Ann. Anim. Sci., Vol. 24, No. 1 (2024) 191–199 *DOI: 10.2478/aoas-2024-0001*

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EFFECT OF LINCOMYCIN AND BUTYRATE GLYCERIDES SUPPLEMENTATION ON PERFORMANCE, BLOOD BIOCHEMICAL CONSTITUENTS, IMMUNE RESPONSE AND NUTRIENT ABSORPTION RELATED GENE EXPRESSION IN BROILERS

Ahmed A. Saleh[1♦], Ibrahim A. Elkhaiat[1], Abdul Rahman A. Alkhamisi[1], Mustafa Shukry[2], Karima El-Naggar[3], Mohammed H. Alzawqari[1, 4], Mahmoud Alagawany[5♦], Abeer A. Kirrella[1]

[1]Department of Poultry Production, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 333516, Egypt
[2]Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh 33516, Egypt
[3]Department of Nutrition and Veterinary Clinical Nutrition, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt
[4]Department of Animal Production, Faculty of Agriculture and Food Sciences, Ibb University, Ibb 70270, Yemen
[5]Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt
♦Corresponding author: ahmed.saleh1@agr.kfs.edu.eg, dr.mahmoud.alagwany@gmail.com

Abstract

Over 35 days feeding trial, the effects of lincomycin (LM) and butyrate glycerides (BG) supplementation on the growth performance, blood biochemical changes, immune response, and nutrient absorption related gene expression in broiler chickens were evaluated. A total of 480 one- day-old broiler chicks (Ross 308) were allotted into 6 groups (8 replicates/group with 10 birds/replicate). Experimental groups were arranged as follows; control group (A) fed the basal diet (BD); B, C, and D groups were fed the BD supplemented with 350, 500 and 1000 g BG/ton of feed respectively. While group E was fed the BD with 350 + 500 + 1000 g BG/ton of feed during starter, grower, and finisher periods, respectively, and finally group F which received lincomycin (LM) antibiotic (100 g/ton) in overall periods. Broilers that received BG in group E (birds supplemented with BG at 350 + 500 + 1000 g/ton feed) and LM (F) supplements both gained more body weight and weight growth (P<0.05). Lower feed intake and improved FCR were observed in all the experimental groups that received either BG or LM compared to control birds (P<0.05). Lower serum total cholesterol, triglycerides, and glutamic oxaloacetic transaminase (GOT) (P<0.05) were recorded in the LM or BG supplemented birds compared to the control. Higher immune response represented by enhanced antibody titer against H9N1, and Newcastle disease (ND) and lower hepatic malondialdehyde (MDA) was obtained in groups D, E and F (P<0.05) compared to the other groups. Higher gene expression of GSH-Px, *Glut 2*, *PepT1* and *FAS* was obtained in groups D, E and F (P<0.05) compared to the control. Supplemental BG or LM increased the unsaturated fatty acids (oleic, linoleic and linolenic acids) while lowered the palmitic acid contents in breast muscle (P<0.05) compared to control. In conclusion, supplementing broilers' diets with BG or lincomycin improved their growth performance, immune response and muscle fatty acid profile as well as modulatory role in lipid metabolism. Supplementing starter, grower, and finisher broiler diets with BG at 350 + 500 + 1000 g/ton each may be a useful substitute for lincomycin.

Key words: butyrate glycerides, lincomycin, broiler, growth, blood parameters

Antibiotics have been commonly used in the poultry industry as growth promoters, to maintain the intestinal microflora balance, enhance bird performance, and reduce morbidity and mortality and treat some intestinal infections (Farag et al., 2021). However, with the increased antibiotic abuse, adverse effects as the potential occurrence of antibiotic resistance as well as residues remaining in poultry products resulted in their ban as growth promoting additives by the European Commission in 2006 (Castanon, 2007). Recognizing these adverse effects of antibiotic growth promoters on human health, has encouraged the specialized researchers on a global scale to find new alternatives which could reduce or replace antibiotics, enhance birds' performance, be safe for the general public health, cost-effective, and environmentally friendly (Bassiony et al., 2021; Lin et al., 2022).

One of the main alternatives which have been approved for use as in-feed supplements is organic acids (Abd El-Hack et al., 2021; Ebeid et al., 2021). These compounds are short chain fatty acids which exerted several beneficial effects including improved gut health and function, enhanced nutrient intake, digestion and metabolism, antimicrobial activity and feed preservation (Broom 2015; Melaku et al., 2021). Among these organic acids, butyric acid (BA) and its derivatives have received considerable attention and become commonly used. Several previous reports documented various positive effects with supplementation of BA and its derivatives in broiler chicken diets; they improve nutrient digestion and growth, can replace antibiotics (Panda et al., 2009; Pearlin et al., 2020; El-Saadony et al., 2022), reduce the spread of infection by *Salmonella* Enteritidis (Fernández-Rubio et al., 2009), provide an important energy source for enterocytes, modulate the intestinal mi-

crobiota and maintain the intestinal barrier, and enhance immune response (Bedford and Gong, 2018; Deepa et al., 2018; Melaku et al., 2021). Aside from being harmful to the equipment, BA has a disagreeable odor. Besides, butyrate is immediately absorbed in the upper part of digestive tract, thus, lower the delivery of a sufficient amount of BA to the target tissues in distal segments of the gut to demonstrate its effects (Bedford and Gong, 2018). Therefore, encapsulation techniques have been developed and used in order to ease the handling and prevent the release of butyrate in the upper GIT. Protected forms of BA, such as coated sodium butyrate and BA glycerides obtained by esterification of BA with glyceride, allowed easier passage of BA through the lower gastrointestinal tract and become available in higher concentrations (Ahsan et al., 2016; Makowski et al., 2022).

Lincomycin is a naturally occurring lincosamide antibiotic obtained from fermentation products of *Streptomyces lincolnensis* (Greenwood et al., 2004). Lincomycin is given orally to poultry as a feed and water additive to treat bacterial enteric infections as well as to improve performance (Chan et al., 2015). According to Ali et al. (2014), lincomycin is beneficial in treating severe bacterial infections caused by bacteria including *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and others. Lincomycin kills bacteria by stopping the production of vital proteins required for survival.

In the current study, it is hypothesized that butyrate glycerides (BG) could replace LM in broiler diet. Therefore, the present study aimed to investigate the effect of lincomycin and BG supplementation in broiler diet on their growth performance, blood biochemical constituents, immune response, muscle fatty acids profile, and the expression of some nutrient transporters related genes.

Material and methods

All the management procedures followed during the course of the study were approved by the Ethics Committee of Local Experimental Animals Care of Kafrelsheikh University, Egypt (Number KFS-IACUC/136/2023).

Birds raising and feeding trial design

A total of 480 one-day-old male broiler chicks (Ross 308) with an average initial body weight (40.9 g/chick) were randomly assigned into 6 experimental treatments (8 replicates/group with 10 birds/replicate). Birds were fed the experimental basal diets (BD) formulated based on the nutritional requirements of Aviagen (2019) for male broilers, with a three-phase feeding program (starter, 0−10 d; grower, 11−24 d; and finisher, 25−35 d) (Table 1). The first control group (A) was fed the BD; B, C, and D groups were fed the BD supplemented with 350, 500 and 1000 g butyrate glycerides (BG)/ton of feed; respectively. The fifth group (E) was fed the BD with 350 + 500 + 1000 g BG/ton of feed during starter, grower, and finisher periods, respectively, while group F was fed the BD supplemented with 100 g/ton of lincomycin (LM) antibiotic in overall periods. The source of BG used was FRA® Butyrin Hybrid Dry (Framelco B.V., The Netherlands), which was composed mainly of mono-, di- and triglycerides of encapsulated butyric acid with a recommended addition level in poultry 0.5–2 kg/ton feed. Birds were housed in pens with a stocking density of 10 birds/m². The starter diets were in crumble form, while the grower and finisher diets were in pellet form. The feed trial took place in a temperature-controlled room with a temperature starting from 32 ± 1°C and decreasing by 1°C every three days until reaching 24 ± 1°C, which was maintained until the end of the experiment (35 days), and proportional humidity was kept between 50% and 70%. The lighting schedule was 23 h light with 1 h dark period during the first 3 days, after which birds were subjected to 16 h light with 8 h dark until the end of the experiment. Throughout the experimental phase, the health status and mortalities were measured regularly each day.

Table 1. Composition of broiler basal diets

Ingredient (g/kg)	Starter (1−10 days)	Grower (11−25 days)	Finisher (26−35 days)
Yellow corn	542	600	664
Soybean meal (46%)	360	308	215
Corn gluten meal (60%)	30	30	60
Soya oil	25	23	22
Calcium carbonate	14.9	12.6	14.1
Dicalcium phosphate	14	13	12
Common salt	3	3	3
Sodium sulfate	2	2	2
DL-methionine (99%)	2	1.5	1.5
L-lysine HCl (8%)	3	3	2.5
L-threonine	0.5	0.5	0.5
Choline chloride (60%)	0.6	0.4	0.4
Premix*	3	3	3
Chemical analysis on DM basis			
AME kcal	3000	3050	3150
Crude protein (%)	23.0	21.0	19.0
Fat (%)	5.00	4.96	5.04
Digestible LYS (%)	1.28	1.24	1.15
Digestible M and C (%)	0.95	0.92	0.87
Digestible THR (%)	0.86	0.83	0.77
Digestible ARG (%)	1.37	1.33	1.25
Digestible ILE (%)	0.90	0.87	0.85
Digestible LEU(%)	1.87	1.83	1.84
Digestible VAL (%)	0.96	0.93	0.91
Calcium (%)	0.96	0.96	0.87
Available P (%)	0.48	0.48	0.44
Sodium (%)	0.19	0.19	0.19
Chloride (%)	0.22	0.22	0.22

*Hero mix® (Hero Pharm, Cairo, Egypt). Composition (per 3 kg): vitamin A 12,000,000 IU, vitamin D_3 2,500,000 IU, vitamin E 10,000 mg, vitamin K_3 2000 mg, vitamin B_1 1000 mg, vitamin B_2 5000 mg, vitamin B_6 1500 mg, vitamin B_{12} 10 mg, niacin 30,000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10,000 mg, manganese 60,000 mg, zinc 50,000 mg, iron 30,000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg.

Birds' growth performance and carcass traits

Bird's BW was recorded individually every week, while FI was measured daily (on a group basis per pen) throughout the experimental period. Birds' weight gain (WG) and feed conversion ratio (FCR) were calculated accordingly. At the end of the experiment, birds (n=10/group) were slaughtered, dissected and the weight of carcass, breast muscle, thigh muscle, abdominal fat, and internal organs (gizzard, liver, spleen and heart) was recorded. The weight of all organs previously mentioned was described as a ratio of the body weight.

Blood biochemical and immune variables

Blood samples were collected from birds (n=10 birds/group), centrifuged at 3000 rpm/10 min for serum separation which was used for analysis of the following blood serum metabolites: total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, glucose, glutamic oxaloacetic transaminase (GOT), creatinine, total protein, and albumin were measured colorimetrically using commercial kits (Diamond Diagnostics, Egypt) following the manufacturer's instructions (Saleh, 2013). Serum antibody titers against Newcastle disease (ND) and avian influenza (H9N1) were determined by the hemagglutination inhibition test using methods described by OIE (2008). Lipid peroxidation indicator, malondialdehyde (MDA) was measured in the liver tissue following Richard et al. (1992).

Fatty acid profile in muscle

Fatty acid profile in muscle tissue (n=10 birds/group) was assessed using gas-liquid chromatography (GLC) following Saleh (2013).

Real-time PCR

In summary, total RNA was extracted from about 50 mg of liver and intestinal tissue (jejunum) using TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA). Nanodrop was used for the measurement of a cDNA synthesis kit; RNA samples with an A260/A280 ratio of 1.8 or greater were used to synthesize DNA, using a cDNA synthesis package (Fermentas, Waltham, MA, United States). The SYBR Green master mix was used with the primers to replicate cDNA given in Table 2. *GAPDH* was used as the housekeeping gene using $2^{-\Delta\Delta T}$ methods (Livak and Schmittgen, 2001).

Statistical analysis

One-way ANOVA was used to analyze the obtained data followed by the Tukey's multiple comparison test to assess the differences in means among treatments. This test was performed using the SAS software (SAS Institute, 2016). Differences were considered to be significant at $P<0.05$.

Results

The overall growth performance of birds over 35 days is illustrated in Table 3. Higher performance in terms of BW and WG was obtained ($P<0.05$) in broilers fed LM (F), and broilers supplemented with BG at 350 + 500 + 1000 g/ton in starter, grower, and finisher periods, respectively (E), compared to the control (A) or those receiving the BG at different doses (B, C and D groups). Lower FI was observed in all groups receiving either BG or LM compared to the control birds ($P<0.05$) with the lowest consumption recorded in D, E (birds supplemented with BG) and F (LM-fed birds), respectively. The effect of the experimental treatments on the WG and FI was reflected on FCR as it was significantly improved ($P<0.05$) in the BG- and LM-supplemented birds which was clear in group E (birds supplemented with BG at 350 + 500 + 1000 g/ton feed) and group F (basal diet with 100 g/ton of LM). No difference was observed in the mortality rate recorded over the course of the feeding trial between groups ($P>0.05$). Carcass and internal organs relative weights followed the same trend as they did not differ ($P<0.05$) between experimental groups in response to supplemental BG or LM (Table 4).

Table 2. Primers sequences used in this study

Gene	Primers sequences	Accession number
PepT1	F:CCCCTGAGGAGGATCACTGTTGGCAGTT T: CAAAAGAGCAGCAGCAACGA	NM_204365
GLUT2	F:CACACTATGGGCGCATGCT T: ATTGTCCCTGGAGGTGTTGGTG	NM_207178
GSH-PX	F: TTGTAAACATCAGGGGCAAA T: ATGGGCCAAGATCTTTCTGTAA	NM_001277853
SOD1	F:ATTACCGGCTTGTCTGATGG T: CCTCCCTTTGCAGTCACATT	NM205064.1
FAS	F: GGTCAGTGCTGCACGAAAT T:CATCTCATACACTCGTCCAAATC	NM_001199487.1
GAPDH	F: CAGGCTTATCCCGTTTAGCA T: AGTCGGAGTCAACGGATTTG	NM_204305

PepT1, peptide transporter 1; GLUT2, glucose transporter 2; SOD1, superoxide dismutase 1; FAS, fatty acid synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 3. Effect of lincomycin (LM) and butyrate glycerides (BG) supplementation on growth performance in broiler chickens

Item	Experimental diets						SEM	P-value
	A	B	C	D	E	F		
Initial body weight (g)	41.04	41.02	40.95	40.96	41.01	41.01	0.05	0.9852
Body weight (35d, g)	1670.63 c	1686.44 c	1740.50 bc	1752.38 ab	1757.00 ab	1776.75 a	19.36	0.0003
Body weight gain (35d, g)	1629.59 c	1645.59 c	1699.51 bc	1711.38 ab	1715.99 ab	1735.74 a	19.36	0.0003
Feed consumption (35d, g)	2831.25 a	2766.50 b	2741.00 bc	2713.25 c	2719.50 c	2730.50 c	0.98	0.0001
FCR (35d)	1.69 a	1.64 b	1.57 b	1.55 b	1.55 b	1.54 b	0.03	0.0014
Mortality (35d, %)	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.8489

a, b, c – the mean values followed by different letters in the same row are significantly different at P<0.05. Values are expressed as means ± standard error. Abbreviations: (A) control group (basal diet), (B) basal diet supplemented with 350 g BG/ton, (C) basal diet supplemented with 500 g BG/ton; (D) basal diet supplemented with 1000 g BG/ton, (E) basal diet supplemented with 350 + 500 + 1000 of BG in starter, grower, and finisher diets, respectively, and (F) basal diet with 100 g/ton of LM.

Table 4. Effect of lincomycin (LM) and butyrate glycerides (BG) supplementation on internal organs weight (%) in broiler chickens

Item	Experimental diets						SEM	P-value
	A	B	C	D	E	F		
Carcass (%)	62.40	61.62	61.61	62.18	62.16	64.37	0.88	0.2940
Breast muscle (%)	22.92	20.20	22.66	21.77	22.69	22.02	1.05	0.4907
Thigh muscle (%)	16.96	15.38	15.88	17.06	16.64	16.61	0.75	0.5801
Gizzard (%)	1.39	1.39	1.39	1.54	1.48	1.41	0.09	0.8058
Liver (%)	2.05	2.05	1.87	2.31	2.58	2.30	0.20	0.2183
Spleen (%)	0.14	0.14	0.13	0.19	0.14	0.15	0.02	0.2715
Heart (%)	0.42	0.47	0.46	0.44	0.46	0.46	0.04	0.9467
Abdominal fat (%)	0.66	0.56	0.52	0.55	0.67	0.48	0.80	0.4258

Values are expressed as means ± standard error. Abbreviations: (A) control group (basal diet), (B) basal diet supplemented with 350 g BG/ton, (C) basal diet supplemented with 500 g BG/ton; (D) basal diet supplemented with 1000 g BG/ton, (E) basal diet supplemented with 350 + 500 + 1000 of BG in starter, grower, and finisher diets, respectively, and (F) basal diet with 100 g/ton of LM.

Table 5. Effect of lincomycin (LM) and butyrate glycerides (BG) supplementation on blood biochemical changes in broiler chickens

Item	Experimental diets						SEM	P-value
	A	B	C	D	E	F		
Total protein (g/dl)	2.63	2.78	2.65	3.28	2.97	3.33	0.27	0.3092
Albumin (g/dl)	1.38	1.38	1.38	1.45	1.35	1.55	0.07	0.3948
Total cholesterol (mg/dl)	136.50 a	122.25 ab	122.00 ab	118.50 ab	114.00 b	112.00 b	5.03	0.0384
Triglyceride (mg/dl)	46.75 a	37.25 ab	31.00 b	30.25 b	32.00 ab	30.00 b	3.61	0.0277
HDL (mg/dl)	63.25	69.25	65.50	63.00	62.00	66.00	5.03	0.9192
Glucose (mg/dl)	138.75	146.00	142.25	122.50	137.00	121.25	17.57	0.8772
GOT, U/I)	265.75 a	260.50 ab	229.50 bc	225.50 c	217.75 c	204.75 c	8.21	0.0003
Creatinine (mg/dl)	0.40	0.40	0.40	0.34	0.33	0.30	0.03	0.1587

a, b, c – the mean values followed by different letters in the same row are significantly different (P<0.05). Values are expressed as means ± standard error. Abbreviations: (A) control group (basal diet), (B) basal diet supplemented with 350 g BG/ton, (C) basal diet supplemented with 500 g BG/ton; (D) basal diet supplemented with 1000 g BG/ton, (E) basal diet supplemented with 350 + 500 + 1000 of BG in starter, grower, and finisher diets, respectively, and (F) basal diet with 100 g/ton of LM.

Table 6. Effect of lincomycin (LM) and butyrate glycerides (BG) supplementation on immune response of broiler chickens

Item	Experimental diets						SEM	P-value
	A	B	C	D	E	F		
ND (titer)	1.68 b	1.94 b	2.45 ab	2.64 ab	2.69 a	2.66 ab	0.22	0.0147
H9N1 (titer)	2.78 b	3.28 b	3.50 ab	3.97 a	3.78 a	3.88 a	0.16	0.0006
Liver MDA (nmol/g)	9.25 a	8.77 a	8.37 a	6.61 b	6.60 b	6.35 b	0.32	0.0001

a, b, c – the mean values followed by different letters in the same row are significantly different (P<0.05). Values are expressed as means ± standard error. Abbreviations: (A) control group (basal diet), (B) basal diet supplemented with 350 g BG/ton, (C) basal diet supplemented with 500 g BG/ton; (D) basal diet supplemented with 1000 g BG/ton, (E) basal diet supplemented with 350 + 500 + 1000 of BG in starter, grower, and finisher diets, respectively, and (F) basal diet with 100 g/ton of LM.

Lack of difference was observed in the serum concentrations of total protein, albumin, HDL-cholesterol, glucose, and creatinine among the different groups (P>0.05) (Table 5). On the other hand, total cholesterol, triglycerides, and GOT responded differently as they showed lower concentrations (P<0.05) in birds supplemented with LM and BG compared to the reference group.

Moreover, the immune response of birds in response to LM and BG supplementation is shown in Table 6. Birds supplemented with BG at 350 + 500 + 1000 g/ton in starter, grower, and finisher periods in group E (birds received BG at 350 + 500 + 1000 g/ton) showed the highest ND titer (P<0.05) compared to other groups. The response obtained with H9N1 immunity titer (P<0.05) of group D (birds supplemented with 1000 g BG/ton feed), E (birds received BG at 350 + 500 + 1000 g/ton in the starter, grower, and finisher periods) and F (birds supplemented with LM lincomycin antibiotic (100 g/ton)) were significantly higher than control and other experimental groups (P<0.05). Furthermore, LM and BG addition in diet (P<0.05) were associated with lower hepatic MDA concentration as the lowest values were obtained in groups D (birds supplemented with 1000 g BG/ton feed), E (birds received BG at 350 + 500 + 1000 g/ton in the starter, grower, and finisher periods) and F (birds supplemented with LM) compared to the experimental groups (P<0.05).

Dietary supplementation of BG or LM significantly modified the fatty acids profile in breast muscle (P<0.05) compared to reference birds (Table 7). Supplemental BG or LM lowered the palmitic acids while increased the unsaturated fatty acid content (oleic, linoleic and linolenic fatty acids) (P<0.05) compared to the control birds. The lowest palmitic acid content was obtained in group D (birds supplemented with 1000 g BG/ton feed) and E (birds received BG at 350 + 500 + 1000 g/ton in the starter, grower, and finisher periods). All birds supplemented with BG in their diets showed the highest content of linoleic acid compared with birds fed diets containing LM or control diet (P<0.05). While, in relation to content of linolenic acid, group C (basal diet supplemented with 500 g BG/ton) and D (birds supplemented with 1000 g BG/ton feed) showed the highest content compared to other groups.

Table 7. Effect of lincomycin (LM) and butyrate glycerides (BG) supplementation on fatty acids in breast muscle of broiler chickens

Fatty acid (g/100 g of fat)	Experimental diets						SEM	P-value
	A	B	C	D	E	F		
Palmitic	1.326 a	1.173 bc	1.1547 bc	1.097 c	1.112 c	1.210 b	0.246	0.0385
Oleic	0.032 c	0.039 b	0.039 b	0.042 a	0.043 a	0.037 bc	0.0032	0.0265
Linoleic	0.176 c	0.186 a	0.188 a	0.189 a	0.188 a	0.182 b	0.054	0.0322
Linolenic	0.192 d	0.216 cd	0.271 a	0.244 ab	0.231 b	0.222 b	0.039	0.0112

a, b, c – the mean values followed by different letters in the same row are significantly different (P<0.05). Values are expressed as means ± standard error. Abbreviations: (A) control group (basal diet), (B) basal diet supplemented with 350 g BG/ton, (C) basal diet supplemented with 500 g BG/ton; (D) basal diet supplemented with 1000 g BG/ton, (E) basal diet supplemented with 350 + 500 + 1000 of BG in starter, grower, and finisher diets, respectively, and (F) basal diet with 100 g/ton of LM.

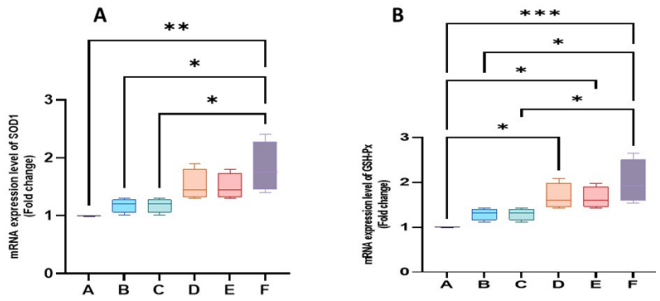


Figure 1. The relative mRNA expression (n=10/treatment) of superoxide dismutase (*SOD*) (A) and glutathione peroxidase (*GSH-PX*) (B) in broiler chicken hepatic tissue. The expressed represents the mean ± SE. * indicates significant difference (P<0.05)

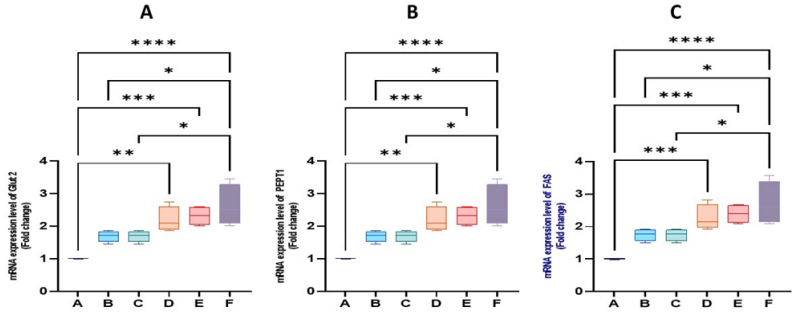


Figure 2. The relative mRNA expression (n= 10/treatment) of glucose transporter 2 (*Glut 2*) (A), peptide transporter 1(*PepT1*) (B) in intestinal tissue, and fatty acid synthase (*FAS*) (C) in liver of broilers. The expressed represents the mean ± SE. * indicates significant difference (P<0.05)

Regarding the gene expression assessed in the current study, LM supplementation in group F (received 100 g lincomycin/ton) stimulated a significant upregulation of SOD mRNA expression level (P<0.05) (Figure 1 A) compared to broilers fed the control or BG supplemented birds (A, B and C groups). The same result was obtained with GSH-Px expression (Figure 1 B), besides higher expression of *GSH-Px* in group D (birds supplemented with 1000 g BG/ton feed) and E (birds received BG at 350 + 500 + 1000 g/ton in the starter, grower, and finisher periods) compared to the control group (P<0.05). Additionally, *Glut 2*and *PepT1* mRNA expression levels demonstrated higher expression (P<0.05) in broiler chickens supplemented with BG in group E (birds received BG at 350 + 500 + 1000 g/ton) and LM (group F) than control group (group A),while *FAS* mRNA expression levels increased in broiler chickens supplemented with BG in group D (birds supplemented with 1000 g BG/ton feed), group E (birds received BG at 350 + 500 + 1000 g/ton) and LM (group F) than control group (group A) (Figure 2 A, B and C).

Discussion

Global concerns for reducing or replacing antibiotics dietary supplementation in poultry expanded the research for finding new alternatives (Ebeid and Al-Homidan, 2022). Supplementation of broiler diet with lincomycin or BG significantly improved their growth performance (higher BW and WG, lower FI and improved FCR). Besides, birds supplemented with BG at 350 + 500 + 1000 g/ton in the starter, grower, and finisher periods as well as those receiving BG at 1000 g/ton continuously during the whole experimental periods demonstrated the highest performance, suggesting that these are the effective supplemental levels of BG. These BG inclusion levels are in quite agreement with previous study (Liu et al., 2017) which documented 0.5 to 1.0 g/kg as the applied levels for microencapsulated sodium butyrate. On the same line Lin et al. (2022) found that dietary addition of a mixture of mono-glycerides of butyric, capric, and caprylic acid showed a positive effect on broilers' growth performance. Also, Almeida et al. (2021) found that a combined mixture of α-monolaurin mono-, di- and triglycerides of butyric acid, and emulsifier enhanced the FCR of broiler chickens. Moreover, Antongiovanni et al. (2007) found that broiler chickens reared on BG mix (mono-, di-, and triglycerides) at 4 different levels (2,000, 3,500, 5,000, and 10,000 mg/kg) had a higher slaughter weight and an improved FCR. The enhanced performance obtained with BG supplementation in the current study might be associated with the positive effects of the butyric acid including; antimicrobial activity against different pathogens, ability to modulate the intestinal microflora, improved gut health and function, increased pancreatic enzyme secretion which consequently improve nutrient digestion and absorption (Leeson et al., 2005; Kaczmarek et al., 2016; Bedford and Gong, 2018; Yang et al., 2018; Letlole et al., 2021). Contradictory findings butyric acid such as enhancing the integrity of the epithelial cell line and the absorptive surface of the intestine through stimulation of intestinal cell proliferation and differentiation, leading to better nutrient utilization, encouraging the gene expression and protein production of tight junctions, making the intestinal epithelial barrier less permeable for pathogens and toxins, which can contribute to intestinal health development and maturity (Peng et al., 2009; Guilloteau et al., 2010). The obtained response suggested that the fat-coated butyrate used in the present study increased release of butyrate in the lower parts of the digestive tract, exerting its antimicrobial function, providing important energy source for enterocytes (Bedford and Gong, 2018; Saleh et al., 2019), helping in improving gut function and nutrient absorption and utilization which was reflected on bird growth. Moreover and supportive to results indicated above, Makowski et al. (2022) found that coated BA or BG dietary addition in turkey positively enhanced their performance which was partly attributed to the improved protein digestibility as a result of increased intestinal absorptive surface area and increased intestinal villi length. Also, Eshak et al. (2016) documented that Na-butyrate encapsulated in palm fat supplementation increased the expression level of insulin-like growth factor-1 (IGF-1) in experimentally induced necrotic enteritis as well as in non-infected birds which could partially contribute to boosted bird growth performance. Unlike the obtained results, Bedford et al. (2017, 2018 a) found that tributyrin (butyrate glyceride) had no growth promoting effect on broiler chickens. Differences between trials might be associated with the BG supplemental level and the duration of feeding.

Additionally, for supporting the obtained results and understanding the mechanism behind the effects of BG on overall performance, the gene expression of some nutrient transporters was assessed. *Glut 2*, and *PepT1* mRNA levels demonstrated higher expression in broilers that received 1000 g BG/ton feed during the whole period; and those fed on different doses (350 + 500 + 1000 g/ton in the starter, grower, and finisher periods) as well as the lincomycin supplemented birds. The nutrient uptake across the plasma membrane is mediated by nutrient transporters. *Glut 2* (glucose transporter 2) is involved in the uptake of fructose and glucose into blood capillaries, in broiler intestine, while *PepT1* is an amino acid transporter. The higher expression of these transporters in the aforementioned groups suggests enhanced uptake of carbohydrates and amino acids in their intestine along with the beneficial impacts of butyric acid derivatives discussed earlier might explain the higher performance obtained. These results are consistent with previous studies revealing that butyric acid upregulated the expression and activity of *SGLT1*, and *GLUT2*, and *PepT1* transporters in different species including mice, grass carp and broiler chickens (Dalmasso et al., 2008; Liu et al., 2017; Raza et al., 2019; Saleh et al., 2018, 2020). In addition, the enhanced broiler performance with lincomycin sup-

plementation was consistent with what was reported in earlier trials (Sun et al., 2005; Mohamed, 2016; Saleh et al., 2022).

Furthermore, birds that received LM or BG showed no differences in their carcass traits or internal organs relative weights. These results are in line with Antongiovanni et al. (2007) who reported no advantage of butyrin supplementation on broiler carcass traits including dressing percentage, breast, thigh, drumstick, and abdominal fat weights. Contradictory findings were obtained with Leeson et al. (2005) who found that carcass weight and breast meat were increased in response to BG supplementation in broiler diets. Also, different forms of BG with different supplemental levels (mono-butyrin, tributyrin or their mixture) in broiler chickens' diet were associated with lower relative abdominal fat (Yin et al., 2016; Bedford et al., 2017, 2018 a). Consistent and contradictory results in this regard suggested that the effects of BG supplementation on carcass traits and organ weight of broilers are highly variable, and that levels evaluated in the present trial may be not able to promote any differences in organs relative weights.

Alterations in the circulating metabolites profile may partly reflect how dietary interventions affect lipid and energy metabolism. In the current study, dietary BG did not affect serum concentrations of total protein, albumin, glucose and HDL-cholesterol, however, the serum total cholesterol and triglycerides were significantly lower in BG- and LM-fed birds. These results are in line with previous reports addressed BG in broilers diet (Yin et al., 2016; Yang et al., 2018; Saleh et al., 2017), while different response was obtained with Almeida et al. (2021) who reported that serum total cholesterol concentration was higher in broilers diets containing the combination of monolaurin and glycerides of butyric acid. Indeed, Yin et al. (2016), Gu et al. (2022) and Saleh et al. (2019) investigated the modulatory effect of BG on lipid metabolism in broiler chickens. Saleh et al. (2019) documented the up-regulation of genes involved in the reduction of synthesis, storage, transportation and secretion of lipids, and those integrated in the oxidation of ingested lipids and fatty acids in the liver (transcriptional regulators of thyroid hormone responsive (THRSP), early growth response gene-1 (*EGR-1*), peroxisome proliferator-activated receptors (PPAR), lipoprotein lipase (*LPL*)). Besides, Bedford et al. (2018 b) found also lower gene expression of acetyl-CoA carboxylase alpha in the adipose tissue of broilers with SILOhealth 104 supplementation (a commercial product mainly containing mono- and di-glycerides of butyrate) ($P<0.05$), which explained the reduced fat deposition obtained in their study. Considering the expression of lipid metabolism associated genes, *FAS* (fatty acid synthase, an enzyme that regulates fatty acid synthesis), showed higher expression in broiler chickens treated with BG either at 1000 g/ton feed or those receiving BG at 350 + 500 + 1000 g/ton in the starter, grower, and finisher periods in the present study. Similarly, Gu et al. (2022) found higher mRNA expression level of FAS besides *PPARa, PPARg*, and *LPL* in broiler chickens that received dietary tributyrin (TB), indicating that TB contribute to balancing the two major pathways of lipid metabolism inside the body (lipogenesis and lipolysis). Unlike the obtained result, Bedford et al. (2018 b) found no changes in the expression of *FAS* in adipose tissue of birds that received SILOhealth 104 (commercial BG containing product). The higher expression of *FAS* in the current study seems contradictory to the lower serum lipid profile parameters detected with BG supplementation. Only FAS expression was assessed in the current study, which in turn did not give a complete picture of the underlying mechanism that occurred in response to BG supplementation. Therefore, further research is required investigating lipolysis and lipogenesis associated genes with the current study design. Additionally, BG supplementation resulted in lower GOT serum level, which indicates its safety and the absence of any adverse effects on liver functioning.

Furthermore, supplemental LM and BG in broiler diet enhanced their immune response which was reflected in the current study by the increased antibody titer against ND and H9N1 besides lower hepatic MDA level. As observed also, additional BG or LM induced higher gene expression of antioxidant enzymes, *SOD* and *GSH-Px*. Clear response was observed with *GSH-Px* enzyme in birds supplemented with 1000 g BG/ton feed and those that received BG at 350 + 500 + 1000 g/ton in the starter, grower, and finisher periods. Higher expression of *GSH-Px* might be associated with the enhanced immune response observed in these groups. Similarly, Eshak et al. (2016) pointed out the efficacy of Na-butyrate encapsulated in palm fat in improving the immune response displayed as high antibody titer against ND and increased weight of immune organs as bursa in experimentally induced necrotic enteritis as well as in non-infected birds. In the same context, Zhang et al. (2011) found that supplemental sodium butyrate enhanced broiler immune response through lowering the proinflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-) and MDA as well as enhanced the activities of antioxidant enzymes (superoxide dismutase and catalase). The same authors attributed these positive effects on immune response to the ability of BA to inhibit NF-kB activation. In the same context, Sunkara et al. (2011, 2012) documented that butyrate derived products could successfully trigger the broiler immune response and disease resistance through increasing the gene expression of intestinal host defense peptides and reduce the proliferation of bacterial pathogens. Collectively, from these studies outcomes, butyrate products are effective in enhancing immune response.

The nutritional aspects including the lipid profile and fatty acids levels in chicken meat, are of increasing concern to consumers, and can be modified in response to dietary changes (Decuypere and Dierick, 2003; Ebeid and Al-Homidan, 2022). Increasing the PUFAs contents and lowering the saturated fats in chicken meat would

improve its nutritional value and quality which will be reflected on the consumer health. Results presented in Table 7 showed that supplemental BG or LM lowered the palmitic acid while increased the unsaturated fatty acids (UFAs) (oleic, linoleic and linolenic acids) ($P<0.05$). All birds that received BG in their diets showed the highest content of linoleic fatty acid. These results are considered positive as the increase in the PUFAs, and reduction of saturated ones are favorable in healthy and functional food used for human consumption. In the current study, the addition of BG showed no difference in birds internal organs weights, however modified the fatty acid composition of muscle which might be associated with its lipid modulatory role discussed earlier.

Conclusions

According to the results indicated above, supplementing broilers' diets with BG or lincomycin positively affected broiler growth performance, immune response, lipid metabolism and muscle fatty acid profile. These effects were mediated through higher gene expression of nutrient transporters, enhancing antibody titers against Newcastle disease and avian influenza, upregulating GSH-Px antioxidant enzyme expression as well as lowering MDA lipid peroxidation marker. BG could be an effective lincomycin alternative with beneficial effect which was more obvious at the supplemental levels of 350 + 500 + 1000 g/ton in the starter, grower, and finisher broiler diets, respectively.

Conflict of interest

Authors have no conflicts of interest.

Acknowledgments

Authors are thankful to all members of Poultry Production Department, Faculty of Agriculture, Kafrelsheikh University, Egypt for their help during the course of the experiment. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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Received: 26 VII 2023
Accepted: 26 X 2023