https://doi.org/10.17221/190/2019-CJAS

Sodium butyrate enhances growth performance and intestinal development in broilers

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Citation: Lan R.X., Li S.Q., Chang Q.Q., An L.L., Zhao Z.H. (2020): Sodium butyrate enhances growth performance and intestinal development in broilers. Czech J. Anim. Sci., 65: 1–12.

Abstract: The aim of this study was to evaluate the effects of sodium butyrate (SB) on growth performance and development of digestive and immune organs in broilers. Dietary treatments had similar compositions but with 0%, 0.03%, 0.06%, and 0.12% SB substituted (weight/weight) for identical amounts of the basal diet. SB supplementation linearly increased ($P < 0.05$) average daily gain for each period, except for days 15–21. SB supplementation linearly increased ($P < 0.05$) the relative weight of proventriculus (day 7), gizzard (days 7 and 14), duodenum (days 21 and 28), jejunum (day 21), ileum (day 21), small intestine (day 21), rectum (day 14), pancreas (days 7 and 21), liver (days 21 and 28), and thymus (days 7, 14, and 21). SB supplementation linearly increased ($P < 0.05$) the relative length of duodenum (day 21), jejunum (days 14 and 21), ileum (days 14 and 21) and small intestine (days 14 and 21), caeca (day 21) and rectum (day 21), as well as it improved intestinal structure by increasing the villus height in jejunum and ileum, and increasing goblet cell counts in duodenum, jejunum, and ileum. Collectively, dietary SB supplementation improved the growth performance of broilers by improving the development and morphological structure of the broilers' intestinal organs.

Keywords: relative weight; relative length; digestive organs; immune organs; broiler

For decades, antibiotics have been used as growth promoters to improve growth performance of poultry because of the low cost of implementation (Fernandez-Rubio et al. 2009). The ban on the use of antibiotic growth promoters (AGP) in feed has forced nutritionists to find alternatives that lack the issues associated with antibiotics, but maintain the positive effects on the functions of the gastrointestinal tract and immune system to improve the digestive efficiency and health status of broilers. Short-chain fatty acids (SCFA) and their salts are considered promising alternatives to AGP (Adil et al. 2011), because a dietary acid lowers the pH of feed and digesta (Olukosi and Dono 2014), and the reduced pH can inhibit the proliferation of acid-intolerant pathogenic bacteria (Islam 2012). Sodium butyrate (SB) has received much attention due to its positive effects on growth performance, intestinal integrity, stimulation of intestinal immune function, inhibition of the growth of pathogens, and enhancement of intestinal barrier function (Guilloteau et al. 2010; Zhang et al. 2011; Cerisuelo et al. 2014; Qaisrani et al. 2015; Song et al. 2017). However, most studies of SB have focused on growth performance and intestinal health, and few studies have focused on its effects on the development of the digestive and immune organs. It has been reported that dietary SB supplementation increased the relative weight of the thymus and the bursa of Fabricius on day 21, as well as of the spleen and thymus on day 35 (Sikandar et al. 2017). Chamba et al. (2014) reported that dietary SB supplementation had no effects on the relative weight of digestive organs in days

Supported by program for scientific research start-up funds of Guangdong Ocean University, China (101402/R18005).

https://doi.org/10.17221/190/2019-CJAS

1–14, 15–28, and 29–42, while the relative length of the jejunum and small intestine was higher on day 14. The effects of SB on the digestive and immune organs are interesting because the effective development of these organs is crucial for optimal digestive efficiency and immune response. However, little is known about the effects of time and about the optimum level for SB supplementation for the best development of digestive and immune organs in broilers. The growth responses of the digestive and immune organs of broilers as affected by dietary SB supplementation should be further studied. Therefore, the aim of this study was to evaluate the effects of SB on growth performance and development of the digestive and immune organs of broilers from day 1 to day 35.

MATERIAL AND METHODS

Ethics. The experimental protocol used in this study was approved by the Animal Care and Use Committee of Guangdong Ocean University, P.R. China.

Sodium butyrate. The product (C4 VFA) used in this study was provided by a commercial company (Beijing Shengtaiyuan Biotechnology Co, Ltd., China), and contained 54% sodium butyrate protected by a physical and chemical matrix of buffer salts.

Experimental design, animals and housing. A total of 288 one-day-old Arbor Acres broilers were purchased from a commercial hatchery (Guangxi Liangshan Company, China) for this 35-day experiment. All broilers were individually weighed and randomly allocated to 4 groups with 6 replication pens (12 broilers per pen) according to their initial BW. The diets had a similar ingredient composition as the basal diet but included 0%, 0.03%, 0.06% and 0.12% SB, substituting for identical amounts of the basal diet (weight/weight). The diet was formulated to meet or exceed the nutrient requirements of broilers during starter (days 1–21) and grower (days 22–35) phases, according to the NRC (1994) recommendation (Table 1). Dietary DM (method 930.15), CP (method 920.39), calcium (method 984.01) and phosphorus (method 965.17) were analysed according to the procedures described by AOAC International (AOAC International 2006). Individual amino acid composition was measured using a Beckman 6300 Amino Acid Analyzer (Beckman Coulter Inc., USA) after 24 h of 6 N-HCl hydrolysis at 110 °C. Performic acid was used before hydrolysis to oxidise Met and Cys to methionine sulfone and cysteic acid. Nitrogen was determined by a Kjeltec 2300 Nitrogen Analyzer (Foss Tecator AB, Sweden).

Table 1. Ingredient composition and nutrient content of diets

Item	Starter (days 1–21)	Grower days 22–35)
Ingredients (%)		
Corn	54.57	62.44
Soybean meal (48% CP)	29.95	25.58
Corn gluten meal (60% CP)	5.90	3.30
Soybean oil	5.50	4.89
Tricalcium phosphate	2.46	2.29
Limestone	0.89	0.75
Salt	0.20	0.20
DL-Met (88%)	0.07	0.07
L-Lys·HCl (78.4%)	0.06	0.08
Vitamin premix[1]	0.20	0.20
Mineral premix[2]	0.20	0.20
Calculated composition (%)		
ME (MJ/kg)	12.95	12.74
CP	21.89	18.90
Ca	1.05	0.96
Lys	1.12	1.01
Met + Lys	0.90	0.86
Available P	0.81	0.73
Analysed composition (%)		
CP	21.12	20.02
Ca	1.03	0.95
Met + Lys	0.89	0.87
Available P	0.44	0.42

[1]provided per kg of complete diet: 128 000 IU vitamin A; 1 600 IU vitamin D_3; 60 IU vitamin E; 1.6 mg vitamin K_3; 0.12 mg biotin; 50 mg choline; 1.2 mg folic acid; 32 mg nicotinic acid; 16 mg pantothenic acid; 4.8 mg riboflavin; 2.4 mg thiamine (B_1); 3.2 mg vitamin B_6; 0.03 mg vitamin B_{12}; [2]provided per kg of diet: Mg, 79 mg as manganese oxide; Zn, 60 mg as zinc oxide; Cu, 100 mg as copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$); Fe, 120 mg as iron sulfate; I, 0.96 mg as potassium iodine; Co, 0.16 mg as cobalt sulfate; Se, 0.24 mg as sodium selenite

All broilers were placed in battery pens (124 cm length × 64 cm width × 40 cm height). The temperature of the room was maintained at 33 ± 1 °C for the first week. After day 8, the temperature was gradually reduced by 0.5 °C per day until it

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declined to 24 °C. Artificial light was provided 24 h/day using fluorescent lights, and the birds had free access to feed in the form of mash and to tap water. Broilers were vaccinated with the combined Newcastle disease virus (NDV) and infectious bronchitis virus vaccine via intranasal and intraocular administration on day 7 and via oral administration on day 21.

Growth performance. After 12 h of fasting, the body weights of broilers and their feed consumption were recorded on a pen basis on days 1, 7, 14, 21, 28, and 35. The ADG, ADFI, and FCR were calculated for each period (days 1–7, 8–14, 15–21, 22–28, and 29–35) and for the overall duration of the experiment (days 1–35).

Sample collection. On days 7, 14, 21, 28, and 35, six broilers from each treatment were randomly selected for the measurement of digestive and immune organs (one broiler per pen). Feed was removed 12 h before slaughter. The broilers were individually weighed and euthanised by cervical dislocation. The length of each intestinal segment was determined with a flexible tape on a glass surface to prevent inadvertent stretching. The lengths of sampled duodenum (from the pyloric junction to the most distal point of insertion of the duodenum mesentery), jejunum (from the most distal point of insertion of the duodenum mesentery to the junction with Meckel's diverticulum), ileum (from the junction with Meckel's diverticulum to the ileo-caecal junction), the sum of two sides of the caeca (from ostium to tip), and rectum (from the ileo-caecal junction to the cloaca) were measured. Following the separation of each of these components from any adherent mesentery, their weights were determined along with those of the proventriculus, gizzard, thymus, pancreas, liver, spleen, and bursa of Fabricius. Digesta were removed by gentle squeezing to measure the empty weight. The data on the weight of digestive organs was recorded and expressed as a percentage of live BW (g/kg). The data on the length of duodenum, jejunum, ileum, caeca, and rectum was recorded and expressed as a percentage of live BW (cm/kg), based on the studies by Mahdzvi and Torki (2009). In addition, samples from the duodenum, jejunum, ileum, and caeca (1 cm at the midpoint) were fixed in 10% buffered formalin for 48 h at room temperature and subsequently dehydrated through a graded ethanol series, then cleared with xylene and finally embedded in paraffin for histological examination.

Analysis of intestinal histomorphology. For the histological analysis to measure villus height (VH) and crypt depth (CD), serial tissue sections of 4 μm were cut and mounted 4 sections per slide. The sections were deparaffinised, rehydrated, and rinsed in distilled water. Finally, the section was stained with haematoxylin for 2 min and eosin for 40 s, then dehydrated and mounted on a slide. The evaluated gastrointestinal morphometric variables were VH, CD, and the ratio of villus height to crypt depth (VCR). Morphological parameters were measured using the Image-Pro Plus software (Version 6.0). Each sample was subjected to 12 replicate measurements for each variable studied. These 12 measurements were then averaged to generate a mean value for each broiler. VH was measured from the top of the villus to the top of the *lamina propria*. CD was measured from the base upward to the region of transition between the crypt and the villus.

For neutral goblet cell histochemistry, serial tissue sections of 5 μm were cut and mounted 4 sections per slide, then processed with Periodic Acid-Schiff (PAS) staining. Following deparaffinisation and dehydration, the slides were incubated in 0.5% periodic acid for 5 min, washed and incubated with Schiff's reagent for 20 min, and then counterstained with haematoxylin for 5 min. The number of PAS positive cells staining red (PAS^+) along the villi was counted using light microscopy as described previously (Uni et al. 2003). The number of goblet cells per villus was counted after staining in 8 well-oriented villus units per broiler.

Statistical analysis. The individual pen was used as the experimental unit and all data were analysed using the MIXED procedure of SAS software (Version 9.1). Treatment means were tested by Duncan's multiple comparisons. In addition, orthogonal comparisons were conducted using polynomial regression to measure the linear and quadratic effects of increasing dietary SB supplementation. $P < 0.05$ was considered to be significant, and $0.05 < P < 0.10$ was considered to be a trend.

RESULTS

Growth performance. As shown in Table 2, on days 7, 14, 28 and 35, there was a linear increase ($P < 0.05$) in BW associated with the inclusion of SB in the diets. In days 1–7, 8–14, 22–28, 29–35, and for the overall period, there was a linear improvement ($P < 0.05$) in ADG associated with the

https://doi.org/10.17221/190/2019-CJAS

Table 2. Effects of sodium butyrate on growth performance of broilers

Item	Dietary sodium butyrate supplementation (%)				SE	*P*-value	
	0	0.03	0.06	0.12		linear	quadratic
BW (g)							
D 1	32.83	31.67	31.83	31.67	0.56	0.39	0.76
D 7	150.00^{c}	151.67^{bc}	161.83^{a}	153.67^{b}	0.85	< 0.00	0.00
D 14	325.33^{c}	343.67^{b}	357.33^{a}	349.50^{ab}	2.93	< 0.00	0.52
D 21	609.57^{ab}	645.13^{a}	625.58^{ab}	591.37^{b}	11.67	0.34	0.07
D 28	1 012.33^{b}	1 036.67^{b}	1 070.33^{a}	1 013.17^{b}	10.15	0.00	0.71
D 35	1 449.67^{c}	1 508.00^{b}	1 549.67^{a}	1 458.67^{c}	5.50	< 0.00	0.23
ADG (g)							
D 1–7	16.74^{d}	17.14^{c}	18.57^{a}	17.43^{b}	0.01	< 0.00	< 0.00
D 8–14	25.05^{b}	27.43^{a}	27.93^{a}	27.98^{a}	0.44	0.00	0.09
D 15–21	40.61^{a}	43.07^{a}	38.22^{ab}	34.55^{b}	1.70	0.35	0.10
D 22–28	57.54^{b}	55.93^{b}	63.54^{a}	60.26^{ab}	1.71	0.02	0.04
D 29–35	62.48^{c}	67.33^{ab}	68.48^{a}	63.64^{bc}	1.37	0.01	0.28
D 1–35	40.48^{c}	42.18^{b}	43.37^{a}	40.77^{c}	0.16	< 0.00	0.20
ADFI (g)							
D 1–7	20.07^{b}	20.17^{b}	21.67^{a}	20.43^{ab}	0.42	0.01	0.19
D 8–14	32.45^{b}	34.48^{a}	34.02^{ab}	34.24^{ab}	0.61	0.08	0.11
D 15–21	73.08	68.85	65.84	72.72	2.85	0.09	0.86
D 22–28	110.68^{b}	110.22^{b}	115.90^{ab}	120.99^{a}	3.06	0.24	0.42
D 29–35	130.76	132.50	137.41	128.63	3.61	0.21	0.72
D 1–35	69.39	69.21	70.64	71.32	0.99	0.39	0.52
FCR							
D 1–7	1.20	1.18	1.17	1.17	0.02	0.30	0.87
D 8–14	1.30^{a}	1.26^{ab}	1.22^{b}	1.23^{ab}	0.03	0.02	1.00
D 15–21	1.81^{b}	1.62^{b}	1.72^{b}	2.13^{a}	0.09	0.52	0.22
D 22–28	1.93^{a}	1.98^{a}	1.83^{b}	2.01^{a}	0.03	0.04	0.02
D 29–35	2.10	1.97	2.01	2.04	0.08	0.43	0.39
D 1–35	1.72^{a}	1.64^{b}	1.63^{b}	1.75^{a}	0.02	0.02	0.28

D = day(s); $^{a–d}$means in the same row with different superscripts differ ($P < 0.05$)

inclusion of SB. In days 1–7, there was a linear improvement ($P < 0.05$) in ADFI associated with the inclusion of SB. Meanwhile, in days 8–14 and 15–21, ADFI showed a linear increasing trend ($P < 0.10$). In days 8–14, 22–28, and for the overall period, there was a linear improvement ($P < 0.05$) in FCR associated with the inclusion of SB.

Relative length and weight of intestinal organs. The effects of dietary SB supplementation on the relative weight and length of digestive organs are shown in Table 3 and Table 4, respectively. There were linear changes ($P < 0.05$) in the relative weight of proventriculus (day 7), gizzard (days 7 and 14), pancreas (days 7 and 21), liver (day 28), duodenum (days 21 and 28), jejunum (day 21), ileum (day 21), small intestine (day 21), and rectum (day 14) associated with the inclusion of SB. There were linear changes ($P < 0.05$) in the relative length of duodenum (day 21), jejunum (days 14 and 21), ileum (days 14 and 21), small intestine (days 14 and 21), caeca (day 21) and rectum (day 21) associated with the inclusion of SB.

Intestinal histological measurements. The effects of dietary SB supplementation on the VH, CD, and VCR of duodenum, jejunum, and ileum are shown in Table 5. On days 7, 14, 21, 28 and 35, there was a linear improvement ($P < 0.05$) in CD associated with the inclusion of SB in duodenum, and VCR showed an increasing trend. On days 7, 14, 21, 28 and 35, there was a linear improvement

https://doi.org/10.17221/190/2019-CJAS

Table 3. Effects of sodium butyrate on relative weight of digestive organs of broilers

Item[1] (g/kg)	Dietary sodium butyrate supplementation (%)				SE	*P*-value	
	0	0.03	0.06	0.12		linear	quadratic
Proventriculus							
D 7	7.02	8.13	8.03	8.74	0.41	0.01	0.64
D 14	5.05	5.64	5.40	5.79	0.36	0.24	0.79
D 21	4.36	4.45	4.90	4.85	0.26	0.12	0.79
D 28	3.41	3.83	3.82	3.71	0.23	0.39	0.26
D 35	3.59	3.44	4.00	3.81	0.26	0.32	0.94
Gizzard							
D 7	27.36^{b}	28.75^{ab}	26.98^{b}	31.05^{a}	0.90	0.03	0.15
D 14	16.21^{b}	19.57^{ab}	19.38^{ab}	22.82^{a}	1.51	0.01	0.98
D 21	14.56	14.33	16.55	16.61	0.98	0.07	0.89
D 28	10.70	12.06	11.97	12.44	0.67	0.10	0.52
D 35	15.21	15.46	15.69	16.32	1.01	0.44	0.85
Pancreas							
D 7	3.16^{b}	3.86^{a}	3.40^{b}	4.20^{a}	0.14	0.00	0.75
D 14	2.90^{ab}	3.11^{ab}	2.49^{b}	3.65^{a}	0.27	0.19	0.09
D 21	2.23^{b}	2.86^{ab}	2.88^{ab}	3.66^{a}	0.30	0.00	0.81
D 28	2.17	2.33	2.79	2.40	0.24	0.29	0.27
D 35	2.39	2.30	2.45	2.04	0.18	0.28	0.38
Liver							
D 7	24.42^{bc}	29.82^{a}	27.31^{ab}	23.33^{c}	1.09	0.25	0.00
D 14	21.66^{ab}	23.39^{ab}	20.65^{b}	25.93^{a}	1.42	0.13	0.23
D 21	19.62	21.13	22.79	22.47	1.16	0.06	0.44
D 28	14.59^{b}	15.05^{b}	18.47^{a}	16.41^{ab}	0.85	0.03	0.16
D 35	15.80	16.73	16.62	17.38	1.09	0.35	0.93
Duodenum							
D 7	7.83	8.17	8.47	8.33	0.61	0.52	0.70
D 14	5.78	6.98	6.88	8.11	0.83	0.08	0.99
D 21	3.76^{b}	3.82^{b}	5.18^{a}	5.58^{a}	0.32	0.00	0.62
D 28	2.74^{b}	3.76^{a}	4.00^{a}	3.37^{ab}	0.23	0.05	0.00
D 35	4.00	4.26	4.73	4.51	0.61	0.48	0.70
Jejunum							
D 7	11.43	13.23	12.39	12.07	0.99	0.81	0.30
D 14	9.53^{b}	12.27^{a}	10.06^{b}	10.185^{ab}	0.71	0.94	0.08
D 21	7.24^{b}	8.45^{ab}	9.27^{a}	8.72^{a}	0.43	0.01	0.06
D 28	5.49	6.78	7.13	5.33	0.59	0.96	0.02
D 35	7.28	8.25	8.82	8.12	0.86	0.43	0.34
Ileum							
D 7	8.99	10.47	10.70	10.01	0.74	0.33	0.16
D 14	7.93	8.00	8.90	9.44	0.83	0.16	0.78
D 21	5.40^{c}	5.62^{bc}	6.81^{ab}	7.68^{a}	0.41	0.00	0.43
D 28	3.68^{b}	4.67^{ab}	5.57^{a}	4.12^{b}	0.42	0.24	0.01
D 35	5.16	6.74	6.18	6.14	0.58	0.37	0.18

https://doi.org/10.17221/190/2019-CJAS

Table 3 to be continued

Item[1] (g/kg)	Dietary sodium butyrate supplementation (%)				SE	*P*-value	
	0	0.03	0.06	0.12		linear	quadratic
Small intestine[2]							
D 7	28.25	31.87	31.55	30.41	1.77	0.45	0.19
D 14	23.24	27.26	25.83	27.74	2.15	0.22	0.63
D 21	16.47^{b}	17.82^{b}	21.26^{a}	21.99^{a}	0.94	< 0.00	0.68
D 28	11.90^{b}	15.21^{ab}	16.71^{a}	12.82^{b}	1.17	0.42	0.01
D 35	16.44	19.25	19.72	18.78	1.87	0.38	0.33
Caeca[3]							
D 7	2.97^{b}	4.91^{a}	4.48^{ab}	3.24^{b}	0.51	0.86	0.01
D 14	2.26	2.91	3.10	2.43	0.44	0.74	0.15
D 21	2.25^{b}	2.38^{b}	2.19^{b}	3.41^{a}	0.33	0.58	0.06
D 28	2.06	2.60	2.47	2.04	0.27	0.88	0.09
D 35	2.57	2.96	4.15	3.89	0.70	0.11	0.65
Rectum							
D 7	1.82^{ab}	2.17^{a}	1.48^{b}	2.14^{a}	0.16	0.68	0.33
D 14	1.04^{b}	1.22^{ab}	1.29^{ab}	1.48^{a}	0.10	0.01	0.95
D 21	0.88	0.91	1.08	0.78	0.10	0.97	0.10
D 28	0.76	1.09	0.79	0.65	0.08	0.10	0.01
D 35	1.05	1.04	1.06	0.98	0.10	0.63	0.75

D = day; [1]data on weight of digestive organs were recorded and expressed as a percentage of live body weight (g/kg); [2]small intestine, the sum weight of duodenum, jejunum, and ileum; [3]caeca, the sum weight of two sides; $^{a–c}$means in the same row with different superscripts differ ($P < 0.05$)

($P < 0.05$) in CD and VCR associated with the inclusion of SB in jejunum, and VH showed an increasing trend. On days 7, 14, 21, 28 and 35, there was a linear improvement ($P < 0.05$) in VH, CD, and VCR associated with the inclusion of SB in ileum.

Dietary SB supplementation increased goblet cell counts in duodenum (linear, $P < 0.05$), jejunum (linear, $P < 0.05$), ileum (linear, $P < 0.05$), and caeca (quadratic, $P < 0.05$) on day 35 (Table 6).

Relative weight of immune organs. As shown in Figure 1, on days 7, 14 and 21, there was a linear increase ($P < 0.05$) in the relative weight of thymus associated with the inclusion of SB. On day 7, there was a quadratic increase ($P < 0.05$) in the relative weight of spleen and an increasing trend ($P < 0.10$) was observed on day 14.

DISCUSSION

Positive effects associated with dietary inclusion of SB on the growth performance of broilers were observed in days 1–7, 8–14, 22–28, 29–35, and 1–35. Similarly, Sikandar et al. (2017) reported that dietary SB supplementation improved weight gain during days 21–28 and 29–35, and decreased FCR during days 1–7 and 21–28. Chamba et al. (2014) reported that SB supplementation improved body weight gain during days 15–28, 29–42, and 1–42, and decreased FCR during days 15–28, 29–42, and 1–42. However, the results were not always consistent. Some studies showed that supplementation with SB or butyrate acid had no beneficial effects on performance in any phase (Mahdzvi and Torki 2009; Aghazadeh and Yazdi 2012; Zou et al. 2019). The inconsistency of these results may be related to differences in age, health status, feed composition, and butyrate concentration in the feed. In the present study, SB improved performance, but no such a benefit was noted with 0.12% SB supplementation at any period. Indeed, it negatively influenced ADG and FCR in days 15–21, which suggested that the overuse of SB had negative effects on growth performance.

The relative weight of digestive organs increased with the dietary inclusion of SB. Gonzalez-Ortiz et al. (2019) indicated that broilers fed butyrate

https://doi.org/10.17221/190/2019-CJAS

Table 4. Effects of sodium butyrate on relative length of intestinal segments of broilers

Item[1] (cm/kg)	Dietary sodium butyrate supplementation (%)				SE	*P*-value	
	0	0.03	0.06	0.12		linear	quadratic
Duodenum							
D 7	109.74	112.77	118.97	107.29	7.43	0.97	0.33
D 14	55.02	62.87	66.53	64.59	4.28	0.11	0.27
D 21	34.05^{b}	39.40^{b}	41.15^{b}	55.00^{a}	2.99	0.00	0.17
D 28	26.44	29.87	27.57	28.08	1.42	0.68	0.32
D 35	19.04	19.02	20.47	18.87	1.31	0.87	0.55
Jejunum							
D 7	215.52	240.61	248.75	209.47	14.80	0.88	0.04
D 14	103.74^{b}	123.76^{ab}	126.67^{a}	127.01^{a}	7.14	0.03	0.18
D 21	74.59^{b}	76.02^{b}	85.12^{ab}	96.07^{a}	5.04	0.00	0.36
D 28	58.14	63.19	63.20	58.31	3.29	0.97	0.15
D 35	38.01	39.20	43.68	41.78	3.09	0.27	0.62
Ileum							
D 7	199.14	200.11	210.36	187.09	12.30	0.64	0.34
D 14	97.79^{a}	117.00^{b}	131.10^{b}	115.38^{b}	5.80	0.02	0.01
D 21	71.99^{b}	72.18^{b}	81.18^{ab}	92.84^{a}	4.75	0.00	0.24
D 28	56.05	60.36	64.84	58.06	3.78	0.54	0.16
D 35	35.63	37.76	43.90	41.05	3.49	0.17	0.48
Small intestine[2]							
D 7	524.40	553.49	578.09	503.84	31.81	0.80	0.12
D 14	256.54^{a}	303.62^{b}	324.30^{b}	306.98^{b}	14.87	0.02	0.04
D 21	180.62^{b}	187.61^{b}	207.44^{b}	243.90^{a}	11.84	0.01	0.23
D 28	140.63	153.42	155.61	144.44	7.57	0.69	0.13
D 35	92.68	95.98	108.04	101.69	7.40	0.23	0.50
Caeca[3]							
D 7	80.39	83.49	94.61	86.04	7.59	0.42	0.45
D 14	48.03	52.05	54.87	52.39	3.04	0.26	0.30
D 21	34.19^{b}	35.75^{ab}	38.73^{ab}	44.31^{a}	3.02	0.02	0.51
D 28	26.60	27.37	27.11	27.05	1.64	0.88	0.81
D 35	19.75^{b}	20.16^{ab}	24.34^{a}	21.66^{ab}	1.40	0.13	0.29
Rectum							
D 7	22.95	23.27	28.55	24.09	1.81	0.30	0.20
D 14	11.41^{b}	13.68^{ab}	16.53^{a}	13.49^{ab}	1.37	0.15	0.07
D 21	8.41	8.79	9.63	10.48	0.71	0.04	0.74
D 28	6.72	7.12	6.20	6.97	0.55	0.95	0.74
D 35	4.67	4.62	4.94	5.34	0.41	0.21	0.59

D = day; [1]data on length of duodenum, jejunum, ileum, caeca, and rectum was recorded and expressed as a percentage of live BW (cm/kg); [2]small intestine, the sum length of duodenum, jejunum, and ileum; [3]caeca, the sum length of two sides; [a–c]means in the same row with different superscripts differ ($P < 0.05$)

had higher relative weights of the total gastrointestinal tract, duodenum and jejunum. Mahdzvi and Torki (2009) stated that the relative weight of small intestine, jejunum and ileum increased with SB supplementation. Aghazadeh and Yazdi (2012) reported the higher relative weight of liver and intestine with butyric acid supplementation, but it had no effects on the relative weight of gizzard. However, other studies reported that butyric acid glycerides or butyric acid had no beneficial

https://doi.org/10.17221/190/2019-CJAS

Table 5. Effects of sodium butyrate on duodenum, jejunum and ileum morphology in broilers

Item		Dietary sodium butyrate supplementation (%)				SE	*P*-value	
		0	0.03	0.06	0.12		linear	quadratic
Duodenum morphology								
Villus height (μm)	D 7	485.80	527.26	546.07	479.67	24.83	0.09	0.61
	D 14	582.95	632.71	655.28	575.61	29.80	0.10	0.50
	D 21	699.54	759.25	786.34	690.73	35.75	0.09	0.70
	D 28	1 049.32^{a}	1 138.87^{ab}	1 179.51^{b}	1 049.32^{ab}	53.63	0.08	0.80
	D 35	1 399.08	1 518.50	1 572.68	1 381.46	71.51	0.08	0.90
Crypt depth (μm)	D 7	166.46^{a}	196.26^{ab}	215.35^{b}	201.08^{b}	11.24	0.00	0.80
	D 14	233.05^{a}	274.77^{ab}	311.70^{b}	281.52^{b}	15.74	0.00	0.90
	D 21	293.64^{b}	346.20^{ab}	392.74^{a}	354.71^{a}	19.84	0.00	0.95
	D 28	308.32^{b}	363.51^{ab}	412.38^{a}	372.45^{ab}	20.83	0.00	0.70
	D 35	323.01^{b}	380.83^{ab}	432.02^{a}	390.18^{a}	21.82	0.00	0.69
Villus height : crypt depth	D 7	3.05	2.78	3.49	2.40	0.21	0.08	0.76
	D 14	2.61	2.39	2.14	2.06	0.18	0.07	0.96
	D 21	2.49	2.28	2.03	1.96	0.17	0.07	0.85
	D 28	3.56	3.25	2.91	2.80	0.25	0.08	0.66
	D 35	4.53	4.13	3.70	3.57	0.32	0.06	0.86
Jejunum morphology								
Villus height (μm)	D 7	426.78^{b}	572.93^{a}	501.93^{ab}	467.25^{b}	28.32	0.07	< 0.00
	D 14	469.45^{b}	630.23^{a}	552.15^{ab}	513.97^{b}	31.16	0.06	0.01
	D 21	676.02^{b}	907.52^{a}	795.10^{ab}	740.12^{b}	44.87	0.09	< 0.00
	D 28	946.42^{a}	1 270.53^{b}	1 113.14^{b}	1 036.18^{ab}	62.81	0.07	0.01
	D 35	1 216.83^{b}	1 431.17^{ab}	1 633.55^{a}	1 332.22^{b}	80.76	0.07	< 0.00
Crypt depth (μm)	D 7	131.02^{a}	198.43^{b}	189.34^{b}	132.90^{a}	10.18	< 0.00	< 0.00
	D 14	157.23^{a}	238.18^{b}	222.53^{b}	159.48^{a}	12.22	< 0.00	< 0.00
	D 21	308.17^{a}	466.83^{b}	436.16^{b}	312.57^{a}	23.95	< 0.00	< 0.00
	D 28	317.41^{a}	480.84^{b}	449.24^{b}	321.95^{ab}	24.67	< 0.00	< 0.00
	D 35	326.65^{a}	494.84^{b}	462.33^{b}	331.33^{a}	25.39	< 0.00	< 0.01
Villus height : crypt depth	D 7	3.30^{ab}	2.93^{bc}	2.72^{c}	3.57^{a}	0.15	0.01	0.69
	D 14	3.03^{ab}	2.69^{bc}	2.49^{c}	3.27^{a}	0.14	0.01	0.58
	D 21	2.23^{ab}	1.97^{bc}	1.83^{c}	2.40^{a}	0.10	0.01	0.67
	D 28	3.02^{a}	2.68^{b}	2.49^{b}	3.27^{a}	0.14	0.01	0.68
	D 35	3.78^{ab}	3.35^{bc}	3.11^{c}	4.08^{a}	0.17	0.02	0.58
Ileum morphology								
Villus height (μm)	D 7	354.42^{c}	450.12^{a}	427.00^{b}	380.56^{c}	10.98	< 0.00	< 0.00
	D 14	496.19^{a}	630.17^{b}	584.47^{c}	532.79^{a}	15.37	< 0.00	< 0.00
	D 21	595.43^{c}	756.20^{a}	701.36^{b}	639.35^{c}	18.45	< 0.00	< 0.00
	D 28	833.60^{a}	1 058.68^{b}	981.91^{ab}	895.09^{b}	25.82	< 0.00	< 0.00
	D 35	1 071.77^{c}	1 361.16^{a}	1 262.45^{b}	1 150.83^{c}	33.21	< 0.00	< 0.00
Crypt depth (μm)	D 7	109.82^{a}	174.04^{b}	167.50^{b}	125.08^{a}	5.53	< 0.00	< 0.00
	D 14	142.77^{a}	226.25^{b}	216.55^{b}	162.60^{a}	7.18	< 0.00	< 0.00
	D 21	157.04^{a}	248.87^{b}	238.20^{b}	178.86^{a}	7.90	< 0.00	< 0.00
	D 28	164.90^{a}	261.32^{b}	250.11^{b}	187.81^{a}	8.30	< 0.00	< 0.00
	D 35	172.74^{b}	273.76^{a}	262.02^{a}	196.75^{b}	8.69	< 0.00	< 0.00
Villus height : crypt depth	D 7	3.25^{a}	2.62^{b}	2.56^{b}	3.07^{a}	0.12	< 0.00	0.07
	D 14	3.50^{a}	2.82^{b}	2.72^{b}	3.31^{a}	0.13	< 0.00	0.07
	D 21	3.82^{a}	3.08^{b}	2.96^{b}	3.61^{a}	0.14	< 0.00	0.07
	D 28	5.09^{a}	4.10^{ab}	3.95^{b}	4.82^{ab}	0.18	< 0.00	0.07
	D 35	6.25^{a}	5.03^{b}	4.85^{b}	5.91^{a}	0.22	< 0.00	0.07

D = day; $^{a–c}$means in the same row with different superscripts differ ($P < 0.05$)

https://doi.org/10.17221/190/2019-CJAS

Table 6. Effects of sodium butyrate on goblet cell counts in different intestinal segments of broilers on day 35

Item	Dietary sodium butyrate supplementation (%)				SE	*P*-value	
	0	0.03	0.06	0.12		linear	quadratic
Duodenum	31.04	36.48	36.04	43.07	2.21	0.04	0.18
Jejunum	46.74^{b}	55.04^{b}	54.37^{b}	65.15^{a}	3.44	0.05	0.18
Ileum	93.11^{b}	109.48^{b}	108.30^{b}	129.22^{a}	6.64	0.04	0.19
Caeca	11.26	13.67	18.30	11.63	0.98	0.15	< 0.00

$^{a–c}$means in the same row with different superscripts differ ($P < 0.05$)

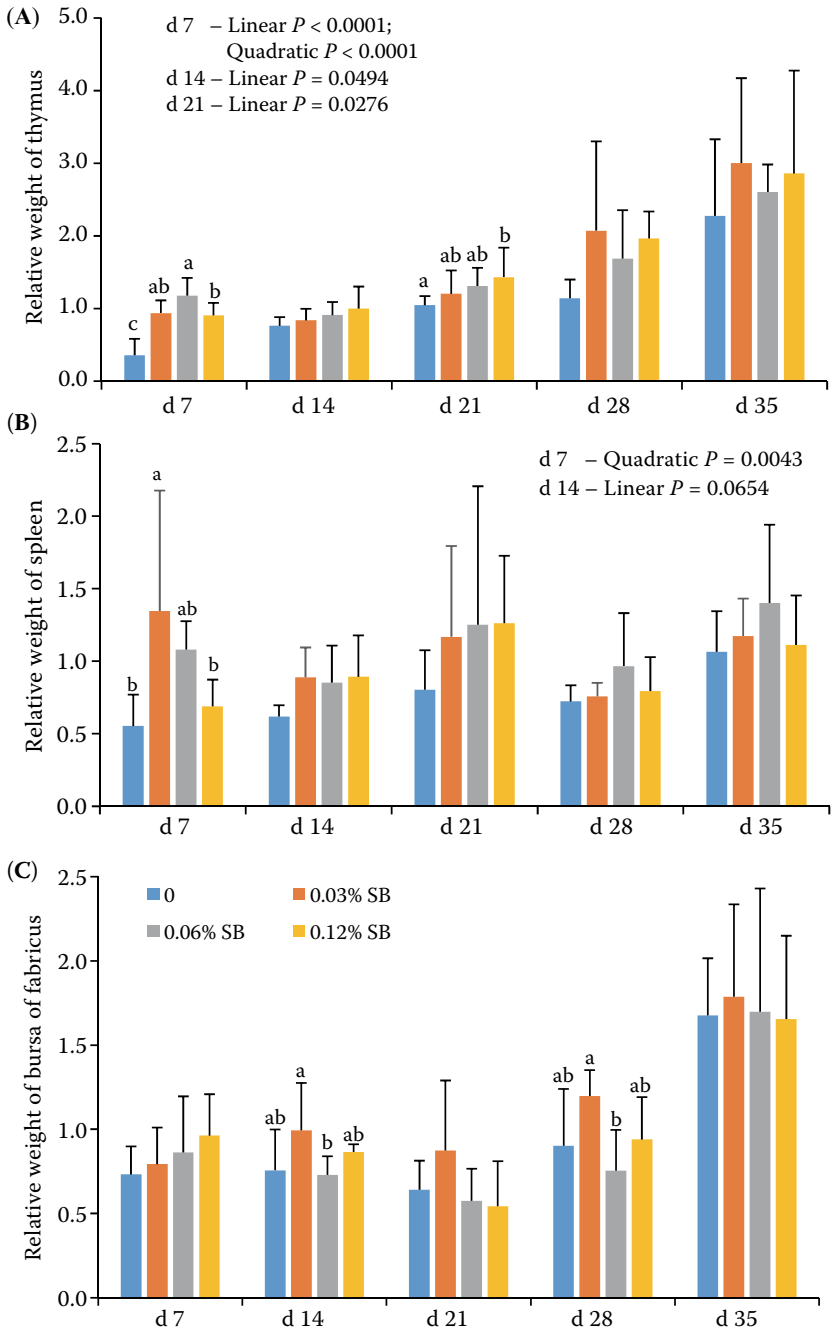

Figure 1. Effects of sodium butyrate on the relative weight of immune organs of broilers. Results are presented as mean ± standard error. The data on weight of immune organs were recorded and expressed as a percentage of live body weight (g/kg)

d = day; $^{a–c}$means in the same row with different superscripts differ ($P < 0.05$)

https://doi.org/10.17221/190/2019-CJAS

effects on the relative weight of liver or gizzard (Antongiovanni et al. 2007; Panda et al. 2009). In this study, dietary SB supplementation linearly increased the relative weight of gizzard on days 7 and 14. A large, well-developed gizzard is beneficial for improving the gut motility (Ferket et al. 2002) and may increase the release of cholecystokinin (Svihus and Hetland 2001), which in turn stimulates the secretion of pancreatic enzymes. A well-developed gizzard will improve nutrient digestibility, further improving the growth performance. The pancreas, duodenum, jejunum and ileum are the major organs for producing and releasing digestive enzymes into the gastrointestinal tract of broilers. The relative length of duodenum, jejunum, and ileum increased with the inclusion of SB in diets on days 21 and 28. Longer jejunum, ileum, and small intestine as an effect of SB supplementation were reported by Chamba et al. (2014). Some studies have revealed that butyrate, in addition to providing epithelial cells with energy, markedly increased epithelial cell proliferation and differentiation, and improved the colonic barrier function (Guilloteau et al. 2010). When butyrate was infused into the colon, it exerted trophic effects on ileum and jejunum epithelial cells. In the small intestine, butyrate enhanced proliferation, differentiation and maturation, and reduced apoptosis of normal enterocytes through its influence on gene expression and protein synthesis (Sengupta et al. 2006). These may be the reasons why SB supplementation enhanced the relative weight and length of intestinal segments. In this study, specially coated SB was used that can deliver the portion of butyrate to the distal intestinal tract due to its slow release during digestion, with beneficial effects on mucosal modulation in the gut (Chamba et al. 2014; Sikandar et al. 2017), then it can be preferentially applied by enterocytes to stimulate intestinal development and function in broilers.

Butyrate, the active ingredient of SB, is absorbed by enterocytes as a main source of energy to promote intestinal development and function, and is beneficial to animal health (Jozefiak et al. 2004; Mahdzvi and Torki 2009). Higher villus height is generally thought to represent a larger surface area for higher absorption capacity and healthy development of the intestine. Our study showed that SB tended to increase the VH of duodenum and jejunum, and linearly increase the VH of ileum, similarly like in previous studies reported by Smulikowska et al. (2009) and Wu et al. (2018). Moreover, a microscopic analysis of the villus indicated that dietary SB supplementation linearly increased goblet cell counts in duodenum, jejunum, and ileum, and quadratically increased goblet cell counts in caeca. The primary function of goblet cells is mucus production and stimulation (Strous and Dekker 1992). The mucus layer is the first line of defence in the intestinal mucosa, which is composed of mucin (Corfield et al. 2000) and is mainly regulated by altering the number of goblet cells. Meanwhile, dietary SB supplementation had beneficial effects on the relative length and weight of duodenum, jejunum, and ileum on day 21. The improved intestinal histomorphology and enhancement of goblet cell counts may contribute to the absorption and reinforcement of the intestinal integrity of broilers (Wu et al. 2018), possibly resulting in increasing relative weight and length of intestinal segments (Choct 2009; Wu et al. 2016) and better growth performance.

Several studies indicated that SB has immunomodulatory capacity (Zhang et al. 2011; Liu et al. 2014), which can decrease inflammatory immune responses. Immune organs are the foundation for achieving the immune function. Thymus, spleen, and bursa of Fabricius are often weighed as indicators because of their critical role in the development and function of the immune system (Kwak et al. 1999). In healthy broilers, an increase in the weight of immune organs is correlated with improved immune response. In this study, the relative weight of thymus linearly increased with SB supplementation on days 7, 14, and 21. In agreement with our study, Sikandar et al. (2017) indicated that dietary SB supplementation resulted in the heavier relative weight of thymus on days 21 and 35, bursa of Fabricius on day 21, and spleen on day 35. The improved weight of thymus may enhance the lymphocyte response (Ochoa et al. 2001), and the production or function of immune cells (Wu et al. 2009). According to current results, it is believed that the improvement of growth performance with dietary SB supplementation may relate to the improvement in the relative weight of intestinal and immune organs, associated with improvement in the growth performance and health of broilers.

CONCLUSION

In conclusion, this study showed that dietary SB supplementation improved growth performance,

https://doi.org/10.17221/190/2019-CJAS

promoted intestinal development by enhancing the relative length and weight of intestinal tract segments, increased VH, VCR and goblet cell counts, and improved the relative weight of thymus and spleen.

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Received September 5, 2019
Accepted December 17, 2019