basis. The laying rate was expressed as the ratio of the total number of eggs

collected to the number of laying hens. The feed conversion ratio (FCR) was calculated daily as grams of feed intake per gram of egg weight on a replication basis. The average egg weight was also determined as an indicator of the hens' daily production.

2.4 Egg quality

To determine egg quality, a total of 144 eggs (12 from each replicate per day) for each treatment were randomly collected and individually weighed on two consecutive days in Week 8. The weights of the shell, albumen, and yolk were recorded to calculate their proportion of the whole egg weight. Then, 12 yolks or 12 albumens from the same replicate were mixed in a beaker and transferred to vacuum tubes. Samples were stored at -80°C and then thawed at 4°C prior to analysis of the nutrient composition. The remaining eggs were used for the measurement of albumen height, Haugh unit, yolk color, and eggshell strength using a digital egg tester (DET-6000, Nabel Co., Ltd., Kyoto, Japan). The eggshell thickness (without inner and outer shell membranes) was measured at the equatorial region of the eggshell using shell thickness vernier calipers (Santo 8012, Santo Co., Ltd., Shanghai, China).

2.5 Yolk composition

Yolk lipid samples were extracted by the chloroform-methanol method with some modification (Chassaing et al., 2015). Lipid extract (60 mg) and hexane (4 mL) were added to a 10-mL test tube and mixed thoroughly. The weight of lipid extract was recorded accurately. Then, 200 μL of KOH-methanol solution (2 mol/L) was added to remove moisture. The samples were analyzed using a gas chromatograph (Agilent GC7890B, Agilent Co., Ltd., Palo Alto, USA). A DB-1HT column (length 15 m, internal diameter 0.25 mm, film thickness 0.25 mm) was used to separate methylated fatty acids. Oven temperature was increased from 200 to 350°C at $8^{\circ}\text{C}/\text{min}$, and then held at 350°C for 5.5 min. The temperature was 320°C at the inlet and 370°C at the detector. All determinations were performed in triplicate. Fatty acids were identified by comparing the retention time of known standards. Relative quantities were expressed as the percentage weight of total fatty acids (Nam et al., 2001). The total egg yolk cholesterol content was determined using commercial kits (Nanjing Jiancheng Biotechnology Co., Ltd., Nanjing, China).

Table 1 Ingredient composition and nutrient levels of basal diets

Component	Proportion (g/kg)
Ingredients	
Corn	615.6
Soybean meal	248.3
Oyster shell power	41.4
Limestone	38.1
Vitamin-mineral premix ¹	49.7
Cod-liver oil	0.3
Rapeseed oil	6.6
Calculated analysis	
Metabolizable energy (kcal/kg)*	2841.6
Crude protein	159.1
Crude fat	28.6
Lys	6.1
Met+Cys	6.3
Met	2.2
Calcium	35.4
Total phosphorus	6.0

¹ Providing, per kg diet: vitamin A (from vitamin A acetate), 9940 IU; vitamin D₃, 4950 IU; vitamin E (from DL- α -tocopheryl acetate), 24 mg; vitamin B₁, 2 mg; vitamin B₂, 5.8 mg; vitamin B₆, 3 mg; vitamin B₁₂, 0.020 mg; biotin, 0.15 mg; Cu, 25 mg; Fe, 746 mg; Mn, 149 mg; Zn, 65 mg; Se, 0.30 mg. * Value is expressed in kcal/kg (1 kcal=4.1868 kJ)

2.6 Albumen composition

The albumen protein content was measured using the method of Sun et al. (2013) with some modification. The amino acid contents were analyzed and quantitatively determined using a Waters 2695 high-performance liquid chromatograph (HPLC; Waters Co., Ltd., Milford, USA) as previously described (Hou et al., 2009) with slight modifications. A 0.4-g albumen sample was weighed into a medium wall Pyrex test tube, and 10 mL of hydrochloric acid (6 mol/L) was added in an oven under a N₂ environment at 110 °C for 24 h. After hydrolysis and re-drying, 200 µL of derivatization reagent was added following 20 min evaporation. About 1 mL of sample diluent (0.015 mol/L disodium hydrogen phosphate solution, using orthophosphoric acid and acetonitrile (95:5, v/v) to adjust to a pH of 7.4) was added. A 10-µL aliquot from the prepared sample was injected into a Pico-Tag column (5 µm, 4.6 mm×250 mm, ZORBAX SB-C18, Agilent Technologies, Palo Alto, USA). All determinations were performed in triplicate. The quantification of each amino acid was determined from a standard curve. Note that Trp is destroyed by acid hydrolysis, and thus its content was not determined.

2.7 Serum biochemical indices and sex hormones

The serum concentrations of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), total protein, albumin, globulin, total bilirubin, creatinine, calcium (Ca), and phosphorus (P) in serum were determined using an automatic biochemical analyzer (Cobas 311, Roche Diagnostica, Basel, Switzerland). The activity of aspartate transaminase (AST), alkaline phosphatase (ALP), γ -glutamyl transferase (GGT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and the concentration of malondialdehyde (MDA) were measured using commercial kits (Jiancheng Co., Ltd., Nanjing, China).

The concentrations of serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Hangzhou Yanhui Biotechnology Co., Ltd., Hangzhou, China).

2.8 Histological morphology

Jejunum samples (about 1 cm) and collected abdominal fat pad samples were fixed in 4% paraformaldehyde, paraffin-embedded, and sectioned at 5–7 µm thickness. Hematoxylin and eosin (H&E) staining was based on the standard method. The villus height and crypt depth (three hens from each replicate) were observed under an optical microscope (DM4000B, Leica Co., Ltd., Wetzlar, Germany). The villus height, crypt depth, and number and size of stained fat droplets in abdominal adipose tissue were quantified with ImageJ 1.45 software (National Institutes of Health, Bethesda, USA). The villus height to crypt depth ratio (VCR) was also calculated.

2.9 Statistical analysis

The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test, using SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA). The results are expressed as mean±standard deviation (SD). $P<0.05$ was considered statistically significant. In all figures and tables, data with different superscript letters represent significant difference based on one-way ANOVA.

3 Results

3.1 Productive performance

The effects of different concentrations of GML supplementation on the laying performance of hens are shown in Table 2. Compared with the control, supplementation with 0.30 or 0.45 g/kg GML significantly increased the laying rate by 4.06% ($P<0.001$) and 1.70% ($P=0.009$), respectively. The average egg

Table 2 Laying performance of hens fed with different levels of GML supplementation

Group	Laying rate (%)	Average egg weight (g/d per hen)	FCR (g/g)
Control	86.22±0.25 ^c	60.78±0.12 ^c	2.26±0.01 ^a
0.15 g/kg GML	87.13±0.25 ^{bc}	61.77±0.13 ^b	2.20±0.01 ^b
0.30 g/kg GML	89.72±0.58 ^a	62.56±0.09 ^a	2.10±0.02 ^c
0.45 g/kg GML	87.69±0.46 ^b	62.23±0.18 ^a	2.18±0.03 ^b

Data are represented by mean±SD ($n=6$). Different letters (comparing all treatments) in the same column indicate values that are significantly different ($P<0.05$) among the groups. GML: glycerol monolaurate; FCR: feed conversion ratio

weight showed a significant increase in all GML-treated groups (1.63%, 2.93%, and 2.39%, respectively) ($P<0.001$ for all), while the FCR showed a significant decrease (2.65% ($P=0.025$), 7.08% ($P<0.001$), and 3.54% ($P<0.001$), respectively).

3.2 Egg quality

The effects of GML treatment on the albumen and yolk of laying hens are presented in Fig. 1. The albumen proportion in the 0.30 and 0.45 g/kg GML groups was significantly reduced by 2.99% ($P=0.016$) and 3.92% ($P=0.003$), respectively, while the yolk proportion was significantly increased by 8.20% ($P=0.001$) and 8.74% ($P=0.001$), respectively. The egg quality of laying hens fed with any of the GML concentrations was improved (Table 3). Generally, supplementation with 0.30 g/kg GML showed the highest increase in albumen height (6.93%, $P=0.007$), Haugh units (3.07%, $P=0.043$), and yolk color (20.97%, $P=0.003$), compared with the control. However, no significant change was observed in the thickness or

strength of eggshells, or in the egg shape index after GML treatment.

3.3 Yolk composition

The effects of different concentrations of GML supplementation on the yolk nutrient composition of eggs from laying hens are shown in Table 4. Compared with the control diet, the yolk lipid content was significantly increased by 12.52% in the 0.30 g/kg GML group ($P=0.007$), while the yolk cholesterol content was highest in hens fed with the diet containing 0.45 g/kg GML (increased by 1.90%, $P=0.001$). Palmitic acid (C16:0) is the predominant saturated fatty acid (SFA) in egg yolk, followed by stearic acid (C18:0). The total SFA concentration in egg yolk was significantly decreased by 9.71% ($P<0.001$), 7.32% ($P<0.001$), and 6.26% ($P<0.001$) in the 0.15, 0.30, and 0.45 g/kg GML groups, respectively. This was attributed mainly to the simultaneous reduction of the C16:0, C18:0, and arachidic acid (C20:0) contents. In terms of total monounsaturated fatty acid (MUFA) concentration, yolks from hens treated with 0.15 or 0.30 g/kg GML showed increases of 6.70% ($P=0.003$) and 5.19% ($P=0.013$), respectively, with significant enhancement in the contents of oleic acid (C18:1) and eicosenoic acid (C20:1). In addition, there were significant effects of dietary GML supplementation on the concentration of total polyunsaturated fatty acid (PUFA) in the yolk, especially for 0.30 and 0.45 g/kg GML, which increased the PUFA content by 16.06% ($P=0.001$) and 11.27% ($P=0.007$), respectively. This was mainly attributed to an increase in linoleic acid (C18:2) content. Moreover, the ratio of PUFA to SFA was remarkably increased, by 16.67%, 23.81%, and 19.05% in the 0.15, 0.30, and 0.45 g/kg GML groups ($P<0.001$ for all), respectively. Thus, 0.30 g/kg GML supplementation showed the greatest potential to improve the lipid nutrition of the egg yolk.

Fig. 1 Proportions of shell, yolk, and albumen in eggs of laying hens fed with different levels of GML supplementation

Proportions are expressed as a percentage (weight to weight) of the whole egg (plus shell). Data are represented by mean $\pm$ SD, $n=36$. Columns with different letters are significantly different ($P<0.05$). GML: glycerol monolaurate

Table 3 Egg quality of laying hens fed with different levels of GML supplementation

Group	Albumen height (mm)	Haugh units	Yolk color	Eggshell strength (kgf/m ²)	Eggshell thickness (mm)	Egg shape index
Control	7.22 $\pm$ 0.26 ^b	85.35 $\pm$ 1.16 ^b	6.20 $\pm$ 0.35 ^b	3.69 $\pm$ 0.43	0.364 $\pm$ 0.009	1.289 $\pm$ 0.048
0.15 g/kg GML	7.26 $\pm$ 0.17 ^b	85.43 $\pm$ 0.51 ^b	6.60 $\pm$ 0.55 ^{ab}	3.70 $\pm$ 0.35	0.372 $\pm$ 0.008	1.321 $\pm$ 0.051
0.30 g/kg GML	7.72 $\pm$ 0.19 ^a	87.97 $\pm$ 1.95 ^a	7.50 $\pm$ 0.50 ^a	3.76 $\pm$ 0.28	0.384 $\pm$ 0.015	1.326 $\pm$ 0.061
0.45 g/kg GML	7.58 $\pm$ 0.18 ^{ab}	86.67 $\pm$ 1.49 ^{ab}	7.06 $\pm$ 0.61 ^{ab}	3.84 $\pm$ 0.36	0.375 $\pm$ 0.024	1.319 $\pm$ 0.045

Data are represented by mean $\pm$ SD ($n=6$). Different letters (comparing all treatments) in the same column indicate values that are significantly different ($P<0.05$) among the groups. kgf: kilogram force; GML: glycerol monolaurate

Table 4 Yolk nutrient composition of laying hens fed with different levels of GML supplementation

Group	Lipid content (%)	Cholesterol content (g/kg)	SFA (g/kg)				
			Total	C14:0	C16:0	C18:0	C20:0
Control	26.63±0.28 ^b	13.71±0.04 ^b	33.89±0.16 ^a	0.44±0.01	26.17±0.04 ^a	7.19±0.11 ^a	0.09±0.01 ^a
0.15 g/kg GML	27.30±0.87 ^b	13.71±0.07 ^b	30.60±0.15 ^c	0.44±0.01	23.77±0.17 ^b	6.33±0.01 ^b	0.05±0.01 ^b
0.30 g/kg GML	30.44±0.82 ^a	13.78±0.04 ^b	31.41±0.42 ^b	0.44±0.01	24.49±0.58 ^b	6.45±0.37 ^b	0.03±0.01 ^c
0.45 g/kg GML	27.86±0.38 ^{ab}	13.97±0.04 ^a	31.77±0.15 ^b	0.45±0.04	24.53±0.22 ^b	6.76±0.09 ^{ab}	0.03±0.01 ^c
Group	MUFA (g/kg)					PUFA (g/kg)	
	Total	C14:1	C16:1	C18:1	C20:1	Total	C18:2
Control	46.45±0.07 ^b	0.08±0.01	4.93±0.07	41.32±0.02 ^b	0.13±0.01 ^c	14.20±0.02 ^c	12.00±0.01 ^c
0.15 g/kg GML	49.56±0.39 ^a	0.11±0.01	4.95±0.06	44.33±0.45 ^a	0.17±0.01 ^{ab}	15.02±0.07 ^{bc}	12.85±0.08 ^{bc}
0.30 g/kg GML	48.86±0.56 ^a	0.12±0.02	4.86±0.09	43.70±0.48 ^a	0.19±0.01 ^a	16.48±0.41 ^a	14.36±0.52 ^a
0.45 g/kg GML	48.11±0.89 ^{ab}	0.10±0.02	4.72±0.02	43.13±1.07 ^{ab}	0.15±0.02 ^{bc}	15.80±0.52 ^{ab}	13.64±0.53 ^{ab}
Group	PUFA (g/kg)					PUFA:SFA	
	C18:3	C20:2	C20:4	C20:5	C22:6		
Control	0.48±0.04	0.16±0.01	0.02±0.01	1.15±0.03	0.40±0.03	0.42±0.01 ^c	
0.15 g/kg GML	0.53±0.09	0.17±0.01	0.02±0.01	1.04±0.02	0.42±0.02	0.49±0.01 ^b	
0.30 g/kg GML	0.48±0.03	0.18±0.02	0.02±0.01	1.06±0.11	0.40±0.01	0.52±0.01 ^a	
0.45 g/kg GML	0.51±0.06	0.16±0.02	0.02±0.01	1.08±0.10	0.39±0.02	0.50±0.01 ^{ab}	

Data are represented by mean±SD ($n=6$). Different letters (comparing all treatments) in the same column indicate values that are significantly different ($P<0.05$) among the groups. GML: glycerol monolaurate; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; C14:0: myristic acid; C16:0: palmitic acid; C18:0: stearic acid; C20:0: arachidic acid; C14:1: myristoleic acid; C16:1: palmitoleic acid; C18:1: oleic acid; C20:1: eicosenoic acid; C18:2: linoleic acid; C18:3: γ -linoleic acid; C20:2: eicosadienoic acid; C20:4: arachidonic acid; C20:5: eicosapentaenoic acid; C22:6: docosahexaenoic acid

3.4 Protein and amino acid analyses of albumen

Results from the analysis of the albumen nutrient composition of laying hens from the four different experimental diet groups are shown in Table 5. The protein content in the 0.30 g/kg GML treatment showed the highest increase (9.59%) compared with the control ($P=0.044$). All the essential amino acids were detected except for Trp, which was infeasible by our detection method. The content of neither the total essential amino acids nor the total non-essential amino acids showed significant differences among these groups. For non-essential amino acids, GML supplementation significantly enhanced the content of His, which was increased by 2.50% ($P=0.020$), 4.55% ($P=0.001$), and 2.22% ($P=0.037$) in the 0.15, 0.30, and 0.45 g/kg GML groups, respectively. Albumen Glu content was highest in hens treated with 0.30 g/kg GML, which showed a significant increase (2.02%, $P=0.002$) compared with the control group.

3.5 Analyses of serum biochemical indices and sex hormones

The effects of dietary GML levels on serum biochemical indices are shown in Table 6. Dietary GML level had significant effects on lipid metabolism. The contents of TG and TC showed significant decreases

in hens fed with 0.30 or 0.45 g/kg GML ($P=0.004$ and $P=0.009$ for TG; $P=0.001$ and $P=0.002$ for TC, respectively), and the level of LDLC was also significantly reduced in these two groups ($P=0.025$ and $P=0.028$, respectively). All the GML supplementation groups showed increased serum HDLC levels, and the 0.45 g/kg GML group showed the highest increase compared with the control ($P=0.044$). As for markers of liver function, the levels of the liver enzyme alanine aminotransferase (ALT) were observed to decrease significantly in the 0.30 and 0.45 g/kg GML supplemented groups ($P=0.003$ and $P=0.001$, respectively). The content of AST was significantly reduced by the 0.45 g/kg GML treatment ($P=0.034$). The serum total bilirubin level was also significantly decreased by 33.75% ($P=0.002$) and 21.25% ($P=0.024$) in hens treated with 0.30 and 0.45 g/kg GML, respectively, compared with the control group.

For the effect of GML on oxidative stress, the lipid peroxidation index was reduced by 39.47% in the 0.30 g/kg GML group ($P<0.001$), and by 45.88% in the 0.45 g/kg GML group ($P<0.001$) compared with the control, because of the lower content of serum MDA. Moreover, significantly elevated activity of SOD and GSH-Px was observed in these two groups ($P=0.006$ and $P<0.001$ for SOD; $P=0.002$ and

Table 5 Albumen nutrient composition of laying hens fed with different levels of GML supplementation

Group	Protein content (%)	Essential amino acid content (mg/g protein)					
		Total	Thr	Val	Met	Ile	Leu
Control	10.64±0.32 ^b	326.00±0.90	39.63±0.21	55.40±0.44	35.50±0.30	46.07±0.23	79.73±0.50
0.15 g/kg GML	11.10±0.61 ^{ab}	327.20±0.85	40.10±0.30	55.73±0.40	35.40±0.40	46.60±0.30	79.97±0.61
0.30 g/kg GML	11.66±0.61 ^a	327.43±0.76	40.23±0.60	55.60±0.52	35.67±0.21	46.43±0.31	80.00±0.46
0.45 g/kg GML	10.76±0.22 ^{ab}	327.60±0.52	40.07±0.21	55.70±0.36	35.63±0.25	46.17±0.25	80.17±0.49

Group	Essential amino acid content (mg/g protein)		Non-essential amino acid content (mg/g protein)				
	Phe	Lys	Total	His	Asp	Glu	Ser
Control	54.83±0.15	60.90±0.53	450.47±3.00	18.02±0.18 ^c	89.86±0.51	127.10±0.61 ^b	58.07±0.50
0.15 g/kg GML	55.13±0.21	60.87±0.60	451.92±2.09	18.47±0.14 ^{ab}	90.41±0.67	128.27±0.55 ^{ab}	57.77±0.72
0.30 g/kg GML	55.12±0.24	60.80±0.50	453.47±0.13	18.84±0.10 ^a	89.89±0.19	129.67±0.64 ^a	58.13±0.31
0.45 g/kg GML	55.17±0.35	60.87±0.35	452.06±0.72	18.42±0.15 ^b	90.01±0.46	128.23±0.42 ^{ab}	58.00±0.20

Group	Non-essential amino acid content (mg/g protein)						Total amino acid content (mg/g protein)
	Gly	Arg	Ala	Pro	Tyr	Cys	
Control	28.29±0.41	54.35±0.60	50.25±0.57	35.90±0.20	34.95±0.24	38.18±0.65	776.47±2.17
0.15 g/kg GML	28.15±0.29	54.24±0.50	49.91±0.84	36.17±0.25	35.16±0.25	37.83±0.40	779.12±2.84
0.30 g/kg GML	28.10±0.26	54.35±0.51	50.35±0.50	36.07±0.25	35.01±0.32	38.01±0.32	780.90±0.88
0.45 g/kg GML	28.40±0.62	54.16±0.19	50.48±0.65	36.10±0.30	35.10±0.30	37.82±0.27	779.66±0.29

Data are represented by mean±SD ($n=6$). Different letters (comparing all treatments) in the same column indicate values that are significantly different ($P<0.05$) among the groups. GML: glycerol monolaurate

Table 6 Effect of dietary GML concentration on serum biochemical indices of laying hens

Group	TG (mmol/L)	TC (mmol/L)	LDLC (mmol/L)	HDLC (mmol/L)	Total protein (g/L)	Albumin (g/L)
Control	11.43±0.77 ^a	3.32±0.08 ^a	0.28±0.01 ^a	2.26±0.07 ^b	49.58±0.47 ^b	18.00±0.83
0.15 g/kg GML	9.60±0.10 ^b	3.40±0.04 ^a	0.26±0.02 ^{ab}	2.79±0.47 ^{ab}	49.68±0.45 ^b	18.23±0.62
0.30 g/kg GML	7.71±0.20 ^c	2.31±0.03 ^c	0.24±0.01 ^b	2.84±0.48 ^{ab}	49.71±0.50 ^b	18.03±0.81
0.45 g/kg GML	9.55±0.39 ^b	2.57±0.03 ^b	0.24±0.01 ^b	3.12±0.29 ^a	54.08±0.34 ^a	18.10±0.71

Group	Globulin (g/L)	Total bilirubin (μmol/L)	ALT (U/L)	AST (U/L)	GGT (IU/L)	Ca (mmol/L)
Control	37.80±1.06	0.80±0.07 ^a	10.46±0.31 ^a	262.42±2.35 ^a	23.25±4.57	5.24±0.08
0.15 g/kg GML	38.03±1.26	0.88±0.05 ^a	10.05±0.51 ^a	264.78±2.93 ^a	21.33±2.52	5.27±0.06
0.30 g/kg GML	37.93±0.99	0.53±0.05 ^b	7.83±0.33 ^b	263.50±1.92 ^a	22.67±3.06	5.24±0.05
0.45 g/kg GML	37.65±0.83	0.63±0.05 ^b	7.05±0.26 ^c	256.15±3.50 ^b	22.33±2.52	5.25±0.06

Group	P (mmol/L)	SOD (U/L)	MDA (nmol/mL)	GSH-Px (U)
Control	2.26±0.05	194.02±3.36 ^c	9.35±0.27 ^a	1847.25±20.62 ^c
0.15 g/kg GML	2.24±0.05	198.04±0.77 ^c	9.22±0.17 ^a	1887.96±14.31 ^c
0.30 g/kg GML	2.24±0.06	204.11±1.87 ^b	5.66±0.31 ^b	2052.94±16.03 ^b
0.45 g/kg GML	2.27±0.06	209.91±3.32 ^a	5.06±0.37 ^b	2243.90±32.09 ^a

Data are represented by mean±SD ($n=15$). Different letters (comparing all treatments) in the same column indicate values that are significantly different ($P<0.05$) among the groups. TG: triglyceride; TC: total cholesterol; LDLC: low-density lipoprotein cholesterol; HDLC: high-density lipoprotein cholesterol; ALT: alanine aminotransferase; AST: aspartate transaminase; GGT: γ -glutamyl transferase; SOD: superoxide dismutase; MDA: malondialdehyde; GSH-Px: glutathione peroxidase; U: unit; IU: international unit

$P=0.001$ for GSH-Px, respectively). As shown in Fig. 2, all dietary GML levels had significant effects on the contents of the serum sex hormones FSH and LH. Compared with the control, the level of serum FSH was significantly increased by 10.00% ($P=0.007$), 17.57% ($P<0.001$), and 10.54% ($P=0.004$) in the 0.15, 0.30, and 0.45 g/kg GML groups, respectively. Additionally, the level of serum LH was significantly increased by 5.05% ($P=0.017$), 11.36% ($P<0.001$), and 6.47% ($P=0.004$), respectively. These results indicate that GML supplementation could positively influence oxidative stress and physiological properties, especially at the 0.30 g/kg dosage.

Fig. 2 Serum sex hormone levels of laying hens fed with different levels of GML supplementation

Data are represented by mean $\pm$ SD ($n=15$). Columns with different letters are significantly different ($P<0.05$). GML: glycerol monolaurate; FSH: follicle-stimulating hormone; LH: luteinizing hormone

3.6 Jejunum morphology analysis

The feed diets containing GML led to significant enhancements in villus height and density compared with the control (Fig. 3). The longest villi were observed in the 0.30 g/kg GML group ($P<0.001$). The jejunum villus length, crypt depth, and VCR of laying hens treated with different levels of GML supplementation are summarized in Figs. 3e and 3f. Compared with the control, the length of villi was significantly increased by 36.25%, 41.43%, and 19.53% in the 0.15, 0.30, and 0.45 g/kg GML groups ($P<0.001$ for all), respectively. Moreover, the VCR was significantly increased by 33.46% ($P=0.002$), 41.31% ($P<0.001$), and 22.06% ($P=0.007$) in the 0.15, 0.30, and 0.45 g/kg GML groups, respectively. The best VCR was observed in hens receiving diets containing 0.30 g/kg GML.

3.7 Abdominal adipose tissue analysis

To evaluate the effect of GML supplementation on the phenotype of adipose tissue, abdominal adipose tissue samples were used for weight and histological analyses. The 0.30 and 0.45 g/kg GML treatments significantly reduced the relative weight of the abdominal fat pad ($P=0.002$ and $P=0.006$, respectively) and the size of adipocytes ($P=0.013$ and $P=0.026$, respectively) compared with the control group (Fig. 4).

4 Discussion

Dietary GML supplementation significantly affected the metabolic status of laying hens. As indicated by serum biochemical parameters, GML treatment can effectively improve lipid metabolism in laying hens. Supplementation with 0.30 or 0.45 g/kg GML significantly decreased the serum TC level. It has been reported that both MCFAs and MCTs are associated with a greater transport of excess cholesterol, resulting in lower serum TC levels (Zhang et al., 2016; Zhou et al., 2017). MCFAs (C8–C10) applied in the feed of broiler chickens resulted in decreased LDLC concentrations and increased HDLC concentrations compared with the control treatment (Shokrollahi et al., 2014). These effects were also found in our GML-treated groups. Our findings differed from those reported by Geng et al. (2016) and Sakanaka et al. (2000) who suggested that dietary MCFAs and MCTs did not influence serum TG concentration. In our study, both 0.30 and 0.45 g/kg GML significantly decreased serum TG levels. The significant improvement of the abdominal fat pad relative weight and adipocyte size also supported the modulation of lipid metabolism by GML. Geng et al. (2016) reported that MCTs significantly prevented abdominal obesity in mice fed a high-fat diet. A meta-analysis of randomized controlled trials showed that MCTs decreased abdominal fat accumulation (Mumme and Stonehouse, 2015). Suppression of lipid absorption, reduction of fatty acid biosynthesis, and enhancement of fatty acid oxidation have been reported to be possible mechanisms for the reduction of body fat accumulation. Therefore, GML may play important roles in the digestion, absorption, and metabolism of lipids, which warrants further study.

Fig. 3 Intestinal morphology of laying hens fed with different levels of GML supplementation

Intestinal morphology of control (a) and hens with 0.15 g/kg GML (b), 0.30 g/kg GML (c), and 0.45 g/kg GML (d) supplementation. Jejunum villus length, crypt depth (e), and VCR of laying hens (f) fed with different levels of GML supplementation. Data are represented by mean±SD ($n=9$). Different letters indicate a significant difference ($P<0.05$). GML: glycerol monolaurate; VCR: villus height to crypt depth ratio

Increases in serum ALT and AST, which are hepatic-specific enzymes, reflect damage to the structural integrity of liver (Coballase-Urrutia et al., 2011). Treatment with 0.30 or 0.45 g/kg GML led to reduced activity of serum ALT and AST in laying hens, indicating a significant improvement in liver function. Such

effects might be related to the amelioration of antioxidant capacity by GML, based on the research of Mirbod et al. (2017) and Famurewa et al. (2017). In our study, feeding 0.30 or 0.45 g/kg GML to laying hens significantly enhanced the activity of antioxidant enzymes (SOD and GSH-Px) and decreased the

Fig. 4 Effects of dietary GML supplementation on abdominal adipose tissue in laying hens

(a) Proportional abdominal fat pad weight is expressed as a percentage of body weight. (b) Statistical analysis of the average size of abdominal adipocytes of laying hens fed with different levels of GML supplementation. Data are represented by mean $\pm$ SD ($n=15$). Different letters indicate significant differences by analysis of variance ($P<0.05$). Representative histological images of abdominal adipose tissues that were hematoxylin and eosin (H&E) stained of control (c), 0.15 (d), 0.30 (e), and 0.45 g/kg (f) glycerol monolaurate (GML) supplementation

content of serum MDA. This reflected an improvement in lipid oxidative stress and may be important in the prevention of chronic diseases associated with lipid peroxidation.

In our study, dietary GML supplementation led to an increase in egg production with elevated egg weight and decreased FCR, which was consistent

with previous reports of improvements in productive performance by MCFA supplementation (Wang and Kim, 2011; Schlievert and Peterson, 2012; Wang et al., 2015). Egg quality also showed significant improvement after GML treatment. Albumen height, Haugh units, and yolk color were significantly increased by 0.30 g/kg GML supplementation. Albumen height

and Haugh units are important for characterizing albumen quality and egg freshness. The improvement of yolk proportion and color might be related to the effects of GML on the absorption of feed lipids and lipid-soluble carotenoid pigments, such as zeaxanthin in corn. Wieland et al. (2015) reported that feed components and conversion affected yolk color, and that the carotenoid content of the egg could affect its yolk color. GML supplementation had no effect on eggshell thickness or strength. This might be related to the mineral metabolism in laying hens and is supported by our finding of no changes in the concentrations of serum Ca or P. Notably, the beneficial effects of GML supplementation on the productivity properties of laying hens were dose-dependent; 0.30 g/kg was the most effective concentration. In the poultry industry, MCFAs and MCTs are usually added to feed as a substitute for soybean oil (Shokrollahi et al., 2014; Zeitz et al., 2015). However, in this study, the purpose of GML supplementation was not to replace macronutrients. The dosage of GML was much lower than the levels of MCFA and MCT additives.

Laying performance and egg quality are profoundly affected by the metabolic status of laying hens. It has been proven that physiological stress, including metabolic rate, energy consumption, and catabolism, directly affects egg quality (Kim and Choi, 2014; Akter et al., 2018). In this study, increases in serum LH and FSH levels were associated with an improvement in the productive performance of laying hens. Zhang et al. (2012) found that dietary γ -aminobutyric acid significantly increased laying performance, with an increasing contents of serum FSH, LH, and other growth hormones. Improvements in intestinal structure also have beneficial effects on production performance. Our morphological analysis found that dietary GML supplementation significantly increased villi height and VCR, which may lead to a better utilization of nutrients with decreased FCR. Moreover, *in vivo* antibacterial effects of GML have also been reported to protect animal health by inhibiting bacterial toxin production, thereby contributing to a healthier and more stable gut environment with better digestive and absorptive capacities (Thormar et al., 1987; Schlievert et al., 1992; Isaacs et al., 1995; Batovska et al., 2009). Lee et al. (2015) reported that dietary MCFA supplementation had positive effects on fecal microbiota content. It has

been proven that the effects of MCFAs are based on their concentrations. MCFAs showed mainly antibacterial effects at high concentrations, while they had more selective effects in the upper digestive tract and changed the composition of gut microbiota (Dierick et al., 2003; Zentek et al., 2012). Thus, the effects of GML on gut microbiota might play a key role in the modulation of the metabolic status of laying hens. This deserves further analysis.

The effect of GML on the lipid metabolism of laying hens was not only shown by the changes in the serum lipid profile, but also related to lipid deposition in the yolk. Eggs with a high ratio of PUFA to SFA are recommended for a healthy diet, with low atherogenic, thrombogenic, and hypercholesterolemic potential, and a low risk of promoting coronary heart disease (Sakanaka et al., 2000). The content of total PUFA and the ratio of total PUFA to total SFA were significantly increased in the egg yolks from GML-treated groups. The amount of C18:1, the major MUFA in chicken eggs, was also significantly increased after GML treatment. It has been reported that higher contents of MUFA and PUFA, as well as a lower content of SFA, as found in the egg yolks from GML-treated groups, could contribute to a decrease in the negative influences of high cholesterol levels in eggs (Milinsk et al., 2003). Fatty acids, which are deposited in yolks, are synthesized from excess carbohydrates and amino acids in hens' livers (Akter et al., 2018). The enrichment of fatty acids in the yolk by GML treatment might be attributed to the reduction of FCR, which could increase the absorption of carbohydrates and protein from feed in laying hens (Jin et al., 2013). Egg proteins are highly digestible and contain all the essential amino acids needed for the human body, and eggs are suitable for people of all ages (Zhang et al., 2012). The total protein content of egg albumen was significantly increased in the 0.30 g/kg GML-treated group. The improvements in the Glu and His contents of albumen in our study are desirable for humans. Glu is the most abundant amino acid and one of the active taste components in egg albumen, and His is essential for optimal growth in infants (Ishibashi and Yonemochi, 2003). The above effects of GML on egg albumen composition might be due to the influence of GML on amino acid metabolism in laying hens induced by decreasing FCR. Consequently, the improvements in fatty acid

composition in egg yolks and protein composition in egg albumen resulting from GML supplementation could result in important nutritional benefits.

5 Conclusions

An improvement in the productive performance of laying hens treated with GML was observed in our study, based on a reduction of FCR and increases in serum sex hormone levels. This result might be attributed to the emulsibility and antibacterial activity of GML, as well as to its modulation function in intestinal morphology, and could be related to the revealed beneficial changes in egg quality and the composition of yolk and album. Interestingly, we also observed effects on the regulation of lipid metabolism by GML supplementation in laying hens, suggesting a role in the improvement of the metabolic status and yolk lipid composition of laying hens. Furthermore, a dose-response relationship between dietary GML supplementation and its beneficial effects on laying hens was found, with 0.30 g/kg being the most effective concentration. In conclusion, our results indicated that dietary GML has positive effects on the productivity properties and metabolic status of laying hens, suggesting that GML has potential to be a promising functional feed additive.

Contributors

Min-jie ZHAO, Hai-ying CAI, and Feng-qin FENG conceived the project. Min-jie ZHAO and Meng-yun LIU performed the experiments, interpreted the results, generated the figures, and wrote the manuscript. Ling-li DENG and Yang LI performed the experiments and interpreted the results. Hui ZHANG and Feng-qin FENG supervised the experiments, interpreted the results, and revised the manuscript. All authors approved the final manuscript and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Min-jie ZHAO, Hai-ying CAI, Meng-yun LIU, Ling-li DENG, Yang LI, Hui ZHANG, and Feng-qin FENG declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed and approved by the Institutional Animal Care and Use Committee of Zhejiang University (Protocol Number ZJU-BEFS-2016004), Hangzhou, China.

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中文概要

题目: 饲料中添加月桂酸单甘油酯对蛋鸡产蛋性能、蛋品质、血清生化指标以及小肠形态的影响

目的: 基于传统食品添加剂月桂酸单甘油酯 (GML) 的抗菌、抗病毒和免疫调节特性, 研究其对蛋鸡产蛋性能、蛋品质和生理健康的影响。

创新点: 将传统食品添加剂 GML 应用于蛋鸡养殖行业, 探究其作为饲料添加剂改善动物营养健康状况的潜能。

方法: 将 360 只海兰褐蛋鸡随机分为 4 组, 每组 6 个重复, 每个重复 15 只蛋鸡, 分别饲喂以玉米-豆粕为主的日粮, 并在日粮中分别添加 0、0.15、0.30 和 0.45 g/kg GML。饲喂 8 周后, 测定蛋鸡的产蛋性能、蛋品质、蛋清以及蛋黄相关营养成分组成、血清生理生化以及激素相关指标, 并对空肠和附睾脂肪切片进行形态学观察, 系统研究 GML 对蛋鸡的影响。

结论: 0.15、0.30 和 0.45 g/kg GML 处理能显著降低饲料转化率, 并显著提高产蛋率和平均蛋重。在鸡蛋品质方面, GML 显著提高了蛋白的高度和哈夫单位, 并增强了蛋黄的颜色。此外, GML 还增加了蛋黄中多不饱和脂肪酸和单不饱和脂肪酸的浓度, 降低了蛋黄中总饱和脂肪酸的浓度。蛋白组成在 GML 作用下也有显著改变, 其中 0.30 g/kg GML 显著增加了总蛋白含量以及组氨酸和谷氨酸的含量。GML 对蛋鸡脂代谢也表现除了积极作用, 能显著降低血清甘油三酯和总胆固醇水平, 减少腹部脂肪组织的沉积。在改善肠道形态方面, GML 增加了绒毛长度以及绒毛高度与隐窝深度的比值。总体来说, GML 对蛋鸡的生产性能和生理特性均有显著的改善作用, 表明 GML 有作为功能性饲料添加剂的潜能, 拓宽了传统食品添加剂 GML 的应用领域。

关键词: 月桂酸单甘油酯 (GML); 蛋鸡; 产蛋性能; 蛋品质; 脂代谢