Changes in the ceca microbiota of broilers vaccinated for coccidiosis or supplemented with salinomycin

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ABSTRACT The objective of this study was to characterize differences in the cecal microbiota of chickens vaccinated for coccidiosis or receiving salinomycin in the diet. In this study, 140 male 1-day-old broiler chickens were divided in 2 groups: vaccine group (live vaccine) vaccinated at the first day and salinomycin group (125 ppm/kg since the first day until 35 d of age). Each treatment was composed for 7 replicates of 10 birds per pen. At 28 d, the cecal content of one bird per replicate was collected for microbiota analysis. The genetic sequencing was conducted by the Miseq Illumina platform. Vaccine group showed lower body weight, weight gain, and poorer feed conversion in the total period ($P < 0.05$). Bacterial 16S rRNA genes were classified as 3 major phyla (Bacteroidetes, Firmicutes, and Proteobacteria), accounting for more than 98% of the total bacterial community. The microbiota complexity in the cecal was estimated based on the α-diversity indices. The vaccine did not reduce species richness and diversity ($P > 0.05$). The richness distribution in the salinomycin group was larger and more uniform than the vaccinated birds. Salinomycin group was related to the enrichment of Bacteroidetes, whereas Firmicutes and Proteobacteria phyla were in greater proportions in the vaccine group. The last phylum includes a wide variety of pathogenic bacteria. The vaccine did not decrease the species richness but decreased the percentage of Bacteroidetes, a phylum composed by genera that produce short-chain fatty acids improving intestinal health. Vaccine group also had higher Proteobacteria phylum, which may help explain its poorer performance.

Key words: broiler chicken, cecal bacterial community, 16S rRNA sequencing

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INTRODUCTION

The concerns about the development of antimicrobial resistance and potential antibiotic residues in meat and eggs were recently increased, pressing the poultry industry to reduce, or even eliminate, the use of anticoccidials in poultry diets. Intensive production system depends on antimicrobials to prevent and treat diseases, as well as to enhance growth performance and the prohibition of antimicrobials as additives may lead to an increase in the incidence of enteric diseases (Castanon, 2007).

Eimeria, an apicomplexan protozoan parasite, is the cause of coccidiosis in the poultry industry. Coccidial infection in broilers results in epithelial cells damage, diarrhea, osmotic stress in the intestine (Perez-Carbajal et al., 2010), and consequently, malabsorption of nutrients (Metzler-Zebeli et al., 2009). As an alternative to the use of anticoccidials, there are vaccines against coccidiosis. However, the performance of the broilers receiving this vaccine is lower than the broilers that receive anticoccidial in the diet (Arczewska-Włosek et al., 2018). The administration of live oocysts through the vaccine results in a low infection level of the intestinal tract, necessary for immunity induction. Mucosal response to vaccination involves an increase in mucin production (Miller et al., 1979; Miller and Nawa, 1979). The mucin layer contains a diversity of

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carbohydrate components, with potentially attachable sites for commensal and pathogenic bacteria (Sonnenburg et al., 2005). Notably, some pathogens utilize mucin as a nutrient source (Adedokun et al., 2012). The increased vaccine-induced mucogenesis may increase the proliferation of pathogenic microbiota, worsening performance.

Therefore, a better characterization of how vaccine impacts the chickens' cecal microbiota is fundamental to improve the use of this alternative method. This study was designed to understand how vaccine changes the intestinal microbiome compared with the common methods of fighting coccidiosis (anticoccidials), in the perspective of explaining the effects in broilers performance.

MATERIALS AND METHODS

All procedures were approved by the Ethics Committee on Animal Use from the Universidade Federal do Rio Grande do Sul, Brazil (35670).

Experimental Procedures

A total of 140 broiler chicks of Cobb strain, with 1 d of age, were raised in a poultry house comprising 14 pens. Each pen housed 10 chicks up to 42 d of age. At the beginning of the trial, the groups were distributed with a 2.5% weight variation of the mean of the total group. The average weight of the 1-day-old chicks was 48.3 g. The broilers were reared in a wood shaving litter that had been reused for 6 times. This approach represents a more realistic concept of the field condition. Environmental temperature management to maintain chickens in thermoneutral conditions during all growth stages were performed using air conditioning; fans and exhaust fans as per the range established by Cobb manual was followed. The temperature (Celsius) and relative humidity (%) were measured daily and the maximum and minimum were registrate and we can be sure there was a humid and hot environment capable of stimulate sporulation and reinfection. We also lightly humidify the litter in the sixth day to assure those conditions.

The basal diets consisted of corn and soybean meal, as main ingredients, and were isonutritive (Table 1). Feed and water were available ad libitum from tubular feeders and nipple drinkers. Half of the chicks received a diet containing 125 ppm of salinomycin since the first day, until 35 d of age, and the other half were vaccinated, via water, against coccidiosis with a live attenuated vaccine (LIVACOX, 30 to 50 thousand oocysts of each attenuated strain *of Eimeria tenella, Eimeria acervulina, and Eimeria maxima*) on the first day of life. Broiler weight and feed intake were measured weekly. These data were used to calculate the average daily gain, daily feed intake, and feed conversion ratio.

Sample Collection, Cecal DNA Extraction, and Library Preparation

At 28 d of age, 1 bird within the average weight of each replicate was euthanized. When the birds were

Table 1. Nutritional composition of the experimental diets.

Ingredients (%)	Prestarter 1–7 d	Starter 8–21 d	Grower 22–33 d	Finisher 34–42 d
Soybean meal	37.66	34.84	31.86	27.77
Maize	56.64	58.91	61.21	65.66
Soybean oil	1.38	2.32	3.45	3.42
L-Lysine	0.30	0.33	0.24	0.27
DL-Methionine	0.36	0.31	0.28	0.27
L-Threonine	0.11	0.07	0.06	0.06
NaCl	0.52	0.5	0.47	0.46
Limestone	0.91	0.94	0.88	0.8
Phosphate	1.89	1.55	1.32	1.11
Choline	0.05	0.05	0.05	0.05
Min premix[1]	0.10	0.10	0.10	0.10
Vit premix[2]	0.034	0.034	0.034	0.034
Salinomycin or kaolin[3]	0.05	0.05	0.05	x
Nutrient composition, calculated				
ME (kcal/kg)	2,960	3,050	3,150	3,200
CP, %	22.40	21.21	19.90	18.400
Ca, %	0.920	0.841	0.758	0.663
Available P, %	0.470	0.401	0.354	0.309
Na, %	0.220	0.21	0.200	0.195
Digestible Arg, %	1.393	1.308	1.221	1.145
Digestible Lys, %	1.324	1.275	1.131	1.060
Digestible Met %	0.651	0.588	0.552	0.525
Digestible Met + Cys, %	0.953	0.876	0.826	0.774
Digestible Trp, %	0.257	0.241	0.225	0.211
Digestible Thr, %	0.861	0.791	0.735	0.689

[1]Mineral premix (per kg/feed) = cobalt 200 mg, 88,000 mg manganese, 95,535 mg zinc, 64,715 mg iron, 15,000 mg copper, 1,795 mg iodine, 200 mg selenium.

[2]Vitamin premix (per kg/feed) = 34.520.000 IU vitamin A, 7.200. 000 IU vitamin D3, 90.000 IU vitamin E, 8.600 mg vitamin K, 6.700 mg vitamin B1, 20.000 mg vitamin B2, 9,000 mg vitamin B6, 72.000 mcg vitamin B12, 34.000 mg pantothenic acid, 140.000 mg niacin, 2.800 mg folic acid, and 240 mg biotin.

[3]Kaolin is considered to have zero nutrient contribution.

slaughtered for the sample collection of the cecal content, scores of the 1 and 2 intestinal lesions (Johnson and Reid, 1970) were observed in the vaccinated birds. Ceca luminal samples were collected from the bottom of the cecum aseptically. All samples were gathered within 30 min after slaughter and immediately transferred into a −80°C refrigerator until use sample analysis.

The cecal samples were thawed and homogenized, and ∼200 mg of each sample was used for extraction of microbial genome DNA using the E.Z.N.A. Stool DNA Kit (Omega Bio-Tek) as per the manufacturer's instructions. The DNA concentration was measured by Qubit 3.0 Flurometer.

The V4 region of bacterial 16S rRNA gene was amplified using F515 (5′CGTCGGCAGCGTCAGATGT GTATAAGAGACAGGTGCCAGCMGCCGCGGA 3′) and R806 (5′GTCTCGTGGGCTCGGAGATGTGT ATAAGAGACAGGGACTACHVGGGTWTCA 3′) primers to characterize the cecal bacterial composition, both modified to contain an Illumina adapter region as described by Caporaso and AuthorAnonymous (2010). Amplification was performed in a 25 μL mixture, consisting of ∼100 ng of genomic DNA, 1.0 mmol $MgCl_2$, 0.5 μmol of each primer, 0.2 mmol of each dNTP, 2U PlatinumTaq DNA Polymerase High Fidelity (Life Technologies), and 1x reaction buffer. Amplification was carried out in a Mastercycler Personal 5332 Thermocycler (Eppendorf) in accordance with the following program: initial denaturation at 94°C for 2 min, followed by 25 cycles of 45 s at 94°C, 45 s at 55°C 1 min at 72°C and a final cycle at 72°C for 6 min. Five microliters of each PCR product was used to verify amplification by gel electrophoresis on a 1% agarose gel.

Amplicons were purified using Agencourt AMPure XP beads following manufacturer instructions. Purified products were again quantified checked in Qubit Fluorometric Quantitation. Indexes were added to DNA libraries following the manufacturer instructions (Illumina Inc., San Diego, CA). Sequencing was conducted on platform Illumina MiSeq with a v2 500 kit, which generates paired-end reads of 250 bp.

Sequences Processing, Classification of Samples and Statistical Analysis of Microbiota and Performance

Bioinformatics analyses of 16S rRNA amplicons were performed using QIIME 2 version 2019.4 (Bolyen and Caporaso, 2019). Raw sequence data were quality filtered, denoised, and chimera-filtered using the q2-dada2 plugin with DADA2 pipeline Callahan (Callahan et al., 2016). The 5′ end 5 nucleotide bases were trimmed from forward and reverse read sequences because of low quality. Reads with a number of expected errors higher than 2 were discarded. Read length filtering was applied and the reads were trimmed at the first instance of a quality score less than or equal to 11. The resulting reads with nucleotide overlap between the forward and reverse reads below 20 and shorter than 250 bp length were discarded. Chimera removal was performed using the consensus method. The amplicon sequence variants (ASV) obtained by DADA2 pipeline were merged into a single feature table using the q2-feature-table plugin. The ASV were aligned with MAFFT (via q2-alignment) (Katoh, 2002) and used to construct a phylogeny with fasttree2 (via q2-phylogeny) (Price et al., 2010). Taxonomy was assigned to ASV using the q2-feature-classifier (Bolyen and Caporaso, 2019) classify-sklearnnaïve Bayes taxonomy classifier. The classifier was trained using extracted Greengenes 13_8 reference sequences with 99% similarity truncated at 250 bp length from 16S rRNA variable region 4 (V4). The resulting feature table, rooted tree from reconstructed phylogeny, and taxonomy classification were imported from Qiime2 to R v3.6.1 environment for further data analysis using Microbiome v1.6.0 (Stevenson and Weimer, 2007) and Phyloseq v1.28.0 R packages (McMurdie and Holmes, 2013). For taxonomic analysis, feature table was transformed to compositional data for taxa bar plot composition visualization of the 10 most abundant genera using plot composition function from Microbiome R package. Community analysis, alpha-diversity metrics (Shannon, Simpson, Chao1, Log Modulo Skewness), beta diversity metrics weighted UniFrac (Lozupone and Knight, 2005), unweighted UniFrac (Lozupone et al., 2007) and Bray-Curtis dissimilarity were estimated using Microbiome and Phyloseq packages in R statistical software. R. Canonical correspondence analysis (CA) and detrended correspondence analysis (**DCA**) were applied to beta diversity chosen metrics using plot ordination function from Phyloseq. Alpha diversity significance was estimated with a pairwise comparison using the nonparametric test Wilcoxon (Wilcoxon, 1946), by Microbiome R package functions. Beta diversity significance were estimated with a permutational multivariate analysis of variance (Anderson et al., 2011) using distance matrices obtained by ordination previously described with Permutational Multivariate Analysis of variance test (PERMANOVA), Adonis function of Vegan R package (Oksanen et al., 2007).

The ASV, with less than 2 samples and less than 20 abundance frequency, were removed from the feature table. The resulting filtered features were grouped collapsed at genus level using q2-taxa plugin for differential abundance analysis. Differential abundance analysis was performed using ANCOM q2-composition plugin, with mean difference as fold difference in feature abundances across groups and centered log-ratio (clr) as transform-function for volcano plot. All sequence data have been deposited in the NCBI Sequence Read Archive (accession no. PRJNA594997).

Data of broilers performance were analyzed by ANOVA using the generalized linear model procedure

Table 2. Performance of broilers vaccinated for coccidiosis or supplemented with salinomycin in diets to 1 – 42 d.

	Treatments			
Variables	Salinomycin	Vaccinated	*P* Value[1]	SE
Body weight (g)	3,213	3,090	0.012	42.7
Body weight gain (g)	3,165	3,042	0.050	32.3
Feed intake (g)	5,003	4,995	0.660	42.8
Feed conversion rate (g/g)	1.58	1.64	0.007	0.01

[1]For the effects of treatments salinomycin or vaccinated.

of SAS (SAS Inst. Inc., Cary, NC) and the level of 5% was the significance level considered.

RESULTS AND DISCUSSION

Performance of Broilers

The performance data are presented in Table 2. Broilers that received salinomycin had higher body weight at 42 d, higher weight gain, and consequently, a better feed conversion rate than the vaccine group ($P < 0.05$). No differences were observed for feed intake ($P > 0.05$). The effects of those drugs are not fully understood, but the potential of the intestinal microbiota in increasing feed efficiency has been shown (Singh et al., 2012; Cox and AuthorAnonymous, 2014) and may be considered in this study. Challenged broilers receiving monensin reduced the bacterial domain and *Escherichia coli* (Moraes et al., 2019) and Ribeiro et al. (2000) observed that broilers receiving monensin, without any microbial challenge, showed better feed conversion in relation to broilers without the drug, especially from 21 to 40 d.

Anticoccidial compounds have been used to control coccidiosis, but *Eimeria* species have developed resistance to both chemical and ionophore drugs over time (Stringfellow et al., 2011). In addition, the use of these substances in animal production can turn bacterial strains resistant to the environment. Vaccines, as an alternative for the control of coccidiosis, provide protection and also help reducing resistance to Eimeria by systematically replacing resistant field strains and inducing specific protective immunity by exposing the broilers immune system to Eimeria antigens (Williams and Gobbi, 2002; Dalloul and Lillehoj, 2005; Stringfellow et al., 2011)

Administration of live oocysts of the vaccine results in a low level of infection necessary for immunity development (Dalloul and Lillehoj, 2005; Li et al., 2005; Stringfellow et al., 2011). This low level of infection is one of the factors responsible for affecting performance. The infection, even low, leads to decreased absorbent intestinal surface area and generates an inflammatory process (Lehman et al., 2009). As the poultry cycle is short, the producer who uses vaccines faces a dilemma: there is not always time enough for broilers to recover the weight. In this experiment, we observed that the vaccine group was not able to achieve the same performance as salinomycin-supplemented broilers.

The pyrogenic effect of the vaccine can increase body temperature and energetic demand, diverting nutritional resources from performance to maintain body homeostasis. In addition to this demand, the intestinal mucosa responds to vaccination by increasing mucin production as a form of protection (Miller et al., 1979; Miller and Nawa, 1979). Mucin is composed of amino acids, such as threonine, glycine, proline, and serine (Faure et al., 2005; Lehman et al., 2009), as well as carbohydrates with possible pathogenic attachable sites (Sonnenburg et al., 2005) and increasing mucin production may benefit pathogenic microbiota proliferation (Adedokun et al., 2012). This feature may also be a predisposing factor for a poor performance. The variation in the growth rate may be associated with differences in the microbiome, which has a considerable effect on nutrient digestion, absorption, and metabolism in animal's body (Turnbaugh et al., 2006; Rinttilä and Apajalahti, 2013), and it is also highly associated with host immune systems and health status in animals (Lan et al., 2005; Kogut, 2013).

Microbiota Cecum

A total of 75,209 reads were randomly subsampled to normalize sequence numbers. The subsampling yielded a coverage of 99.9%, indicating that it was representative of the total population.

Alpha Diversity

There was no statistical difference between groups regarding to the estimated richness (Chao1) or diversity (Simpson, Shannon, and Skewness indices) (Figure 1). The vaccine did not decrease the species richness estimated by the Chao index ($P = 0.94$) compared with the salinomycin group. In addition, there were no differences for diversity species through the Simpson index ($P = 0.84$), Shannon ($P = 0.95$) and Skewness ($P = 0.73$). On the other hand, independently the statistical significance, Chao1 index showed a larger and more uniform distribution in salinomycin broilers, whereas Shannon graphic showed a lower α-diversity species for the microbiota of vaccinated broilers. According to Yegani and Korver (2008), several factors such as diet, environment, and genetics induce changes in the

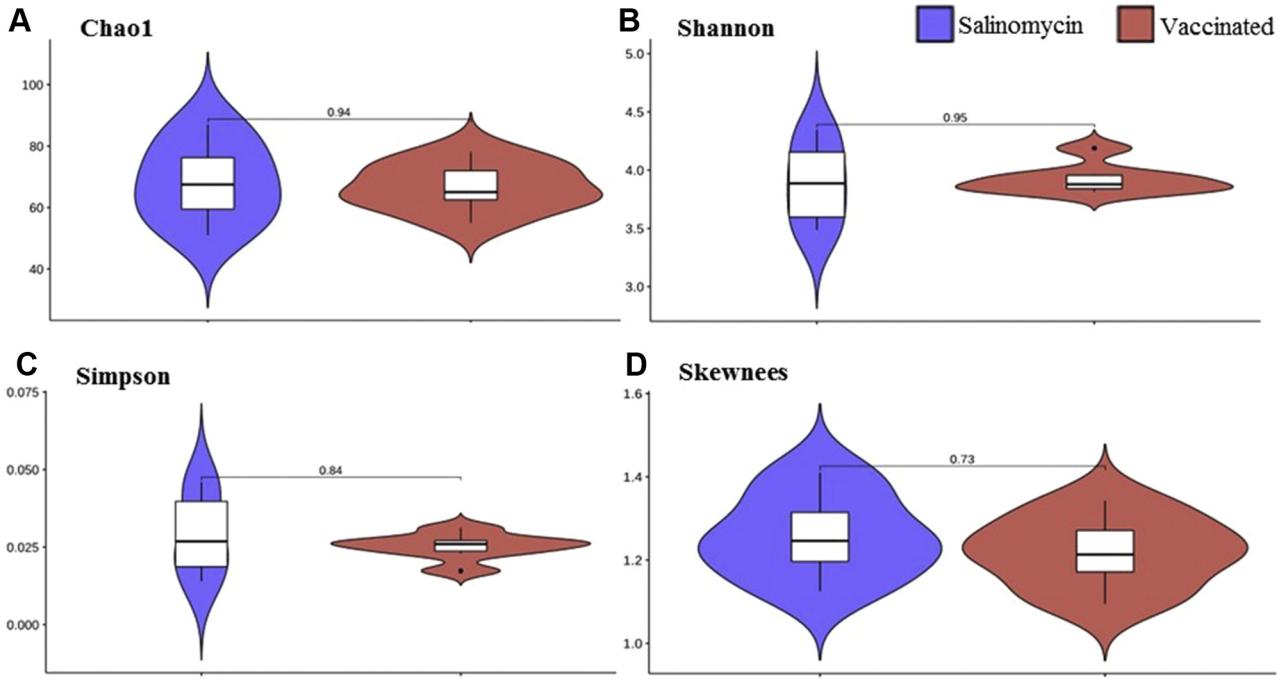

Figure 1. Analysis of alpha diversity in the cecum of broiler chicken treated with salinomycin or vaccinated.

intestinal microbiota; the use of antimicrobials is one of the most important factors.

Beta Diversity

Detrended correspondence analysis of genes was used to evaluate functional structure changes in the microbial communities. Detrended correspondence analysis did not clearly separate populations, with a 27.7% and a 37% variation explained for DCA1 and DCA2, respectively (Figure 2A). This indicates that the structures of the bacterial communities are very similar between both groups. The ordering of bacterial communities (CA1 13,2% and CA2 16,1%) showed a nondistinct cecum microbiota in accordance with intervention (Figure 2B), contrarily to the findings of Czerwiński et al. (2012) who showed that salinomycin supplementation suppressed microbial activity and alter the microbial community structure.

Relative Abundances

As it was seen, none of the treatments caused significant changes in the cecal microbial structure (related to the genera that form a community and how they are distributed), but it affected the microbial participation (the different percentages of phyla and genera in the cecum). In both treatments, 5 phyla were more common (>1%), highlighting the predominance of Firmicutes and Bacteroidetes, followed by a small proportion of Cyanobacteria, Proteobacteria, and Tenericutes. Within the groups, salinomycin had higher Bacteroidetes proportions (54.6 vs. 44.5%), whereas Firmicutes

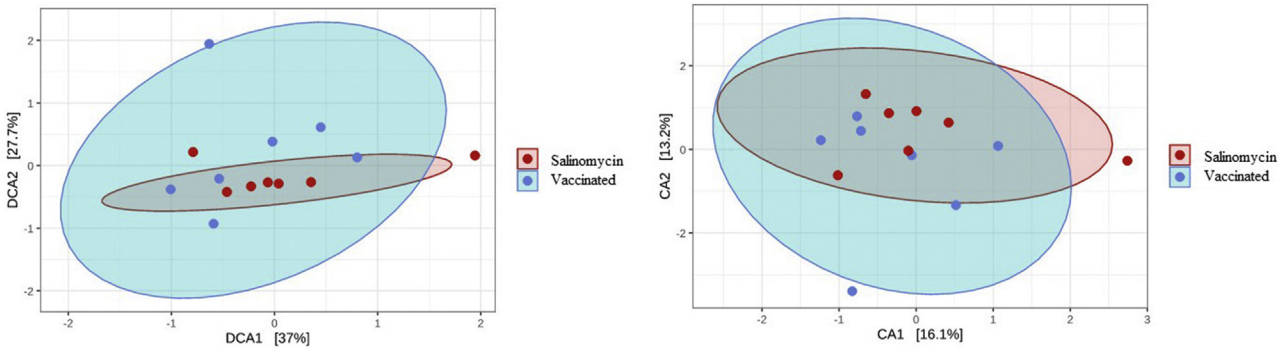

Figure 2. First two dimensions of the detrended correspondence analysis (DCA) and canonical correspondence analysis (CA) and the amount of variation explained are shown. Each circle represents the populations, and colors represent the groups.

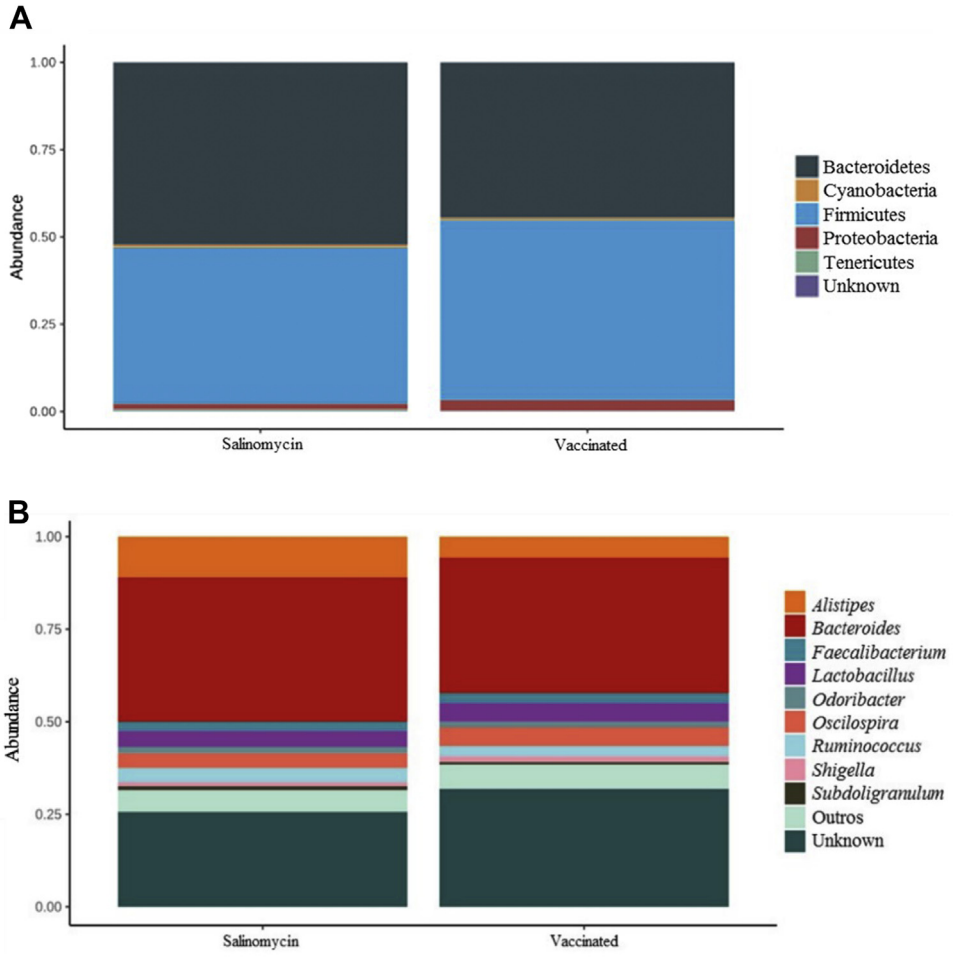

Figure 3. Relative abundances at the phylum (A) and genus (B) level of the main bacteria found in the cecum of broiler chicken treated with salinomycin or vaccinated.

were in lower proportions (42.8 vs. 51.4%; Figure 3), than the vaccine group. Firmicutes and Bacteroidetes have been associated with higher energy uptake bacterial profiles capacity (Xiao et al., 2017) and the last one has been associated with short-chain acid metabolism (Pandit et al., 2018). Short-chain fatty acids (**SCFA**) are considered stimulators of broiler performance and intestinal epithelium growth as well as modulators of composition and activity of the gastrointestinal microbiota (Czerwiński et al., 2012) attributed the best performance of chickens to the increase of the Bacteroidetes phylum.

Firmicutes are in smaller proportions in the salinomycin group. Most of this phylum of bacteria has a gram-positive cell wall and the results can be explained as salinomycin exhibit high activity against gram-positive bacteria. Firmicutes are associated with the fermentation of undigestible feeds in the ceca, improving the digestibility of nutrients. However, Lee et al. (2017) did not associate or correlate this group with better broiler performance. The Proteobacteria more than doubled its percentage in the vaccine group (1.2 vs. 3.1%). This phylum is formed by gram-negative bacteria and includes a wide variety of pathogenic species such as *Escherichia spp., Campylobacter spp., Salmonella spp., Pseudomonas spp.* The lower percentage of Proteobacteria phylum in the salinomycin group may indicate a healthy intestinal environment (Dai et al., 2018), and probably contributed for the better performance observed in this chickens. Tenericutes phylum (0.8 vs. 0.1%) and Cyanobacteria (0.5 vs. 0.7%) were also classified, but these bacteria were present at relatively low abundance.

At the genus level, genera whose proportion exceeds 1% corresponds to 68.5 and 61.5% of the total genera found for the the salinomycin and vaccine groups, respectively. Within the phylum Bacteroidetes, *Bacteroides* genus was found in the highest percentages in the ceca (39% in the salinomycin *vs.* 36% in the vaccine group). This genus is related to the ability to degrade indigestible fiber in the cecum (Lee et al., 2017). The fermentation of indigestible fibers may increase the production of SCFA, helping the host-beneficial cecal microbiota. A higher relative abundance of Alistipes in the salinomycin group was observed in relation to the vaccine group (10 vs. 5%). Alistipes belong to the same Bacteroidetes phylum and is the main member within Rikenellaceae family. They are resistant organisms with the ability of fermenting carbohydrate to produce acetic acid and are generally considered as beneficial

bacteria (Rautio et al., 2003). Odoribacter, the mainly detected genus in the Porphyromonadaceae family was found in a similar percentual between both groups (1.6 vs. 1.4%). This genus is very important for both microbial and host epithelial cell growth (Meehan and Beiko, 2014).

As for Firmicutes phylum, the genus *Oscillospira* was observed in a percentage of 4.1% for salinomycin group and 5.1% for the vaccine group. In the same phylum, the Ruminococcus genus was found in a proportion of 3.8% in the salinomycin and 2.6% in the vaccine group. As members of Ruminococcaceae family, this genus can produce SCFA through glucose metabolism and digest cellulose in feed (Liu et al., 2008).

The Lactobacillus genus was observed at 4.5 vs. 5.2%, for the salinomycin and vaccine groups, respectively. This genus is considered a crucial member of the commensal microbiota regarding health of the host. In a study developed by Czerwinski et al. (2012), the authors noted a lower value of total bacteria and Lactobacillus in the cecum of chickens fed a salinomycin-supplemented diet. The authors justified that this drug can suppress the dominant lactic acid bacteria, potentially Lactobacillus. As seen, in our experiment, although no statistical difference was found between the treatments, it was observed a lower percentage of Lactobacillus in the salinomycin group. Faecalibacterium genus showed similar amount in both groups (2.3 vs. 2.4%). Male chickens with higher body weight were associated to the enrichment of Faecalibacterium (Lee et al., 2017). *Subdoligranulum* genus, member of Firmicutes family was presented at 1.2 vs. 0.6% for the salinomycin and vaccine groups, respectively. This genus comprises species that produce butyrate in the ceca (Lund et al., 2010). *Shigella* affiliated to Proteobacteria, potentially pathogenic, was found in similar percentages in both groups (1.1 vs. 1.6%), showing no treatment influence.

One of the major concerns using vaccine to control coccidiosis is the predisposition of opportunistic bacteria proliferation such as *Clostridium*, which may benefit from mucus produced by the epithelium and cause disease such as necrotic enteritis. In our study, the genus *Clostridium* appeared at level <1% and did not show statistical difference between groups.

The relationship between the genus found in cecal microbiota shows that groups of *Bacteroides* have a strong relationship to each other, and also to the genus *Alistipes* (distance 0.1) (Figure 4), possibly because they are formed by beneficial microorganisms, responsible for degrading insoluble fibers and generating SCFA. *Bacteroides* also have a close relationship to the genus *Lactobacillus*, *Subdoligranulum,* and *Faecalibacterium* (distance 0.1). The genus *Alistipes* has a moderate relationship to *Defluviitalia* and *Oscillospira* (distance 0.2), and a strong relationship to *Faecalibacterium* (distance 0.1).

The genus of *Ruminoccocus* has a strong relationship (distance 0.1) to *Shigella* and *Streptococcus,* which are pathogenic bacteria causing harm to healthy birds. This relationship is still poorly understood and it's not possible to describe in what sense it goes: we cannot describe in what sense it goes: whether it is beneficial or maleficent. Undoubtedly one of the most important and discussed groups of bacteria is the C*lostridium* genus. This genus was shown to be moderately related to *Lactobacillus* and *Oscillospira* (distance 0.2), but strongly correlated with *Shigella*, a pathogenic group (distance 0.1). In this case, Lactobacillus and Oscillospira could control the population growth of the genus Clostridium and in the last case, a correlation in the same direction, that is, causing damage to the birds.

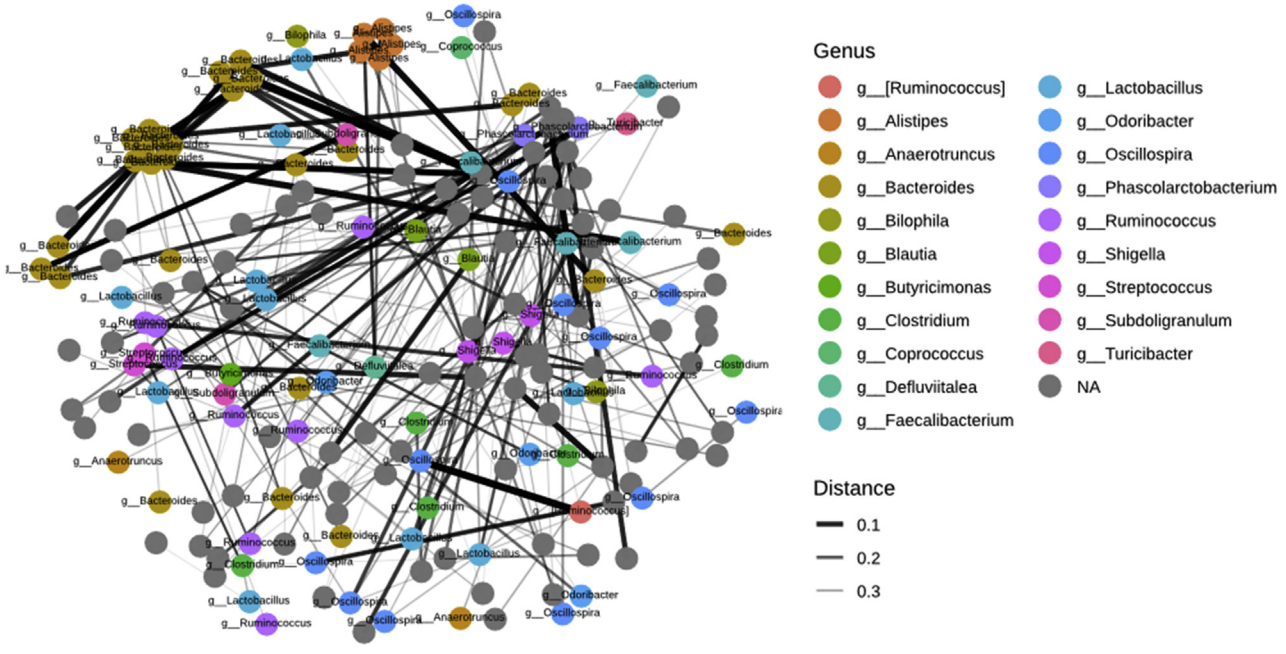

Figure 4. Total relationship between genera found in the cecum of broilers.

The difference between coccidiosis control methods, salinomycin vs. vaccine, lays in the fact that birds receiving salinomycin have a higher percentage of phyla and genera related to SCFA production, resulting in the improvement of intestinal health besides controlling pathogenic bacteria growth. This is confirmed because the vaccine group had a lower percentage of Bacteroidetes phylum and a higher percentage of Proteobacteria phylum. For future studies, we suggest the association of organic acids or prebiotics for the benefit of the SCFA producer bacteria in chickens receiving coccidiosis vaccine to improve those groups of bacteria.

DISCLOSURES

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. The authors confirm that they have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing, they confirm that they have followed the regulations of their institutions concerning intellectual property.

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