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Effects of protected sodium butyrate and reduced energy content in diets for broiler chickens

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Abstract

Effects of protected sodium butyrate (PSB) in broiler diets with reduced metabolizable energy (ME) content on production performance, intestinal histomorphometry and nutrient metabolizability were evaluated in two experiments. In one experiment, 384 one-day-old male chicks with an average weight of 44 ± 0.2 g were distributed into four treatment groups in a completely randomized design with six replications of 16 birds. The treatments consisted of i) a basal diet without reduced ME or PSB (BD); ii) BD with a 100 kcal/kg reduction in ME, without PSB; iii) BD with a 100 kcal/kg reduction in ME + 105 g/t PSB; and iv) BD with a 100 kcal/kg reduction in ME + 225 g/t PSB. Performance and carcass yield were evaluated. In the second experiment, 280 one-day-old male chicks were also distributed into four treatment groups in a completely randomized design with seven replications of 10 birds to evaluate metabolizability coefficients and intestinal development. The birds that received diet 3 had the best feed conversion ratio (FCR), final weight (FW) and weight gain (WG). Inclusion of PSB (105 and 225 g/t) in reduced-ME diets increased the metabolizability of crude protein coefficient at 35 days old. Birds that received diet 3 also had greater jejunal villus height at 21 and 35 days old. It is recommended to feed 105 g/t of PSB with a 100 kcal/kg reduction in ME until broilers were 35 days old.

Keywords: intestinal histomorphometry, nutrition, organic acids, performance
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Introduction

Poultry farming is facing the challenge of reducing broiler production costs while delivering a quality product. Nutrition accounts for about 70% of the total costs of poultry production, with the ME content constituting the largest portion of the feed. Thus, higher percentages of energy ingredients are included in the formulation (Ahiwe *et al*., 2018, Ayasan et al., 2021). In view of the high proportion that feed costs represent in total production cost, increasing nutrient utilization is essential to ensure higher performance rates as and greater profitability in the production of broiler chickens. Therefore, the interest of nutritionists in the use of feed additives in broiler farming has increased in recent years, especially additives that could enhance performance and intestinal health while reducing costs.

Butyrate, a four-carbon-chain salt derived from butyric acid, is an acidifying additive that can be used in animal feed without restrictions and without the risk of contaminating the final product. The supply of synthetic butyrate sources as additives in poultry feed has been widely researched in broiler farming (Sunkara *et al*., 2011; Qaisrani *et al*., 2015; Bortoluzzi *et al*., 2017; Sikandar *et al*., 2017; Wu *et al*., 2018). When administered orally, sodium butyrate can be used in its free form or microencapsulated. Free sodium butyrate is absorbed rapidly and metabolized by mucosal cells in the initial part of the gastrointestinal tract, reducing significantly the amount of butyrate that reaches the intestine (Moquet, 2018; Layter, 2019).

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Microencapsulation in a lipid matrix, however, prevents the rapid absorption of butyrate in the upper part of the gastrointestinal tract, increasing its action at the intestinal level (Moquet *et al*., 2016).

Butyrate is recognized mainly for its trophic action in the intestine, where it acts as a readily available source of energy for enterocytes, favouring cell multiplication and differentiation (Lan et al., 2020). As a result, it can increase the size of intestinal villi and thus broaden the intestinal nutrient absorption area, improving the metabolization of dietary nutrients (Chamba *et al*., 2014). In addition to its trophic effect, butyrate is known to have bactericidal and bacteriostatic action on pathogenic bacteria in the intestine (Van Immerseel *et al*., 2004). When microbial activity is reduced, so is competition with the host for nutrients and the degradation of the intestinal mucosa, which culminates in reduced energy expenditure for cell repair (Ahsan *et al*., 2016). The decrease in gastric pH caused by feeding butyrate increases the retention of feed in the digestive system and activates pepsinogen, thus improving the digestion of proteins. When released in the duodenum, pepsinogen lowers the pH of the digesta, stimulating pancreatic secretion of bicarbinate and consequently improving nitrogen retention and nutrient metabolizability (Bellaver & Scheurman, 2004; Ahsan *et a*l., 2016). Thus, inclusion of butyrate in rations can improve the metabolizability of nutrients in the diet, increase ME and thus might allow for a strategic reduction in dietary energy, provided it does not affect performance, carcass yield and production costs. Therefore, this study examined the effects of PSB in broiler diets with reduced ME content on production performance, intestinal histomorphometry and metabolizability of dietary nutrients.

Material and Methods

Two experiments were developed at the Animal Science Department at the School of Veterinary and Animal Science (EVZ) of the Federal University of Goiás (UFG), located in Goiânia, Brazil. The research project was approved by the Ethics Committee on Animal Use (CEUA) at UFG (approval no. 044/16).

The first experiment was carried out at the School Aviary at EVZ/UFG (16°35'48.3" S and 49°17'08.8" W) in an industrial shed with production of 21 000 broilers, with 1500 m² (12 x 125 m), side walls of masonry 0.40 m, wire mesh 2.80 m high, ceiling height of 3.20 m, and east-west orientation. The experimental period was 42 days, which were divided into two phases, namely starter (1 to 21 days) and grower/finisher (22 to 42 days). The diets were based on maize and soybean meal, with vitamin and mineral supplements, and were formulated according to Rostagno *et al*. (2017). A total of 384 one-day-old male Cobb 500® chicks with an average weight of 44 ± 0.2 g were used. The birds were distributed into four treatment groups in a completely randomized design with six replications of 16 birds each. The dietary treatments consisted of i) a basal diet (BD) without ME reduction and no butyrate; ii) BD with a 100 kcal/kg reduction in ME; iii) BD with a 100 kcal/kg reduction in ME and 105 g/t protected sodium butyrate; and iv) BD with a 100 kcal/kg reduction in ME and 225 g/t protected sodium butyrate. The PSB used in the treatments was included in a commercial feed additive that contained 30% butyrate. The additive was included to replace the inert (kaolin) in the diets in which ME was reduced by 100 kcal/kg. The birds were housed in 28 experimental 1.80 × 1.60 m boxes made of plastic mesh and PVC pipe inside a shed to mimic conditions under which broilers are raised in the industry. The boxes were located inside an industrial brick shed with clay tiles, concrete floors and screened walls. In addition, the facility was equipped with a negative ventilation system, a diesel heater, a pad cooling system and misters. The boxes had nipple drinkers, poultry feeders and rice husk litter. Water and feed were available ad libitum throughout the experimental period. Internal heating in the shed was monitored by checking the air temperature and relative humidity. Constant lighting was provided by fluorescent lamps. Table 1 shows the composition of the basal diet and Table 2 shows its nutritional content.

Table 1 Composition of broiler diets formulated for starter and grower/finisher phases

Ingredients (%)	Starter diet (1-21 days)		Grower/finisher diet (22-42 days)	
	No ME reduction	Reduced ME	No ME reduction	Reduced ME
Grain maize	59.60	61.95	59.91	62.20
Soybean meal, 45%	34.65	34.22	31.81	31.43
Dicalcium phosphate	1.43	1.43	1.21	1.21
Limestone	0.96	0.98	0.90	0.91
Common salt	0.48	0.48	0.45	0.46
Dl-methionine	0.29	0.28	0.26	0.26
L-lysine HCL	0.22	0.23	0.18	0.19
Soybean oil	2.01	0.07	4.94	3.00
L-threonine	0.06	0.06	0.04	0.04
Choline chloride	0.05	0.05	0.05	0.05
Anticoccidial[1]	0.05	0.05	0.05	0.05
Mineral supplement[2]	0.05	0.05	0.05	0.05
Vitamin supplement[3]	0.05	0.05	0.05	0.05
Inert[4]	0.1	0.1	0.1	0.1

[1]Anticoccidial: Maxiban™ (narasin + nicarbazin) used only until 35 days old
[2]Mineral content per kg; copper: 16.25 g; iron: 100 g; iodine: 2.000 g; manganese: 150 g; zinc 125 g
[3]Vitamin content per kg of 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
Vitamin content per kg of grower and finisher; vitamin A: 16.000.000 IU, vitamin D3: 3.800.000 IU, vitamin E: 40.000 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
[4]The additive the inert (kaolin) in the diets in which ME was reduced by 100 kcal/kg
ME: metabolizable energy

Table 2 Calculated nutritional composition of broiler diets formulated for starter and grower/finisher phases

Calculated nutritional composition	Starter diet (1–21 days)		Grower/finisher diet (22–42 days)	
	No ME reduction	Reduced ME	No ME reduction	Reduced ME
Metabolizable energy, kcal/kg (as fed)	3.000	2.900	3.200	3100
Crude protein, %	20.8	20.8	19.5	19.5
Calcium, %	0.819	0.819	0.732	0.732
Choline, mg/kg	330	330	300	300
Available phosphorus, %	0.391	0.391	0.342	0.342
Digestible lysine, %	1.174	1.174	1.078	1.078
Digestible methionine + cystine, %	0.846	0.846	0.787	0.787
Digestible methionine, %	0.563	0.563	0.52	0.52
Sodium, %	0.21	0.21	0.2	0.2
Digestible threonine, %	0.763	0.763	0.7	0.7
Digestible tryptophan, %	0.231	0.229	0.214	0.214

ME: metabolizable energy

Performance was evaluated on the 7th, 21st, 35th and 42nd days old, by measuring feed intake (g), average weight (g) and weight gain (g), and calculating FCR (kg/kg). Feed intake (g) was calculated per

experimental unit as the weight difference between the feed provided and orts. Average weight was calculated as the total weight of the chickens divided by the number of birds in the plot. Weight gain was determined as the difference between the average initial and final weights of the birds in each period. Feed conversion ratio was the result of weight gain divided by feed intake. The performance variables were calculated considering the mortality rate, which was recorded daily. Viability was determined by subtracting the observed mortality, in percentage values, from 100%.

To determine the yields of carcass, breast, drumsticks, wings and abdominal fat, two birds representing the average weight of the plot were selected per replicate on the 42nd day of life. These were fasted for eight hours, stunned by electric shock, and slaughtered. Subsequently, they were weighed again and eviscerated. Then the major cuts (breast, drumsticks and wings) were extracted and the abdominal fat that was present in the entire cavity and that adhered to the bursa was collected and weighed individually on a precision scale. Carcass yield was calculated relative to the live weight before slaughter and expressed in percentage terms. The yields of the carcass parts and abdominal fat were determined relative to the weight of the carcass including head and legs.

The second experiment was carried out at the Experimental Aviary at the Poultry Section of EVZ/UFG (16°35'33.0" S, 49°16'51.4" W) to evaluate the addition of PSB to broiler diets with reduced ME content and its effects on the metabolizability coefficients and intestinal development of the birds. In total, 280 one-day-old male Cobb 500® chicks with an average weight of 44 ± 0.2 g were used. The birds were distributed into four treatments in a completely randomized design with seven replications of four animals each.

Treatments were the same as those tested in the first experiment. Two metabolism trials were conducted: one in the starter phase, from 18 to 21 days, and another in the grower phase, from 32 to 35 days old. The total excreta collection method was used in both trials (Sakomura & Rostagno, 2016).

In the second experiment, the birds were housed in cages – in Experiment 1 the birds were housed in boxes – to collect excreta to evaluate the metabolizability of nutrients in the diets. All birds were housed in batteries with 28 experimental cages made of galvanized steel, with dimensions of 0.80 × 0.75 m. The cages were equipped with drinkers, trough feeders and an excreta collection tray lined with plastic sheeting. The cages were located inside an experimental masonry shed, and ventilation was controlled through curtain management. Water and feed were available ad libitum throughout the experimental period. Internal heating in the shed was monitored by checking the air temperature and relative humidity, and constant lighting was provided by incandescent lamps in each experimental unit.

Excreta were collected twice daily, packed in labelled plastic bags, and frozen. For chemical analyses, the samples were pre-dried in a rectilinear forced-air oven at 55 ± 5 ºC and then ground in a Wiley mill. The analyses were performed according to Silva & Queiroz (2002). Nutritional balances were calculated as proposed by Matterson *et al*. (1965) and metabolizability coefficients as proposed by Batal & Parsons (2002) and Noy & Sklan (2002). The metabolizability coefficients were calculated as the percentage ratio between the retained (excretion minus intake) and ingested amounts of each nutritional component or energy, following Sakomura & Rostagno (2016).

For the histomorphometric analyses of the intestinal mucosa, one bird per experimental unit was euthanized by cervical dislocation at 21 and 35 days old to collect intestinal fragments. Samples 5 cm in length were taken from the small intestine segments (duodenum and jejunum). To prepare the histological slides, these segments were fixed in a 10% buffered formaldehyde solution for 24 hours. After fixation, they were stored in 70% alcohol, processed according to Luna (1968) and haematoxylin-eosin (HE) stained. The images were acquired under 5 × magnification, using a Leica DM 4000B optical microscope coupled to a microcomputer. The images were analysed using Image J software, where villus height, crypt depth and villus to crypt ratio were measured. Seventy villus readings and 70 crypt readings were taken per treatment, totalling 560 readings for each age.

The data were checked for outliers using box-and-whisker plots, homogeneity of variances using Bartlett's test, and normality of the residuals with Cramér-von Mises test. The data were then subjected to analysis of variance and the means were compared with the Student-Newman-Keuls test (P <0.05), using R statistical software.

Results and Discussion

In the evaluation of performance (Table 3), from one to seven days old, there were no differences between treatments for any of the variables (P >0.05). Through 21 days old, the chickens fed Diet 3 had a better FCR (P = 0.0357) than those fed Diet 2, which also produced less efficient growth than Diet 1 (BD). Thus, inclusion of 105 g/t of protected butyrate in Diet 3 compensated for the 100 kcal/kg reduction in dietary energy during this period. However, Diet 4 did not produce any difference in FCR when compared with the other diets. In addition, FW, WG and FI showed no statistical differences among treatments when the birds were from one to 21 days old (P >0.05). From 1 to 35 days old (Table 2), the chickens fed Diet 3 did not

differ from the birds fed the other diets in FW or WG (P <0.05). In addition, the broilers fed Diet 1 were most efficient during this period and no differences were observed in FI. In the evaluation of performance from one to 42 days old, Diet 1 produced the best results for FW, WG, FCR. Thus, the addition of sodium butyrate to the diet did not compensate for the reduced energy intake over the entire rearing period. Viability was 100% throughout the experiment.

Table 3 Performance of chickens fed diets with or without reduction in metabolizable energy content and including two levels of protected sodium butyrate, in the various rearing periods

Time period Treatment	Final weight, kg	Weight gain, kg	Feed intake, kg	Feed conversion ratio, kg/kg
Days 1 to 7				
No ME reduction	0.183	0.139	0.166	1.197
Reduced ME	0.175	0.132	0.173	1.315
Reduced ME + 105 butyrate	0.178	0.134	0.172	1.290
Reduced ME + 225 butyrate	0.180	0.137	0.177	1.296
P-value	0.474	0.550	0.707	0.339
CV, %	4.56	6.27	9.22	9.19
Days 1 to 21				
No ME reduction	0.924	0.879	1.274	1.449[b]
Reduced ME	0.879	0.853	1.266	1.517[a]
Reduced ME + 105 butyrate	0.903	0.849	1.250	1.456[b]
Reduced ME + 225 butyrate	0.902	0.865	1.278	1.488[ab]
P-value	0.599	0.606	0.951	0.036
CV, %	5.85	6.18	6.86	2.61
Days 1 to 35				
No ME reduction	2.422[a]	2.378[a]	3.635	1.528[b]
Reduced ME	2.305[b]	2.261[b]	3.563	1.576[a]
Reduced ME + 105 butyrate	2.348[ab]	2.304[ab]	3.596	1.561[a]
Reduced ME + 225 butyrate	2.320[b]	2.276[b]	3.588	1.576[a]
P-value	0.035	0.036	0.813	0.010
CV, %	2.90	2.98	3.58	1.59
Days 1 to 42				
No ME reduction	3.248[a]	3.204[a]	5.106	1.594[b]
Reduced ME	3.069[b]	3.026[b]	4.978	1.646[a]
Reduced ME + 105 butyrate	3.073[b]	3.029[b]	5.032	1.661[a]
Reduced ME + 225 butyrate	3.081[b]	3.037[b]	4.985	1.641[a]
P-value	0.002	0.002	0.435	0.002
CV, %	2.51	2.55	2.92	1.64

[a,b] Within a column a period, means with a common superscript did not differ by the Student-Newman-Keuls test at 5% probability
ME: metabolizable energy

No differences (P >0.05) between the treatments were observed for any of the carcass traits (Table 4).

Table 4 Yields of carcass and cuts of chickens fed diets with or without a reduction in ME content and including two levels of protected sodium butyrate, at 42 days old

Traits	Treatments				*P*-value	CV, %
	Basal diet	Reduced ME	Reduced ME + 105 butyrate	Reduced ME + 225 butyrate		
Carcass, %	70.5	70.7	67.4	69.4	0.54	6.21
Breast, %	29.3	28.2	27.6	28.7	0.55	7.52
Thigh & drum, %	29.3	29.2	29.2	29.1	0.99	5.53
Wing, %	10.1	10.8	10.9	10.7	0.58	10.36
Abdominal fat, %	1.7	1.5	1.6	1.5	0.49	18.44
Gizzard, %	2.3	2.3	2.3	2.5	0.67	11.73
Liver, %	2.7	2.4	2.9	2.7	0.20	14.98
Heart, %	0.6	0.6	0.6	0.6	0.61	17.15

The metabolism trial conducted from 18 to 21 days old revealed a lack of differences (P >0.05) between groups for dry matter metabolizability coefficient (DMMC) and crude protein metabolizability coefficient (CPMC) and nitrogen balance (NB) (Table 5). The basal diet provided the highest (P <0.05) apparent metabolizable energy (AME) and nitrogen-corrected AME (AMEn) and up to 21 days old, the addition of butyrate at the tested levels did not improve AME or AMEn.

Table 5 Results from metabolism trials for chickens fed diets with or without a reduction in ME content and including two levels of protected sodium butyrate, from 18 to 21 and 32 to 35 days old

Time period Treatment	DMMC, %	CPMC, %	NB, g/day	AME, kcal/kg DM	AMEn, kcal/kg DM
18 to 21 days					
Basal diet	77.0	68.7	14.1	3614.7[a]	3436.0[a]
Reduced ME	76.6	71.4	15.1	3424.7[b]	3231.9[b]
Reduced ME + 105 butyrate	75.4	69.5	14.9	3402.4[b]	3214.3[b]
Reduced ME + 225 butyrate	77.0	69.7	14.7	3444.7[b]	3258.3[b]
P-value	0.406	0.306	0.261	<0.001	<0.001
CV, %	2.53	3.77	6.98	1.58	1.70
32 to 35 days					
Basal diet	74.4	62.9[a]	16.1	3655.1[a]	3481.0[a]
Reduced ME	73.8	56.8[b]	14.4	3404.6[b]	3248.2[b]
Reduced ME + 105 butyrate	75.2	62.4[a]	16.0	3475.6[b]	3303.7[b]
Reduced ME + 225 butyrate	74.8	63.4[a]	16.8	3434.4[b]	3259.3[b]
P-value	0.536	0.022	0.199	<0.001	<0.001
CV, %	2.55	6.78	13.58	2.11	1.98

[a,b] Within a column and period, means with a common superscript did not differ by the Student-Newman-Keuls test at 5% probability

ME: metabolizable energy; DMMC: dry matter metabolizability coefficient, CPMC: crude protein metabolizability coefficient, NB: nitrogen balance, AME: apparent metabolizable energy, AMEn: nitrogen-corrected apparent metabolizable energy

From 32 to 35 days old, there were no significant differences among the treatments for DMMC or NB (P >0.05) (Table 5). However, CPMC differed (P = 0.0121), with Diet 2 producing a lower value than the other three diets, which were similar to each other. Thus, it seems the addition of butyrate compensated for the reduced ME level of CPMC in Diet 2 In this age interval, the basal diet produced the highest AME and AMEn values (P <0.05).

At 21 days old, the histomorphometric evaluation of the duodenum showed no significant differences among the treatments for villus height and villus to crypt ratio (P >0.05). However, BD and Diet 4 produced

shallower crypts that Dets 2 and 3 (Table 6). In the jejunum, villus height was greater in the birds fed Diet 3 than those fed the other diets (P = 0.0022). As for crypt depth in the jejunum, dietary supplementation with butyrate produced intermediate values between the high value produced by Diet 2 and the low value produced by BD (P <0.001). Diets 1, 3 and 4 produced higher villus to crypt ratios in the jejunum than Diet 2.

When the broilers were 35 days old, no differences were found between the groups for villus height or villus to crypt ratio in the duodenum (P >0.05). However, Diet 2 produced the greatest duodenal crypt depth (P = 0.0344). In the jejunum, diet 3 resulted in the greatest villus height among the treatments (P <0.001). Crypt depth was similar (P >0.05) for all treatments. However, the villus to crypt ratio in the jejunum was higher (P = 0.022) in the animals fed Diet 3 than those fed Diet 4.

Table 6 Histomorphometric analysis of the duodenum and jejunum of chickens fed diets with or without a reduction in metabolizable energy content and including two levels of protected sodium butyrate, at 21 and 35 days old

Time / Treatment	DUODENUM			JEJUNUM		
	VH, µm	CD, µm	V:C	VH, µm	CD, µm	V:C
21 days						
No ME reduction	1789.2	81.1^{b}	23.8	1116.9^{b}	69.3^{c}	16.3^{a}
Reduced ME	1767.1	97.5^{a}	19.1	1069.0^{b}	104.7^{a}	11.0^{b}
Reduced ME + 105 butyrate	1734.3	95.1^{a}	18.6	1203.3^{a}	81.6^{b}	14.6^{a}
Reduced ME + 225 butyrate	1627.7	77.2^{b}	21.3	1108.2^{b}	83.2^{b}	13.7^{a}
P-value	0.169	0.006	0.107	0.002	<0.001	<0.001
CV (%)	10.67	24.27	30.44	9.76	23.02	26.75
35 days						
No ME reduction	1768.2	86.0^{b}	22.9	1102.2^{b}	86.7	13.4^{ab}
Reduced ME	1731.6	115.8^{a}	15.1	1008.8^{b}	78.0	14.1^{ab}
Reduced ME + 105 butyrate	1780.7	88.5^{b}	22.6	1235.1^{a}	76.2	17.5^{a}
Reduced ME + 225 butyrate	1741.6	83.7^{b}	21.6	1102.2^{b}	90.6	13.0^{b}
P-value	0.910	0.034	0.112	<0.001	0.133	0.022
CV (%)	11.30	27.42	34.14	10.78	25.85	32.80

^{a,b,c} Within a column and period, means with a common superscript did not differ by the SNK test at 5% probability
ME: metbolizable energy; VH: villus height, CD: crypt depth, V:C: ratio of villus height to crypt depth

The inclusion of PSB in poultry feed is related to possible improvements in intestinal development and in the absorption and digestion of dietary nutrients, and increased availability of ME, which results in better broiler performance. Pires *et al.* (2020) stated that the inclusion of butyrate in poultry diets could improve intestinal development and increase the metabolizability coefficients of dietary nutrients and ME, thereby increasing production rates. In this respect, decreasing the energy content of the diet could be considered strategic, as it allows a reduction of feed costs while maintaining performance, carcass characteristics and intestinal development.

In the period from one to seven days old, performance was not influenced by the inclusion of PSB. Likewise, Chamba *et al*. (2014) reported that the use of PSB in chicken diets (700 g/t of feed) did not influence performance in the starter phase (up to 14 days).

In the present study, reducing the ME level of the diet by 100 kcal/kg did not worsen performance in the starter phase. Abudabos *et al*. (2014) did not observe an effect of reducing the dietary ME (by up to 75 kcal/kg of feed) on the performance of chickens in the starter phase. Oliveira (2018) reported the same lack of effects after reducing ME in the starter diet by 90 kcal/kg.

According to Ravindran *et al*. (2016), young birds have limited capacity to utilize energy because of their low bile and lipase production and limited nutrient-absorption capacity, as their intestine is still developing (Wang *et al*., 2017). Because PSB is coated with a lipid matrix, the data suggested that sodium butyrate might not have been completely released in the birds' intestine owing to the low lipase production of chicks in the first week of life.

From one to 21 days old, feed conversion was similar between Diets 3 and 4, and the BD This observation agreed with Bortoluzzi *et al*. (2017), who found that despite the reduction of the energy and CP

levels of chicken diets in the phase of 1 to 28 days old, supplementation with sodium butyrate (1,000 g/t of the commercial product) neutralized the effect of nutritional reduction. The authors reported that the birds that received butyrate-supplemented diets exhibited similar performances to the control group, whose nutritional levels were not reduced.

The observed improvement in feed conversion following the use of Diet 3 compared with Diet 2 could be attributed to a number of biological effects of butyrate, which resulted in optimized utilization of energy by the bird. One such effect was a reduction in the activity of pathogenic bacteria (Van Immerseel *et al*., 2004), which leads to decreased competition for nutrients with the host and reduced desquamation of epithelial cells and epithelial turnover, ultimately reducing energy expenditure for epithelial repair and increasing the availability of nutrients for absorption and digestion (Dibner & Butin, 2002).

Sodium butyrate also has a trophic effect, in which it provides direct energy for enterocytes in the intestines of birds (Dibner & Butin, 2002), resulting in increased development of the intestinal mucosa (Chamba *et al*., 2014). In addition, it has an epigenetic effect, that is, it interferes with the gene expression of cells, promoting the proliferation of epithelial cells, and consequently broadening the nutrient absorption area (Hamer *et al*., 2008).

The better feed conversion obtained during the period from days 1 to 35 with the BD compared with Diets 3 and 4 corroborated the findings of Oliveira (2018), who described better feed conversion at 21 days old in chickens that received diets without ME reduction than in those in which ME was reduced by up to 90 kcal/kg. In the periods from 1 to 35 days and 1 to 21 days, Diet 3 resulted in similar live weight and WG to the BD, demonstrating a positive effect of PSB. However, Diet 4 did not prove as efficient.

Performance results from 1 to 42 days old were worse in the chickens fed the reduced-ME diets, regardless of butyrate inclusion, compared with the BD. Thus, it was concluded that the use of PSB had no positive impacts in the finisher phase. The superior performance achieved with the BD corroborates results described by other authors (Nogueira *et al*., 2013; Ferreira *et al*. 2015; Infante-Rodriguez *et al*. 2016; Oliveira, 2018), indicating that there is a positive relationship between increases in ME and performance, in broilers.

The improved performance shown by the birds that received the BD may be related to the higher net energy content of the diet, since the addition of oils reduced heat increment and the rate of passage of feed through the gastrointestinal tract, improving the absorption of dietary nutrients and, consequently, production performance (Braga & Baião, 2001).

Feeding Diets 2, 3 and 4 did not change feed intake in any of the periods in the present study as has been previously described in other studies (Plumstead *et al*., 2007; Richards & Proszkowiec-Weglarz, 2007). Likewise, Oliveira (2018) compared the positive-control treatment with diets with reduced ME levels (reductions of 30, 60 and 90 Kcal/kg) and did not detect significant differences for feed intake. According to Bertechini (2011), the modern broiler is selected for rapid growth and to consume feed according to the physical capacity of its gastrointestinal tract, with adjustments in feed intake being limited to meeting its energy requirements, exhibiting a hyperphagic behaviour.

The use of PSB also did not influence feed intake. This finding corroborated the reports of Zhang *et al.* (2011) and Pascual *et al*. (2020), who described no difference in intake in an experiment testing various levels of sodium butyrate in chicken feed.

Neither butyrate inclusion nor the reduction in the ME content of diet affected the yields of carcass or cuts. Similarly, Gopinger *et al*. (2017) tested ME variations of 200 kcal/kg more or less in the diet and did not find an effect of the dietary energy level on the yields of carcass or cuts of chickens at the end of the rearing period. Zhang *et al*. (2011) used 400 g/t PSB and did not observe differences between the treatment with butyrate and the control, for the yields of carcass or cuts.

Based on the results of the metabolic trial, the use of PSB(105 and 225 g/t) in the reduced-ME diets increased the metabolizability coefficient of dietary crude protein, providing a result similar to that achieved with the BD in the period from 32 to 35 days old. In terms of AME and AMEn, the BD provided the highest values for both variables in the chickens at 21 and 35 days old.

The higher ME value (AME and AMEn) and metabolizability of dietary nutrients seen in BD can be explained by the addition of oils and fats to the diet, which, in addition to increasing energy density, reduced the rate of passage of feed through the gastrointestinal tract, thereby improving the absorption and digestion of dietary nutrients (Braga & Baião, 2001). According to Martins *et al*. (2016), the gradual increase in dietary energy content provided by the inclusion of increasing oil levels, which reduces heat increment, is associated with the probable increase in protein deposition, which in turn increases the energy to protein ratio of the diets and hence the feed conversion ability of birds.

The positive effect of the use of PSB in the energy-reduced diets on CPMC can be explained by the action of butyrate in the intestinal microbiota of birds (Carvalho *et al*., 2021). Dibner & Putin (2001) suggested that organic acids, which include butyrate, improve the digestibility of protein and energy by

reducing microbial competition with the host for nutrients and endogenous nitrogen losses, in addition to lowering the pH of the digesta and favouring the action of proteolytic enzymes (Ahsan *et al*., 2016).

Smulikowska *et al*. (2009) reported that the dietary inclusion of PSB (300 g/t) increased the apparent digestibility and retention of nitrogen, but did not influence the AME content of the diet. Qaisrani *et al*. (2015) reported a trend of increased proventricular proteolytic activity in chickens fed butyrate. Pires *et al*. (2021) worked with laying hens and stated that PS improved the energy metabolizability of the diet. According to Moquet *et al*. (2018), butyrate improved protein digestibility, whereas improvements in energy digestibility were uncertain.

In the present study, using PSB in Diets 3 and 4 increased villus height and villus to crypt ratio in the jejunum, which resulted in a larger surface area for nutrient absorption and a lower rate of cell turnover. Once ingested, butyrate is converted to butyric acid, in an undissociated form that is readily absorbed by enterocytes. After being transported into the cell, the butyric acid in the mitochondria is metabolized to acetyl-CoA, which enters the citric acid cycle, producing energy (ATP) and carbon dioxide. In this way, it contributes to the growth of the villi and consequently increases the area where nutrients are absorbed by enterocytes (Hamer *et al*. 2008).

The results in the current study are similar to those observed by Pires *et al*. (2021), who evaluated the inclusion of PSB in diets for laying hens and concluded that it improved intestinal parameters. Chamba *et al*. (2014) observed greater villus height in the jejunum resulting from feeding sodium butyrate compared with the control treatment. Sikandar *et al*. (2017) showed that villus height and villus surface area in the duodenum and jejunum were greater in the groups that were fed diets with butyrate than in the control. Similarly, Adil *et al*. (2010) found greater villus heights in the duodenum and jejunum of broilers supplemented with 3% sodium butyrate compared with the control. Wu *et al*. (2018) reported that chickens fed butyrate showed longer and wider villi and more goblet cells than the control group.

Conclusions

The inclusion of PSB at 105 g/t in the diet of broilers aged up to 35 days allowed savings of 100 kcal/kg in ME, based on the performance evaluation. Further, this dietary augmentation improved intestinal histomorphometry at 21 and 35 days old and protein metabolizability at 35 days old. Thus, the use of PSB at 105 g/t in diets with ME reduced by 100 kcal/kg could be recommended until broilers are 35 days old.

Authors' Contributions

MFP, NSML and DVJ conceived and designed the experiments. MFP, RANM, ISF, DPC and HFO conducted the experiments. MFP and NSML analysed the data. NSML, DVJ and MBC contributed reagents, materials, and analytical tools. MFP, NSML and HFO wrote the paper. HFO edited the manuscript.

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Conflict of interest declaration

The authors have no conflict of interest to declare.

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