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#### **Original Article**

### ■Author(s)

| Pires MF <sup>I</sup>      | (D) https://orcid.org/0000-0003-4806-6466 |
|----------------------------|---|
| Leandro NSM <sup>1</sup>   | (D) https://orcid.org/0000-0002-6525-9975 |
| Oliveira HF <sup>I</sup>   | (D) https://orcid.org/0000-0003-4109-1087 |
| Jacob DV <sup>∥</sup>      | (D) https://orcid.org/0000-0002-0985-9677 |
| Carvalho FB <sup>I</sup>   | (D) https://orcid.org/0000-0001-5900-5437 |
| Stringhini JH <sup>I</sup> | (D) https://orcid.org/0000-0002-3710-6963 |
| Carvalho DP <sup>I</sup>   | (D) https://orcid.org/0000-0002-7954-235X |
| Andrade CL <sup>I</sup>    | (D) https://orcid.org/0000-0002-8735-5386 |

- <sup>1</sup> Department of Animal Science, Veterinary and Animal Science College, Federal University of Goiás, Goiânia, Brazil.
- Department of Animal Science, Adisseo South America, Campinas, Brazil.

#### Mail Address

Corresponding author e-mail address Helder Freitas de Oliveira Rodovia Goiânia - Nova Veneza, km 8, Campus II Samambaia, Goiânia, GO -74690-900 - Brasil. Phone: +62 982728873 Email: helder@zootecnista.com.br

#### ■Keywords

Additive; intestinal health; metabolizability; nutrition; organic acids.



Submitted: 06/December/2020 Approved: 04/January/2021 *Effect of Dietary Inclusion of Protected Sodium Butyrate on the Digestibility and Intestinal Histomorphometry of Commercial Laying Hens* 

### ABSTRACT

The objective of this study was to evaluate the effects of dietary inclusion of protected sodium butyrate (PSB) on the intestinal development and feed nutrient metabolizability of commercial laying hens. The birds started to receive the treatment rations at 58 weeks of age. At 76 weeks of age the laying hens were distributed in a randomized block design to four treatments (0, 105, 210 and 300 g t<sup>-1</sup>/ PSB), six replicates, and two birds/replicate. The nitrogen balance (NB), ether extract balance (EEB), dry matter metabolizability coefficient, nitrogen, ether extract, ash, apparent metabolizable energy (AME), and AME corrected by nitrogen (AMEn) were evaluated. For assessment of intestinal development, we evaluated the duodenum, jejunum, ileum, cecum, and colorectal lengths, the relative intestine weight and villus height, crypt depth, and villus:crypt ratio of the duodenum, jejunum, and ileum. A decreasing linear effect was observed in the duodenum length, while an increasing linear effect was observed in the height of the duodenum and jejunum villi. A guadratic effect was found in the jejunum crypt depth. A linear increasing effect was found on the villus:crypt ratio of the duodenum and jejunum, and a quadratic effect was observed in the ileum. Quadratic and increasing linear effects were observed in the NB and EEB, respectively. Additionally, increasing linear effects were observed in the AME and AMEn. The dietary addition of 300 g t<sup>-1</sup> of PSB improved intestinal development and energy metabolizability of the diet.

### INTRODUCTION

As laying hens age, they lay larger eggs with reduced shell thickness owing to the larger egg surface area; additionally, the calcium carbonate deposition per unit surface area decreases because the egg weight increase rate is higher than the shell volume increase rate in eggs laid by ageing birds (Carvalho *et al.*, 2013; Oliveira *et al.*, 2020). In addition, older birds have reduced ability to absorb calcium through the intestine owing to a reduction in their capacity to convert the inactive form of vitamin D into its active form; moreover, their retention rate of absorbed calcium is lower, as is their ability to mobilize bone calcium, owing to the lower enzymatic activity of carbonic anhydrase, which leads to reduced eggshell calcification (Vilela *et al.*, 2016). However, according to Sengor *et al.* (2007), as the birds get older, the intestinal mucosa cells weaken, thereby leading to a reduction in duodenal villus height, which hampers the absorption of the nutrients required for eggshell formation.

Along with the reduction in shell quality, the high cost of bird feed ingredients has led to research advances towards developing performance-enhancing additives that maximize nutrient uptake and, consequently, improve the egg quality, performance, and intestinal



mucosa structure of laying hens, thereby promoting better nutrient absorption (Lemos *et al.*, 2017).

Sodium butyrate  $[Na(C_3H_7COO)]$ , is a four-carbon short-chain organic acid in the form of a soluble salt derived from butyric acid; it can be used in its free or protected form in animal feeds. In its free form, it is absorbed in the proximal parts of the gastrointestinal tract, therefore it does not reach the distal parts. In its protected form, sodium butyrate is gradually released into the digestive tract, thereby increasing its mode of action at several parts of the intestine (Vallejos *et al.*, 2015; Santos Junior *et al.*, 2016).

Sodium butyrate is readily transformed into butyric acid within the digestive tract of birds, where it improves intestinal health through various mechanisms. Its inclusion in the diet provides a readily available source of energy for the enterocytes of the intestinal mucosa, thereby increasing the cell proliferation rate and nutrient absorption from the feed. It improves the beneficial bacterial populations and reduces the colonization of harmful bacteria in the digestive tract (Van et al., 2004; Chamba et al., 2014; Ahsan et al., 2016). Regarding improvements in dietary nutrient metabolism, sodium butyrate acts by acidifying the digestive tract, thereby increasing the duration of food retention in the stomach, improving the activity of the digestive enzymes, and increasing pancreatic secretions (Machinsky et al., 2010).

The objective of the present study was to investigate the effect of increasing levels of dietary protected sodium butyrate on the digestibility and intestinal histomorphometry of light laying hens aged 76 weeks.

## **MATERIAL AND METHODS**

The research was approved by the Ethics Committee on Animal Use (protocol n° 74/14). The experiment was conducted in Goiânia, Goiás, Brazil (16°40'43"S, 49°15'14"W), in an area that has an altitude of 749 m.

A total of 400, 58-week-old, Dekalb White light laying hens were obtained from a commercial farm. They were all weighed individually, and 320 laying hens were selected. Birds with a live weight above or below the breed standard (average weight of 1.660 kg), the standard deviation of which was 10 %, were discarded. There was a 21-day adaptation period, which was followed by egg production in order to select birds according to their egg production capacity (maximum variation of 10 %). The laying hens received experimental feed when they were between 58 and 76 weeks old. The birds were housed in pairs, in galvanized wire cages with linear feeders and nipple drinkers, in a brick laying house. Throughout the experimental period, feed was supplied twice daily, at 8 a.m. and 6 p.m., while ensuring that the birds received water and feed in accordance with the recommended daily consumption values established for the breed. The lighting schedule was adjusted to 16 h of light per day, in accordance with the recommendations for the breed, and was controlled by an automated clock.

We used a randomized block design for the experiment, the hens were assigned to blocks based on their bodyweight (light birds weighing 1.494 - 1.660 kg and heavy birds weighing 1.660 - 1.826 kg), with four treatments, and eight repetitions with ten birds each. When the birds became 76 weeks old, the metabolic test was performed, with six repetitions and two birds in each repetition. The treatments included 0 g.t<sup>-1</sup>, 105 g.t<sup>-1</sup>, 210 g.t<sup>-1</sup>, and 300 g.t<sup>-1</sup> of protected sodium butyrate in the feed.

The statistical model used was:

 $y_{ij} = u + b_i + t_j + e_{ij}$ 

y<sub>ii</sub>: an observation in treatment i and block j;

 $\mu$ : the overall mean;

b,: the fixed effect of the i-th treatment;

t: the fixed effect of the j-th block;

 $\dot{e_{ii}}$ : random error with a mean of 0 and variance  $\sigma 2$ .

The experimental feed was prepared from a baseline feed consisting of maize and soybean meal in accordance with the Brazilian nutritional standards for birds and pigs Rostagno *et al.* (2011) (Table 1); the only difference among the diets was the protected sodium butyrate level. The commercial additive Adimix Precision<sup>®</sup> (30 % protected sodium butyrate) was used as a source of protected sodium butyrate. In order to obtain the aforementioned protected sodium butyrate levels in the treatments, we added 0 g.t<sup>-1</sup>, 350 g.t<sup>-1</sup>, 700 g.t<sup>-1</sup>, and 1.000 g.t<sup>-1</sup> of Adimix Precision<sup>®</sup> in the hens' rations.

The metabolic test was performed using the total excrement collection method for four consecutive days. During this period, the feed consumption monitoring and excrement collection were performed twice daily (morning and afternoon). The excreta were stored in plastic bags identified and stored in a freezer until the end of the collection period. Subsequently, the samples were thawed, homogenized, and divided in aliquots that were weighed. Then, they were predried in ventilated greenhouses at 55 °C for 72 h.



**Table 1** – Composition and calculated nutritional value of the basal diet.

| Ingredient  | g.kg <sup>-1</sup> as fed |  |  |  |
|---|---------------------------|--|--|--|
| Corn  | 635.7                     |  |  |  |
| Soybean meal 45%                                      | 235.1                     |  |  |  |
| Limestone   | 94.2                      |  |  |  |
| Dicalcium phosphate                                   | 13.4                      |  |  |  |
| Inert   | 1.0                       |  |  |  |
| Sodium butyrate                                       | 0                         |  |  |  |
| Common salt   | 4.7                       |  |  |  |
| Soybean oil   | 12.9                      |  |  |  |
| DL-methionine   | 1.3                       |  |  |  |
| Vitamin supplement <sup>1</sup>                       | 1.0                       |  |  |  |
| Mineral supplement <sup>2</sup>                       | 0.5                       |  |  |  |
| L-Threonine   | 0.1                       |  |  |  |
| L-Lysine HCl  | 0.1                       |  |  |  |
| Total   | 1000                      |  |  |  |
| Nutrient  | Calculated composition    |  |  |  |
| Gross energy (kcal.kg <sup>-1</sup> )                 | 2.800                     |  |  |  |
| Crude protein (g.kg-1)                                | 160.0                     |  |  |  |
| Digestible Methionine + Cystine (g.kg <sup>-1</sup> ) | 6.0                       |  |  |  |
| Digestible Methionine (g.kg <sup>-1</sup> )           | 3.7                       |  |  |  |
| Digestible Lysine (g.kg <sup>-1</sup> )               | 7.4                       |  |  |  |
| Calcium (g.kg <sup>-1</sup> )                         | 40.2                      |  |  |  |
| Available phosphorus (g.kg-1)                         | 3.4                       |  |  |  |
| Linoleic acid (g.kg <sup>-1</sup> )                   | 20.1                      |  |  |  |
| Digestible Arginine (g.kg <sup>-1</sup> )             | 9.8                       |  |  |  |
| Chlorine (g.kg <sup>-1</sup> )                        | 3.2                       |  |  |  |
| Potassium (g.kg <sup>-1</sup> )                       | 6.1                       |  |  |  |
| Digestible Phenylalanine (g.kg <sup>-1</sup> )        | 7.4                       |  |  |  |
| Digestible Isoleucine (g.kg <sup>-1</sup> )           | 6.2                       |  |  |  |
| Digestible Leucine (g.kg <sup>-1</sup> )              | 13.7                      |  |  |  |
| Digestible Threonine (g.kg <sup>-1</sup> )            | 0.55                      |  |  |  |
| Digestible Tryptophan (g.kg <sup>-1</sup> )           | 0.17                      |  |  |  |
|   |                           |  |  |  |

<sup>1</sup>Mineral Mix for poultry. Content/kg: Copper 18 mg, Zinc 120 mg, Iodine 2 mg, Iron 60 mg, Manganese 120 mg. <sup>2</sup>Mineral vitamin supplement for laying hens. Content/kg: Folic Acid 500 mg, Ash 500 g, Niacin 25 g, Calcium Pantothenate 10 g, Selenium 330 mg, Vit. A 8.600 IU, Vit. B1 1.500 IU, Vit. B12 13.000 mcg, Vit. B2 4.000 IU, Vit. B6 1.700 IU, Vit. D3 2.000 IU, Vit. E 10.000 IU, Vit. H 52 mg, Vit. K 1.800 mg.

Subsequently, the dry matter, nitrogen, and ethereal extract were analyzed according to the methodology described by Silva & Queiroz (2002). The crude energy was determined using a calorimeter pump (model 5000; Ika).

Based on the bromatological analyses, we calculated the nitrogen balance (NB), ether extract balance (EEB), dry matter metabolizability coefficient (DMMC), nitrogen metabolizability coefficient (NMC), ether extract metabolizability coefficient (EEMC), ash metabolizability coefficient (AMC), apparent metabolizable energy (AME), and AME corrected by nitrogen (AMEn) using the equations proposed by Sakomura & Rostagno (2016).

After the metabolic test was performed, one bird per repetition was euthanized by cervical dislocation

to analyze its intestinal morphometry. The entire intestine was collected and weighed, and mensured lengths the duodenum (from the pylorus to the distal portion of the duodenal loop), jejunum (from the distal duodenal loop to the ileumcecal junction), ileum (from the anterior portion of the caecum to the ileumcecal junction), cecum (from its base to the summit), and colorectal (junction ileumcecal to the anterior portion of the cloaca). The relative weight of the intestine was obtained by the equation (gut weight/live weight of the bird) × 100.

For the histological analyses of the intestinal mucosa, we collected 5-cm samples from the segments of the small intestine (duodenum, jejunum, and ileum). The small intestine samples were opened, fixed in polystyrene, and immersed in 10 % formalin for 24 h. Next, they were stored in 70 % alcohol, processed according to the methodology described by Luna (1968), and stained by the hematoxylin-eosin (HE) method. The images of the blades were obtained with the aid of an optical microscope (DM 4000B; Leica) coupled to a microcomputer, and were then analyzed with the aid of ImageJ software, via which five measurements of villus height and crypt depth per blade were obtained, totaling 360 readings. The villus:crypt ratio was obtained by dividing the height of the villus by the depth of the crypt.

The data collected were subjected to analysis of variance with  $\alpha = 0.05$ ; polynomial regression was performed for all the protected sodium butyrate levels, using R statistical software.

## RESULTS

The regression analysis showed that the addition of dietary protected sodium butyrate did not have an effect on the intestinal morphometry (relative weight of the intestine and length of the duodenum, ileum, cecum, and colon) of laying hens at 76 days of age (Table 2). However, the length of the duodenum decreased linearly (p<0.05) with increasing levels of sodium butyrate in the diet (Figure 1).

The histological analysis of the small intestine (Table 3) showed an increasing linear effect (p<0.05) on the villus height of the duodenum and jejunum with increasing sodium butyrate levels in the diet (Figure 2). The crypt depth of the duodenum and ileum was not influenced by the treatments. A quadratic effect was observed in the crypt depth of the jejunum, which decreased with protected sodium butyrate levels of up to 151 g t<sup>-1</sup> (p<0.05) (Figure 3).



**Table 2** – Relative weight and intestine length of laying hens aged 76 weeks that were fed with different levels of protected sodium butyrate (PSB).

| Variables            |      | Dietary levels o | f PSB (g.kg <sup>-1</sup> ) | $C \setminus (0/)$ | <i>p</i> -value |                    |       |
|----------------------|------|------------------|-----------------------------|--------------------|-----------------|--------------------|-------|
|                      | 0    | 105              | 210                         | 300                | - CV (%) -      | L                  | Q     |
| Intestine weight (%) | 4.2  | 3.9              | 4.3                         | 3.9                | 15.14           | 0.695              | 0.918 |
| Length (cm)          |      |                  |                             |                    |                 |                    |       |
| Duodenum             | 39.9 | 39.6             | 35.3                        | 29.2               | 19.64           | 0.012 <sup>1</sup> | 0.288 |
| Jejunum              | 80.1 | 79.7             | 78.6                        | 75.1               | 11.26           | 0.336              | 0.646 |
| lleum                | 21.5 | 19.0             | 17.6                        | 17.4               | 24.93           | 0.132              | 0.532 |
| Cecum                | 13.9 | 12.1             | 11.7                        | 13.6               | 12.04           | 0.609              | 0.970 |
| Colon-rectum         | 8.1  | 7.0              | 5.6                         | 7.3                | 24.11           | 0.226              | 0.378 |

 $^{1}Y = 41.49833 - 0.03563X; R^{2} = 0.85$ 

**Table 3** – Villus height ( $\mu$ m), crypt depth ( $\mu$ m) and villus/crypt ratio in the duodenum, jejunum and ileum of light laying hens aged 76 weeks that were fed with different levels of protected sodium butyrate (PSB).

| Variables -        |       | Dietary levels o | $C \setminus (0)$ | <i>p</i> -value |        |                      |                    |
|--------------------|-------|------------------|-------------------|-----------------|--------|----------------------|--------------------|
|                    | 0     | 105              | 210               | 300             | CV (%) | L                    | Q                  |
| Villus height (µm) |       |                  |                   |                 |        |                      |                    |
| Duodenum           | 770.9 | 932.4            | 1132.8            | 1203.1          | 23.79  | 0.018 <sup>1</sup>   | 0.791              |
| Jejunum            | 550.2 | 591.4            | 657.2             | 856.4           | 20.50  | < 0.001 <sup>2</sup> | 0.144              |
| lleum              | 474.5 | 652.8            | 621.5             | 627.7           | 19.28  | 0.089                | 0.113              |
| Crypt depth (µm)   |       |                  |                   |                 |        |                      |                    |
| Duodenum           | 145.6 | 144.9            | 130.              | 128.6           | 19.24  | 0.235                | 0.988              |
| Jejunum            | 104.1 | 76.3             | 84.8              | 100.8           | 17.87  | 0.004                | 0.004 <sup>3</sup> |
| lleum              | 96.1  | 90.3             | 77.2              | 102.2           | 20.94  | 0.959                | 0.095              |
| Villus/crypt ratio |       |                  |                   |                 |        |                      |                    |
| Duodenum           | 4.8   | 6.5              | 8.8               | 9.3             | 31.91  | 0.0134               | 0.738              |
| Jejunum            | 5.4   | 7.7              | 7.9               | 8.8             | 26.58  | 0.0105               | 0.426              |
| lleum              | 5.0   | 7.4              | 8.2               | 6.2             | 23.28  | 0.003                | 0.007 <sup>6</sup> |

 $^{1}$  Y = 812.4557 + 1.398X; R<sup>2</sup> = 0.96

 $^{2}$  Y = 515.4423 + 0.9649X; R<sup>2</sup> = 0.84

 $^3$  Y = 102.969082 - 0.340313X + 0.001126X2; R2 = 0.94; Pmax = 151 g  $t^{\text{-}1}$ 

 $^{4}$ Y = 5.244239 + 0.01488X; R<sup>2</sup> = 0.94

 $^{5}$  Y = 5.900341 + 0.0103X; R<sup>2</sup> = 0.86

 $^{6}\,\text{Y}=4.9904404+0.0365269\text{X}$  - 0.0001068X2;  $\text{R}^{2}=0.98;$  Pmax = 171 g t  $^{1}$ 

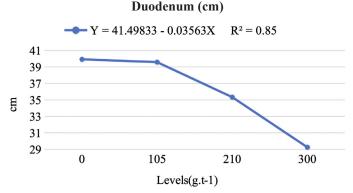


Figure 1 – Linear effect for duodenal length (cm) of laying hens fed with different levels of protected sodium butyrate.

The villus:crypt ratio of the duodenum and jejunum increased linearly (p<0.05) with the inclusion of sodium butyrate in the ration (Figure 4). On the other hand, a quadratic effect (p<0.05) was observed in the villus:crypt ratio of the ileum, which peaked at a protected sodium butyrate level of 171 g t<sup>-1</sup> (Figure 5).

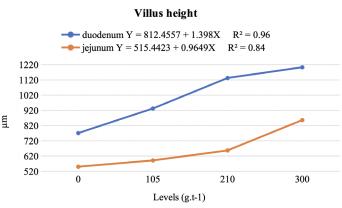


Figure 2 – Linear effect for duodenal and jejunal villus height ( $\mu$ m) of laying hens fed with different levels of protected sodium butyrate.

In terms of the dietary nutrient metabolism (Table 4), significant regression was observed for the NB, EEB, AME, and AMEn. The regression analysis did not show any differences in terms of the feed nutrient metabolizability coefficients (DMMC, NMC, EEMC, and AMC) (*p*>0.05).

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Crypt depth ----- jejunum Y =  $102.969 - 0.340313X + 0.001126X^2$  R<sup>2</sup> = 0.94110 100 90 80 70∟ 0 105 210 Levels (g.t-1)

Figure 3 – Quadratic effect for jejunal crypt depth (µm) of laying hens fed with different levels of protected sodium butyrate.

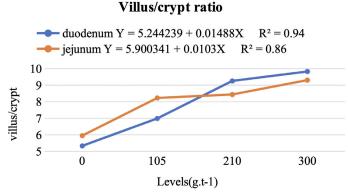


Figure 4 - Linear effect for the duodenal and jejunal villus/crypt ratio of laying hens fed with different levels of protected sodium butyrate.

A guadratic effect was observed in the NB (p < 0.05), as this increased with sodium butyrate levels of up to 93 g t<sup>-1</sup>. The EEB, AME, and AMEn increased linearly (p < 0.05) with increasing sodium butyrate levels.

# DISCUSSION

In the present study, the relative weight and length of the intestinal segments (duodenum, ileum, cecum, colon, and rectum) were not influenced by Butyrate on the Digestibility and Intestinal Histomorphometry of Commercial Laying Hens

Effect of Dietary Inclusion of Protected Sodium



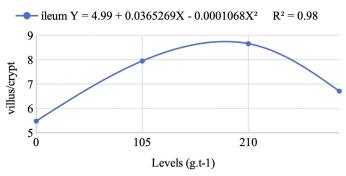


Figure 5 – Quadratic effect for the ileal villus/crypt ratio of laying hens fed with different levels of protected sodium butyrate.

the treatments. Silva (2013) evaluated the use of 300 g.t<sup>-1</sup> of sodium butyrate in the feed of nursery-phase pigs and found that the relative weight of their small intestines was lower than that of the control group. According to Silva (2013), a better dietary nutrient uptake can reduce the feed bolus viscosity and the functional load of the intestine, thus decreasing the relative weight of the small intestine. However, this was not observed in the present study.

The length of the duodenum decreased with increasing sodium butyrate levels in the diet. Similarly, Viola et al. (2008) evaluated the addition of 4.5 kg.t-<sup>1</sup> of an organic acid mixture (50 % lactic acid, 8 % formic acid, and 7 % acetic acid) in the diet of broiler chickens; their results showed that the organic acid treatment resulted in shorter jejunum lengths in 21-day-old chickens than in the control group.

The inclusion of protected sodium butyrate in the laying hens' rations resulted in increased villus height in the duodenum and jejunum. According to Niwińska

Table 4 – Nitrogen balance (NB), ether extract balance (EEB), dry matter metabolizability coefficient (DMMC), nitrogen metabolizability coefficient (NMC), ether extract metabolizability coefficient (EEMC), ash metabolizability coefficient (AMC), apparent metabolizable energy (AME) and apparent metabolizable energy corrected by nitrogen (AMEn) of the feed of light laying hens containing protected sodium butyrate (PSB).

| Variables - |      | Dietary levels of PSB (g.kg <sup>-1</sup> ) |      |      |          | <i>p</i> -value    |                    |
|-------------|------|---|------|------|----------|--------------------|--------------------|
|             | 0    | 105   | 210  | 300  | – CV (%) | L                  | Q                  |
| NB (g)      | 8.34 | 8.91  | 7.96 | 7.00 | 11.91    | 0.012              | 0.050 <sup>1</sup> |
| EBB (g)     | 2.42 | 2.65  | 2.50 | 2.73 | 5.34     | 0.005 <sup>2</sup> | 0.940              |
| DMMC (%)    | 72.6 | 74.3  | 75.7 | 73.6 | 4.02     | 0.392              | 0.142              |
| NMC (%)     | 52.2 | 51.9  | 51.6 | 46.2 | 11.90    | 0.123              | 0.302              |
| EEMC (%)    | 80.1 | 80.1  | 81.5 | 80.6 | 3.18     | 0.532              | 0.711              |
| AMC (%)     | 88.8 | 88.7  | 88.6 | 87.1 | 2.48     | 0.244              | 0.455              |
| AME (kcal)  | 3166 | 3088  | 3346 | 3322 | 2.50     | < 0.0013           | 0.251              |
| AMEn (kcal) | 3060 | 2984  | 3247 | 3238 | 2.26     | < 0.0014           | 0.126              |

 $^{1}$ Y = 8.365 + 0.007931X - 0.00004245X<sup>2</sup>; R<sup>2</sup> = 0.95; Pmax: 93 g t<sup>1</sup>

<sup>2</sup>Y = 2.4531504 + 0.0007912X; R<sup>2</sup> = 0.47

 $^{3}$  Y = 3120.1888 + 0.7221X; R<sup>2</sup> = 0.53

<sup>4</sup> Y = 3011.7774 + 0.7895X; R<sup>2</sup> = 0.58



et al. (2017), this effect may have occurred because butyric acid is a source of energy readily available and rapidly absorbed by the intestinal mucosa, in order to produce adenosine triphosphate (ATP). Thus, sodium butyrate is able to accelerate the mitosis process that occurs in the crypt-villus region (Górka et al., 2014), thereby resulting in an increase in the cell proliferation rate and lower energy loss through turnover. The higher the cell number, the greater the intestinal villus height, thus, the larger the nutrient absorption surface area (Maiorka et al., 2004). In addition, sodium butyrate can influence positively the intestinal integrity because of its bactericide and bacteriostatic effects, since it decreases the intestinal cell desquamation, thereby favoring the structure and growth of intestinal villi (Ahsan et al., 2016) and reducing the enterocyte turnover owing to the reduction of bacterial toxins and improved colonic barrier function (Guilloteau et al., 2010).

The results of the present study are corroborated by Herrera *et al.* (2009) who verified an increase in the duodenal villus height of laying hens that had been fed diets containing 300 g.t<sup>-1</sup> and 500 g.t<sup>-1</sup> of sodium butyrate for 73 weeks. In addition, Panda *et al.* (2009) evaluated the addition of increasing levels of sodium butyrate (200 g.t<sup>-1</sup>, 400 g.t<sup>-1</sup>, and 600 g.t<sup>-1</sup>) in broiler rations and observed higher duodenal villus heights in all sodium butyrate treatments compared to the control treatment.

No significant differences were found in the crypt depth of the duodenum and ileum; however, in the jejunum, the lowest crypt depth was found at the 151 g.t<sup>-1</sup> dose. The jejunum crypt depth increased with increasing protected sodium butyrate levels. These results do not corroborate those of (Santos, 2013), who did not observe jejunum crypt depth increases with the use of sodium butyrate in the feed of Japanese quails. According to Oetting *et al.* (2006), a crypt depth increase can indicate higher cell proliferation activity, thereby ensuring an appropriate cell renewal rate in the apical region of the villus.

A linear effect was observed in the villus:crypt ratio of the duodenum and jejunum. Therefore, as the levels of protected sodium butyrate in the feed increased, the intestinal absorption area also increased. In the ileum, a quadratic effect was observed in the villus:crypt ratio, as this peaked at a dose of 171 g.t<sup>-1</sup> of protected sodium butyrate in the diet, thus indicating an increase in the nutrient absorption area. Santos (2013) did not detect any differences in the villus:crypt ratio in any of the segments of the small intestine of Japanese quails that were fed diets with 150 g.t<sup>-1</sup> of protected sodium butyrate, compared with the control group.

According to Arouca *et al.* (2012), the higher the villus:crypt ratio, the better the nutrient absorption and the lower the energy losses owing to cell renewal. Similarly, the results of the present study showed that there was an increase in the nutrient absorption capacity and lower energy losses when protected sodium butyrate was used. According to the small-intestine histomorphometric analysis, the use of 300 g.t<sup>-1</sup> of sodium butyrate was the most efficient, as it resulted in the greatest duodenum and jejunum villus heights as well as the highest villus:crypt ratios in the three segments of the small intestine.

The NB increased with the inclusion of up to 93 g.t<sup>-1</sup> of sodium butyrate in the diet; as the sodium butyrate level increased, the NB decreased. However, the EEB increased with increasing sodium butyrate levels in the diet. An increasing linear effect was observed on the AME and the AMEn. Likewise, Riboty *et al.* (2016) observed that broiler chickens that had been fed diets with sodium butyrate had increased AMEn values compared with the control group.

The improved nutrient retention and increase in metabolizable energy (AME and AMEn) can be explained by the action of protected sodium butyrate in the intestine. According to Van *et al.* (2004), protected sodium butyrate can reach the bird's intestine without undergoing dissociation. Once in the intestine, the matrix that protects sodium butyrate is emulsified and hydrolyzed through the action of hepatic and pancreatic secretions, thus releasing the acid in a dissociated form and diminishing the pH of the digesta in the duodenum.

The low pH of the digesta stimulates the secretion of the hormone secretin by the duodenal cells. This stimulates the pancreas to secrete bicarbonate, thereby neutralizing the acidic pH in the duodenum and increasing the action of pancreatic enzymes. In addition, according to Niwińska *et al.* (2017), the presence of sodium butyrate in the duodenum stimulates the secretion of the hormone cholecystokinin (CCK), which stimulates the secretion of pancreatic enzymes. This also stimulates the secretion of bile by the gallbladder, which acts as a lipid emulsifier, thus facilitating the action of the enzyme pancreatic lipase as well as lipid transportation and absorption (Lan *et al.*, 2020).

The positive effects of protected sodium butyrate on the nutrient metabolizability of the feed can also be correlated with the antimicrobial effect of



the organic acid. According to Costa *et al.* (2011), sodium butyrate acts on the pathogenic microbiota owing to its bactericidal and bacteriostatic effects, thereby reducing the competition for dietary nutrients, decreasing fermentation, and increasing the available energy that can be metabolized in the intestinal mucosa. Decreasing pathogenic microbiota levels result in a lower enterocyte turnover owing to the reduction of bacterial toxins (Ahsan *et al.*, 2016; Guilloteau *et al.*, 2010).

Sodium butyrate also provides readily available energy to enterocytes, which can favor cell proliferation and, consequently, increase the villus size, thereby increasing the nutrient absorption area in the intestine. In the present study, we verified that protected sodium butyrate increased the villi of the duodenum and jejunum and resulted in a greater villus:crypt ratio in the duodenum, jejunum, and ileum, which may have favored nutrient retention and increased metabolizable energy (AME and AMEn).

With the results of the present study, that is, with improved intestinal development and energy metabolizability of the diet, it is possible to explain a better response in performance and eggshell quality. Pires et al. (2020) observed that the inclusion of protected sodium butyrate in the laying hens diets did not appear to affect the productive performance of the birds, but it did improve the eggshell quality of laying hens at 61-76 weeks of age, improving the thickness, resistance and percentage of shell and reducing the percentage of broken and dirty eggs. According to the authors, the observed improvement can be explained by the effects of the additive on the intestinal mucosa, as an energy source for the intestinal cells, and also in the increase of metabolizability and nutrient retention, as confirmed in the present study.

## CONCLUSION

The addition of protected sodium butyrate in the diet of commercial laying hens improved the intestinal parameters. A level of 300 g t<sup>-1</sup> of protected sodium butyrate is recommended for the improvement of the intestinal development and the energy metabolizability of the diet.

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# **CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

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