R. Bras. Zootec., 53:e20220150, 2024
https://doi.org/10.37496/rbz5320220150

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Effects of encapsulated butyric acid on general performance, intestinal health, and colonization in organs of poultry infected with *Salmonella* Enteritidis

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Received: January 31, 2023
Accepted: January 16, 2024

How to cite: Leonídio, A. R. A.; Minafra, C.; Andrade, M. A.; Nascente, E. P.; Nascimento, G. M.; Santos, J. B.; Stringhini, J. H. and Almeida, A. M. S. 2024. Effects of encapsulated butyric acid on general performance, intestinal health, and colonization in organs of poultry infected with *Salmonella* Enteritidis. Revista Brasileira de Zootecnia 53:e20220150. https://doi.org/10.37496/rbz5320220150

Editors:
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ABSTRACT - This experiment was carried out to evaluate the effects of organic acids on the intestinal integrity and productive performance of broiler chickens experimentally inoculated with *Salmonella* Enteritidis. Additionally, *Salmonella* colonization levels in internal organs were evaluated. The study included 576 one-day-old Cobb™ male broilers, distributed into eight treatment groups and six replicates. A completely randomized experimental design (encapsulated butyric acid × inoculation by *Salmonella* Enteritidis) was used. Poultry was orally inoculated with *Salmonella* Enteritidis in the first day (0 or 2.0 × 10^6 CFU/mL) and after 22 days of age (0 or 1.0 × 10^9 CFU/ mL). The butyric acid was added to the feed in different concentration (treatments 0.03, 0.075, and 0.15%), and the chickens were raised up to 42 days old. Encapsulated butyric acid at 0.03% increased the body weight gain and the average weight of the chickens up to 21 days old. After 14 days old, the butyric acid had a positive effect on jejunum villus. Encapsulated butyric acid at 0.03% in feed improves the performance and intestinal integrity of chickens.

Keywords: additives, chicks, histomorphometry, organic acids, salmonellosis

1. Introduction

Salmonella sp. is a problem in poultry farming worldwide. In poultry, it induces intestinal damage, which affects the function of the digestive tracts, resulting in diarrhea and low feed intake (Remus et al., 2014; El-Saadony et al., 2022). In addition, some bacteria serovars such as *Salmonella* Enteritidis (SE) can cause disease in humans, and infected chickens and poultry products are considered the main sources of infection (Dunkley et al., 2009; Vandeplas et al., 2010; Li et al., 2021).

One of the current methods to control *Salmonella* infection in poultry is by adding organic acids to their feed and water (Pickler et al., 2012; Ruvalcaba-Gómez et al., 2022). Organic acids, such as butyric acid, provide carbon sources for villi growth, reduce enteropathogenic bacteria, and improve intestinal health (Nava et al., 2009; Berge and Wierup, 2012; Abd El-Hack et al., 2022). In their undissociated form, the acids can diffuse through the bacterial cell membrane. Once inside the cell, they dissociate and reduce the cytoplasmic pH, which can directly affect enzymatic reactions, cell growth, or even kill the bacteria (Sikandar et al., 2017).

In birds, organic acids, including butyric acid, are rapidly absorbed and metabolized by the cells of the ingluvius mucosa, limiting the amount of acid that reaches the small intestine; consequently, its action on pathogenic bacteria is also limited. Microencapsulation techniques such as ButiPEARL™ (Kemin®) promote a slow release of the acid after uptake by the bird, thereby allowing it to reach the lower intestinal tract, which contains the bacteria to be combated (Poshadri and Kuna, 2010; Kaczmarek et al., 2016).

Robust research has been done regarding the use of feed additives to improve the performance of farm animals (Gois et al., 2023). The use of microencapsulated carvacrol and cinnamaldehyde can influence in the metabolizability of nutrients, increasing metabolizable energy in broilers (Facchi et al., 2023). Fish and sacha inchi oils (oil mixture), selenium yeast, and chromium yeast in hen feed increase egg production and decrease feed conversion (Morales-Suárez et al., 2022). Also, the feed additives, such as organic acid can maintain animal health, but more studies are necessary.

Therefore, the present study aimed to evaluate the efficacy of butyric acid microcapsules in controlling SE infection in broiler chickens by determining its effects on growth performance, liver and spleen *Salmonella* carrier load, and intestinal morphological changes in broilers experimentally inoculated.

2. Material and Methods

The project was approved by the local Ethics Committee for the Use of Animals in the year 2021 (CEUA; approval no. 070/12) and was carried out in Goiânia, GO, Brazil (−16.67926° N, −49.25629° W).

2.1. Experimental design

The study involved 576 one-day-old Cobb™ male poultry supplied by a local commercial hatchery that vaccinates the broiler breeders against SE. To confirm that the chickens were free from *Salmonella* contamination prior to infection, cloacal and fecal samples after hatching were collected from all the chickens and tested (PCR and conventional culture method) for the presence of bacteria. The results were negative for all the samples.

The birds were distributed in a completely randomized 4 × 2 factorial design (four levels of encapsulated butyric acid (EBA) × non-inoculation or inoculation with *Salmonella* Enteritidis), composed of eight treatments with six replicates each, containing groups of 12 chickens each: control group (placebo); 300 g encapsulated butyric acid/ton (chicken feed) (0.03%); 750 g encapsulated butyric acid/ton (0.075%); 1,500 g encapsulated butyric acid/ton (0.15%); orally inoculated with SE (positive control); orally inoculated with SE and treated with 300 g encapsulated butyric acid/ton (0.03%); orally inoculated with SE and treated with 750 g encapsulated butyric acid/ton (0.075%); orally inoculated with SE and treated with 1,500 g encapsulated butyric acid/ton (0.15%).

2.2. Inoculation of *Salmonella* Enteritidis

Before lodging, swabs of the facilities, as well as samples of the feeds supplied to the chickens were collected and tested for the presence of *Salmonella* by the conventional culture method, and it was not detected in any of the samples.

The inoculum was prepared using SE isolated from samples from broilers and characterized by a reference laboratory. The concentrations of inoculum solution were 2.0×10^6 CFU/mL (first inoculation) and $> 1.0 \times 10^9$ CFU/mL (second inoculation). We deposited 0.3 mL of the inoculum solution in the oral cavity of the chickens at one day old (before housing them) and at 22 days old.

2.3. Experimental tests: performance, intestinal health, and colonization in organs

The feeding program (Table 1) was designed in accordance with the feed composition and nutritional requirements proposed by Rostagno et al. (2011).

Table 1 - Percentage composition of the experimental diets offered to the birds during the experimental period (1 to 42 days old)

Ingredient	Diet (g kg^{-1} as fed)			
	Pre-starter (1-7 d)	Starter (8-21 d)	Grower (22-35 d)	Finisher (36-42 d)
Corn grain	600.0	641.01	725.0	773.0
Soybean meal (45%)	333.0	265.0	175.0	136.0
Soybean oil	15.0	-	-	-
Meat and bone meal (45%)	-	45.0	45.0	35.0
Offal meal	-	15.2	15.2	15.0
Poultry fat	-	10.5	10.0	10.0
Limestone	8.1	4.5	3.1	3.8
Dicalcium phosphate	19.5	-	-	-
Salt	4.5	3.4	3.2	3.3
L-threonine	3.6	1.6	7.3	6.9
L-lysine HCL	4.9	4.3	5.9	6.4
DL-methionine	4.1	3.3	3.6	3.5
Vitamin premix[1]	1.0	1.0	1.0	1.0
Mineral premix[2]	0.5	0.5	0.5	0.5
Inert/EBA[3]	5.0	5.0	5.0	5.0
Total	1,000.00	1,000.00	1,000.00	1,000.00
Nutrient (on dry matter)				
Metabolizable energy (kcal kg^{-1})	2,960	3,050	3,150	3,200
Crude protein	22.40	21.20	19.80	18.40
Calcium (%)	0.92	0.841	0.75	0.66
Available phosphorus (%)	0.47	0.40	0.38	0.32
Sodium (%)	0.22	0.21	0.20	0.19
Lysine (%)	1.34	1.21	0.82	1.06
Methionine + cysteine (%)	0.96	0.87	0.82	0.77
Methionine (%)	0.68	0.60	0.59	0.56

[1] Vitamin supplement (levels guaranteed per kg of product): vitamin A, 1,680,000 IU; vitamin D3, 400,000 IU; vitamin E, 3500 mg; vitamin K, 360 mg; vitamin B1, 436.50 mg; vitamin B2, 1200 mg; vitamin B6, 624 mg; vitamin B12, 2400 mcg; folic acid, 200 mg; pantothenic acid, 3120 mg; niacin, 8400 mg; biotin, 10,000 mcg.
[2] Mineral supplements (levels guaranteed per kg of product): zinc, 17,500 ppm; iron, 12,500 ppm; copper, 2,000 ppm; iodine, 187.50 ppm; selenium, 75 ppm.
[3] Digestible minimum concentration of calcium butyrate in the microcapsules: 45%. Encapsulated butyric acid was added to the feed instead of the inert material (starch) at 0.03, 0.075, and 0.15%.

The encapsulated source of butyric acid (ButiPEARL; Kemin Industries, São Paulo, SP) used in the experiment consisted of 45% butyrate salt. The butyric acid is manufactured using a spray freezing process (MicroPEARLS™ patented technology), which envelops butyric acid in a fatty matrix, reducing odor and also allowing slow release of organic acid along the poultry intestine.

Chickens and their feed were weighed at 14, 21, and 42 days to calculate average weight (AW), weight gain (WG), feed intake (FI), and feed conversion (FC) (corrected with dead weight). Dead chickens were identified and weighed to adjust FI and FC. Mortality was recorded daily and considered for performance test (done weekly).

At 14 and 42 days old, one chicken per group (total of six chickens per treatment) was euthanized, and 1 cm of duodenum and jejunum were immediately collected after slaughter and fixed with 10% neutral buffered formalin solution for histological examination. After hematoxylin-eosin staining, the fragments were subjected to a histomorphometry analysis to measure villus height and crypt depth, using the ImageJ 1.41 software program (Rasband, 2015). Villus height was measured from the tip of the villus to the base where it joins the crypt, and crypt depth was defined as the depth of invagination of the crypt with adjacent villi.

Cloacal swabs were collected from chickens (8, 20, and 40 days old) to verify the excretion of *Salmonella* fecal excretion. Liver and spleen fragments from chickens at 8, 15, 28, and 42 days old were also collected to assess the invasiveness of the bacteria in extra-intestinal tissues.

To test for the presence of *Salmonella* in cloacal swabs, liver and spleen fragments were collected from six chickens per treatment, immediately after slaughter. The samples were analyzed with technique adaptation by methods proposed by the Georgia Poultry Laboratory methods (1997) and the Ministério da Agricultura, Pecuária e Abastecimento (Brasil, 2018).

Samples biochemically confirmed as *Salmonella* were subjected to serological tests using anti-O polyvalent serum. Samples confirmed as positive both by biochemical and serological tests were subjected to a reference laboratory for serotyping.

2.4. Statistical analysis

All data were subjected to simple regression analysis. Variables presenting a level of significance higher than 5% (P>0.05) were subjected to an analysis of variance (ANOVA), and the means were compared by Tukey's test at 5%. The statistical program SAS® (Statistical Analysis System, version 8) was used in all the statistical analyses. To evaluate colonization by SE, a descriptive test that considered the frequency was used. The following model was used for the ewe-related variables:

$$Y_{ijk} = \mu + S_i + \alpha_{ij} + T_k + TS_{ik} + \beta_{ijk},$$

in which Y_{ijk} = value observed with encapsulated butyric acid with or without SE inoculation, μ = overall constant (population mean), S_i = effect of supplementation with encapsulated butyric acid (i = 0.03; 0.075; 0.15), α_{ij} = random error associated with each observation Y_{ijk}, T_k = effect of SE inoculation, TS_{ik} = effect of interaction between encapsulated butyric acid and SE inoculation, and β_{ijk} = random error associated with each observation Y_{ijk}.

3. Results

In the performance analysis, we observed that the SE infection had a negative effect (P<0.05) on AW, WG, FI, and FC during the period of 1 to 14 days after birth (Table 2). At this same age, the highest feed conversion was achieved with the highest rate of butyric acid inclusion in inoculated and non-inoculated groups (P<0.05) when compared with the values in the placebo group.

Table 2 - Performance of 14-day-old broilers inoculated with *Salmonella* Enteritidis (SE) and treated with doses of butyric acid

EBA level	1 to 14 days			
	AW (g)	WG (g)	FI (g)	FC (g/g)
Placebo	462.78	417.80	536.26	1.29b
0.03%	460.43	415.49	542.49	1.30b
0.075%	459.36	414.47	541.26	1.31ab
0.15%	456.56	411.59	550.08	1.36a
Regression	NS	NS	NS	NS
Contamination with SE				
Non-inoculated	475.90a	430.92a	564.12a	1.31
Inoculated	443.66b	398.75b	523.96b	1.32
		P-value		
Levels	0.777	0.785	0.164	0.012
Contamination	<0.0001	<0.0001	<0.0001	0.731
Levels × contamination	0.535	0.524	0.148	0.748
CV (%)	3.20	3.55	2.78	4.09

EBA - encapsulated butyric acid; AW - average weight; WG - weight gain; FI - feed intake; FC - feed conversion; NS - not significant; CV - coefficient of variation (%).
a,b - Lowercase letters (ANOVA followed by Tukey's test) in the same column indicate significant differences (P<0.05).

During the period from 1 to 21 days old (Table 3), the chickens that received the lowest dose of acid (0.03%), both inoculated and non-inoculated with SE, attained the best AW and WG (P<0.05). The SE infection also affected FI rate and FC (P<0.05). In 42-day-old poultry, no interaction was detected between the studied factors (EBA × SE inoculation) in AW, WG, FI, and FC (P>0.05) (Table 4). Over the 42 days of age, there was no significant difference (P>0.05) in mortality among treatments.

Histomorphometry of the duodenum in 14-day-old poultry (Table 5) revealed that the inoculation with *Salmonella* reduced the villus height and the villus to crypt ratio (P<0.05). Similarly, SE reduced the villus height in jejunum at 14 days in the inoculated groups (P<0.05) (Table 6). The supplementation with the lowest dosage (0.03%) of EBA produced beneficial effects on villus height, regardless of inoculation (P<0.05). At 42 days, the inoculation affected crypt depth in the jejunum of poultry (P<0.05) (Figure 1).

Table 3 - Performance of 21-day-old broilers inoculated with *Salmonella* Enteritidis (SE) and treated with doses of butyric acid

EBA level	1 to 21 days			
	AW (g)	WG (g)	FI (g)	FC (g/g)
Placebo	911.76b	866.76b	1414.40	1.63
0.03%	955.50a	910.56a	1448.20	1.59
0.075%	912.95b	868.06b	1435.50	1.65
0.15%	923.00b	878.03b	1426.40	1.63
Regression	NS	NS	NS	NS
Contamination with SE				
Non-inoculated	929.85	884.86	1458.87a	1.65a
Inoculated	922.19	887.28	1402.91b	1.60b
		P-value		
Level	0.040	0.040	0.203	0.167
Contamination	0.436	0.440	<0.0001	0.028
Levels × contamination	0.089	0.089	0.105	0.152
CV (%)	4.5	4.73	2.93	4.04

EBA - encapsulated butyric acid; AW - average weight; WG - weight gain; FI - feed intake; FC - feed conversion; NS - not significant; CV - coefficient of variation (%).
a,b - Lowercase letters (ANOVA followed by Tukey's test) in the same column indicate significant differences (P<0.05).

Table 4 - Performance of 42-day-old broilers inoculated with *Salmonella* Enteritidis (SE) and treated with doses of butyric acid

EBA level	1 to 42 days			
	AW (g)	WG (g)	FI (g)	FC (g/g)
Placebo	2112.55	2067.60	4212.10	2.07
0.03%	2114.41	2069.50	4230.40	2.04
0.075%	2109.40	2064.50	4203.60	2.02
0.15%	2082.88	2037.90	4153.50	2.04
Regression	NS	NS	NS	NS
Contamination with SE				
Non-inoculated	2113.92	2068.90	4214.10	2.03
Inoculated	2097.24	2052.30	4187.70	2.05
		P-value		
Levels	0.798	0.798	0.758	0.429
Contamination	0.559	0.562	0.673	0.183
Levels × contamination	0.738	0.736	0.635	0.903
CV (%)	4.11	4.19	4.47	2.85

EBA - encapsulated butyric acid; AW - average weight; WG - weight gain; FI - feed intake; FC - feed conversion; NS - not significant; CV - coefficient of variation (%).

Table 5 - Intestinal histomorphometry, average of villus height (VH), crypt depth (CD), and villi to crypt ratio (V:C) of the duodenum of 14- and 42-day-old chickens inoculated with *Salmonella* Enteritidis (SE) and treated with doses of encapsulated butyric acid (EBA)

EBA level	14 days			42 days		
	VH (µm)	CD (µm)	V:C	VH (µm)	CD (µm)	V:C
Placebo	1,104.82	199.92	5.52	1,272.56	243.48	5.22
0.03%	1,112.57	225.20	4.94	1,264.06	228.62	5.52
0.075%	1,104.03	212.63	5.19	1,395.99	205.57	6.79
0.15%	1,031.74	221.67	4.65	1,287.15	213.42	6.03
Regression	NS	NS	NS	NS	NS	NS
Contamination with SE						
Non-inoculated	1,176.12a	209.65	5.60a	1,259.93	221.16	5.85
Inoculated	1,013.92b	221.95	4.56b	1,352.69	224.91	6.01
			P-value			
Levels	0.402	0.737	0.158	0.519	0.054	0.095
Contamination	0.002	0.227	0.0007	0.136	0.599	0.183
Levels × contamination	0.231	0.842	0.055	0.382	0.208	0.128
CV (%)	12.66	15.36	16.88	15.94	15.44	15.33

NS - not significant; CV - coefficient of variation (%).
a,b - Lowercase letters (ANOVA followed by Tukey's test) in the same column and in the same age group indicate significant differences (P<0.05).

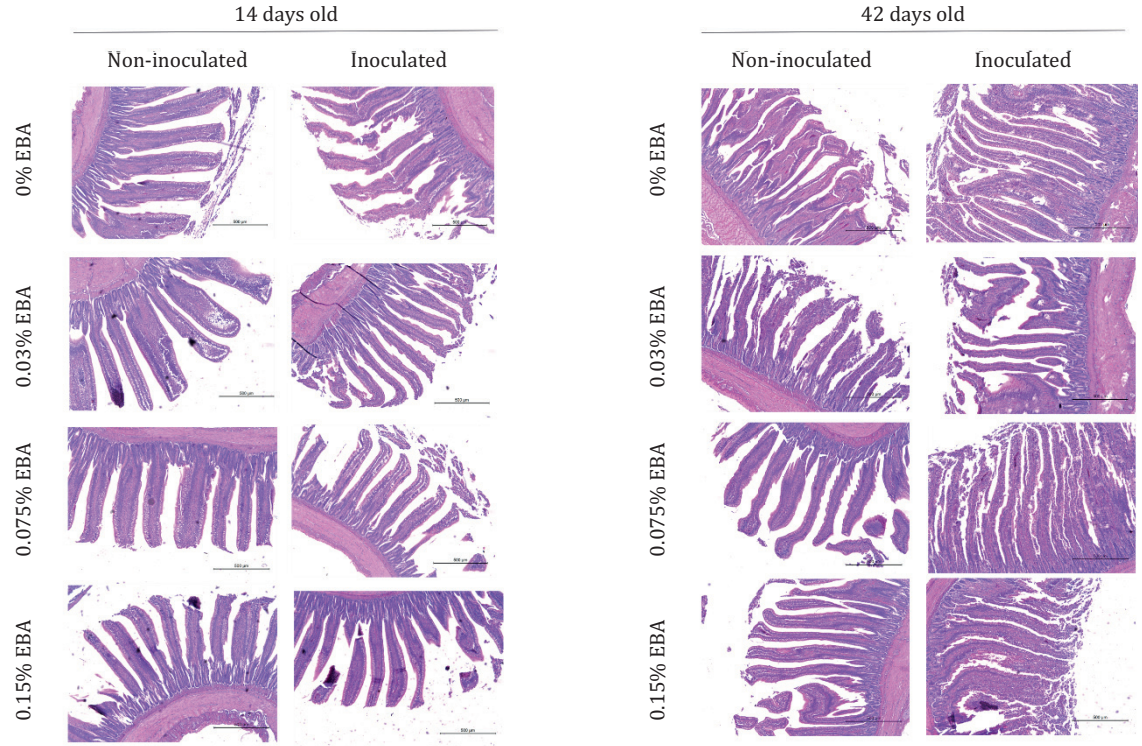

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Figure 1 - Histomorphometry of villi height and crypt depth in the jejunum of 14- and 42-day-old chicks non-inoculated and inoculated with *Salmonella* Enteritidis, fed diet supplemented with different levels of encapsulated butyric acid (EBA).

The presence of *Salmonella* in the liver and spleen at 8-15 days was not observed in any of the treatments (Table 7). At 28 days, SE was detected in the organs of the inoculated group that received 0.15% of EBA. At 42 days, the bacteria was not isolated in any of the inoculated groups. *Salmonella* sp. was detected in cloacal swabs of positive control group at eight days (Table 7). In 20-day-old chickens, the bacteria was recovered of inoculated groups treated with 0.03 and 0.15% EBA.

Table 6 - Intestinal histomorphometry, average of villus height (VH), crypt depth (CD), and villi to crypt ratio (V:C) of the jejunum of 14- and 42-day-old chickens inoculated with *Salmonella* Enteritidis (SE) and treated with doses of encapsulated butyric acid (EBA)

EBA level	14 days			42 days		
	VH (µm)	CD (µm)	V:C	VH (µm)	CD (µm)	V:C
Placebo	734.62ab	172.15	4.26	926.79	162.39	5.70
0.03%	822.99a	181.32	4.53	742.72	148.59	4.99
0.075%	740.21ab	171.12	4.32	946.73	160.37	5.90
0.15%	692.71b	160.19	4.32	837.64	157.61	5.31
Regression	NS	NS	NS	NS	NS	NS
Contamination with SE						
Non-inoculated	790.96a	171.71	4.60a	839.88	145.90b	5.75
Inoculated	711.74b	170.30	4.17b	904.34	170.61a	5.30
			P-value			
Levels	0.017	0.159	0.942	0.051	0.794	0.110
Contamination	0.008	0.860	0.044	0.225	0.016	0.676
Levels × contamination	0.484	0.397	0.635	0.484	0.397	0.635
CV (%)	11.18	12.49	17.17	11.18	12.49	17.17

NS - not significant; CV - coefficient of variation (%).
a,b - Lowercase letters (ANOVA followed by Tukey's test) in the same column and in the same age group indicate significant differences (P<0.05).

Table 7 - Frequency of *Salmonella* Enteritidis isolation in organs and cloacal swabs of inoculated chicks treated with doses of encapsulated butyric acid

Treatment	Contamination by *Salmonella* Enteritidis (%)						
	Organs (liver and spleen)				Cloacal swabs		
	8 d	15 d	28 d	42 d	8 d	20 d	40 d
PC[1]	0	0	0	0	16.6	0	0
0.03%	0	0	0	0	0	16.6	0
0.075%	0	0	0	0	0	0	0
0.15%	0	0	16.6	0	0	16.6	0

[1] PC: positive control (inoculated with *Salmonella* Enteritidis; not treated).

4. Discussion

The inoculation with SE negatively influenced performance of poultry at 14 days. Probably, these results observed in the inoculated animals may have been caused by lesions in the intestinal mucosa caused by the bacterium. Injuries in the intestinal mucosa caused by bacteria of the genus *Salmonella* alter the intestinal physiology, reducing the absorption of nutrients and, consequently, the performance of the birds. Moreover, the competition between *Salmonella* and the host for nutrients, associated with the inflammatory response and the activation or dysregulation of the immune system, costs energy that may reduce FI and WG (Vandeplas et al., 2009; Quinteiro-Filho et al., 2012; Zhen et al., 2018; Sikandar et al., 2022).

The best AW and WG was observed during the period from 1 to 21 days in the birds treated with the lowest dose of EBA (0.03%). A similar result was reported by Chamba et al. (2014), who found the best mean weight gain rates in chickens in the growth phase supplemented with partially protected butyric acid. Dietary acidification may influence the microflora of the gastrointestinal tract, making the intestinal lumen less favorable to the growth of pathogenic bacteria sensitive to acid (Dibner and Buttin, 2002). Thus, the inhibitory effect on the bacterial population increases the availability of energy and nutrients to the animal, improving its growth rate and feed efficiency (Øverland et al., 2000; Canibe et al., 2001).

Feed intake of 21-day-old chicks was reduced in inoculated groups, which can be justified by the anorexia that these animals develop (Halavatkar and Barrow, 1993). The inoculated birds showed a higher feed conversion rate than the placebo group, which can be explained by compensatory WG, because there is a reduction maintenance requirement during the refeeding period (Yu and Robinson, 1992). Jia et al. (2009) reported a similar effect in birds inoculated with *Clostridium perfringens*.

In the period of 1-42 days, the inoculation with SE did not affect the performance, corroborating the findings by Ribeiro et al. (2007) and Zhen et al. (2018). The addition of butyric acid to the diet was also found to not have any significant effect on the performance in the same period. Moreover, it did not affect the FI rate. Conflicting results were found by Levy et al. (2015), who also included 0.03% of ButiPearl™ in the feed and found an increase in WG and better feed conversion of birds at this same age, which indicates that other variables, such as the buffering effect of the diet, may interfere with the action of the product.

The SE infection reduced the villus height and the villus to crypt ratio in both the duodenum and jejunum. This result is similar to those found by Moharreri et al. (2022) and Liu et al. (2023), who identified significant morphological changes in the ileum and jejunum portions, respectively, in birds challenged with SE. In oral infection, *Salmonella* promotes an influx of heterophils and monocytic phagocytes, which results in damage including fusion and shortening of villi and damage of follicle-associated epithelium (Iqbal et al., 2005; Wigley, 2014). The overproduction of reactive oxygen species caused by SE can also cause damage to epithelial cells and other histopathological changes (Liu et al., 2023).

The shorter length of the duodenal villi negatively affected the performance of birds, as was demonstrated in this study. A high villus to crypt ratio indicates a long villus in which the epithelium is sufficiently matured and functionally active, in combination with a shallow crypt, resulting in better digestive efficiency (Nabburs, 1995; Kaczmarek et al., 2016). On the other hand, a lower ratio may indicate the presence of destroyed villi and greater cell proliferation in the crypts, resulting from the attempt to restore the intestinal epithelium (Viola and Vieira, 2007).

In the jejunum, supplementation with the lowest dosage (0.03%) of butyric acid produced beneficial effects on villus height, regardless of inoculation, similar result observed by Czerwiński et al. (2012) and Yang et al. (2019). Chamba et al. (2014) and Wu et al. (2018) also reported that the length of jejunal villi was greater in chickens supplemented with partially protected butyric acid.

Since organic acids can provide energy substrates for the intestinal epithelium, they may have a trophic effect on the mucosa of the small intestine, improving their absorptive capacity. Other mechanisms by which short chain fatty acids enhance the development of enterocytes include the production of pancreatic secretions, enterotrophic gastrointestinal hormones, and stimulation of blood flow and autonomic nervous system (Rombeau et al., 1995).

As observed in this study, at 42 days, the inoculation increased crypt depth in the jejunum. Similar findings were also reported by Andrade et al. (2012), who found deepest crypts in turkeys inoculated with SE. According to these authors, the crypt depth is correlated with cell replacement rate (cellular turnover), and this indicates a compensatory response to cell destruction caused by *Salmonella*.

At eight days, SE was found only in fecal samples of the positive control group, which suggests the effects of EBA on these bacteria. However, this result infers an effect limited to the intestinal environment, since SE was found only in the liver and spleen of 28-day-old chickens that received the highest dosage of organic acid. Van Immerseel et al. (2004) also found similar results, although such supplementation did not influence the invasion of pathogens in deeper tissues, such as the liver and spleen.

At 20 days, it was possible to isolate bacteria from cloacal swabs from chickens supplemented with 0.03 and 0.15% of the commercial product. This may be due to the phenomenon known as acid tolerance response. *Salmonella* can become more resistant to acidic environments after a short period of adaptation to a moderately acidic pH, process that involves the action of several regulons, especially those controlled by RpoS, Fur, PhoPQ, and OmpR/EnvZ (Rychlik and Barrow, 2005).

For the development of ATR, it is necessary that the bacteria be exposed to an adaptation pH (4.5-6.0) for 1 to 4 h (Ye et al., 2019). Thus, *Salmonella* is able to carry out this pre-adaptation in the birds' ingluvium, where the pH is between 4-5, due to bacterial lactic acid fermentation, inducing acid tolerance prior to entry in the gizzard. This natural resistance can be further potentiated by contact with bile and short chain fatty acids present in the intestine (Rychlik and Barrow, 2005).

The absence of the bacterium in liver and spleen samples at 8-15 days in the treatments can be explained by the presence of antibodies against SE that the poultry received from their mothers that were immunized (Inoue et al., 2008). According to Si et al. (2014), poultry of mothers vaccinated against SE acquire high levels of antibodies of maternal origin, which can significantly reduce the infection in these chickens. Additionally, *Salmonella* was not isolated in any of the treatments at 40-42 days. These results are justified by the development of immune system and gradual installation of gut microbiota that contribute to the reduction of the susceptibility of the host to *Salmonella*, since the microbiota starts to compete for substrates and prevents the survival of *Salmonella* (Chung et al., 2012).

The form and intensity at which the supplementation with butyric acid may influence the composition and diversity of the intestinal microbiota of broilers (Wu et al., 2018) are still poorly understood. Previous studies have revealed a marked reduction of Lactobacillaceae in the intestinal lumen of animals treated with butyric acid (Huang et al., 2015; Wu et al., 2018). The bacteria of the family Lactobacillaceae, such as *Lactobacillus* sp., are considered beneficial to the host, converting glucose into lactic acid in the poultry intestine, causing the inhibition of pathogenic bacteria such as *Salmonella* sp. and *Escherichia coli* (Ashan et al., 2016; Al-Khalaifa et al., 2019; Merino et al., 2019; Mustafa et al., 2022).

5. Conclusions

The inclusion of 0.03% encapsulated butyric acid in the chicken feed controls the *Salmonella* Enteritidis colonization in the intestine, favors the growth performance of broiler chickens up to 21 days old, and produces beneficial effects on jejunum villus height.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Leonídio, A. R. A. and Andrade, M. A. **Data curation:** Leonídio, A. R. A.; Minafra, C. and Stringhini, J. H. **Formal analysis:** Leonídio, A. R. A. and Santos, J. B. **Funding acquisition:** Leonídio, A. R. A. and Nascimento, G. M. **Investigation:** Leonídio, A. R. A. and Nascimento, G. M. **Methodology:** Leonídio, A. R. A.; Minafra, C. and Andrade, M. A. **Project administration:** Andrade, M. A. and Almeida, A. M. S. **Resources:** Leonídio, A. R. A. and Andrade, M. A. **Software:** Leonídio, A. R. A. and Santos, J. B. **Supervision:** Leonídio, A. R. A.; Andrade, M. A. and Stringhini, J. H. **Validation:** Leonídio, A. R. A.; Andrade, M. A.; Stringhini, J. H. and Almeida, A. M. S. **Visualization:** Leonídio, A. R. A.; Minafra, C.; Nascente, E. P. and Almeida, A. M. S. **Writing – original draft:** Leonídio, A. R. A. and Andrade, M. A. **Writing – review & editing:** Leonídio, A. R. A.; Nascente, E. P. and Almeida, A. M. S.

Acknowledgments

The authors would like to express their gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Kemin do Brasil Ltda., and Escola de Veterinária e Zootecnia of the Universidade Federal de Goiás.

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