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Original Research Article

Evaluation of dynamic effects of dietary medium-chain monoglycerides on performance, intestinal development and gut microbiota of broilers in large-scale production

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ABSTRACT

Medium-chain monoglycerides (MG) have been reported to affect the productive performance, gut microbiota and health of broiler chickens reared in ideal experimental conditions at home and abroad. However, the effects of MG on performance, intestinal development and gut microbiota of chickens in large-scale farms during different feed stages remain unknown. The present study was conducted on a modern farm with a total of 12,000 yellow feathered broiler chicks that were randomly allotted to 2 groups (1000 chicks/replicate, 6 replicates/group) for a 70-day trial. The control group (CON group) received a basal diet, and the treated group (MG group) was fed a basal diet containing 300 mg/kg mixed MG. The results revealed that dietary MG significantly ($P < 0.05$) increased the body weight and average feed intake, but notably reduced the feed conversion and mortality of chickens in large-scale production during the starter phase. The villus height of the duodenum in the MG group at 1, 2 and 7 wk of age increased notably, and the villus height to crypt depth ratio at 1, 2, 5 and 10 wk of age was improved. Dietary MG decreased the serum insulin content of chickens at 5, 7 and 10 wk of age, and decreased the serum lipopolysaccharide at 3 and 7 wk of age. The triglyceride level of chickens at 3, 5 and 10 wk of age and the low-density lipoprotein cholesterol level of chickens at 7 and 10 wk of age in the MG group decreased notably, while the high-density lipoprotein cholesterol increased significantly. Moreover, MG supplementation selectively increased the relative abundance of genus *Bacteroides* (family Bacteroidaceae) and *Lachnospiraceae_NK4A136_group*, but decreased the content of genus *Rikenellaceae_RC9_gut_group*, *Collinsella* and family Barnesiellaceae in the cecum of chickens at 3, 7 and 10 wk of age. Conclusively, these findings showed that dietary MG notably enhanced chicken performance, health and feed nutrient utilization at early ages by regulating gut microbiota, intestinal development and serum biochemical indices.
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1. Introduction

Medium-chain fatty acids (MCFA) are a group of saturated fatty acids with 8 to 12 carbons, which naturally occur in coconut oil, palmetto oil, camphor seed oil and breast milk in the form of triglycerides (Dayrit, 2015; Batovska et al., 2009; Zhao et al., 2020; Liu et al., 2020d). Medium-chain monoglycerides (MG) are the 1-monoglycerides of MCFA, including glycerol monocaprylin (GMC, C8:0), glycerol monodecanoate (GMD, C10:0) and glycerol

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monolaurate (GML, C12:0) (Liu et al., 2021b; Jackman et al., 2020). It has been reported that most of the GMC and GMD are rapidly absorbed in the digestive tract and transported to the liver via the portal vein and further produce ketone bodies. GML can not only be directly transported to the liver via the portal vein, but also be absorbed by the lymphatic system in lauric acid (Aw and Grigor, 1980; Ramírez et al., 2001; Guillot et al., 1994). There is growing evidence that demonstrates dietary supplementation of MG improves the intestinal barrier function and blood lipid balance, and will not cause excessive fat accumulation in mice (Zhao et al., 2019, 2020; Mo et al., 2019; Zhang et al., 2021). In poultry production, MG can be used as promising feed additives to improve the growth performance, egg and meat quality, as well as gut health of chickens. Broiler chickens fed diets with GML showed significant increases in body weight (Fortuoso et al., 2019), feed intake (Liu et al., 2020c), muscle monounsaturated fatty acid and total antioxidant capacity (Mustafa, 2018; Valentini et al., 2020), and notable decreases in feed conversion (Fortuoso et al., 2020; Saleh et al., 2021). Laying hens receiving diets with MG exhibited significantly higher laying rate, eggshell strength, eggshell thickness, egg total amino acids and flavor (Liu et al., 2020a, 2020d).

The chicken digestive tract harbors a large number of microorganisms that closely and intensively interact with both the host and ingested feed, playing primary roles in productive performance, nutrient utilization and prevention of pathogen invasion in chickens (Liu et al., 2021a). MG pass through the gastrointestinal tract with relative stability and long residence time (Dierick et al., 2003), being able to modulate the composition and function of gut microbiota due to their wide spectrum of antibacterial effects, suppressing the growth of gram-positive bacteria, gram-negative bacteria, yeast and mold (Wang et al., 2020; Bunkova et al., 2011). Gut microbiota manipulation has been proven the main pathway that dietary MG exerts beneficial effects on mice (Zhao et al., 2019, 2020; Zhang et al., 2021; Mo et al., 2021). Inclusion of MG improves the productive performance and intestinal development of broiler chickens by selectively increasing the relative abundance of beneficial intestinal bacteria (*Bifidobacteriaceae*, *Bacteroides* and an unclassified genus of Lachnospiraceae family), but decreased the proportion of harmful bacteria (Liu et al., 2020c, 2021a; Kong et al., 2021). Adding GML to the diets of broilers significantly enhances chicken gut health by suppressing the growth of pathogenic bacteria (*Eimeria* spp. oocysts and *Escherichia coli*) (Fortuoso et al., 2019), or by increasing beneficial bacteria (Lan et al., 2021). Improvements in reproductive performance, egg quality and albumen amino acid composition in laying hens showed a close positive relationship with gut microbiota modulation by MG supplementation (Liu et al., 2020a, 2020d).

Gut microbiota including *Lactobacillus* spp., *Ruminococcaceae*, *Bacteroidales* and *Lachnospiraceae* was positively associated with production performance and feed conversion ratio in broilers (Torok et al., 2011a, 2011b). Stanley found that 24 unclassified gut bacterial species could potentially be considered target populations for improving animal growth performance (Stanley et al., 2012). An unknown class of Firmicutes was negatively correlated with performance (Stanley et al., 2013), while *E. coli* and *Clostridium perfringens* were closely related to chicken health, productivity and disease (Stanley et al., 2014). The improvements in weight gain, feed conversion efficiency, the efficiency of energy extraction, health, productivity and disease in broilers could be achieved by gut microbiota manipulation (Angelakis, 2016). Although modulation of gut microbiota by MG supplementation has been investigated before, the dynamic effects of dietary MG on the gut microbiota of broilers in large-scale production during the entire rearing phase are unknown. Moreover, the dynamic effects of dietary MG on growth performance, intestinal development and nutrient utilization of broilers in large-scale poultry farming have not been reported. In our previous study, dietary MG at 300 mg/kg was revealed to be the most effective dose in increasing the body weight, feed intake, feed efficiency and intestinal beneficial bacteria in broilers reared in ideal experimental conditions (Liu et al., 2020b, 2021a). Therefore, the present study was conducted to determine the dynamic effects of dietary MG supplementation on growth performance, intestinal development, gut microbiota as well as serum parameters in broilers on large-scale farms at both starter and finisher stages.

2. Materials and methods

2.1. Animal ethics statement

The use of all the broilers and experimental protocols in this study were approved by the Animal Care and Use Committee of Zhejiang University (Protocol Number ZJU-BEFS-2016004).

2.2. Experimental design, animals and management

Mixed monoglycerides (MG) with 95% purity containing GML (CAS No. 142-18-7) and GMD (CAS No. 26402-22-2) were provided by Hangzhou Longyu Biotechnology Co., Ltd (Hangzhou, Zhejiang, China), and supplemented in the basal diet by replacing the same amount of energy in oil. The experimental design, animals and management are clearly described in our previous study (Liu et al., 2021b, 2023). A total of 12,000 one-day-old male yellow feathered broiler chicks (provided by a local hatchery) were weighed and randomly allotted to 2 groups (1000 chicks/replicate, 6 replicates/group) on a modern farm for a 70-day experiment. The chickens in the control group (the CON group) received a basal diet (Table S1), and the treated group (the MG group) were fed a basal diet containing 300 mg/kg MG. The chickens were raised on the ground, fed ad libitum and given free access to water during the whole experiment. Replicates were randomly distributed in the farmhouse with constant lighting for 24 h. The room temperature was controlled using heaters and gradually decreased from 36 to 25 °C and then kept roughly constant.

2.3. Growth performance measurement and sample collection

All the chickens were weighed by replicates at 0 and 10 wk of age, and some of the chickens were weighed at 1, 2, 3, 5 and 7 wk of age (Fig. 1). Feed consumption for each replicate was recorded weekly throughout the entire experimental period, and the mortality was recorded to adjust the feed conversion rate (FCR). The average daily feed intake of each replicate was calculated subsequently.

Two randomly selected chickens from each replicate (2 chickens/replicate, 6 replicates/group) were slaughtered and sampled at 1, 2, 3, 5 and 7 wk of age after fasting for 12 h. Blood samples were drained from the wing vein by 5-mL vacuum tubes containing coagulant gel, and the serum was obtained by centrifugation (2000 × *g*, 15 min, 4 °C) and stored at −80 °C. Part of the liver, abdominal fat, an approximately 2 cm length of the duodenum (at the middle of the duodenal loop) and jejunum (at the midway between the point of entry of the bile duct and Meckel's diverticulum) of each chicken was collected and fixed in 4% paraformaldehyde for 24 h at 25 °C. Cecal digesta was promptly isolated and rapidly frozen in liquid nitrogen, then transported to the laboratory in a dry-ice pack and stored at −80 °C.

2.4. Serum biochemical analysis

Serum leptin, insulin, peptide YY, free fatty acids, total amino acids, lipopolysaccharides and adiponectin were measured using

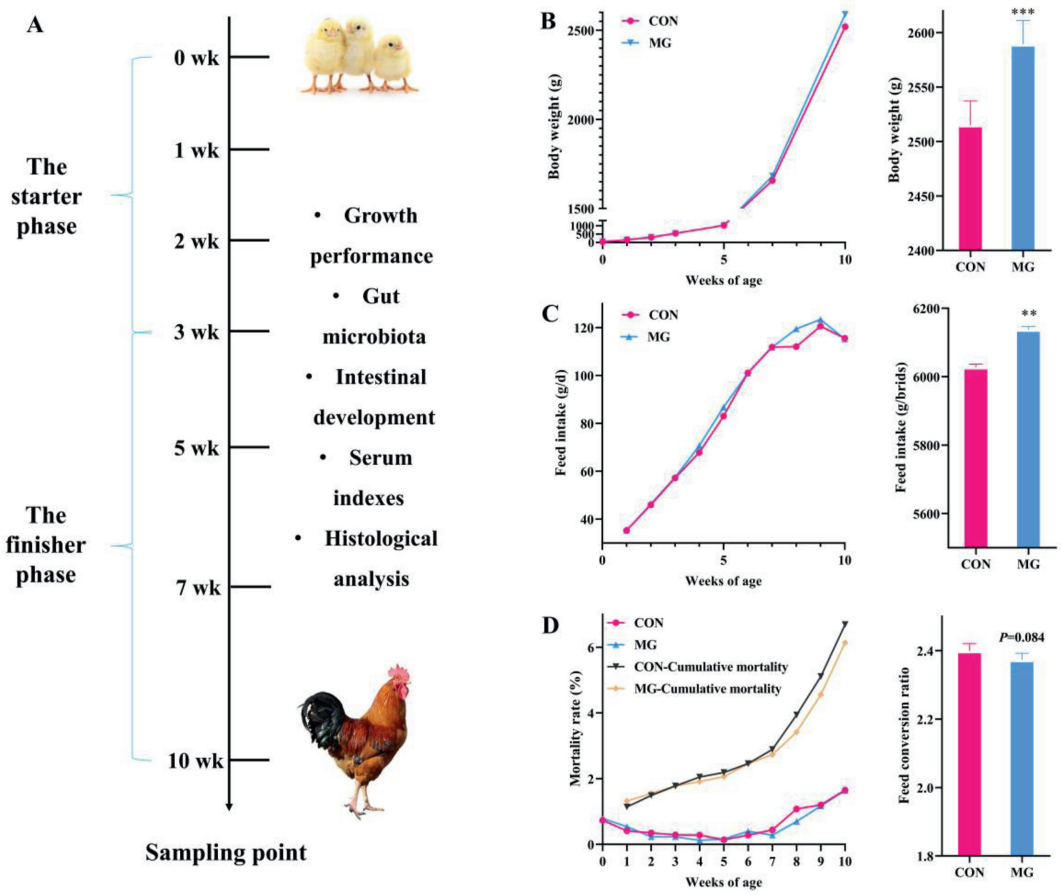

Fig. 1. The experimental design and effect of monoglycerides (MG) on productive performance of broilers in large-scale production. The control group (CON group) received a basal diet, and the treated group (MG group) were fed a basal diet containing 300 mg/kg mixed MG. (A) Experimental design. (B) Body weight. (C) Feed intake. (D) Mortality. Part of the growth performance was also described in our previous study (Liu et al., 2023). Asterisks indicate significant differences according to Student's *t*-test (**$P < 0.01$, ***$P < 0.001$).

commercial ELISA kits following the manufacturer's instructions. Serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glutamic-pyruvic transaminase (GPT), glutamic oxalacetic transaminase (GOT), uric acid, and glucose were measured using kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) by following the manufacturer's instructions.

2.5. Cecal short-chain fatty acids measurement

Cecal digesta was weighed (0.1 g) and mixed with 0.5 mL 2-ethylbutyric acid (1 mmol/L), then the pH was adjusted to 2.5. The supernatant was obtained after centrifugation (10,000 × *g*, 20 min), and analyzed by gas chromatography (GC-2014Shimadzu Corporation, Japan) with a DB-FFAP column. The gas chromatography settings, oven temperature program, identification and quantification of short chain fatty acid (SCFA) was the same as our previous study (Liu et al., 2020c).

2.6. Histomorphology analysis

The paraformaldehyde-fixed tissues were embedded in paraffin and sliced at 5 μm, then stained with hematoxylin and eosin (H&E) following the standard method. The frozen sections of embedded liver tissues were sectioned at 6 μm for oil red O staining (Gao et al., 2021). The hepatic lipid accumulation, intestinal villus length and crypt depth were quantified by Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD).

2.7. 16S rRNA sequencing and analysis

2.7.1. DNA extraction, PCR amplification and 16S rRNA gene sequencing

Bacterial genomic DNA was extracted from chicken cecal digesta collected at 3, 7 and 10 wk of age by QIAamp DNA Stool Mini Kits (QIAGEN, Venlo, The Netherlands) according to the manufacturer's instructions. The DNA concentration of each sample was diluted to 1 ng/μL with double distilled water with the help of concentration determination (Thermo Nano-Drop 2000 spectrophotometer, Wilmington, DE, USA). Then, the bacterial 16S rRNA gene in the V3–V4 region was amplified using the universal primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). The amplicons were purified and quantified, then they were sequenced on an Illumina HiSeq2500 platform (Illumina, San Diego, CA, USA), and the raw paired-end reads were generated (Liu et al., 2022).

2.7.2. Processing of sequencing data

After the assembly (FLASH software, version 1.2.11, https://ccb.jhu.edu/software/FLASH/index.shtml) and quality control (QIIME, version 1.9.1, http://qiime.org/install/index.html) of the raw reads, the clean reads were obtained. The operational taxonomic units (OTU) with ≥97% similarity were picked using the UPARSE software

package (version 7.0.1009, http://www.drive5.com/uparse/) and their taxonomic information was annotated referring to the Silva128/16S_bacteria database (http://www.arb-silva.de/) (Yu et al., 2022). Then the microbial structure and β-diversity (unweighted UniFrac distance-based principal coordinate analysis [PCoA]) were analyzed. The characteristic bacteria between groups were picked by linear discriminant analysis effect size algorithm (LEfSe, http://huttenhower.sph.harvard.edu/galaxy/). The raw sequence data was uploaded to NCBI sequence read archive (PRJNA638502).

2.8. Statistical analysis

Significant differences were detected using Student's *t*-test or Wilcoxon rank-sum test. A *P*-value of < 0.05 was recognized as significant ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) and $0.05 < P < 0.1$ were discussed as tendencies.

3. Results

3.1. Effect of dietary MG on productive performance of broilers

As shown in Fig. 1B, C and D, the average body weight and feed intake were significantly higher in the MG group than the CON group at the end of the experiment, but the feed conversion ratio was not significantly lower in the MG treated group. Specifically, the body weight increased notably at 5 wk of age in the broiler chickens fed with MG, and the feed intake increased obviously at the end of the starter phase (3 wk of age). The mortality of the chickens in the CON group at 2, 3, 4, 7, 8, 9 and 10 wk of age was higher relative to that of the MG group, showing a notably increased cumulative mortality from 2 to 10 wk of age.

3.2. Effect of dietary MG on biochemical parameters of broilers

Compared with the CON group, dietary MG decreased the serum insulin content of chickens at 5 ($P = 0.06$), 7 ($P = 0.085$) and 10 ($P < 0.001$) wk of age (Fig. 2B). MG supplementation showed a trend toward decreased serum free fatty acids (Fig. 2E, $P = 0.102$), but increased the total amino acid level (Fig. 2F, $P = 0.100$). The serum uric acid level at 10 wk of age in the MG group decreased significantly relative to that of the CON group (Fig. 2G), and the serum lipopolysaccharide at 3 and 7 wk of age decreased significantly (Fig. 2H, $P < 0.05$). No significant changes were observed in the serum content of leptin, peptide YY and glucose between the 2 treatments (Fig. 2A, C and D).

3.3. Effect of dietary MG on lipid metabolism and fat deposition of broilers

As shown in Fig. 3, the TG level of chickens at 5 and 10 wk of age and the LDL-C level of chickens at 7 and 10 wk of age in the MG group decreased notably (Fig. 3A and D), while the HDL-C increased significantly (Fig. 3E, $P < 0.01$). Dietary MG decreased the serum GOT activity of chickens at 10 wk of age (Fig. 3J, $P < 0.05$), but exerted no significant effects on TC (Fig. 3B), adiponectin and GPT (Fig. 3F and I). No significant changes were observed in the morphology of abdominal fat and liver between the 2 groups (Fig. 3C, G and H).

3.4. Effect of dietary MG on intestinal morphology of broilers

Compared with the CON group, the villus height of the duodenum in the MG group at 1 ($P < 0.05$), 2 ($P = 0.095$) and 7 ($P < 0.05$) wk of age increased notably, and the villus height to crypt depth ratio (V/C ratio) of the MG group at 1 ($P = 0.093$), 2 ($P = 0.086$), 5 ($P = 0.103$) and 10 ($P < 0.05$) wk of age improved (Fig. 4A–C). In the jejunum, the crypt depth of the MG group at 10 wk of age decreased significantly ($P < 0.05$), while the V/C ratio increased notably (Fig. 4E and F).

3.5. Effect of dietary MG on cecal short-chain fatty acid content of broilers

As shown in Fig. 5, compared to the CON group, the cecal butyric acid and valeric acid content of the MG-fed chickens at 7 wk of age increased significantly (Fig. 5C and E), but the isobutyric acid at 3 wk of age was significantly reduced (Fig. 5D). No significant changes were observed in other cecal short-chain fatty acids.

3.6. Effect of dietary MG on diversity and composition of gut microbiota

The Venn diagram shows that a total of 819 OTU were detected in the broiler chickens at 3 wk of age (Fig. 6A), and 46 and 209 OTU were only found in the CON and MG groups, respectively. The PCoA plot shows that the data points of both the CON group and the MG group were intensively clustered and distinctly drifted away from the CON group without any overlaps. The distance calculated by unweighted UniFrac displayed notable differences between the CON group and the MG group in both the PC1 ($P < 0.001$) and PC2 ($P = 0.074$) coordinates with an explanation of 50.47% observed total variance (Fig. 6D), suggesting alterations of the gut microbiota community in the MG group. The gut microbiota of chicken cecum at 3 wk of age was dominated by Firmicutes and Bacteroidetes (Fig. 6E), including Ruminococcaceae, Rikenellaceae, Lachnospiraceae, Bacteroidaceae, Clostridiales_vadinBB60_group, Barnesiellaceae and Lactobacillaceae (Fig. 6F). The relative abundance of family Lachnospiraceae and Bacteroidaceae was notably increased in the MG group, but the content of family Barnesiellaceae and Lactobacillaceae was reduced.

A total of 1023 OTU were observed in the gut microbiota of whole samples at 7 wk of age. Among them, 111 and 127 OTU were unique to the CON group and the MG group, respectively, while 785 OTU commonly existed in the 2 groups (Fig. 6B). Clear separations between the sample points of the 2 groups are observed in the PCoA plot (Fig. 6G). The distances calculated by unweighted UniFrac showed significant differences in the PC1 coordinates ($P < 0.001$) with an explanation of 26.64% observed total variance (Fig. 6G), suggesting overall structural alterations of gut microbiota caused by MG supplementation. Taxonomic profiling showed that Bacteroidetes and Firmicutes were the 2 main phyla in the gut microbiota of broilers, mainly consisting of family Bacteroidaceae, Ruminococcaceae, Lachnospiraceae, Rikenellaceae, Tannerellaceae, Prevotellaceae, Muribaculaceae and Clostridiales_vadinBB60_group (Fig. 6H and I). Dietary supplementation of MG increased the abundance of Bacteroidaceae, Tannerellaceae and Muribaculaceae, while reducing the Lachnospiraceae and Rikenellaceae content (Fig. 6I).

The total OTU and unique OTU in the CON group and the MG group were 1006 and 1000, 119 and 113 (Fig. 6C), respectively. The sample points of the 2 groups clustered intensively according to the treatment in the PCoA plot, where their distance in the PC1 coordinates ($P < 0.001$) was significant with an explanation of 22.57% observed total variance (Fig. 6G), indicating distinct changes in the gut microbiota community of the MG group. The dominant phyla of the gut microbiota were Bacteroidetes and Firmicutes in broilers (Fig. 6K), and the dominant families were Bacteroidaceae, Ruminococcaceae, Rikenellaceae, Lachnospiraceae, Muribaculaceae, Tannerellaceae, Prevotellaceae, an unclassified family of

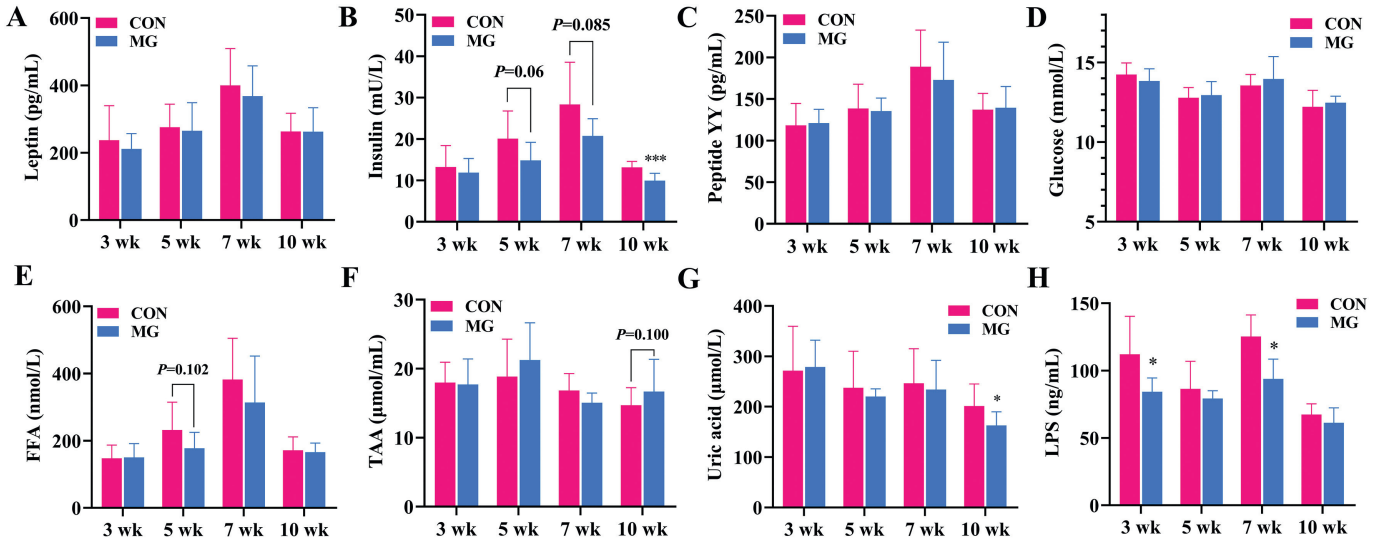

Fig. 2. Effect of monoglycerides (MG) on serum indices of broilers at different feeding ages. (A) Leptin. (B) Insulin. (C) Peptide YY. (D) Glucose. (E) Free fatty acid (FFA). (F) Total amino acid (TAA). (G) Uric acid. (H) Lipopolysaccharide (LPS). The control group (CON group) received a basal diet, and the treated group (MG group) were fed a basal diet containing 300 mg/kg mixed MG. Asterisks indicate significant differences according to Student's *t*-test (*$P < 0.05$).

Bacteroidales order and Clostridiales_vadinBB60_group (Fig. 6L). Compared to the CON group, the relative abundance of Bacteroidaceae was increased in the MG group, but the content of Rikenellaceae, Muribaculaceae and Prevotellaceae was decreased (Fig. 6L).

3.7. Differential chicken gut microbiota between the CON and MG groups

The taxonomic profiling showed that the relative abundance of family Bacteroidaceae ($P < 0.05$) and Lactobacillaceae at 3, 7 and 10 wk of age was notably increased in the MG group (Fig. 7A and B), while the content of Barnesiellaceae and Victivallaceae decreased obviously (Fig. 7C and D). At the genus level, dietary MG supplementation increased *Bacteroides* and *Lachnospiraceae_NK4A136_group* numbers in chicken cecum compared to that of the CON group at 3, 7 and 10 wk of age (Fig. 7E and F), but reduced the relative content of *Rikenellaceae_RC9_gut_group*, *Collinsella* and *CHKCI002* (Fig. 7G–I).

4. Discussion

Medium-chain monoglycerides are generally recognized as a promising growth promoter in the diets of broiler chickens according to recently published papers at home and abroad. The body weight of the MG group was observed to increase faster than that of the CON group from the early finisher phase of the broiler chickens in the present study. Similar to our previous study, dietary supplementation of 300 to 600 mg/kg MG notably increased the body weight from 5 wk of age in broiler chickens on small-scale farms (Liu et al., 2020b). In our other study, the body weight of broiler chickens showed no obvious differences at the starter phase, while the treated broiler chickens presented with significantly increased average body weight at the end of the experiment (Liu et al., 2020c). In white feathered broilers, Saleh reported chickens fed diets with 0 to 1000 mg/kg GML showed increased body weight at 28 to 33 d of age (Saleh et al., 2021), while dietary GML combined with oregano essential oil in 0 to 750 mg/kg only exerted beneficial effects on body weight in the grower and finisher phase (Amer et al., 2021). It has been reported that 300 to 1200 mg/kg GML in the diets of broilers didn't affect the body weight of Arbor Acres chicks at both 7 and 14 d of age (Kong et al., 2021). Apart from the results from Lan et al. (2021) where the body weight of broiler chickens was significantly higher at both the end of the starter and the finisher phase in the 500 and 1000 mg/kg GML supplementation groups, it seems that dietary supplementation of medium-chain monoglycerides only influenced the fattening broilers with significantly increased average body weight. We speculated that these outcomes mainly resulted from the fast growth rate, lower feed conversion and small body weight gain of brooding broiler chicks.

Average daily feed intake is the primary factor driving the growth rate of broiler chickens (Abdollahi et al., 2018), thus the alterations of feed intake by MG supplementation could act as a sensitive and instant signal in searching for the accurate action time of MG on improving chicken productive performance. In the current study, the notable increase in feed intake in MG-treated chicks was firstly observed at 3 wk of age, which was 2 wk earlier than that of the increased body weight. Similar to our previous study, the increase in feed intake was found at 29 to 35 d of age in chicks fed with MG, while a significantly improved body weight was obtained 2 wk later (Liu et al., 2020b). Kong stated that dietary supplementation of 600 to 1200 mg/kg GML significantly increased the feed intake of Arbor Acres chicks at 7 to 14 d of age (Kong et al., 2021). Wu also reported Ross 308 broiler chickens fed diets with 500 to 1000 mg/kg lauric acid exhibited increased feed intake from 1 to 21 d, as well as from 21 to 42 d compared to the CON group (Wu et al., 2021). The feed intake regulation caused by MG supplementation could also be supported by the notably decreased serum insulin at 3, 5, 7 and 10 wk of age in the current study. Similar to mammals, the long-term regulation of appetite and energy balance in poultry is controlled by peripheral tissue and central nervous system circuits with hormones, neuropeptides, receptors, enzymes, transcription factors, binding/transport protein, nutrient signals, etc (Richards, 2003; Richards and Proszkowiecweglarz, 2007). The circulating leptin and insulin present potential energy sensing signals to the hypothalamus that determine the appropriate level of activity in the anabolic and catabolic pathways. The plasma leptin and insulin levels rise with increases in energy state, causing decreased feed intake and increased energy expenditure allowing chickens to maintain body weight and achieve energy balance,

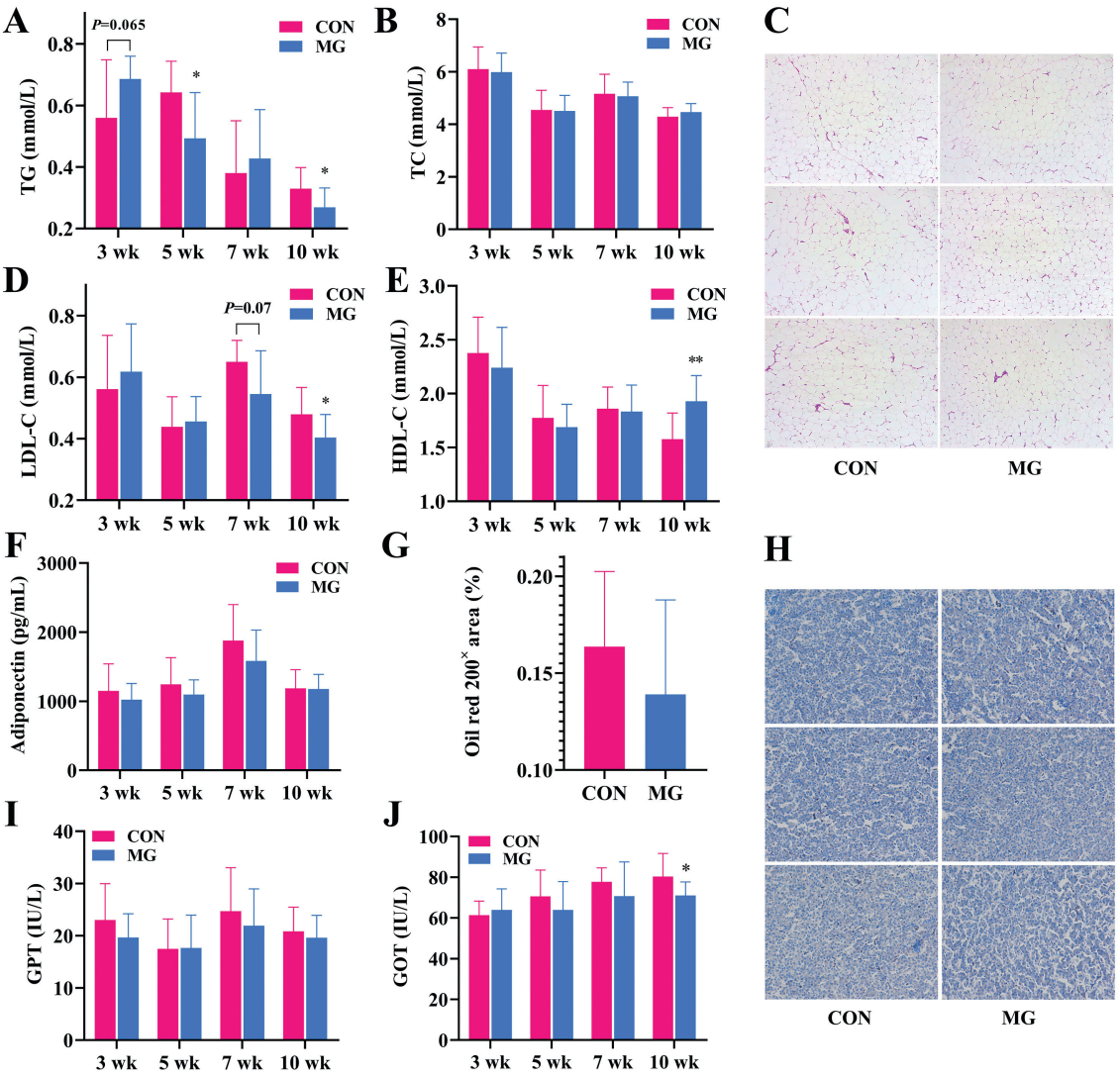

Fig. 3. Effect of monoglycerides (MG) on serum profile and morphology of broilers at different feeding ages. (A) Triglycerides (TG). (B) Total cholesterol (TC). (C) Morphology of abdominal fat (200× magnification). (D) Low-density lipoprotein cholesterol (LDL-C). (E) High-density lipoprotein cholesterol (HDL-C). (F) Adiponectin. (G) The liver lipid area in panel. (H) Oil red O staining of liver were estimated by the Image J software (200× magnification). (I) Glutamic-pyruvic transaminase (GPT). (J) Glutamic oxalacetic transaminase (GOT). The control group (CON group) received a basal diet, and the treated group (MG group) were fed a basal diet containing 300 mg/kg mixed MG. Asterisks indicate significant differences according to Student's *t*-test (*$P < 0.05$, **$P < 0.01$).

while the plasma leptin and insulin levels fall with decreases in energy state which result in increased feed intake and decreased energy expenditure. Therefore, the long-term relatively low plasma level of insulin in treated broiler chickens demonstrated that dietary MG supplementation increased the feed intake via hormone level.

In the present study, dietary supplementation of MG showed positive trends in reducing serum TG and LDL-C levels of broilers, but increased the HDL-C level. The H&E and oil red staining revealed that MG supplementation exerted no detectable effect on both hepatic and abdominal fat deposition in broilers. Serum leptin tended to decrease in the MG-treated chickens, while the serum adiponectin showed no changes compared to the CON group. These findings indicated that dietary MG promoted fat digestion and absorption, but exerted no adverse effects on fat deposition in broilers. The present results were in accordance with our previous study, where significantly decreased TG concentration and elevated HDL-C levels were observed in broiler chickens fed with 300 to 600 mg/kg MG (Liu et al., 2020b). Similarly, a commercial dietary medium-chain fatty acid (MCFA, C8–C10) mixture was reported to significantly reduce the serum TG and LDL-C content, while increasing the HDL-C level in both broiler chickens and Japanese quail (Saeidi et al., 2016; Shokrollahi et al., 2014). Similar results were obtained from adding GML to HFD-diet-fed mice in which the serum TG, TC and LDL-C content was significantly decreased, and the HDL-C level was increased in comparison with the CON group (Zhao et al., 2020).

Intestinal morphology indices including villus height, crypt depth and villus height/crypt (V/C) ratio are important indicators of health status, recovery and function of the intestine, playing important roles in nutrient digestion and absorption (Pham et al., 2020). Intestines with higher villus height and V/C ratio, as well as lower crypt depth have a greater mucosal surface area, which is positively related to the digestive capacity of chickens (Zeitz et al., 2015; Wang et al., 2017). In the current study, the H&E staining demonstrated that the inclusion of MG notably increased the

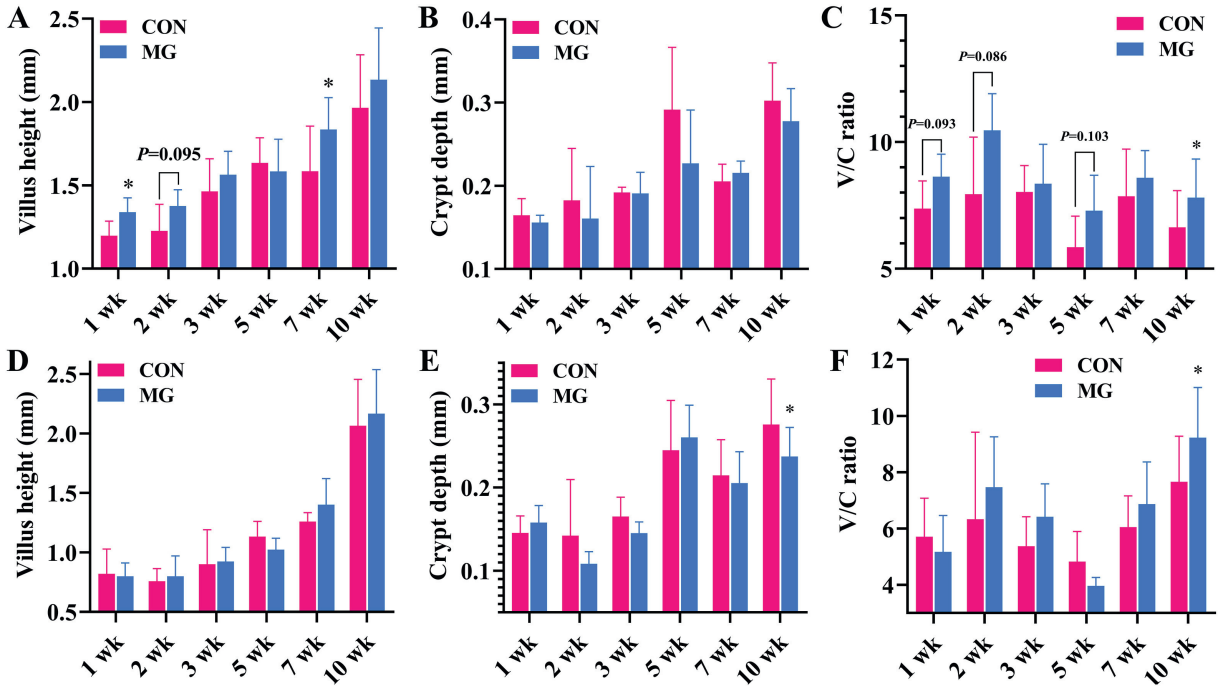

Fig. 4. Effect of monoglycerides (MG) on the intestinal morphology of broilers at different feeding ages. (A) Villus height of duodenum. (B) Crypt depth of duodenum. (C) Villus height/crypt depth (V/C) ratio of the duodenum. (D) Villus height of jejunum. (E) Crypt depth of jejunum. (F) Villus height/crypt depth ratio of jejunum. The control group (CON group) received a basal diet, and the treated group (MG group) were fed a basal diet containing 300 mg/kg mixed MG. Asterisks indicate significant differences according to Student's *t*-test (*$P < 0.05$).

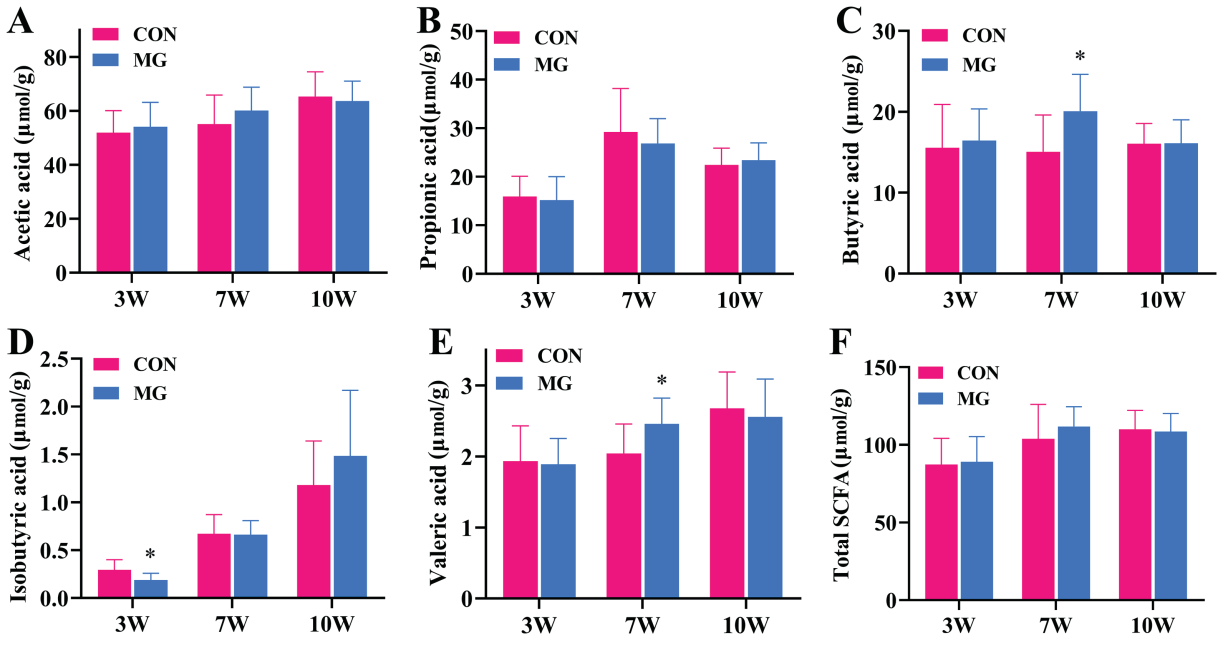

Fig. 5. Effect of monoglycerides (MG) on cecal short-chain fatty acid content in broilers at different feeding ages. (A) Acetic acid content. (B) Propionic acid content. (C) Butyric acid content. (D) Isobutyric acid content. (E) Valeric acid content. (F) Short chain fatty acids (SCFA). The control group (CON group) received a basal diet, and the treated group (MG group) were fed a basal diet containing 300 mg/kg mixed MG. Asterisks indicate significant differences according to Student's *t*-test (*$P < 0.05$).

duodenal villus height and V/C ratio of chickens at 1 week of age, suggesting that the MG supplementation rapidly exerted significant beneficial effects on intestinal development. In the jejunum, the significantly reduced crypt depth and enhanced V/C ratio were observed in the chickens at 10 wk of age, indicating that dietary MG improved jejunal development mainly in the finisher phase. Similar to our previous study, broilers fed diets with MG notably increased villus height and V/C ratio in both the duodenum and jejunum of broilers at the end of the experiment (Liu et al., 2020b). GML supplementation showed a tendency to increase the V/C ratio of the duodenum and jejunum in yellow-feathered broiler chickens at 8 wk of age (Liu et al., 2020c). Similarly, administration of graded

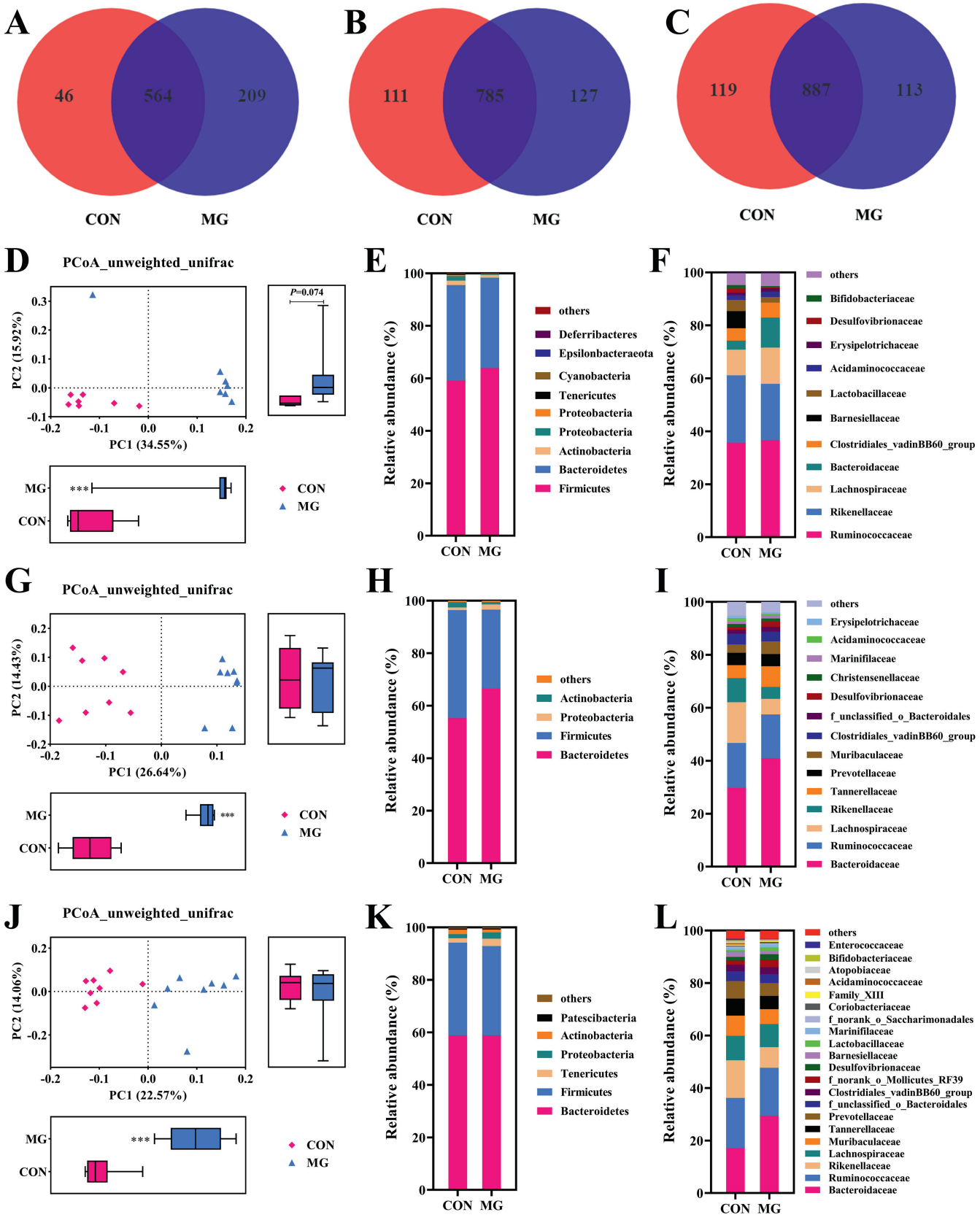

Fig. 6. Effect of monoglycerides (MG) on diversity and composition of gut microbiota in broilers at different feeding ages. (A), (B) and (C) Venn diagrams of gut microbiota at 3, 7 and 10 wk of age, respectively. (D) (G) and (J) Beta-diversity of gut microbiota at 3, 7 and 10 wk of age, respectively. (E), (H) and (K) Relative abundance of gut microbiota at the phylum level at 3, 7 and 10 wk of age, respectively. (F) (I) and (L) Relative abundance of gut microbiota at the family level at 3, 7 and 10 wk of age, respectively. Part of the data was also described in our previous study (Liu et al., 2023). The control group (CON group) received a basal diet, and the treated group (MG group) were fed a basal diet containing 300 mg/kg mixed MG. Asterisks indicate significant differences according to Wilcoxon rank-sum test (***$P < 0.001$).

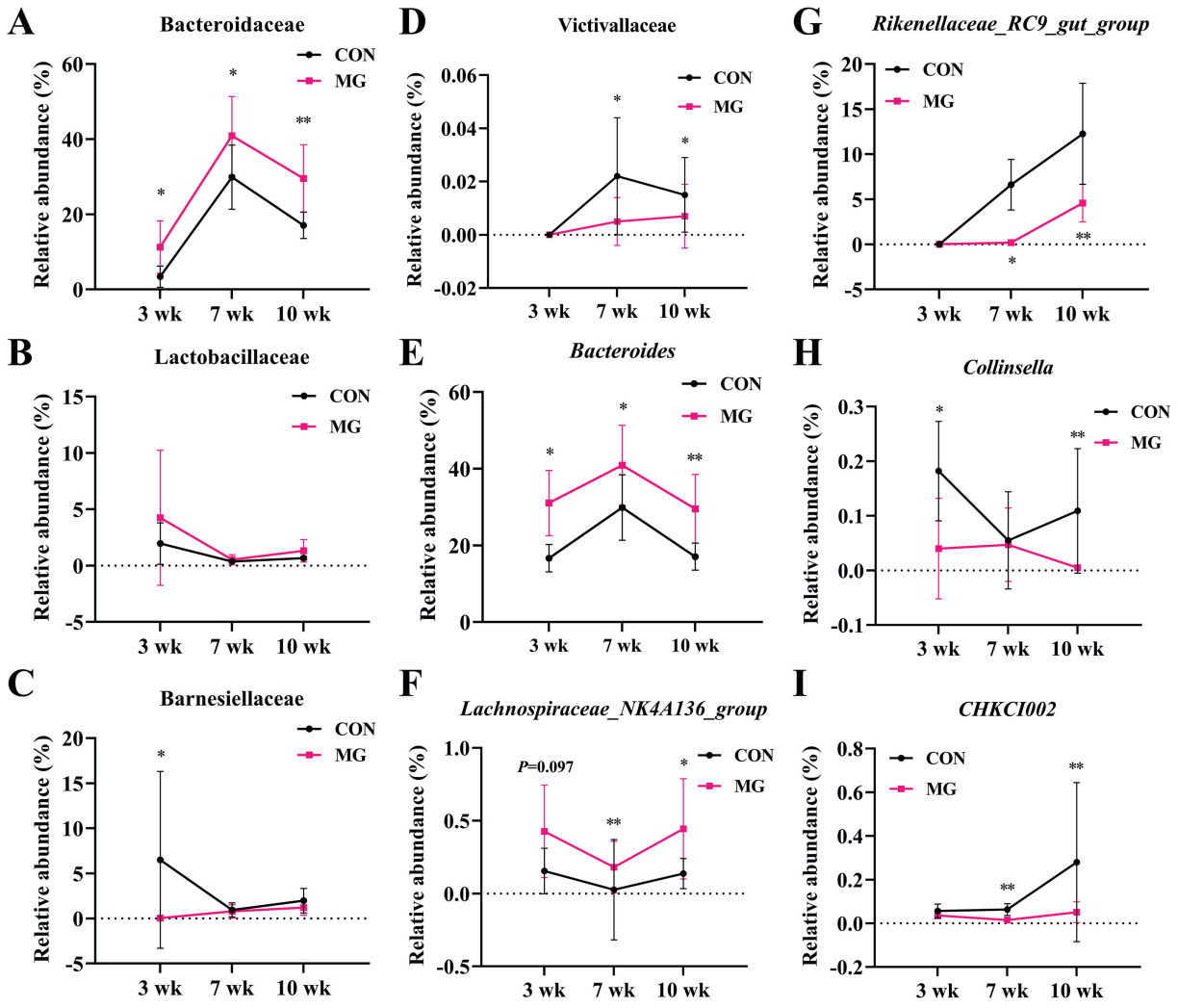

Fig. 7. Differential gut microbiota of broilers at different feeding age. (A), (B), (C) and (D) Differential gut microbiota at the family level. (E), (F), (G), (H) and (I) Differential gut microbiota at the genus level. The control group (CON group) received a basal diet, and the treated group (MG group) were fed a basal diet containing 300 mg/kg mixed MG. Asterisks indicate significant differences according to Wilcoxon rank-sum test (*$P < 0.05$, **$P < 0.01$).

levels of GML (300, 600, 900 and 1200 mg/kg) remarkably reduced the crypt depth and increased the V/C ratio of the duodenum and jejunum in Arbor Acres chicks at 1 and 2 wk of age (Kong et al., 2021). Wu observed significant improvements in the villus height, crypt depth and V/C ratio of the jejunum and cecum of Ross 308 broiler chickens fed diets with different concentrations of lauric acid (500 and 1000 mg/kg) from 0 to 6 wk of age (Wu et al., 2021). Conclusively, dietary supplementation of MG in the diet of broilers notably improved intestinal development, which was partially responsible for increased body weight, feed efficiency and protein digestibility (from 51.40% to 54.49%, data not shown) in the current study.

The relative abundance profiles of cecal microbiota at phylum and family level showed distinct features between the samples collected at 3 and 7 wk of age, as well as 3 and 10 wk of age. However, the samples collected at 7 and 10 wk of age represented high similarity in cecal microbiota community and composition. The present study demonstrated that the gut microbiota of chickens in the starter phase was highly variable and notably different from that of the finisher phase. Consistent with a previously published study (Huang et al., 2018), the gut microbiota experienced successional development as the age of chickens increased, and the structure and composition remained stable from 7 to 10 wk of age in this study. Firmicutes was the dominant phylum in the gut microbiota of chickens at the end of the starter phase, whereas their relative abundance decreased from 3 to 10 wk of age and the content of phylum Bacteroidetes was increased. These findings were inconsistent with our previous studies where Firmicutes was the main phylum of cecal microbiota in the MG and GML-fed chickens at the end of the finisher phase (Liu et al., 2020c, 2021a). The rearing conditions were the main difference, where the chickens in the current study were reared on the ground and the chickens in the previous articles were reared in cages, suggesting that the phylum Bacteroidetes was helpful for chickens to adapt to the more complex environment (Seidlerova et al., 2020). Interestingly, in our previous study, dietary MG at 300 to 600 mg/kg significantly changed the gut microbiota community, showing a tendency to decrease the phylum Firmicutes content and increase the relative abundance of phylum Bacteroidetes (Liu et al., 2021a). Linglian demonstrated that dietary GML in the diets of broilers at 600 mg/kg significantly increased the relative abundance of phylum Bacteroidetes in the cecal microbiota of chickens at both 7 and 14 d of age (Kong et al., 2021). The broiler chickens receiving diets containing lauric acid showed an increased abundance of

phylum Bacteroidetes but decreased phylum Firmicutes in cecal microbiota at 42 d of age (Wu et al., 2021). Similarly, the present study showed that dietary supplementation of 300 mg/kg MG tended to promote the colonization of *Bacteroidetes* during the finisher phase.

The relative abundance profiles of chicken cecal microbiota differed notably among different feeding stages, while the changes in the relative content of some gut microbiota such as Bacteroidaceae, *Bacteroides* and *Rikenellaceae_RC9_gut_group* in the MG-containing diet fed chickens showed similar trends. *Bacteroides* was the primary member of the family Bacteroidaceae and accounted for over 40% of the chicken cecal microbiota. *Bacteroides* have been reported to play a critical role in decomposing complex molecules into simpler compounds that are absorbed by both the host and the gut microbiota, particularly the utilization of nitrogenous substances. The increased *Bacteroides* in the MG group in the present study was probably responsible for the lowered FCR, which was supported by the significantly enhanced protein digestibility from 51.40% to 54.49% by MG supplementation (data not shown). Lachnospiraceae can utilize complex plant-derived carbohydrates, especially in the readily degraded less recalcitrant indigestible polysaccharides and starch, to release sugars for both gut microbiota and host (Meehan and Beiko, 2014). Apajalahti and Singh stated that a higher relative abundance of the Lachnospiraceae represented a close correlation with lower FCR and increased body weight of commercial broiler chickens (Singh et al., 2012; Apajalahti and Vienola, 2016). As a member of Lachnospiraceae, the increase of *Lachnospiraceae_NK4A136_group* may contribute to the reduction of FCR and increased body weight in the present study. Pitta et al. demonstrated that *Rikenellaceae_RC9_gut_group* was involved in structural carbohydrate degradation (Si et al., 2021). However, it also has been proven that an increase in the abundance of *Rikenellaceae_RC9_gut_group* was positively associated with intestinal permeability and oxidative stress, subsequently impairing the intestinal barrier function and stimulating gut inflammation (Shen et al., 2022). *Collinsella* is the gas-producing bacteria in the chicken gastrointestinal tract, which has been reported to be positively related to serum cholesterol (Wu et al., 2020), abnormal lipid metabolism and type 2 diabetes (Li et al., 2022). The increased relative abundance of family Barnesiellaceae in the cecal microbiota might be associated with tibial dyschondroplasia which has been recognized as one of the most severe nutritional and metabolic disorders in chickens (Tong et al., 2018). The reduction in the relative abundance of *Rikenellaceae_RC9_gut_group*, *Barnesiellaceae* and *Collinsella* in the cecal microbiota of the MG group suggested a relatively more host-friendly gastrointestinal tract environment.

Coincidentally, in our previous study, dietary MG supplementation selectively increased the relative abundance of Bacteroidaceae (*Bacteroides*) and Lachnospiraceae (an unclassified genus of the Lachnospiraceae family), but reduced the content of cecal Barnesiellaceae (an unclassified genus of Barnesiellaceae) in the chickens at the end of finisher phase (Liu et al., 2021a). Inclusion of MG in the diets of aged laying hens significantly increased the proportion of genera *Lachnospiraceae_NK4A136_group* in cecal microbiota (Liu et al., 2020d). Also, dietary GML was found to selectively increase the colonization of Lachnospiraceae (an unclassified genus of Lachnospiraceae family) and decreased the family Barnesiellaceae in another study (Liu et al., 2020c). Similarly, Kong demonstrated that dietary GML at 0 to 1200 mg/kg in the diets of broilers significantly increased the relative abundance of genus *Lachnospiraceae* and *Bacteroides* (Kong et al., 2021). Conclusively, the families of Bacteroidaceae (genera *Bacteroides)*, Lachnospiraceae (an unclassified genus of Lachnospiraceae family), and Barnesiellaceae (an unclassified genus of Barnesiellaceae) were the specific gut microbiota in response to MG supplementation throughout the entire rearing stage, acting as the functional bridge of the beneficial effects on chicken performance conferred by MG. These results were closely in line with Torok's (Torok et al., 2011a, 2011b), who stated that the proportion of *Lachnospiraceae*, *Bacteroidales*, *Lactobacillus*, etc, were closely associated with chicken productive performance and feed conversion ratio, and the composition of these gut microbiota can be considered as promising indicators for feed utilization efficiency evaluation and formula optimization (Torok et al., 2011a, 2011b). Moreover, according to the previous study (Torok et al., 2011b; Stanley et al., 2012; Angelakis, 2016), the present findings provide us with a promising and reliable approach to directly remodeling the chicken gut microbiome to improve the chicken's productive performance and gut health.

Lipopolysaccharides (LPS) are a kind of endotoxin derived from the cell wall of Gram-negative bacteria in the gastrointestinal tract, and an elevated circulating level of which is an important parameter for systemic low-grade inflammation characterization (Jiang et al., 2018; Zhao et al., 2020). The relatively lower serum LPS level induced by MG supplementation indicated a better health status of broilers than that of the CON group, which could partially explain the reduction of chicken mortality in the treated group. It has been reported that the inclusion of graded levels of GML (100, 200 and 300 mg/kg) reduced the *Eimeria* spp. oocysts count at 6 wk of age, and decreased the total bacterial count and *E. coli* count at both 3 and 6 wk of age, shaping a healthier gut in broilers (Fortuoso et al., 2019). Gabriela recently reported that dietary GML blended with curcuminoids and cinnamaldehyde significantly reduced the proportion of *Eimeria* spp. oocysts and total bacterial counts in oocyst-challenged broilers at 6 wk of age (Galli et al., 2021). Similarly, GML blended with cinnamaldehyde has remarkable inhibition against infectious bronchitis virus by inhibiting the multiplication of infectious bronchitis virus and promoting the immune function of broilers (Zhang et al., 2022). Additionally, though the SCFA-producing gut microbiota increased significantly, the SCFA content at 3, 7 and 10 wk of age did not show notable differences due to the breeding mode and complex environment. The increased cecal butyric acid content at 7 wk of age in the MG group still indicated a host-friendly gastrointestinal tract environment. These findings proved that dietary MG improved gut health and balance from a biochemical point of view.

5. Conclusions

In the present study, the body weight and average feed intake were significantly increased in the chickens fed with MG, while the feed conversion and mortality were notably reduced. The intestinal morphology was notably improved by MG supplementation with enhanced feed protein utilization ability. The unchanged hepatic and abdominal fat deposition, decreased TG level and LDL-C, as well as increased HDL-C in chickens suggested no adverse effects of MG supplementation on fat metabolism. Genus *Bacteroides*, *Lachnospiraceae_NK4A136_group*, *Rikenellaceae_RC9_gut_group*, *Collinsella* and family Barnesiellaceae were recognized as the specific chicken cecal bacteria in response to MG supplementation at different feeding stages. The present study revealed that dietary MG improved performance, health and feed efficiency through regulating gut microbiota, intestinal development and serum biochemical indices at an early stage. Moreover, the present findings suggested that productive performance improvement and gut health can be obtained by directly remodeling the chicken gut microbiome.

Author contributions

Tao Liu: Investigation, Data curation, Formal analysis, Writing - original draft preparation. **Shengyue Ruan:** Investigation, Data curation, Formal analysis. **Qiufen Mo:** Investigation, Data curation. **Minjie Zhao:** Investigation, Data curation. **Jing Wang:** Methodology, Formal analysis. **Zhangying Ye:** Methodology, Writing - review editing, Supervision. **Li Chen:** Conceptualization, Writing-Reviewing and Editing, Supervision. **Fengqin Feng:** Conceptualization, Formal analysis, Writing - original draft preparation, Writing - review editing, Supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2023.05.003.

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