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Broiler Microbiological and Histological Response to Diets Varying in Sodium and Calcium Butyrate

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Abstract
The aim of the study was to determine the effect of supplementing broiler diets with different levels of sodium and calcium butyrate on certain histological and microbial traits. This study was conducted in the poultry field of the Department of Animal Production at the College of Agricultural Engineering Sciences / University of Baghdad (Baghdad, Iraq) during November - December (2020) in a total of 308 one-day-old chicken utilized in the study, and each treatment had three replicates consisting of 10 chicks each. Duodenal villi height increased significantly ($p<0.05$) for treatments T2, T3, T4, and T5, villi thickness increased significantly ($p<0.05$) for treatment T3, and crypt depth increased significantly ($p<0.05$) for treatment T2. Treatment T2 significantly increased villi height and treatment T5 significantly increased villi thickness in the jejunum, in comparison to the other treatments. In the ileum, treatment T2 resulted in significantly greater increases in villi height, treatment T6 in villi thickness, and treatment T4 in crypt depth compared to the other treatments. In the duodenum and jejunum, there were no statistically significant variations in the numbers of *E. coli* and Lactobacilli bacteria, but in the ileum, the number of E. coli increased significantly in treatments T3 and T4. In this respect, lactobacilli did not differ noticeably.

Keywords: Lactobacilli, Chicken, Ileum, Duodenum, Jejunum

Introduction

Antibiotics and other additives have long been used in poultry feed as growth promoters; their addition has been shown to stabilize the intestinal microbial flora, improve growth performance, and prevent some intestinal diseases (Hassan et al., 2010). These are two requirements of the modern poultry industry (Javid and Khan, 2016).Because of rising antibiotic resistance in chickens (Kabir 2009) and the risk of spreading disease from infected animals to humans, the European Union banned the use of antibiotics in poultry feed in 2006 (Singer and Hofacre 2006;Tokić et al 2007), requiring the poultry industry to develop methods for raising birds without antibiotics (Castanon, 2007). Organic acids and their salts, like butyric acid, are a byproduct of microbial fermentation of dietary fibers (Hamer et al., 2008) and are considered natural products with no harmful effect; they can be used with all species and ages of birds and do not interact with most medicines and feed additives in bird feed or drinking water. They extend the life of feed and make it easier to store (Zhang et al., 2014; Coban, 2020). Since organic acids and their salts reduce the pH of the digestive tract, they have attracted a lot of attention for their potential to inhibit the growth of harmful bacteria (Eidelsburger et al., 1992; Boling et al., 2000; Partanen et al., 2001; Kill et al., 2011a). These compounds have also been shown to have beneficial effects on growth performance and intestinal integrity (Chamba et al., 2014) Na, K, Mg, and Ca salts of butyric acid can be utilized as food additives because, unlike free acids, butyric acid has no odor and is less volatile, making it safer to work with during the feed manufacturing process (Ahsan et al., 2016). In addition to improving intestinal health and preventing the emergence of cancer cells in the colon epithelial cells by regulating cell proliferation, differentiation, and apoptosis (Da Zhou et al. 2017), the salts have other benefits, such as stimulating intestinal blood flow, mucin secretion, and water absorption. The capacity of acid salt to inhibit the development of pathogenic bacteria has led to its use as a feed additive (Flint et al., 2012). The aim of the study was to determine the effect of different levels of sodium butyrate and calcium butyrate on broilers' histological and microbiological characteristics.

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Materials and methods

From November 1, 2020, to November 12, 2020, the University of Baghdad's Department of Animal Production's poultry farm served as the site for a field experiment in the College of Agricultural Engineering Sciences. The experiment used 210 Ross-308 broiler chicks that were one days old and had not yet been sexed from the Iraqi Society hatchery in Abu Ghraib, Baghdad. Chicks typically weighed around 42,372 grammes on average. The chicks were given food and water ad libitum and were grown on a layer of sawdust that was 3 to 5 centimeters deep. The lighting was set up so that there was 23 hours of light and one hour of darkness to help the chicks adjust to the dark. Diets were prepared in a laboratory dedicated to the chicken industry, and chicks were randomly assigned to one of seven treatments, with three replicates per treatment (10 birds per replicate). Statistical analysis was carried out using One-Way ANOVA in the SPSS Software. Differences were considered significant at $P<0.05$ (Al-Gharban, 2017)

Table 1. Starter diet (1-10 days) ingredients and computed chemical composition

Ingredients	Starter diet						
	T1	T2	T3	T4	T5	T6	T7
Yellow corn	43.8	43.8	43.6	43.4	43.5	43.5	43.5
Wheat	14	14	14	13.5	14	14	13.7
Soybean meal	32.7	32.5	32.5	32.6	32.7	32.7	32.7
Protein Center (1)	5	5	5	5	5	5	5
oil	2.2	2.2	2.2	2.4	2.4	2.3	2.3
Di-Calcium Phosphite	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Limestone	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Vitamins and Minerals Blend (2)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Calcium butyrate	-	-	-	-	0.2	0.4	0.8
Sodium butyrate	-	0.2	0.4	0.8	-	-	-
Salt	0.3	0.3	0.3	0.3	0.2	0.1	0.0
Total	100	100	100	100	100	100	100
Calculated Chemical Analysis (3)							
Crude protein %	23.0	22.9	22.9	22.8	23.0	23	22.9
Represented energy (Kg/ Kcal feed)	3005.3	3000.4	2993.7	2991.8	3013.2	3004.2	2994.9
Methionine + Cysteine %	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Lysine %	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Calcium %	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Phosphorous %	0.48	0.48	0.48	0.48	0.48	0.48	0.48

(1)Dutch protein center BROCON-5 SPECIAL W. Each kg contains: 40% crude protein, 5% fat, 2.81% fiber, 3.14% calcium, 2.65% phosphorous, 2.50% sodium, 3.88% chloride, 3.85% lysine, 3.70% methionine, 4.12% methionine + cysteine, 2107 Kilo Calories/Kg Represented Energy, 20,000 IU Vitamin A, 80,000 IU Vitamin D3, 600 mg Vitamin E, 50 mg Vitamin K3, 50 mg Vitamin B1, 140 mg Vitamin B2, 80 mg Vitamin B6, 700 µg B12, 20 mg Acid Folic, 5 mg citric acid, 2 mg biotin, 800 mg niacin, 1 mg iron, 200 mg copper, 1,600 mg manganese, 1,200 mg zinc, 20 mg iodine, 5 mg selenium, 6 mg cobalt, 33.50 mg antioxidant (BHT).

(2)A mixture of vitamins and minerals, each kg of which contains: 5000 IU Vitamin A, 600 IU D3, 10 mg E, 2 mg K3, 2 mg B1, 2 mg B2, 2 mg B6, 5 micrograms B12, 10 mg C, 15 mg niacin, 500 µg folic acid, 5 mg D-calcium phosphate, 40 mg zinc, 100 µg cobalt, l50 mg lysine.

(3)According to the chemical analysis of the ration according to the NRS (1994)

Table 2. Growth diet (11 – 22 days) ingredients and computed chemical composition

Ingredients	Growth diet						
	T1	T2	T3	T4	T5	T6	T7
Yellow corn	44.5	44.5	44.3	44	44.6	44.5	44.2
Wheat	15.9	15.7	15.7	15.7	15.7	15.7	15.5

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Soybean meal	**29**	**29**	**29**	**28.5**	**29**	**29**	**29**
Protein Center (1)	**5**	**5**	**5**	**5**	**5**	**5**	**5**
oil	**3.4**	**3.4**	**3.4**	**3.4**	**3.4**	**3.4**	**3.6**
Di-Calcium Phosphite	**0.6**	**0.6**	**0.6**	**0.6**	**0.6**	**0.6**	**0.6**
Limestone	**1.1**	**1.1**	**1.1**	**1.1**	**1.1**	**1.1**	**1.1**
Vitamins and Minerals Blend (2)	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**
Calcium butyrate	-	-	-	-	0.2	0.4	0.8
Sodium butyrate	-	0.2	0.4	0.8	-	-	-
Salt	**0.3**	**0.3**	**0.3**	**0.3**	**0.2**	**0.1**	**0.0**
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Chemical Analysis (3)							
Crude protein %	**21.5**	**21.5**	**21.4**	**21.2**	**21.5**	**21.5**	**21.4**
Represented energy (Kg/ Kcal feed)	**3105.5**	**3099.1**	**3092.4**	**3106.2**	**3102.5**	**3099.1**	**3100.9**
Methionine + Cysteine %	**0.84**	**0.84**	**0.82**	**0.83**	**0.84**	**0.84**	**0.83**
Lysine %	**1.2**	**1.2**	**1.2**	**1.2**	**1.2**	**1.2**	**1.2**
Calcium %	**0.85**	**0.85**	**0.85**	**0.85**	**0.85**	**0.85**	**0.85**
Phosphorous %	**0.46**	**0.46**	**0.46**	**0.46**	**0.46**	**0.46**	**0.46**

(1)Dutch protein center BROCON-5 SPECIAL W. Each kg contains: 40% crude protein, 5% fat, 2.81% fiber, 3.14% calcium, 2.65% phosphorous, 2.50% sodium, 3.88% chloride, 3.85% lysine, 3.70% methionine, 4.12% methionine + cysteine, 2107 Kilo Calories/Kg Represented Energy, 20,000 IU Vitamin A, 80,000 IU Vitamin D3, 600 mg Vitamin E, 50 mg Vitamin K3, 50 mg Vitamin B1, 140 mg Vitamin B2, 80 mg Vitamin B6, 700 µg B12, 20 mg Acid Folic, 5 mg citric acid, 2 mg biotin, 800 mg niacin, 1 mg iron, 200 mg copper, 1,600 mg manganese, 1,200 mg zinc, 20 mg iodine, 5 mg selenium, 6 mg cobalt, 33.50 mg antioxidant (BHT).

(2)A mixture of vitamins and minerals, each kg of which contains: 5000 IU Vitamin A, 600 IU D3, 10 mg E, 2 mg K3, 2 mg B1, 2 mg B2, 2 mg B6, 5 micrograms B12, 10 mg C, 15 mg niacin, 500 µg folic acid, 5 mg D-calcium phosphate, 40 mg zinc, 100 µg cobalt, l50 mg lysine.

(3)According to the chemical analysis of the ration according to the NRS (1994).

Table 3. Growth diet (23 - 42 days) ingredients and computed chemical composition.

Ingredients	**Final diet**						
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Yellow corn	**47.6**	**47.6**	**47.3**	**47.1**	**47.4**	**47.2**	**47.2**
Wheat	**15**	**14.8**	**14.8**	**14.2**	**15**	**15**	**14.5**
Soybean meal	**26**	**26**	**26**	**26.2**	**26**	**26**	**26.2**
Protein Center (1)	**5**	**5**	**5**	**5**	**5**	**5**	**5**
oil	**4.2**	**4.2**	**4.3**	**4.5**	**4.3**	**4.3**	**4.4**
Di-Calcium Phosphite	**0.6**	**0.6**	**0.6**	**0.6**	**0.6**	**0.6**	**0.6**
Limestone	**1.1**	**1.1**	**1.1**	**1.1**	**1.1**	**1.1**	**1.1**
Vitamins and Minerals Blend (2)	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**
Calcium butyrate	-	-	-	-	0.2	0.4	0.8
Sodium butyrate	-	0.2	0.4	0.8	-	-	-
Salt	**0.3**	**0.3**	**0.3**	**0.3**	**0.2**	**0.1**	**0.0**
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Chemical Analysis (3)							
Crude protein %	**20.2**	**20.2**	**20.2**	**20.2**	**20.2**	**20.2**	**20.2**
Represented energy (Kg/ Kcal feed)	**3180.0**	**3173.7**	**3172.7**	**3170.1**	**3182.3**	**3184.6**	**3173.8**
Methionine + Cysteine %	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**	**0.80**
Lysine %	**1.1**	**1.1**	**1.1**	**1.1**	**1.1**	**1.1**	**1.1**
Calcium %	**0.84**	**0.84**	**0.84**	**0.84**	**0.84**	**0.84**	**0.84**
Phosphorous %	**0.45**	**0.45**	**0.45**	**0.45**	**0.45**	**0.45**	**0.45**

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(1)Dutch protein center BROCON-5 SPECIAL W. Each kg contains: 40% crude protein, 5% fat, 2.81% fiber, 3.14% calcium, 2.65% phosphorous, 2.50% sodium, 3.88% chloride, 3.85% lysine, 3.70% methionine, 4.12% methionine + cysteine, 2107 Kilo Calories/Kg Represented Energy, 20,000 IU Vitamin A, 80,000 IU Vitamin D3, 600 mg Vitamin E, 50 mg Vitamin K3, 50 mg Vitamin B1, 140 mg Vitamin B2, 80 mg Vitamin B6, 700 µg B12, 20 mg Acid Folic, 5 mg citric acid, 2 mg biotin, 800 mg niacin, 1 mg iron, 200 mg copper, 1,600 mg manganese, 1,200 mg zinc, 20 mg iodine, 5 mg selenium, 6 mg cobalt, 33.50 mg antioxidant (BHT).

(2)A mixture of vitamins and minerals, each kg of which contains: 5000 IU Vitamin A, 600 IU D3, 10 mg E, 2 mg K3, 2 mg B1, 2 mg B2, 2 mg B6, 5 micrograms B12, 10 mg C, 15 mg niacin, 500 µg folic acid, 5 mg D-calcium phosphate, 40 mg zinc, 100 µg cobalt, l50 mg lysine.

(3)According to the chemical analysis of the ration according to the NRS (1994).

Results and discussion

Histological traits

According to Table (4), adding different level of sodium and calcium butyrate to broiler diets from 1 to 42 days old led to a statistically significant (P0.05) rise in duodenal villi height for treatments T2, T3, T4, and T5 compared with treatments T1, T6, and T7.Treatment T3 had the greatest villi thickness compared to the other experimental treatments, while treatment T2 had the greatest significant depth of crypts.

Table 4. Effect of sodium and calcium butyrate supplementation on the histological characteristics of the broiler duodenum at 6 weeks of age

Treatments	Duodenum		
	Villi height (µm)	Villi thickness (µm)	Crypt depth (µm)
T1	C 2.66 ±175.94	B 2.266 ±48.02	BC 3.34 ±57.92
T2	A 8.88 ±338.91	B 3.25 ±51.42	A 2.54 ±73.08
T3	A 8.28 ±340.66	A 1.54 ±58.64	B 2.33 ±61.55
T4	A 4.80 ±335.85	B 2.00 ±49.49	C 2.22 ±54.13
T5	A 9.08 ±317.20	B 1.46 ±50.69	BC 2.00 ±59.24
T6	C 3.01 ±187.60	B 1.70 ±47.09	BC 1.05 ±58.94
T7	B11.43 ±244.43	B 1.21 ±45.97	BC 1.39 ±55.66
Leave of significant	*	*	*

*Different letters within the same column indicate significant differences at the level (P<0.05), N.S. There is no significant difference.

** treatments: T1 is a control treatment free of addition, T2, T3, T4, adding sodium butyrate at levels 0.2, 0.4, 0.8%, respectively, and T5, T6, and T7 adding calcium butyrate at levels 0.2, 0.4, 0.8%, respectively.

Treatment T2 showed a significant increase (P<0.05) in jejunal villi height compared to the other experimental treatments, while treatment T5 showed a significant increase in villi thickness compared to the other treatments. The depth of the crypts significantly increased with the treatments T1, T2, and T5 (table 5).

Table 5. The effect of sodium and calcium butyrate supplementation on the histological characteristics of the broiler Jejunum at 6 weeks of age

Treatments	Jejunum		
	Villi height (µm)	Villi thickness (µm)	Crypt depth (µm)
T1	AB 9.41 ±330.39	BC 2.41 ±52.28	A 2.91 ±74.18
T2	A 9.35 ±359.91	AB 2.52 ±57.63	A 7.73 ±72.08
T3	E 4.45 ±227.41	D 3.06 ±42.62	BC 1.77 ±53.60
T4	CD 11.22 ±276.93	CD 1.39 ±45.26	C 2.27 ±46.13
T5	BC 7.27 ±302.08	A 2.78 ±61.26	A 1.60 ±74.62
T6	CD 12.25 ±272.37	BC 1.17 ±52.49	BC 2.27 ±56.24
T7	DE 9.10 ±253.06	BC 1.71 ±50.48	B 1.93 ±57.11
Leave of significant	*	*	*

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*Different letters within the same column indicate significant differences at the level (P<0.05), N.S. There is no significant difference.
** treatments: T1 is a control treatment free of addition, T2, T3, T4, adding sodium butyrate at levels 0.2, 0.4, 0.8%, respectively, and T5, T6, and T7 adding calcium butyrate at levels 0.2, 0.4, 0.8%, respectively.

Treatment T2 showed a significant increase (P<0.05) in villi compared to the other treatments, while treatment T6 showed a significantly higher level of villous thickness and treatment T4 showed a significantly higher level of crypt depth (Table 6).

Table 6. The effect of sodium and calcium butyrate supplementation on the histological characteristics of the broiler ileum at 6 weeks of age

Treatments	Ileum		
	Villi height (µm)	Villi thickness (µm)	Crypt depth (µm)
T1	B 8.07 ±340.15	ABC 2.59 ±49.65	C 3.59 ± 58.39
T2	A 11.20 ±406.33	ABC 1.84 ±49.87	C 2.35 ± 74.5
T3	BC 9.61±324.27	AB 2.62 ±53.87	C 2.91 ±53.47
T4	BC 10.90 ±323.31	BC 2.56 ±47.38	A 10.90 ±323.31
T5	C 7.20 ±294.27	C 2.38 ±45.47	B 29.68 ±136.69
T6	D 10.45 ± 238.90	A 1.80 ±55.61	C 3.52 ± 53.07
T7	D 11.05 ±229.16	ABC1.45 ±52.43	C 1.42 ±55.76
Leave of significant	*	*	*

*Different letters within the same column indicate significant differences at the level (P<0.05), N.S. There is no significant difference.
** treatments: T1 is a control treatment free of addition, T2, T3, T4, adding sodium butyrate at levels 0.2, 0.4, 0.8%, respectively, and T5, T6, and T7 adding calcium butyrate at levels 0.2, 0.4, 0.8%, respectively.

Butyrate is a source of energy for the intestinal cells and thus contributes to the growth and development of the villi, which may explain why adding sodium and calcium butyrate causes changes in the histological characterization (Lesson et al., 2005; Ahsan et al., 2016). Enterocyte proliferation and villus elongation were promoted by calcium butyrate in broiler diets, as reported by Yang et al. (2009) and Abd El Wahab et al. (2019).
The majority of the energy and physiological requirements for intestinal cells are met by short-chain fatty acids (Nagy, 2007), so supplementing with salts of these acids, such as sodium and calcium butyrate, may promote the development of goblet cells and mucin production, as well as the height of villi, the thickness of crypts, and the depth of crypts (Martinez et al., 2016). In addition to stimulating the development of goblet cells and myosin production, this also accounts for the increase in crypt height, width, and depth (Martinez et al., 2016). The intestinal epithelial tissues are protected by butyric acid, which also enhances their histological properties and proliferation. The histological features of the jejunum and duodenum are enhanced by sodium and calcium butyrate. Increased differentiation of epithelial cells enhanced intestinal epithelial function, and preservation of villus structure have all been linked to mucosal tensing and protection (Hu et al., 2007; Al Fataftah and Abdelqader, 2016). This explains for the increased villi height, width, and crypt depth, as well as the encouragement of goblet cell proliferation and mucin production (Martinez et al., 2016).
Butyric acid protects the mucous membrane, promotes intestinal growth, and enhances the histological characteristics of the intestinal epithelium, just as sodium and calcium butyrate do for the jejunum and duodenum (Hu et al., 2007; Al Fataftah and Abdelqader, 2016). In addition to enhancing epithelial cell differentiation, enhancing epithelial cell function, and protecting villus structure (Kinoshita et al. 2002; Elnesr et al. 2019).Butyric acid also promotes intestinal cell proliferation, which aids in the repair of injured mucosa and increases villus height, as well as improves blood flow to the intestines and the secretion of digestive enzymes (Elnesr et al. 2017b; Sikandar et al., 2018).

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Table 7 reveals that the number of E. coli was significantly higher in treatments T3, T4, T6, and T7 compared to T1, whereas there were no statistically significant differences between treatments T2, T5, and T1. As the pH of the intestine is lowered by calcium and sodium butyrate, it becomes an unfavourable environment for the growth of harmful bacteria and the activity of acid-loving bacteria (Hodin, 2000; Rezaei, and Guilloteau, 2010). The composition of the intestinal microbiota is profoundly influenced by acidity (Warnecke and Gill, 2005).

Table 7. Effect of sodium and calcium butyrate supplementation on the on microbial traits (logarithmic cycle / g) in different parts of the intestine at 6 weeks of age

Treatment	Duodenum		Jejunum		Ileum	
	E. coli	Lacto	*E. coli*	Lacto	*E. coli*	Lacto
T1	0.11±4.74	0.18±4.99	0.13±4.87	0.08±4.93	C 0.16±4.73	0.07±5.18
T2	0.06±4.88	0.02±5.27	0.50±5.22	0.29±4.85	BC 0.05±4.92	0.45±5.56
T3	0.31±5.33	0.20±4.86	0.45±5.19	0.29±4.68	A 0.05±5.24	0.20±5.06
T4	0.07±5.19	0.27±4.72	0.01±4.90	0.12±5.06	AB 0.09±5.12	0.04±5.26
T5	0.11±4.95	0.32±5.25	0.09±494	0.32±5.43	C 0.13±4.78C	0.32±5.47
T6	0.37±5.42	0.12±5.20	0.23±5.10	0.47±5.72	BC 0.02±4.90	0.22±5.11
T7	0.26±5.40	0.08±5.16	0.36±5.50	0.61±4.92	BC 0.10±4.90	0.39±5.42
Significance	N. S	N. S	N. S	N. S	*	N. S

*Different letters within the same column indicate significant differences at the level (P<0.05), N.S. There is no significant difference.

** treatments: T1 is a control treatment free of addition, T2, T3, T4, adding sodium butyrate at levels 0.2, 0.4, 0.8%, respectively, and T5, T6, and T7 adding calcium butyrate at levels 0.2, 0.4, 0.8%, respectively.

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