Effects of a mixture of mono-glycerides of butyric-, capric-, and caprylic acid with chlortetracycline on the growth performance, intestine morphology, and cecal microflora of broiler birds

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ABSTRACT This study aimed to investigate the effects of a mixture of mono-glycerides of butyric-, capric-, and caprylic acid (**MMG**) on the growth performance, intestinal morphology, and cecal microflora of broilers. A total of 960 male Arbor Acre broilers were offered basal diets with or without Chlortetracycline additive (**CA**) at 500 g/t, and MMG at 3,000, 1,000, or 650 g/t, with 8 replicates of 20 birds per treatment. The results confirmed 500 g/t CA with/without 1,000 g/t MMG increased the average daily weight gain (**ADG**) of birds compared to the control group 1 during the 42-d experimental period ($P < 0.05$). Comparing to the control group 1, 500 g/t CA with either 650 g or 1,000 g/t MMG or 1,000 g MMG alone increased the ADG of birds during the late growth stage (22−42 d) ($P < 0.05$). On d 42, the serum triglyceride levels were higher ($P < 0.05$) in groups supplemented with CA and CA + 1,000 g/t MMG comparing to the control group; while urea nitrogen level was higher in the control group comparing to the rest of treatment groups Compared to the control group 1, 1,000 g/t MMG alone without CA decreased the abundance of *Faecalibacterium* and *Bacteroides* but increased the abundance of *Escherichia/Shiegella.* About 500 g/t CA alone treatment group had higher abundance of *Lactobacillus* comparing to the rest of groups. In conclusion, dietary supplement with MMG showed beneficial efficacy on the growth and intestinal function of broilers, demonstrating the potential value of MMG to poultry industry. In terms of dosage, the current trial shows that 3,000 g/t (1−21 d) and 1,000 g/t (22−42 d) MMG without CA was the appropriate dietary supplemented rate for broilers. And the mixed use of 500 g/t CA and 1,000 g/t MMG was benefit for broilers at 22 to 42 d.

Key words: mono-glyceride, broiler, growth performance, intestinal development, cecal microbiota

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INTRODUCTION

For the past 60 yr, the global meat production has increased dramatically. Poultry meat alone contributed to 36.7% of world's whole meat production in 2018, surpassing the pig meat becoming the most produced meat type in the world market (Ritchie, 2017). In order to meet the boosting demand for poultry meat, the antibiotic additives have been widely used in livestock feed for growth promotion purpose since 1940s. But the abuse of antibiotic additives induced transferable antibiotic resistance in pathogenic bacteria in both birds and human beings (Witte, 2000). Reducing or replacing the antibiotics in poultry feed has been accepted and promoted worldwide, which urges the scholars and enterprises finding alternative feed additives for the poultry industry.

Short chain fatty acids (**SCFA**) and medium-chain fatty acids (**MCFA**) are metabolites of gut anaerobes fermenting undigested polysaccharides (Tan et al., 2014). These fatty acids not only provide energy for the gut epithelial cells, but also have antibacterial effect (Dai et al., 2020). It is known that butyrate acid (**BA**) is one of the primary SCFA generated by microbes in the small intestine and large intestine, and is considered the

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prime source of energy for enterocytes (Kau et al., 2011). It can significantly stimulate colonic epithelial cell proliferation, increase microvilli length and cell turnover, thereby promoting nutrient absorption, enhancing intestinal immune function, preventing enteritis and diarrhea, and ultimately reducing mortality and improving health and growth performance of animal. It was reported that chicken body weight gain was increased with sodium butyrate as feed additive at concentrations of 0.5 and 2 g/kg from 0 to 21 d (Hu and Guo, 2007). In addition, BA shows bactericidal and bacteriostatic effect depending on the physiological status of the bacteria and the physicochemical characteristics of the external environment (Ricke, 2003). It showed that after BA entering bacterial cells, it disassociates the hydrogen and butyrate ions, and the accumulation of hydrogen ion lead to the death of some pathogenic bacteria (Guilloteau et al., 2010). In vitro data proved that 12.5 mM BA was effective agianst *C. jejuni* (Deun et al., 2008) and 10 mM BA downregulated the *Salmonella* pathogenic gene expression at pH 6.0 (Gantois et al., 2006).

However, the results of supplement free BA in poultry feed on pathogenic bacteria suppression in vivo were not consistent in different research. It may due to the reason that free BA were consumed rapidly by the cecal enterocytes creating a strong concentration gradient, which resulted in an insufficient rate at the site of colonization (Deun et al., 2008). Moreover, free BA has an offensive odor at 0.05 to 2.5% incorporation level in feed that deters animal from consuming feed supplemented with free BA (Pituch et al., 2013). In this regard, butyrate glycerides, butyrate salts and different encapsulation techniques have been developed in order to deliver BA as feed additive to animal's lower intestine, which is the targeting location (Van Immerseel et al., 2005; Bedford and Gong, 2017a).

The mixture of mono-glycerides of butyric-, capric-, and caprylic acid (**MMG**) can pass through the stomach of human and livestock animals and enter the intestine to function (Sampugna et al., 1967). Part of the mono-glycerides of butyrate will be directly absorbed in jejunum and ileum, and be hydrolyzed to BA in the intestinal mucosa cells (Watt and Steinberg, 2008). The remaining MMG will reach the hindgut, and be decomposed into BA under the action of microbe, which provides energy to intestinal mucosa epithelial cells, thus improving intestinal mucosa morphology and promoting intestinal immunity (Guo, 2008). The efficacy of soybean oil containing 2, 3.5, 5, and 10 g/kg (mixed feed weight) MMG were tested as additive in broiler diets (Antongiovanni et al., 2007). Birds treated with higher MMG mix gained a higher live weight at slaughter and showed improved feed conversion rate. Zhang et al. (2005) showed that MMG at 2 g/kg rate in diets can promote the ileal mucosal development and increase the epithelial cell turnover.

Current experiment was designed to study the effects of several supplement strategies of MMG, and a defined dose of antibiotic growth promoter (chlortetracycline) as comparison on Arbor Acre (**AA**) birds. By analyzing the growth performance and related gut morphology of AA birds, so the present study aimed to investigate the potential of MMG to improve broiler performance and gut health of broilers.

MATERIALS AND METHODS

The MMG used in our experiment is SILOhealth 104 P, which was kindly provided by BASF SE, Germany. SILOhealth 104 P is a commercial product with mono- and di-, triglycerides of butyrate as the main active ingredient, mixed with a portion of the mono- and di-, triglycerides of other medium chain fatty acids. Chlortetracycline produced by CP Group with a purity of 15% was chosen as the antibiotic additive in this experiment.

Animals and Diets

A total of 960 healthy male AA broilers at 1 d of age with the same batch and genetic background were selected and transferred to the animal experiment base of the Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences. All the experimental procedures were conducted in accordance with the guidelines for animal welfare and approved by the Animal Care and Use Committee, Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences. The whole experiment period lasted 42 days with two stages: 1 to 21 d and 22 to 42 d. The birds were individually weighed and randomly divided into 6 groups with similar average initial weight among groups. Birds in Group 1 to 6 were fed with basal diets (**BD**), BD supplemented with 500 g/t Chlortetracycline additive (**CA**), BD supplemented with 500 g/t CA and 1000 g/t MMG, BD supplemented with 500 g/t CA and 1,000 g/t MMG at 1 to 21 d and BD supplemented with 500 g/t CA and 650 g/t MMG at 22 to 42 d, BD supplemented with 500 g/t CA and 1,000 g/t MMG at 1 to 21 d and BD supplemented with 500 g/t CA at 21 to 42 d, and BD supplemented with 3,000 g/t MMG at 1 to 21 d and BD supplemented with 1,000 g/t MMG at 21 to 42 d, respectively. Each group (160 AA broilers) was further subdivided into 8 cages (20 broilers/cage) the dimension of 120 cm × 120 cm.

All broilers were housed in environmentally controlled cages with hard plastic mesh flooring. Artificial illumination was used during the entire trial period. When brooding, infrared light was used to maintain temperature at 33 to 34°C in the first week and 27 to 31°C in the second week, and the light was used as needed in following weeks. The chicken house was naturally ventilated and regularly cleaned. The relative humidity of the house was maintained at 55 to 65%. The broilers were supplied ad libitum access to feed and water throughout the trial period. The basal diet (Table 1) was formulated according to the Nutrient Requirements of Broilers (NRC, 1994).

Table 1. Composition and nutrient levels of basal diets (air-dry basis, %).

Items	Diets	
	1−21 days old	22−42 days old
Ingredients		
Corn	47.25	49.90
Soybean meal	30.35	27.77
Corn protein powder (CP 60%)	4.30	3.00
Wheat middling and reddog	11.20	10.00
Limestone	1.62	1.49
$CaHPO_4 \bullet 2H_2O$	1.44	1.24
Soybean oil	2.00	4.95
NaCl	0.30	0.30
78.5% *L*- Lys	0.35	0.20
98.5% *DL*- Met	0.19	0.15
1% Premix[1]	1.00	1.00
Total	100.00	100.00
Nutrient levels[2]		
ME/(MJ/kg)	12.14	12.96
CP	21.01	19.01
CF	3.82	3.60
Ca	1.00	0.90
Total phosphorus	0.70	0.65
Availablephosphorus	0.43	0.38
Lys	1.33	1.13
Met	0.55	0.47
Met+Cys	0.89	0.79

[1]The premix provided the following micronutrients (per kilogram of complete diet):VA 12,000 IU, VD_3 2,500 IU, VE 20 mg, VK_3 3 mg, VB_1 3 mg, VB_2 8 mg, VB_6 7 mg, VB_{12} 0.03 mg, *D*-pantothenic acid 20 mg, nicotinic acid 50 mg, biotin 0.1 mg, folic acid 1.5 mg, Cu (as copper sulfate) 9 mg, Zn (as zinc sulfate) 110 mg, Fe (as ferrous sulfate) 100 mg, Mn (as manganese sulfate) 100 mg, Se (as sodium selenite) 0.16 mg, I (as potassium iodide) 0.6 mg.

[2]Nutrient levels are calculated values.

Data Collection

Growth Performance Body weight of AA broilers was individually measured at the d 1, 21, and 42. Feed intake and mortality per cage were recorded daily. Postmortem was performed to identify the cause of death. The average daily feed intake (**ADFI**), average daily body weight gain (**ADG**), feed/gain ratios (**F/G**), and mortality were calculated according to the data from each cage.

Measurement of Serum Biochemical Parameters On d 42, after 12 h fasting, 8 broilers in each group (1 broiler in each cage) with body weight close to the mean were chosen to collected blood samples from wing vein. The serum was separated from blood samples by centrifugation at 3,000 × *g* for 15 min at 4°C by Cence centrifuge L550 (Hunan Xiangyi Laboratory Instrument Development Co., Ltd., China), then stored at −20°C until further analysis.

The levels of glucose (**GLU**), triglyceride (**TG**), total cholesterol (**TCHO**), uric acid (**UA**), urea nitrogen (**BUN**), creatinine (**Cr**), total protein (**TP**), albumin (**ALB**), and the activities of alkaline phosphatase (**ALP**), alanine transaminase (**ALT**), and aspartate aminotransferase (**AST**) in serum were measured with the commercial assay kits (Guilin Urit Medical Electronics Co., LTD, Guilin, China) by an automatic biochemical analyzer (URIT-8000, Guilin Urit Medical Electronics Co., LTD,).

Measurement of Intestinal Mucosal Morphology On d 42, after the collection of blood samples, the broilers were slaughtered and the small intestine was promptly removed and divided into 3 parts: duodenum, jejunum, and ileum. After that, 2-cm segments were cut from the midpoint of each intestinal section. These intestinal tissue samples were lightly flushed with physiological saline (154 mmol/L), blotted dry with filter paper and fixed into 10% neutral buffered formalin (Lin et al., 2017). A microtome (RM-2235, Leica microsystems AG., Hessen, Germany) was used to make 5 or 6 μm slices that were mounted in glass slides and subsequently stained with hematoxylin and eosin (HE staining). Finished slides were observed under an Olympus Van-Ox S microscope (Opelco, Washington, DC) and the typical microscopic fields were selected for photos. Ten sections of the proper microscopic fields were chosen from each sample for analysis of villus height, crypt depth, villi height over crypt depth (**V/C**), intestinal wall thickness, and number of lymphocytes and goblet cells in the intestinal villi epithelium, using an image analysis system (Image-Pro, Media Cybernetics, Inc., Silver Springs, MD).

Analysis of Cecal Microflora The cecum of each selected bird was separated and the chyme samples were collected and stored at −80°C for future analysis. 16S rDNA sequencing technology was used for exploration of the microflora construction and the changes in the cecum microbiology of birds in different groups (Best et al., 2017).

Statistical Analysis

Statistical analysis of data was done with Statistical Package for the Social Sciences (**SPSS**) 19.0 (IBM, Armonk, NY). One-way ANOVA model was performed to test all data. Replicate was used as the experimental unit. Results were presented as means and pooled standard errors of the means (**SEM**). When the main effects were significant, the differences among means were further determined using Duncan's multiple range. Differences between means of all groups were considered significant at $P < 0.05$.

RESULTS

Growth Performance

The effects of MMG supplement strategies on the final BW, ADG, ADFI, and F/G ratio of broilers are showed in Table 2. No differences ($P > 0.05$) were found on final weight, ADG, ADFI, and F/G ratio in broilers among groups at 1 to 21 d. On d 42, Group 1 had lowest final BW among all groups, and was significantly lower than Group 2 and 3. The same trend was observed in ADG at 22 to 42 d and 1 to 42 d of the experiment. These data revealed that, MMG supplement in diet could improve the ADG of AA broilers at 22 to 42 d compared with Group 1.

Table 2. Effect of MMG supplement strategies in diet on growth performance of AA broiler.

Item	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	SEM	*P*-value
Average initial weight of 1 d/(g)	47.74	47.79	47.74	47.74	47.83	47.78	0.011	0.151
Average final weight of 21 d/(g)	607.02	643.75	631.05	621.80	615.00	615.53	5.671	0.456
Average final weight of 42 d/(g)	2,330.79[c]	2,513.70[ab]	2,574.69[a]	2,446.53[abc]	2,375.90[bc]	2,487.18[abc]	24.291	0.028
1−1 d								
ADG/(g)	26.63	28.38	27.78	27.34	27.01	27.04	0.270	0.457
ADFI/(g)	43.79	47.23	44.99	45.16	44.06	45.15	0.542	0.500
F/G	1.65	1.66	1.62	1.66	1.63	1.68	0.020	0.974
22−42 d								
ADG/(g)	82.09[c]	89.05[ab]	93.57[a]	90.32[ab]	86.58[bc]	89.45[ab]	0.989	0.016
ADFI/(g)	143.97	148.66	148.12	147.10	144.33	144.09	1.664	0.936
F/G	1.76	1.67	1.58	1.63	1.67	1.61	0.020	0.168
1−42 d								
ADG/(g)	54.36[c]	58.71[ab]	60.16[a]	57.12[abc]	55.43[bc]	58.08[abc]	0.578	0.028
ADFI/(g)	93.88	98.24	96.70	95.03	93.84	94.66	0.978	0.763
F/G	1.73	1.67	1.61	1.67	1.69	1.63	0.014	0.183

Abbreviations: AA, Arbor Acre; ADG, average daily weight gain; ADFI, average daily feed intake; F/G, feed/gain ratios; MMG, mono-glycerides of butyric-, capric-, and caprylic acid.

[a-c]Means in a row not sharing a same superscript letter are different ($P < 0.05$).

Serum Biochemical Parameters

On d 42, the serum level of TG was higher ($P < 0.05$) in Group 2 and 3 than that in Group 1, and BUN were higher ($P < 0.05$) in Group 1 than Group 3, 5 and 6 (Table 3). There was no difference ($P > 0.05$) among groups on serum levels of GLU, TCHO, UA, Cr, TP, ALB, GLB, ALB, ALP, ALT, AST, AST/ALT during the whole experiment period. These data demonstrated that MMG might partly improve serum biochemical parameters of AA broilers.

Intestinal Mucosal Morphology

Broilers fed diets supplemented with MMG had more intact intestinal mucosa than that with BD group at the duodenum, jejunum and ileum in 42 d (Figure 1). As showed in Table 4, the majority of measured parameters were not influenced by the MMG supplement strategies, but the number of intraepithelial lymphocytes of ileum was higher ($P < 0.05$) in Group 6 than that in Group 2 and 3.

Cecal Microflora

A total of 3,569,080 pairs of raw reads, in which 3,260,563 were clean reads in 48 samples (with an average of 67,928 clean reads each sample) were revealed.

The 15 most predominant genera in cecal microflora of AA broilers in each treatment were showed in the bar chart (Figure 2). The 10 dominant genera included *Faecalibacterium, Alistipes, Escherichia/Shigella, Lactobacillus, Bacteroides, Ruminococcaceae* UCG-014, Ruminococcus torques group, uncultured bacterium f Lachnospiraceae, uncultured bacterium f Ruminococcaceae and *Subdoligranulum. Faecalibacterium, Escherichia-Shigella, Alistipes, Bacteroides* and *Lactobacillus* were the 5 genera that varied the most among 6 groups, Compared to Group 1, MMG in Group 6 decreased the abundance of *Faecalibacterium* and *Bacteroides* by 10.5 and 13.4%, respectively, and increased the abundance of *Escherichia/Shiegella* by 10.5%. Compared to Group 2, multiple strategies of adding MMG as feed supplement in Group 3, 4, and 5 showed decreased abundance of *Lactobacillus* by 8.3, 10.7 and 9.0%, respectively. As the data showed, with or without the exist of CA, different

Table 3. Effect of MMG in diet on serum biochemical indices of 42 d AA broiler.

Item	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	SEM	*P*-value
GLU/(mmol/L)	11.24	10.82	10.17	11.07	11.57	11.49	0.194	0.324
TG/(mmol/L)	0.35[b]	0.48[a]	0.51[a]	0.42[ab]	0.41[ab]	0.42[ab]	0.016	0.050
TCHO/(mmol/L)	5.51	5.70	6.12	5.93	5.99	5.78	0.148	0.883
UA/(μmol/L)	389.07	358.63	350.26	400.26	374.63	409.99	11.999	0.691
BUN/(mmol/L)	1.24[a]	1.15[ab]	0.88[c]	1.03[abc]	1.00[bc]	0.92[bc]	0.035	0.018
Cr/(μmol/L)	82.95	86.27	91.70	102.07	107.20	96.33	2.759	0.083
TP/(g/L)	40.57	46.45	45.22	41.39	39.60	38.59	1.405	0.536
ALB/(g/L)	16.12	16.93	18.13	17.88	18.30	17.53	0.319	0.360
GLB/(g/L)	24.45	29.52	27.10	23.51	21.30	21.07	1.251	0.327
ALB/GLB	0.67	0.67	0.75	0.80	0.88	0.86	0.027	0.089
ALP/(U/L)	267.04	264.88	219.70	269.73	267.83	327.11	10.603	0.116
ALT/(U/L)	1.94	2.79	2.38	2.35	2.55	2.86	0.111	0.178
AST/(U/L)	171.63	208.53	222.17	210.73	196.48	228.16	6.274	0.114
AST/ALT	94.31	79.99	102.45	99.01	80.13	86.54	4.138	0.495

Abbreviations: AA, Arbor Acre; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, urea nitrogen; Cr, creatinine; GLU, glucose; MMG, mono-glycerides of butyric-, capric- and caprylic acid; TG, triglyceride; TCHO, total cholesterol; TP, total protein; UA, uric acid.

[a-c]Means in a row not sharing a same superscript letter are different ($P < 0.05$).

Figure 1. Effect of MMG in diet on intestinal mucosal morphology of 42 d AA broiler (40 × magnification). Abbreviations: AA, Arbor Acre; MMG, mono-glycerides of butyric-, capric- and caprylic acid.

strategies of MMG supplement in feed could modify the cecal microflora community construction in AA broilers.

DISCUSSION

In present study, we performed several feeding strategies to evaluate the effects of dietary supplement with MMG on growth performance, serum biochemical parameters, intestinal morphology, and intestinal microbial construction. The main active ingredients of MMG are a portion of glycerides of medium chain fatty acids.

The data showed that, tested feeding strategies exhibited significant influence on the average final weight on the 42 d, but no significant influence in growth performance indicators in all stages, except for the ADG at 22 to 42 d or 1 to 42 d of the AA broilers. Group 2 and Group 3 showed significantly higher average final weight on the 42 d, ADG at 22 to 42 d and at 1 to 42 d than BD group ($P < 0.05$), which demonstrated that the 500 g/t CA as well as the combination of CA and MMG could promote the weight gain at 22 to 42 d of AA broiler feeding. Chlortetracycline additive as an antimicrobial compounds, showed not only good disease control but also excellent growth promotional effects on swine and birds (Dong et al., 2011; Williams et al., 2018). Interestingly, we also found that the ADG of Group 3 were higher than that of Group 5 at both 22 to 42 d and 1 to 42 d ($P < 0.05$), from which we concluded that 1,000 g/t MMG may had an important role in the broilers' weight

Table 4. Effect of MMG in diet on intestinal mucosal morphology of 42 d AA broiler.

Item	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	SEM	*P*-value
Duodenum								
Villus height/(μm)	600.29	615.58	626.01	626.60	597.30	591.81	15.055	0.978
Crypt depth/(μm)	71.16	66.23	70.13	71.28	66.17	66.93	1.095	0.536
V/C	8.51	9.31	8.96	8.84	9.05	8.89	0.216	0.950
Intestinal wall thickness/(μm)	93.60	94.16	106.97	96.17	95.79	104.07	2.132	0.333
Number of goblet cells (Entries/100 absorptive cells)	16.63	17.20	16.78	15.00	17.60	16.60	0.408	0.577
Number of Intraepithelial lymphocyte (Entries/100 absorptive cells)	31.15	33.38	36.45	34.80	36.63	38.43	0.860	0.173
Jejunum								
Villus height/(μm)	473.72	505.36	534.97	514.39	484.59	452.37	12.191	0.430
Crypt depth/(μm)	60.05	55.53	59.04	54.13	57.21	55.48	0.955	0.458
V/C	8.13	9.36	9.09	9.55	8.47	8.17	0.256	0.446
Intestinal wall thickness/(μm)	94.91	79.85	89.41	84.40	89.51	93.07	2.420	0.508
Number of goblet cells (Entries/100 absorptive cells)	17.55	15.83	17.58	14.88	16.40	16.15	0.555	0.731
Number of Intraepithelial lymphocyte (Entries/100 absorptive cells)	42.20	38.53	38.85	36.43	33.95	38.33	0.924	0.194
Ileum								
Villus height/(μm)	359.49	379.47	357.71	374.02	354.99	360.33	9.654	0.975
Crypt depth/(μm)	54.91	57.82	55.57	53.74	57.19	53.26	1.093	0.820
V/C	6.62	6.55	6.44	6.90	6.22	6.74	0.124	0.715
Intestinal wall thickness/(μm)	98.51	92.11	96.08	93.84	95.38	101.35	2.243	0.887
Number of goblet cells (Entries/100 absorptive cells)	22.23	21.35	21.05	21.00	19.93	19.78	0.328	0.260
Number of Intraepithelial lymphocyte (Entries/100 absorptive cells)	30.80^{ab}	25.20^{c}	25.60^{bc}	29.10^{abc}	29.73^{abc}	32.03^{a}	0.774	0.047

Abbreviations: AA, Arbor Acre; MMG, mono-glycerides of butyric-, capric- and caprylic acid; V/C, villi height over crypt depth.
$^{a-c}$Means in a row not sharing a same superscript letter are different ($P < 0.05$).

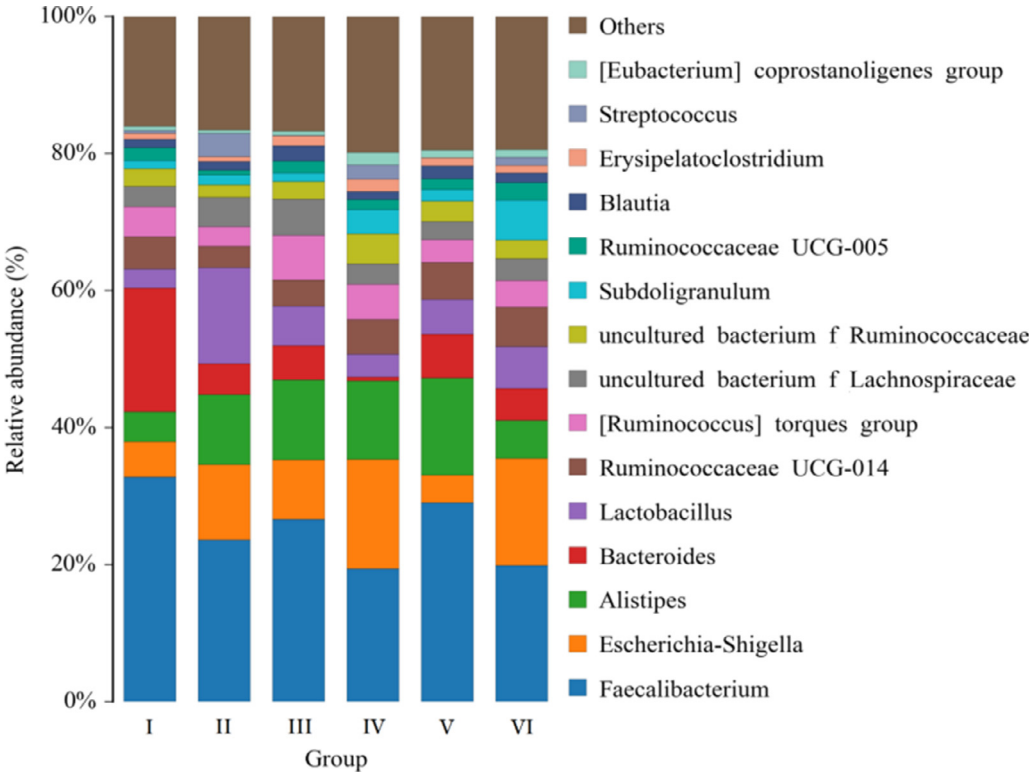

Figure 2. Effect of MMG in diet on cecal microbial community (genus-level) of 42 d AA broiler. Abbreviations: AA, Arbor Acre; MMG, monoglycerides of butyric-, capric- and caprylic acid.

gain mainly at 22 to 42 d of the growth period with 500 g/t CA. This finding was partially supported by previous research that the supplementation with MMG could improve ADG of broilers, and the benefit effect had certain rate pattern with the mixture (Bedford et al., 2017b). It was also proved that the MMG showed some effect on the performance of broilers, particularly on lipid catabolism (Yin et al., 2016). The possible explanation for this effect may be that butyrate inhibited histone deacetylase so that the muscle fiber in cross-sectional area increased and the intramuscular fat accumulation decreased (Walsh et al., 2015).

In order to find out the metabolic processes involved in MMG promoting broilers' growth performance, we tested the serum biochemical parameters associated with nutrition metabolism. According to our data, the parameters of GLU, TG, TCHO, UA, Cr, TP, ALB, GLB, ALB/GLB, ALP, ALT, AST, AST/ALT were not influenced by the feeding strategies. However, that supplementation of 1,000 g/t MMG in Group 3 reduced the BUN content in the serum compared to the that in Group 2 ($P < 0.05$), and the similar result were showed in Group 6 compared to Group 1 ($P < 0.05$). Urea nitrogen is a byproduct of protein metabolism, and it is an indicator for kidney function (Huang et al., 2017). The present data showed that MMG may strengthen kidney function to excrete more urea formed by the liver and carried by the blood, as the BUN drops in the blood samples. The above results were inconsistent with previous researches. Bedford et al. (2017b) showed that the combination of 500 ppm monobutyrin and 500 ppm tributyrin significantly influenced serum phosphorus, AST, carbon dioxide, calcium and cholesterol levels of AA broilers. Yang et al. (2018) compared the effect of 3,000 ppm MMG supplementation on blood metabolism with that with the basal diet in broilers. He stated that LDL/VLDL, lipids, lactate, alanine, asuccinate, di- and trimethylamine, choline, Glycerophosphorylcholine, and Trimethylamine N-oxide in broilers serum were significantly increased under MMG supplementation in diets, compared to the basal diet. None of these researches emphasized the changes of BUN with MMG supplementation in diets. Therefore, whether improved kidney function was the main reason for the effect of MMG on the promotion of growth performance reminded to be determined.

Multiple reports proved that the development of the gastrointestinal tract and the gut microbiota play critical role in broiler's productivity, because the GI tract is the main location for nutrient utilization and the microflora within were essential participator of the metabolism (Prandini et al., 1997; Montagne et al., 2003; Choct, 2009). The mucosal morphologies of intestinal sections (duodenum, jejunum and ileum) were investigated in the research. The number of intraepithelial lymphocytes in ileum was lower in Group 2 than Group 1 and 6, as well as Group 3 than Group 6 ($P < 0.05$). The possible reason could be that additive of CA and the combination of CA and MMG in the diet helped ameliorating the immune status in the intestine, so that the amount of lymphocyte cell as participator in immune response decreased. However, it was difficult to tell if MMG alone had the function of improving the immune condition in the present study. Zou et al. (2019) found

that birds receiving 300 mg/kg diet of sodium butyrate reduced the expression of inflammatory genes in the ileum, suggesting butyrate derivatives like sodium butyrate and MMG may improve the intestinal immune condition and promote the intestinal development. In addition, MMG were proved to reduce the expression of proinflammatory cytokines and improve tight junction in the colon of weaning pigs (Hou et al., 2014; Tugnoli et al., 2014). Therefore, it is highly possible that MMG had some synergy effect with CA on relieving the immunoreaction in the intestine, especially the ileum.

16S rDNA sequencing technology was applied to check if MMG had effect on the microbiota composition in cecal. The dominant genera in cecal microbiota were similar among different groups, which meant treatment effects on the diversity of ileum microbiota was similar ($P > 0.05$). However, the composition of microbiota in cecal were different among groups. Compared to the basal diet group, MMG supplementation decreased the abundance of *Faecalibacterium* and *Bacteroides*. With the existence of CA in the basal diet, MMG could decrease the abundance of *Lactobacillus* in the cecal. *Faecalibacterium* is an obligate anaerobe and butyrate producer providing energy to the colonic mucosa (Luo et al., 2013). The decrease in the *Faecalibacterium* population implied function suppression by butyrate released from MMG, possibly as the result of feedback from the high level of butyrate in the broiler cecum. This result was consistent with previous research by Yang et al. (2018). *Bacteroides* is also obligate anaerobe that can influence host function such as immune system development. There were several fatal pathogenic bacteria fell into this genera, such as *B.fragilis, B.ovatus, B. distasonis, B.vulgatus*, and *B.thetaiotaomicron* (Mandell et al., 2010). The reduction of *bacteroides* spp. because of MMG could possibly contribute to lower rate of disease and higher production of the broilers.

In conclusion, MMG supplementation at 3,000 g/t (1 to 21 d) and 1,000 g/t (22 to 42 d) without 500 g/t CA could enhance the growth performance of broilers in the present study. In addition, mixed supplementations of 500 g/t CA and 1,000 g/t MMG could improve the birds' growth performance at 22 to 42 d period. The possible explanation for the effect is that MMG improved the intestinal morphology and cecal microbiota composition of the broilers, which might ameliorate the immune condition and the nutrition metabolism. This study provided information for the application of MMG as the potential replacement to antibiotic additives in broiler production.

DISCLOSURES

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, 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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