Canadian Science Publishing | Canadian Journal of Animal Science

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Journal:	*Canadian Journal of Animal Science*
Manuscript ID	CJAS-2023-0002
Manuscript Type:	Article
Date Submitted by the Author:	16-Mar-2023
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Keywords:	butyric acid, blood profile, coccidiosis, gut health, Poultry

Supplementation of microencapsulated sodium butyrate on the performance, haemato-biochemical profile and intestinal microbiota composition of broiler chickens challenged with *Eimeria* spp.

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Declaration of interests: none.

Abstract

The aim of this study was to evaluate the effect of microencapsulated sodium butyrate (MSB) in substitution to antibiotics on performance, haematology, intestinal injury scores and oocyst count in the excreta of broilers challenged with Eimeria spp. 1,050 male Ross chicks were used, distributed in completely randomized design, with six treatments: unchallenged control diet (UC); challenged control diet (CCD) ; challenged and supplemented (CS) with 1,000 mg/kg of MSB; CS with 1,500 mg/kg of MSB; CS with 2,000 mg/kg of MSB; CS with avilamycin, with five repetitions. At 16 days of age the birds were inoculated orally and individually with Eimeria. Higher average weigh gain (AWG) and average feed intake (AFI) were observed in the UC, and in the final breeding phase, better feed conversion ratio (FCR) and productive efficiency factor (PEF). The microbiota that had received additives showed higher relative abundance of the phylum Bacteroidota, as well as that of the unchallenged birds; however, the CCD had higher relative abundance of the phylum Firmicutes. It is concluded that MSB can be used as an alternative to antibiotics, assisting in the recovery of performance, haematology, allometry of organs of the GIT.

Keywords: butyric acid, blood profile, coccidiosis, gut health, poultry.

1- Introduction

The increase in productivity and growth of broiler breeding have prompted the need to use antibiotics to protect animal organism from pathogens, and consequently, improve its performance. However, due to restrictions on the use of these antibiotics by the European Union in 2006, alternatives that could replace them had to be sought (Salazar *et al.,* 2008; Widiastuti *et al.,* 2019).

In animal nutrition, organic acids and their salts are viable options that improve performance and exert antibacterial and pH-lowering effects with the function of providing energy to intestinal cells (Melaku *et al*., 2021). The use of substances that can substitute growth promoters has been widely studied. Microencapsulated butyric acid is known to decrease the pH of the intestinal mucosa, creating an acidic environment for the growth of microorganisms harmful to the health of the host. It is one of the most effective food additives to be used in animal nutrition to improve morphology, small intestine functions, and as a result, performance. In addition, it reduces the effects of losses caused by stress or diseases that affect animals (Abdel-Fattah *et al*., 2008; Santos *et al*., 2012).

Nutrition is one of the tools used by nutritionists to modulate the immune system of poultry in order to produce an ideal state in the organism, as immunity changes require feed energy and nutrients to form cells and other substances involved in the defence system (Cardoso and Tessari, 2015). Immune improvement through nutrition can help to reduce the use of chemicals, such as performance enhancers (Qureshi, 2002).

When nutrition is combined with immunity, it facilitates modulating animal health through the dietary nutrients provided in the adequate amount for maintenance and homeostasis, allowing the animal to respond to any infections and diseases (Grimble, 2002).

When there is high competition with pathogenic bacteria, the intestinal mucosa

is damaged, leading to epithelial lesions, and thus poor digestion and absorption of nutrients. Nevertheless, when this first line of defence – the intestinal mucosa – fails, the immune cells act as a second line to fight the offending agent (Kim and Lillehoj, 2019). Such cells include leucocytes and NK (natural killer) cells, which destroy the infected ones. In some cases, this second line of defence alone is not enough to help the organism to fight the invading agent; then, a third line of defence comes into play, in which the lymphocytes intervene along with the leucocytes so that the animal can recover from damage caused by the pathogens (Cruvinel *et al*., 2010).

In view of the above circumstances, the objective of this study was to evaluate the effect of microencapsulated sodium butyrate (MSB) to replace antibiotics on the performance, haematology, allometry of gastrointestinal (GIT) organs, oocyst count, intestinal epithelium lesion scores, and characterization of the intestinal microbiota in broilers challenged with *Eimeria acervulina*, *E. maxima*, and *E. tenella*.

2- Material and Methods

The experiment was carried out in accordance with the principles and rules of the Ethics Committee on the Use of Animals – CEUA of São Paulo State University – Unesp, Campus of Dracena (protocol number 18/2018).

2.1 Birds, design, and experimental diet

A total of 1,050 male Ross® broiler chicks were reared from one to 42 days of age (d.o.) in 30 pens with rice husks bedding, each containing 35 animals/pens. The birds were distributed in a completely randomized design with six treatments and five repetitions. The treatments consisted of: UC - unchallenged control diet (corn-soybean meal); CC- challenged control diet; 1,000 - challenged and supplemented with 1,000 mg/kg of MSB; 1,500 - challenged and supplemented with 1,500 mg/kg of MSB; 2,000 -

challenged and supplemented with 2,000 mg/kg of MSB; AVL - challenged and supplemented with avilamycin, with five repetitions.

The butyric acid used in this study is a commercial product which contains 30% sodium butyrate (SB) coated with palm oil. The antibiotic used was avilamycin 20% (20 g/t, with 10g avilamycin activity).

Water and feed were provided *ad libitum*. The feeding program was divided into four phases: pre-starter (1 – 7 days), starter (8 – 21 days), grower (22 – 33 days), and finisher (34 – 42 days). The isoenergetic and isoaminoacidic diets were formulated with corn and soybean meal, as recommended by Rostagno *et al*. (2017) (Table 1). Additives were included to substitute the inert material (kaolin). Antibiotics and coccidiostats were removed from the vitamin-mineral supplement to avoid interfering with the treatments.

(INSERT TABLE 1)

2.2 Challenge with *Eimeria* spp.

An adapted protocol consisted of inoculating 0.5 mL suspension containing *Eimeria acervulina*, *E. maxima* and *E. tenella* orally at 16 d.o. to cause moderate lesions in the intestinal epithelium of the broilers (Freitas *et al*., 2008). The concentrations used were $2x10^5$ sporulated oocysts/broiler *E. acervulina* and $2x10^4$ sporulated oocysts/broiler *E. maxima* and *E. tenella* (Holdsworth *et al*., 2004), all of which were stored in a glass beaker and diluted in distilled water. The inoculation process occurred individually. Each broiler was restrained manually, and with the aid of an automatic pipette they received the suspension, which improved the veracity of the challenge. The unchallenged treatment received 0.5 mL saline solution (placebo). The three species were chosen for their importance in the broiler production because of their high incidence and the economic losses that they can promote (Williams, 1999, 2005).

2.3 Response variables

2.3.1 Performance

The performance variables evaluated were average weight gain (AWG), average feed intake (AFI), feed conversion ratio (FCR) and viability (VB) for periods 1 to 21 and 1 to 42 days of rearing, as well as productive efficiency factor (PEF) at the final of the total period. The VB of each experimental unit was obtained by subtraction: 100 - mortality, which was calculated as a percentage. The PEF was calculated for the total rearing period by the formula:

$$PEF = \frac{(VB \times AWG)}{(FCR)/10}$$

2.3.2 Haematological and biochemical variables

The broilers were sampled at 21 and 42 d.o., and 3 mL blood were collected from 10 birds/treatment through brachial vein puncture. A microhematocrit centrifuge was used to determine haematocrit in heparinised whole blood, in which capillary was used and centrifuged at 1,200 rpm for 5 min; afterwards, the results were estimated in percentages using a reading ruler that is part of the centrifuge (Microhematocrit Centrifuge - Model 1180 - BENFER).

Haemoglobin concentration was determined using the cyanomethemoglobin method (Campbell, 1998), and standardised formulas were used to calculate the Wintrobe indices, which are MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), and MCHC (mean corpuscular haemoglobin concentration).

$$\text{Leucocyte count (per µl)} = \frac{\text{Leucocyte count x erythrocyte count (per µl)}}{\text{2,000 (erythrocyte count)}}$$

$$MCV\ (fl) = \frac{\text{Haematocrit}}{}$$

$$\text{MCH (pg/cell)} = \frac{\text{Haemoglobin x 10}}{\text{Total erythrocyte Erythrocyte count}}$$

$$\text{MCHC (g/dL)} = \frac{\text{Haemoglobin concentration x 100}}{\text{Haematocrit}}$$

The counting of the number of total and differential cells in blood extensions (Charles Noriega, 2000) was performed on a blood sample containing Natt-Herrick stain solution (1:200 dilution). The erythrocyte count was carried out in a Neubauer chamber, and the leucocyte one in blood extension on glass slides stained with Rapid Panoptic. Total proteins, albumin, glucose, and haemoglobin were determined using photocolorimetric techniques with reagent kits (Labtest Diagnóstica, Brazil).

2.3.3 Relative organ weight

At 21 and 42 d.o., two broilers per pen were sacrificed by atlanto-occipital joint dislocation, followed by bleeding to assess the reactions that occur in their organism and GIT, so as to understand the mechanisms affecting the digestion of nutrients in the diets tested. Therefore, the relative organ weight and the length of the small and large intestines were determined. The spleen, pancreas, gizzard, proventriculus, liver, small intestine and large intestine were removed and weighed to determine the relative weight, and the length of the small and large intestines were measured. Spleen, pancreas, proventriculus, and liver were weighed immediately after being withdrawn, and the gizzard was opened and weighed after its contents were eliminated. Following their removal, the small and large intestines were separated by sections exactly where the duodenum emerges from the gizzard and at the beginning of the caecum, being subsequently weighed and measured. The measurement of the large intestine was considered by the length of the colon and rectum added to the length of the caeca.

189

190 **2.3.4 Oocyst count and intestinal epithelium lesion scores**

191 At 4, 5, 6, and 7 days after inoculation, samples of excreta were collected for
192 oocyst count. The freshest faeces present in the bed of each experimental unit were
193 collected and homogenized, and two grams of each sample were diluted in 28 mL
194 saturated sodium chloride (NaCl) solution (Gordon and Withlock, 1939). Counting was
195 performed in a MacMaster chamber, and the results are expressed as the number of
196 oocysts per gram of faeces (OPG) (Hodgson, 1970).

197 At 21 and 42 d.o., the lesion scores in the small intestine were evaluated
198 according to the Johnson and Reid methodology (1970).

199

200 **2.3.5 Intestinal Microbiota (PCR and DGGE)**

201 At 21 d.o., 20 intestinal samples were aseptically removed and stored in sterile
202 microtubes, where they were immediately frozen in a freezer at -80ºC and, soon
203 afterwards, sent to the company NGS Soluções Genómicas (Piracicaba, SP) for
204 sequencing (paired library) on an Illumina MiSeq.

205 For this, primers for the V3-V4 regions containing adapters for Illumina MiSeq
206 sequencing were used for PCR amplification of the 16S rRNA gene. A first PCR (16S
207 rRNA V3-V4) was performed under the following conditions: 95 °C for 3 min, followed
208 by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension
209 at 72 °C for 5 min. A second PCR was subsequently performed with the index
210 sequences under the following conditions: 95 °C for 3 min, followed by 12 cycles of 95
211 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min.
212 PCRBio Ultra Mix (PCR Biosystems, London, United Kingdom) was used in both PCR
213 reactions, while AMPure XP beads (Beckman Coulter, Brea, CA, USA) were used for
214 purification. The samples were grouped into sequencing libraries, and the amplicons

were sequenced with an Illumina MiSeq platform using the paired-end, 250-cycle V3 MiSeq Reagent Kit.

The libraries were prepared following the Illumina recommendations (Figure 1). The primers used for *locus*-specific amplification of *archaea* or bacteria flank specific regions of the 16S rRNA. The Illumina linker sequences that were hybridized to the sequences immobilized on the sequencing slide are:

Forward overhang: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus-specific sequence].

Reverse overhang: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus-specific sequence].

The sequencing was performed on the Illumina Miseq system, as described in Figure 1, and the readings produced were 2x250 bp.

INSERT FIGURE 1

2.4 Statistical analysis

Data analysis was performed using the statistical analysis system (SAS) (2012), with 5% significance criterion. Firstly, analysis of normality of the residues by the Shapiro-Wilk test (PROC UNIVARIATE), as well as analysis of homogeneity of variances were conducted. Data that did not show residual normality by the Shapiro-Wilk test were submitted to logarithmic transformation. Successively, following the aforementioned premises, the data were submitted to analysis of variance (ANOVA) by PROC GLM, and when there was a significant effect, the means were compared by Tukey test with 5% significance.

Microbiota data were analysed as previously published (Callahan et al., 2016), using a set of packages implemented in the R language (Team, 2013) and available from the BioConductor project (Gentleman et al, 2004; Huber et al., 2015):

Bioconductor Workflow for Microbiome Data Analysis: from raw reads to community analyses.

After the initial processing of the sequencing data by DADA2, the taxonomies were assigned to each ASV (Amplicon Sequencing Variants) using a DADA2 program implementation of the Naive Bayes classifier for this purpose (Wang *et al*., 2007). The taxonomic classifications generated by DADA2, and their quantifications were imported into the phyloseq program (Mcmurdie and Holmes, 2013), also implemented in R. Each sample richness and abundance were used to calculate the Shannon and Simpson diversity indices of bacteria.

3- Results

3.1 Performance

In the period from 1 to 21 d.o. (Table 2), a difference was observed between diets for AWG and AFI. It is noted that the supply of a diet with no additives or without inoculation of Eimeria species provided the birds with a higher AWG ($P < 0.001$) compared to the other treatments, which emphasizes that the sanitary challenge was efficient and that the inoculated birds suffered a drop in performance. These chickens also presented the highest AFI, although similar to the other ones, differing ($P = 0.0103$) only from the birds that received 2,000 mg/kg of MSB.

(INSERT TABLE 2).

At 42 d.o. (Table 2) the chickens differed only in FCR and PEF. The best FCR ($P = 0.0108$) and highest production factor ($P = 0.0055$) occurred in birds that were not inoculated neither received any additive in the ration, showing again the power of the imposed challenge.

3.2 Haematological and biochemical variables

At 21 d.o. (Table 3), differences (P = 0.0512) were observed only in the haematocrit variable, in which challenged broilers had values lower than those unchallenged, indicating possible anaemia because of blood loss as a result of injuries caused in the intestine.

(INSERT TABLE 3)

For the challenged control group and those that received 1,500 mg/kg and 2,000 mg/kg MSB, a high leucocyte count was also identified. As it is known that such cells have a crucial role in the defence of the organism in cases of infection, this finding demonstrates that the immune system of the broiler responded to the effects caused by Eimeria species inoculation.

At the end of the rearing period, 42 d.o. (Table 3), there were no differences in the variables analysed. The haematocrit values found in the challenged and unchallenged broilers were similar (P = 0.7049), and an increase in erythrocyte values was also observed with age, showing that the birds were able to recover from the lesions caused by the protozoa. Moreover, it was possible to detect that all broilers that received either MSB or avilamycin presented higher MCV values compared to unchallenged control, which reveals that these additives contributed to the recovery of the organism.

The data presented in Table 4 show that at the ages 21 and 42 d.o. there were no differences between the variables studied.

(INSERT TABLE 4)

At 21 d.o., high concentrations of the albumin variable were found in all challenged treatments, but the glucose and protein levels remained normal. Albumin – a plasma protein – has many functions, with the most important ones related to the maintenance of plasma osmotic pressure, the transport of substances such as hormones and minerals around the body, immunity, buffer action, and regulation of enzymes (Swenson and O'reece, 1996; Boettcher, 2004).

At the end of the rearing period, the immunity of the broilers was noticed to have been improved by the increase in protein levels. This finding can demonstrate the immune system recovering, since proteins act in the defence of the organism through the modulation of antibodies and defence cells (Melillo, 2013).

Glucose and protein levels were within the standard value indicating homeostasis, which suggests that the broilers were in normal condition. Birds regulate glucose metabolism similarly to the way mammals do, but there are quantitative differences; while the glucose concentration in a cow varies from 40 to 80 mg/dL, laying hens can have values from 130 to 270 mg/dL, ranging from 200 to 500 mg/dL in healthy birds (Swenson and O'Reece, 1996; Schmidt *et al.,* 2007).

3.3 Relative organ weight

Differences were found in the relative liver and pancreas weight at 21 and 42 d.o., respectively (Table 5).

(INSERT TABLE 5)

At 21 d.o., no differences between diets for the variables tested were observed, except for relative liver weight (P = 0.0023). The highest liver weights occurred in broilers that received additives (antibiotic or MSB), yet not differing from the unchallenged ones.

At the end of the rearing period (42 d.o.), differences (P = 0.0266) were verified only in pancreas relative weight. Broilers fed a diet containing antibiotics showed a higher pancreas weight, similar to that in broilers that received MSB, which shows its potential as an additive to replace the chemical growth promoter in the variable analysed.

3.4 Oocyst count and intestinal epithelium lesion scores

When analysing the OPG excreted by the chickens (Table 6), no significant difference between treatments (P = 0.5599) was noted, neither an interaction between treatments nor collection periods (P = 0.3674). Differences were only found between the different periods of excreta collection (P < 0.0001).

Regardless of the treatment, chickens that were inoculated with Eimeria species had significantly higher oocyst excretion on the fourth day after the procedure. (INSERT TABLE 6)

When the intestinal lesion scores were evaluated (Table 7), no significant differences were found between treatments, neither interaction between treatments nor collection periods. There was a difference (P < 0.0001) only between periods of excreta collection. Marked lesions caused by *E. maxima* and *E. tenella* were observed in the intestine of chickens at 5 days post-inoculation (21 d.o.). Nevertheless, at the end of the rearing period (42 d.o.) all the birds presented a decrease in the lesion score, which shows that the additives – MSB and/or antibiotics – contributed to the recovery of the birds.

(INSERT TABLE 7)

3.5 Intestinal Microbiota (PCR and DGGE)

The relative abundances of bacterial phylum and genus are shown in Figures 2 and 3, where variations in bacterial communities are observed between treatments. There was no difference in the Shannon (P = 0.7694) or Simpson (P = 0.7197) indices used to assess the diversity of the microbiota.

INSERT FIGURE 2

It was observed that the most abundant classes in all treatments were *Firmicutes*, and that in challenged control birds there was a great abundance of *Bacteroidota*.

INSERT FIGURE 3

It was observed in all treatments that the most abundant genera were *Lactobacillus*, *Staphylococcus*, and *Clostridia_sensu_strico_1*, with emphasis on *Bacteroides* in the intestine of challenged control birds.

4- Discussion

Good intestinal health status and high nutrient digestibility are important factors in achieving better performance in broilers. The use of substances that can replace growth promoters is richly studied. Microencapsulated butyric acid is widely known to lower the pH of the intestinal mucosa, creating an acidic environment for the growth of microorganisms harmful to the health of the host. It is one of the most effective food additives to be used in animal nutrition to increase weight gain and improve FCR (Abdel-Fattah *et al*., 2008; Pearlin *et al*., 2020).

Sodium butyrate is known to prevent the proliferation of bacteria, thus increasing the possibility of improving the performance of animals as they will no longer compete with the normal microbiota to obtain nutrients (Song *et al.,* 2017).

It is worth mentioning that when additives are included in the diet, it is essential to consider the health challenge imposed to understand their mechanism of action in relation to the responses that will be obtained.

At 21 d.o., the performance decrease in inoculated birds was evident, unlike the non-inoculated ones, which showed higher AWG. This increase in weight reflects the higher consumption exhibited by these birds, which only differed from those supplemented with 2,000 mg/kg MSB, showing lower AFI. This fact may be linked to the amount of butyrate in the ration, which may have been high, affecting the organoleptic characteristics of the ration and causing the intake to reduce. It is known that, although SB is used as a substitute for butyric acid in rations for being solid, more stable and causing less discomfort when handled due to its less intense odour (Zhang

369 *et al*., 2011), it still has a typical smell of cheese, as in some there is butyric
370 fermentation, where such smell comes from (Perry, 2004).

371 In both variables – GPM and CRM – butyrate showed results like those of
372 antibiotics; thus, it can be used as an alternative to the worldwide ban.

373 When analysing the performance of 21-d.o. broilers fed with 0, 100, 200, 300,
374 400, and 500 mg/kg encapsulated butyric acid, Levy *et al*. (2015) verified lower
375 consumption of the diet with the highest dose of acid as well as worse FCR. Likewise,
376 Imran *et al*. (2018), when testing different doses (250, 350, and 450 mg/kg) of
377 microencapsulated butyric acid in the diet of broilers, found a decrease in their intake
378 when they received diets with the highest amount of acid, which was also observed in
379 the present study.

380 Wu *et al*. (2018) found no differences in the performance of broilers at 21 d.o. fed
381 different doses of SB (200, 400, 800, and 1,000 mg/kg) and antibiotic. Such fact was
382 observed in the current study, as well, in which at this stage, the birds that received
383 different doses of acid showed no difference compared to the antimicrobial.

384 Some authors (Boling *et al,* 2000; Abdel-Fattah *et al.*, 2008; Kana *et al.*, 2011;
385 Goiri *et al*., 2021) have reported that the higher levels of acids incorporated into the diet
386 reduce feed intake by the birds, which may because of the palatability of acidified diets
387 during the first few days. However, in the subsequent stages of rearing, birds tend to
388 adapt to the diet acidification.

389 At 42 d.o., a difference was detected only for the variables FCR and PEF. The
390 best FCR and highest production factor occurred in birds that had not been inoculated
391 neither received any additive in the ration, which reveals the power of the imposed
392 challenge once more. Regarding PEF, which considers daily weight gain, feed
393 conversion and viability, the unchallenged birds had the highest index for it, possibly
394 because of the best results obtained in the variables involved in this indicator. The PEF

considered to be excellent has a value above 280, which was the index found in all treatments in the present study (Mendes and Patricio; 2004).

Coccidiosis infection causes disorders in the metabolism of carbohydrates and lipids and may contribute to a worse performance in the zootechnical variables. In addition, the activation of serum enzymes linked to ATP synthesis and utilization may be a potential pathway involved in body weight reduction in broilers challenged with Eimeria. Such fact was observed in the present study, in which all challenged treatments showed worse productive performance (Furtoso *et al*., 2019).

In the analysis of the total rearing period, in view of the conditions imposed in this study and regardless of the level of inclusion, it can be inferred that the replacement of the antibiotic by the MSB proved to be possible given the statistical similarity between the diets in the variables tested.

Nutrition combined with immunity has become increasingly important, as a well-balanced diet contributes positively to the resistance of the animal organism, leading to efficient immune responses against diseases, thus promoting good performance and well-being (Cardoso and Tessari, 2015; Souza and Vargas Junior, 2020).

At 21 d.o., haematocrit levels of challenged birds were lower than those of the unchallenged birds, emphasising that the health challenge was efficacious, weakening the immune system of the birds.

The erythrocyte count was below normal (2.5 to 3.5 x 10^6 μl^{-1}) at 21 d.o., and there were higher concentrations in the number of leucocytes (normal = 11,900 ± 15,6 cel μl^{-1}) and heterophiles (normal = 3,000 to 6,000 cell/mm^3) (Bounous and Stedman, 2000; Borsa, 2009; Lindenwald *et al*., 2021) after challenge, confirming that the immune system of the broilers was affected. The causes of an increased leucocyte count in birds can be linked to inflammations due to infections, haemorrhage, toxicity, fast growing tumours, and so forth (Miguel, 2017).

At 42 d.o., haematocrit levels were higher than they had been in previous rearing phases in broilers challenged with Eimeria species, highlighting that both MSB – regardless of level – and antibiotic were efficacious in helping the body to recover from damage caused by inoculation. Erythrocyte levels increased and were found within normal limits. Such cells tend to be more numerous in males, just as they are likely to increase with age in birds (Mitchell and Johns, 2008; Oliveira *et al*., 2021).

As the broilers grew, they also had a rise in the number of leucocytes as well as in the heterophil/lymphocyte ratio (H/L), which is one based on changes in the blood system and stressors, such as temperature changes, indicating chronic stress (Beirão *et al*., 2012). However, it is not always a viable indicator, owing to the lack of correlation between this ratio and the plasma concentrations of corticosterone, a hormone with immunosuppressive action (Campbell, 2012).

When analysing 7 – 42-day-old broilers, Tessari *et al*. (2006) and Borsa (2009) detected reference values for MCV ranging from 145.65 fL to 170.48 fL, values higher than those found in this research. The MCV values obtained in present study corroborate the normal parameters considered by Tag El-Din *et al*. (2020), who reported similar values in broilers fed SB at doses of 200 and 300 mg/kg. Such fact may be associated to the protective action that butyric acid exerts on GIT, favouring iron absorption and improving the production of B vitamins that positively influence the processes of blood cell formation, since there was intestinal mucosa recovery after the challenge with Eimeria species.

The haematological variables measured at the end of the rearing period point to an improvement – though not significant – in the immunity of the broilers, showing that they recovered from the challenge with Eimeria.

No differences were identified between treatments for the biochemical variables studied at any of the ages tested. At 21 d.o., glucose (standard = 200 to 500

mg/dL) and protein (standard = 2.5 to 4.5 g/dL) levels were within the reference values (Campbell, 2004).

At the end of the rearing period, an increase in blood protein concentration and a decrease in serum albumin were found in the challenged broilers, which may suggest that their intestine managed to recover from the injuries caused by Eimeria species. The albumin represents 40 to 50% of the total plasma protein of birds and is synthesised in the liver, being essential for plasma osmotic pressure regulation (Kaneko *et al*., 1997). It is responsible for maintaining the osmotic balance of the body, regulating the amount of water in the blood. Thus, it can be inferred that the broilers could be dehydrated at 21 d.o. because of the challenge with *Eimeria* spp., causing them haemorrhagic diarrhoea, which triggered haemoconcentration, that is, an elevation in plasma albumin (Thrall *et al*., 2012; Chen *et al*., 2020; López-Osorio *et al*., 2020).

Raza *et al*. (2019), in their research with butyric acid in broiler diets, reported a rise in serum protein and a reduction in albumin in those that received 3,000 and 4,000 mg/kg butyric acid compared to the control treatment.

Butyric acid promotes intestinal health improvement resulting in the absorption of more nutrients throughout the GIT, as it is used as an energy source for enterocytes, which are absorptive cells present in the intestine (Imran *et al*., 2018).

At 21 d.o., the diets were revealed to be different only for relative liver weight. Among the inoculated broilers, the use of additives enabled recovery, since they increased the weight of this organ – similarly to the unchallenged birds – which suggests the effectiveness of the product used in this investigation.

The liver is responsible for detoxification and bile production, indicating that the increase in this organ weight depends on the amount of activity it performs (Ogwuegbu *et al*., 2021). Brenes *et al*. (1993) stated that, after absorption, the liver is the main site for the metabolism of short-chain fatty acids; therefore, it is suggested

that liver weight depends on the GI microbiota and/or its fermentation products. This important organ is also responsible for synthesising blood clotting proteins as well as proteins involved in immunity, such as albumin (Zaefarian *et al*., 2019). This fact confirms the higher levels of blood albumin found at 21 d.o., in which the broilers were in critical period because of the challenge with Eimeria.

In the present study, it was verified that the highest relative liver weights were obtained in broilers that received MSB, regardless of its level. This may indicate that the additive was efficient in promoting efficacious improvement in the organism of challenged birds, demonstrating that it helped to recover this essential organ that regulates the metabolism of nutrients and the excretion of toxic substances.

At 42 d.o., the diets differed only for relative pancreas weight. In comparison with the previous phase, the use of an additive – either MSB (1,500 mg/kg) or antibiotic – led the broilers to recover, once the weight of this organ increased, unlike that of broilers which did not receive any additive. A greater proportion (size) of the pancreas is expected to be associated to increased pancreatic enzyme activity (Silva *et al*., 2014).

Sodium butyrate works by maintaining the beneficial balance of the intestinal microbiota as it decreases the pH, and it also serves as an energy source for the development of the intestinal epithelium (Silva *et al*., 2020). This was observed by the greater length of the small intestine with the inclusion of 1,000 mg/kg and 2,000 mg/kg, which reflected in better animal performance (Silva and Nörnberg, 2003). The intestine is also the largest immune organ in the body; consequently, it is often clear that a more developed intestine will make the animal healthier, which in turn will digest and use nutrients more efficiently (Chen *et al*., 2022).

Higher organ weight is related to greater organ activity. This also occurs when the weight of immune organs increases, suggesting that there is an improvement in the body responses of healthy animals (Ahsan *et al.,* 2016).

Butyrate is known to supply epithelial cells with energy, elevating their proliferation and differentiation in the intestine. Several studies (Sengupta *et al*., 2006; Guilloteau *et al*., 2010; Chamba *et al*., 2014; Sikandar *et al*., 2017; Lan *et al*., 2020) show that butyrate provides energy to intestinal cells with a trophic effect, in addition to reducing enterocyte apoptosis through its influence on gene expression and protein synthesis. This makes the supplied energy stimulate the development of the intestinal mucosa, promoting an increase in the organ size.

At 42 d.o., the broilers that had received 1,000 mg/kg MSB were observed to have an increase in the relative weight of the spleen, which is an indispensable organ linked to the organism defence in cases of infections, injuries, and diseases, acting in the production of defence cells. Sikandar *et al*. (2017) found higher spleen weight in broilers fed 1,000 mg/kg SB compared to control values. Makled *et al*. (2019), when using 6,000 mg/kg SB in broiler diets, detected higher spleen weight compared to animals that had received prebiotics and yogurt. There are no studies available in the literature highlighting the effects of SB on morphological changes in organs of the immune system to which the results of the present investigation can be compared. Therefore, further research in this area is needed.

When analysing the OPG, higher excretion was detected on the fourth day after inoculation (20 d.o.), when compared to the other periods of excreta collection (5, 6 and 7 days after inoculation). According to Bowman and Georgis (2010), the period between infection and the sign of its detectable stage is called prepatent, and this period can be approximately 5 days for *E. maxima* (Kawazoe, 2009). At 21 d.o., a drop in AWG was observed in all inoculated birds, since they had been intensely infected with oocysts of *E. maxima* and *E. tenella*.

This can be confirmed by analysing the intestinal lesion score in these chickens. At 21 d.o. (five days postinoculation), their intestines were severely damaged due to the action of Eimeria species, indicating that the challenge had been effective and had

529 impaired the intestinal activity of the birds. Such fact can be confirmed by the decrease
530 in consumption and weight gain of the challenged birds. However, at 42 d.o. (26 days
531 postinoculation), there was a significant decrease in lesion scores in all birds, which
532 demonstrates that MSB and/or antibiotics were able to contribute to the recovery of the
533 intestinal mucosa, providing benefits to the immunology of the birds, as observed by
534 the increase in haematocrit levels when comparing the starter phase (21 d.o.) and the
535 end of the rearing period (42 d.o.).

536 Taherpour *et al*. (2012), when supplementing diets for broilers with butyric acid,
537 reported a positive effect by *Eimeria* spp. in reducing the duodenal and caecal lesion
538 score as they verified recovery of the intestinal mucosa, the same way it occurred in
539 the present study.

540 One of the challenges in using SB as an additive is getting it to the intestinal tract
541 to deliver the molecule to the preferred location. Thus, to regulate the population
542 balance of intestinal bacteria, acidification of the intestinal environment is essential, as
543 it will control potential pathogens (Onrust *et al.,* 2020; Dai *et al.,* 2021).

544 In the relative abundance of the phyla, an increase in *Firmicutes* is associated
545 with an increase in nutrient absorption, whereas an increase in *Bacteroidota* is
546 associated with a decrease in nutrient absorption (Jumpertzet *et al.,* 2011; Oakley *et*
547 *al.,* 2014).

548 When Zhou *et al*. (2017) included SB in the diet of chickens challenged with
549 *Eimeria tenella*, they did not have a significant effect on the microbiota analysis either.
550 Nevertheless, the inclusion of the additive increased the abundance of *Firmicutes* and
551 decreased that of *Bacteroidota* in birds infected with E. tenella, a fact observed in the
552 present study.

553 Bortoluzzi *et al*. (2018) verified that the frequency of *Firmicutes* decreased, while
554 *Bacteroidota* increased both in birds challenged with *Eimeria maxima* and *Clostridium*
555 *perfringens* and in those that did not receive any additive, which was also observed in

the present research.

This fact proves that among the treatments challenged with *Eimeria* spp. (challenged control, 2,000 mg/kg MSB, and avilamycin), the birds that did not receive any additives had greater abundance of the phylum *Bacteroidota*. These microorganisms are important in the development and maintenance of the intestinal health and the immune system. Still, they can be an important pathogenic agent, especially when there are predisposing conditions, such as damage to the intestinal mucosa (Hampson *et al*., 2010) caused by the sanitary challenge imposed on chickens. This fact was confirmed when the lesion score analysis was performed at 21 d.o. in the present study.

Using additives that favour the preservation of the morphofunctional integrity of the digestive system is essentially important for the good development of production animals, as well as to avoid production losses (Souza *et al*., 2020). At 21 d.o., the challenged birds that had not received any additives were observed to have a lower relative liver weight, which corroborates the fact that the composition of the intestinal microbiota interferes with the integrity of the digestive system, and it may lead to poor well-being of the birds.

Sodium butyrate supplementation in diets for broilers influences the microbiota composition in a way that is beneficial for health and growth performance. Such fact was observed in the zootechnical performance variables when the microbiota was unbalanced by the challenge with Eimeria species and yet remained healthy.

By analysing the intestinal microbiota of birds, it is possible to verify that SB use preserves the diversity of microorganisms as well as antibiotic use does (Deepa *et al*., 2020). Using such tool for more refined analyses could expand the fundamental knowledge about the regulatory role of SB in intestinal health and be a viable alternative to the use of antibiotics.

583 **5- Conclusion**

584 It is concluded that microencapsulated sodium butyrate can be used as an
585 alternative to the chemical antimicrobial because it resembles avilamycin. It is evident
586 that the use of MSB can help in the recovery of performance, its immunological status,
587 intestinal lesions, and in the maintenance of the intestinal microbiota of broilers post-
588 challenge with *Eimeria* spp. at the doses used in this study.

589

590 **6- Acknowledgment**

591 The authors would like to thank the São Paulo Research Foundation
592 (FAPESP) for the grant of a technical training scholarship number 2019/20261-0 and
593 financial support number 2018/21792-6.

594 Competing interests: The authors declare there are no competing interests.

595 Data availability: Data generated or analyzed during this study are provided in full
596 within the published article.

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Table 1 - Composition and calculated values of the experimental diets.

Ingredients (g/kg)	Diets[1]			
	Pre-starter (1 - 7 d)	Starter (8 - 21 d)	Grower (22 - 33 d)	Finisher (34 - 42 d)
Corn	570.1	585.8	637.7	695.6
Soybean meal	360.7	340.6	286.2	239.0
Soybean oil	24.69	32.45	38.20	34.08
Choline chloride 60	0.72	0.63	0.58	0.43
Salt	5.34	5.17	4.93	4.67
Dicalcium phosphate	16.45	14.49	15.21	9.94
Limestone	11.51	10.49	7.30	7.83
L-lysine	2.38	2.39	2.45	2.53
DL- methionine	3.20	3.02	2.57	2.16
L-threonine	0.45	0.42	0.36	0.28
L-valine	0.50	0.50	0.50	0.50
Mineral supplement[2]	0.50	0.50	0.50	0.50
Vitamin supplement[3]	2.00	2.00	2.00	2.00
Inert[4]	2.00	2.00	2.00	2.00
Total	1,000.0	1,000.0	1,000.0	1,000.0
Calculated Values				
ME (MJ/kg)	12.46	12.77	13.19	13.40
CP (g/kg)	212.9	204.8	184.0	166.9
Methionine [5] (g/kg)	6.28	6.01	5.32	4.72
Methionine+cystine[5] (g/kg)	9.67	9.29	8.32	7.50
Lysine [5] (g/kg)	13.07	12.56	11.24	10.14
Threonine [5] (g/kg)	8.63	8.29	7.42	6.69
Tryptophan [5] (g/kg)	2.65	2.53	2.21	1.95
Valine [5] (g/kg)	10.06	9.67	8.65	7.81
Calcium [5] (g/kg)	9.71	8.78	7.58	6.34
Phosphorus [5] (g/kg)	4.07	3.68	3.74	2.71
Sodium (g/kg)	2.25	2.18	2.08	1.97
Choline (g/kg)	0.375	0.330	0.300	0.225
Linoleic acid (g/kg)	26.33	30.57	34.22	32.84

[1] Pre-starter, 1-7 d.o; starter, 8-21 d.o; grower, 22-33 d.o; finisher, 34-42 d.o.
[2] Mineral premix provided per kg of feed: Cu, 9 mg; I, 1 mg; Zn, 60 mg; Fe, 30 mg; Mn, 60 mg.
[3] Vitamin supplement for broilers provided per kg of diet in pre-starter and starter phases: vitamin A, 11,000,00 UI; vitamin D3, 2,000,00 UI; vitamin E, 16.00 UI; vitamin K3, 1.50 mg; vitamin B1, 1.20 mg; vitamin B2, 4.50 mg; vitamin B6, 2.00 mg; vitamin B12, 16.00 mcg; folic acid, 0.40 mg; pantothenic acid, 9.20 mg; biotin, 0.06 mg; niacin, 35 mg; Se, 0.25 mg. Vitamin supplement for broilers provided per kg of diet in grower phase: vitamin A, 9,000,00 UI; vitamin D3, 1,600,00 UI; vitamin E, 14.00 UI; vitamin K3, 1.50 mg; vitamin B1, 1.00 mg; vitamin B2, 4.00 mg; vitamin B6, 1.80 mg; vitamin B12, 12.00 mcg; folic acid, 0.30 mg; pantothenic acid, 8.28 mg; biotin, 0.05 mg; niacin, 30 mg; Se, 0.25 mg. Vitamin supplement for broilers provided per kg of diet in finisher phase: vitamin A, 3,000,00 UI; vitamin D3, 500.00 UI; vitamin E, 5.00 UI; vitamin K3, 0.50 mg; vitamin B1, 0.30 mg; vitamin B2, 1.00 mg; vitamin B6, 0.40 mg; vitamin B12, 3.00 mcg; pantothenic acid, 3.68 mg; biotin, 0.015 mg; niacin, 5 mg; Se, 0.20 mg.
[4] Treatments were obtained by replacement of inert by additives: Diet without additive: 2,000 mg/kg inert. Diet with 1,000 mg/kg MSB (microencapsulated sodium butyrate): 1,000 mg/kg MSB + 1,000 mg/kg inert. Diet with 1,500 mg/kg de MSB: 1,500 mg/kg of MSB + 500 mg/kg of inert. Diet with 2,000 mg/kg of MSB: 2,000 mg/kg of MSB. Diet with antibiotic: 50 mg/kg avilamycin + 1,950 mg/kg inert.
[5] Digestible values.

Table 2 - Performance of broilers fed with diets supplemented with microencapsulated sodium butyrate as an alternative to antibiotics from 1 to 21 and 1 to 42 d.o.[1]

Variables[2]	UC[2]	Challenged[3]					SEM[4]	P
		CC	1,000	1,500	2,000	AVL		
				21 d.o				
AWG (g)	839.96a	783.45b	760.60b	756.14b	745.98b	761.02b	7.039	<0.001
AFI (g)	1129.71a	1097.24ab	1073.60ab	1078.22ab	1047.15b	1086.32ab	6.956	0.0103
FCR	1.40	1.47	1.44	1.45	1.47	1.44	0.008	0.1983
VB (%)	98.86	97.14	96.00	94.86	97.14	92.57	0.793	0.2837
				42 d.o				
AWG (g)	2914.35	2847.26	2775.60	2761.80	2767.80	2768.20	19.507	0.1212
AFI (g)	4776.69	4742.38	4730.55	4623.46	4659.83	4665.20	27.408	0.6066
FCR	1.73b	1.76ab	1.87a	1.85ab	1.83ab	1.88a	0.014	0.0108
VB (%)	93.14	90.86	88.57	86.28	90.86	84.00	1.168	0.2264
PEF	373.31a	349.53ab	312.71b	308.56b	328.06ab	297.27b	6.994	0.0055

[1]Values are means ± SEM. n = 5 replicate pens of 35 birds each.
[2] AWG. average weight; AFI. average feed intake; FRC. feed conversion ratio; VB. viability; PEF. productive efficiency factor.
[3] UC, negative control (no additives); CC, challenged control (no additives); 1,000, inclusion of 1,000 mg/kg microencapsulated sodium butyrate (MSB); 1,500, inclusion of 1,500 mg/kg MSB; 2,000, inclusion of 2.000 mg/kg MSB; AVL, inclusion of antibiotic (avilamycin). [3]Broilers inoculated at 16 d.o. with *Eimeria acervulina* ($2x10^5$ sporulated oocysts/mL) and *E. maxima* and *E. tenella* ($2x10^4$ sporulated oocysts/mL) at a dosage of 0.5 mL per bird.
[4]SEM. standard error of the mean.

Table 3 - Haematological variables of broilers fed diets supplemented with microencapsulated sodium butyrate as an alternative to antibiotics at 21 and 42 d.o.[1]

Variables	UC[2]	Challenged[3]					SEM[8]	P
		CC	1,000	1,500	2,000	AVL		
				21 d.o.				
Haematocrit (%)	28.60	22.70	22.10	20.88	22.90	21.10	0.818	0.0512
Erythrocytes(x10^6/mm^3)	1.90	1.80	1.73	1.67	1.46	1.42	0.058	0.0882
Haemoglobin (g/dl)	5.59	6.54	5.98	6.36	6.42	6.13	0.277	0.8785
Leukocytes (cell/mm^3)	48.201.50	64.715.60	47.579.72	56.690.50	58.113.00	48.345.00	3.752.57	0.7457
MCV (fl^3)[4]	153.83	150.91	127.33	138.46	120.10	162.15	6.919	0.4885
MCH (pg/cell)[5]	29.90	39.08	36.76	37.60	45.70	47.05	1.975	0.1168
MCHC (g/dL)[6]	19.89	34.54	31.56	34.13	42.32	33.14	2.497	0.2150
Heterophile(cells/mm^3)	5.707.27	7.173.54	4.159.35	6.909.32	7.220.11	3.749.44	714.97	0.6195
Lymphocyte(cells/mm^3)	6.296.50	7.377.55	8.566.78	7.366.62	11.115.04	8.277.44	746.07	0.5504
H:L[7]	0.76	0.73	0.67	0.86	0.68	0.50	0.064	0.7531
				42 d.o.				
Haematocrit (%)	27.50	27.20	27.80	28.60	26.60	26.00	0.467	0.7049
Erythrocytes(x10^6/mm^3)	2.86	2.81	2.78	2.65	2.65	2.93	0.095	0.9609
Haemoglobin (g/dl)	5.08	5.44	5.14	5.84	5.93	5.39	0.183	0.7258
Leukocytes (cell/mm^3)	68.266.64	79.178.75	51.016.00	68.911.50	59.985.00	72.363.50	5.578.46	0.7457
MCV (fl^3)[4]	99.36	93.56	102.36	111.54	119.60	102.90	4.794	0.7116
MCH (pg/cell)[5]	17.86	18.96	20.64	20.02	24.22	22.12	0.896	0.3921
MCHC (g/dL)[6]	18.68	20.76	21.22	18.68	20.65	22.42	0.741	0.6905
Heterophile(cells/mm^3)	4.264.96	5.823.71	2.825.08	3.714.94	3.330.69	5.917.11	456.091	0.2361
Lymphocyte(cells/mm^3)	7.196.91	12.140.27	6.617.20	7.781.85	3.391.50	5.780.98	940.783	0.1897
H:L[7]	0.73	0.72	0.83	0.90	1.16	1.04	0.077	0.5238

[1]Values are means ± SEM of 5 replicates (n = 2 per replicate) in a treatment group.
[2] UC, negative control (no additives); CC, challenged control (no additives); 1,000, inclusion of 1,000 mg/kg of microencapsulated sodium butyrate (MSB); 1,500, inclusion of 1,500 mg/kg of MSB; 2,000, inclusion of 2,000 mg/kg of MSB; AVL, inclusion of antibiotic (avilamycin).
[3]Broilers inoculated at 16 d.o. with *Eimeria acervulina* ($2x10^5$ sporulated oocysts/mL) and *E. maxima* and *E. tenella* ($2x10^4$ sporulated oocysts/mL) at a dosage 0.5 mL per bird.
[4]MCV. mean corpuscular volume.
[5]MCH. mean corpuscular haemoglobin.
[6]MCHC. mean corpuscular haemoglobin concentration.
[7]H:L. relationship heterophile: lymphocyte.
[8]SEM. standard error of the mean.

Table 4 - Biochemical variables of broilers fed diets supplemented with microencapsulated sodium butyrate as an alternative to antibiotics at 21 and 42 d.o.[1]

Variables	UC[2]	Challenged[3]					SEM[4]	P
		CC	1,000	1,500	2,000	AVL		
				21 d.o.				
Glucose (g/dL)	236.23	220.58	228.97	231.87	213.43	238.58	4.425	0.5915
Protein (g/dL)	3.43	2.54	3.18	3.15	3.01	3.21	0.092	0.0951
Albumin (g/dL)	2.95	3.49	3.20	3.09	3.20	3.35	0.098	0.7183
				42 d.o.				
Glucose (g/dL)	255.40	261.22	248.83	251.33	267.89	241.22	4.944	0.7301
Protein (g/dL)	6.06	5.18	5.01	5.81	4.93	5.30	0.168	0.3016
Albumin (g/dL)	2.36	2.77	2.07	1.85	2.46	2.09	0.102	0.1017

[1]Values are means ± SEM of 5 replicates (n = 2 per replicate) in a treatment group.
[2] UC, negative control (no additives); CC, challenged control (no additives); 1,000, inclusion of 1,000 mg/kg microencapsulated sodium butyrate (MSB); 1,500. inclusion of 1,500 mg/kg MSB; 2,000, inclusion of 2,000 mg/kg MSB; AVL. inclusion of antibiotic (avilamycin).
[3]Broilers inoculated at 16 d.o. with *Eimeria acervulina* ($2x10^5$ sporulated oocysts/mL) and *E. maxima* and *E. tenella* ($2x10^4$ sporulated oocysts/mL) at a dosage 0.5 mL per bird.
[4]SEM. standard error of the mean.

Table 5- Relative weight and length of organs of the digestive system of broilers fed diets supplemented with microencapsulated sodium butyrate as an alternative to antibiotics at 21 and 42 d.o.[1]

		Challenged[3]						
RW[4]	**UC[2]**	**CC**	**1,000**	**1,500**	**2,000**	**AVL**	**SEM[5]**	**P**
				21 d.o.				
Spleen (%)	0.18	0.19	0.14	0.14	0.18	0.14	0.112	0.7071
Pancreas (%)	0.33	0.27	0.30	0.26	0.32	0.29	0.009	0.1308
Gizzard (%)	2.64	2.66	2.32	2.54	2.37	2.53	0.061	0.5341
Liver (%)	2.44ab	1.80b	2.65a	2.84a	2.62a	2.79a	0.089	0.0023
PV (%)	0.47	0.53	0.44	0.47	0.47	0.44	0.015	0.5693
SI (%)	4.30	6.36	5.94	5.87	5.59	5.02	0.229	0.1068
LI (%)	1.37	1.75	1.84	1.70	1.96	1.90	0.077	0.2971
Length								
SI (cm)	125.20	134.60	131.20	130.40	132.80	126.00	2.217	0.8919
LI (cm)	51.80	44.20	48.20	46.20	52.40	51.00	0.888	0.5763
				42 d.o.				
Spleen (%)	0.08	0.09	0.11	0.08	0.09	0.09	0.002	0.0575
Pancreas (%)	0.21ab	0.18b	0.22ab	0.24a	0.22ab	0.24a	0.006	0.0266
Gizzard (%)	1.74	1.64	1.66	1.62	1.81	1.80	0.028	0.1564
Liver (%)	1.70	1.59	1.66	1.75	1.88	1.74	0.032	0.2272
PV (%)	0.35	0.33	0.38	0.37	0.32	0.30	0.011	0.3265
SI (%)	3.05	3.17	2.90	3.07	3.33	3.31	0.090	0.7725
LI (%)	0.77	0.88	0.95	0.78	0.96	0.87	0.030	0.4397
Length								
SI (cm)	164.00	160.20	174.60	157.60	171.00	176.80	2.944	0.3154
LI (cm)	64.00	66.00	68.20	59.80	66.80	62.00	1.079	0.2081

[1] Values are means ± SEM of 5 replicates (n = 2 per replicate) in a treatment group.

[2] UC, negative control (no additives); CC, challenged control (no additives); 1,000, inclusion of 1,000 mg/kg microencapsulated sodium butyrate (MSB); 1,500, inclusion of 1,500 mg/kg MSB; 2,000, inclusion of 2,000 mg/kg MSB; AVL, inclusion of antibiotic (avilamycin).

[3] Broilers inoculated at 16 d.o. with *Eimeria acervulina* ($2x10^5$ sporulated oocysts/mL) and *E. maxima* and *E. tenella* ($2x10^4$ sporulated oocysts/mL) at a dosage of 0.5 mL per bird.

[4] RW, relative weight; PV, pro-ventricle; SI, small intestine; LI, large intestine.

[5] SEM. standard error of the mean.

Table 2 - Oocysts count per gram in faeces at 4, 5, 6, and 7 days post-inoculation with *Eimeria* spp.

Effects		**OPG[2]**
Treatments[1]	UC	0
	CC	135.67
	1,000	140.45
	1,500	160.25
	2,000	197.71
	AVL	115.88
Periods	4 days	336.26 a
	5 days	69.84 c
	6 days	65.22 c
	7 days	127.84 b
	SEM[3]	18.878
Variation Source		**Probability**
Treatments		0.5599
Periods		<0.0001
Treatments*Periods		0.3674

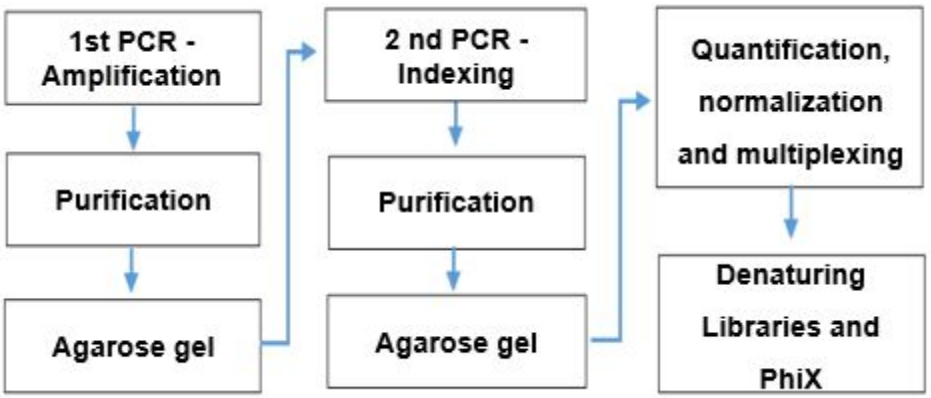
[1] UC, negative control (no additives); CC, challenged control (no additives); 1,000, inclusion of 1,000 mg/kg of microencapsulated sodium butyrate (MSB); 1,500, inclusion of 1,500 mg/kg of MSB; 2,000, inclusion of 2,000 mg/kg of MSB; AVL, inclusion of antibiotic (avilamycin).
[2]OPG, Oocysts per gram in faeces
[3]SEM, standard error of the mean

Table 7 - Intestinal mucosal lesion scores at 5 and 26 days post- inoculation with *Eimeria* spp.

Effect		Lesion scores		
		E. acervulina	*E. maxima*	*E tenella*[1]
	UC	0	0	0
Treatments [3]	CC	0.800	1.300	0.599
	1,000	0.667	1.400	0.680
	1,500	0.500	1.000	0.300
	2,000	0.500	1.600	0.409
	AVL	0.800	1.800	0.668
Periods	5 days	0.4667	2.0000 a	0.9518 a
	26 days	0.6800	0.8400 b	0.1109 b
	EPM[2]	0.569	1.420	1.160
Variation Source		**Probability**		
Treatments		0.4724	0.4198	0.4479
Periods		0.1642	0.0001	<0.0001
Treatments*Periods		0.2607	0.9571	0.5796

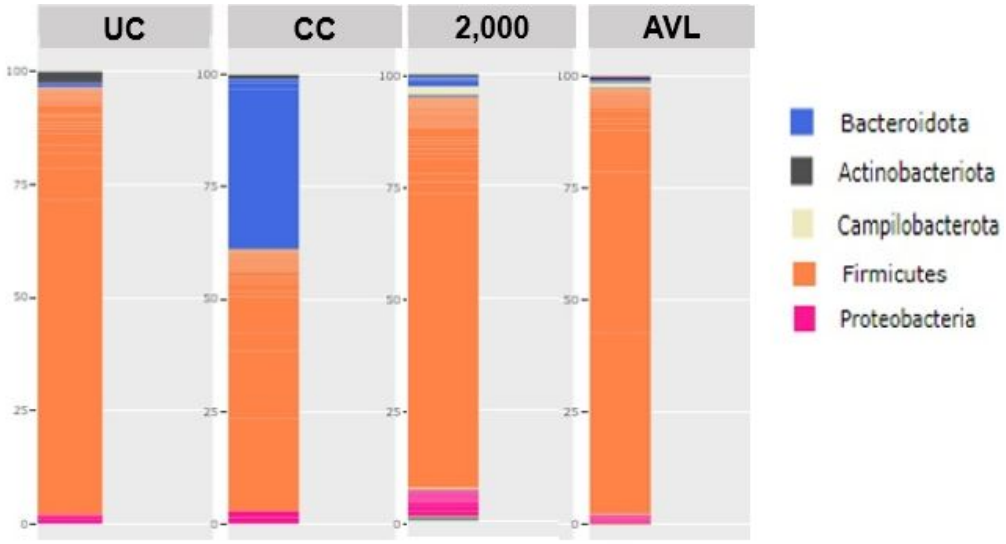
[1] The data of *E. tenella* were submitted to logarithmic transformation.
[2] SEM. standard error of the mean.
[3] UC, negative control (no additives); CC, challenged control (no additives); 1,000, inclusion of 1,000 mg/kg microencapsulated sodium butyrate (MSB); 1,500, inclusion of 1,500 mg/kg of MSB; 2,000, inclusion of 2,000 mg/kg MSB; AVL, inclusion of antibiotic (avilamycin).

Figure 1- Library preparation.



Source: By the own author.

Figure 2- Relative abundance of the bacterial phyla, according to the treatments studied.



UC - unchallenged control diet (corn-soybean meal)

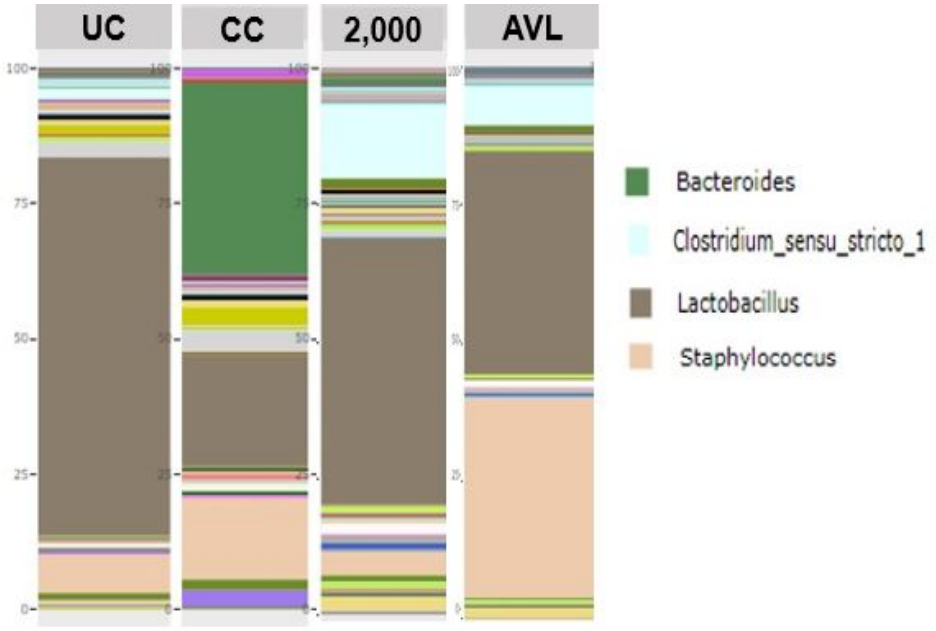
CC- challenged control diet

2,000 - challenged and supplemented with 2,000 mg/kg of MSB

AVL - challenged and supplemented with avilamycin

Source: By the own author.

Figure 3- Relative abundance of bacterial genera, according to the treatments studied.



UC - unchallenged control diet (corn-soybean meal)

CC- challenged control diet

2,000 - challenged and supplemented with 2,000 mg/kg of MSB

AVL - challenged and supplemented with avilamycin

Source: By the own author.