Effect of Partially-Protected Sodium Butyrate and Virginiamycin on Nutrient Digestibility, Metabolizable Energy, Serum Metabolites and Performance of Broiler Chickens

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Abstract: Continuous use of antibiotics has generated the need of looking for new alternatives in order to decrease emergence of resistant bacteria. The aim of this study was to evaluate the effect of partially-protected sodium butyrate (PPSB) and virginiamycin (VM) on nutrient digestibility, metabolizable energy, serum metabolites and performance of broiler chickens. A complete randomized block design was used with 1071 one-day old Cobb 500® chickens. Three treatments were established: without antibiotics (T1), with 20 ppm of VM (T2) and with 700 ppm of PPSB (T3). Three mash diets were made: starter (1-14 days), grower (15-28 days) and finisher (29-42 days). In experiment 1, performance and serum metabolites were assessed in 805 chicks distributed in 3 treatments of 7 replicates. One chick per replicate was bled at 11 and 31 days. In experiment 2, nutrient digestibility and metabolizable energy were determined in 168 chicks allocated in 3 treatments of 8 replicates. All excreta were collected between 10-13 and 30-33 days. Dry matter, crude protein, fat digestibility, true metabolizable energy and true metabolizable energy corrected by nitrogen were higher with PPSB (p<0.01). Compared with control, VM improved dry matter and crude protein digestibility at 11 days as well as fiber at 31 days (p<0.01) but decreased glucose, cholesterol (p<0.01) and triglycerides (p = 0.04) at 31 days. PPSB also increased cholesterol at 11 days (p = 0.04) and lowered uric acid at 31 days (p = 0.02) respect to control and VM, respectively. Body weight gain and feed conversion ratio were significantly better with PPSB than other treatments. These results suggest that PPSB can be used as a growth promoter in broiler chicken diets.

Key words: Sodium butyrate, digestibility, energy, metabolite, broiler

INTRODUCTION
There are many feed additives used as antibiotic growth promoters. The goal is to improve gut health and performance (Ni et al., 2012; Chan et al., 2015). Virginiamycin (VM) is commonly used in the commercial broiler industry as a growth promoter. VM is a cyclic polypeptide antibiotic complex obtained from Streptomyces virginiae cultures. VM is effective against gram-positive bacteria and it has been used for decades as a growth promoter in broiler chicken diets. Indeed, VM effect is based on the modulation of bacterial microbiota, allowing enterocytes to absorb more nutrients contained in the diet (Eysen et al., 1962; Eysen and De Somer, 1963a,b; Vervaek et al., 1979). Also, VM reduced production of bacterial metabolites such as lactic acid, amines, ammonia and some toxins that affect enterocyte nutrient absorption (Ahmadi, 2011; Parks et al., 2001; Cummings, 2003).

On the other hand, acidifiers have been used extensively as food preservatives and in recent years, as growth promoters. More recently, a new group of acidifiers was introduced in poultry diets, volatile short-chain fatty acids (VSCFA). They are weak organic acids used in low volumes that reduce pathogenic microorganisms because their undissociated form diffuses across bacterial membranes (Huyghebaert et al., 2011; Dharma et al., 2014). Among these and other natural alternatives, butyric acid is the organic acid with the most potential use in broiler chickens because of its better antimicrobial activity against enteric pathogens (Fernandez-Rubio et al., 2009), enterocyte growth (Chamba et al., 2014) and immune system stimulation (Zhang et al., 2011a).

There are several products based on glicerides (Leeson et al., 2005), sodium salts (Mallo et al., 2012, 2010), encapsulated (Levy et al., 2015), partially-protected (Fernandez-Rubio et al., 2009; Chamba et al., 2014) and pure (Panda et al., 2009) butyric acid that have been evaluated under different experimental conditions around the world. Butyrate molecule form and dose were the main differences between reported experiments (Moquet et al., 2016). PPSB in vegetable fat has produced better results compared to other butyric acid forms. Its effect is produced by its slow release and better physical and chemical properties (Cortyl, 2012). PPSB is slowly released in the
digestive tract until reaching the distal parts of jejunum and ileum, modulating effectively the morphology of the mucosa and microflora (Mallo et al., 2012; Fernandez-Rubio et al., 2009). Moreover, this form has less odor, corrosivity and instability when elaborating feed (Cortyl, 2012).

However, butyric acid effect varies according to diet composition and flock health. In Canada (Leeson et al., 2005), its effect was lesser than in Europe (Mallo et al., 2010; Smulikowska et al., 2009) and Asia (Yang et al., 2010; Hu and Guo, 2007; Yang, 2010). In Latin America, experiments with PPSB are scarce. The aim of this study was to evaluate the effect of partially-protected sodium butyrate on nutrient digestibility, metabolizable energy, serum metabolites and performance of 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 of broiler male chickens published by Rostagno et al. (2011). Animal protein sources (fish meal, meat and bone meal, blood meal and avian meal) were added to all diets between 6.5-6.6%. Diets were fortified with complete vitamin and trace mineral mixes. Diet 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 and available phosphorus requirements were similar to average recommended industry values. Mash diets were given according to feed intake tables for Ecuadorian highland conditions. These tables included a feed intake restriction to avoid asches, that represent about 20% below recommended by genetic house (Cobb, 2012). Finally, no antibiotics were provided through drinking water or diets.

Housing and management: Two commercial facilities for broilers, located at 2500 meters above sea level, were used in these trials. Chicks were obtained from a local hatchery and were vaccinated against Newcastle and Infectious Bronchitis diseases post hatch via spray. Temperature and natural ventilation were controlled by gas heaters and manually set curtains. Temperature was maintained around 32±1°C during first week and then gradually reduced to 22±1°C at the end of the fifth week. Management, health and biosecurity measures were similar to conventional poultry practices to avoid asches development.

In Experiment 1, performance and serum metabolites were measured in 903 one-day-old mixed Cobb 500® chicks randomly distributed in 3 treatments of 7 replicates. New rice husk served as litter over concrete floors. Each pen was equipped with one tubular feeder and one automatic water font. In Experiment 2, nutrient digestibility and metabolizable energy were measured in 168 chicks distributed in 3 treatments of 8 replicates. These chicks were from the same batch of experiment 1 and reared under similar temperature and ventilation conditions but in metallic cages. Experimental design and diets were the same as experiment 1.

In both experiments, a complete randomized blocking design was chosen. Chicks were blocked according to initial live body weight and barn location. First treatment (negative control) was a basal diet without antibiotics; second treatment was the basal diet plus virginiamycin (VM, Stafac®) at 20 ppm as a growth promoter because this is the recommended level of VM to applied nutrition (Ahmadi, 2011; Miller, 2012). Third treatment was the basal diet plus 700 ppm of partially-protected sodium butyrate (PPSB, Gustor BP70®), which was 30% protected in vegetable fat and 40% in free form.

Measurements: In Experiment 1, data collection was based on 100% of experimental population. Chicks were weighed on a group basis in each pen. Each pen corresponded to a replicate. Live body weight, feed intake and number of death chicks were recorded in each pen on days 1, 13, 21 and 42. Before weighing, the birds were fasted for 12 h. Death, culled chicks removed for, sampling, asches and other reasons and feed residue were weighed and recorded daily before taking them out from the pens. Adjusted feed conversion ratio was calculated as feed intake divided by weight gained of live birds plus dead and culled birds (kg feed: kg of weight gain).

For serum metabolites measurement, feed was removed 12 h prior to sampling. 2 ml blood samples were collected by venipuncture of the brachial vein of one chick per pen. Blood was collected in pressured tubes without anticoagulant (Vacutainer® tubes; BD Inc., Oakville, ON, Canada). Blood samples were kept at room temperature for serum accumulation for at least 30 min. Then, samples were kept refrigerated to 4°C and submitted to the laboratory where were centrifuged at 3000 rpm for 10 min collected in cryogenic tubes and used for metabolite measurement. Finally, DiaSys® kits (DiaSys Diagnostics Systems GmbH, Holzheim, Germany) were used to measure total protein (FS 10), total cholesterol (FS 10), total triglycerides (FS 10), total glucose (GOD FS 10) and uric acid (FS TOOS).

In Experiment 2, chicks were fed same diets as in experiment 1. Nutrient digestibility was assessed by the total excreta collection method (Sibbald, 1976) during 10-13 and 30-33 age days. Only these periods evaluation feeding was ad libitum, feed intake was registered and excreta were collected twice a day during 4 days. Remaining feed in each pen feeder was collected and weighed daily. Excreta and feed samples were stored in hermetic plastic bags, identified and then kept frozen at -20°C until testing. Nutrient content in excreta and feed samples was measured by the AOAC methods (925.9; 920.85; 920.87; 923.03; 1990). Coefficient of digestibility was calculated with the following formula:
Metabolizable energy was assessed by using same samples of the nutrient digestibility assay. Samples were completely burned in a calorimetric adiabatic bomb to measure the gross energy content per gram of dry matter. Then, true metabolizable energy (TME) values were obtained using the gross energy contents of feed, excreta, as well as the F.E.m + U.E.e losses of fasting birds (Sibbald, 1976). True metabolizable energy was corrected to zero nitrogen balance (TMEa) using $8.22 \text{ kcal/g N}$ retained (Hill and Anderson, 1958).

**Statistical analysis:** Data were analyzed using two-way analysis of Variance (ANOVA) test with diet and blocks as the factors. The significance of difference between means was determined by Tukey test. Results were considered significant when $p<0.05$. In the case of non-normal distributions for variables like mortality and serum metabolites, a natural logarithm transformation was used before ANOVA. A Kruskal-Wallis test was applied when non-normal distribution. Statistical analysis was done using SAS 9.4® (SAS Foundation, Cary, North Carolina, USA).

**RESULTS AND DISCUSSION**

**Digestibility and metabolizable energy:** Nutrient digestibility coefficients and true metabolizable energy and true metabolizable energy corrected by nitrogen are shown in Table 2. PPBS increased digestibility of all nutrients respect to the control at 11 and 31 days except fat digestibility at 11 days. VM increased dry matter and crude protein digestibility at 11 days and crude fiber digestibility at 31 days compared to the control. PPBS increased crude protein, crude fiber and ether extract digestibility at 11 days and dry matter and ether extract digestibility at 31 days compared to VM. VM increased crude fiber digestibility at 31 days compared to PPBS. PPSB increased TME and TMEa compared to control and VM treatments. VM and control were similar in the two types of energy.

In this study, PPSB improved nutrient digestibility and true metabolizable energy compared to VM and control. These findings are in agreement with the positive effect of sodium butyrate (SB) found in other studies at different ages (Zou et al., 2010; Mallo et al., 2011). However, the dose and form of SB affect nutrient digestibility and energy (Smulikowska et al., 2009). Improvement in these outcomes was reached using higher doses of another form of SB (Mallo et al., 2011).

Better nutrient digestibility is accomplished through efficient endocrine regulation and digestive enzyme secretion, a balanced intestinal microbial population and maintenance of mucosal integrity which is ensured by a proper balance between mitotic rates of crypt stem cells and tip villi apoptosis (Guilloteau et al., 2010; Hassan and Iqbal, 2016; Moquet et al., 2018). In this regard, butyrate is an apoptosis inhibitor of mucosal cells, energy source for enterocytes and has direct effect on mucosal cell proliferation, intestinal morphology and immune function (Cuff et al., 2002; Guilloteau et al., 2010). SB effect on metabolizable energy also depends on SCFA concentrations in the cecum and lipase concentrations in the small intestine (Cortyl, 2012). According to Kaczmarek et al. (2016), it can be speculated that additional increased intestinal absorptive area by increasing villus height, butyrate can increase pancreatic fluid secretion and consequently improve fat, starch and other nutrient digestibility and that this in turn, may increase diet AMEn.

The improvement in digestibility observed after antibiotic growth promoters' supplementation is related to reduction in microbial use of nutrients or/and enhanced nutrients' absorption because of thinner intestinal wall. VM effect on
Table 2: Nutrient digestibility (%) and true metabolizable energy (TME) and corrected by nitrogen (TME\(_n\)) (Kcal/kg DM) of diets with virginiamycin (VM) and partially-protected sodium butyrate (PPSB)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>VM</th>
<th>PPSB</th>
<th>SEM</th>
<th>p-value</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicates</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birds/replicate</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>11 days age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter (%)*</td>
<td>80.7(^a)</td>
<td>82.2(^a)</td>
<td>83.2(^a)</td>
<td>0.3</td>
<td>&lt;0.01</td>
<td>0.79</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>83.3(^b)</td>
<td>85.5(^b)</td>
<td>87.6(^b)</td>
<td>0.3</td>
<td>&lt;0.01</td>
<td>0.88</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>65.5(^a)</td>
<td>65.3(^a)</td>
<td>69.6(^a)</td>
<td>0.3</td>
<td>&lt;0.01</td>
<td>0.91</td>
</tr>
<tr>
<td>Ether extract (%)</td>
<td>81.8(^a)</td>
<td>77.7(^a)</td>
<td>81.8(^a)</td>
<td>0.6</td>
<td>&lt;0.01</td>
<td>0.73</td>
</tr>
<tr>
<td>TME (Kcal/kg DM)</td>
<td>3.011(^b)</td>
<td>3.016(^b)</td>
<td>3.101(^b)</td>
<td>9</td>
<td>&lt;0.01</td>
<td>0.65</td>
</tr>
<tr>
<td>TME(_n) (Kcal/kg DM)</td>
<td>2.994(^b)</td>
<td>3.003(^b)</td>
<td>3.087(^b)</td>
<td>9</td>
<td>&lt;0.01</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>31 days age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter (%)*</td>
<td>80.4(^b)</td>
<td>79.8(^b)</td>
<td>81.1(^b)</td>
<td>0.2</td>
<td>&lt;0.01</td>
<td>0.71</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>79.3(^c)</td>
<td>80.1(^c)</td>
<td>81.4(^c)</td>
<td>0.4</td>
<td>&lt;0.01</td>
<td>0.69</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>76.1(^c)</td>
<td>80.9(^c)</td>
<td>78.9(^c)</td>
<td>0.5</td>
<td>&lt;0.01</td>
<td>0.82</td>
</tr>
<tr>
<td>Ether extract (%)</td>
<td>76.1(^c)</td>
<td>75.5(^c)</td>
<td>82.5(^c)</td>
<td>0.4</td>
<td>&lt;0.01</td>
<td>0.95</td>
</tr>
<tr>
<td>TME (Kcal/kg DM)</td>
<td>3.219(^c)</td>
<td>3.208(^c)</td>
<td>3.300(^c)</td>
<td>7</td>
<td>&lt;0.01</td>
<td>0.89</td>
</tr>
<tr>
<td>TME(_n) (Kcal/kg DM)</td>
<td>3.174(^c)</td>
<td>3.170(^c)</td>
<td>3.246(^c)</td>
<td>7</td>
<td>&lt;0.01</td>
<td>0.86</td>
</tr>
</tbody>
</table>

SEM: Standard error of the mean. R\(^2\): Coefficient of determination

*Means in the same row with different superscript letter are statistically different (p<0.05) according to ANOVA two-way set repetition and comparison of Tukey-Kramer

Table 3: Levels of some serum metabolites (mg/dl) of chicks fed Virginiamycin (VM) and partially protected sodium butyrate (PPSB)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>VM</th>
<th>PPSB</th>
<th>SEM</th>
<th>p-value</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>11 days age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)*</td>
<td>177.3</td>
<td>179.6</td>
<td>160.9</td>
<td>12</td>
<td>0.96</td>
<td>0.23</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>7.1</td>
<td>4.8</td>
<td>5.2</td>
<td>0.9</td>
<td>0.18</td>
<td>0.46</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)**</td>
<td>88.5(^a)</td>
<td>106.3(^a)</td>
<td>166.1(^a)</td>
<td>21</td>
<td>0.04</td>
<td>0.53</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>50.8</td>
<td>110.4</td>
<td>78.8</td>
<td>17</td>
<td>0.36</td>
<td>0.49</td>
</tr>
<tr>
<td>Total protein (g/(\text{dl}))</td>
<td>3.7</td>
<td>3.5</td>
<td>3.9</td>
<td>0.2</td>
<td>0.25</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>31 days age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)*</td>
<td>276.1(^c)</td>
<td>195.4(^c)</td>
<td>287.9(^c)</td>
<td>17</td>
<td>&lt;0.01</td>
<td>0.71</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>8.2(^a)</td>
<td>11.0(^a)</td>
<td>7.4(^a)</td>
<td>0.8</td>
<td>0.02</td>
<td>0.73</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)**</td>
<td>218.1(^a)</td>
<td>124.8(^a)</td>
<td>250.7(^a)</td>
<td>14</td>
<td>&lt;0.01</td>
<td>0.81</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>222.2(^b)</td>
<td>133.8(^b)</td>
<td>183.8(^b)</td>
<td>21</td>
<td>0.04</td>
<td>0.58</td>
</tr>
<tr>
<td>Total protein (g/(\text{dl}))</td>
<td>3.7</td>
<td>3.6</td>
<td>3.4</td>
<td>0.2</td>
<td>0.51</td>
<td>0.50</td>
</tr>
</tbody>
</table>

SEM: Standard error of the mean. R\(^2\): Coefficient of determination

*Means in the same row with different superscript letter are statistically different (p<0.05) according to ANOVA two-way set to repeat and Tukey adjusted to the number of comparisons

**ANOVA and Tukey Cholesterol at 11 days of age were performed in logarithmic scale. The SEM Cholesterol to 11 days of age was calculated with the original data.

Nutrient digestibility and metabolizable energy obtained in this study is different from others that found positive effect (Odunsi et al., 1999; Bartov, 1992; March et al., 1978; Singh et al., 2000). Diet ingredients, gut microflora composition, health challenge environments, better health status of GIT and VM spectrum and mode of action may account for this difference (Odunsi et al., 1999; Ahmadi, 2011; La Vorgna et al., 2013).

**Serum metabolites:** Serum metabolites measured in this study are presented in Table 3. At 11 days, PPSB increased total cholesterol levels compared to control group. The other metabolites were not affected by VM or PPSB at this age. At 31 days, any of the measured metabolites were affected by PPSB compared to the control group. However, total glucose, total cholesterol and total triglycerides were lowered with VM compared to control and PPSB treatments. Finally, uric acid was higher with VM compared to PPSB. The results of all serum metabolites studied in this trial are reliable since it guaranteed its stability from sampling (fasting hours before collecting, storage temperature in the room, cooling) and the hours before measurement in the laboratory according to previous studies (Marjani, 2008; Rajman et al., 2008; Cuhadar et al., 2012; Oddo et al., 2012; Demir et al., 2004). The biochemical profile of young growing broilers examined in several studies are extremely variable. When broiler chickens are fed on standard mixtures, the breeding lines and age of bird seem to be the main factors influencing metabolism's intensity and changes of the blood parameters (Meluzzi et al., 1992; Piotrowska et al., 2011) although the environment, season and exposure to antigens also have a great influence (Bowes et al., 1989; Meluzzi et al., 1992).
Table 4: Growth performance of fed chickens with virginiamycin (VM) and partially-protected sodium butyrate (PPSB)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>VM</th>
<th>PPSB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicates</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Birds/Replicate</td>
<td>43</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>Initial weight (g)</td>
<td>33.4</td>
<td>33.5</td>
<td>33.4</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>2.040</td>
<td>2.048</td>
<td>2.125</td>
</tr>
</tbody>
</table>

**0-21 days**

| Weight gain (g) | 568<sup>a</sup> | 561<sup>a</sup> | 580<sup>a</sup> |
| Feed consumption (g) | 800    | 804  | 812  |
| Feed conversion (g/g) | 1.41  | 1.43 | 1.40 |
| Mortality (%) | 10.4    | 6.7  | 9.0  |

**22-42 days**

| Weight gain (g) | 1.415<sup>b</sup> | 1.440<sup>b</sup> | 1.509<sup>b</sup> |
| Feed consumption (g) | 2.774   | 2.751 | 2.603 |
| Feed conversion (g/g) | 1.96<sup>c</sup> | 1.91<sup>c</sup> | 1.86<sup>c</sup> |
| Mortality (%) | 5.6    | 6.3  | 6.5  |

**0-42 days**

| Weight gain (g) | 1.990<sup>d</sup> | 2.008<sup>d</sup> | 2.098<sup>d</sup> |
| Feed consumption (g) | 3.574   | 3.555 | 3.615 |
| Feed conversion (g/g) | 1.80<sup>e</sup> | 1.77<sup>e</sup> | 1.73<sup>e</sup> |
| Mortality (%) | 16.0    | 13.0 | 15.4 |

SEM: Standard error of the mean. R<sup>2</sup>: Coefficient of determination

*Means in the same row with different superscript letter are statistically different (p<0.05) according to ANOVA two-way set to repeat and Tukey adjusted to the number of comparisons*

Chickens slaughtered for blood sampling were considered in the calculations of mortality.

In this study, attempts were made to determine if PPSB and VM affect the level of certain serum metabolites related to energetic and protein metabolism. However, SB and VM effect on serum metabolites vary between reported studies due to different forms of butyric acid (Mahdavi and Torki, 2009), diet composition (Belay and Teeter, 1996; Odusui et al., 1999; Ahmadi, 2011), gut microbota composition (Taherpour et al., 2009) and flock health (Zhang et al., 2011a).

In this sense, it has also been reported that total cholesterol levels can be modified by the intestinal microbota (Taherpour et al., 2009) which depends on protein and mineral alkalizing effect as well as the dose and type of addittives used in the diet (Brzoska et al., 2013; Rinttila and Apajalaiti, 2013). It has been proposed that some Lactobacillus and Bifidobacterium can use cholesterol reducing its absorption (Mohan et al., 1995; Mohan et al., 1996). Moreover, age-related enzyme development can also affect cholesterol absorption. The younger chicks are more immature enzymatically and therefore have less capacity to digest saturated fat through lipases (Bartov, 1987; Ketels and De Groote, 1989). Also, it has been documented that gut health challenges due to bacterial endotoxins and lipopolysaccharides can impact nutrient absorption and its related serum metabolites (Zhang et al., 2011a).

Nevertheless, it is generally known that under regimen of food restriction or fasting there falls in the total protein concentration of plasma and lipogenesis, also increased uric acid and lipolysis, while glucose levels remains stable and appear to be markedly resistant prolonged food deprivation (Rajman et al., 2006). In our study, discriminating methodology and measurement equipment, type of food, ages, line genetics and sex of birds found similar trends and values close all serum parameters (Bowes et al., 1989; Meluzzi et al., 1992; Demir et al., 2004, Piotrowska et al., 2011). We think the increased level of cholesterol in PPSB (p<0.05) could respond better intestinal integrity reported in previous studies (Moquet et al., 2016) which would enhance the intestinal absorption of young birds which is particularly poor. At 31 days of age, obtained with VM (low glucose, elevated UA, decreased TG and cholesterol) correlate with what was found in various tests under fasting end or high catabolism and high amino acid requirements of birds although values total protein are not different from control. It is important to further evaluate serum parameters to better understand the effect of both growth promoters.

**Performance:** Performance results are shown in Table 4. PPSB increased weight gain at 21 days compared to VM. PPSB improved weight gain and feed conversion ratio between 22-42 days compared to the control. There was a trend for VM on performance between 22 to 42 days and at 42 days compared to the control. Overall, PPSB improved performance at 42 days compared to VM and control. There were no differences in feed intake and percentage of mortality between treatments.

Performance in chickens fed PPSB or VM were assessed to associate increase nutrient digestibility and metabolizable energy with better performance. SB effect on performance has been documented in previous studies with different butyrate molecules and doses (Leeson et al., 2005; Panda et al., 2008; Mahdavi y Torki, 2009;
Smulkowska et al., 2009; Mallo et al., 2010; Antongiovanni et al., 2010; Adil et al., 2011, Zhang et al., 2011a; Aghazadeh and Taha Yazdi, 2012; Jerzsele et al., 2012; Shahir et al., 2013; Chamba et al., 2014; El-Sawy et al., 2015; Kaczmarek et al., 2016). Overall, better performance was attributed to better nutrient digestibility and metabolizable energy. SB improved gut development, energy sourcing for enterocytes and health when the protected form and high doses were used (Kotunia et al., 2004; Guilloteau et al., 2010; Zhang et al., 2011a,b; Jerzsele et al., 2012; Chamba et al., 2014; Levy et al., 2015) even attenuating the intestinal oxidative stress and inhibiting the release of proinflammatory cytokines in broilers with immune challenges (Li et al., 2015). Also, SB increases Lactobacillus and Bilidobacterium and has the highest bacterical against the acidi-intolerant species such as Escherichia coli and Salmonella (Taherpour et al., 2009; Antongiovanni et al., 2007; Hassan and Iqbal, 2016; Wafaa et al., 2016). These effects together can explain the better nutrient digestibility and energy obtained in this study.

The positive effect of VM on performance has been reported previously (March et al., 1978; Bartov, 1992; Belay and Teeter, 1996; Odunsi et al., 1999; Singh et al., 2000; Ahmad, 2011). In this trial, 20 ppm of VM were used as manufacturer recommendations, however no performance improvement was obtained when compared to the control similar to that reported by Proudfoot et al. (1990) with 11 ppm VM. Variation in the susceptibility of certain gut bacteria (Dumonceaux et al., 2006; La Vorgna et al., 2013) and less effect on enterocytes growth can related to the lack of VM response (Baurhoo et al., 2009). VM effect on performance is also affected by diet, gut microbiota composition (Eyssen et al., 1962; Harms et al., 1986; Odunsi et al., 1999; Parks et al., 2001; Cervantes et al., 2008) and flock health and management (Belay and Teeter, 1996; Bray et al., 2009; La Vorgna et al., 2013). Moreover, this inconsistent result, could be explained by trials conducted in low health challenge environments because the growth-enhancing effects of antimicrobial additives become apparent when chickens are subjected to suboptimal conditions (Hassan and Iqbal, 2016).

This study contributes to the current knowledge of PPSB and VM effect on nutrient digestibility, metabolizable energy and performance and nutrient utilization. According to the authors’ knowledge, in the literature there are no data available on the effects of PPSB and VM combination on these parameters. Future studies are necessary to explain lack of VM response as well as about ileal digestibility in birds feeding PPSB. PPSB effect on performance could be explained partially by the result of better nutrient digestibility and metabolizable energy. Additionally, this better digestibility according to findings in other studies could be mediated by be one the major enterocytes energy source, by its effectiveness in controlling bacteria like E. coli and Salmonella reducing microbial competition with the host and reducing toxic compounds by pathogenic microbiota, ensuring adequate integrity of the intestinal villi. This study provides additional evidence that partially-protected sodium butyrate enhances performance of broiler chickens through a better nutrient digestibility, higher metabolizable energy and hence increases availability of nutrients for growth. PPSB can be used as an alternative growth promoter in broiler chicken diets.

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