Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Oct - Dec 2018 / v.20 / n.4 / 707-716
http://dx.doi.org/10.1590/1806-9061-2018-0787

Effects of Dietary Supplementation with Tributyrin and Coated Sodium Butyrate on Intestinal Morphology, Disaccharidase Activity and Intramuscular Fat of Lipopolysaccharide-Challenged Broilers

■Author(s)

Xiong J[I,II] https://orcid.org/0000-0001-8624-9621
Qiu H[I,II] https://orcid.org/0000-0001-7610-7275
Bi Y[I,II] https://orcid.org/0000-0002-3238-2379
Zhou HL[III] https://orcid.org/0000-0002-2904-677X
Guo S[I,II] https://orcid.org/0000-0003-4852-5124
Ding B[I,II] https://orcid.org/0000-0001-6935-0269

[I] Hubei Collaborative Innovation Center for Animal Nutrition and Feed Safety, Wuhan Polytechnic University, Wuhan 430023, China.
[II] Hubei Key Laboratory of Animal Nutrition and Feed Science, Wuhan Polytechnic University, Wuhan 430023, China.
[III] Department of Bioengineering, Xiangyang Vocational and Technical College, Xiangyang 441050, China.

■Mail Address

Corresponding author e-mail address
Binying Ding
Hubei Collaborative Innovation Center for Animal Nutrition and Feed Safety, 68 Xuefu south road, Changqing Garden, Dongxihu district Wuhan Polytechnic University, Wuhan 430023, China.
Phone: +86-27-83956175
Email: dbying7471@126.com

■Keywords

Broilers, coated sodium butyrate, intestine, lipopolysaccharide, tributyrin.

Submitted: 29/March/2018
Approved: 23/May/2018

ABSTRACT

The objectives of this study were to investigate the effects of tributyrin (TB) and coated sodium butyrate (CSB) on intestinal morphology, disaccharidase activity and intramuscular fat of broilers challenged with lipopolysaccharide (LPS). A total of 160 1-day-old healthy Cobb broilers were randomly allocated into four groups: (1) control; (2) LPS, in which broilers were fed a basal diet and intraperitoneally injected with 500 μg/kg LPS on days 38, 40 and 42; (3) TB, in which LPS-challenged broilers were fed basal diet supplemented with 500 mg/kg TB; and (4) CSB, in which LPS-challenged broilers were fed basal diet supplemented with 877 mg/kg CSB. Addition of TB and CSB inhibited (p<0.05) the decrease in villus height in the duodenum and ileum of LPS-challenged broilers, respectively. Both TB and CSB increased (p<0.05) activity of maltase in the small intestine, and TB increased (p<0.05) activity of isomaltase in the ileum. Additionally, dietary addition of TB and CSB decreased (p<0.05) the content of intramuscular fat. In conclusion, dietary supplementation of TB was more effective than CSB in improving intestinal morphology and disaccharidase activity of LPS-challenged broilers, and they both reduced intramuscular fat in the breast and legs.

INTRODUCTION

Broilers are raised under modern farming conditions characterized as large scale and high intensity stocking, and suffer from considerable stress, with sensitivity to different types of stressor (Yalcin *et al*., 2001). Previous studies have indicated that immunological stress adversely affects production performance and physiological function in the intestine (Kang *et al*., 2014; Li *et al*., 2015). Thus, some feed additive is required to reduce stress from high-intensity stocking, and to increase production performance in broilers. Butyric acid is reported to act as energy sources for animals (Mahdavi & Torki, 2009; Li *et al*., 2015), increase anti-oxidative capacity (Li *et al*., 2015), and reduce expression of pro-inflammatory cytokines (Zhang *et al*., 2011). Hu and Guo (2007) reported that dietary supplementation with 500 mg/kg sodium butyrate enhanced growth performance and stimulated growth of duodenal mucosa in broilers. Other studies have shown that dietary butyrate supplementation improves growth performance, enhances immune function (Jang *et al*., 2017; Bedford & Gong, 2017), reduces production of pro-inflammatory cytokine such as interleukin (IL)-1β and IL-6 (Li *et al*., 2015), modulates intestinal microbiota (Yang et al., 2018) and promotes intestinal development (Mazzoni *et al*., 2008; Zhang *et al*., 2011). Nonetheless, butyrate is hardly used in animal production due to the fact that it is volatile and strong smelling, and quickly absorbed in the upper part of the digestive tract.

Therefore, new forms of butyrate products including tributyrin (TB) and microencapsulated butyrate have been developed to facilitate its application in animal feed. TB is degraded by intestinal lipase to release butyrate, which affects intestinal morphology and is then absorbed by the small intestine (Li *et al.*, 2015). Coated sodium butyrate (CSB) can pass through the stomach and influence the hind gastrointestinal tract by sustained release of butyrate (Van Immerseel *et al.*, 2004). TB and CSB may be differentiated by their butyrate release rate in the gastrointestinal tract, which may lead to different effects on intestinal function.

Lipopolysaccharide (LPS) is a membrane glycolipid typically found in Gram-negative bacteria that can cause intestinal damage, immune response and physiological changes, and is usually used as a tool to achieve disease conditions in animals. We used a model of LPS-challenged broilers to hypothesize that dietary TB and CSB supplementation would affect intestinal morphology and disaccharidase activities, and change blood lipid profile, thus affecting muscle lipid metabolism. This was because oral butyrate is reported to decrease fat synthesis by downregulation of insulin receptor β, phosphatidyl-inositol-3-kinase and atypical protein kinase Cζ in live broiler chicks (Mátis *et al.*, 2015).

The objective of the present study was to investigate the effects of dietary supplementation of TB and CSB on intestinal morphology, disaccharidase activity, and intramuscular fat of LPS-challenged broilers. Also, we determined the changes in DNA and protein levels of intestinal mucosa and blood lipid content in broilers.

MATERIALS AND METHODS

Experimental Procedure

The experiment was conducted for 42 days at the Animal Center of Wuhan Polytechnic University, China, and was in accordance with the guidelines established by the Animal Care and Use Committee of Hubei Province.

A total of 160 (80 male + 80 female) 1-day-old healthy Cobb chicks (initial body weight 45.1±0.5 g; Wuhan Chia Tai Co. Ltd., China) were allocated into 16 stainless-steel cages with 10 birds (5 male + 5 female) per cage. Each cage was 200 × 140 × 70 cm (length × width × height). The room temperature was set by air conditioner at 33, 31 and 25°C during the first, second and remaining weeks, respectively. The chickens had free access to feed and water during the feeding trial. However, the feed intake was restricted to 150 g/day for broilers at 38, 40 and 42 days of age, which aimed to unify nutrient intake for chickens with all treatments. The amount of feed intake was determined according to Cobb broiler management guide (Beijing Poultry Breeding, 2014). Diets were formulated according to the nutrient requirements (National Research Council, 1994) for broiler chickens. Diet composition in the basal diet is shown in Table 1. Body weight and feed intake of broilers were recorded weekly.

Table 1 – Ingredients and nutritional composition of the basal diet.

Item	Period	
	Week 1 to 3	Week 4-6
Ingredients (%)		
Corn	53.81	61.25
SBM, 44	34.90	26.75
Wheat middling	5.00	5.00
Soybean oil	2.00	2.60
DL-methionine	0.24	0.25
L-Lysine	-	0.22
Salt	0.41	0.37
Limestone	1.60	1.63
Dicalcium phosphate	1.14	0.93
Premix[I]	1.00	1.00
Total	100	100
Nutrition composition (%)[II]		
Metabolisable energy (MJ/kg)	12.10	12.53
Crude protein	19.50	16.78
Methionine	0.53	0.50
Methionine+cystine	0.84	0.78
Lysine	1.04	1.07
Tryptophane	0.23	0.19
Threonine	0.74	0.64
Calcium	0.91	0.87
Available phosphorus	0.40	0.34

[I]Formulated to provide (per kg of diet): 50 000 IU vitamin A, 13 750 IU vitamin D_3, 100 of vitamin E, 10 mg vitamin K, 11 mg vitamin B1, 30 mg vitamin B2, 300 mg vitamin B3, 55 mg vitamin B5, 20 mg vitamin B6, 0.75 mg vitamin B7, 0.05 mg vitamin B12, 5000 mg choline chloride, 75 mg manganese, 50 mg zinc, 80 mg iron, 7.5 mg copper, 0.3 mg iodine, and 0.2 mg selenium.

[II]Calculated value.

The broilers randomly received one of four dietary treatments with four replicates of 10 birds (5 male + 5 female). Four treatment groups were as follows: (1) control, ingesting the basal diet and receiving an intraperitoneal injection of sterile saline; (2) LPS, ingesting the basal diet and receiving an intraperitoneal injection of LPS (L2880; Sigma, St Louis, MO, USA); (3) TB, ingesting the basal diet supplemented with 500 mg/kg 45% TB (Wuhan Pan-China Biotechnology Co. Ltd., Wuhan, China), ~194.22 mg/kg butyrate, and receiving an intraperitoneal injection of LPS; and (4) CSB, ingesting the basal diet supplemented with 877 mg/kg 30% CSB (Hangzhou King Techina Feed Co.

Ltd., Hangzhou, China), approximately equivalent to 194.22 mg/kg butyrate and receiving intraperitoneal injection of LPS. The doses of TB and CSB were chosen on the basis that 500 mg/kg sodium butyrate stimulated growth of duodenal mucosa in broilers (Hu & Guo, 2007), and 1000 mg/kg CSB improved growth performance of nursery pigs (Jang *et al*. 2017). On days 38, 40 and 42 of the trial, broilers in the LPS, TB and CSB groups were intraperitoneally administered 500 μg/kg LPS, which was determined according to our previous study (Li *et al*., 2015), and the control group received the same volume of sterile saline. TB and CSB were mixed thoroughly with the feed ingredients.

Broilers were immunized against Newcastle disease and infectious bronchitis at age 7 and 21 days and against infectious bursal disease at 14 and 28 days. The vaccine and immunization program were provided by Pulike Biological Engineering. All practical procedures were approved and supervised by Hubei Province Institutional Animal Care and Use Committee (Wuhan, China).

Sample collection

On day 42 of the trial, a total of 48 blood samples (3 mL) with three samples in each replicate were collected from the wing vein puncture at 4 h after LPS or saline injection, and then centrifuged at 3000 × *g* for 10 min at 4°C to obtain serum, which was frozen at -80°C until later analysis of serum lipid metabolites. On day 43 of the trial, all broilers were euthanized by cervical dislocation to collect pectoralis and thigh muscles for analysis of fat content. Abdominal fat lying in the peritoneum and around the gizzard was collected for determination of abdominal fat ratio. The 2-3-cm segments were collected from the mid-duodenum, mid-jejunum and mid-ileum, and then fixed in 4% paraformaldehyde for histological examination (Hou *et al*., 2010). According to the previous methods (Dahlqvist, 1964), the remaining intestines were opened longitudinally and flushed with cold physiological saline and scraped by a sterile glass microscope slide to obtain mucosae, which were collected in 1.5-mL sterile centrifuge tubes, and then stored at -80°C until later analysis of disaccharidase activity.

Intestinal morphology observation

The intestinal segments soaked in 4% paraformaldehyde were removed within 72 h, cut, and embedded in paraffin. Sections of 4-μm thickness were cut and stained with hematoxylin and eosin. According to the study by Frankel *et al*. (1993), the 10 longest villi with structural integrity, clear dye and smoothness in each section were observed with a microscope using a computer-assisted morphometric system (Optimetric; BioScan Inc., Edmonds, WA, USA). The height of the villi was measured from the villus tip to the valley between adjacent villi, and the crypt depth from the valley between adjacent villi to the basolateral membrane. Each index was expressed with the average values of measured data.

Abdominal and intramuscular fat

The pectoralis and thigh muscles were analysed by Soxhlet extraction method for fat level. The abdominal fat ratio was calculated as follows:

abdominal fat ratio (%) = weight of abdominal fat (g)/ live body weight (g) × 100% (Mahdavi & Torki, 2009)

DNA and protein in intestinal mucosa

Intestinal mucosa was frozen in liquid nitrogen and ground into powder, which was then homogenised by a microhomogenizer (T 10 basic ULTRA-TURRAX®, IKA Works Guangzhou, Guangdong, China) for 2 min at cold physiological saline (w/v, 1:9). The homogenate was centrifuged at 3000 × *g* for 10 min at 4°C to obtain the supernatant, which was again diluted with physiological saline at a ratio of 1:9 (w/v) and obtained 1% mucosal homogenate. The homogenate was analysed for protein content using the Coomassie Brilliant Blue method described by Bradford (1976). DNA was extracted from the same 1% mucosal homogenate as described by Johnson and Chandler (1973), and the concentration was analysed with ultraviolet visible spectrophotometry. Finally, the measurement was used for calculating the total protein (TP)/DNA ratio.

Measurement of disaccharidase activity

Disaccharidase activity was detected using the glucose oxidase–peroxidase coupling method as described by Zhu *et al*. (2014). The 10% mucosal homogenate was centrifuged at 3000 × *g* for 10 min at 4°C to obtain the supernatant, and 100 μL diluted supernatant was incubated with the same volume of 0.056 mol/L sucrose, maltose or isomaltose at 37°C for 1 h. The enzymatic reaction was terminated in a boiling water bath for 2 min. The released glucose was determined by commercial kits (Shanghai Rongsheng Biotech Co. Ltd., Shanghai, China). Coomassie Brilliant Blue G-250 reagent was used for determination of protein concentrations with bovine serum albumin as

a standard. Disaccharidase activity was expressed as U/mg protein.

Measurement of lipid metabolites in serum

The content of triglyceride (TG), total cholesterol (TCHO), high-density lipoprotein–cholesterol (HDL-C) and low-density lipoprotein–cholesterol (LDL-C) was determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The enzymolysis approach was used for analysis of TCHO and TG, and the selective precipitation method for HDL-C and LDL-C.

Statistical analysis

The data were analysed by one-way analysis of variance using SPSS version 19.0 software (SPSS, 2010; SPSS, Inc., Chicago, IL). Differences among treatment means were determined by Tukey's multiple range test. The level of confidence required for significance was set at $p<0.05$.

RESULTS

Growth performance

During the 42-day experimental period, the daily feed intake for the control, LPS, TB and CSB groups averaged 98.53 ± 2.12, 96.61 ± 2.76, 99.24 ± 2.14 and 97.93 ± 2.51 g/day, respectively, and daily gain averaged 47.37 ± 1.83, 45.18 ± 2.03, 47.59 ± 2.18 and 46.18 ± 1.87 g/day, respectively. There was no significant difference in growth performance among the four groups (data not shown).

Intestinal morphology

Compared with the control group, LPS challenge decreased ($p<0.05$) the villus height in the duodenum and ileum (Table 2). However, compared with the LPS group, challenged birds fed CSB- and TB-supplemented diets had higher villus height in the duodenum and ileum, respectively ($p<0.05$). The jejunal villus height was not significantly affected by treatment.

Table 2 – Effect of treatment on intestinal morphology of broilers.

Items	Control Group	LPS Group	TB Group	CSB Group
Villus height,μm				
Duodenum	1032.77 ± 6.70a	834.73 ± 14.87c	893.70 ± 69.48bc	935.67 ± 32.71b
Jejunum	685.73 ± 8.42	698.47 ± 18.24	696.43 ± 24.56	711.53 ± 6.75
Ileum	524.83 ± 2.31b	479.13 ± 1.93c	642.93 ± 45.33a	463.67 ± 15.63c
Crypt depth,μm				
Duodenum	206.83 ± 10.01c	285.67 ± 5.55b	323.03 ± 21.77a	285.03 ± 16.92b
Jejunum	206.13 ± 6.25c	235.00 ± 24.98a	238.41 ± 8.97a	246.87 ± 5.46a
Ileum	156.23 ± 3.72c	182.57 ± 0.64b	215.03 ± 10.86a	151.07 ± 8.45c
Villus: Crypt				
Duodenum	5.37 ± 0.07a	3.07 ± 0.11b	2.88 ± 0.68b	3.19 ± 0.02b
Jejunum	3.34 ± 0.07a	3.12 ± 0.17b	2.91 ± 0.15bc	2.77 ± 0.01c
Ileum	3.34 ± 0.08a	2.86 ± 0.07c	3.16 ± 0.07ab	3.10 ± 0.20b

Control Group, ingesting the basal diet and receiving an intraperitoneal injection of sterile saline. LPS Group=Lipopolysaccharide group, ingesting the basal diet and receiving an intraperitoneal injection of LPS. TB Group=Tributyrin Group, ingesting the basal diet supplemented with 500 mg/kg TB. CSB Group=Coated sodium butyrate Group, ingesting the basal diet supplemented with 877 mg/kg CSB. Within rows, values with different letters are statistically different ($p<0.05$), and values without letter are not significant ($p>0.05$).

The LPS-challenged birds had greater ($p<0.05$) duodenal, jejunal and ileal crypt depth than that in the control group. Only CSB supplementation decreased ($p<0.05$) the ileal crypt depth in the challenged birds. Compared with the control group, LPS challenge decreased ($p<0.05$) the ratio of villus height to crypt depth in the duodenum, jejunum and ileum. Both TB and CSB supplementation increased ($p<0.05$) the ratio of villus height to crypt depth in the ileum of challenged birds.

DNA and protein levels in intestinal mucosa

There were no significant differences in the intestinal protein content or the DNA content in the duodenum and ileum ($p>0.05$) (Table 3). Compared with the control group, LPS challenge decreased ($p<0.05$) the DNA content in the jejunum. However, the CSB group had higher ($p<0.05$) jejunal DNA content than the control and LPS groups had. Compared with the control group, LPS challenge increased ($p<0.05$) the ratio of protein to DNA in the jejunum and ileum. TB supplementation reduced ($p<0.05$) this ratio only in the ileum, and CSB decreased ($p<0.05$) the ratio in both jejunum and ileum.

Table 3 – Effect of treatments on the protein and DNA levels and the protein/DNA ratio in the intestine of broilers.

Items	Control Group	LPS Group	TB Group	CSB Group
Protein (mg/g)				
Duodenum	75.02 ± 2.31	66.24 ± 1.75	66.47 ± 5.60	69.79 ± 4.93
Jejunum	70.04 ± 4.44	75.85 ± 1.72	72.61 ± 2.42	71.38 ± 3.60
Ileum	63.59 ± 6.21	73.12 ± 5.32	66.45 ± 6.36	67.49 ± 5.07
DNA(mg/g)				
Duodenum	0.54 ± 0.05	0.54 ± 0.02	0.50 ± 0.02	0.54 ± 0.03
Jejunum	0.50 ± 0.02b	0.45 ± 0.02c	0.47 ± 0.02bc	0.55 ± 0.03a
Ileum	0.48 ± 0.04	0.46 ± 0.04	0.51 ± 0.06	0.53 ± 0.02
Protein/DNA				
Duodenum	141.24 ± 11.31	144.64 ± 5.95	142.48 ± 13.51	144.01 ± 17.81
Jejunum	144.48 ± 4.26b	157.78 ± 8.58a	158.76 ± 2.13a	130.14 ± 1.65c
Ileum	137 ± 6.12b	159 ± 9.13a	134 ± 4.36b	136 ± 6.87b

Control Group, ingesting the basal diet and receiving an intraperitoneal injection of sterile saline. LPS Group=Lipopolysaccharide group, ingesting the basal diet and receiving an intraperitoneal injection of LPS. TB Group=Tributyrin Group, ingesting the basal diet supplemented with 500 mg/kg TB. CSB Group=Coated sodium butyrate Group, ingesting the basal diet supplemented with 877 mg/kg CSB. Within rows, values with different letters are statistically different ($p<0.05$), and values without letter are not significant ($p>0.05$).

Disaccharidase activity

Compared with the control group, LPS challenge decreased ($p<0.05$) the activity of sucrase in the jejunum and ileum (Table 4). Inclusion of TB in the diet increased ($p<0.05$) sucrase activity in the jejunum and ileum of challenged birds, while CSB supplementation elevated ($p<0.05$) ileal sucrase activity. LPS challenge also reduced ($p<0.05$) maltase activity in the three segments of the small intestine. However, the intestinal maltase activity was increased ($p<0.05$) by both TB and CSB supplementation in the challenged birds. LPS challenge decreased ($p<0.05$) isomaltase activity in the ileum and dietary inclusion of TB increased ($p<0.05$) ileal maltase activity.

Table 4 – Effect of treatments on activity of disaccharidase in intestine of broilers.

Items	Control Group	LPS Group	TB Group	CSB Group
Sucrase (U/mg prot)				
Duodenum	0.34 ± 0.03	0.34 ± 0.03	0.32 ± 0.09	0.40 ± 0.05
Jejunum	0.81 ± 0.01b	0.58 ± 0.01c	1.13 ± 0.19a	0.74 ± 0.09bc
Ileum	0.53 ± 0.09a	0.37 ± 0.01b	0.61 ± 0.03a	0.52 ± 0.07a
Maltase (U/mg prot)				
Duodenum	2.21 ± 0.14a	1.76 ± 0.04b	2.19 ± 0.06a	2.38 ± 0.42a
Jejunum	3.46 ± 0.46b	2.55 ± 0.28c	4.48 ± 0.23a	3.21 ± 0.34b
Ileum	3.75 ± 0.55a	2.38 ± 0.34b	3.87 ± 0.47a	3.51 ± 0.66a
Isomaltase (U/mg prot)				
Duodenum	21.70 ± 1.22	25.10 ± 2.36	22.60 ± 2.62	21.67 ± 2.98
Jejunum	24.56 ± 1.85	22.24 ± 0.57	23.04 ± 0.53	22.73 ± 1.29
Ileum	28.32 ± 3.68ab	23.24 ± 2.15b	29.24 ± 2.06a	23.86 ± 2.20b

Control Group, ingesting the basal diet and receiving an intraperitoneal injection of sterile saline. LPS Group=Lipopolysaccharide group, ingesting the basal diet and receiving an intraperitoneal injection of LPS. TB Group=Tributyrin Group, ingesting the basal diet supplemented with 500 mg/kg TB. CSB Group=Coated sodium butyrate Group, ingesting the basal diet supplemented with 877 mg/kg CSB. Within rows, values with different letters are statistically different ($p<0.05$), and values without letter are not significant ($p>0.05$).

Blood lipid content

LPS administration decreased ($p<0.05$) TCHO and HDL-C concentration in the serum and increased ($p<0.05$) TG level (Table 5). Compared with the LPS

Table 5 – Effect of treatments on blood lipids of broilers.

Items	Control Group	LPS Group	TB Group	CSB Group
HDL-C (mmol/L)	2.567 ± 0.122a	2.278 ± 0.248b	2.710 ± 0.073a	2.283 ± 0.067b
LDL-C (mmol/L)	1.010 ± 0.232	0.982 ± 0.267	0.611 ± 0.060	0.801 ± 0.061
TCHO (mmol/L)	3.684 ± 0.191a	2.824 ± 0.184b	3.594 ± 0.040a	3.459 ± 0.087a
TG (mmol/L)	0.136 ± 0.012b	0.216 ± 0.005a	0.136 ± 0.004b	0.131 ± 0.009b

Control Group, ingesting the basal diet and receiving an intraperitoneal injection of sterile saline. LPS Group=Lipopolysaccharide group, ingesting the basal diet and receiving an intraperitoneal injection of LPS. TB Group=Tributyrin Group, ingesting the basal diet supplemented with 500 mg/kg TB. CSB Group=Coated sodium butyrate Group, ingesting the basal diet supplemented with 877 mg/kg CSB. HDL-C=high density lipoprotein-cholesterol. LDL-C=low density lipoprotein-cholesterol. TCHO=total cholesterol. TG=triglyceride. Within rows, values with different letters are statistically different ($p< 0.05$), and values without letter are not significant ($p>0.05$).

group, dietary TB supplementation increased (p<0.05) serum HDL-C level, and both TB and CSB elevated (p<0.05) serum TCHO level and decreased (p<0.05) TG.

Fat accumulation in carcass

The intramuscular fat content of the breast in the TB and CSB groups was lower (p<0.05) than that in the LPS group and did not significantly differ from that in the control group (Table 6). LPS challenge increased (p<0.05) intramuscular fat content of the legs, which was decreased by both TB and CSB (p<0.05). The abdominal fat was not significantly affected by the treatments (p>0.05).

DISCUSSION

Butyrate and its derivatives have aroused interests among researchers as they improve animal production. Hu and Guo (2007) found that sodium butyrate improved growth performance, facilitated the growth of duodenal mucosa, and inhibited lactobacilli in broilers. Other studies have demonstrated the effects of sodium butyrate on growth performance, immune state, energy state and mucosal composition in chickens or pigs (Manzanilla *et al*., 2006; Claus *et al*., 2007; Mazzoni *et al*., 2008; Jang *et al*. 2017). In our study, dietary supplementation with TB and CSB

Table 6 – Effect of treatments on deposition of abdominal and intramuscular fat of broilers.

Items	Control Group	LPS Group	TB Group	CSB Group
Abdominal fat (%)	1.35 ± 0.31	1.55 ± 0.05	1.37 ± 0.11	1.16 ± 0.15
Intramuscular fat in breast (%)	2.20 ± 0.32ab	2.98 ± 0.78a	1.64 ± 0.20b	1.83 ± 0.08b
Intramuscular fat in leg (%)	7.71 ± 0.75bc	12.52 ± 0.33a	8.82 ± 0.83b	6.68 ± 0.58c

Control Group, ingesting the basal diet and receiving an intraperitoneal injection of sterile saline. LPS Group=Lipopolysaccharide group, ingesting the basal diet and receiving an intraperitoneal injection of LPS. TB Group=Tributyrin Group, ingesting the basal diet supplemented with 500 mg/kg TB. CSB Group=Coated sodium butyrate Group, ingesting the basal diet supplemented with 877 mg/kg CSB. Within rows, values with different letters are statistically different ($p<0.05$), and values without letter are not significant ($p>0.05$).

at levels equivalent to 194.22 mg/kg butyrate did not affect feed intake and daily gain, which was in accordance with dietary supplementation with 500 mg/kg TB in the study by Li *et al*. (2015). Bedford *et al*. (2017a, b; 2018) also reported that butyrate glyceride supplementation did not affect average daily gain or feed:gain in broidler. The reason for this may be the low level of TB and CSB supplementation. However, Li *et al*. (2015) did find that TB affected intestinal function and immune response. We also further studied the effect of TB and CSB on intestinal morphology, digestive enzyme activity, blood lipids and intramuscular fat.

The villus height and crypt depth in the small intestine are related to nutrient adsorption (Manzanilla *et al*., 2006), and the villus:crypt ratio is in proportion to the capacity for digestion and absorption (Hou *et al*., 2010). In this study, both villus height and villus:crypt ratio decreased in the duodenum and ileum, and the villus:crypt ratio also reduced in the jejunum, but the crypt depth increased in the duodenum, jejunum and ileum of LPS-challenged broilers. Li *et al*. (2015) also found that LPS decreased the villus height and villus:crypt ratio in the duodenum and increased the crypt depth of the ileum. Zhang *et al*. (2012) showed that duodenal and jejunal villus height decreased in early LPS-challenged chicks. However, Li *et al*. (2015) did not observe that LPS affected the jejunum, which was not consistent with our study. The reason for this may be related to the time of LPS administration (day 22–26 vs. 38–42). The addition of 500 mg/kg TB did increase both villus height and villus:crypt depth ratio in the ileum as compared with LPS-only administration and CSB supplementation, which showed that TB was more effective than CSB in alleviating the harmful effect of LPS. The different effect of TB and CSB on the intestine may be related to the concentration of released butyrate. Davis (1930) has indicated that the average digestibility of TB in poultry was ~86.9%, whereas the butyrate-released rate of CSB was 41.6% in the artificial small intestine (Chen *et al*., 2011).

The reduction of mucosal protein and DNA level, which is related to cell growth and repair, suggests reduced anabolism in enterocytes (Sukhotnik *et al*., 2004). The protein: DNA ratio has been used as an indicator of cell hypertrophy (Jin *et al*., 1994). In the present study, the LPS challenge decreased the DNA content in the jejunum and increased the protein: DNA ratio in the jejunum and ileum, which showed damage in enterocytes. The addition of TB and CSB decreased the ratio of protein to DNA, which showed that TB and CSB alleviate mucosal damage.

Disaccharides are hydrolysed by intestinal disaccharidase into monosaccharides that are adsorbed in the small intestine. Claus *et al*. (2007) indicated that the disaccharidase level is in direct proportion to the digestive activity of the small intestine. In the present study, the activities of sucrase and maltase decreased but the activity of isomaltase was not

affected in the jejunum and ileum of LPS-challenged broilers, indicating type-specific disaccharidase in response to LPS challenge. In the present study, dietary supplementation with TB or CSB significantly increased the activity of maltase in the small intestine, and TB also increased isomaltase activity in the ileum. Our study showed that TB elevated disaccharidase activity in a wider range of the intestine, compared with the previous study (Li *et al*., 2015), showing that TB only increased sucrase activity in the duodenum of broilers, which may be related to the consecutive days of TB administration. Previous studies have indicated that butyrate enhances sucrase activity in swine, alleviates the reduction of sucrase activity caused by LPS (Claus *et al*., 2007), and favours disaccharidase secretion in intestinal epithelial cells (Fusunyan *et al*., 1999). Based on this, the inclusion of TB or CSB in the diet may improve the digestive activities of the small intestine in broilers.

TCHO, in relation to animal health, includes HDL-C (transfers peripheral blood cholesterol into the liver) and LDL-C (transfers cholesterol synthesised in the liver to peripheral tissue). LPS decreases HDL level in the blood through reducing synthesis of core carrier proteins (Hardardottir, 1997), which is in accordance with our study. A high level of TG may induce ascites syndrome (Yang *et al*., 2007). Our study showed that LPS injection decreased serum HDL-C level, indicating that LPS restrains metabolism of blood cholesterol in the liver. However, dietary TB supplementation significantly elevated serum HDL-C level, which may be related to butyrate induction of phospholipid transfer protein. Other studies have shown that phospholipid transfer protein, in proportion to HDL-C, is induced by butyrate (Guo *et al*., 1999; Albers *et al*., 1996). However, dietary CSB supplementation did not alleviate the decrease of serum HDL-C caused by LPS, which may be related to intestinal absorption and the transfer route of CSB. Serum TCHO level increased after intraperitoneal injection (on days 21, 23 and 25) of 250 or 500 μg/kg LPS in broilers, but 1 mg/kg did not cause any further significant change, compared with sterile saline administration (Dong *et al*., 2007). In contrast, our study found that LPS administration decreased serum TCHO concentration. Serum TCHO in response to LPS stress may be associated with time of administration and dose of LPS. The underlying mechanisms need to be further studied. Dietary TB and CSB supplementation can alleviate the decrease of serum TCHO caused by LPS, indicating that butyrate released from TB and CSB may ameliorate LPS stress.

In the present study, LPS administration increased serum TG, which is consistent with the study of Dong *et al*. (2007). Feingold et al. (1999) has found that LPS can significantly decrease expression of hepatic lipase mRNA, which accelerates hydroxylation of TG in mice (75% reduction) and hepatic cells, indicating that LPS decreases serum TG level. Our study showed that dietary supplementation of TB and CSB lowered serum TG in LPS-challenged broilers. Liu et al. (2007) has reported that short-chain fatty acid, including butyrate, reduces serum insulin level, which then increases activity of lipoprotein lipase and accelerates TG degradation.

A higher level of abdominal fat decreases productivity and adversely affects carcass quality of broilers. Intramuscular fat is strongly related to meat quality and flavour of chickens. Metabolic activity of broilers upon exposure to stress shows decreased protein synthesis but increased fat deposition (Siegel *et al*., 1984). Ain *et al*. (1996) found that chronic heat exposure enhanced abdominal fat deposition, and modified muscle and fat partition in broiler carcasses. As a stressor, LPS administration does not change the abdominal fat percentage, but significantly enhances intramuscular fat in breast and legs. Dong *et al*. (2007) have also shown that LPS stress significantly increases the fat content of breast muscle. This might be because LPS stress significantly increases serum TG content and then promotes fat deposition.

In the present study, the dietary supplementation of TB and CSB decreased the intramuscular fat in breast and legs, compared with the LPS group, indicating that they alleviate the adverse effect of fat deposition induced by LPS stress. Butyrate released from TB and CSB is absorbed into the blood and participates in gluconeogenesis and the formation of ketone bodies, which indirectly affect lipid metabolism (Engelhard *et al*., 1997). The addition of TB and CSB reduces intramuscular fat percentage, which may be related to a decrease in serum TG. Yin *et al*. (2016) demonstrated that butyrate glycerides reduces body fat deposition via regulation of gene expression for lipid catabolism in broilers. The exact mechanism for this need to be further investigated.

In conclusion, dietary supplementation with TB (500 mg/kg) was more effective than CSB (877 mg/kg) in improving intestinal morphology and disaccharidase activity of LPS-challenged broilers, and they both reduced intramuscular fat in breast and legs. A full understanding of the underlying mechanisms requires further study.

ACKNOWLEGEMENTS

This work was financially supported by Hubei Provincial Technology and Innovation Program (2016ABA121) and by the Research Program of the Education Department of Hubei Province (B2016078).

REFERENCES

Ain BH, Geraert PA, Padilha JC, Guillaumin S. Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcasses. Poultry Science 1996;75:505-513.

Albers JJ, Tu AY, Paigen B, Chen H, Cheung MC, Marcovina SM. Transgenic mice expressing human phospholipid transfer protein have increased HDL/non-HDL cholesterol ratio. International Journal of Clinical and Laboratory Research 1996;26:262-267.

Bedford A, Gong J. Implications of butyrate and its derivatives for gut health and animal production. Animal Nutrition 2017;4(2):151-159.

Bedford A, Yu H, Hernandez M, Squires EJ, Leeson S, Gong J. Effects of fatty acid glyceride product SILOhealth 104 on the growth performance and carcass composition of broiler chickens. Poultry Science 2018;97:1315–1323.

Bedford A, Yu H, Hernandez M, Squires EJ, Leeson S, Hou Y, Gong J. Response of Ross 308 and 708 broiler strains in growth performance and lipid metabolism to diets containing tributyrate glycerides. Canadian Journal of Animal Science 2017b;98:98-108.

Bedford A, Yu H, Squires EJ, Leeson S, Gong J. Effects of supplementation level and feeding schedule of butyrate glycerides on the growth performance and carcass composition of broiler chickens. Poultry Science 2017a;96:3221–3228.

Beijing poultry breeding. The revised edition of Cobb broiler management guide. Beijing: Beijing Poultry Breeding; 2014.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 1976;72:248–254.

Chen GS, Xu ZF, Yu R, Zhao FF, Xu XF. Investigation on stability of different types of sodium butyrate in the process of production and processing. Chinese Journal of Animal Science 2011;47:62-66.

Claus R, Günthner D, Letzguss H. Effects of feeding fat-coated butyrate on mucosal morphology and function in the small intestine of the pig. Journal of Animal Physiology & Animal Nutrition 2007;91:312-318.

Dahlqvist A. Method for assay of intestinal disaccharidases. Analytical Biochemistry 1964;7:18-25.

Davis RE. The metabolism of tributyrin. Journal of Biological Chemistry 1930;88:67-75.

Dong XL, Liu GH, Cai HY, Zheng AJ, Zhang S, Chen GL, et al. Effect of acute immunological stress induced by LPS on meat quality of broilers. Chinese Journal of Animal Nutrition 2007;19:622-626.

Engelhard HH, Duncan HA, Dal CM. Molecular characterization of glioblastoma cell differentiation. Neurosurgery 1997;41:886-897.

Feingold KR, Memon RA, Moser AH, Shigenaga JK, Grunfeld C. Endotoxin and interleukin-1 decrease hepatic lipase mRNA levels. Atherosclerosis 1999;142:379-387.

Frankel WL, Zhang W, Afonso J, Klurfeld DM, Don SH, Laitin E, et al. Glutamine enhancement of structure and function in transplanted small intestine in the rat. Journal of Parenteral and Enteral Nutrition 1993;17:47-55.

Fusunyan RD, Quinn JJ, Fujimoto M, Macdermott RP, Ian RS. Butyrate switches the pattern of chemokine secretion by intestinal epithelial cells through histone acetylation. Molecular Medicine 1999;5:631-640.

Guo Z, Yuan C, Weilavery T, Fang Y, Garvin RA, Nishida HI, et al. Secretion of phospholipid transfer protein by human hepatoma cell line, Hep G2, is enhanced by sodium butyrate. Journal of Nutrition 1999;129:1984-1991.

Hardardottir I, Sipe J, Maser AH. LPS and cytokines regulate extra hepatic mRNA levels of apolipoprotein during the acute phase response in Syrian hamsters. Biochimica et Biophysica Acta 1997;1344:210-220

Hou Y, Wang L, Ding B, Liu Y, Zhu H, Liu J, et al. Dietary α-ketoglutarate supplementation ameliorates intestinal injury in lipopolysaccharide-challenged piglets. Amino Acids 2010;39:555-564.

Hu ZH, Guo YM. Effects of dietary sodium butyrate supplementation on the intestinal morphological structure, absorptive function and gut flora in chickens. Animal Feed Science and Technology 2007;132:240–249

Jang YD, Lindemann MD, Monegue HJ, Monegue JS. The effect of coated sodium butyrate supplementation in sow and nursery diets on lactation performance and nursery pig growth performance. Livestock Science 2017;195:13-20.

Jin L, Reynolds LP, Redmer DA, Caton JS, Crenshaw JD. Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. Journal of Animal Science 1994;72:2270-2278.

Johnson LR, Chandler AM. RNA and DNA of gastric and duodenal mucosa in antrectomized and gastrin-treated rats. American Journal of Physiology 1973; 224:937-940.

Kang P, Zhang LL, Hou YQ, Ding BY, Yi D, Wang L, et al. Effects of L-proline on the Growth Performance, and Blood Parameters in Weaned Lipopolysaccharide (LPS)-challenged Pigs. Asian-Australasian Journal of Animal Sciences 2014;27:1150-1156.

Li JL, Hou YQ, Yi D, Zhang J, Wang L, Qiu HY, et al. Effects of tributyrin on intestinal energy status, antioxidative capacity and immune response to lipopolysaccharide challenge in broilers. Asian-Australasian Journal of Animal Sciences 2015;28:1784-1793.

Liu YX, Hu ZH, Guo YM. The effect of SCFAs combination on growth performance of broiler chickens and its mechanism. Chinese Feed Industry 2007;28:42-45.

Mahdavi R, Torki M. Study on usage period of dietary protected butyric acid on performance, carcass characteristics, serum metabolite levels and humoral immune response of broiler chickens. Journal of animal and veterinary advances 2009;8:1702-1709.

Manzanilla EG, Nofrarías M, Anguita M, Castillo M, Perez JF, Martín-Orúe SM, et al. Effects of butyrate, avilamycin, and a plant extract combination on the intestinal equilibrium of early-weaned pigs. Journal of Animal Science 2006;84:2743-2751.

Mátis G, Kulcsár A, Turowsk V, Fébel H, Neogrády Zs, Huber K. Effects of oral butyrate application on insulin signaling in various tissues of chickens. Domestic Animal Endocrinology 2015;50:26-31.

Mazzoni M, Le GM, De FS, Minieri L, Trevisi P, Wolinski J, et al. Supplemental sodium butyrate stimulates different gastric cells in weaned pigs. Journal of Nutrition 2008;138:1426-1431.

National Research Council. Nutrient requirements of poultry. 9th ed. Washington: National Academy Press; 1994.

Siegel HS, Van Kampen M. Energy relationships in growing chickens given daily injections of corticosterone. British Poultry Science 1984;25:477-485.

Sukhotnik I, Mogilner J, Krausz MM, Lurie M, Hirsh M, Coran AG. Oral arginine reduces gut mucosal injury caused by lipopolysaccharide endotoxemia in rat. Journal of Surgical Research 2004;122:256-262.

Van Immerseel F, Fievez V, Buck J, Pasmans F, Martel A, Haesebrouck F, et al. Microencapsulated short-chain fatty acids in feed modify colonization and invasion early after infection with Salmonella enteritidis in young chickens. Poultry Science 2004;83:69-74.

Yalcin S, Özkan S, Türkmut L, Siegel PB. Responses to heat stress in commercial and local broiler stocks. 1. Performance traits. British Poultry Science 2001;42:149-152.

Yang X, Yin F, Yang Y, Lepp D, Yu H, Ruan Z, et al. Dietary butyrate glycerides modulate intestinal microbiota composition and serum metabolites in broilers. Scientific Reports 2018;8:4940.

Yang XJ, Yang Y, He X, Yuan JM, Wang Z, Guo YM. Dietary different kinds of polyunsaturated fatty acids improve lipid metabolism in broiler chicks. In: Advances in poultry nutrition and feed science. Beijing: China agricultural Science and Technology Press; 2007.

Yin F, Yu H, Lepp D, Shi X, Yang X, Hu J, et al. Transcriptome analysis reveals regulation of gene expression for lipid catabolism in young broilers by butyrate glycerides. PLoS ONE 2016;11:e0160751.

Zhang WH, Jiang Y, Zhu QF, Gao F, Dai SF, Chen J, et al. Sodium butyrate maintains growth performance by regulating the immune response in broiler chickens. British Poultry Science 2011;52:292-301.

Zhang X, Zhao L, Cao F, Ahmad H, Wang G, Wang T. Effects of feeding fermented Ginkgo biloba leaves on small intestinal morphology, absorption, and immunomodulation of early lipopolysaccharide-challenged chicks. Poultry Science 2012;92:119-130.

Zhu HL, Hu LL, Hou YQ, Zhang J, Ding BY. The effects of enzyme supplementation on performance and digestive parameters of broilers fed corn-soybean diets. Poultry Science 2014;93:1704-1712.