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Performance and intestinal microbiota of chickens receiving probiotic in the feed and submitted to antibiotic therapy

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Abstract

The purpose of this study was to verify the ability of a probiotic in the feed to maintain the stability of the gut microbiota in chickens after antibiotic therapy and its association with growth performance. One thousand six hundred twenty 1-day-old Cobb male were housed in floor pens (36 pens, 45 birds/pen) and were fed corn-/soya bean meal-based diets supplemented with or without probiotic (*Bacillus subtilis*) during the entire rearing phase. From 21 to 24 days of age (three consecutive days), the chickens were submitted to antibiotic therapy via drinking water (bacitracin and neomycin) in order to mimic a field treatment and induce dysbiosis. Growth performance was monitored until 42 days of age. At 2, 4 and 6 days after antibiotic therapy, three chickens from each pen were euthanized and the contents of the small intestine and caeca were collected and pooled. The trial was conducted with four treatments and nine replicates in a 2 × 2 factorial arrangement for performance characteristics (with and without probiotic × with and without antibiotic therapy); for the intestinal microbiota, it was in a 2 × 2 × 3 factorial arrangement (with and without probiotic × with and without antibiotic therapy × 2, 4 and 6 days after the antibiotic therapy) with three replicates per treatment. Terminal restriction length polymorphism (T-RFLP) analysis showed that the structure of gut bacterial community was shaped by the intestinal segment and by the time after the antibiotic therapy. The number of 16S rDNAs copies in caecum contents decreased with time after the therapeutic treatment. The antibiotic therapy and dietary probiotic supplementation decreased richness and diversity indexes in the caecal contents. The improved performance observed in birds supplemented with probiotic may be related to changes promoted by the feed additive in the structure of the intestinal bacterial communities and phylogenetic groups. Antibiotic therapy modified the bacterial structure, but did not cause loss of broiler performance.

KEYWORDS
antibiotic therapy, broiler chicken, DNA, gut, microbiota, probiotic, sequencing

1 | INTRODUCTION

Poultry producers indicate increasing mortality and diseases (mainly intestinal health problems) in chickens are raised without antibiotic growth promoters (AGPs). It is likely that intestinal health problems have been partly masked by the routine use of AGPs and that, paradoxically, it is increasing the use of antibiotics on therapeutic administration (antibiotic therapy) (Huyghebaert, Ducatelle, & Van Immerseel, 2011). In the European Union, the use of AGPs was banned in 2006 while, in the United States, the Center of Veterinary Medicine of the Food and Drug Administration, issued a document in 2012 ("Guidance for Industry") recommending that antibiotics should be used only in case of specific diseases and not for growth promotion (Onrust et al., 2015). In chicken production, antibiotic therapy as a management tool consists of administering specific drugs during a limited period, which is important for controlling certain diseases caused by harmful bacteria. It is important to distinguish the use of antibiotics for therapy (which uses therapeutic doses) from that with the purpose of promoting good productive performance of animals (which uses subtherapeutic doses of AGPs). The use of antibiotics as a therapeutic measure frequently causes disturbance in the human gut microbiota (Lindberg, Jarnheimer, Olsen, Johansson, & Tysklind, 2004; Van Der Waaij & Nord, 2000). In addition, antibiotic therapy is known to cause change of microbial consortia structure, thus catalysing dysbiosis with a consequent detrimental impact on physiology and metabolic performance of the host that may ultimately result in the development of gut disorders (Allen & Stanton, 2014). Dysbiosis or dysbacteriosis is defined as a change in the intestinal microbiota composition resulting in an imbalance between beneficial and harmful bacteria (Ducatelle, Eeckhaut, Haesebrouck, & Van Immerseel, 2015). Disturbance of the microbiota is frequently associated with villous atrophy, decrease in the thickness of the tunica *muscularis* and increase in T lymphocyte infiltration in the gut mucosa of broiler chickens (Teirlynck et al., 2013).

There is a great interest worldwide to develop feed additives with the ability to positively modulate the microbiota, improving broiler performance and controlling pathogens, especially those associated with zoonotic implications (Thomke & Elwinger, 1998; Verstegen & Williams, 2002; Yang, Iji, & Choct, 2009). Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Reid, Jass, Sebulsky, & McCormick, 2003), and it seems that this terminology is adequate for the use in animal production (Hill et al., 2014). Positive effects have been observed when probiotics were used for the prevention and treatment of gastrointestinal disturbance in broilers challenged with *Clostridium perfringens* infection (Li, 2017) or *Campylobacter jejuni* (Mañes-Lázaro et al., 2017). The bacterial community of the gastrointestinal tract plays a major role in the physiology of the chicken, modulating metabolic and immunological processes (Pan & Yu, 2014).

In the industrial chicken production, farmers have described negative effects on bird performance after the therapeutic treatment with antibiotics. Even though there is an extensive number of studies describing the negative effect of antibiotic therapy on human gut microbiota (Macfarlane, 2014; Ferrer, Santos, Ott, & Moya, 2014; Robinson & Young, 2010), this has not been, to our knowledge, reported in chickens. On the other hand, it has been demonstrated that the use of probiotic during and post-antibiotic therapy reduces the extent of disruption of the intestinal microbiota in humans (Plummer et al., 2005).

In this study, we hypothesized that dietary probiotic supplementation is able to prevent dysbiosis in chicken gut following antibiotic therapy and that antibiotic therapy may impair the performance of the chickens. The purpose of this study was to verify the ability of a probiotic (*Bacillus subtilis)* in the feed to maintain the stability of the gut microbiota in chickens after antibiotic therapy and its correlation with the growth performance of the birds.

2 | MATERIAL AND METHODS

2.1 | Birds and dietsi

All the procedures used in this experiment were approved by the institutional animal care and use committee of the College of Agriculture "Luiz de Queiroz," University of Sao Paulo. A total of 1,620 1-day-old male Cobb-500 broiler chickens were raised in floor pens from 1 to 42 days of age. Chicks were weighed by pen for equal weight distribution and placed randomly into 36 pens (45 birds/pen). The nutritional programme consisted of four diets: pre-starter (1–7 days), starter (8–21 days), grower (22–35 days) and finisher (36–42 days). For each phase, a basal corn–soya bean meal–soya bean oil diet was formulated and supplemented with or without a probiotic (*B. subtilis*). The same batch of feed, in mash form, was used to produce the basal diet and the probiotic-supplemented diet in order to guarantee that difference between batches was not an interfering factor. The ingredient composition and the nutritional specifications of the diets were based on the Brazilian Tables (Rostagno et al., 2011, Table 1). The probiotic was constituted by live spores of *B. subtilis*, strain C-3102 (Calpis, Japan), containing 10^9 cfu/kg of product and was included at a rate of 30 g/ton of diet (in the concentration of 3×10^7 cfu/kg of feed). Chickens had ad libitum access to water and feed during the entire experimental period. The diets were not supplemented with any anticoccidial agent or antimicrobial growth promoter.

The experiment was divided into two parts. From 1 to 21 days, the chickens received only the dietary treatments, diet without and with probiotic (two treatments and 18 replicates); after 21 days, the chickens were assigned to the combination of dietary and therapeutic treatments (four treatments and nine replicates).

2.2 | Antibiotic therapy and sample collection

The chickens were weighed weekly by pen to calculate body weight gain (WG), feed intake (FI) and feed conversion ratio (FCR); mortality was registered daily to calculate viability (VB).

TABLE 1 Composition and calculated contents of the experimental diets

Item, %	Pre-starter	Starter	Grower	Finisher
Corn	48.59	53.32	56.48	60.31
Soya bean meal	43.30	40.00	35.31	31.88
Soya bean oil	3.46	3.92	4.81	4.80
Dicalcium phosphate	1.86	1.53	1.31	1.10
Limestone	0.91	0.95	0.89	0.80
Salt	0.51	0.48	0.46	0.44
MHA	0.45	0.40	0.38	0.34
L-Lysine.HCl	0.14	0.15	0.15	0.16
L-Threonine	0.04	0.04	0.03	0.03
Vitamin premix[a]	0.10	0.10	0.08	0.06
Mineral premix[b]	0.05	0.05	0.05	0.05
Choline chloride (60%)	0.08	0.08	0.06	0.04
Total (kg)	100	100	100	100
Calculated composition				
MEn (kcal/kg)	2.960	3.050	3.150	3.200
CP (%)	23.80	22.00	20.58	19.34
Calcium (%)	0.92	0.84	0.76	0.66
Available phosphorus (%)	0.47	0.40	0.35	0.31
Sodium (%)	0.22	0.21	0.20	0.19
Digestible Met (%)	0.63	0.58	0.54	0.51
Digestible Met + cys (%)	0.95	0.88	0.83	0.77
Digestible Lys (%)	1.32	1.22	1.13	1.06
Digestible Thr (%)	0.86	0.79	0.74	0.69

Notes. MHA: methionine hydroxy analogue.
[a]Vitamin premix provided per kilogram of diet: pre-starter and starter phases: Vit. A (retinyl palmitate)—10.000 UI; Vit. D_3—3.000 UI; Vit. E (D-L-α-tocopheryl acetate)—40 UI); Vit. K_3—3 mg; Vit. B_1—2 mg; Vit. B_2—6 mg; Vit. B_6—4 mg; Vit. B_{12}—12 μg; niacin—40 g; pantothenate—12 g; biotin—100 mg; folic acid—1 mg; Se—0.25 mg. Vitamin premix provided per kilogram of diet: grower: Vit. A (D-L-α-tocopheryl acetate)—8.000 UI; Vit. D3—2.400 UI; Vit. E (D-L-α-tocopheryl acetate)—32 UI; Vit. K3—2.4 mg; Vit. B1—1.6 mg; Vit. B2—4.8 mg; Vit. B6—3.2 mg; Vit. B12—16 μg; niacin—32 mg; pantothenate—7.2 mg; biotin—0.12 mg; folic acid—0.8 mg; Se—0.2 mg. Vitamin premix provided per kilogram of diet: finisher: Vit. A (D-L-α-tocopheryl acetate)—6.000 UI; Vit. D3—1.800 UI; Vit. E (D-L-α-tocopheryl acetate)—24 UI; Vit. K3—1.8 mg; Vit. B1—1.2 mg; Vit. B2—3.6 mg; Vit. B6—2.4 mg; Vit. B12—12 μg; acido nicotínico—24 mg; pantothenate—7.2 mg; biotin—0.09 mg; folic acid—0.6 mg; Se—0.15 mg. [b]Mineral premix provided per kilogram of diet: Mn—80 mg; Fe—50 mg; Zn—50 mg; Cu—10 mg; Co—1 mg; I—1 mg.

Starting at 21 days of age, the pens on each dietary treatment (without and with probiotic) were randomly assigned or not to the therapeutic treatment with antibiotics. The antibiotic therapy consisted of 200 mg/L of bacitracin methylene disalicylate (effective against Gram-positive bacteria, Hooge, Sims, Sefton, Connolly, & Spring, 2003) and 1,000 mg/L of neomycin sulphate (effective against Gram-negative bacteria, Jao & Jackson, 1964) in the drinking water for three consecutive days (from 21 to 24 days). The period of the antibiotic therapy followed the manufacturer's protocol. For the medication, the antibiotics were supplied in one stainless steel water trough per pen; during this period, all the bell drinkers were suspended. Water troughs were also installed in the pens in which the birds were not medicated. At 2, 4 and 6 days after the completion of the antibiotic therapy (on days 26, 28 and 30), three chickens from each pen were sacrificed by cervical dislocation with no feed withdrawal and the contents of the small intestine (duodenum, jejunum and ileum) and caeca were collected separately and pooled by pen. At each collection time, pens from three replicates were chosen to provide the samples. The intestinal contents were immediately frozen at −20°C for subsequent analysis.

A 2 × 2 factorial arrangement was adopted to study the performance characteristics (with and without probiotic × with and without antibiotic therapy):

T1: Basal diet (−P−A).
T2: Basal diet + antibiotic therapy (−P + A).
T3: Basal diet + 3×10^7 CFU/kg probiotic (+P−A).
T4: Basal diet + 3×10^7 CFU/kg probiotic +antibiotic therapy (+P + A).

And a 2 × 2 × 3 factorial arrangement for intestinal microbiota analysis (with and without probiotic [P] × with and without antibiotic therapy [A] × time after the antibiotic therapy [T]):

T1: Basal diet (-P-A)
T2: Basal diet + antibiotic therapy (-P+A)
T3: Basal diet +3×10^7 cfu/kg probiotic (+P-A)
T4: Basal diet + 3×10^7 cfu/kg probiotic + antibiotic therapy (+P+A)
] At 2d, 4d and 6d after the antibiotic therapy

And a 2 × 2 × 3 factorial arrangement for intestinal microbiota analysis (with and without probiotic [P] × with and without antibiotic therapy [A] × time after the antibiotic therapy [T]):

2.3 | DNA extraction

In order to analyse the microbiota of the small intestine, the bacterial pellet was obtained according to the procedure proposed by Apajalahti, Sarkilahti, Heikkinen, Nyrminen, and Holben, (1998). Caecal samples were not submitted to separation of the bacterial pellet. The bacterial DNA isolation was conducted according to Lu et al. (2003). DNA presence was verified using agarose gel (1.5%) electrophoresis.

2.4 | Bacterial community structure by terminal restriction length polymorphism (T-RFLP)

Bacterial rDNA gene sequences were amplified with universal 16S bacterial primers 8fm (AGAGTTTGATCMTGGCTCAG) and 926 R (CCGTCAATTCCTTTRAGTTT) (Schütte et al., 2009). The forward primer was 5′ labelled with 6-carboxyfluorescein (FAM). PCRs were done in 25 µl volume containing 1× PCR buffer, 3 mM $MgCl_2$, 0.2 mM concentrations of each deoxynucleoside triphosphate, 0.05 µl concentrations of each primer at 0.2 pmol, 0.01 µl of Taq DNA polymerase at 0.02 U, 3 µl of template DNA and completed with Milli-Q water. All PCRs were done and run in a thermal cycler following amplification conditions: initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 45 s, with a final extension step at 72°C for 10 min. The specificity of PCR products was analysed by gel electrophoresis in agarose gel 1.0% (w/v). A volume of 10 µl of purified PCR product was digested with 5 U of H*ha*I (Fermentas Life Sciences), according to the manufacturer's instructions. After the restriction reaction, the products were precipitated by the addition of 2 µl of EDTA (125 mM), 3 µl of sodium acetate (3 M) and 50 µl of ethanol (100%) according to the method suggested by the manual of Big Dye® Terminator v3.1 Cycle Sequencing Kit. Samples were stored at −20°C until they were analysed. Terminal restriction fragments (T-RFs) analysis was performed using ABI PRISM 3500 Genetic Analyzer (Applied Biosystems). Data obtained from sequencing were analysed with Gene Mapper v.4.1 (Applied Biosystems) software. Peak heights were transformed to relative data (percentage of detection). T-RFLP profiles were compared among different samples using T-RF relative abundance (>1%), in which each T-RF was considered to be one operational taxonomic unit (OTU).

2.5 | Quantification of total bacteria by quantitative PCR

The real-time PCR (qPCR) analysis was used for quantification of the number of copies of total domain *Bacteria* (16S rDNA) in the small intestine and caecum contents. The reactions were done in duplicate in Rotor-Gene 6000 equipment (Corbett Life Science, Australia). Detection system used was by SYBR Green I. All reactions of qPCR consisted of 5 µl of Platinum® Quantitative *PCR* SuperMix-UDG kit (Invitrogen, Brazil), 1 µl of each primer at 10 nM, 1 µl of template DNA and 2 µl of Milli-Q water in a 10 µl total volume. Amplifications of 16S rDNA fragments were done using the primers P1 (5′-CCT ACG GGA GGC AGCAG-3′) and P2 (5′-ATT ACC GCG GCT GCT GG-3′) (Muyzer, Dewaal, & Uitterlinden, 1993). The cycling conditions were 95°C for 15 s, then 35 cycles of 94°C for 1 min, 55°C for 1 min then 72°C for 1 min. A standard curve of the gene 16S rDNA

TABLE 2 Sequences of primers forward (f) and reverse (r) to isolate the gene 16S rDNA to the sequencing

Primer name	Sequence
16SV4FPCR1_1f	5′ TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG AYT GGG YDT AAA GNG 3′
16SV4FPCR1_2f	5′ TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG NAY TGG GYD TAA AGN G 3′
16SV4FPCR1_3f	5′ TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG NNA YTG GGY DTA AAG NG
16SV4FPCR1_4f	5′ TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG NNN AYT GGG YDT AAA GNG
16SV4RPCR1_1r	5′ GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GCC GTC AAT TCM TTT RAG T
16SV4RPCR1_2r	5′ GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GNC CGT CAA TTC MTT TRA GT
16SV4RPCR1_3r	5′ GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GNN CCG TCA ATT CMT TTR AGT
16SV4RPCR1_4r	5′ GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GNN NCC GTC AAT TCM TTT RAG T

was constructed to quantify the number of the target gene copies. The Ct, or fractional cycle number at which the fluorescence passes the threshold, was determined for each sample and compared with the standard curves.

2.6 | Bacterial community analysis by sequencing

After the DNA extraction, the 16S rRNA gene was amplified by PCR using the primers described by Bell (2011) (Table 2). The sequencing was conducted in the MiSeqTMSystem (Illumina, California, USA), with "Kit MiSeq Reagent v2 (500 cycles)." Bioinformatics analysis of the sequences was conducted using the QIIME (Quantitative Insights Into Microbial Ecology) software, applying specific command lines (Caporaso et al, 2010). Briefly, the FastQ files were separated into **.fasta* and **qual* files. The quality of the sequences was checked (Q > 20), and the primers and barcodes were removed using the command "*slitlibraries.py*". The sequences were binned into operational taxonomic units (OTUs) using a 97% similarity threshold with *unclust* method. The taxonomic classification was conducted using the RDP classifier. Following this step, the OTU tables were obtained with the frequencies of all desired groups. The richness and diversity were calculated using the command *alpha_diversity.py*. Chao and Shannon indexes were estimated. The Chao index estimates the number of species (OTUs) comprising the microbiota, and the Shannon index estimates the biodiversity on the basis of the uniformity of the sequences among the various OTUs (Hill, Walsh, Harris, & Moffett, 2003).

2.7 | Statistical analysis

The data of productive performance, real-time PCR, phylogenetic classification and richness and diversity indexes were analysed by ANOVA with procedures appropriate for a completely randomized design in a factorial model using the GLM procedure of SAS (2006). For data of T-RFLP, the multivariate statistical technique was used with principal coordinate analysis (PCoA) and performed with Bray–Curtis. Analyses were done using Past 2.12 (Hammer, Harper, & Ryan, 2001) software. To analyse the productive performance data, the model included probiotic, antibiotic therapy and its interaction. The quantities of 16S rDNA genes were logarithmically transformed to log base 10 for use as a dependent variable. For the phylogenetic classification, richness and diversity indexes the model included the probiotic, antibiotic therapy and time after the antibiotic therapy (age) and its interactions. The normality of the residues and homogeneity of variances were checked. The data that did not meet the normality and homogeneity of variances were transformed by square root (variable + 1).

3 | RESULTS

3.1 | Growth performance results

3.1.1 | Before the antibiotic therapy

Performance results from 1 to 7 days and 1 to 21 days refer only to the dietary treatments (diets with and without probiotic; Table 3). In the pre-starter phase (1–7 days), there was no statistical difference in performance characteristics between birds fed with and without probiotic in the diets ($p > 0.05$). From 1 to 21 days, there was no statistical effect of treatments on FI, FCR and VB, with average values of 1,319 g, 1.325 and 99.0%, respectively, but probiotic increased ($p = 0.04$) the WG of the chickens (1,002 g) compared to those not supplemented (990 g).

3.1.2 | After the antibiotic therapy

From 1 to 28 and 1 to 35 days of age, there was no interaction between dietary probiotic supplementation and antibiotic therapy on the performance traits (Table 4). However, there was an effect of probiotic increasing WG and an effect of antibiotic therapy improving FCR at 28 days. Probiotic supplementation also improved WG, FI and FCR at 35 days (Table 4). From 1 to 42 days, there was no interaction or main effect of probiotic and antibiotic therapy on the performance traits, which averaged 2,973 g for WG, 4,859 g for FI, 1.634 for FCR and 96.6% for VB (Table 5).

3.2 | Intestinal microbiota

3.2.1 | T-RFLP

In the T-RFLP analysis, it is evident that the gut community structure is shaped primarily by the intestinal segment (small intestine or

TABLE 3 Performance of broiler chickens submitted the treatments without probiotic (−P) or with probiotic (+P)

	WG (g)		FI (g)		FCR (g:g)		VB (%)	
	1–7 days	1–21 days	1–7 days	1–21 days	1–7 days	1–21 days	1–7 days	1–21 days
−P	141	990[b]	153	1,315	1.092	1.328	99.74	99.35
+P	141	1,002[a]	153	1,322	1.087	1.319	99.30	98.71
p value	0.99	0.04	0.63	0.23	0.67	0.14	0.16	0.19
SEM	0.74	2.87	0.85	3.07	0.005	0.003	0.16	**0.24**

Notes. Results of the periods 1–7 and 1–21 days.

Means within the same column with different superscript a, b differ significantly ($p < 0.05$).
FCR: feed conversion rate; FI: feed intake; VB: viability; WG: weight gain.

TABLE 4 Performance of broiler chickens submitted the treatments without probiotic (−P) or with probiotic (+P) in the diet and with (+A) or without antibiotic therapy (−A)

	WG (g)		FI (g)		FCR (g:g)		VB (%)	
	1–28 days	1–35 days	1–28 days	1–35 days	1–28 days	1–35 days	1–28 days	1–35 days
−P−A	1,674	2,235	2,309	3,482	1.416	1.559	99.0	98.5
+P−A	1,697	2,332	2,340	3,581	1.416	1.536	97.7	97.4
−P + A	1,678	2,246	2,306	3,474	1.413	1.546	97.5	96.6
+P + A	1,704	2,293	2,320	3,496	1.397	1.525	98.7	98.1
SEM	11.34	18.02	12.07	23.86	0.0051	0.008	0.70	0.92
Main effect probiotic (P)								
−P	1,632[b]	2,240[b]	2,307	3,477[b]	1.414	1.553[b]	98.2	97.5
+P	1,657[a]	2,313[a]	2,330	3,538[a]	1.407	1.530[a]	98.2	97.7
Main effect antibiotic therapy (A)								
−A	1,642	2,283	2,325	3,531	1.416[b]	1.547	98.4	97.9
+A	1,647	2,270	2,314	3,485	1.405[a]	1.536	98.1	97.3
Significance (main effects and interactions)								
P	0.044	0.001	0.079	0.029	0.146	0.027	1.0	0.872
A	0.651	0.495	0.375	0.091	0.042	0.226	0.746	0.561
P × A	0.819	0.193	0.509	0.153	0.152	0.922	0.111	0.212

Notes. Results for the periods 1–28 and 1–35 days.
FCR: feed conversion rate; FI: feed intake; VB: viability; WG: weight gain.

caecum) and secondly by the time after the antibiotic therapy (or age of birds; Figure 1).

In the small intestine contents, it was noticed higher similarity of microbiota profiles at 26 and 30 days in relation to 28 days. The caecum contents revealed a clear separation of bacterial community composition and homogeneity of the groups at 30 days compared to those at 26 and 28 days.

3.2.2 | Total bacteria by qPCR

There was no interaction among the factors (probiotic, antibiotic therapy and time after the therapy) or single effect of the probiotic and antibiotic therapy in the copy number of the gene 16S rDNA in small intestine and caecum contents (Table 6). However, there was main effect of time after the antibiotic therapy (T) in the gene copy number representative of the domain *Bacteria* in the caecum contents ($p = 0.001$), but not in the small intestine. The number of 16S rDNA copies in caecum contents at 28 and 30 days was similar but lower than 26 days of age of birds (Table 6).

3.2.3 | Sequencing

The profile of the bacterial phyla in the small intestine and caecum contents of the chickens is shown in Table 7. The analysis of the microbiota revealed a predominance of the phyla *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes* and *Tenericutes*, which accounted for 99% and 96% of the population in the small intestine and caecum, respectively, on average. There was a main effect of antibiotic therapy reducing the *Tenericutes* group in the caecum contents of birds ($p < 0.01$; Table 7). An interaction P × A × T was observed in the caecum contents for *Firmicutes* ($p = 0.03$) and *Bacteroidetes* ($p = 0.02$). At 28 days, but not at the other dates, the treatment with antibiotic without probiotic in the feed caused an increase in the frequency of the phylum *Firmicutes*, while this group

TABLE 5 Performance of broiler chickens submitted the treatments without probiotic (−P) or with probiotic (+P) in the diet and with (+A) or without antibiotic therapy (−A)

	WG (g)	FI (g)	FCR (g:g)	VB (%)
−P−A	2,957	4,829	1.634	97.3
+P−A	3,009	4,946	1.644	95.6
−P + A	2,963	4,836	1.632	95.8
+P + A	2,963	4,824	1.628	97.8
SEM	24.51	39.27	0.006	1.18
Main effect probiotic				
−P	2,960	4,833	1.633	96.5
+P	2,986	4,885	1.636	97.4
Main effect antibiotic therapy				
−A	2,983	4,888	1.639	97.0
+A	2,963	4,830	1.630	96.8
P value				
P	0.341	0.238	0.622	0.484
A	0.468	0.198	0.202	0.888
P × A	0.347	0.155	0.280	0.485

Notes. Results for the periods 1–42 days.
FCR: feed conversion rate; FI: feed intake; VB: viability; WG: weight gain.

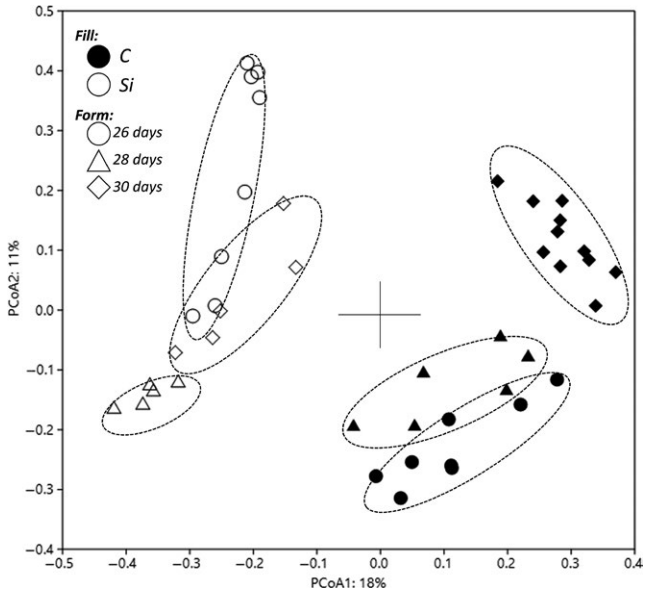

FIGURE 1 Principal coordinate analysis ordination of gut microbial communities identified by gut section and time after the antibiotic therapy. Gut microbial communities from the small intestine—SI (open figures) and caecum contents—C (filled figures). Inside of each gut section, 26 days (circle), 28 days (triangle) and 30 days (diamond)

was maintained higher in the treatments with probiotic in the feed. A corresponding effect of the same treatment (antibiotic without probiotic in the feed) was a decrease in the phylum *Bacteroidetes* in caecum contents at 28 days, but not at the other dates. P × A interactions were detected in the small intestine for *Firmicutes* and *Tenericutes* (Table 8). In the absence of antibiotic therapy, probiotic increased the frequency of the phylum *Firmicutes*, but there was no effect when the birds were medicated. On the other hand, dietary probiotic supplementation resulted in lower frequency of *Tenericutes* in the small intestine in the absence of antibiotic therapy, but not when the medication was applied.

The main groups detected in the small intestine contents were *Lactobacillus*, *Staphylococcus*, *Streptococcus*, *Faecalibacterium* and *Enterobacteriaceae* (Table 9). *Lactobacillus* was the most predominant genus in the small intestine contents of the chickens. Significant P × A × T interactions were observed on the frequency of *Lactobacillus* ($p = 0.03$) and *Faecalibacterium* ($p = 0.02$). Overall, there was an increase of *Lactobacillus* with age for all treatments, except for the control treatment. When the diets were supplemented with probiotic, the frequency of *Lactobacillus* was lower at 26 days but there was a trend for increasing at 28 and 30 days; this trend was not observed for the chickens not supplemented. There was no clear effect of the antibiotic therapy on the frequency of *Lactobacillus* (Table 9). The population of *Faecalibacterium* was lower in the small intestine of the birds fed diets with probiotic and medicated with antibiotic at 26 days; however, at 30 days, this population was lower in birds supplemented with probiotic without antibiotic therapy.

The predominant groups at the genus level in the caecum contents were *Lactobacillus*, *Streptococus*, *Faecalibacterium*, *Ruminococcus* and

TABLE 6 Copy numbers of the gene 16S rDNA per gram of small intestine and caecum contents

Treatments	Small intestine	Caecum
Main effect probiotic (P)		
−P	7.51×10^{8}	1.33×10^{11}
+P	8.79×10^{8}	1.21×10^{11}
Main effect antibiotic therapy (A)		
−A	6.37×10^{8}	1.39×10^{11}
+A	1.01×10^{9}	1.21×10^{11}
Main effect time after the antibiotic therapy (T)		
26 days	1.13×10^{9}	3.70×10^{11a}
28 days	2.17×10^{8}	1.19×10^{10b}
30 days	1.06×10^{9}	9.18×10^{9b}
Significance (main effects and interactions)		
P	0.33	0.59
A	0.18	0.96
T	0.78	<0.001
P × A	0.72	0.81
P × T	0.87	0.45
A × T	0.47	0.73
P × A × T	0.50	0.44
SEM	0.18	0.081

Notes. Means within the same column with different superscript "a, b" differ significantly ($p \leq 0.05$). Values are means of nine pens/treatment (40 birds/pen).

Bacteroides (Table 10). There was single effect of antibiotic therapy on the population of *Lactobacillus* ($p = 0.05$) and P × A × T interaction on *Bacteroides* population in the caecum ($p < 0.01$). The antibiotic therapy promoted higher number of *Lactobacillus* in the caecum contents of birds. At 28 days, chickens receiving probiotic or antibiotic therapy had the frequency of *Bacteroides* in the caecum reduced compared to the control birds.

There was no main effect of P, A, T or interaction between the factors on richness and diversity indexes in the small intestine contents of chickens. In general, richness and diversity indexes were lower in the small intestine when compared to caecum microbiota (Table 11). A significant P × A interaction in the richness ($p = 0.039$) and diversity ($p = 0.035$) and main effect of age ($p < 0.001$) were observed in the caecum contents (Table 11). The age of sampling, irrespective of whether the birds were submitted or not to antibiotic therapy, showed a reduction in the richness and diversity indexes of the microbiota of the caecum contents. At 28 and 30 days, the richness and diversity of caecum microbiota were similar, but lower than at 26 days of age of the chickens.

The P × A interaction encountered for richness and diversity in the caecum ($p < 0.05$) revealed that, compared to the control, probiotic and antibiotic therapy individually decreased those characteristics, but no further reduction occurred when the treatments were combined (Table 12).

TABLE 7 Frequency (%) of main bacterial phyla in the small intestine (SI) and caecum (C) contents of chickens

	Firmicutes		*Actinobacteria*		*Proteobacteria*		*Bacteroidetes*		*Tenericutes*	
Treatments	**SI**	**C**	**SI**	**C**	**SI**	**C**	**SI**	**C**	**SI**	**C**
26 days										
−P										
−A	86.7	82.7	2.0	1.7	2.3	1.0	2.3	1.0	5.0	6.6
+A	86.0	93.3	7.7	0.3	2.0	0.6	1.7	1.3	2.0	2.3
+P										
−A	92.0	82.7	5.5	1.0	2.0	1.0	0.5	2.3	0.5	6.3
+A	87.0	88.0	9.0	0.3	2.7	3.3	0	2.7	0.6	1.7
28 days										
−P										
−A	89.0	69.0^{b}	7.3	2.0	2	1	0.7	9.5^{a}	1.0	12.5
+A	94.0	89.7^{a}	3.3	1.3	2	1.7	0	2.0^{b}	0	3.7
−A	94.0	87.5^{a}	3.7	1.0	1.7	2.5	0	1.0^{b}	0	6.5
+P										
+A	89.0	86.5^{ab}	6.3	0	1	1	1.7	3.5^{ab}	0	6.0
30 days										
−P										
−A	85.3	86.5	3.0	0.5	2.3	0.5	4.0	2.0	3.6	9.0
+A	88.0	85.3	8.5	0.3	2.0	1.3	0	1.3	0	8.3
+P										
−A	93.0	86.3	5.0	0.3	1.5	1.0	0	1.7	0	6.7
+A	89.0	88.5	6.3	1.0	1.0	2.0	1.7	1.5	2.3	4.0
SEM	1.76	1.60	1.31	0.35	0.38	0.42	0.69	0.51	0.75	0.97
Main effect probiotic (P)										
−P	88.2	84.4	5.3	1.0	2.1	1.0	1.4	2.8	1.9	7.1
+P	90.4	86.5	6.4	0.6	1.7	1.8	0.4	2.1	0.6	5.1
Main effect antibiotic therapy (A)										
−A	90.1	82.4	4.4	1.0	1.9	1.1	1.2	2.9	1.7	7.9^{a}
+A	88.4	88.5	7.3	0.5	1.8	1.6	0.6	2.0	0.8	4.3^{b}
Main effect time after antibiotic therapy (T)										
26 days	87.9	86.7	6.0	0.8	2.2	1.5	1.2	1.8	2.0	4.3
28 days	91.7	83.2	5.8	1.1	1.7	1.5	0.1	4.0	0.2	7.2
30 days	88.3	86.7	5.7	0.5	1.9	1.2	1.4	1.6	1.5	7.0
Significance (main effects and interactions)										
P	0.25	0.23	0.44	0.29	0.38	0.06	0.19	0.59	0.13	0.09
A	0.39	<0.01	0.06	0.18	0.84	0.28	0.29	0.43	0.34	<0.01
T	0.22	0.22	0.98	0.55	0.50	0.68	0.38	0.09	0.18	0.06
P × A	0.04	0.03	0.73	0.61	0.74	0.99	0.10	0.01	0.04	0.36
P × T	0.78	0.07	0.78	0.36	0.66	0.64	0.97	0.06	0.63	0.54
A × T	0.86	0.11	0.51	0.36	0.88	0.35	0.98	0.39	0.96	0.45
P × A×T	0.77	0.03	0.14	0.81	0.69	0.09	0.39	0.02	0.42	0.15

Note. Means within the same column with no common superscript "a, b" differ significantly ($p \leq 0.05$).

4 | DISCUSSION

In this study, we combined different molecular techniques (T-RFLP, qPCR and sequencing) to investigate chicken gut microbial communities from the small intestine and caecum contents and associated these results with performance parameters. The first technique used was T-RFLP which is not a tool for the absolute characterization and identification of microbial communities but,

instead, allows changes in community structure to be studied. Real-time PCR was used to quantify total *Bacteria*, and the sequencing allowed phylogenetic classification at the phylum and genera level as well as estimation of microbial richness and diversity indexes in the chicken gut. 16S rRNA gene sequencing has been employed in a range of studies to assess the diversity and phylogenetic relationships of gut microbes, and this has proven to be a powerful tool for understanding the factors that shape microbial communities, due to both its informative and predictive potential (Waite & Taylor, 2014). A number of studies describe the effect of antibiotic therapy altering the balance of gut microbiota in humans (Ferrer et al., 2014; Macfarlane, 2014; Robinson & Young, 2010), but a similar effect, or its association with performance, has not been demonstrated for chickens. In humans, Sullivan, Barkholt and Nord (2003) demonstrated the capacity of dietary probiotic supplementation in preventing ecological disturbances during the antibiotic therapy. In the original definition as proposed by Fuller (1984), probiotics exerted their effect by improving the intestinal microbial balance of the host. The non-pathogenic *Bacillus* species of Gram-positive bacteria have been studied and used as additives for their capacity of sporulation in poultry rearing (Griggs & Jacob, 2015). This characteristic favours the use of *Bacillus* as a probiotic, since it is resistant to heat produced during the feed pelleting process (Shivaramaiah et al., 2011).

The improvement in performance observed in this study in birds supplemented with probiotic is in agreement with other studies that also reported the positive effects of probiotics on performance parameters (Geier, Torok, Allison, Ophel-Keller, & Hughes, 2009; Patterson & Burkholder, 2003; Zhang & Kim, 2014). In addition, others studies have shown improvement in performance using probiotic in the diets when chickens were under challenge conditions, such as *C. perfringens* (Jayaraman et al., 2013), *Escherichia coli* (Zhang et al., 2014), *Eimeria* sp. (Giannenas et al., 2014) or simply injection of LPS (Jiang, Schatzmayr, Mohnl, & Applegate, 2010). However, taking together the results of many different trials, there is not always a beneficial effect of the probiotic to chickens (Huyghebaert et al., 2011; Willis, Isikhuemhen, & Ibrahim, 2007).

TABLE 8 P × A interaction in the frequency of *Firmicutes* and *Tenericutes* in the small intestinal contents

	Antibiotic therapy (A)	
Probiotic (P)	**−A**	**+A**
Firmicutes		
−P	87.00^{B}	89.33
+P	93.22^{A}	87.56
Tenericutes		
−P	3.22^{Aa}	0.67^{b}
+P	0.17^{B}	1.0

Notes. Means within a column and main effects not sharing a common superscript "A, B" are different ($p \leq 0.05$).
Means within a row and main effects not sharing a common superscript "a, b" are different ($p \leq 0.05$).

The major phyla observed in the intestinal contents in this study (*Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria*) coincide with those reported by Singh et al. (2014) in faecal samples of chickens. *Firmicutes*, *Bacteroidetes* and *Tenericutes* were more severely affected by antibiotic therapy or probiotic than other bacteria phyla. For Zhu, Zhong, Pandya, and Joerger (2002), *Firmicutes* is the most predominant phylum in the chicken gut, including the main classes *Bacilli* and *Clostridia* which contain beneficial and pathogenic groups to broiler chickens. A reduction in *Bacteroidetes* and an increase in *Firmicutes* in the gut of animals supplemented with probiotic was also observed by Cui, Shen, Jia, and Wang (2013) and Zhao et al. (2013). Studies in some species have related alteration in gut microbiota to an increase in BW mainly involving a shift in two major bacterial phyla *Firmicutes* and *Bacteroidetes* (Guo et al., 2008; Ley, Turnbaugh, Klein, & Gordon, 2006). Singh et al. (2013) observed a significant increase in the body weight associated with an increase in *Firmicutes*/*Bacteroidetes* ratio in chickens that were supplemented with penicillin as AGP in comparison with chickens from control group. We also detected this effect in chickens submitted to the antibiotic therapy and those that were supplemented with probiotic in the diet.

In this study, the dietary probiotic supplementation or the therapeutic antibiotics, when used alone or in combination, increased the proportion of *Lactobacillus* and decreased the proportion of *Faecalibacterium* in the chicken gut when compared to the control treatment in the small intestine contents. In contrast to our results, Singh et al. (2012) showed higher proportion of the genus *Faecalibacterium* in faecal samples of chickens which had a good feed conversion in comparison with those of poor feed conversion.

In agreement with our results, Videnska et al. (2013) also detected an increase in *Lactobacillus* in the faecal samples of layer hens submitted to antibiotic therapy (tetracycline and streptomycin). On the other hand, Bortoluzzi et al. (2015) observed that zinc bacitracin, when used as AGP, increased the percentage of *Enterococcus* at 21 days of age and tended to increase the percentage of *Clostridiales* and decrease the amount of *Lactobacillales* at 35 days in the small intestine of chickens.

Terminal restriction length polymorphism analysis revealed that the gut section and the time after the antibiotic therapy had significant impact on the profile of the gut microbiota. We found that the time after the antibiotic therapy shifts the gut microbiota composition in the small intestine and caecum contents independently. The bacterial structure in the caecum contents of birds at 30 days was changed in relation to 26 and 28 days. In addition, qPCR analysis of the intestinal microbiota demonstrated that there was a decrease in the total bacterial population and in the number of different bacterial species in the caecal contents of the birds with the time after the antibiotic therapy (the bacterial population was lower at 28 and 30 days of age in relation to 26 days). On the other hand, the percentage of *Lactobacillus* increased in the gut of chickens in all treatments except for the control treatment. When the diets were supplemented with probiotic, the frequency

TABLE 9 Frequency (%) of *Lactobacillus* (LAC), *Staphylococcus* (STAPHY), *Streptococcus* (STREPTO), *Faecalibacterium* (FAECA) and *Enterobacteriaceae* (ENTERO) in the small intestine contents of chickens

Treatments	LAC	STAPHY	STREPTO	FAECA	ENTERO
26 days					
−P					
−A	47.6^{ab}	1.3	4.3	2.3^{a}	1.0
+A	36.0^{b}	1.0	7.3	2.7^{a}	0.3
+P					
−A	19.5^{b}	1.5	5.5	4.0^{a}	0.5
+A	28.7^{b}	3.0	31.7	0.3^{b}	1.0
28 days					
−P					
−A	61.7^{a}	1.3	10.3	0.7	0.6
+A	65.0^{a}	1.3	11.7	0.6	0.6
−A	49.7^{ab}	2.3	12.7	0.7	0.33
+P					
+A	67.5^{a}	1.0	4.5	0.5	0.5
30 days					
−P					
−A	24.0^{b}	0.5	2.5	4.5^{a}	0.5
+A	77.5^{a}	1.5	2.0	1.0^{ab}	0.5
+P					
−A	74.5^{a}	1.0	2.0	0.5^{b}	0
+A	50.0^{ab}	3.0	5.7	2.5^{ab}	0.5
SEM	5.77	2.73	0.58	0.42	0.27
Main effect probiotic (P)					
−P	52.0	1.2	6.4	2.0	0.6
+P	48.3	2.0	10.0	1.4	0.5
Main effect antibiotic therapy (A)					
−A	46.1	1.3	6.2	2.1	0.6
+A	54.1	1.8	20.4	1.3	0.5
Main effect time (T)					
26 days	33.0	1.7	12.2	2.3	0.7
28 days	61.0	1.5	9.7	0.6	0.5
30 days	56.5	1.5	3.0	2.1	0.4
Significance (main effects and interactions)					
P	0.92	0.19	0.25	0.40	0.88
A	0.11	0.43	0.086	0.79	0.46
T	<0.01	0.82	<0.01	0.03	0.81
P × A	0.18	1.0	0.43	0.81	0.63
P × T	0.40	0.96	0.06	0.32	0.92
A × T	0.89	0.66	0.01	0.63	0.53
P × A×T	0.03	0.40	0.07	0.02	0.57

Note. Means within the same column with no common superscript "a, b" differ significantly ($p \leq 0.05$).

of *Lactobacillus* was lower at 26 days but there was a trend for increasing at 28 and 30 days. Lactobacilli are nonpathogenic Gram-positive inhabitants of animal intestinal microbiota that are widely used as probiotics (Brisbin et al., 2011). The positive effect of probiotic in comparison with the control diet on performance in this study may be associated with higher *Lactobacillus* number in the small intestine content. It has been documented that *Lactobacillus* species produce bacteriocins that are active against

TABLE 10 Frequency (%) of *Lactobacillus* (LAC), *Streptococcus* (STREPTO), *Faecalibacterium* (FAECA), *Ruminococcus* (RUMINO) and *Bacteroides* (BACTERO) in the caecum contents of chickens

Treatments	LAC	STREPTO	FAECA	RUMINO	BACTERO
26 days					
−P					
−A	7.7	2.7	10.0	2.0	0.3
+A	9.3	9.0	8.7	2.7	0.3
+P					
−A	4.7	3.7	12.0	2.0	0.7
+A	7.7	4.0	8.3	3.3	0.7
28 days					
−P					
−A	4.5	2.0	10	2.5	3.5[a]
+A	10.0	6.7	7.3	2.3	1.0[b]
+P					
−A	9.5	3.0	8.5	3.5	0.7[b]
+A	11.0	5.0	9.0	4.5	1.0[b]
30 days					
−P					
−A	8.5	4.5	9.5	4.0	0.5
+A	7.3	3.0	11.0	3.7	0.3
+P					
−A	7.7	6.0	11.3	3.3	0.7
+A	7.0	3.0	7.0	2.5	0.7
SEM	2.0	1.05	1.75	0.40	0.2
Main effect probiotic (P)					
−P	10.9	4.6	9.4	2.9	1.0
+P	13.3	4.1	9.4	3.1	0.5
Main effect antibiotic therapy (A)					
−A	7.1[b]	3.6	10.2	2.9	0.9
+A	17.1[a]	5.1	8.5	3.2	0.6
Main effect time (T)					
26 days	7.3	4.8	9.8	2.5	0.5
28 days	15.3	4.2	8.7	3.2	1.4
30 days	13.3	4.1	9.7	3.4	0.4
Significance (main effects and interactions)					
P	0.75	0.67	0.98	0.47	0.04
A	0.05	0.24	0.40	0.55	0.09
T	0.36	0.85	0.88	0.63	<0.01
P × A	0.74	0.18	0.67	0.24	0.04
P × T	0.31	0.63	0.92	0.13	<0.01
A × T	0.72	0.12	0.95	0.36	0.39
P × A × T	0.14	0.71	0.66	0.76	<0.01

Note. Means within the same column with no common superscript "a, b" differ significantly ($p \leq 0.05$).

other Gram-positive bacteria such as *C. perfringens* (Fassina, Newman, Stough, & Liles, 2016). In agreement with our results, Angelakis and Raoult (2010) observed that chickens inoculated with *Lactobacillus* (4×10^{10}) at 4 days of age had higher weight gain in comparison with control treatment (without *Lactobacillus* inoculum). However, it is important to consider that the distinction between beneficial and harmful bacteria is not clear. Indeed, a large array of intestinal microorganisms, which normally are commensals, can, at some point, become a potential threat to the host (Ducatelle et al., 2015).

TABLE 11 Richness and diversity indexes in the small intestine (SI) and caecum (C) contents

	Richness		Diversity	
	SI	C	SI	C
26 days				
−P				
−A	928	7,012	6.1	10.5
+A	1,054	4,141	5.4	8.5
+P				
−A	585	4,989	4.4	9.1
+A	657	4,807	4.9	9.4
28 days				
−P				
−A	903	5,019	5.0	9.3
+A	687	1,987	4.5	6.6
+P				
−A	768	1,413	4.6	6.6
+A	871	4,472	4.8	8.94
30 days				
−P				
−A	998	2,016	5.8	7.5
+A	792	2,026	4.4	7.5
+P				
−A	820	2,426	4.8	7.3
+A	1,130	1,619	6.2	6.3
SEM	72.28	546	0.33	0.43
Main effect probiotic (P)				
−P	894	3,700	5.2	8.3
+P	805	3,287	5.0	7.9
Main effect antibiotic therapy (A)				
−A	833	3,812	5.1	8.4
+A	865	3,175	5.0	7.9
Main effect time (T)				
26 days	806	5,237^{a}	5.2	9.4^{a}
28 days	807	3,223^{b}	4.7	7.9^{b}
30 days	935	2,022^{b}	5.3	7.1^{b}
Significance (main effects and interactions)				
P	0.30	0.49	0.50	0.44
A	0.70	0.30	0.77	0.25
T	0.37	<0.001	0.39	0.023
P × A	0.13	0.039	0.054	0.035
P × T	0.08	0.88	0.27	0.89
A × T	0.72	0.54	0.99	0.83
P × A × T	0.40	0.10	0.51	0.059

Note. Means within the same column with no common superscript "a, b" differ significantly ($p \leq 0.05$).

Considering the chicken performance at 28 days (the first record of performance after the antibiotic therapy), it was observed that the birds submitted to antibiotic therapy did not show impairment in performance, as we have hypothesized. Indeed, the FCR in medicated birds was improved at 28 days. Probably, the changes that the 3 days of therapeutic treatment caused in the gut microbiota

TABLE 12 Effects of probiotic and antibiotic therapy on the index of richness and diversity in the caecum contents

	Antibiotic therapy	
Probiotic (P)	**- A**	**+ A**
Richness		
–P	4,682^{Aa}	2,718^{b}
+P	2,943^{B}	3,633
Diversity		
–P	9.12^{Aa}	7.50^{b}
+P	7.69^{B}	8.20

Notes. Means within a column not sharing a common superscript "A, B" are different ($p \leq 0.05$).
Means within a row not sharing a common superscript "a, b" are different ($p \leq 0.05$).

of chickens were not sufficiently harmful to induce negative effects on performance. Plummer et al. (2005) described that the extent of the disturbance of the chicken microbiota depends on the nature of the antimicrobial agent, the absorption, the route of elimination and any potential enzymatic degradation and/or binding to faecal material.

Finally, the supplementation of the dietary probiotic *B. subtilis* resulted in lower richness and diversity indexes in the caecum contents, indicating a more stable microbiota which can be beneficial for the host, resulting in better performance of the chickens. Thus, the control treatment had greater richness and diversity parameters of the microbial community in the caecum contents in relation to the others treatments. Greater diversity of the microbial community is associated with greater activation of immune cells and inflammatory process (Bai et al., 2016). Inflammation of the gut mucosal epithelium has been shown to be a key mechanism for mucosal colonization by several pathogens (Vuong, Chou, Hargis, Berghman, & Bielke, 2016). Although the antibiotic therapy did not affect the OTU number (richness) and diversity in the small intestine, we observed that the therapeutic treatment decreased the richness and diversity indexes in the caecum community.

5 | CONCLUSIONS

The improved performance of broiler chickens supplemented with probiotic in the diet is associated with changes promoted on the gut microbiome such as an increase in the phylum *Firmicutes* and in the genus *Lactobacillus* in the small intestine and a reduction in the OTUs in the caecum. This may be an indicative that the probiotic *B. subtilis* promoted selection of specific groups in the intestinal microbiota.

The antibiotic therapy modulated the structure of the gut microbiota community in chickens; however, the changes were not detrimental enough to impair the performance of the broilers.

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