INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

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

R. Riboty[1], F. Chamba[2], M. Puyalto[3] and J.J. Mallo[3]
[1]Animal Production Graduate Program, Equinoctial Technological University, Sto. Domingo, Ecuador
[2]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA
[3]Norel S.A., c/Jesus Aprendiz 19, 28007 Madrid, Spain

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 903 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 (Eyssen et al., 1962; Eyssen and De Somer, 1963a,b; Vervaeke 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 glycerides (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

---

Corresponding Author: R. Riboty, Animal Production Graduate Program, Equinoctial Technological University, Sto. Domingo, Ecuador

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 6-8.5%. 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 ascites, 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 ascites 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 batteries. 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, 21 and 42. Before weighing, the birds were fasted for 12 h. Death, culled chicks removed for, sampling, ascites 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 cull 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 GmbHInc., 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:

$$\text{Coefficient of digestibility} = \frac{\text{(Nutrient intake-Nutrient excreted)}}{\text{Nutrient intake}}$$

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 (TMEn) using 8.22 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 TMEn 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

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

	Starter	Grower	Finisher
Ingredient	0-14 d	15-28 d	29-42 d
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 bonemeal	-	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	0.15	0.15	0.15
Sodium chloride	0.31	0.26	0.26
DL methionine	0.26	0.24	0.22
L-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)	2.975	3.050	3.132
Crude protein (%)	21.50	21.00	18.50
Lysdig (%)	1.24	1.13	1.03
Metdig (%)	0.48	0.45	0.41
TSAAdig (%)	0.90	0.82	0.76
Thredig (%)	0.81	0.73	0.67
Trypdig (%)	0.21	0.20	0.19
Crude fiber (%)	3.27	3.31	3.32
Calcium (%)	1.00	0.95	0.85
Non-phytate P (%)	0.50	0.45	0.42
Sodium (%)	0.22	0.18	0.17

*One vitamin and mineral premix kg provides: vitamin A 12MIU, vitamin D_3 5 KIU, vitamin E 35 KIU, 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, Cu 15 g, I 1 g, Fe 55 g, Mn 110 g, Se 0.28 g, Zn 90 gcsp1 kg

proper balance between mitotic rates of crypt stem cells and tip villi apoptosis (Guilloteau *et al.*, 2010; Hassan and Iqbal, 2016; Moquet *et al.*, 2016). 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 (TMEn) (Kcal/kg DM) of diets with virginiamycin (VM) and partially-protected sodium butyrate (PPSB)

Treatments	Control	VM	PPSB			
Replicates	8	8	8			
Birds/replicate	7	7	7	SEM	p-value	R^2
11 days age						
Dry matter (%)*	80.7^b	82.2^a	83.2^a	0.3	<0.01	0.79
Crude protein (%)	83.3^c	85.5^b	87.6^a	0.3	<0.01	0.88
Crude fiber (%)	65.5^b	65.3^b	69.6^a	0.3	<0.01	0.91
Ether extract (%)	81.6^a	77.7^b	81.8^a	0.6	<0.01	0.73
TME (Kcal/kg DM)	3.011^b	3.016^b	3.101^a	9	<0.01	0.85
TMEn (Kcal/kg DM)	2.994^b	3.003^b	3.087^a	9	<0.01	0.85
31 days age						
Dry matter (%)*	80.4^b	79.8^b	81.1^a	0.2	<0.01	0.71
Crude protein (%)	79.3^b	80.1^{ab}	81.4^a	0.4	<0.01	0.69
Crude fiber (%)	76.1^c	80.9^a	78.9^b	0.5	<0.01	0.82
Ether extract (%)	76.1^b	75.5^b	82.5^a	0.4	<0.01	0.95
TME (Kcal/kg DM)	3.219^b	3.208^b	3.300^a	7	<0.01	0.89
TMEn (Kcal/kg DM)	3.174^b	3.170^b	3.246^a	7	<0.01	0.86

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)

Treatments	Control	VM	PPSB	SEM	p-value	R^2
11 days age						
Glucose (mg/dl)*	177.3	179.6	180.9	12	0.98	0.23
Uric acid (mg/dl)	7.1	4.6	5.2	0.9	0.18	0.46
Cholesterol(mg/dl)**	88.5^b	106.0^{ab}	166.1^a	21	0.04	0.53
Triglycerides (mg/dl)	80.8	110.4	78.0	17	0.36	0.49
Total protein (g/dL)	3.7	3.5	3.9	0.2	0.25	0.39
31 days age						
Glucose (mg/dl)*	276.1^a	195.4^b	287.9^a	17	<0.01	0.71
Uric acid (mg/dl)	8.2^{ab}	11.0^a	7.4^b	0.8	0.02	0.73
Cholesterol (mg/dl)**	218.1^a	124.8^b	250.7^a	14	<0.01	0.81
Triglycerides (mg/dl)	222.2^a	133.8^b	183.8^{ab}	21	0.04	0.58
Total protein (g/dL)	3.7	3.6	3.4	0.2	0.51	0.50

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, 2006; Rajman *et al.*, 2006; Cuhadar *et al.*, 2012; Oddoze *et al.*, 2012; Demir *et al.*, 2004). The biochemical profile of young growing broilers examined in several studies are extremaly 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)

Treatments	Control	VM	PPSB			
Replicates	7	7	7			
Birds/replicate	43	43	43	SEM	p-value	R^2
Initial weight (g)	33.4	33.5	33.4	0.1	0.78	0.91
Final weight (g)	2.040	2.048	2.125	25	0.07	0.63
0-21 days						
Weight gain (g)	568[ab]	561[b]	580[a]	4	<0.01	0.76
Feed consumption (g)	800	804	812	7	0.50	0.42
Feed conversion (g:g)	1.41	1.43	1.40	0.01	0.15	0.50
Mortality (%)[1]	10.4	6.7	9.0	1.3	0.18	0.36
22-42 days						
Weight gain (g)	1.415[b]	1.440[ab]	1.509[a]	21	0.02	0.68
Feed consumption (g)	2.774	2.751	2.803	21	0.27	0.56
Feed conversion (g:g)	1.96[a]	1.91[ab]	1.86[b]	0.02	0.01	0.72
Mortality (%)[1]	5.6	6.3	6.5	0.6	0.50	0.40
0-42 days						
Weight gain (g)	1.990[b]	2.006[b]	2.096[a]	23	0.01	0.70
Feed consumption (g)	3.574	3.555	3.615	26	0.29	0.51
Feed conversion (g:g)	1.80[a]	1.77[a]	1.73[b]	0.01	<0.01	0.71
Mortality (%)[1]	16.0	13.0	15.4	1.1	0.19	0.53

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[1]
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; Odunsi *et al.*, 1999; Ahmadi, 2011), gut microbiota 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 microbiota (Taherpour *et al.*, 2009) which depends on protein and mineral alkalizing effect as well as the dose and type of additives used in the diet (Brzoska *et al.*, 2013; Rinttila and Apajalahti, 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 regimens 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.*, 2009; Mahdavi y Torki, 2009;

Smulikowska *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 *Bifidobacterium* and has the highest bactericidal against the acid-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; Ahmadi, 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.

ACKNOWLEDGMENTS

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.

Ahmadi, F., 2011. The effect of different levels of virginiamycin on performance, immune organs and blood metabolite of broiler chickens. Ann. Biolog. Res., 2: 291-298.

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.

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.

AOAC, 1990. Official methods of analysis of the AOAC, 15th ed. Methods 925.09, 920.85, 920.87, 923.03. Association of official analytical chemists. Arlington, VA, USA.

Bartov, I., 1987. Combined effect of age and ambient temperature on the comparative growth of broiler chicks fed tallow and soybean oil. Poult. Sci., 66: 273-279.

Bartov, I., 1992. Lack of effect of dietary energy-to-protein ratio and energy concentration on the response of broiler chickens to virginiamycin. Br. Poult. Sci., 33: 381-391.

Baurhoo, B., P.R. Ferket and X. Zhao, 2009. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations and carcass parameters of broilers. Poult. Sci., 88: 2262-2272.

Belay, T. and R. Teeter, 1996. Virginiamycin and caloric density effects on live performance, blood serum metabolite concentration and carcass composition of broilers reared in thermoneutral and cycling ambient temperatures. Poult. Sci., 75: 1383-1392.

Bowes, V., R.J. Julian and T. Stirtzinger, 1989. Comparison of serum biochemical profiles of male Broilers with female broilers and white leghorn chickens. Can. J. Vet. Res., 53: 7-11.

Bray, J.L., C.S. Taylor, T.E. Cherry and J. Carey, 2009. Performance comparison between the use and nonuse of a enteric health medication program across five consecutive commercial broiler flocks. J. Appl. Poult. Res., 18: 165-171.

Brzóska, F., B. Sliwinski and O. Michalik-Rutkowska, 2013. Effect of dietary acidifier on growth, mortality, post-slaughter parameters and meat composition of broiler chickens. Ann. Anim. Sci., 13: 85-96.

Cervantes, H.M., K.W. Bafundo, G.M. Pesti and R.I. Bakalli, 2008. Live and processing performance responses of broilers fed low and extra-low nutrient density withdrawal diets supplemented with virginiamycin. J. Appl. Poult. Res., 17: 87-92.

Chamba, F., M. Puyalto, A. Ortiz, H. Torrealba, J.J. Mallo and R. Riboty, 2014. Effect of partially protected sodium butyrate on performance, digestive organs, intestinal villi and *E. coli* development in broilers chickens. Int. J. Pout. Sci., 13: 390-396.

Chan, G., A. Guthrie, T. Sivaramalingam, J. Wilson, D. Vancraeynest, R. Moody and S. Clarck, 2015. A framework for assessing the efficacy of antimicrobials in the control of necrotic enteritis in broiler chickens. J. Appl. Poult. Res., 24: 246-256.

Cortyl, M., 2012. Sodium butyrate in poultry-the importance of proper protection. Nortel Animal Nutrition. FEED Business Worldwide-November 2012. www.efeedlink.com/publication/issue_55.html.

Cuhadar, S., A. Atay, M. Koseoglu, A. Dirican and A. Hur, 2012. Stability studies of common biochemical analytes in serum separator tubes with or without gel barrier subjected to various storage conditions. Biochem. Med., 22: 202-214.

Cuff, M.A., E.W. Lambert and S.R. Shirazi-Beechey, 2002. Substrate induced regulation of the human colonic monocarboxylate transporter, MCTI. J. Physiol., 539: 361–371.

Cummings, T.S., 2003. Understanding competitive exclusion and the rationale for using growth promotant antibiotics in microflora management. http://www.cvm.msstate.edu/Poultry/Documents/Microflora%20Management.pdf.

Demir, E., S. Sarica, A. Sekeroglu, M.A. Ozcan and Y. Seker, 2004. Effects of early and late feed restriction or feed withdrawal on growth performance, ascites and blood constituents of broiler chickens. Acta Agriculturae Scandinavica, Section A-Anim. Sci., 54: 152-158.

Dharma, K., R. Tiwari, R.U. Khan, S. Chakraborty, M. Gopi, K. Karthik, M. Saminathan, P.A. Desingu and L.T. Sunkara, 2014. Growth Promoters and Novel Feed Additives Improving Poultry Production and Health, Bioactive Principles and Benefical Applications: The Trends and Advances-A Review. Int. J. Pharmacol., 10: 129-159.

Dumonceaux, T.J., J.E. Hill, S.M. Hemmingsen and A.G. Van Kessel, 2006. Characterization of Intestinal Microbiota and Response to Dietary Virginiamycin Supplementation in the Broiler Chicken. Appl. Environ. Microbiol., 72: 2815-2823.

El-Sawy, A.E.F., Z.K. El-Maddawy and H.S.I. Bo-Ghazel, 2015. The growth promoting effect of beta-glucan in comparison with sodium butyrate in broiler chicks. AJVS, 44: 23-37.

Eyssen, H., V. De Prins and P. De Somer, 1962. The growth-promoting action of virginiamycin and its influence on the crop flora in chickens. Poult. Sci., 41: 227-233.

Eyssen, H. and P. De Somer, 1963a. The mode of action of antibiotics in stimulating growth of chicks. J. Exp. Med., 117: 127-137.

Eyssen, H. and P. De Somer, 1963b. Effects of antibiotics on growth and nutrient absorption of chicks. Poult. Sci., 42: 1373-1379.

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

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.

Hassan, S. and J. Iqbal, 2016. Recent advances in the role of organic acids in poultry nutrition. J. Appl. Anim. Res., 44: 359-369.

Harms, R.H., N. Ruiz and R.D. Miles, 1986. Influence of virginiamycin on broilers fed four levels of energy. Poult. Sci., 65: 1984-1986.

Hill, F.W. and D.L. Anderson, 1958. Comparison of metabolizable energy and productive determinations with growing chicks.J. Nutr., 64: 587-603.

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

Huyghebaert, G., R. Ducatelle and F. Van Immerseel, 2011. An update on alternatives to antimicrobial growth promoter for broilers. Vet. J., 187: 182-188.

Jerzsele, A., K. Szeker, R. Csizinszky, E. Gere, C. Jakab, J.J. Mallo and P. Galfi, 2012. Efficacy of protected sodium butyrate, a protected blend of essential oils, their combination and Bacillus amyloliquefaciens spore suspension against artificially induced necrotic enteritis in broilers. Poult. Sci., 91: 837-843.

Kaczmarek, S.A., A. Barri, M. Hejdysz and A. Rutkowski, 2016. Effect of different doses of coated butyric acid on growth performance and energy utilization in broilers. Poult. Sci., 00: 1-9.

Ketels, E. and G. De Groote, 1989. Effect of ratio of unsaturated to saturated fatty acids of the dietary lipid fraction on utilization and metabolizable energy of added fats in young chicks. Poult. Sci., 68: 1506-1512.

Kotunia, A., J. Wolinski, D. Laubitz, M. Jurkowska, V. Rome, P. Guilloteau and R. Zabielski, 2004. Effect of sodium butyrate on the small intestine development in neonatal piglets feed by artificial sow. J. Physiol. Pharmacol., 55: 59-68.

La Vorgna, M., J.L. Schaeffer, D. Bade, J. Dickson, K. Cookson and S.W. Davis, 2013. Performance of broilers fed a broader spectrum antibiotic (virginiamycin) or a narrower spectrum antibiotic (bacitracin methylene disalicylate) over 3 consecutive grow-out cycles. J. Appl. Poult. Res., 22: 574-582.

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.

Levy, A.W., J.W. Kessler, L. Fuller, S. Williams, G.F. Mathis, B. Lumpkins and F. Valdez, 2015. Effect of feeding an encapsulated source of butyric acid (ButiPEARL) on the performance of male Cobb broilers reared to 42 d of age. Poult. Sci., 00: 1-7.

Li, J., Y. Hou, D. Yi, J. Zhang, L. Wang, H. Qiu, B. Ding and J. Gong, 2015. Effects of tributyrin on intestinal energy status, antioxidative capacity and immune response to lipopolysaccharide challenge in broilers. Asian Aust. J. Anim. Sci., 28: 1784-1793.

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. Vet. Adv., 8: 1702-1709.

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

Mallo, J.J., M. Puyalto and S.V. Rama Rao, 2011. Evaluation of the effect of sodium butyrate addtition to broiler diets on energy and protein digestibility, productive parameters and size of intestinal villi of animals. 48th AECA Scientific Poultry Symposium, pp: 343-349.

Mallo, J.J., A. Balfagon, M.I. Gracia, P. Honrubia and M. Puyalto, 2012. Evaluation of different protections of butyric acid aiming for release in the last part of the gastrointestinal tract of piglets. J. Anim. Sci., 90: 227-229.

Marjani, A., 2006. Effect of storage time and temperature on some serum analytes. Int. J. Lab. Med., 2: 1-6.

March, B., R. Soong and C. MacMillan, 1978.Growth rate, feed conversion and dietary metabolizable energy in response to virginiamycin supplementation of different diets. Poult. Sci., 57: 1346-1350.

Meluzzi, A., G. Primiceri, R. Giordani and G. Fabris, 1992. Determination of blood constituents reference values in broilers. Poult. Sci., 71: 337-345.

Miller Publishing, Co., 2012. Feed additive compendium. Miller publishing company, Minnetonka, MN.

Mohan, B., R.Kadirvel, M. Bhaskaran and A. Natarajan, 1995. Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. Br. Poult. Sci., 36: 779-803.

Mohan, B., R. Kadirvel, M. Bhaskaran and A. Natra Jan, 1996. Effect of probiotic supplementation on growth, nitrogen utilization and serum cholesterol in broilers. Br. Poult. Sci., 37: 395-401.

Moquet, P.C.A., L. Onrust, F. Van Immerseel, R. Ducatelle, W.H. Hendriks and R.P. Kwakkel, 2016. Importance of release location on the mode of action of butyrate derivates in the avian gastrointestinal tract. World's Poult. Sci. J., 72: 61-80.

Ni, J.J., T.T. Ju and X.S. Piao, 2012. Effect of flavomycin on performance, gut morphology and intestinal microflora in broilers. J. Anim. Vet. Adv., 11: 1669-1673.

Oddoze, C., E. Lombard and H. Portugal, 2012. Stability study of 81 analytes in human whole blood, in serum and in plasma. Clin. Biochem., 45: 464-469.

Odunsi, A., A. Onifade and G. Babatunde, 1999. Response of broiler chicks to virginiamycin and dietary protein concentrations in the humid tropics. Arch. Zootec., 48: 317-325.

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-Australasian J. Anim. Sci., 22: 1026-1031.

Parks, C.W., J.L. Grimes, P.R. Ferket and A.S. Fairchild, 2001. The effect of mannanoligosaccharides, bambermycins and virginiamycin on performance of large white male market Turkeys. Poult. Sci., 80: 718-723.

Piotrowska, A., K. Burlikowska and R. Szymeczko, 2011. Changes in blood chemistry in broiler chickens during the fattening period. Foliabiologica (Krakow), 59: 3-4.

Proudfoot, F.G., E.D. Jackson and H.W. Hulan, 1990. The Response of male chicken broilers to the dietary addition of virginiamycin. Poult. Sci., 69: 1713-1717.

Rajman, M., M. Jurani, D. Lamosova, M. Macajova, M. Sedlackova, L. Kost'al, D. Jezova and P. Viboh, 2006. The effects of feed restriction on plasma biochemistry in growing meat type chickens (Gallus gallus). Comp. Biochem. Physiol. Part A, 145: 363-371.

Rinttila, T. and J. Apajalahti, 2013. Intestinal microbiota and metabolites-implications for broiler chicken health and performance. J. Appl. Poult. Res., 22: 647-658.

Rostagno, H., L. Teixeira, J. Lopes, P. Gomes, R. Oliveira and D. Lopes, 2011. Tablas brasilenas para aves y cerdos. 3. ed. Vicosa, MG:UFV, DZO.

Shahir, M.H., S. Moradi, O. Afsarian and O. Esmaeilipour, 2013. Effects of cereal type, enzyme and sodium butyrate addition on growth performance, carcass traits and intestinal morphology of broilers. Revista Brasileira de Ciencia Avicola, 15: 181-189.

Sibbald, I.R., 1976. A bioassay for true metabolizable energy in feedingstuffs. Poult. Sci., 55: 303-308.

Singh, M., R. Srivastava, S. Chauhan and K. Singh, 2000. Responses of virginiamycin and bacitracin methylene disalicylate on the weight gains and nutrient utilization of broiler chicken. Ind. J. Poult. Sci., 35: 272-275.

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. Feed Sci., 18: 478-489.

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. Biotechnol., 8: 2329-2334.

Vervaeke, I.J., J.A. Decuypere, N.A. Dierick and H.K. Henderickx, 1979. Quantitative *in vitro* evaluation of the energy metabolism influenced by virginiamycin and spiramycin used as growth promoters in pig nutrition. J. Anim. Sci., 49: 846-856.

Wafaa, A. Abd El-Ghany, M.H. Awaad, S.A. Nasef and A.F. Gaber, 2016. Effect of sodium butyrate on Salmonella enteritidis infection in broiler chickens. Asian J. Poult. Sci., 10: 104-110.

Yang, Z., Y. Zaibin and Y. Weiren, 2010. Effects of different levels of sodium butyrate on nutrient utilization of broilers.China Feed, 8: 007.

Yang, Z., 2010. Effect of sodium butyrate on growth-promoting mechanism and application efficacy in broilers. Agricultural Science Paper. Abstract. http://www.agrpaper.com/effects-of-sodium-butyrate-on-growth-promoting-mechanism-and-application-efficacy-in-broilers.htm.

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

Zhang, W.H., F. Gao, Q.F. Zhu, C. Li, Y. Jiang, S.F. Dai and G.H. Zhou, 2011b. Dietary sodium butyrate alleviates the oxidative stress induced by corticosterone exposure and improves meat quality in broiler chickens. Poult. Sci., 90: 2592-2599.

Zou, Y., Z. Yang, W. Yang, S. Jiang and X. Zhao, 2010. Effects of different levels of sodium butyrate on nutrient utilization of broilers. China Feed 2010-08. http://en.cnki.com.cn/Article_en/CJFDTOTAL-SLGZ 201008007. htm (Abstr.).