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Association of sodium butyrate and phytase on the performance, bone quality and intestinal development in broilers

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Abstract: This study assessed the effect of the combination of microencapsulated sodium butyrate (SB) and phytase (PH) on the performance, intestinal integrity, and bone development of broilers. The experiment comprised 736 chicks distributed in a completely randomised design in a 2 × 2 factorial scheme (with and without inclusion of SB and with the inclusion of PH at the recommended dose and superdosing) totalling four treatments and eight repetitions of 23 birds each. SB was added at 0.750 kg/tonne and PH was included at 750 phytase units (FTU)/kg and 1 500 FTU/kg of diet. The live weight (LW), feed intake (FI) and feed conversion (FC) were evaluated weekly. Bone measurements of the tibia and femur, densitometry, length, the Seedor index, and diameter were performed at seven and 28 days. In the same ages, fragments of the jejunum and ileum segments were collected and subjected to a morphometry analysis. At seven days of age, the birds supplemented with the phytase superdosing showed a better FI and LW, and the microencapsulated SB in the diet showed a better LW and FC. In the period from one to 28 days, the treatment supplemented with SB provided a higher FI and LW. The SB supplementation resulted in greater bone measurements at seven and 28 days. The use of the superdosing phytase increased the villus length and width of the jejunum at seven days and at 28 days. There was an increase in the villus length, villus-to-crypt ratio, absorption area, and muscular layer of the jejunum and a decrease in the crypt width of the jejunum. The SB supplementation increased the ileum crypt width at seven days and there was no effect on any intestinal segment at 28 days. The supplementation of SB or PH 1 500 FTU/kg in the broiler diets' growth promoter or antibiotic-free resulted in a better performance and bone measurements, while the association of the additional PH 1 500 FTU/kg and SB supplementation resulted in the greater depth and width of the jejunum crypt and villus : ileum crypt at seven days and greater villus length and ileum absorption area at 28 days. The association of SB and PH may be a strategy to improve the performance and bone quality and intestinal integrity in broilers.

Keywords: densitometry; enzymes; gut health; intestinal morphometry; organic acids

Due to the removal of the antimicrobial growth promoters from the diet of broiler chickens due to bio-security threats for human and animal health, the search for gut health or other non-therapeutic alternatives is becoming increasingly important as the incidence of enteric diseases has grown in commercial flocks submitted to antibiotic-free programmes. (Mehdi et al. 2018). Organic acids and

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enzymes could be used in the animal feed as an alternative to antibiotics to make use of the nutrients in feed ingredients more efficiently without posing risks to consumers' health.

Short-chain fatty acids (SCFAs) constitute a group of molecules that contain from one to seven carbon atoms and which exist as straight or branched-chain compounds. SCFAs such as acetate, propionate and butyrate are produced in the cecum and colon of animals via the fermentation of carbohydrates, such as dietary fibres and unabsorbed starch. SCFAs are generally inhibitory to microorganisms depending on the type of fatty acid, type of microorganism and environmental pH. A low pH increases the concentration of dissociated SCFAs, which, in that conformation, can pass into bacterial cells where the intercellular pH is higher. This higher pH dissociates the SCFAs, so the intracellular pH decreases and subsequently changes the bacterial cell's metabolism, thus, by this mode of action, they can effectively replace the antibiotics and improve the production performance (Adil et al. 2011; Wu et al. 2018). Supplementing diets with SCFAs increases the gastric proteolysis, protein and amino acid digestibility and utilisation of minerals and, thus, improving the performance of the birds (Haque et al. 2009).

Among the SCFAs, butyric acid has an increasing importance in maintaining human and animal intestinal health. Butyric acid is available in the salt form of Na, K, Mg or Ca and can be used in free or protected (microencapsulated) forms. In the free mode, the compound acts only in the proximal part of the digestive tract, while the microencapsulated SB acts in the posterior portion of the gastrointestinal tract, mainly at the ileum and cecum level (Van Immerseel et al. 2004; Sikandar et al. 2017).

Numerous beneficial effects of SB have been demonstrated through an increased mucus production, an elevated intestinal integrity, resistance to immunological stress, that controls the intestinal barrier function and promotes the pathogen control (Guilloteau et al. 2010; Zou et al. 2019). Indeed, SB can be an energy source for epithelial cells and may directly modulate the intestinal microbiota, through its bactericidal effect, or indirectly by stimulating the growth of beneficial lactic acid bacteria (Ahsan et al. 2016; Bortoluzzi et al. 2017; Zou et al. 2019).

Antinutritional factors present in the diet can affect the intestinal health, as phytates have clearly been shown to increase the endogenous protein flow (Cowieson et al. 2011; Cowieson et al. 2016) whose effects are not only relevant for the protein status of the animal, but also for the availability of a substrate for the putrefactive organisms in the distal gastrointestinal tract. Phytase supplementation is used in commercial broiler diets to improve the phosphorus (P) availability, diet cost, environmental impact, and its effectiveness can be even greater in reducing the anti-nutrient effect of phytates (Walk et al. 2014; Manobhavan et al. 2016; Sommerfeld et al. 2018). The improvement of P availability is achieved by the cleavage of P from the phytate by the phytase enzyme. The complete dephosphorylation of phytate [myo-inositol hexakis(dihydrogen phosphate)] produces a myo-inositol ring (Sommerfeld et al. 2018). Therefore, improvements due to the addition of high doses of phytase in a poultry diet can be indicated by an increased weight gain, tibia mineralisation, nutrient digestibility, and blood plasma myo-inositol concentration (Cowieson et al. 2013). Additionally, the use of phytase superdosing would be expected to hydrolyse the phytate more quickly and completely in the upper part of the digestive tract, and, thus, more efficiently reduce the antinutritional effect of phytate on the intestinal mucosa (Cowieson et al. 2016).

Thus, the interaction between the phytase and the organic acids in diets for broilers can have additional effects on the performance, intestinal integrity, and bone quality in broilers.

MATERIAL AND METHODS

The experiment was carried out in cages in the experimental vivarium at the Federal University of Paraná – Palotina Sector. The Committee for Ethical Conduct in the Use of Experimental Animals approved all the procedures for raising animals and collecting biological material (01/2014).

The study comprised 736 one-day-old Ross AP 95 male chicks. The birds were distributed in a completely randomised design, in a 2 × 2 factorial scheme (with and without the inclusion of SB vs with the inclusion of PH at the recommended dose and superdosing), obtaining four treatments with eight repetitions and 23 birds per experimental unit. The experimental diets comprised: experimental diet 1: basal diet + 750 phytase units (FTU) PH; experimental diet 2: basal diet + 750 FTU PH + SB;

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experimental diet 3: basal diet + 1 500 FTU PH; experimental diet 4: basal diet + 1 500 FTU PH + SB.

The PH was used at a dose of 750 FTU/kg of diet in diets 1 and 2 [PH activity: 10 000 FTU/g; Nutritional contribution: P: 0.175%; Ca: 0.192%; Na: 0.040%; crude protein: 0.491%; metabolisable energy: 61 kcal/kg]. For treatments 3 and 4 (1 500 FTU/kg), the enzyme supplementation was in addition to the nutritional matrix used in treatments 1 and 2. The commercial product is based on the SB used at a dose 0.750 kg/tonne in diets 2 and 4 (physical appearance of micro pearls with white colouring, granulated and an SB concentration of 320 g/kg).

The experimental diets, based on corn and soybean meal, were formulated to meet the nutritional requirements of broilers, divided into two phases: initial (1–14 days old) and growth (15–28 days old), according to the recommendations of the local poultry companies (Table 1). The diets were also prepared without the inclusion of a growth promoter or anticoccidials.

The birds' breeding environment consisted of an acclimatised room (air conditioning and exhaust fans), with four batteries of cages, each composed of eight cages (0.55 × 0.80 m), totalling 32 experimental units. The temperature of the birds' thermal comfort was regulated according to their age. Water and feed were provided *ad libitum* throughout the trial period. The vaccination programme was carried out in the hatchery (Marek's disease, Gumboro disease and infectious bronchitis).

To evaluate the performance (average weight, feed consumption, and feed conversion), the birds and leftover diet from each experimental unit were weighed weekly, expressed from 1–7 and 1–28 days old. The feed conversion was corrected for the weekly bird mortality, according to the methodology described by Sakomura and Rostagno (2016).

At seven and 28 days of age, three birds/repetition (24 birds/treatment) were randomly picked, sacrificed and, after removing all adherent tissue from the left leg, the tibia and femur of each bird, were weighed and the length and diameter were measured with digital callipers (mm). The Seedor index (Seedor et al. 1991) was obtained by dividing the weight of the bones (mg) by their length (mm).

Similarly, the adherent tissue of the right leg was removed and the tibia was subjected to radiographic bone densitometry (bone mineral density). Initially, the bones were placed under a photographic film, all in the anteroposterior position, and then radiographed using a Procion Ion 70× – Mobile Column X-ray device (Procion Indústria e Comércio Ltda, Sao Paulo, Brazil), calibrated with a focus of 0.8 × 0.8 mm and an exposure time of 0.3 seconds. The voltage, current, and exposure time values were considered ideal after previous analyses. The radiographs were processed in an automatic developer. The reading of radiographs was performed using the histogram tool of the Adobe Photoshop v8.0 software (Adobe, San Jose, CA, USA) to determine the density of the bone pieces. As a radiographic reference, in the radiographic shots, we used an aluminium penetrometer with 10 steps with 1 mm thickness between each step. The densitometric readings were performed at six points in the central portion of the radiographic image and on each degree of the aluminium penetrometer.

The data obtained in grey values were converted into values related to the thickness of the aluminium penetrometer (aluminium mm), indicating the bone mineral density.

From the same birds sacrificed for the bone collection, we obtained fragments from the jejunum and ileum, approximately 5 cm in length, which were attached and opened longitudinally on Styrofoam plates, and washed with saline. The samples were fixed in a buffered formaldehyde solution and later embedded in paraffin. Each fragment was subjected to 5 µm thick semi-serial sections and stained with haematoxylin-eosin. For the morphometric study, the images were captured using light microscopy (10× objective), using a computerised image analyser system (Image-Pro® Plus, v5.2; Media Cybernetics, Bethesda, MD, USA). The length and width of 20 villi and the depth and width of 20 crypts of each slide were measured. These morphometric measurements were used to calculate the absorption surface area of the intestinal mucosa, using the formula proposed by Kisielinski et al. (2002).

$$\text{Absorption area} = [(WV \times HV) + (WV/2 + WC/2)^2 - (WV/2)^2]/[(WV/2 + WC/2)^2] \quad (1)$$

where:

WV – the width of the villus;

HV – the height of the villus;

WC – the width of the crypt.

The results obtained in the experiment were analysed using the analysis of variance (ANOVA) from the General Lineal Model (GLM) procedure

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Table 1. Composition and nutritional profile of the experimental diets

Ingredients (kg)	Initial (1–14 days of age)				Growing (15–28 days of age)			
	diet 1	diet 2	diet 3	diet 4	diet 1	diet 2	diet 3	diet 4
Corn	555.65	554.39	555.52	554.00	588.76	587.23	588.61	587.08
Soybean meal	351.08	351.08	351.08	351.34	307.73	307.99	307.75	308.01
Soybean oil	19.37	19.88	19.42	19.93	33.74	34.26	33.79	34.31
Dicalcium phosphate	7.90	7.90	7.90	7.90	7.21	7.21	7.21	7.21
Limestone	11.01	11.01	11.01	11.01	11.52	11.52	11.52	11.52
Salt	3.26	3.26	3.26	3.26	3.27	3.27	3.27	3.27
Initial[1]/growing[2] premix	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Choline chloride	1.75	1.75	1.75	1.75	1.50	1.50	1.50	1.50
Tryptophan	0.14	0.14	0.14	0.14	–	–	–	–
L-threonine (98%)	1.31	1.31	1.31	1.31	0.58	0.58	0.58	0.58
DL-methionine (99%)	3.47	3.47	3.47	3.47	2.38	2.38	2.38	2.38
L-lysine (80%)	4.99	4.99	4.99	4.99	3.24	3.24	3.24	3.24
Phytase	0.075	0.075	0.150	0.150	0.075	0.075	0.150	0.150
Sodium butyrate	–	0.750	–	0.750	–	0.750	–	0.750
Nutritional levels								
ME (kcal/kg)	3.120	3.120	3.120	3.120	3.250	3.250	3.250	3.250
Crude protein (%)	21.8	21.8	21.8	21.8	19.8	19.8	19.8	19.8
Total calcium (%)	0.950	0.950	0.950	0.950	0.94	0.94	0.94	0.94
Total phosphorus (%)	0.681	0.681	0.681	0.681	0.65	0.65	0.65	0.65
Available phosphorus (%)	0.450	0.450	0.450	0.450	0.43	0.43	0.43	0.43
Sodium (%)	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Chlorine (%)	0.31	0.31	0.31	0.31	0.25	0.25	0.25	0.25
Choline (mg/kg)	784	784	784	784	–	–	–	–
Dig. lysine (%)	1.30	1.30	1.30	1.30	1.10	1.10	1.10	1.10
Dig. methionine (%)	0.63	0.63	0.63	0.63	0.51	0.51	0.51	0.51
Dig. methionine + cystine (%)	0.96	0.96	0.96	0.96	0.81	0.81	0.81	0.81
Dig. threonine (%)	0.86	0.86	0.86	0.86	0.73	0.73	0.73	0.73
Dig. tryptophan (%)	0.26	0.26	0.26	0.26	0.22	0.22	0.22	0.22
Dig. valine (%)	0.92	0.92	0.92	0.92	0.84	0.84	0.84	0.84

Dicalcium phosphate = 20% P, 23% Ca; Dig. = digestibility; Limestone = 37% Ca; ME = metabolisable energy

[1]Cu (as copper sulfate) 4 mg, Fe (as ferrous sulfate) 16 mg, I (as potassium iodide) 0.28 mg, Mn (as manganese sulfate) 16 mg, Se 0.12 mg, Zn 32 mg, vitamin A 3 200.40 IU, vitamin D_3 800.28 IU, vitamin E 12 IU, vitamin K_3 0.8 mg, vitamin B_1 1.2 mg, vitamin B_2 2.4 mg, vitamin B_6 1.0 mg, vitamin B_{12} 4.8 mg, nicotinic acid 14 mg, D-pantothenic acid 6 mg, folic acid 0.4 mg, biotin 0.03 mg

[2]Cu (as copper sulfate) 6.67 mg, Fe (as ferrous sulfate) 26.67 mg, I (as potassium iodide) 0.47 mg, Mn (as manganese sulfate) 26.67 mg, Se 0.20 mg, Zn 53.33 mg, vitamin A 5 334 IU, vitamin D_3 1 333.80 IU, vitamin E 20 IU, vitamin K_3 1.33 mg, vitamin B_1 2.00 mg, vitamin B_2 4.00 mg, vitamin B_6 1.67 mg, vitamin B_{12} 8.0 mg, nicotinic acid 23.33 mg, D-pantothenic acid 10 mg, folic acid 0.67 mg, biotin 0.05 mg

with the statistical program SAS/STAT® v9 (SAS Institute, Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Performance data from 1–7 days and 1–28 days are shown in Table 2. There was no significant interaction between the SB and PH supplementation ($P > 0.05$) on the performance of the birds.

In the first age period (1–7 days), the birds that received the SB supplementation had a higher live weight ($P = 0.052\ 0$) and better feed conversion ($P = 0.042\ 5$). The positive impact of the SCFAs on the growth performance can be attributed to the better digestibility and reduction in the pH values

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Table 2. Performance of the broilers from one to seven days of age and one to 28 days of age supplemented with sodium butyrate (SB) and phytase (PH)

	Feed intake (g)			Live weight (g)			Feed conversion (g/g)		
	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean
	750	1 500		750	1 500		750	1 500	
SB 1–7 days									
0	126.95 ± 7.88	135.60 ± 6.98	131.28 ± 7.22	154.09 ± 8.56	166.25 ± 9.02	160.17^{B} ± 7.98	1.124 ± 0.01	1.120 ± 0.01	1.122^{A} ± 0.02
0.750 g/tonne	130.73 ± 6.77	135.94 ± 7.77	133.33 ± 7.30	160.95 ± 7.67	170.91 ± 8.89	165.93^{A} ± 8.67	1.105 ± 0.02	1.076 ± 0.03	1.090^{B} ± 0.02
Mean	127.3^{b} ± 7.02	135.7^{a} ± 7.03	–	157.53^{b} ± 8.11	168.5^{a} ± 8.65	–	1.115 ± 0.01	1.098 ± 0.02	–
CV (%)		5.82			6.09			3.99	
Analysis of variance									
PH		0.002 9			0.000 6			0.252 5	
SB		0.339 0			0.052 0			0.042 5	
PH × SB		0.422 3			0.702 2			0.409 0	
SB 1–28 days									
0	1 647.23 ± 56.34	1 675.38 ± 49.34	$1\,661.30^{B}$ ± 53.02	1 211.36 ± 73.34	1 230.34 ± 72.45	$1\,228.22^{B}$ ± 72.06	1.418 ± 0.07	1.421 ± 0.08	1.419 ± 0.08
0.750 g/tonne	1 719.18 ± 59.76	1 726.46 ± 58.98	$1\,722.82^{A}$ ± 57.78	1 283.96 ± 69.85	1 289.34 ± 71.34	$1\,286.65^{A}$ ± 71.00	1.395 ± 0.09	1.436 ± 0.07	1.415 ± 0.07
Mean	1 683.20 ± 58.02	1 700.91 ± 52.33	–	1 255.03 ± 71.23	1 259.83 ± 71.67	–	1.406 ± 0.07	1.428 ± 0.08	–
CV (%)		5.49			5.96			3.10	
Analysis of variance									
PH		0.587 8			0.827 9			0.163 7	
SB		0.067 2			0.012 9			0.796 7	
PH × SB		0.749 1			0.979 2			0.233 0	

CV = coefficient of variation; FTU = phytase unit

^{A,B,a,b}Different capital letters in the same column and different lowercase letters in the same line differ by the *F*-test

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in the feed and intestine. Lowering the dietary pH by weak organic acids, such as butyric acid has been reported to be helpful in reducing the microbial competition for nutrients by lowering the risk of subclinical infections, decreasing the intestinal immune response and the pathogenic organisms which are sensitive to a low pH (Ghazala et al. 2011). The improvement in the feed conversion could be possibly due to the better utilisation of the nutrients resulting in an increased body weight gain in the birds fed SB in the diet. The use of SB in poultry nutrition is well accepted due to its pH reduction effect that limits the pathogen development and helps in the protein digestion (Sikandar et al. 2017).

In the period from one to seven days, the birds that received the phytase superdosing presented a higher live weight (P = 0.000 6) and feed consumption (P = 0.002 9). The growth-promoting effect in the present study can be partially attributed to the increase in feed intake and better utilisation of P. The improved performance arising from superdosing with phytase can be attributed to the increased energy digestibility or nutrient digestibility from the amino acids (Cowieson et al. 2016). The phytate breakdown by phytase is associated with a change in the pattern of lower inositol phosphate esters such as IP5, IP4, IP3, IP2, and IP1, some of which might exert antinutritive effects by binding other nutrients and impairing their digestibility (Cowieson et al. 2011).

Cowieson et al. (2013) reported improvements in the growth performance and insulin levels in broilers supplemented with myo-inositol. Inositol is involved in the phosphoinositide family of lipids. Phosphoinositides are stored in the plasma membrane and are a source of lower derivative inositols which are used as secondary messengers in insulin signalling and intra-cellular Ca signalling (Croze and Soulage 2013). The presently observed improvements in the growth performance can possibly be attributed to the increased inositol availability for use in these pathways. Furthermore, the PH can reduce the binding of the phytate to the dietary protein and eliminate its antinutritional effect as well as reducing any endogenous amino acid losses (Cowieson et al. 2016).

In the evaluation of the data collected in the total period of the experiment, from one to 28 days of age of the birds, the addition of SB resulted in a greater feed consumption (P = 0.067 2) and live weight (P = 0.012 9); however, without altering the feed conversion of the birds.

Mansoub (2011) reported that dietary SB increased the weight gain up to 28 days of age. Likewise, the partially protected and microencapsulated SB positively affected the performance of broilers during the grower and finisher periods. The improvement in the performance of broilers is considered due to the different functions accomplished by the SB. Butyric acid increases the villi length in the small intestine (Chamba et al. 2014) and stimulates the pancreatic exocrine, thus, increasing the secretions of digestive enzymes, such as amylase and lipase. Consequently, the feed digestion and nutrient absorption is improved.

The results of the bone analysis at seven days of age are shown in Table 3. There was no significant interaction between the SB and PH supplementation for the bone measurements. The supplementation with SB increased the tibia diameter (P = 0.038 4), femur length (P = 0.058 0), femur Seedor index (P = 0.020 1) and femur diameter (P = 0.042 1). This positive effect was also observed at this same age for the birds' live weight and feed conversion.

It has been suggested that SB promotes differentiation into the osteoblasts and their maturation, stimulates the bone formation by osteoblasts, especially with respect to the formation of hydroxyapatite crystals or the extracellular matrix and by increasing the production of bone sialoprotein and osteoprotegerin (Katono et al. 2008). However, few reports have looked into the effects of SB on the bone formation of birds.

At 28 days of age (Table 4), there was also no significant interaction between the SB and PH supplementation for the bone measurements. A greater tibia length (P < 0.023 2) to the SB supplementation and a greater femur diameter (P = 0.009 4) with PH 750 FTU/kg were observed. For the bone densitometry, there was no effect of the SB and PH supplementation at seven or 28 days of age. PH acts on the release of P and Ca, which can increase the concentration of these minerals in the blood flow and influence the bone quality (Walk et al. 2014; Lee et al. 2017). An improved phosphorus digestibility and Ca digestibility in the PH supplemented diet have been reported by several researchers (Lalpanmawia et al. 2014).

Bone responses to PH supplementation showed an increase in the bone mineralisation, a reduced risk of femoral fracture, thigh and upper thigh rupture, cartilage separation, and haemorrhagic lesions in the flesh (Walk et al. 2014). This is probably relat-

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Table 3. Bone densitometry, length, Seedor index and diameter of the bones of the broilers at seven days of age supplemented with sodium butyrate (SB) and phytase (PH)

	Bone densitometry (mmAl)			Lenght (mm)			Seedor index			Diameter (mm)		
	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean
	750	1 500		750	1 500		750	1 500		750	1 500	
TIBIA												
SB												
0	0.98 ± 0.14	0.99 ± 0.14	0.98 ± 0.13	44.24 ± 1.68	44.30 ± 1.59	44.27 ± 1.77	33.79 ± 3.69	33.34 ± 3.66	33.57 ± 3.71	2.92 ± 0.29	2.90 ± 0.27	2.91^{B} ± 0.28
0.750 g/tonne	1.05 ± 0.15	1.02 ± 0.16	1.03 ± 0.14	43.95 ± 1.76	44.31 ± 1.68	44.13 ± 1.75	35.36 ± 3.68	33.87 ± 4.02	34.62 ± 3.98	3.00 ± 0.28	3.08 ± 0.29	3.04^{A} ± 0.26
Mean	1.01 ± 0.12	1.03 ± 0.13	–	44.10 ± 1.69	44.31 ± 1.66	–	34.58 ± 3.54	33.60 ± 4.11	–	2.96 ± 0.27	2.99 ± 0.28	–
CV (%)		14.36			3.82			11.61			9.86	
Analysis of variance												
PH		0.721 1			0.488 8			0.232 3			0.569 2	
SB		0.093 2			0.650 4			0.199 8			0.038 4	
PH × SB		0.588 7			0.629 1			0.519 9			0.385 3	
FEMUR												
SB												
0		–		31.87 ± 1.90	31.55 ± 1.96	31.71^{B} ± 1.91	29.88 ± 4.23	30.92 ± 4.11	30.84^{B} ± 4.18	3.15 ± 0.36	3.22 ± 0.35	3.19^{B} ± 0.35
0.750 g/tonne		–		32.31 ± 1.78	32.66 ± 1.93	32.48^{A} ± 1.89	33.09 ± 4.10	31.72 ± 3.90	32.43^{A} ± 4.06	3.33 ± 0.37	3.36 ± 0.36	3.34^{A} ± 0.36
Mean		–		32.09 ± 1.82	32.10 ± 1.89	–	31.48 ± 4.01	31.32 ± 3.94	–	3.24 ± 0.37	3.29 ± 0.38	–
CV (%)		–			6.13			13.22			11.49	
Analysis of variance												
PH		–			0.973 2			0.846 8			0.478 2	
SB		–			0.058 0			0.020 1			0.042 1	
PH × SB		–			0.404 0			0.156 9			0.821 3	

CV = coefficient of variation; FTU = phytase unit

^{A,B}Different capital letters in the same column differ by the *F*-test

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Table 4. Bone densitometry of the broilers at 28 days of age supplemented with sodium butyrate (SB) and phytase (PH)

	Bone densitometry (mmAl)			Lenght (mm)			Seedor index			Diameter (mm)		
	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean
	750	1 500		750	1 500		750	1 500		750	1 500	
TIBIA												
SB												
0	1.09 ± 0.18	1.08 ± 0.17	1.08 ± 0.18	–	84.83 ± 3.20	85.23 ± 3.77	85.03^{B} ± 3.56	142.94 ± 15.16	145.75 ± 15.51	144.35 ± 15.41	8.19 ± 0.98	8.00 ± 0.87
0.750 g/tonne	1.15 ± 0.19	1.10 ± 0.17	1.13 ± 0.18	–	86.70 ± 3.87	86.40 ± 3.69	86.55^{A} ± 3.72	144.51 ± 15.09	137.46 ± 14.89	140.98 ± 15.52	8.31 ± 0.92	7.74 ± 0.88
Mean	1.12 ± 0.19	1.09 ± 0.18	–	–	85.76 ± 3.04	85.82 ± 3.72	–	143.72 ± 14.32	141.61 ± 15.02	–	8.25 ± 0.89	7.87 ± 0.94
CV (%)		16.91			3.71			10.53			12.04	
Analysis of variance												
PH		0.503 9			0.934 1			0.498 5			0.062 3	
SB		0.262 4			0.023 2			0.282 9			0.718 5	
PH × SB		0.559 7			0.598 0			0.116 6			0.346 1	
FEMUR												
SB												
0		–		62.01 ± 2.73	63.13 ± 2.08	62.57 ± 2.58	128.06 ± 12.45	127.08 ± 13.88	127.57 ± 14.74	8.18 ± 0.88	7.90 ± 0.78	8.04 ± 0.84
0.750 g/tonne		–		62.95 ± 2.54	63.35 ± 2.01	63.15 ± 2.74	131.42 ± 14.65	125.36 ± 15.22	128.39 ± 14.36	8.44 ± 0.91	7.79 ± 0.85	8.11 ± 0.90
Mean		–		62.48 ± 2.32	63.24 ± 2.04	–	129.74 ± 12.98	126.22 ± 14.44	–	8.31^{a} ± 0.89	7.84^{b} ± 0.82	–
CV (%)		–			4.40			11.15			10.44	
Analysis of variance												
PH		–			0.193 9			0.240 7			0.009 4	
SB		–			0.319 6			0.784 8			0.671 1	
PH × SB		–			0.536 6			0.396 4			0.285 0	

CV = coefficient of variation; FTU = phytase unit

^{A,B,a,b}Different capital letters in the same column and different lowercase letters in the same line differ by the *F*-test

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ed to the release of minerals from the phytate bond as well as the prevention of bounding with phytate. Supplementing PH in a poor P poultry diet increases the growth, relative retention of the total P, Ca, Cu, and Zn and improves the bone mineralisation in broilers (Manobhavan et al. 2016). The results possibly mean that the evaluated diet contained sufficient nutrients for bone mineralisation.

The supplementation of the mixture of SCFAs in the broiler diet can produce an increase in the digestibility and availability of nutrients (such as Ca and P) (Ziaie et al. 2011). Some SCFAs produced by the gut microbiota, like butyrate acid, play an important role in bone formation and bone mineralisation by influencing the osteoprotegerin signalling pathways (Katono et al. 2008).

The data on the intestinal morphometry of the jejunum at seven days of age are shown in Table 5. For the results of the villus length and villus width, a significant difference was observed for PH supplementation only. The addition of PH 1 500 FTU increased the length (P = 0.054 9) and width (P = 0.019 1) of the jejunum villi. There was significant interaction (P = 0.029 1) for the crypt depth and crypt width (P = 0.028 1). The addition of PH 750 FTU/kg to the SB supplemented diet reduced the depth and width of the crypt. There was no effect of the PH or butyrate on the absorption area, villus crypt ratio and muscle layer.

The supplementation of SB and phytase in the superdosing increased the crypt depth and width. It has been demonstrated that SCFAs stimulate the proliferation of normal crypt cells, improving healthy tissue turnover and maintenance (Diao et al. 2019). An increase in the crypt depth can be used as a predictor of an increased crypt cell production rate and overall stimulation of cell turnover in the small intestine, which are generally associated with reduced digestive and absorptive capacity (Montagne et al. 2007).

Sayrafi et al. (2011) studied the responses of the histo-morphometrical parameters to the antibiotic growth promoter and alternative additives and reported that an increase in the crypt depth indicates accelerated villi renewal which occurs as a result of damaged mucosal cells.

An increase in the surface of the villi implicates an augmentation in the intestinal absorptive capacity. These results show that although the SB supplementation increases the proliferative capacity of the crypt (Ahsan et al. 2016), there was no increase

Table 5. Intestinal morphometry (jejunum) of the broilers at seven days of age supplemented with sodium butyrate (SB) and phytase (PH)

	Villus lenght (µm)			Villus width (µm)			Crypt depth (µm)			Crypt width (µm)			Relation vile : crypt			Absorption area (µm^2)			Muscle layer (µm)		
	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean
	750	1 500		750	1 500		750	1 500		750	1 500		750	1 500		750	1 500		750	1 500	
SB																					
0	431.66 ± 86.32	432.99 ± 82.41	432.32 ± 85.32	115.88 ± 18.45	118.21 ± 19.12	117.05 ± 18.78	49.99^a ± 7.89	45.93^a ± 6.92	47.96 ± 6.55	43.27^a ± 5.23	39.89^a ± 5.45	41.58 ± 6.08	8.69 ± 1.63	9.62 ± 1.75	9.15 ± 1.72	8.44 ± 1.46	8.36 ± 1.65	8.40 ± 1.58	83.03 ± 16.73	81.24 ± 16.02	82.14 ± 17.00
0.750 g/ tonne	438.15 ± 85.12	508.68 ± 83.03	473.41 ± 85.08	109.44 ± 17.32	129.24 ± 20.42	119.34 ± 19.02	47.41^b ± 7.02	51.92^a ± 8.90	49.66 ± 8.03	43.14^b ± 6.01	46.67^a ± 6.72	44.90 ± 5.89	9.39 ± 1.78	9.84 ± 1.66	9.62 ± 1.75	8.62 ± 1.52	8.76 ± 1.44	8.69 ± 1.51	88.97 ± 17.08	90.90 ± 18.59	89.94 ± 17.31
Mean	434.90^b ± 84.44	470.83^a ± 82.89	–	112.66^b ± 17.71	123.73^a ± 18.94	–	48.70 ± 7.50	48.92 ± 7.63	–	43.20 ± 5.78	43.28 ± 6.14	–	9.04 ± 1.69	9.73 ± 1.73	–	8.53 ± 1.55	8.56 ± 1.58	–	86.00 ± 17.15	86.07 ± 17.14	–
CV (%)	18.92			15.89			15.60			14.61			18.16			18.21			20.66		
Analysis of variance																					
PH	0.054 9			0.019 1			0.908 8			0.959 0			0.109 5			0.944 2			0.987 6		
SB	0.092 1			0.619 4			0.378 0			0.034 3			0.282 5			0.474 0			0.090 1		
PH × SB	0.104 5			0.062 1			0.029 1			0.028 1			0.582 8			0.779 5			0.682 5		

CV = coefficient of variation; FTU = phytase unit; ^{a,b}different lowercase letters in the same line differ by the F-test

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in the villus, as observed in the jejunum mucosa of the birds that received the phytase superdosing.

In Table 6, the evaluation of the ileum mucosa at seven days of age shows a significant interaction between the supplementation of butyrate and PH for the villus-to-crypt ratio only (P = 0.006 7). The unfolding of the interaction demonstrated that the PH supplementation at the recommended dose (750 FTU) associated with the SB supplementation resulted in the highest villus-to-crypt ratio. The use of SB increased the crypt width compared to the control diet (P = 0.034 5). This result could be attributed to the direct trophic effect of butyrate on the ileum mucosa, since the dietary butyrate supplementation contributes to the energy requirement of the ileum cells, due to the small fermentation rates in this region (Onrust et al. 2015; Ahsan et al. 2016).

The positive results of the supplementation with the SB and PH on the intestinal mucosa may explain the better performance observed at seven days. Onrust et al. (2015) cite favourable responses to the use of protected organic acids in maintaining the intestinal health as a source of energy for intestinal cells. These acids also act positively on the proliferation, differentiation, and maturation of the intestinal cells, in addition to ensuring the integrity of the intestinal barrier and, therefore, greater food efficiency and weight gain.

The intestinal morphometry of the jejunum at 28 days of age showed no significant interaction between the PH and butyrate (Table 7). PH supplementing above the recommended dose (1 500 FTU) increased the villus length (P = 0.002 4), the villus-to-crypt ratio (P = 0.043 1), absorption area (P = 0.002 5), muscle layer (P = 0.050 7), and decreased the crypt width (P = 0.028 7).

Despite the improvement in the intestinal conditions of the birds, the addition of PH at higher doses did not alter the performance in the same period. The best intestinal integrity observed could be attributed to the PH supplementation effects on the dephosphorylation of phytic acid in its smaller esters of myo-inositol phosphate (IP5 to IP1) by reducing its anti-nutritional effects and its benefits due to the use of myo-inositol. There were possible vitamin-lipotropic effects, such as the regulation of cell morphogenesis and histogenesis, maintenance of cell membrane structure, lipid synthesis, and cell growth (Lee et al. 2017).

The intestinal morphometry of the ileum in broilers at 28 days is shown in Table 8. The vil-

Table 6. Intestinal morphometry (ileum) of the broilers at seven days of age supplemented with sodium butyrate (SB) and phytase (PH)

	Villus lenght (µm)			Villus width (µm)			Crypt depth (µm)			Crypt width (µm)			Relation vile:crypt			Absorption area (µm^2)			Muscle layer (µm)		
	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean
	750	1 500		750	1 500		750	1 500		750	1 500		750	1 500		750	1 500		750	1 500	
SB																					
0	303.95 ± 69.11	339.63 ± 77.32	321.79 ± 71.21	109.24 ± 15.13	112.54 ± 13.92	110.89 ± 14.77	50.15 ± 9.87	47.69 ± 9.11	48.92 ± 9.36	41.29 ± 5.32	40.51 ± 5.28	40.90^{b} ± 5.35	6.15^{Ba} ± 1.20	7.25^{Aa} ± 1.32	6.70 ± 1.28	6.26 ± 1.53	6.60 ± 1.62	6.43 ± 1.50	96.96 ± 22.97	107.48 ± 24.78	102.22 ± 23.89
0.750 g/tonne	325.81 ± 73.41	312.32 ± 70.58	319.06 ± 73.69	112.97 ± 14.58	114.38 ± 14.01	113.68 ± 13.92	49.44 ± 8.44	50.39 ± 9.67	49.92 ± 8.44	43.96 ± 5.77	42.92 ± 5.78	43.44^{a} ± 6.02	6.61^{Aa} ± 1.21	6.24^{Ab} ± 1.28	6.42 ± 1.27	6.58 ± 1.48	6.31 ± 1.39	6.44 ± 1.49	96.28 ± 22.91	99.72 ± 21.54	98.00 ± 22.75
Mean	314.88 ± 71.21	325.97 ± 73.69	–	111.10 ± 15.11	113.46 ± 13.78	–	49.80 ± 8.97	49.04 ± 9.25	–	42.63 ± 5.64	41.72 ± 5.37	–	6.38 ± 1.25	6.75 ± 1.29	–	6.42 ± 1.49	6.45 ± 1.45	–	96.62 ± 20.78	103.60 ± 22.75	–
CV (%)	22.81			13.88			19.74			13.42			19.92			24.35			23.20		
Analysis of variance																					
PH	0.476 7			0.480 7			0.720 2			0.444 9			0.169 5			0.928 0			0.159 4		
SB	0.860 6			0.404 6			0.636 9			0.034 5			0.300 3			0.966 9			0.392 3		
PH × SB	0.116 9			0.777 3			0.419 9			0.913 4			0.006 7			0.371 2			0.473 7		

CV = coefficient of variation; FTU = phytase unit; ^{A,B,a,b}different capital letters in the same column and different lowercase letters in the same line differ by the F-test

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Table 7. Intestinal morphometry (jejunum) of the broilers at 28 days of age supplemented with sodium butyrate (SB) and phytase (PH)

	Villus lenght (μm)			Villus width (μm)			Crypt depth (μm)			Crypt width (μm)			Relation vile : crypt			Absorption area (μm^2)			Muscle layer (μm)		
	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean
	750	1 500		750	1 500		750	1 500		750	1 500		750	1 500		750	1 500		750	1 500	
SB																					
0	656.95 ± 123.75	743.68 ± 157.45	700.32 ± 146.78	656.95 ± 98.89	642.47 ± 94.58	649.71 ± 95.75	71.35 ± 12.45	73.73 ± 13.04	72.54 ± 12.99	49.14 ± 4.12	45.03 ± 3.99	47.09 ± 4.70	9.22 ± 1.68	10.24 ± 1.74	9.73 ± 1.65	11.35 ± 2.43	12.73 ± 2.87	12.04 ± 2.77	115.52 ± 25.02	128.00 ± 26.99	121.76 ± 25.08
0.750 g/ tonne	642.47 ± 122.78	758.13 ± 159.02	700.30 ± 149.45	743.68 ± 100.04	758.13 ± 97.63	750.90 ± 99.79	65.03 ± 12.16	71.13 ± 12.85	68.08 ± 12.41	48.20 ± 4.89	47.43 ± 4.21	47.82 ± 4.69	10.10 ± 1.72	10.71 ± 1.69	10.40 ± 1.75	11.07 ± 2.65	13.17 ± 2.99	12.12 ± 2.82	117.97 ± 26.41	128.13 ± 27.02	123.05 ± 26.85
Mean	649.71^{b} ± 121.47	750.90^{a} ± 158.78	–	700.32 ± 99.05	700.30 ± 95.78	–	68.19 ± 13.02	72.43 ± 12.99	–	48.67^{a} ± 4.77	46.23^{b} ± 4.33	–	9.66^{b} ± 1.70	10.47^{a} ± 1.68	–	11.21^{b} ± 2.68	12.95^{a} ± 2.87	–	116.74^{b} ± 25.89	128.07^{a} ± 26.74	–
CV (%)		22.09			14.51			18.86			10.85			18.69			22.16			21.45	
Analysis of variance																					
PH		0.002 4			0.063 4			0.140 2			0.028 7			0.043 1			0.002 5			0.050 7	
SB		0.999 6			0.215 8			0.121 6			0.508 4			0.093 1			0.886 7			0.821 4	
PH × SB		0.654 8			0.978 4			0.516 1			0.130 2			0.606 1			0.528 1			0.839 3	

CV = coefficient of variation; FTU = phytase unit; ^{a,b}different lowercase letters in the same line differ by the *F*-test

Table 8. Intestinal morphometry (ileum) of the broilers at 28 days of age supplemented with sodium butyrate (SB) and phytase (PH)

	Villus lenght (μm)			Villus width (μm)			Crypt depth (μm)			Crypt width (μm)			Relation vile : crypt			Absorption area (μm^2)			Muscle layer (μm)		
	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean	PH (FTU/kg)		mean
	750	1 500		750	1 500		750	1 500		750	1 500		750	1 500		750	1 500		750	1 500	
SB																					
0	436.72^{Aa} ± 132.85	362.77^{Ba} ± 122.42	399.74 ± 127.02	138.14 ± 21.28	142.67 ± 22.25	140.40 ± 21.34	62.90 ± 11.02	57.08 ± 10.36	59.99 ± 11.03	50.53 ± 4.98	50.09 ± 4.66	50.31 ± 4.58	6.90^{Aa} ± 1.43	6.51^{Aa} ± 1.39	6.71 ± 1.40	7.29^{Aa} ± 2.17	5.80^{Bb} ± 1.72	6.54 ± 1.90	152.25 ± 31.78	139.79 ± 28.69	146.02 ± 29.05
0.750 g/ tonne	345.57^{Ba} ± 112.45	444.28^{Aa} ± 129.56	394.92 ± 128.56	140.01 ± 20.45	129.61 ± 20.37	134.81 ± 20.87	58.21 ± 10.58	61.71 ± 11.45	59.96 ± 10.56	53.58 ± 4.77	50.80 ± 4.45	52.19 ± 3.99	5.95^{Ba} ± 1.29	7.11^{Aa} ± 1.45	6.53 ± 1.38	5.40^{Bb} ± 1.63	7.56^{Aa} ± 2.08	6.48 ± 1.87	150.86 ± 29.98	157.65 ± 31.14	154.25 ± 29.56
Mean	391.14 ± 126.39	403.53 ± 127.68	–	139.07 ± 21.36	136.14 ± 22.08	–	60.56 ± 10.97	59.39 ± 10.95	–	52.06 ± 4.06	50.45 ± 4.09	–	6.42 ± 1.41	6.81 ± 1.33	–	6.34 ± 1.87	6.68 ± 1.92	–	151.56 ± 30.05	148.72 ± 29.87	–
CV (%)		31.38			15.67			17.95			9.76			23.86			30.21			20.49	
Analysis of variance																					
PH		0.695 5			0.607 8			0.685 6			0.225 4			0.354 5			0.479 4			0.733 9	
SB		0.878 9			0.329 5			0.991 4			0.157 9			0.669 1			0.894 0			0.326 1	
PH × SB		0.008 2			0.194 9			0.108 6			0.376 4			0.065 4			0.000 3			0.251 6	

CV = coefficient of variation; FTU = phytase unit; ^{A,B,a,b}different capital letters in the same column and different lowercase letters in the same line differ by the *F*-test

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lus length (P = 0.008 2) and intestinal absorption area (P = 0.000 3) showed a significant interaction between the SB supplementation and the PH. The villus length and absorption area demonstrated a reduction in the diets supplemented with the lowest PH dose (750 FTU) added with the SB. The opposite effect was observed when the diet was supplemented with the phytase superdosing (1 500 FTU) and the SB, that is, a greater villus length and absorption area.

The association of sodium butyrate supplementation with the phytase superdosing resulted in a greater depth and width of the jejunum crypt and the villus : ileum crypt at seven days and the villus length and ileum absorption area at 28 days.

Further studies are needed on phytase superdosing supplementation on poultry performance and bone growth and development, using diets deficient in Ca and P. Moreover, other studies should investigate the concept of superdosing on the intestinal health in enteric challenge situations and their interaction with the SB supplementation.

CONCLUSION

The supplementation of SB or PH 1 500 FTU/kg in broilers diets growth promoter free resulted in better performance and bone measurements.

The association of the addition PH 1 500 FTU/kg and SB supplementation resulted in greater depth and width of the jejunum crypt and villus : ileum crypt at seven days and greater villus length and ileum absorption area at 28 days.

The association of SB and PH may be a strategy to improve the performance and bone quality, and intestinal integrity in broilers.

Conflict of interest

The authors declare no conflict of interest.

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