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Probiotics composed of *Bacillus* spp. in the litter and feed of broiler chickens

Probióticos à base de Bacillus *spp. na cama e na ração de frangos de corte*

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ABSTRACT: Adding probiotic microorganisms to broiler diets can help improve health status and zootechnical performance throughout the production period. This study aimed to evaluate the effects of *Bacillus amyloliquefaciens* probiotic strains and the composition of *Bacillus subtilis* and *Bacillus toyoi* on their ability to colonize the intestine and litter of broilers. The morphometric aspects of the duodenal villi and zootechnical performance were also evaluated. The treatments were assigned in a complete randomized design. A total of 300 one-day-old male Cobb broiler chicken was used in five treatments, with four replicates each. The birds were housed in batteries of experimental cages (100 x 80 cm), and the leftover feed was weighed weekly to calculate body weight gain, intake, and feed conversion ratio. Two birds from each replicate were euthanasied to determine the *Bacillus* spp. count in the feces of the intestinal content and samples were also collected from the poultry litter (wood shavings of *Pinnus elliottii*) to determine *Bacillus* spp. in different days. The duodenal segments of birds were analyzed to evaluate intestinal morphometry (crypts and villi). There was no change in microbiota characteristics or zootechnical performance between the treatments. In conclusion, commercial probiotic control treatment showed positive results, such as better intestinal colonization and greater presence of probiotic strains in the litter. These effects can result in better performance of birds in situations where field challenges occur.

KEYWORDS: poultry farming; poultry; zootechnical performance.

RESUMO: Adicionar microrganismos probióticos às dietas de frangos de corte pode ajudar a melhorar o estado de saúde e o desempenho zootécnico durante todo o período de produção. Este estudo teve como objetivo avaliar os efeitos das cepas probióticas de *Bacillus amyloliquefaciens* e a composição de *Bacillus subtilis* e *Bacillus toyoi* sobre sua capacidade de colonizar o intestino e a cama de frangos de corte. Os aspectos morfométricos das vilosidades duodenais e o desempenho zootécnico também foram avaliados. Os tratamentos foram distribuídos em um delineamento inteiramente casualizado. Um total de 300 frangos de corte machos Cobb de um dia de idade foram usados em cinco tratamentos, com quatro repetições cada. As aves foram alojadas em baterias de gaiolas experimentais (100 x 80 cm), e a ração restante foi pesada semanalmente para calcular o ganho de peso corporal, ingestão e taxa de conversão alimentar. Duas aves de cada repetição foram eutanasiadas para determinar a contagem de *Bacillus* spp. nas fezes do conteúdo intestinal e amostras também foram coletadas da cama de frango (aparas de madeira de *Pinnus elliottii*) para determinar *Bacillus* spp. em dias diferentes. Os segmentos duodenais das aves foram analisados para avaliar a morfometria intestinal (criptas e vilosidades). Não houve alteração nas características da microbiota ou no desempenho zootécnico entre os tratamentos. Em conclusão, o tratamento controle probiótico comercial apresentou resultados positivos, como melhor colonização intestinal e maior presença de cepas probióticas na cama. Esses efeitos podem resultar em melhor desempenho das aves em situações onde ocorrem desafios de campo.

PALAVRAS-CHAVE: avicultura; frango; desempenho zootécnico.

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INTRODUCTION

Several products have been used to modulate the intestinal microbiota of birds, highlighting antimicrobials as performance-enhancing additives. However, the use of these compounds has been restricted in the European Union since 2006 by the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA). Since 2009, the search for effective alternative products that can improve the microbiota of birds has intensified (European Communities, 2003). Among the options for zootechnical performance-enhancing additives that act on the intestinal microbiota are probiotics, prebiotics, enzymes, and organic acids (Brasil, 2015).

Probiotics are living microorganisms that, when administered adequately, confer health benefits to the host (FAO/WHO, 2002). Probiotics act by modulating the microbiota competitive exclusion, adherence to sites of action in the intestine, and competition for nutrients. They produce substances with antibacterial activity, such as bacteriocins and organic acids, and promote immunomodulation (Gaggia; Mattarelli; Biavati, 2010; Chambers; Gong, 2011). The most used probiotics in bird diets are *Lactobacillus* spp., *Enterococcus* spp., *Saccharomyces* spp., *Bifidobacterium* spp., and *Bacillus* spp. (Gaggia; mattarelli; Biavati, 2010).

Bacillus spp. are gram-positive Bacillus-shaped bacteria. It is a ubiquitous bacterial genus in nature and quite heterogeneous, harmful, innocuous, and beneficial to health. Under stressful conditions, they sporulate and remain indefinitely in the environment (Konemann *et al.,* 2001). These spores are widely used as probiotics and competitive exclusion agents in humans and animals, differentiating them from other species of microorganisms in their vegetative form. The sporulated form of bacteria can withstand the low pH of the digestive tract and reach the intestine in large amounts, where they germinate and are then eliminated in the fecal content. Once in the intestinal environment, they colonize and multiply, thus promoting competitive exclusion and probiotic effects (Casula; Cutting, 2002).

Therefore, the objective of the present work was to evaluate the effects of *Bacillus amyloliquefaciens* probiotic strains and the composition of *Bacillus subtilis* and *Bacillus toyoi* on their ability to colonize the intestine and poultry litter.

MATERIAL AND METHODS

This study was approved by the Committee on Ethics and Use of Animals (CEUA), under opinion no. 01/2015 - CEUA/Universidade Federal do Paraná – Setor Palotina in accordance with ethical principles in animal experimentation adopted by the National Council for the Control of Animal Experimentation (CONCEA).

A total of 300 one-day-old male Cobb Slow broilers from 53-week-old breeders were used. These birds were healthy and vaccinated at the hatchery against Marek's disease, fowl pox, Gumboro disease, and infectious bronchitis.

The birds were housed in batteries of experimental cages composed of four overlapping cages with a diameter of approximately 100 × 80 cm. Each cage was lined with 15 cm of sealed wood shavings of *Pinnus elliottii*. The experimental room was preheated for 2 h before placing the birds for thermal comfort. Throughout the experiment, the heating was maintained. The air was renewed using two exhaust fans.

The experimental design was entirely randomized, with five treatments and four replications of 15 birds each. Treatments with probiotics were carried out using commercial food provided ad libitum from the first day of housing until the end of the experiment. We used a probiotic compound based on *Bacillus subtilis* and *Bacillus toyoi* (4 × 108 CFU/g) at a dose of 2 kg/ton and a commercial probiotic based on *Bacillus amyloliquefaciens* (1 × 109 CFU/g) at a dose of 1 kg/ton. The litter treatment was prepared by diluting 4 g of probiotic in 300 mL of sterile water and spraying on wood shavings (5 g/m2), in a single dose, directly in the litter immediately before placing the birds. The water was supplied with the aid of manual and nipple drinkers without interruption in all treatments.

Probiotics

The commercial feed used in the experiment was nutritionally balanced according to age (Table 1). Among its components, antimicrobial enramycin of the cyclodepsipeptide class is used as an additive to improve zootechnical performance in birds (Palermo-Neto; Almeida, 2002; MAPA, 2015).

The probiotic products were previously tested at the Microbiology Laboratory of the YY, to check the quality of the inoculum and the absence of possible infectious agents. The treatments were described as follows: negative control treatment, birds treated with commercial feed without the addition of any probiotics and housed in the litter without the addition of probiotics; probiotic compound treatment in the litter (probiotic compound based on *Bacillus subtilis* and *Bacillus toyoi* (4 × 108 CFU/g) at a dose of 2 kg/ton and a commercial probiotic based on *Bacillus amyloliquefaciens* (1 × 109 CFU/g) at a dose of 1 kg/ton), birds treated with commercial feed without the addition of probiotics and housed in the litter with the application of a probiotic compound based on *B. subtilis* and *B. toyoi*; probiotic compound treatment in the litter, and in the feed, birds treated with commercial feed with the addition of a probiotic compound based on *B. subtilis* and *B.* toyoi and housed in the litter with application of a probiotic compound based on *B. subtilis* and *B. toyoi*; probiotic compound treatment in the feed, birds treated with commercial feed with the addition of a probiotic compound based on *B. subtilis* and *B. toyoi*, and housed in the litter without probiotic compound; commercial probiotic control treatment, birds treated with commercial feed with the addition of a probiotic based on *B. amyloliquefaciens* and housed in the litter without the addition of the probiotic compound.

Table 1. Ingredients used in the feed of broiler chickens fed from 1 to 28 days.

Ingredients	Initial phase (1 to 14 days)	Final phase (15 to 28 days)
Corn (7,87%)	57, 4578	63,3019
Soybean meal(46%)	35,1898	30,0600
Calcitic limestone	0,9767	1,1859
Dicalcium phosphate	1,8820	1,3756
Common salt	0,3500	0,3500
Methionine 99%	0,1618	0.0373
Vit/mim suplement	0,5000	0,5000
Soy oil	3,4818	3,1893
Total	100,00	100,00
Metabolizable energy (Kcal/Kg)	3.050,00	3.100,00
Crude protein (%)	21,0000	19,0000
Calcium (%)	0,9600	0,9000
Methionine + Cystine (%)	0,8000	0,6304
Total methionine (%)	0,6000	0,3350
Available phosphorus (%)	0,4500	0,3500
Sodium (%)	0,1575	0,1576

Count of *Bacillus* spp.

At 7, 14, 21, and 28 days of housing, eight birds per treatment (two per repetition) were sacrificed, and the intestines were collected aseptically and individually packed in sterile plastic bags (Nasco Whirl-Pak®). In the laboratory, a longitudinal incision was made for each sample, and feces were collected following the protocol proposed by Souza (2011). Subsequently, 1 mL of the solution was transferred to tubes containing 9 mL of 1% peptone water and serially diluted to 10-4 CFU/mL. The serial samples were plated in duplicate, using 0.1 mL of the solution in Petri dishes with TSA medium (2% tryptone soy broth agar). The plates were then incubated at 37 °C for 24 h. After the incubation period, colonies in plates with uniform growth were counted and identified by their morphology, Gram staining, and catalase production, according to Konemann *et al.* (2001).

To count *Bacillus* spp. in the poultry litter at 7, 14, 21, and 28 days of housing, individual aliquots of wood shavings from the cages were collected at five equidistant points and placed in sterile plastic bags. In the laboratory, the material from the five points collected was homogenized, removing an aliquot of 10 g, which was processed according to the methodology described for the intestine.

Intestinal Morphometry and Absorption Area of the Duodenum Mucosa

At 14 and 28 days, duodenum samples were collected from eight birds per treatment (two per replicate) to evaluate the morphometry of the crypts and villi.

Individual samples of approximately 5 cm in length opened at the mesenteric border, distended by the serous tunic, and longitudinally attached were fixed in 10% buffered formalin for 18 h. Subsequently, they were cut, washed in 70% ethyl alcohol, and dehydrated in an increasing series of ethyl alcohol. After dehydration, the samples were cleared in xylene and embedded in paraffin. Five semi-serial 5 µm thick slices were placed on each histological slide, of which six slices were discarded (Silz *et al.,* 2013). The slides were stained according to the technique using hematoxylin and eosin.

For the morphometric study, the images were captured by light microscopy (Olympus BX 50) with a 10× objective, using a computerized image analyzer system (Image Pro Plus, version 5.2 – cybernetic average). The length and width of 20 villi were measured, and the depth and width of 20 crypts of each bird were collected for the duodenum segment. Morphometric measurements were used to calculate the absorption surface of the intestinal mucosa, using the formula proposed by Kisielinski *et al.* (2002).

Evaluation of Zootechnical Parameters

The birds and leftover feed were collected and weighed weekly to calculate weight gain, feed intake, and feed conversion ratio (feed given/animal weight gain).

Statistical Analysis

The results of bacterial counts were transformed into log10, as well as other data, and subjected to statistical analysis

using the GLM-SAS procedure (2002). The data means were compared using the Tukey test at the 5% probability level. To evaluate the relationship between *Bacillus* spp. colony counts, Pearson's correlation, and regression analysis were used to evaluate the bacterial count collections.

RESULTS

The colony count of *Bacillus* spp. in feces and litter samples at 7, 14, 21, and 28 days of the experiment is shown in Table 2, represented in Log10/g.

The *Bacillus* spp. count in feces evaluated the ability of probiotic bacteria to survive and resist the conditions of the bird's gastrointestinal tract. The results during the experimental period showed a significant interaction ($p<0.05$) between the treatments and collection times.

In the breakdown of the interaction (Table 3), it can be observed that in the collection carried out at 7 days, no significant difference ($p>0.05$) was observed between the treatments, although the probiotic strains were recovered from the feces in all treatments that received probiotics.

The probiotic compound treatment in the litter, which was housed in shavings inoculated or sprayed with a probiotic strain, did not show a count of microorganisms in the feces at 7 days, showing that there was no relationship between the inoculum in the litter and the intestinal colonization of the birds in the first week. As expected, there was no recovery of *Bacillus* spp. in the feces of the animals in the negative control treatment, as they did not receive probiotics via any route.

At 14 days, there was a significant difference ($p<0.05$) in the number of microorganisms. The probiotic compound treatment in poultry litter and ration resulted in the highest count; however, it did not differ from the commercial probiotic control, which had intermediate counts, together with the probiotic compound treatment in poultry litter. The lowest values were attributed to negative control and probiotic compound treatments.

Table 2. Counts of *Bacillus* spp. by count of broiler feces and litter as a function of treatment and time (Log10/g).

	***Bacillus* spp. / feces**	***Bacillus* spp. / litter**
Treatments		
Negative control	0,02	0,00[b]
Probiotic compound in the litter	2,05	1,03[b]
Probiotic compound in the litter, and in the feed	20,02	3,87[b]
Probiotic compound in the feed	14,08	13,54[b]
Commercial probiotic control	84,84	116,87[a]
Time		
07 days	14,20	19,11
14 days	9,35	44,79
21 days	29,24	20,63
28 days	44,03	23,75
Analysis of Variance		
Treatment	0,0096	0,0013
Time	0,0004	0,7919
Treatment x Time	<0,0001	0,9763
CV, %	122,26	142,73

Table 3. Average counts of *Bacillus* spp. on days 7, 14, 21, and 28, isolated from feces of broilers submitted to different treatments with probiotics (Log10/g).

Treatments	**07 days**	**14 days**	**21 days**	**28 days**	**Regression**
Negative control	0,00 [a]	0,06 [c]	0,00 [b]	0,00 [b]	NS
Probiotic compound in the litter	0,00 [a]	7,58 [bc]	0,62 [b]	0,00 [b]	NS
Probiotic compound in litter and ration	27,50 [a]	25,00 [a]	8,83 [b]	18,77 [b]	NS
Probiotic compound in the ration	25,00 [a]	0,98 [c]	1,50 [b]	28,85 [b]	NS
Commercial probiotic control	18,50 [a]	13,12 [ab]	135,25 [a]	172,50 [a]	Linear [1]
Value P	0,6015	< 0,0001	0,0002	< 0,0001	

* Averages followed by different letters differ significantly ($p>0,05$) NS: Not significant; [1] $y= 611875 + 83446x$; $R^2= 0,6238i$.

At 21 and 28 days, *Bacillus* spp. in feces was significantly higher ($p<0.05$) in commercial probiotic control treatment than in other treatments. Among all treatments evaluated, only the commercial probiotic control showed increased counts over time, with a continuous linear increase compared to the other treatments.

At no age evaluated (7, 14, 21, and 28 days), the counts of *Bacillus* spp. in the feces of the probiotic compound treatment in the litter were significantly superior to the negative control, although microorganisms were present in the feces of these birds at 14 and 21 days.

Regarding *Bacillus* spp. in the poultry litter, no significant interaction ($p>0.05$) was observed between the treatments and collection times. However, there was a significant difference ($p<0.05$) in *Bacillus* spp. growth in the poultry litter to the treatments, and the commercial probiotic control showed higher weekly counts ($p<0.05$) regardless of the treatment time (Table 3).

Although none of the products used in the experiment had this label indication, the administration of the probiotics used in the commercial probiotic control group in the diet proved to be more effective for environmental colonization. Meanwhile, the probiotic product (compound) containing the strains of B. subtilis and B. toyoi was not recovered from the litter in greater amounts than the negative control treatment.

The analysis of intestinal morphological characteristics did not show a significant difference ($p>0.05$) between treatments at 14 days of age. However, at 28 days, the lowest crypt depths were obtained in the negative control and commercial probiotic control treatments, and intermediate depths were recorded for the probiotic compound in the poultry litter and feed and the probiotic compound in the ration treatments. The deepest crypts were recorded for the probiotic compound treatment in the poultry litter (Table 4).

There were no significant differences in zootechnical performance between treatments at the different ages evaluated (Table 5).

DISCUSSION

The results shown in the count of *Bacillus* spp. are consistent with expectations, as the development of the microbiota in the first days after hatching follows an exponential pattern, the balance of this ecosystem can vary with time and intestinal segment, and the microbiota of the small intestine of birds is balanced only at 2 weeks post-hatch. The initial exponential growth is soon followed by a lag phase caused by nutrient depletion and the accumulation of toxic metabolites (Apajalahti; Kettunen, 2006). Therefore, the transition phase and immaturity of the ecosystem at this time may explain the variation found in the different treatments at 14 days of age.

At 21 and 28 days, *Bacillus* spp. in feces was significantly higher in the commercial probiotic control treatment than in the other treatments. This may be related to the fact that with the microbiota stabilized and evolved to maturity (Lee *et al.,* 2010), the presence of the commercial strain of *B. amyloliquefaciens* in the control probiotic group and increasing amounts showed the ability to resist, survive, and be metabolically active under gastroenteric conditions.

Table 4. Villus length, crypt depth, villus: crypt ratio (V: C), and absorption area (AA) of the duodenal mucosa of broilers at 14 and 28 days of age.

Treatments	Villus, µm	Crypt, µm	V:C	AA, µm²
14 days				
Negative control	1589,50 [a]	259,00 [a]	6,25 [a]	27,66 [a]
Probiotic compound in the litter	1661,00 [a]	280,50 [a]	6,16 [a]	27,38 [a]
Probiotic compound in litter and ration	1668,25 [a]	253,00 [a]	6,69 [a]	27,93 [a]
Probiotic compound in the ration	1687,50 [a]	251,25 [a]	6,80 [a]	28,90 [a]
Commercial probiotic control	1703,33 [a]	245,00 [a]	7,07 [a]	30,29 [a]
CV, %	5,73	9,93	10,13	6,54
Value P	0,1597	0,5846	0,1539	0,0924
28 days				
Negative control	2059,00 [a]	302,75 [b]	7,00 [a]	29,53 [a]
Probiotic compound in the litter	2099,00 [a]	372,25 [a]	5,90 [a]	28,74 [a]
Probiotic compound in litter and ration	2244,00 [a]	367,50 [ab]	6,51 [a]	29,95 [a]
Probiotic compound in the ration	2156,25 [a]	332,50 [ab]	6,69 [a]	29,12 [a]
Commercial probiotic control	2119,00 [a]	286,00 [b]	7,58 [a]	31,29 [a]
CV, %	7,90	10,23	11,86	6,10
Value P	0,6226	0,0038	0,0729	0,6057

* Averages with the same letter do not differ significantly (p>0,05).

Table 5. Weekly productive performance of broiler chickens supplemented with probiotics in the ration or on poultry litter from 1 to 28 days of age.

Treatments	Weight gain /g	Consumption ration/g	Feed conversion
1 a 7 days			
Negative control	162,87 [a]	190,13 [a]	1,168 [a]
Probiotic compound in the litter	158,70 [a]	194,80 [a]	1,229 [a]
Probiotic compound in litter and ration	157,33 [a]	202,57 [a]	1,290 [a]
Probiotic compound in the ration	162,77 [a]	190,70 [a]	1,173 [a]
Commercial probiotic control	157,20 [a]	180,10 [a]	1,147 [a]
CV, %	3,37	6,59	8,07
Value P	0,3878	0,2105	0,2710
1 a 14 days			
Negative control	457,97 [a]	539,65 [a]	1,179 [a]
Probiotic compound in the litter	465,00 [a]	529,22 [a]	1,139 [a]
Probiotic compound in litter and ration	457,83 [a]	534,31 [a]	1,169 [a]
Probiotic compound in the ration	472,42 [a]	553,19 [a]	1,172 [a]
Commercial probiotic control	464,05 [a]	555,93 [a]	1,198 [a]
CV, %	4,97	5,05	4,31
Value P	0,8913	0,5871	0,5871
1 a 21 days			
Negative control	961,06 [a]	1199,82 [a]	1,249 [a]
Probiotic compound in the litter	979,09 [a]	1224,05 [a]	1,250 [a]
Probiotic compound in litter and ration	967,20 [a]	1156,48 [a]	1,250 [a]
Probiotic compound in the ration	984,11 [a]	1173,73 [a]	1,193 [a]
Commercial probiotic control	1011,17 [a]	1229,93 [a]	1,216 [a]
CV, %	2,74	4,75	4,35
Value P	0,1473	0,3362	0,4599
1 a 28 days			
Negative control	1524,45 [a]	2249,25 [a]	1,477 [a]
Probiotic compound in the litter	1542,05 [a]	2206,95 [a]	1,432 [a]
Probiotic compound in litter and ration	1493,31 [a]	2172,84 [a]	1,458 [a]
Probiotic compound in the ration	1562,48 [a]	2230,99 [a]	1,427 [a]
Commercial probiotic control	1605,28 [a]	2218,90 [a]	1,382 [a]
CV, %	4,03	4,21	3,97
Value P	0,1793	0,8213	0,2294

* Averages with the same letter do not differ significantly (p>0,05).

This may have occurred due to some characteristics of this bacterial species, such as the production of extracellular enzymes, including α-amylase, cellulase, metalloproteases, proteases (Ahmed *et al.,* 2014), α-acetolactate, decarboxylase, endoglucanase, hemicellulases, phytase, maltogenic amylase, and xylanase (Supriyati *et al.,* 2015). Ahmed *et al.* (2014) evaluated strains of the same species under the characteristics of the cecal microbiota of broilers and, unlike the results obtained in this study, found no interference of *Bacillus* spp. in the intestine.

The use of the probiotic compound with strains of B. subtilis and B. toyoi did not result in higher intestinal counts; however, the germination capacity of B. subtilis spores in the intestinal tract and its probiotic activity was characterized by Casula; Cutting (2002) in a murine model. The results of the studies by Appelt *et al.* (2010), Traldi *et al.* (2009), Gracia *et al.* (2009), and Leandro *et al.* (2010) demonstrated a beneficial effect of this probiotic strain in chickens.

The effectiveness of the probiotic depends on factors such as the dosage and type of vehicle used (Gaggia; Mattarelli; Biavati, 2010). Because there was no indication in the package insert for application on the poultry litter, the dose used in this study was performed by empirical extrapolation (5 g/m2), and it was probably not enough to cause the probiotic effect of the bacteria, such as surviving, colonizing, and being

metabolically active at the target site (Guarner *et al.,* 2008). The possibility that the tested litter did not act as a vehicle to guarantee the stability of the probiotic strain was ruled out, as, at 14 and 21 days of age, the strain was recovered from the feces of the birds. However, at 28 days, this event did not occur, probably due to the maturation process of the birds' intestinal microbiota and the non-permanence of the probiotic strain in this ecosystem (Apajalahti; Kettunen, 2006).

The *Bacillus* spp. count in the poultry litter did not show a significant interaction between the treatments and collection times. There was only one difference in the growth of *Bacillus* spp. in the poultry litter. According to Chasula and Cutting (2002), the spores of *Bacillus* spp. used as probiotics germinate in the intestine, colonize, multiply, and are eliminated in the fecal content. The host then acts as a multiplying agent for the microorganism, which, being sporulated, would have better conditions for survival in the environment.

Wadud *et al.* (2012) showed that litter might contain this microorganism in its microbiota. However, the results of this study indicate that the use of these probiotic microorganisms administered in the ration, or even applied directly to poultry litter, did not guarantee interference in the litter microbiota and the return of its count.

The probiotic compounds of *B. subtilis* and *B. toyoi* were not recovered from the litter in amounts greater than those of the negative control treatment. The possibility of the interaction of other microorganisms presents in the substrate and competition with other microorganisms present in the litter microbiota in a model of competitive exclusion may justify this point (Gaggia; Mattarelli; Biavati, 2010). In addition to these effects, there is a possibility that the constitution and characteristics of the litter act as suspension vehicles for probiotic strains in a way that reduces their viability (Guarner *et al.*, 2008).

Pearson's correlation values showed a positive association between *Bacillus* spp. in feces and poultry litter. However, the correlation coefficient was low. However, it is important to note that the presence of probiotics is just one of the factors that interfere with the formation and composition of the microbiota. It can also be influenced by other factors, such as the characteristics of microorganisms that already exist in the intestinal environment before the introduction of the probiotic strain, the type of diet provided, the age of the birds, and even the conditions of the housing environment, with greater or lesser stress challenges (Guarner *et al.,* 2008).

According to the analysis of intestinal morphological characteristics, it can be concluded that there was no significant difference between treatments at 14 days of age, with only a difference at 28 days. Smaller crypts are associated with better intestinal health (Viola; Vieira, 2007) and lower energy expenditure to maintain the physiological conditions of the intestinal mucosa (Maiorka, 2004).

In similar evaluations, Pelicano *et al.* (2003) showed no changes in villus height or crypt depth in birds supplemented with *Bacillus* spp. Sen *et al.* (2012) demonstrated a linear increase in the height of the villus and villus × crypt ratio with increasing doses of *B. subtilis* as a probiotic. Using a probiotic strain of *B. amyloliquefaciens*, Lei *et al.* (2015) found positive results in the increase in villus height and the ratio of villus: crypt compared to the control group.

There was no significant difference in zootechnical performance between the treatments at the evaluated ages. Corroborating previous analyses showed no differences between the negative control, probiotic compound in poultry litter, probiotic compound in poultry litter and chow, and probiotic compound in ration treatments. Although it showed positive results in some evaluations, commercial probiotic control treatment was not influenced by these changes to improve its performance indices.

Several studies have shown contradictory results regarding the evaluation of zootechnical parameters using probiotics. In contrast, An *et al.* (2008), who evaluated weight gain, feed intake, and feed conversion in broilers supplemented with *B. amyloliquefaciens*, found better weight gain and a tendency toward better feed conversion. Furthermore, Ahmed *et al.* (2014), with the same strain, showed better growth performance and feed efficiency in broilers raised for up to 35 days.

Similarly, using *B. subtilis*, Gracia *et al.* (2009) evaluated broilers at 21 and 42 days of age, and Sen *et al.* (2012) observed better weight gain and fed conversion during their experiments with broilers and evaluated at 35 days of age. Lei *et al.* (2015), using two doses of *B. amyloliquefaciens*, reported greater weight gain and lower feed conversion.

CONCLUSIONS

The different treatments did not change the microbiota characteristics or zootechnical performance of the probiotic compounds (*B. subtilis* and *B. toyoi*). However, treatment with a commercial probiotic (*B. amyloliquefaciens*) results in better intestinal colonization and a greater presence of probiotic strains in the litter.

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