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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>0-14 day</th>
<th>15-28 day</th>
<th>29-42 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow corn</td>
<td>57.99</td>
<td>59.25</td>
<td>63.60</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>28.30</td>
<td>28.00</td>
<td>22.50</td>
</tr>
<tr>
<td>Fish meal, 65%</td>
<td>5.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blood meal</td>
<td>3.50</td>
<td>2.00</td>
<td>-</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>-</td>
<td>3.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Poultry meal</td>
<td>-</td>
<td>3.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Palm oil</td>
<td>1.60</td>
<td>3.20</td>
<td>3.80</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.00</td>
<td>1.00</td>
<td>1.10</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>1.20</td>
<td>1.00</td>
<td>1.30</td>
</tr>
<tr>
<td>Vitamins and minerals premix</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Salt</td>
<td>0.31</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>DL methionine</td>
<td>0.26</td>
<td>0.24</td>
<td>0.22</td>
</tr>
<tr>
<td>Lysine sulphate</td>
<td>0.02</td>
<td>0.22</td>
<td>0.42</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>Antifungal</td>
<td>0.25</td>
<td>0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>Choline chloride 60%</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Mycotoxin adsorbent</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Anticoccidial</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Calculated analyses

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Starter</th>
<th>Grower</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME, Kcal/kg</td>
<td>2975</td>
<td>3050</td>
<td>3132</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>21.50</td>
<td>21.00</td>
<td>18.50</td>
</tr>
<tr>
<td>Lys dig (%)</td>
<td>1.24</td>
<td>1.13</td>
<td>1.03</td>
</tr>
<tr>
<td>Met dig (%)</td>
<td>0.48</td>
<td>0.45</td>
<td>0.41</td>
</tr>
<tr>
<td>TSAA dig (%)</td>
<td>0.90</td>
<td>0.82</td>
<td>0.76</td>
</tr>
<tr>
<td>Thr dig (%)</td>
<td>0.81</td>
<td>0.73</td>
<td>0.67</td>
</tr>
<tr>
<td>Trp dig (%)</td>
<td>0.21</td>
<td>0.20</td>
<td>0.19</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>3.27</td>
<td>3.31</td>
<td>3.32</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>1.00</td>
<td>0.95</td>
<td>0.85</td>
</tr>
<tr>
<td>Nonphytate P (%)</td>
<td>0.50</td>
<td>0.45</td>
<td>0.42</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>0.22</td>
<td>0.18</td>
<td>0.17</td>
</tr>
</tbody>
</table>

1Vitamin and mineral premix contain the following ingredients/kg: vitamin A 12MIU, vitamin D, 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, cyanocobalamin 19 mg, pantothenic acid 17 g, biotin 170 mg, copper 15 g, iodine 11 g, iron 55 g, manganese 110 g, selenium 0.28 g, zinc 90 g, calcium 1200 mg, sodium 1900 mg, potassium 4500 mg, calcium 200 mg, magnesium 100 mg, copper 15 g, manganese 110 g, selenium 0.28 g, zinc 90 g, calcium 1200 mg, sodium 1900 mg, potassium 4500 mg, calcium 200 mg, magnesium 100 mg.

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 weights (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 Zeiss 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, jejunum 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
colonies were counted. Colony counts were multiplied 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 glyceroles (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 ingestion. Chicks were 20% feed restricted during second week to prevent ascites development and butyric acid dose was not adjusted to this feed intake.

Colistin 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 (Bozorgmehr, 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.

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
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
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 jejunum 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.
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