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Protected sodium butyrate in chicken diets until 21 days of age improves intestinal development and performance

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Abstract

This study was developed to examine the performance, carcass and cut yields, nutrient digestibility, and intestinal histomorphometry of chickens fed diets supplemented with protected sodium butyrate until 21 days of age. Two experiments were conducted, both of which involved the following treatments: basal diet with inclusion of an antibiotic; basal diet without antibiotic or protected sodium butyrate (PSB; control); basal diet with inclusion of 225 g/t PSB in the pre-starter and starter phases; and basal diet with inclusion of 300 g/t PSB in the pre-starter and starter phases. In the first experiment, 784 male broiler chicks were distributed into the four treatments, with seven replicates of 28 birds, to evaluate performance and carcass and cut yields. In experiment II, 280 male broiler chicks were distributed into the four treatments, with seven replicates of 10 birds, to evaluate intestinal digestibility and histomorphometry. At 42 days of age, the broilers supplemented with 225 g/t PSB had a higher average final weight than the control group. At seven days, the chickens supplemented with 300 g/t PSB exhibited the highest duodenal villus height; those supplemented with 225 or 300g/t PSB or antibiotic showed the greatest jejunal villus height; and those treated with 225 g/t PSB exhibited the highest jejunal villus/crypt ratio. At 21 days of age, the broilers that received 225 g/t PSB showed the highest duodenal and jejunal villus height. The use of protected sodium butyrate in chicken diets up to 21 days of age improves intestinal development and performance until slaughter age.

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Introduction

Studies aimed at improving nutrient utilisation in birds, and, consequently, their performance, point to the importance of development and maintenance of intestinal integrity and balance of the intestinal microbiota, which impede the fixation and multiplication of pathogenic microorganisms, preventing enteric diseases (Celi *et al*., 2017; Diaz Carrasco *et al*., 2019; Oviedo-Rondón, 2019).

A commonly employed nutritional strategy to increase poultry production rates by maintaining integrity and manipulating the intestinal microbiota is the use of antibiotics as growth promoters, at subtherapeutic levels. However, despite all the improvements achieved in intestinal physiology, in January 2006, the European Union — one of the world's largest importers of poultry products — decided to completely ban the use of growth-promoting antibiotics due to growing concerns about the presence of residues in products for human consumption, which can produce allergic reactions and toxicity or induce resistance in pathogenic bacteria (Huyghebaert *et al*., 2011).

This new scenario, coupled with market demands, has driven the search for substitutes for growth-promoting antibiotics that do not reduce productivity in poultry farming or increase its production costs. Among these alternatives, organic acids stand out. Graham Solomons & Fhyhle (2011) defined organic acids as any substance with a general R-COOH structure, known as derivatives of carboxylic acids, e.g., amino acids, fatty acids, coenzymes, and intermediate metabolites. Those associated with antimicrobial activity are short-chain fatty acids that produce fewer protons per molecule upon dissociation, which can be either monocarboxylic, such as formic, acetic, propionic and butyric acids; or carboxylated with the hydroxyl group, e.g., lactic, malic, tartaric, benzoic and citric acids, which are natural constituents of plants and animals (Picker *et al*., 2012).

Butyric acid, or butyrate, is known to be a safe alternative to antibiotics and, as such, has received great attention in the poultry industry. However, because butyrate is odorous and unstable, sodium butyrate is used as a substitute due to its stable and non-odoriferous properties (Lan *et al*., 2020); when administered in animal feed, it can be used in its free or protected form (micro-encapsulated). In its free form, it is rapidly absorbed in the early parts of the gastrointestinal tract, which substantially reduces the amount of butyrate reaching the distal parts of the intestine (Kaczmarek *et al*., 2016). Butyrate, in its protected form, is gradually released into the gastrointestinal tract, increasing its action throughout the intestine (Ogwuegbu *et al*., 2021).

The use of sodium butyrate in the diet of broiler chickens has shown positive effects on their intestinal health. This is mainly due to its trophic action, whereby it can increase nutrient absorption area by increasing the height of the villi (Adil *et al*., 2011; Chamba *et al*., 2014; Sikandar *et al*., 2017) and the number of goblet cells (Sikandar *et al*., 2017), and its ability to control pathogenic microorganisms (Ahsan *et al*., 2016). Sodium butyrate has been shown to play an important role as an energy source for gastrointestinal epithelial cells and to have antimicrobial, anti-inflammatory, and antioxidant properties (Liu *et al*., 2014; Song *et al*., 2017). As a result, improvements are seen in the digestibility of dietary nutrients (Kaczmarek *et al*., 2016; Riboty *et al*., 2016) and production performance (Chamba *et al*., 2014; Riboty *et al*., 2016; Bortoluzzi *et al*., 2017; Liu *et al*., 2019; Lan *et al*., 2020). In addition, butyrate in its protected form, when released into the duodenum, decreases the pH of the digesta, stimulating pancreatic and bicarbonate secretion, improving nitrogen retention, and nutrient digestibility coefficients of the diet (Ahsan *et al*., 2016; Kaczmarek et al., 2016).

Aiming at economic savings with the use of sodium butyrate only in the pre-starter and starter phases and considering the importance of the establishment of the microbiota and intestinal development until 21 days of age, this study investigated the performance, carcass and cut yields, digestibility of dietary nutrients, and intestinal histomorphometry of broilers fed diets supplemented with protected sodium butyrate up to 21 days of age.

Material and Methods

Two experiments were conducted. All procedures performed were previously approved by the Ethics Committee on Animal Use (CEUA) at the Federal University of Goiás (UFG) (approval no. CEUA/UFG 044/16).

The first experiment was carried out in an industrial shed with dimensions (W × L) of 12 × 125 m (1.500 m²), where the environment was controlled by a negative pressure system with the use of misters and evaporative cooling pads for air inlets. A total of 784 one-day-old male broiler chicks of the Cobb 500®

commercial line, with an average initial weight of 46 ± 0.2 g were distributed into four treatments in a completely randomized design with seven replicates of 28 birds each. Treatments consisted of a basal diet with the inclusion of a performance-enhancing antibiotic; basal diet without antibiotic or protected sodium butyrate (PSB) (control); basal diet with inclusion of 225 g/t PSB in the pre-starter and starter phases; and basal diet with inclusion of 300 g/t PSB in the pre-starter and starter phases.

The experimental period was 42 days, which were divided into four phases: pre-starter (1 to 7 days), starter (8 to 21 days), grower (22 to 35 days) and finisher (36 to 42 days). The experimental diets were formulated based on maize, soybean meal, and animal-derived meals, in accordance with the recommendations of Rostagno *et al*. (2017). All diets included a variable portion of 0.165% to include the antibiotic or sodium butyrate and/or inert substance (kaolin), according to the treatments.

In the treatment involving the use of antibiotics, 165 g/t Stafac 100® (10% virginiamycin) was used in all rearing phases, which corresponds to 16.5 g/t virginiamycin. For the treatments with PSB (225 and 300 g/t), the commercial product Adimix® Precision (Nutriad, Groupe Adisseo's), containing 30% sodium butyrate, was used as a source, i.e., 750 and 1,000 g/t of the commercial product was added. The antibiotic and butyrate were added to the feed replacing the inert ingredient (Table 1).

Table 1 Ingredients and calculated nutritional composition of the diets used in the pre-starter (1 to 7 days old), starter (8 to 21 days old), grower (22 to 35 days old), and finisher (36 to 42 days old) phases

Ingredient (%)	**Pre-starter**	**Starter**	**Grower**	**Finisher**
Grain maize	54.647	60.005	63.989	70.215
Soybean meal (45.5%)	35.280	30.885	23.902	15.240
Poultry fat	1.134	1.405	1.876	2.078
Meat and bone meal (47%)	3.683	4.415	3.141	6.897
Offal meal (62.5%)	3.010	1.134	3.542	1.807
Feather meal (84.81%)	-	-	1.535	2.008
Calcitic limestone	0.532	0.532	0.632	0.231
Salt	0.391	0.351	0.271	0.211
Sodium bicarbonate	0.080	0.050	0.100	0.151
Choline chloride (75%)	0.050	0.080	0.050	0.060
Dl-methionine (99%)	0.411	0.351	0.271	0.251
L-lysine HCl (64%)	0.331	0.341	0.281	0.452
L-threonine (98%)	0.070	0.080	0.050	0.090
L-valine (96.5%)	0.020	0.010	-	-
[1]Vitamin supplement	0.050	0.050	0.050	0.050
[2]Mineral supplement	0.050	0.050	0.050	0.050
[3]Anticoccidial agent	0.050	0.050	0.050	0.000
[4]Phytase	0.010	0.010	0.010	0.010
[5]Antifungal agent	0.030	0.030	0.030	0.030
[6]Antioxidant	0.004	0.004	0.004	0.004
[7]Variable portion	0.165	0.165	0.165	0.165
Calculated nutritional composition				
Metabolizable energy (kcal/kg)	3,000	3,050	3,150	3,200
Crude protein (%)	25.00	22.50	21.60	19.53
Calcium (%)	0.98	0.98	0.95	0.86
Available phosphorus (%)	0.49	0.48	0.46	0.44
Sodium (%)	0.22	0.21	0.20	0.19
Chlorine (%)	0.30	0.27	0.23	0.20

Potassium (%)	0.90	0.82	0.70	0.57
Digestible methionine + cystine (%)	1.03	0.92	0.85	0.75
Digestible methionine (%)	0.73	0.64	0.56	0.49
Digestible lysine (%)	1.36	1.21	1.10	1.00

[1]Vitamin supplement (composition per kg of product): pre-starter and starter (vitamin A - 20,000,000 IU; vitamin D3 - 5,000,000 IU; vitamin E - 50,000 IU; vitamin K3 - 4,000 mg; vitamin B1 - 5,000 mg; vitamin B2 - 13,000 mg; vitamin B6 - 7,000 mg; vitamin B12 - 36 mg; niacin - 84,000 mg; pantothenate - 30,000 mg; folic acid - 2,400 mg; biotin - 160 mg; selenium - 600 mg); grower and finisher (vitamin A - 16,000,000.00 IU; vitamin D3 - 3,800,000.00 IU; vitamin E - 40,000.00 IU; vitamin K3 - 3,600 mg; vitamin B1 - 3,600 mg; vitamin B2 - 11,000 mg; vitamin B6 - 5,200 mg; vitamin B12 - 30 mg; niacin - 70,000 mg; pantothenate - 26,000 mg; folic acid - 1,800 mg; biotin - 100 mg; selenium - 600 mg). [2]Mineral supplement (composition per kg of product): copper - 16.25 g; iron - 100 g; iodine - 2000 g; manganese - 150 g; zinc - 125 g. [3]Anticoccidial: Maxiban® (narasin + nicarbazin). [4]Phytase: Microtech. [5]Antioxidant: Endox® (ethoxyquin and butylated hydroxyanisole). [6]Antifungal agent: copper sulphate. [7]Variable portion: kaolin and/or performance-enhancing antibiotic, and/or protected sodium butyrate. Antibiotic: Stafac 100® (10% virginiamycin) 165 g/t. Protected sodium butyrate: Adimix® Precision (30% butyric acid) 0.5, 0.75 or 1.00 g/t

All birds were housed in 28 experimental cages measuring 3.24 m², at a housing density of 11 birds/m². The boxes were set up in the central part of the shed and built using PVC pipes and 2-mm plastic mesh screens. Each cage, which housed 28 birds, was equipped with a line of nipple drinkers (10 nipples/cage) and a tube-type chick feeder until the seventh day of age and a tube-type adult poultry feeder from the 8th to the 42nd day of age.

Performance variables (average final weight [AFW], average weight gain [AWG], average feed intake (AFI), feed conversion ratio (FCR) and viability) were measured from 1 to 7, 1 to 21, 1 to 35 and 1 to 42 days of age, whereas the “production factor” was evaluated from 1 to 42 days of age. To this end, the chickens, the feed supplied, and orts were weighed weekly and the number and weight of dead chickens were recorded daily. Average final weight consisted of the average weight of chickens in each plot at the end of each experimental period. Weight gain was calculated as the difference between the final and initial weights of the chickens. Average feed intake was determined as the difference in the weight of the feed supplied and orts in each period, divided by the number of chickens (the number of dead chickens was used as a criterion for correcting intake values). Feed conversion was calculated as the ratio between average feed intake and AWG, which was later corrected for mortality according to Sakomura & Rostagno (2016). Viability was expressed as the percentage of surviving chickens relative to the initial number of housed animals. Finally, the production factor was calculated as an index that considers live weight, viability, age, and feed conversion.

At 42 days of age, two birds that represented the average weight of the plot (± 5%) were selected in each plot, fasted for 8 h, and slaughtered to measure the yields of carcass, breast, drumsticks + thighs, wings, abdominal fat, gizzard, and liver. The yield of the eviscerated carcass without head, neck and feet was calculated relative to the pre-slaughter body weight, as follows:

%CY = (carcass weight*100/live weight),

whereas the yield of the carcass parts, namely, breast, drumsticks + thighs, wings and abdominal fat were calculated as a function of carcass weight:

%PY = (part weight*100/carcass weight).

The second experiment involved 280 one-day-old male broiler chicks of the Cobb 500® commercial line, with an average initial weight of 46 ± 0.2 g. The birds were distributed into four treatments in a completely randomized design with seven replicates and 10 animals per replicate.

The treatments were the same as in Experiment I, as follows: basal diet with inclusion of a performance-enhancing antibiotic; basal diet without antibiotic or PSB (Control); basal diet with inclusion of 225 g/t PSB in the pre-starter and starter phases; and basal diet with inclusion of 300 g/t PSB in the pre-starter and starter phases. In all rearing phases, 165 g/t Stafac 100® (10% virginiamycin) was used, corresponding to 16.5 ppm virginiamycin. The diets used in Experiment II were the same as those formulated for the pre-starter and starter phases in Experiment I (Table 1).

Two digestibility trials were carried out, in two periods: the first from 4 to 7 days of age, and the second from 18 to 21 days of age. The digestibility coefficients of dietary nutrients and energy were determined using the total excreta collection method, as described by Sibbald & Slinger (1963) and adapted by Sakomura & Rostagno (2016). Feed intake, weight gain, and total excreta produced by the birds were measured throughout the experimental period. Excreta were collected twice daily (08h00 and 16h00) to avoid fermentation.

The chicks were housed in galvanised-wire battery cages with dimensions of 0.25 × 0.75 × 0.80 m (H × W × L), with mesh floors and equipped with excreta-collection trays and trough-type drinkers and feeders. The battery cages were located in a brick shed with internal dimensions of 12.96 × 2.96 m (38.36 m^2), covered with clay tiles, with concrete flooring and sides with a short wall, screen, and curtains.

Excreta were packed in properly identified plastic bags and stored in a freezer until the end of the collection period. Afterwards, the samples were thawed, homogenised, and aliquoted. Then, they were pre-dried in an air oven at 55 ºC, for 72 h. Next, the dry matter was obtained using a rectilinear oven at 105 °C and the nitrogen content was determined using in a nitrogen distiller using the Kjeldahl method (INCT-CA N-001/1), as proposed by Detmann *et al*. (2012). The 6.25 factor was used to convert the nitrogen value into crude protein, due to the widespread use of this value by nutrition laboratories. Gross energy was determined using a calorimeter. The nutritional composition of the experimental diets was analysed in terms of dry matter, gross energy, and crude protein contents according to the aforementioned methodologies.

Once the results of the chemical analyses of excreta and feed were obtained, the digestibility coefficients of dry matter and crude protein as well as the nitrogen balance were calculated using equations proposed by Sakomura & Rostagno (2016). Apparent metabolizable energy and nitrogen-corrected apparent metabolizable energy were calculated as proposed by Matterson *et al*. (1965).

At 7 and 21 days of age, one bird that represented the average weight of the plot (± 5%) was selected per replicate, totalling seven birds per treatment. The selected bird was stunned by electronarcosis and later euthanized by cervical dislocation to collect intestinal fragments for a morphometric assessment of the intestinal mucosa. To make the histological slides, 2.0-cm segments of the duodenum (in the distal portion of the duodenal loop) and the jejunum (2.0 cm before the ileal diverticulum) were collected and fixed in a 10% buffered formaldehyde solution for 24 h. After fixation, they were stored in 70% alcohol, processed according to the methodology of Luna (1968), and stained using the Haematoxylin-Eosin method. Semi-serial sections of 5-µm thickness were performed with an electronic rotary microtome.

Images were obtained at 5x magnification, using an optical microscope connected to a computer. The images were analysed using ImageJ software, where 20 villus height and 20 crypt depth measurements were taken in each segment, per replicate. Villus height measurements were taken from the basal region of the villi to their apex and crypt measurements from their base to the villous–crypt transition region (Fukayama *et al*., 2005). The villus/crypt ratio was calculated by dividing villus height by crypt depth.

All data were checked for the presence of outliers (box-and-whisker plot), homogeneity of variances (Bartlett test), and normality of residuals (Cramér–von Mises). Subsequently, they were subjected to analysis of variance and the means were compared using the SNK test (P <0.05), using R statistical software (2019).

Results

In the pre-starter phase (one to seven days of age), the use of PSB in the diet did not influence (P >0.05) broiler performance. However, from 1 to 21 days of age, the inclusion of 225 g/t PSB and the use of the performance-enhancing antibiotic provided higher AFW and AWG (P <0.05), than the control treatment. The chickens fed 225 g/t PSB showed 59-g and 60-g higher AFW and AWG, respectively, than those which received the control treatment (Table 2).

In the evaluation of performance from 1 to 35 days of age (Table 2), the broilers that consumed PSB (225 or 300 g/t) up to 21 days of age had superior AFW and AWG results than the chickens in the control and antibiotic treatment groups (P < 0.05). The diets with 225 and 300 g/t PSB increased AFW by 78 and 56 g and AWG by 80 and 57 g, respectively.

When performance was evaluated for the total period (1 to 42 days of age), the broilers fed the diet supplemented with 225 g/t PSB showed higher AFW and AWG than the control group (P <0.05) but were statistically similar to the group fed 300 g/t PSB and the growth-promoting antibiotic (Table 3). In other words, the supply of 225 g/t PSB up to 21 days of age improved performance until 42 days of age.

Table 2 Average final weight (AFW), average weight gain (AWG), average feed intake (AFI), feed conversion ratio (FCR), and viability of broilers fed diets supplemented or unsupplemented with antibiotic or protected sodium butyrate, from 1 to 7, 1 to 21, and 1 to 35 days of age

Treatment	AFW (kg)	AWG (kg)	AFI (kg)	FCR (kg/kg)	Viability (%)
			1 to 7 days of age		
ANTIBIOTIC	0.188	0.142	0.158	1.110	99.5
CONTROL	0.186	0.140	0.155	1.109	100.0
225 PSB	0.189	0.143	0.161	1.118	99.5
300 PSB	0.186	0.139	0.159	1.146	100.0
P value	0.4461	0.3252	0.8907	0.8873	0.6444
CV %	2.12	2.82	7.89	8.52	1.00
			1 to 21 days of age		
ANTIBIOTIC	0.993[a]	0.947[a]	1.300	1.401	97.5
CONTROL	0.946[b]	0.900[b]	1.281	1.366	98.5
225 PSB	1.005[a]	0.960[a]	1.330	1.371	97.0
300 PSB	0.970[ab]	0.924[ab]	1.280	1.386	99.5
P value	0.0089	0.0091	0.1641	0.4406	0.354
CV %	3.14	3.32	3.36	3.59	2.72
			1 to 35 days of age		
ANTIBIOTIC	2.375[b]	2.328[b]	3.648	1.555	97.0
CONTROL	2.371[b]	2.324[b]	3.658	1.547	95.2
225 PSB	2.449[a]	2.404[a]	3.686	1.528	97.4
300 PSB	2.427[a]	2.381[a]	3.644	1.522	98.0
P value	0.0051	0.0052	0.7988	0.1628	0.3585
CV %	1.74	1.79	2.35	1.90	2.86

ANTIBIOTIC: basal diet with performance-enhancing antibiotic. CONTROL: basal diet without antibiotic or protected sodium butyrate. 225 PSB: basal diet with inclusion of 225 g/t protected sodium butyrate in the pre-starter and starter phases. 300 PSB: basal diet with inclusion of 300 g/t protected sodium butyrate in the pre-starter and starter phases. [a,b]Means with different superscript letters in the column differ from each other using the SNK test at 5% probability. CV: coefficient of variation

Table 3 Average final weight (AFW), average weight gain (AWG), average feed intake (AFI), feed conversion ratio (FCR), viability, and production factor (PF) of broilers fed diets supplemented or unsupplemented with antibiotic or protected sodium butyrate, from 1 to 42 days of age

Treatment	AFW (kg)	AWG (kg)	AFI (kg)	FCR (kg/kg)	Viability (%)	PF (%)
ANTIBIOTIC	3.217[ab]	3.171[ab]	5.164	1.620	96.4	456.3
CONTROL	3.162[b]	3.117[b]	5.219	1.647	94.6	432.6
225 PSB	3.301[a]	3.255[a]	5.401	1.626	94.3	455.6
300 PSB	3.238[ab]	3.192[ab]	5.271	1.628	97.0	459.4
P value	0.0216	0.022	0.1418	0.3608	0.4941	0.0806
CV %	2.07	2.10	3.22	1.68	3.69	4.17

ANTIBIOTIC: basal diet with performance-enhancing antibiotic. CONTROL: basal diet without antibiotic or protected sodium butyrate. 225 PSB: basal diet with inclusion of 225 g/t protected sodium butyrate in the pre-starter and starter phases. 300 PSB: basal diet with inclusion of 300 g/t protected sodium butyrate in the pre-starter and starter phases. [a,b]Means with different superscript letters in the column differ from each other using the SNK test at 5% probability. CV: coefficient of variation

There were no differences (P >0.05) between the treatments for the yields of carcass, breast, drumsticks + thighs, wings, abdominal fat, gizzard, or liver at 42 days of age (Table 4). In the period from 4 to 7 days of age, the use of PSB in the diet did not influence (P >0.05) the dietary metabolizable energy content, the digestibility coefficient of crude protein, or nitrogen balance. However, the inclusion of 300 g/t PSB in the diet induced a higher digestibility coefficient of dry matter than 225 g/t (P = 0.0328). The control and antibiotic treatments did not differ from each other or from the treatments with butyrate addition, for these parameters (Table 5). From 18 to 21 days of age, the digestibility coefficients of dry matter and crude

protein, nitrogen balance, apparent metabolizable energy, and nitrogen-corrected apparent metabolizable energy did not differ (*P* >0.05) between the treatment groups (Table 5).

Table 4 Yields of carcass and cuts of broilers fed diets supplemented or unsupplemented with antibiotic and protected sodium butyrate, at 42 days of age

Treatment	Yield (%)						
	Carcass	Breast	Drumsticks + thighs	Wings	Abdominal fat	Gizzard	Liver
ANTIBIOTIC	71.5	33.5	32.8	11.6	2.2	1.5	1.5
CONTROL	71.8	33.3	32.9	11.7	2.2	1.4	1.8
225 PSB	71.4	32.1	33.3	11.8	2.0	1.4	1.9
300 PSB	72.3	33.4	32.6	11.8	2.1	1.4	1.8
P value	0.3746	0.6479	0.8503	0.9049	0.8781	0.8337	0.2163
CV %	1.40	6.85	4.44	5.00	25.74	8.94	17.47

ANTIBIOTIC: basal diet with performance-enhancing antibiotic. CONTROL: basal diet without antibiotic or protected sodium butyrate. 225 PSB: basal diet with inclusion of 225 g/t protected sodium butyrate in the pre-starter and starter phases. 300 PSB: basal diet with inclusion of 300 g/t protected sodium butyrate in the pre-starter and starter phases. CV: coefficient of variation

Table 5 Digestibility coefficient of dry matter (DCDM), digestibility coefficient of crude protein (DCCP), nitrogen balance (NB), apparent metabolizable energy (AME), and nitrogen-corrected apparent metabolizable energy (AMEn) in broilers fed diets supplemented or unsupplemented with antibiotic and protected sodium butyrate

Treatment	DCDM	DCCP	NB (g/day)	AME	AMEn
	(%)			(kcal/kg DM)	
			4 to 7 days of age		
ANTIBIOTIC	76.5^{ab}	71.5	7.5	3540.1	3324.2
CONTROL	76.9^{ab}	70.0	6.9	3557.8	3347.0
225 PSB	75.4^{b}	68.9	7.1	3514.4	3306.2
300 PSB	77.0^{a}	71.3	7.3	3572.1	3357.2
P value	0.0328	0.2175	0.1914	0.1136	0.116
CV (%)	1.37	3.58	6.85	1.25	1.23
			18 to 21 days of age		
ANTIBIOTIC	70.6	77.1	15.3	3644.1	3453.8
CONTROL	66.3	77.0	14.1	3614.7	3436.0
225 PSB	68.0	75.7	14.5	3590.7	3407.3
300 PSB	66.8	76.4	14.3	3616.8	3436.8
P value	0.0637	0.4874	0.0795	0.2467	0.3083
CV (%)	4.51	2.30	6.15	1.32	1.32

ANTIBIOTIC: basal diet with performance-enhancing antibiotic. CONTROL: basal diet without antibiotic or protected sodium butyrate. 225 PSB: basal diet with inclusion of 225 g/t protected sodium butyrate in the pre-starter and starter phases. 300 PSB: basal diet with inclusion of 300 g/t protected sodium butyrate in the pre-starter and starter phases. ^{a,b}Means with different superscript letters in the column differ from each other by the SNK test at 5% probability. CV: coefficient of variation

At seven days of age, the broilers supplemented with 300 g/t PSB had the highest duodenal villus height (*P* <0.001). Jejunal villus height was greater in the chickens supplemented with butyrate (225 and 300 g/t) and the performance-enhancing antibiotic than in those fed control treatment (*P* <0.001). The broilers supplemented with antibiotic exhibited a higher jejunal crypt depth than those supplemented with 225 g/t PSB (*P* =0.0097). Villus height/jejunal crypt depth ratio was highest in the broilers supplemented with 225 g/t PSBN (*P* <0.001) (Table 6).

At 21 days of age, the broilers fed the diet with 225 g/t PSB showed the greatest duodenal villus height (*P* <0.001). Villus height/crypt depth in the duodenum differed between the groups (*P* = 0.0146), with the lowest result seen in the antibiotic-treated group, which did not differ from the animals on the 300 g/t butyrate treatment, whereas the control, 225 g/t PSB and 300 g/t PSB treatment groups did not differ from

each other. The use of antibiotic or inclusion 225 g/t PSB in the diet provided the greatest jejunal villus height (*P* <0.001) (Table 6).

Table 6 Villus height (VH), crypt depth (CD), and villus height/crypt depth (VH/CD) ratio in the duodenum and jejunum of broiler chickens fed diets supplemented or unsupplemented with antibiotic or protected sodium butyrate, at 7 and 21 days of age

Treatment	Duodenum			Jejunum		
	VH (µm)	CD (µm)	VH/CD	VH (µm)	CD (µm)	VH/CD
			7 days of age			
ANTIBIOTIC	1086.4^{b}	76.5	13.1	727.2^{a}	82.5^{a}	9.4^{b}
CONTROL	1088.2^{b}	71.3	15.4	620.8^{b}	73.6^{ab}	8.7^{b}
225 PSB	1163.3^{b}	77.5	15.4	778.1^{a}	64.0^{b}	12.6^{a}
300 PSB	1364.1^{a}	87.4	15.9	753.9^{a}	78.2^{ab}	10.2^{b}
P value	<0.001	0.0809	0.2407	<0.001	0.0097	<0.001
CV (%)	9.00	19.61	20.09	12.02	23.56	25.97
			21 days of age			
ANTIBIOTIC	1725.9^{bc}	92.0	19.3^{b}	1206.6^{a}	85.3	13.6
CONTROL	1765.5^{b}	81.3	22.7^{a}	1091.5^{b}	69.5	15.8
225 PSB	1869.5^{a}	83.5	22.9^{a}	1192.7^{a}	87.4	14.6
300 PSB	1640.0^{c}	84.9	20.1^{ab}	1125.2^{b}	87.8	13.7
P value	<0.001	0.2149	0.0146	<0.001	0.0722	0.2848
CV (%)	8.40	21.13	19.94	7.77	28.93	25.86

ANTIBIOTIC: basal diet with performance-enhancing antibiotic. CONTROL: basal diet without antibiotic or protected sodium butyrate. 225 PSB: basal diet with inclusion of 225 g/t protected sodium butyrate in the pre-starter and starter phases. 300 PSB: basal diet with inclusion of 300 g/t protected sodium butyrate in the pre-starter and starter phases. ^{a,b}Means with different superscript letters in the column differ from each other by the SNK test at 5% probability. CV: coefficient of variation

Discussion

The inclusion of PSB as an additive in the broiler diet until 21 days of age proved to be able to improve final weight and average weight gain from 21 days of age to slaughter age. This result was likely due to the positive effect of butyrate on the development and maintenance of the intestinal epithelium in the early stages of life.

According to Obst & Diamond (1992) the development and maturation of the gastrointestinal tract in the starter phase can substantially affect production performance, since it is correlated with the growth rate of chickens. These processes are also important for the development of other tissues and organs, because it is in this phase that the broilers exhibit rapid development, which is marked by important physiological changes such as intestinal development; development of the thermoregulatory system; beginning of the development of immunocompetence; as well as development of muscle, bone system and fat (Abreu, 2021). The villi are well-developed within 14 days of hatching, whereas the intestine completes its development in the first 20 to 30 days of age (Ito *et al*., 2004)

Bortoluzzi *et al*. (2017) reported similar performance results in broilers in the starter phase. The authors found no difference in feed conversion using 700 g/t sodium butyrate, but observed greater weight gain. Ogwuegbu *et al*. (2021), when evaluating the inclusion of 2 and 4g/kg of partially-protected sodium butyrate in the ration of broiler chickens, also verified an increase in weight gain in relation to chickens in the control group, in the finisher phase of rearing. The authors attributed this result to the increase in nutrient digestibility in chickens that received feed containing sodium butyrate. In contrast, Lan *et al*. (2020) found no differences in live weight between broilers fed a diet containing a commercial PSB product (54% butyrate) at the product levels of 300 and 600 g/t (162 and 324 g of sodium butyrate/t), and the control group.

The present results show that the PSB level of 225 g/t had a positive effect in the starter phase; however, the 300 g/t level did not differ from the control or antibiotic treatments, which shows that the dose was not adequate for this phase. Similar results were observed by other researchers that used higher doses of sodium butyrate. González-Ortiz *et al*. (2019) used 1 kg/t of a PSB product (30% butyrate), i.e., 300 g of sodium butyrate/t, and observed a reduction in feed intake and weight gain in broilers in comparison to the control group. Similarly, Lan *et al*. (2020) evaluated PSB levels and found that the highest level (648 g/t)

provided the lowest final weight at 21 days of age. It is suggested that the use of higher rates of butyrate may impair nutrient absorption due to a negative effect of butyrate on the intestinal epithelium. In a study examining the effect of butyrate at different concentrations in an *in vitro* assay with Caco-2 cells (human colon epithelial cell line), Peng *et al*. (2007) stated that high concentrations of butyrate have a detrimental effect on the intestinal barrier function, which is related to apoptosis of intestinal epithelial cells by mechanisms not yet fully understood.

In terms of production performance, from 1 to 35 days of age, the use of PSB in the diet (supplied until 21 days of age), at both tested levels (225 and 300 g/t), improved AFW and AWG when compared with the control treatment and the use of antibiotic. Similarly, at 42 days of age, the treatment with 225 g/t PSB provided higher AFW and AWG; however, the treatment with 300 g/t did not differ from the control or antibiotic treatments. Therefore, these results suggest that the PSB level of 225 g/t in the pre-starter (1 to 7 days of age) and starter (8 to 21 days of age) diets was conducive to improved weight gains until 42 days of age.

In the evaluation of carcass and cut yields, there were no effects of PSB at the different tested levels. Similarly, Zhang *et al*. (2011) used 400 g/t of PSB and found no differences between the treatment with butyrate and the control treatment. The present findings differ from the results described by Panda *et al*. (2009), who evaluated unprotected butyrate supplementation (200, 400 and 600 g/t) and obtained reduced abdominal fat and increased carcass weight.

The greater duodenal and jejunal villus height seen at 7 and 21 days of age can be explained by the fact that, once ingested, butyrate is converted to butyric acid due to the acidic pH. Butyric acid, in turn, is readily absorbed by enterocytes and used in cellular metabolism as a source of energy, contributing to the growth of villi and, consequently, increasing the area of nutrient absorption by enterocytes (Chamba *et al*., 2014). Butyrate is able to supply energy to intestinal cells after being transported into the cell, and, in the mitochondria, it is metabolised to Acetyl-CoA, which enters the citric acid cycle, producing ATP and CO_2 (Donohoe *et al*., 2012). According to Kawamata *et al*. (2007), butyrate ions, in dissociated form, can also be absorbed as an energy source, but are transported by diffusion, by exchange with the bicarbonate ion (HCO^{3-}), or by active transport using membrane transporters (MCT1 and SMCT1).

The improvement in intestinal development can also be explained by the ability of butyrate to reduce the pathogenic microbiota in the intestine, thereby reducing competition with the host for nutrients, epithelial cell desquamation and epithelial turnover, and, consequently, energy and nutrient expenditure for repair (Dibner & Buttin, 2002; Moquet *et al*., 2016). By reducing the pH of the proventriculus, the gizzard and the upper part of the intestine, butyrate has a bacteriostatic effect, as it favours the growth of lactic acid-producing bacteria, such as *Lactobacilli* and *Bifidobacteria* spp., which need an acidic medium to grow (Rolfe, 2000). Lactic acid-producing bacteria compete for space and nutrients with pathogenic bacteria within the intestine, thus reducing the population of pathogenic bacteria. In addition, lactic acid-producing bacteria produce bacteriocins, organic acids and bactericidal substances, maintaining a healthy environment. After sodium butyrate is converted to butyric acid, it is able to enter the bacterial cell wall by diffusion due to its lipid solubility, in a bactericidal effect. Inside the cell, the acid dissociates, lowering the internal pH, which causes toxicity within the bacterial cell. As a consequence, the purine bases are affected, which leads to denaturation of essential enzymes within the cell and bacterial death (Ahsan *et al*., 2016). Sodium butyrate has been shown to play an important role as an energy source for gastrointestinal epithelial cells and to have antimicrobial, anti-inflammatory and antioxidant properties (Ahsan *et al*., 2016; Bortoluzzi *et al*., 2017).

The results found in the histomorphometric evaluation corroborate those reported by Sikandar *et al*. (2017), where the use of PSB (500 and 1000 g/t) increased the length of duodenal and jejunal villi when compared with control treatment and the treatment including performance-enhancing antibiotics. In contrast with our study, Liu *et al*. (2019) found no effect of sodium butyrate on the intestinal histomorphometry of broiler chickens up to 21 days of age. Pascual *et al*. (2020) also observed no effects of using 500 g/t of a PSB product (30% butyrate) on intestinal histomorphometry at 45 days of age.

Although PSB provided an increase in intestinal villus height, butyrate did not improve nutrient digestibility or increase metabolizable energy. This may be due to the lack of challenges in experiment II (use of cages, clean environment, excreta removed frequently), since the positive effects of butyrate can be attributed to a lower pro-inflammatory response when birds experience nutritional, environmental, and immunological challenges (Moquet *et al*., 2016).

Disagreeing with the present results, Kaczmarek *et al*. (2016) observed that the use of protected calcium butyrate (300 g/t) increased the ileal digestibility of crude protein and total fat digestibility at 14 days

of age, as well as the apparent ileal digestibility of threonine, serine, proline and histidine, and nitrogen-corrected apparent metabolizable energy at 35 days of age. According to the authors, this result may be related to the capacity of butyrate salt to stimulate increased secretion of pancreatic fluid, which, in turn, can improve the digestibility of nutrients and AMEn. Riboty *et al*. (2016) reported that the use of partially protected sodium butyrate (700 g/t) increased the digestibility coefficients of fat, dry matter and crude protein, apparent metabolizable energy, and nitrogen-corrected apparent metabolizable energy. Liu *et al*. (2017) found higher ileal digestible energy and energy digestibility coefficients with the use of PSB (500 and 1000 g/t), at 42 days of age, in comparison to the control treatment. Pires *et al*. (2020) verified that the apparent metabolizable energy and the apparent metabolizable energy corrected for nitrogen increased linearly with increasing protected sodium butyrate levels (0, 105, 210, and 300 g/kg) in the diet of commercial laying hens. According to the authors, the improvement observed may be related to the action of proteginous sodium butyrate in the intestine, stimulating the secretion of pancreatic enzymes, as well as the increase in the height of the duodenum and jejunum villi, favouring the increase in metabolizable energy (AME and AMEn).

The literature may feature discrepant data on the effect of butyrate on performance, nutrient digestibility, and intestinal histomorphometry due to variations between studies, e.g., in terms of animal health status, diet composition, environmental conditions, use of free or protected butyrate, effects of the levels used, and also the matrix used in the coating (Moquet *et al*., 2016).

The advantage obtained from the use of butyrate in the pre-starter and starter phases is the reduction in expenditure on additives, as it has proven to be efficient when used up to the starter phase and to influence performance until slaughter age.

Conclusion

The 225 g/t level of protected sodium butyrate can be used in diets for broilers in the starter rearing phase, since it increases weight gain until slaughter age (42 days) and improves intestinal development at seven and 21 days old.

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Authors' Contributions

MFP, NSML, and DVJ conceived and designed the experiments. MFP, RANM, SDA, and JMSM performed the experiments. MFP analysed the data. NSML, MBC, and FBC contributed reagents, materials, and analytical tools. MFP and JMSM wrote the paper. JMSM edited the manuscript.

Conflicts of Interest Declaration

The authors declare they have no conflicts of interest regarding the work presented in this report.

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