ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 13 (7): 390-396, 2014
ISSN 1682-8356

Effect of Partially Protected Sodium Butyrate on Performance, Digestive Organs, Intestinal Villi and *E. coli* Development in Broilers Chickens

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Abstract: The aim of this study was to evaluate the effect of partially protected sodium butyrate (PSB) on performance, digestive organs, intestinal villi and *E. coli* development in broilers chickens. Nine hundred twenty four one-day-old mixed Cobb® chicks were divided in 3 treatments with 7 replicates each in a randomized block design. Treatment T1 was a control diet without any growth promoter, treatment T2 was the control diet plus colistin at 100,000 IU/kgBW and treatment T3 was the control diet with PSB at 700 ppm. Chicks were fed in mash form for 3 phases: starter (1-14 days), grower (15-28 days) and finisher (29-42 days). There were no significant differences on performance among all treatments in starter phase. Chicks fed PSB in grower and finisher phases had the highest weight gain and the best feed conversion ratio. Relative digestive organs weights were not affected by treatment in any phase. Jejunum and small intestine relative lengths of birds fed PSB and colistin at 14 days were longer than those of birds fed control diet. Jejunal villi of birds fed PSB and colistin at 42 days were higher than those in birds fed the control diet. Colistin produced the deepest crypts and the lowest villi height/crypt depth ratios in all intestinal segments at 14 days. Intestinal *E. coli* growth was not affected by any treatment. These data indicate that partially protected sodium butyrate and colistin improves performance, colistin as an antibiotic growth promoter and PSB by improving intestinal villi development in broilers chickens.

Key words: Sodium butyrate, performance, villi, *Escherichia coli*, broilers

INTRODUCTION

Antibiotics have been used for decades to improve poultry performance with low cost of implementation and ease adding to feed and water (Fernandez-Rubio *et al*., 2009). However, currently concern about possible antibiotic residues and resistance has arisen restrictions of antibiotics use in poultry (Jan *et al*., 2007; Saberfar *et al*., 2008). Because of this fact, industry and researchers have had to look for natural alternatives such as oily plant extracts (Hernandez *et al*., 2004; Mitsch *et al*., 2004), yeast cell walls (Gajewska *et al*., 2012), probiotics (Dankowiakowska *et al*., 2013; Patterson and Burkholder, 2003) and prebiotics (Hajati and Rezaei, 2010).

According to Adil *et al*. (2011), short chain fatty acids (SCFA) are promissory and potentially alternatives to antibiotic growth promoters. Of these acids, butyric acid has shown to have effects on promoting colonic epithelial cell proliferation, stimulating immune system, modulating intestinal microflora composition against harmful bacteria and preventing from colonic cancer factor. However, other researchers have not found any proved benefits, possibly due to influence of butyric acid chemical form and doses (Irani *et al*., 2011; Mahdavi and Torki, 2009; Mansoub *et al*., 2011; Mansoub, 2011; Sayrafi *et al*., 2011). Butyric acid products have to be included in the diet in conjunction or encapsulates with other chemicals in order to decrease its unpleasant odor and increase ration palatability (Antongiovanni *et al*., 2010).

Despite many studies have been published about different butyric acid forms and their effects on broiler chickens performance, little is known about partially protected sodium butyrate. The aim of this experiment was to check efficacy of partially protected sodium butyrate on performance, digestive organs, intestinal villi and *E. coli* development in broiler chickens.

MATERIALS AND METHODS

Diet formulation: Basal diets without antibiotic growth promoters were formulated for starter (1-14 days), grower (15-28 days) and finisher (29-42 days) phases. Diets were based on corn and soybean meal to meet nutritional standards for medium performance broiler male chickens published by Rostagno *et al*. (2011). Animal protein sources were added to all diets between 6-8.5%, which is a common animal protein source level in poultry industry. All diets were fortified with complete vitamin and trace mineral mixes. Diets composition and calculated analysis are shown in Table 1. Diets were formulated using Brill Formulation® (Feed Management Systems Inc.) based on digestible amino acids. Calcium

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Table 1: Experimental diet composition (%) and calculated nutritional content

	Starter	Grower	Finisher
Ingredient	0-14 day	15-28 day	29-42 day
Yellow corn	57.99	59.25	63.60
Soybean meal	28.30	26.00	22.50
Fish meal, 65%	5.00	-	-
Blood meal	3.50	2.00	-
Meat and bone meal	-	3.00	1.00
Poultry meal	-	3.00	5.00
Palm oil	1.60	3.20	3.80
Limestone	1.00	1.00	1.10
Monocalcium phosphate	1.20	1.00	1.30
Vitamins and minerals premix[1]	0.15	0.15	0.15
Salt	0.31	0.26	0.26
DL methionine	0.26	0.24	0.22
Lysine sulphate	0.02	0.22	0.42
L-Threonine	0.01	0.02	0.05
Antifungal	0.25	0.25	0.20
Choline chloride 60%	0.15	0.15	0.15
Mycotoxin adsorbent	0.20	0.20	0.20
Anticoccidial	0.05	0.05	0.05
Antioxidant	0.02	0.02	0.02
Calculated analyses			
ME, Kcal/kg	2975	3050	3132
Crude protein (%)	21.50	21.00	18.50
Lys dig (%)	1.24	1.13	1.03
Met dig (%)	0.48	0.45	0.41
TSAA dig (%)	0.90	0.82	0.76
Thr dig (%)	0.81	0.73	0.67
Trp dig (%)	0.21	0.20	0.19
Crude fiber (%)	3.27	3.31	3.32
Calcium (%)	1.00	0.95	0.85
Nonphytate P (%)	0.50	0.45	0.42
Sodium (%)	0.22	0.18	0.17

[1]Vitamin and mineral premix contain the following ingredients/kg: vitamin A 12MIU, vitamin D_3 5MIU, vitamin E 35KIU, vitamin K 3,8 g, thiamin 3,5 g, Riboflavin 9 g, Pyridoxine 4 g, niacin 65 g, folic acid 1,8 g, Cyanocobalamine 19 mg, Pantothenic acid 17 g, biotin 170 mg, Cu 15 g, I 1 g, Fe 55 g, Mn 110 g, Se 0,28 g, Zn 90 g csp 1 kg

and available phosphorus requirements were similar to average industry values. All diets were given as mash and according to feed intake tables for this geographical area. These tables included a feed intake restriction to avoid ascites. Also, no antibiotics were provided through drinking water.

Housing and management: Nine hundred twenty four one-day-old mixed Cobb 500® chicks were obtained from a local hatchery and were distributed in three treatments with 7 replicates and forty four chicks randomly assigned each. Experiment was arranged in a randomized block design according to starting live body weight and barn location. First treatment (negative control) (T1) was a basal diet, second treatment (T2) was the basal diet with colistin sulfate (Colival®) at 100,000 IU/kgBW as a growth promoter. Third treatment (T3) was the basal diet plus 700 ppm of partially protected sodium butyrate (Gustor BP70®), which is 30% protected in vegetable fat and 40% in free form.

Chicks were vaccinated against Newcastle disease and Infectious Bronchitis via spray post hatch. New rice husk served as litter over concrete floors. A commercial designed broiler facility, located at 2,600 meters above sea level, was used. Each pen was equipped with one tubular feeder and an automatic water font. Temperature and ventilation were controlled by gas heaters and manually set curtains. Temperature was maintained at 32±1°C during first week, then gradually reduced to 22±1°C at the end of fifth week. Management, health and biosecurity measures were similar to conventional practices for this region to avoid ascites development.

Measurements: Data collection was based on 100% of experimental population. The method of weight was conducted on a group basis in each of seven repetitions. Live body weight, feed intake and death chicks were recorded for each repetition on days 1, 14, 28 and 42. Death chicks or removed for any reason (for sampling, ascites) and feed residue were weighed before taken them out from pens. Adjusted feed conversion was calculated as feed consumed divided by gain weight of live birds plus dead or cull birds (kg feed/kg of gain).

Digestive organ measurements were conducted on days 14, 28 and 42. One chick per repetition was randomly selected and slaughtered after electrical stunning. Proventriculus, gizzard, liver, spleen and pancreas were weighed. Intestines were extended on a table and each segment was measured with a flexible meter. Data summaries were reported as relative weighs (g/kgLBW) and lengths (cm/kgLBW), respectively (Aghazadeh and TahaYazdi, 2012; Mahdavi and Torki, 2009).

After length measurement, intestines were dissected to cut one-centimeter pieces from distal portions of duodenum (one centimeter before descending loop), jejunum (one centimeter before Meckel's diverticulum) and ileum (one centimeter before ileocecal junction) (Jaramillo, 2011). These pieces were fixed with 10% saline buffered formalin and then, processed by Paraffin inclusion method. Pieces were sliced by cryotome on 5 micrometer thickness laminates and finally, stained by Hematoxylin-Eosin method (Shiva *et al*., 2012).

Villi height and crypt depth were measured under a Carl Ziess microscopy (Jena 742797 RDA model) and measured with graduated optic lenses (Olympus 10x/18). Villi height was measured from villi apex until crypt mouth. Crypt depth was measured from crypt bottom until crypt mouth. One villi and one crypt classified as normal were measured in each intestinal segment (Itza *et al*., 2008).

Intestinal *E. coli*, jejunal content samples were taken from the same slaughtered chicks for digestive organs evaluation to follow the methodology of dilution and incubation by Mahdavi *et al*. (2010). Here, one gram of jejunal content was diluted in 9 mL of saline solution to get a 10^1 dilution. Then, one mL of this dilution was mixed with 9 mL of saline solution to get another ten-fold dilution. These serial dilutions were carried out until

getting a 10^6 dilution. For *E. coli* growing, 1 mL of each dilution was spread on a Petrifilm® (3M Inc.) plate. After a 24 h incubation at 37°C, air-bubble-associated blue colonies were count Colony counts were multiply by dilution to obtain the number of *E. coli* unit forming colonies per gram of jejunal content (CFU/g).

Data were processed with SAS® (SAS Institute Inc.) statistical software and analyzed with a two-way analysis of variance. Multiple comparisons were conducted with Duncan method at 0.05 alpha level.

RESULTS AND DISCUSSION

Live performance: Treatment effect on performance is shown in Table 2. Sodium butyrate or colistin sulfate supplementation in starter phase did not affect weight gain, feed intake and feed conversion ratio. Similar results at this age were found in other studies (Antongiovanni *et al*., 2007; Liu, 2009; Mahdavi and Torki, 2009). In contrast to these findings, Mansoub (2011) reported that up to 0.2% of sodium butyrate increased weight gain and feed conversion ratio during the first 28 days.

In growing and finishing phases, chicks fed partially protected sodium butyrate diet showed better weight gain and feed conversion ratio than chicks fed control diet. Similar findings in these phases were found in other studies with butyric acid glycerides (Antongiovanni *et al*., 2007; Leeson *et al*., 2005; Taherpour *et al*., 2009), butyrate (Panda *et al*., 2009), partially protected sodium butyrate (Mallo *et al*., 2010) and encapsulated butyrate (Zou *et al*., 2010).

Butyric acid benefits are caused by its effects on promoting intestinal epithelium cell development and modulating intestinal symbiotic bacteria growth (Antongiovanni *et al*., 2007). It reduces pathogenic bacteria growth and increases beneficial ones. In this study, lack of butyric acid effect on performance during first two weeks appears to be due to its total ingested Chicks were 20% feed restricted during second week to prevent ascites development and butyric acid dose was not adjusted to this feed intake.

Colistin sulfate improved weight gain and feed conversion ratio in last two weeks when compared to control group. Colistin sulphate did not produce better performance than sodium butyrate in any phase. Previous studies with other growth promoters like such as bacitracin (Liu, 2009) bacitracin plus colistin (Zou *et al*., 2010) bacitracin methylene disalicylate (Sayrafi *et al*., 2011) and colistin sulfate (Bozorgmehri, 2004; Teo and Tan, 2006) reported no effects on weight gain and feed conversion ratio on days 1-21 but they found better performance on days 22-42 when compared to control treatment.

Mortality ratio and feed intake were not affected by the treatment in all phases. In this study, butyrate and colistin sulphate effect on feed intake was not properly assessed due to feed restriction to avoid ascites.

Table 2: Effects of dietary partially protected sodium butyrate and colistin on performance of broilers

Days	Negative control	Colistin	Sodium butyrate	p-value
Body weight gain (g)				
0-14	281±7	283±5	283±8	0.7251
15-28	684±14^b	680±9^b	725±10^a	<0.0001
29-42	1019±66^b	1072±50^a	1082±88^a	0.0232
0-42	1984±84^c	2035±53^b	2090±87^a	0.0021
Feed intake, g/bird				
0-14	367±7	370±3	369±6	0.5588
15-28	1257±29	1262±16	1274±11	0.2273
29-42	1950±72	1952±36	1972±67	0.4481
0-42	3574±103	3584±44	3615±72	0.2972
Feed conversion, g fed/g gain				
0-14	1.31±0.03	1.31±0.02	1.30±0.02	0.9665
15-28	1.84±0,02^a	1.86±0.03^a	1.76±0.03^b	0.0001
29-42	1.92±0.06^a	1.82±0.06^b	1.83±0.10^b	0.0052
0-42	1.80±0.03^a	1.76±0.03^b	1.73±0.04^c	0.0004
Mortality (%)				
0-14	3.9±3.2	3.3±5.3	3.4±1.1	0.9121
15-28	2.2±2.6	2.1±2.9	1.6±1.7	0.8672
29-42	1.3±1.8	1.7±1.8	1.9±1.5	0.7993
0-42	7.3±3.9	6.9±7.1	6.8±2.3	0.9713

Values are means±standard error. Means values with different letters at the same row differ significantly at p<0.05

Butyric acid products, forms, types and doses could explain possible results reported in other studies with no effects on performance in any phase (Aghazadeh and Taha Yazdi, 2012; Irani *et al*., 2011; Mahdavi and Torki, 2009; Mallo *et al*., 2012; Sayrafi *et al*., 2011). Zhang *et al*. (2011) suggested that lack of effect of sodium butyrate on performance could be attributable to different types and concentrations of bacteria that chicks are exposed in different rearing conditions, for this reason anti-inflammatory and anti-catabolic effects of butyric acid are more evident when chicks are reared in low health status conditions than in high ones.

Digestive organs: Relative weights of evaluated organs were not affected by butyrate or colistin addition in any phase. These findings are shown in Table 3. Other authors have reported higher relative weights of proventriculus gizzard or liver (Liu, 2009; Aghazadeh and Taha Yazdi, 2012). However, other studies have not found effect of sodium butyrate on relative weights of liver or gizzard (Antongiovanni *et al*., 2007; Adil *et al*., 2011; Panda *et al*., 2009; Mallo *et al*., 2012; Aghazadeh and Taha Yazdi, 2012). Spleen and pancreas (Jang *et al*., 2007; Mahdavi and Torki, 2009; Mallo *et al*., 2012).

Only pancreas relative weight was lowest with sodium butyrate that control treatment at 14 days ($p<0.05$). According to Guilloteau *et al*. (2010) butyrate supplementation increases mitotic index and decreases apoptotic index of exocrine pancreatic cells in calves. This means that butyrate speeds up pancreatic cellular refill without increasing pancreas relative weight. Also these authors found that by the time pancreatic Elastase II reach its 50% activity, the majority of gastric content gets duodenal lumen. This phenomenon takes 2-3 h

Table 3: Digestive organ relative weight (g/kg) of each treatment

Days	Negative control	Colistin	Sodium butyrate	p-value
Proventriculus				
14 d	7.5±0.6	7.4±0.9	7.4±0.7	0.9664
28 d	4.7±0.6	4.9±0.5	5.0±0.7	0.6279
42 d	3.1±0.6	3.0±0.3	3.5±0.6	0.3105
Gizzard				
14 d	36.4±4.4	38.7±5.5	36.3±3.4	0.4167
28 d	22.7±4.4	23.8±0.9	22.0±3.6	0.5623
42 d	16.8±2.7	17.2±2.6	16.5±1.7	0.8873
Liver				
14 d	42.4±7.4	43.4±8.1	42.1±4.3	0.9075
28 d	35.7±2.2	34.7±3.7	35.6±3.1	0.8510
42 d	25.6±3.6	24.1±2.5	25.1±2.2	0.6562
Pancreas				
14 d	5.0±1.6^{a}	4.5±0.8^{ab}	3.9±0.4^{b}	0.0966
28 d	3.3±0.2	3.0±0.6	3.4±0.2	0.1864
42 d	2.0±0.4	2.1±0.3	2.0±0.3	0.7057
Spleen				
14 d	1.1±1.1	0.8±0.6	0.9±1.2	0.8315
28 d	1.2±0.4	1.0±0.1	1.1±0.2	0.4451
42 d	1.2±0.4	1.5±0.3	1.3±0.4	0.4397

Values are means±standard error. Means values with different letters at the same row differ significantly at p<0.05

Table 4: Small intestine relative lengths (cm/kg) of each treatment

Days	Negative control	Colistin	Sodium butyrate	p-value
Duodenum*				
14 d	75±22	83±6	88±8	0.2908
28 d	28±4	26±3	27±4	0.6858
42 d	15±2	14±1	16±1	0.4266
Jejunum				
14 d	162±23^{b}	185±16^{a}	190±11^{a}	0.0192
28 d	63±10	62±9	61±6	0.9434
42 d	35±5	35±6	38±6	0.8123
Ileum				
14 d	154±28^{b}	182±19^{a}	176±9^{ab}	0.0529
28 d	61±8	61±10	59±6	0.8116
42 d	35±5	33±5	34±5	0.8042
Small intestine				
14 d	391±74^{b}	450±38^{a}	455±23^{a}	0.0609
28 d	152±19	150±20	147±12	0.8678
42 d	86±16	83±11	87±11	0.7950

Values are means±standard error. Means values with different letters at the same row differ significantly at p<0.05

and it seems to explain better digestibility and performance observed in calves supplemented with sodium butyrate.

Intestinal relative lengths are summarized on Table 4. Duodenum relative length was not affected by the treatments in any phase. Jejunum and small intestine relative lengths were longer with butyrate or colistin at 14 days as compared with control treatment. Also, ileum relative length was longer with colistin at 14 days than control.

Heavier (Aghazadeh and TahaYazdi, 2012) and longer small intestines (Adil *et al*., 2011) have been reported with sodium butyrate. Small chain fatty acids like acetic propionic and butyric stimulated intestinal epithelial cell proliferation by increasing intestinal levels and expressions of Glucagon Like Peptide 2 (GLP-2), ileal proglucagon mRNA, Glucose Transporter Type 2 (GLUT-2) and other proteins (Adil *et al*., 2011). Also, high dose of sodium butyrate increased jejunum and small intestines lengths (Mahdavi and Torki, 2009). It seems that colonic SCFA induce autonomic neural signals that go to central nervous system, which responds with a second neural signal, a hormone or a growth factor (Gastrin) that stimulate jejunum development. Besides, sodium butyrate increases blood flow to intestines that leads to better tissue oxygenation and growth (Reilly *et al*.,1995).

Intestinal villi: Villi height, crypt depth and villi height/crypt depth ratio (VH/CD ratio) are shown in Table 5. In duodenum, crypts were deeper and VH/CD ratio was lower with colistin at 14 days. There were no differences by treatments in duodenum villi characteristics in the others phases. Higher duodenal villi have been reported using 3% but not with 2% of sodium butyrate at 42 days (Adil *et al*., 2011).

In jejunum, villi height was higher (p<0.05) in butyrate and control treatments at 14 days and butyrate or colistin at 42 days. Crypt depth was lower and VH/CD ratio was higher (p<0.05) in butyrate and control treatments at 14 days. In this study, colistin produced the deepest crypts as in duodenum. Increased jejunal villi with addition of 2-3% of butyric acid in birds at 42 days (Adil *et al*., 2011) indicated that butyric acid modulates intestinal bacteria and stimulates intestinal epithelial growth.

In ileum, there were no differences in villi height between treatments or any phases. Crypts were less deep and VH/CD ratio was higher in butyrate and control treatment at 14 days and with colistin at 42 days. Some studies have shown beneficial effects of sodium butyrate on villi and crypts at different ages (Antongiovanni *et al*., 2007; Mallo *et al*., 2012; Panda *et al*., 2009; Sayrafi *et al*., 2011; Smulikowska *et al*., 2009) but others have not found any effect (Adil *et al*., 2011; Leeson *et al*., 2005). These different responses are attributable to dose, form and concentration of butyric acid and level of microbial exposure that chicks were subjected.

Intestinal mucosa function and structure may depend on balance between cell proliferation, migration and apoptosis (Murakami *et al*., 2007). This cell intestinal refill takes place every 90-96 h (Macari, 1998). In this study, deeper crypts obtained with colistin at 14 days could be a result of a high cell refill ratio due to either by increasing in villi dimensions or by maintenance because of high destruction ratio (Antongiovanni *et al*., 2007; Sayrafi *et al*., 2011). Cao *et al*. (2013) has shown significant decrease in villus height and increased crypt depth when chickens are challenged by bacteria such as *E. coli*. However, the lower VH/CD ratio obtained with colistin in this study conditions confirms that colistin produced deep crypts because of maintenance rather than increased villus height, this is due antibiotics are

Table 5: Villi height (VH, um), crypt depth (CD, um) and VH/CD ratio in broiler chickens fed either Colistin or partially protected sodium butyrate

	Villus height (um)				Crypt depth (um)				VH/CD ratio			
Days	Negative control	Colistin	Sodium butyrate	p-value	Negative control	Colistin	Sodium butyrate	p-value	Negative control	Colistin	Sodium butyrate	p-value
Duodenum												
14 d	436±24	471±76	464±80	0.6339	183±37^{b}	279±39^{a}	217±49^{b}	0.0039	2.47±0.39^{a}	1.7±0.3^{b}	2.2±0.4^{a}	0.0069
28 d	457±53	457±84	514±103	0.2814	336±56	371±57	400±115	0.3249	1.40±0.41	1.3±0.3	1.4±0.4	0.4756
42 d	507±121	550±65	557±84	0.5045	371±45	407±73	400±58	0.4488	1.36±0.30	1.4±0.3	1.4±0.2	0.9280
Jejunum												
14 d	821±86^{a}	686±63^{b}	771±99^{a}	0.0075	230±37^{b}	371±49^{a}	257±67^{b}	0.0002	3.61±0.48^{a}	1.9±0.2^{b}	3.2±1.1^{a}	0.0019
28 d	779±104	736±99	736±107	0.4910	414±56	429±39	464±69	0.2642	1.91±0.38	1.7±0.4	1.6±0.4	0.4204
42 d	836±90^{b}	957±61^{a}	957±73^{a}	0.0311	486±56	514±75	507±61	0.6986	1.76±0.30	1.9±0.3	1.9±0.2	0.6224
Ileum												
14 d	1121±91	1086±125	1079±122	0.7833	279±70^{b}	421±76^{a}	300±71^{b}	0.0022	4.24±1.07^{a}	2.6±0.3^{b}	3.8±1.0^{a}	0.0076
28 d	1229±236	1229±160	1307±146	0.3242	486±63	486±85	493±61	0.9506	2.51±0.25	2.5±0.2	2.7±0.3	0.5136
42 d	1221±138	1357±110	1314±184	0.1764	529±39^{a}	450±50^{b}	500±76^{ab}	0.0299	2.34±0.37^{b}	3.0±0.4^{a}	2.7±0.4^{ab}	0.0205

Values are means±standard error. Means values with different letters at the same row differ significantly at p<0.05

Table 6: Jejunal *E. coli* growth (Log CFU/g) of each treatment

Days	Negative control	Colistin	Sodium butyrate
14 d	3.1±2.2	2.9±1.9	2.9±1.9
28 d	3.3±0.7	3.2±0.6	3.4±0.7
42 d	4.3±0.4	4.2±0.4	4.1±0.2

Values are means±standard error

ineffective modulating intestinal bacteria. Beneficial bacteria decrease produces more cell refill to replace more cell destruction. This needed is satisfied by deeper crypts (Sayrafi *et al*., 2011). Also, anticoccidials like salinomycin reduce jejunal villi height and crypt depth. Besides, they decrease SCFA, lactic acid and the amount of lacto bacilli and total bacteria (Czerwinski *et al*., 2012).

***Escherichia coli*:** Jejunal *E. coli* growth was not different among treatments (p>0.05). Table 6 shows results of *E. coli* quantification. Similarly, Hu and Guo (2007) did not find any effect of sodium butyrate at three different levels on jejunal *E. coli* growth in McConkey and EMB agar. It has been shown that intestine segments have different microbial populations and therefore different SCFA concentrations and pH (Sun and O'Riordan, 2013). Beneficial bacteria produce bactericides and can compete against pathogen bacteria for space and nutrients as *E. coli* and *C. difficile* (Sun and O'Riordan, 2013). Beneficial bacteria are highly host adapted to physic and nutritional characteristics.

In this study, although diets included animal protein sources like fish, meat and bone, blood or chicken by-product meals. *E. coli* growth was not influenced by SCFA presence neither by competence of the other jejunal bacteria. Here, *E. coli* growth in control treatment was similar to other control diets results obtained with corn-soybean meal diets (Diarra *et al*., 2007; Mahdavi *et al*., 2010).

Intestinal bacteria in broiler chickens are established 2-3 weeks post hatch. Antibiotics can alter this process. Colistin sulfate increased Enterobacteria population in jejune and ceca at 2 and 4 weeks of age (Ohya and Sato, 1983). Some *E. coli* strains did not decrease although they were *in vitro* sensible (Kikuchi *et al*., 1969). And most recently, Yang *et al*. (2012) found an increment in the population of cecal *E. coli* from 21 to 42 days in birds treated with colistin sulfate.

Several mechanisms may explain beneficial effects of butyrate as examples: control of pathogenic bacteria and improved performance, increased digestibility and reduced oxidative stress and inflammatory processes (Van Inmersel *et al*., 2004; Panda *et al*., 2009; Fernandez-Rubio *et al*., 2009; Guilloteau *et al*., 2010, Mallo *et al*., 2012), however there are still details to be clarified in poultry. Taking into account other factors that may influence the response of butyric acid as form, protected or not, concentration and dosage, pH of intestinal environment, exposure time, sensitivity of the type of specific pathogens, environmental rearing, different proportions of protein raw materials, raw material digestibility and buffer capacity of the diet have to be conducted to assess the effect of this additive in broiler production.

Conclusions: The results of this study support that supplementation with partially protected sodium butyrate improves performance of broilers due to the stimulating effect of butyric acid on the development and regeneration of intestinal villi, mainly in the jejune and ileum. Apparently it may require higher oral doses of sodium butyrate protected early when birds are restricted feeding. Therefore, the partially protected sodium butyrate can be used as growth promoter in broiler diets and is a viable alternative to antibiotic growth promoter rotation.

Conflict of interest: Authors declare that do not have any conflict of interest while conducting this trial.

ACKNOWLEDGEMENTS

Authors would immensely like to thank to Integracion Avicola Oro Cia. Ltda. for funding and Dr. Sandro Cerrate for technical help to this project.

REFERENCES

Adil, S., G. Banday, M. Bhat, M. Salahuddin, T. Ahmad Raquib and S. Shanaz, 2011. Response of broiler chicken to dietary supplementation of organic acids. J. Central Eur. Agric., 12: 498-508.

Aghazadeh, A.M. and M. Taha Yazdi, 2012. Effect of butyric acid supplementation and whole wheat inclusion on the performance and carcass traits of broilers. S. Afr. J. Anim. Sci., 42: 241-248.

Antongiovanni, M., A. Buccioni, S. Minieri, I. Galigani and S. Rapaccini, 2010. Monobutyrine: a novel feed additive in the diet of broiler chickens. Italian J. Anim. Sci., 9: 369-371.

Antongiovanni, M., A. Buccioni, F. Petacchi, S. Leeson, S. Minieri, A. Martini and R. Cecchi, 2007. Butyric acid glycerides in the diet of broiler chickens: effects on gut histology and carcass composition. Italian J. Anim. Sci., 6: 19-25.

Bozorgmehri, F., 2004. The effect of Colistin Sulfate in feed on the controlling of Salmonella enteritidis contamination in a broiler farm. Arch. Razi Ins., 58: 105-110.

Cao, G.T., X.F. Zeng, A.G. Chen, L. Zhou, L. Zhang, Y.P. Xiao and C.M. Yang, 2013. Effects of a probiotic. Enterococcus faecium. on growth performance. intestinal morphology. immune response. and cecal microflora in broiler chickens challenged with *Escherichia coli* K88. Poult. Sci., 92: 2949-2955.

Czerwinski, J., O. Hojberg, S. Smulikowska, R.M. Engberg and A. Mieczkowska, 2012. Effects of sodium butyrate and salinomycin upon intestinal microbiota. mucosal morphology and performance of broiler chickens. Arch. Anim. Nutr., 66: 102-116.

Dankowiakowska, A., I. Kozlowska and M. Bednarczyk, 2013. Probiotics prebiotics and symbiotics in poultry. Mode of action limitation and achievements. J. Central Eur. Agric., 14: 467-478.

Diarra, M.S., F.G. Silversides, F. Diarrassouba, J. Pritchard, L. Masson, R. Brousseau and E. Topp, 2007. Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens. *Clostridium perfringens* and *enterococcus* counts. and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Escherichia coli* isolates. Appl. and Environ. Microbiol., 73: 6566-6576.

Fernandez-Rubio, C., C. Ordonez, J. Abad-Gonzalez, A. Garcia-Gallego, M. Pilar Honrubia, J. Jose Mallo and R. Balana-Fouce, 2009. Butyric acid-based feed additives help protect broiler chickens from *Salmonella enteritidis* infection. Poult. Sci., 88: 943-948.

Gajewska, J., J. Riedel, A. Bucka, J. Zabik and M. Michalczuk, 2012. Influence of prebiotics and butyric acid on the composition of intestinal microflora of broiler chickens. Annals of Warsaw University of Life Sciences-Sggw Anim., 51: 47-53.

Guilloteau, P., L. Martin, V. Eeckhaut, R. Ducatelle, R. Zabielski and F. Van Immerseel, 2010. From the gut to the peripheral tissues: the multiple effects of butyrate. Nutr. Res. Rev., 23: 366-384.

Hajati, H. and M. Rezaei, 2010. The application of prebiotics in Poultry production. Int. J. Poult. Sci., 9: 298-304.

Hernandez, F., J. Madrid, V. Garcia, J. Orengo and M.D. Megias, 2004. Influence of two plant extracts on broilers performance. digestibility. and digestive organ size. Poult. Sci., 83: 169-174.

Hu, Z. and Y. Guo, 2007. Effects of dietary sodium butyrate supplementation on the intestinal morphological structure. absorptive function and gut flora in chickens. Anim. Feed Sci. and Tech., 132: 240-249.

Irani, M., S. Gharahveysi, M. Zamani and R. Rahmatian, 2011. The effect of butyric acid glycerides on performance and some bone parameters of broiler chickens. Afr. J. Biotech., 10: 12812-12818.

Itza, M., C. Lopez, E. Avila, S. Gomez, P. Velasquez and J. Arce, 2008. Efecto de la fuente energetica y el nivel de energía sobre la longitud de las vellosidades intestinales. la respuesta inmune y el rendimiento productivo en pollos de engorda. Vet. Mexico, 39: 357-376.

Jan, I.S., Y.H. Ko, S.Y. Kang and C.Y. Lee, 2007. Effect of a commercial essential oil on growth performance. digestive enzyme activity and intestinal microflora population in broiler chickens. Anim. Feed Sci. and Tech., 134: 304-315.

Jaramillo, A., 2011. Evaluacion de la mezcla de un prebiotico y un acido organico en la salud intestinal y parametros productivos de pollos de engorde. Tesis de grado Magster en Produccin Agrcola Universidad nacional de Colombia Ibagu Colombia.

Kikuchi, T., K. Inoue, M. Sakai, N. Hara, K. Sunagawa, Y. Ichihashi and T. Oikawa, 1969. The effect of antibiotics against the intestinal bacterial flora. In eds Germ Free Biol. Exp. and Clini. Aspects Plenum Press New York, 355-359.

Leeson, S., H. Namkung, M. Antongiovanni and E.H. Lee, 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poult. Sci., 84: 1418-1422.

Liu, L., 2009. Effects of sodium butyrate on growth. digestion. intestinal histology and immunity in broiler chickens. Tesis de Master Nanjing Agricultural College S8315.

Macari, M., 1998. Aspectos? Siologicos do sistema digestivo das aves. Pages in VIII SACAVET Semana Acad Med Vet FMVZUSP Sao Paulo Brazil, 4-18

Mahdavi, A.H., H.R. Rahmani, N. Nili, A.H. Samie, S. Soleimanian-Zad and R. Jahanian, 2010. Effects of dietary egg yolk antibody powder on growth performance. intestinal *Escherichia coli* colonization. and immunocompetence of challenged broiler chicks. Poult. Sci., 89: 484-494.

Mahdavi, R. and M. Torki, 2009. Study on Usage Period of Dietary Protected Butyric Acid on Performance. Carcass Characteristics. Serum Metabolite Levels and Humoral Immune Response of Broiler Chickens. J. Anim. and Vet. Adv., 8: 1702-1709.

Mallo, J.J., M. Puyalto and S.V. Rama Rao, 2012. Evaluation of the effect of sodium butyrate addition to broiler diet on energy and protein digestibility productive parameters and size of intestinal villi of animals. Feed Livest., 8: 26-30.

Mallo, J.J., M. Gracia, P. Honrubia and M. Puyalto, 2010. Effect of butyrate on broiler performance. S9-Nutrition. Digestion. XIIIth European Poultry Conference.

Mansoub, N., K. Rahimpour, L. Majedi, M. Mohammad, M.S. Zabihi and M. Kalhori, 2011. Effect of different level of butyric acid glycerides on performance and serum composition of broilers chickens. World J. Zool., 6: 179-182.

Mansoub, N.H., 2011. Comparative effect of butyric acid. probiotic and garlic on performance and serum composition of broilers chickens. Version Electronica Am. Eur. J. Agric. and Environ. Sci., 11: 507-511.

Mitsch, P., K. Zitterl-Eglseer, B. Kohler, C. Gabler, R. Losa and I. Zimpernik, 2004. The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. Poult. Sci., 83: 669-675.

Murakami, A.E., M.I. Sakamoto, M.R.M. Natali, L.M.G. Souza and J.R.G. Franco, 2007. Supplementation of glutamine and vitamin E on the morphometry of the intestinal mucosa in broiler chickens. Poult. Sci., 86: 488-495.

Ohya, T. and S. Sato, 1983. Effects of dietary antibiotics on intestinal microflora in broiler chickens. Nat. Inst. Anim. Health Quarterl., 23: 49-60.

Panda, A.K., S.V.R. Rao, M.V.L.N. Raju and C.S. Sunder, 2009. Effect of Butyric Acid on Performance. Gastrointestinal Tract Health and Carcass Characteristics in Broiler Chickens. Asian-Austr. J. Anim. Sci., 22: 1026-1031.

Patterson, J.A. and K.M. Burkholder, 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci., 82: 627-631.

Reilly, K.J., W.L. Frankel, A.M. Bain and J.L. Rombeau, 1995. Colonic short-chain fatty-acids mediate jejunal groth by increasing gastrin. I. Gut., 37: 81-86.

Rostagno, H., L. Teixeira, J. Lopes, P. Gomes, R. Oliveira and D. Lopes, 2011. Tablas brasileñas para aves y cerdos.

Saberfar, E., B. Pourakbari, K. Chabokdavan and F. Taj Dolatshahi, 2008. Antimicrobial Susceptibility of *Escherichia coli* Isolated from Iranian Broiler Chicken Flocks, 2005-2006. J. Appl. Poult. Res., 17: 302-304.

Sayrafi, R., F. Soltanalinejad, R. Shahrooz and S. Rahimi, 2011. Effects of butyric acid glycerides and antibiotic growth promoter on the performance and intestinal histomorphometry of broiler chickens. J. Food Agric. and Environ., 9: 285-288.

Shiva, C., S. Bernal, M. Sauvain, J. Caldas, J. Kalinowski, J. and N. Falcon, 2012. Evaluacion del aceite esencial de orégano y (*Origanunvulgari*) y extracto deshidratado de jengibre (*Zingiberofficinali*) como potenciales promotores de crecimiento en pollos de engorde[version electronica]. Revista de Investigaciones Vet. del Peru, 23: 160-170.

Smulikowska, S., J. Czerwinski, A. Mieczkowska and J. Jankowiak, 2009. The effect of fat-coated organic acid salts and a feed enzyme on growth performance. nutrient utilization. microflora activity. and morphology of the small intestine in broiler chickens. J. Anim. and Feed Sci., 18: 478-489.

Sun, Y. and M.X.D. O'Riordan, 2013. Regulation of Bacterial Pathogenesis by Intestinal Short-Chain Fatty Acids. In S. Sariaslani and G. M. Gadd (Eds.). San Diego: Elsevier Academic Press Inc. Adv. in Appl. Microbiol., 85: 93-118.

Taherpour, K., H. Moravej, M. Shivazad, M. Adibmoradi and B. Yakhchali, 2009. Effects of dietary probiotic. prebiotic and butyric acid glycerides on performance and serum composition in broiler chickens. Afr. J. Biotech., 8: 2329-2334.

Teo, A.Y.L. and H.M. Tan, 2006. Effect of *Bacillus subtilis* PB6 (CloSTAT) on broilers infected with a Pathogenic Strain of *Escherichia coli*. J. Appl. Polt. Res., 15: 229-235.

Van Immerseel, F., V. Fievez, J. de Buck, F. Pasmans, A. Martel, F. Haesebrouck and R. Ducatelle, 2004. Microencapsualted ShortChain Fatty Acids in Feed Modify Colonization and Invasion Early After Infection with Salmonella Enteritidis in Young Chickens. Poult. Sci., 83: 69-74.

Yang, C.M., G.T. Cao, P.R. Ferket, T.T. Liu, L. Zhou, Y.P. Xiao and A.G. Chen, 2012. Effects of probiotic. *Clostridium butyricum*. on growth performance. immune function. and cecal microflora in broiler chickens. Poult. Sci., 91: 2121-2129.

Zhang, W.H., Y. Jiang, Q.F. Zhu, F. Gao, S.F. Dai, J. Chen and G.H. Zhou, 2011. Sodium butyrate maintains growth performance by regulating the immune response in broiler chickens. Br. Poult. Sci., 52: 292-301.

Zou, Y., Z.B. Yang, W.R. Yang, S.Z. Jiang, G.G. Zhang and R. Yu, 2010. Effects of Sodium Butyrate on gut pH value. gut VFA and cecal microflora of broilers. Technical article on line of Hangzhou King Techina Technology Co Ltd.