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Effects of xylanase and probiotic supplementation on broiler chicken diets

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ABSTRACT - The objective of the present study was to evaluate the effects of xylanase and probiotic supplementation on the performance, carcass characteristics, intestinal pH, intestinal viscosity, and ileal microbiota of broiler chickens fed diets containing wheat bran. The study animals were kept in metal cages, and the study was performed using a completely randomized design, with four treatments, six birds per treatment, and six replicates. The four treatments included a control group, a probiotic-supplemented group, a xylanase-supplemented group, and a group that received both xylanase and probiotic supplementation. The diets of all four groups contained wheat bran (50 and 30 g/kg for the starter and grower phases, respectively) and phytase, and at 10 d after hatching, the experimental birds were challenged orally with *Eimeria* sp commercial vaccine. During the initial phase, supplementation with xylanase, probiotics, or their combination yielded greater weight gains than the control diet; however, considering the period from 10-35 d, the chickens receiving xylanase + probiotic and the diet without the additives showed lower weight gain (2.746 and 2.600 kg, respectively). All the supplemented diets reduced cecum viscosity, and supplementation with probiotic showed a significantly lower pH (6.11). The ileal microbiota was also influenced by xylanase and probiotic supplementation, modulating the frequencies of the genera *Lactobacillus* and *Clostridium*. The positive effects of supplementation with xylanase or probiotics alone were similar to those of co-supplementation, and no associative effect was observed.

Keywords: additive, enzyme, ileal microbiota, instestinal viscosity

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1. Introduction

Studies with feed additives in broiler diets gained strength from the ban on the use of antibiotics, in which there was a need to find safe alternatives (Roofchaei et al., 2019). In addition, broiler diets are generally composed of plant-derived ingredients, and such components can contain high levels of non-starch polysaccharides (NSP), which are considered antinutritional factors in broiler nutrition. Thus, the use of feed additives can represent excellent nutritional strategies to minimize these antinutritional effects and, therefore, improve the performance of broilers in a scenario of banning antibiotics as growth promoters. Several additives have been shown to be beneficial, such

as the use of essential oils in feed conversion improvement (Hajiaghapour and Rezaeipour, 2018), prebiotics in microbial modulation (Hazrati et al., 2020), phytogenics acting in the hematological profile (Trindade et al., 2019), and organic acids in the nutrient digestibility (Nguyen et al., 2018).

Considering the NSP present in diets, the dietary administration of enzymes, such as xylanases, can improve the nutrient availability of plant-derived ingredients by favoring fiber hydrolysis (Adeola and Cowieson, 2011). In addition, xylanase could provide prebiotic in the intestinal lumen, from the breakdown of NSP, which would modulate the intestinal microbiota (Craig et al., 2020).

Meanwhile, the administration of probiotics has been reported to enhance the humoral immune response and maintain the intestinal barrier (Huang et al., 2019), antioxidant capacity (Wu et al., 2019), nutrient digestibility, and intestinal morphology of broilers (He et al., 2019), and can, thereby, favor microbial modulation (Rodrigues et al., 2020).

Therefore, the simultaneous administration of exogenous enzymes and probiotics can represent an excellent alternative to mitigate the deleterious effects of antinutritional factors of soluble carbohydrates and provide better microbial environment. However, previous reports of such co-supplementation have yielded divergent results (Vandeplas et al., 2009; Praes, 2013), evidencing that studies on the effects of combined xylanase and probiotic supplementation are needed.

Accordingly, the objective of the present study was to evaluate the effects of supplementary xylanase and probiotics on the performance, carcass characteristics, intestinal pH, intestinal viscosity, and ileal microbiota of broiler chickens fed diets containing wheat bran.

2. Material and Methods

Research on animals was conducted according to the institutional committee on animal use (number 30/2019, according to Brazilian law).

Male Cobb broiler chicks (n = 144), obtained from a commercial hatchery, were vaccinated against Marek, avian Bouba, and Gumboro before the start of the experimental period (March 1 to April 5, 2019). The chicks were initially housed in the experimental poultry house in Manaus, Amazonas, Brazil (latitude 3° 06'13" S, longitude 59° 58' 48" W, 260 m elevation) in a litter that contained infant drinkers and tray feeders. During the first 9 d, the chicks were raised according to the pedigree manual.

At 10 d of age, the chicks were weighed and transferred to metal cages (100 × 45 × 45 cm), which contained nipple drinkers and gutter feeders, with six chicks allocated to each cage, thereby ensuring an area 750 cm^2 per chick. Each experimental chick was challenged with attenuated *Eimeria* oocysts, following the methodology of Rafael (2015). Briefly, the commercial vaccine Bio-Coccivet R, which is a suspension of *E. acervulinas*, *E. brunette*, *E. maxima*, *E. necatrix*, *E. praecox*, *E. tenella*, and *E. mitis*, was orally administered at a dosage of 10 times the manufacturer's recommendation. The experimental period was divided into two rearing phases, starter (10–21 d) and grower (22–35 d), using different supplemented and non-supplemented diets. The ambient temperature during the experimental period ranged from 23 to 31 °C.

The treatments included a control group, a probiotic-supplemented group, xylanase-supplemented group, and a group supplemented with both xylanase and probiotic (Tables 1 and 2).

The probiotic (COLOSTRUM MIX, Biocamp, composed of competitive exclusion probiotic microorganisms and probiotic components obtained from the intestinal microbiota of specific pathogen-free adult poultry, BR) was added at a rate of 0.10 g/kg (manufacturer's recommendation), and the xylanase (Smizyme Xylanase, Saulus, produced from genetically modified yeast *Pichia pastoris*) was added at a rate of 0.10 g/kg. In addition, all the diets were supplemented with a 6-phytase (Smizyme Phytase, Saulus, 500 FTU, 0.10 g/kg), and the inclusion of this enzyme considered its beneficial effects on chicken nutrition and the solidification of its use in the poultry industry.

The nutritional matrix of each enzyme was fulfilled, according to the manufacturer's recommendation, in the diet formulations. Xylanase contributed 150 kcal/kg of feed, whereas phytase contributed 0.12% of available phosphorus, 0.11% calcium, 0.300% crude protein, 0.012% digestible lysine, 0.005% digestible methionine, 0.018% digestible threonine, 0.002% digestible tryptophan, and 0.015% digestible valine. All the diets were formulated according to the nutritional recommendations of Rostagno et al. (2017). Wheat bran was also added to the diets, to increase enzyme substrates (i.e., NSP), at rates of 50 and 30 g/kg during the starter and grower phases, respectively.

At the end of each experimental phase (starter and grower), the broilers and feed leftovers were weighed to calculate weight gain, feed intake, and feed conversion, and at the end of the growth phase, four birds were removed from each replicate cage with average weight representative of the experimental

Table 1 - Experimental diets in the starter phase (10 to 21 d of age)

Item	Treatment (g/kg)			
	Control	Xylanase	Probiotic	Xylanase + probiotic
Ingredient				
Corn grain (7.86% CP)[1]	455.50	464.70	455.50	464.70
Soybean meal (46.50% CP)[1]	399.85	410.20	399.85	410.20
Soybean oil	62.09	43.16	62.09	43.16
Wheat bran	50.00	50.00	50.00	50.00
Calcitic limestone	14.60	14.58	14.60	14.58
Dicalcium phosphate	5.21	5.20	5.21	5.20
Salt	2.43	3.42	2.43	3.42
DL-methionine	2.27	2.00	2.27	2.00
Inert[2]	2.20	1.91	2.20	1.91
L-lysine HCL	2.00	1.44	2.00	1.44
Vitamin mixture[3]	1.00	1.00	1.00	1.00
Mineral mixture[4]	1.00	1.00	1.00	1.00
Choline chloride	0.82	0.82	0.82	0.82
L-threonine	1.02	0.56	1.02	0.56
Butylated hydroxytoluene	0.01	0.01	0.01	0.01
Nutrient	Calculated composition (g/kg)			
Linoleic acid	31.86	16.98	32.03	17.15
Calcium	7.97	7.97	7.97	7.97
Chlorine	2.97	2.97	2.97	2.97
Metabolizable energy (Mcal/kg)	3.100	2.950	3.100	2.950
Available phosphorus	3.12	3.12	3.12	3.12
Digestible lysine	12.94	12.94	12.94	12.94
Digestible methionine and cysteine	9.66	9.66	9.66	9.66
Digestible methionine	5.30	5.30	5.30	5.30
Potassium	8.98	8.98	8.98	8.98
Crude protein	240.0	240.0	240.0	240.0
Sodium	2.21	2.21	2.21	2.21
Digestible threonine	8.44	8.44	8.44	8.44

[1] Crude protein value determined in laboratory.
[2] Washed sand.
[3] Guaranteed analysis (per kg of product): vitamin A, 6,000,000 IU; vitamin D3, 2,000,000 IU; vitamin E, 12,000 mg; vitamin K3, 800 mg; vitamin B1, 1,000 mg; vitamin B2, 4,500 mg; vitamin B6, 1,500 mg; vitamin B12, 12,000 mg; niacin, 30,000 mg; calcium pantothenate, 10,000 mg; folic acid, 550 mg; biotin, 50 g; antioxidant, 5,000 mg; excipient q.s., 1,000 g.
[4] Guaranteed analysis (per kg of product): iron (ferrous sulphate), 60,000 mg; copper (copper sulphate), 13,000 mg; manganese (manganese sulphate), 120,000 mg; zinc (zinc oxide), 100,000 mg; iodine (calcium iodine), 2,500 mg; selenium (sodium selenite), 500 mg; excipient q.s., 1,000 g.

unit for further analysis. The chickens were numbed by cervical dislocation, bled via the jugular vein, scalded, plucked, eviscerated, and weighed. Carcass yield was calculated from live weight after fasting and carcass weight, which was measured after removing the viscera, head, feet, and abdominal fat.

To assess intestinal viscosity, the intestines of two birds per repetition were separated, sectioned in the portions duodenum, jejunum, ileum, and cecum, and their respective contents collected by mechanical compression in the cranial-caudal direction in an appropriate and identified container. The contents were analyzed using the methodology described by Morgado (2013). A portion (0.5 g) of each intestinal

Table 2 - Experimental diets in the grower phase (22 to 35 d of age)

Item	Treatment (g/kg)			
	Control	Xylanase	Probiotic	Xylanase + probiotic
Ingredient				
Corn grain (7.86% CP)[1]	502.53	527.30	502.53	527.30
Soybean meal (46.50% CP)[1]	375.91	369.82	375.91	369.82
Soy oil	60.22	41.45	60.22	41.45
Wheat bran	30.00	30.00	30.00	30.00
Limestone	10.25	10.31	10.25	10.31
Dicalcium phosphate	5.77	5.73	5.77	5.73
Salt	4.98	4.97	4.98	4.97
DL-methionine	3.30	3.27	3.30	3.27
Inert[2]	2.00	2.00	2.00	2.00
L-lysine HCL	1.75	1.87	1.75	1.87
Vtamin mixture[3]	1.00	1.00	1.00	1.00
Mineral mixture[4]	1.00	1.00	1.00	1.00
Choline chloride	0.65	0.65	0.65	0.65
L-threonine	0.63	0.62	0.63	0.62
Butylated hydroxytoluene	0.01	0.01	0.01	0.01
Nutrient	Calculated composition (g/kg)			
Linoleic acid	34.59	27.51	34.59	27.51
Calcium	7.12	7.12	7.12	7.12
Chlorine	3.67	3.77	3.67	3.77
Metabolizable energy (Mcal/kg)	3.200	3.050	3.200	3.050
Available phosphorus	2.64	2.64	2.64	2.64
Digestible lysine	12.23	12.23	12.23	12.23
Digestible methionine and cysteine	9.14	9.14	9.14	9.14
Digestible methionine	5.01	5.01	5.01	5.01
Potassium	8.92	8.92	8.92	8.92
Crude protein	223.2	223.2	223.2	223.2
Sodium	2.21	2.21	2.21	2.21
Digestible threonine	7.97	7.97	7.97	7.97
Digestible tryptophan	2.20	2.20	2.20	2.20
Digestible valine	9.36	9.36	9.36	9.36

[1] Crude protein value determined in laboratory.
[2] Washed sand.
[3] Guaranteed analysis (per kg of product): vitamin A, 6,000,000 IU; vitamin D3, 2,000,000 IU; vitamin E, 12,000 mg; vitamin K3, 800 mg; vitamin B1, 1,000 mg; vitamin B2, 4,500 mg; vitamin B6, 1,500 mg; vitamin B12, 12,000 mg; niacin, 30,000 mg; calcium pantothenate, 10,000 mg; folic acid, 550 mg; biotin, 50 g; antioxidant, 5,000 mg; excipient q.s., 1,000 g.
[4] Guaranteed analysis (per kg of product): iron (ferrous sulphate), 60,000 mg; copper (copper sulphate), 13,000 mg; manganese (manganese sulphate), 120,000 mg; zinc (zinc oxide), 100,000 mg; iodine (calcium iodine), 2,500 mg; selenium (sodium selenite), 500 mg; excipient q.s., 1,000 g.

content sample was separately mixed with 1.5 mL of distilled water and centrifuged at 3000 rpm, and 0.5 mL of each resulting supernatant was diluted to 15 mL, using water, and subjected to viscosity measurement using a Cannon-Fenske viscometer (capillary 150 mm) in a 37 °C water bath. Two measurements were performed for each sample, and viscosity was calculated from the mean of the two sample flow times, according to equation 1:

$$\eta = k \cdot T \quad (1)$$

in which η = the viscosity of the sample, T = sample runoff time (s), and k = the viscometer constant.

For pH analysis, the intestinal content of one bird by repetition was collected similarly to that previously described. Intestinal pH was measured using a Gehaka Model PG 1800 Digital Microprocessor pH meter, as described by Reis et al. (2017). Briefly, the intestinal contents of each sample were weighed, diluted at a 1:10 (m:v) ratio using distilled water, and homogenized in containers. The electrode was then inserted into the solution, and data were collected from the samples after the pH reading stabilized.

The ileal content of one bird from each replicate was collected and then a pool was composed where it was frozen and stored. Sterile materials were used throughout the collection process, and different utensils were used for each treatment group. The bacteria in the samples were identified using high-throughput sequencing of the V3/V4 region of the 16S rRNA gene and a pipeline (Neoprospecta Microbiome Technologies, Brazil). The resulting sequences with 100% identity were grouped and compared to a 16S rRNA sequence database for identification.

The study was performed using a completely randomized design, with four treatments and six replicates with six birds each. The treatments consisted of different diets, subjected to the statistical model (2):

$$Y_{ij} = \mu + T_i + e_{ij}, \quad (2)$$

in which Y_{ij} = observed value for treatment i, in repetition j; μ = average of the experiment; T_i = effect of different diets; and e_{ij} = random error associated to each observation.

Data were submitted to analysis of variance by using SISVAR (Statistical and Genetic Analysis System, 2010; Ferreira, 2011), with the criterion of 5% probability, and the group means were compared using the Tukey test. Meanwhile, for analysis of the intestinal microbiota, Cluster Analysis was performed using a multivariate statistics in the free software package PAST to separate the population averages into groups, using Eucledian distance, based on the variables considered, so that they have the most similar characteristics possible within the group in which they were classified, and that are as heterogeneous as possible among the groups formed. To observe the relationships of microbial populations in the different treatments, non-metric multidimensional scale (NMDS) plotting was performed.

3. Results

During the starter phase (10-21-days-old), dietary supplementation affected (P<0.05) weight gain and feed conversion, but not feed intake (Table 3), and during the grower phase (10-35-days-old), dietary supplementation affected (P<0.05) weight gain and feed intake, but not feed conversion. In regards to carcass yield, only thigh + drumstick was significantly affected by diet, whereas in regard to relative weight, only abdominal fat was significantly affected (Table 4).

Dietary supplementation affected (P<0.05) the viscosity of all the evaluated intestinal segments, except that of the jejunum, and only affected the pH of the duodenum, cecum, and ileum segments (Table 5). In the duodenal and cecal regions, the control diet yielded the highest viscosity (1.42 and 1.36 cP, respectively), with no significant differences between the values of the other groups, and in the ileal region, xylanase supplementation yielded the lowest viscosity (1.30 cP), with no significant differences between the values of the other groups. In the duodenum, the control diet yielded the

greatest pH (6.54), whereas probiotic and xylanase + probiotic supplementation yielded lower pH values in the ileum, and supplementation with only the probiotic yielded the lowest pH value in the cecum (6.11).

In regards to ileal microbial diversity, cluster analysis separated the taxa into three groups: one group composed of the result obtained with the control diet, another group comprised of diets supplemented with probiotic and xylanase + probiotic, and a group formed by the diet supplemented with xylanase (Figures 1 and 2). For frequencies, members of the *Firmicutes*, *Proteobacteria*, and

Table 3 - Performance of birds fed diets supplemented or not with xylanase and probiotic

Treatment	Weight gain (g)	Feed intake (g)	FCR
	Performance 10 to 21 d old age		
Control	577.82a	927.16	1.60a
Xylanase	705.77b	914.89	1.30b
Probiotic	743.83b	920.22	1.27b
Xylanase + probiotic	715.85b	950.99	1.40b
Probability	0.0023	0.0952	0.0009
CV	3.95	1.72	4.79
	Performance 10 to 35 d old age		
Control	2600.62a	3600.20a	1.38
Xylanase	2852.00ab	3856.47ab	1.35
Probiotic	3019.33b	4019.67b	1.33
Xylanase + probiotic	2746.40a	3671.93ab	1.33
Probability	0.0024	0.0311	0.8249
CV	2.64	3.87	3.15

CV - coefficient of variation.
a-b - Means followed by distinct letters (columns) differ significantly (P≤0.05) by Tukey's test.

Table 4 - Carcass characteristics of broilers fed diets supplemented or not with xylanase and probiotic slaughtered at 36 d of age

Treatment	Yield (%)				
	Carcass	Breast	Thigh and drumstick	Wing	Back
Control	71.03	36.48	28.96a	18.80	11.17
Xylanase	71.34	37.23	31.43a	19.56	11.36
Probiotic	70.20	35.11	31.43ab	19.17	10.99
Xylanase + probiotic	69.89	38.08	34.75b	19.83	11.25
Probability	0.2827	0.3767	0.0008	0.5741	0.8755
CV	2.02	8.05	6.30	6.91	7.13
	Relative weights (%)				
	Gizzard	Heart	Liver	Abdominal fat	
Control	2.44	0.50	28.96	2.34a	
Xylanase	2.43	0.49	31.43	1.82b	
Probiotic	2.47	0.46	31.43	1.68b	
Xylanase + probiotic	2.58	0.49	34.75	2.72a	
Probability	0.7331	0.3800	0.3656	0.0010	
CV	9.24	5.67	14.10	20.10	

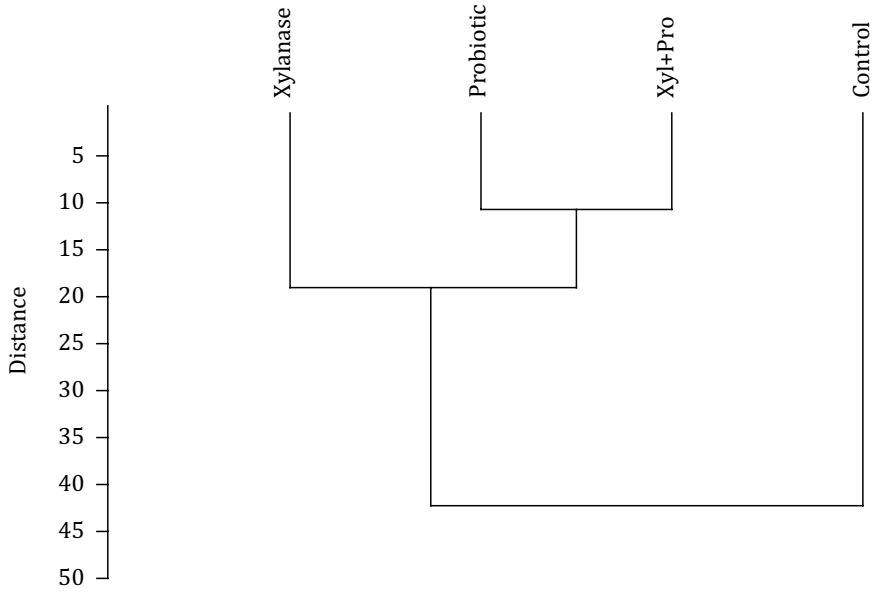
CV - coefficient of variation.
a-b - Means followed by distinct letters (columns) differ significantly (P≤0.05) by Tukey's test.

Actinobacteria were most abundant (Table 6). The *Firmicutes* were the most frequently represented phylum, regardless of diet, although the group was least abundant (81.03%) in the ilea of chickens fed the xylanase-supplemented diet. Accordingly, the ileal microbiota of the xylanase group exhibited relatively higher frequencies of *Proteobacteria* (11.80%) and *Actinobacteria* (7.17%). The most prevalent genera in all treatments were *Lactobacillus*, *Enterococus*, and *Clostridium* (Table 7), whereas the most prevalent species were *C. ruminantium*, *E. faecalis*, *L. agilis*, *L. aviarius*, *L. helveticus*, and *L. salivarius* (Table 8).

Table 5 - Viscosity of different segments of broilers' intestine

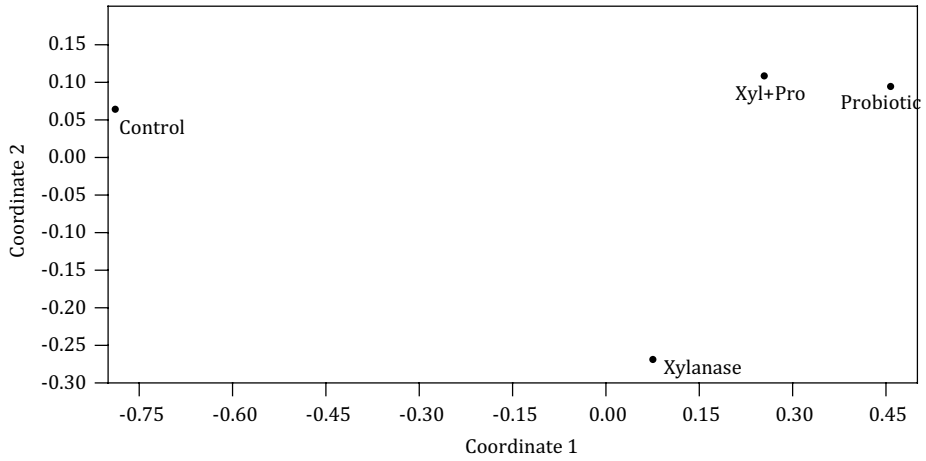
Treatment	Viscosity (cP)			
	Duodenum	Jejunum	Ileum	Cecum
Control	1.42b	1.28	1.37b	1.36a
Xylanase	1.34a	1.23	1.14a	1.29b
Probiotic	1.40b	1.24	1.30b	1.28b
Xylanase + probiotic	1.37b	1.33	1.33b	1.27b
Probability	0.0189	0.0701	0.0031	0.0123
CV	4.39	6.83	3.40	2.88
	Intestinal pH			
Control	6.64b	6.48	6.38b	6.38b
Xylanase	6.34a	6.78	6.31b	6.46b
Probiotic	6.44a	6.54	6.17a	6.11a
Xylanase + probiotic	6.28a	6.32	6.19a	6.49b
Probability	0.0358	0.4504	0.0008	0.0009
CV	2.57	3.96	3.01	1.91

CV - coefficient of variation.
a-b - Means followed by distinct letters (columns) differ significantly (P≤0.05) by Tukey's test.



Similarity data analyzed by Euclidean index.

Figure 1 - Cluster analysis for ileal microbiota.



The point represents one pooled sample comprising all Operational Taxonomic Units.

Figure 2 - Representative graph considering non-metric multidimensional scaling (NMDS).

Table 6 - Diversity of phylum, class, and order found in ileum of broilers fed diets supplemented or not with xylanase and probiotic

Identification	Diversity (%)[1]			
	Control	Xylanase	Probiotic	Xyl+Pro
Phylum				
Actinobacteria	0.330	7.170	0.070	0.080
Firmicutes	98.80	81.03	98.99	99.57
Proteobacteria	0.870	11.80	0.940	0.350
Class				
Actinobacteria	0.320	7.160	0.070	0.080
Alphaproteobacteria	0.040	1.700	0.010	0.010
Bacilli	71.03	77.22	98.87	90.64
Betaproteobacteria	0.000	0.000	0.050	0.000
Clostridia	27.53	3.820	0.110	8.930
Epsilonproteobacteria	0.110	0.000	0.010	0.100
Erysipelotrichia	0.240	0.000	0.000	0.000
Gammaproteobacteria	0.730	10.10	0.880	0.240
Order				
Actinomycetales	0.080	7.160	0.070	0.070
Bacillales	0.240	0.820	0.030	0.210
Bifidobacteriales	0.240	0.000	0.000	0.010
Burkholderiales	0.000	0.000	0.050	0.000
Campylobacterales	0.110	0.000	0.010	0.100
Clostridiales	27.53	3.810	0.110	8.920
Enterobacteriales	0.710	9.740	0.870	0.230
Erysipelotrichales	0.240	0.000	0.000	0.000
Lactobacillales	70.80	76.39	98.84	90.44
Pseudomonadales	0.010	0.360	0.010	0.010
Rhizobiales	0.000	0.000	0.010	0.000
Rhodobacterales	0.000	1.720	0.000	0.000
Sphingomonadales	0.040	0.000	0.000	0.010

[1] Percentage of occurrence of the microorganism in the microbiological profile.

Table 7 - Diversity of genera found in ileum of chicken fed diets supplemented or not with xylanase and probiotic

Identification	Diversity (%)[1]			
	Control	Xylanase	Probiotic	Xyl+Pro
Genera				
Acinetobacter	0.000	0.000	0.000	0.010
Bacillus	0.030	0.360	0.010	0.030
Bifidobacterium	0.240	0.000	0.000	0.010
Blautia	0.020	0.000	0.000	0.010
Burkholderia	0.000	0.000	0.030	0.000
Campylobacter	0.040	0.000	0.000	0.000
Citrobacter	0.010	0.000	0.000	0.000
Clostridium	27.51	3.810	0.110	8.920
Corynebacterium	0.000	0.100	0.010	0.010
Cryobacterium	0.000	0.260	0.000	0.000
Dietzia	0.000	0.050	0.000	0.000
Enterobacter	0.010	0.000	0.000	0.000
Enterococcus	15.75	0.260	4.510	1.380
Escherichia	0.320	5.940	0.460	0.130
Faecalitalea	0.230	0.000	0.000	0.000
Globicatella	0.000	0.000	0.050	0.000
Helicobacter	0.070	0.000	0.010	0.100
Klebsiella	0.010	0.000	0.000	0.000
Kocuria	0.000	0.100	0.000	0.000
Lactobacillus	55.01	76.09	94.20	89.02
Lactococcus	0.040	0.050	0.010	0.010
Leifsonia	0.000	0.050	0.000	0.000
Leucobacter	0.000	0.360	0.000	0.000
Lysinibacillus	0.000	0.100	0.000	0.010
Paenibacillus	0.000	0.050	0.010	0.000
Paracoccus	0.000	1.700	0.000	0.000
Pseudomonas	0.010	0.360	0.010	0.010
Ralstonia	0.000	0.000	0.020	0.000
Rhizobium	0.000	0.000	0.010	0.000
Rothia	0.080	6.240	0.060	0.060
Serratia	0.350	3.710	0.400	0.100
Shigella	0.010	0.100	0.000	0.000
Sphingomonas	0.040	0.000	0.000	0.010
Staphylococcus	0.210	0.310	0.020	0.170
Streptococcus	0.000	0.000	0.040	0.010
Turicibacter	0.010	0.000	0.000	0.000
Weissella	0.000	0.000	0.030	0.000

[1] Percentage of occurrence of the microorganism in the microbiological profile.

Table 8 - Main species found in ileum of broilers fed diets supplemented or not with xylanase and probiotic

Identification	Diversity (%)[1]			
	Control	Xylanase	Probiotic	Xyl+Pro
Species				
Clostridium ruminantium	27.16	3.81	0.11	8.93
Enterococcus faecalis	15.58	0.00	0.00	0.37
Escherichia coli	0.30	5.93	0.47	0.13
Lactobacillus agilis	15.09	16.55	14.30	34.35
Lactobacillus aviarius	6.02	57.16	70.52	36.10
Lactobacillus helveticus	10.04	0.46	2.32	1.27
Lactobacillus salivarius	22.72	1.60	5.96	17.17
Paracoccus aminovorans	0.00	1.44	0.00	0.00
Rothia endophytica	0.08	5.93	0.01	0.03
Serratia liquefaciens	0.21	2.37	0.18	0.07

[1] Percentage of occurrence of the microorganism in the microbiological profile.

4. Discussion

The results of the present study indicate that supplementing broiler feed with xylanase, probiotics, or both can improve weight gain and feed conversion by 20 and 17.5%, respectively, during the starter phase, when compared with the control diet. Considering the period up to 35 d of age, the probiotic diet yielded greater weight gain (3019.33 g), which was greatly influenced by the higher feed intake.

When administered together, xylanase and probiotic supplementation yielded better results than the control diet. However, their combined action did not yield greater gains than the diets supplemented with the individual additives, thus indicating the absence of an additive effect. This finding contrasts with the results reported by Nusairat et al. (2018), who studied the combined use of xylanase and *Bacillus* as an alternative to growth promoters and concluded that co-supplementation significantly improved feed conversion and intestinal injury score.

The gains obtained from dietary supplementation may be associated with their mechanisms of action in improving the intestinal environment. Furthermore, xylanase and probiotic supplementation stimulated feed intake, which influenced weight gain. Such an effect was also reported by Abdel-Hafeez et al. (2017), who reported that probiotic supplementation can stimulate feed intake.

To infer about the physiological responses that occurred in the intake in relation to supplementation of additives, it is necessary to highlight that the biochemical mechanisms of satiety are not easy to understand. However, probiotic additives contribute to intestinal microbial modulation, and the mechanisms by which microbiota affect satiety has been discussed. For example, Fetissov (2017) reported that bacteria metabolize undigested fibers and can produce a variety of energy substrates, such as ATP, lactate, and butyrate, or bioactive molecules, such as lipopolysaccharide (LPS) and 5-hydroxytryptamine (5HT), which may activate enteroendocrine cells (EEC) and, thus, trigger the local and systemic release of the tyrosine tyrosine (PYY) and glucagon 1-like (GLP1) peptides.

As for the action of xylanase on intake, it is important to highlight that, among several effects, the best feeding passage (Bedford and Schulze, 1998) can be highlighted, due to reduced viscosity, since high intestinal viscosity can reduce the passage rate of the digesta (Gohl and Gohl, 1977). Thus, changes in intestinal viscosity and rate of food passage may affect feed intake.

Meanwhile, in regards to carcass parameters, the treatments influenced ($P<0.05$) only the variable thigh and drumstick, which presented the highest yield value for the treatment of the group of broilers fed xylanase + probiotic. Chen et al. (2018) suggested that the addition of xylanase could significantly improve broiler carcass characteristics. The greater carcass weights of chickens fed xylanase or

probiotic diets can be partly attributed to the greater utilization of nutrients, especially amino acids. Indeed, Jasek et al. (2018) investigated the effect of a multicarbohydrase-containing α-galactosidase and xylanase on the ileal digestible energy, crude protein, and ileal amino acid digestibility of broilers and found that enzyme supplementation improved the digestibility of individual amino acids, including aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and tryptophan. In this assumption, among these amino acids, lysine stands out because it comprises ~7.5% of all carcass protein (Sklan and Noy, 2004) and, thus, directly influences carcass parameters (Brasil et al., 2018).

In regards to viscosity, the highest viscosity of the control group could reflect the supply of wheat bran, which includes a large amount of arabinoxylan and, thus, increases digesta hydrophilia. When xylanase is involved, such reductions in viscosity are mainly achieved by reducing the molecular weight of xylan, through hydrolysis into smaller compounds, which subsequently reduces the viscous effects of the feed, the digestibility of which is directly proportional to the molecular weight of the wheat arabinoxylans (Bedford and Classen, 1993). Furthermore, the positive effects of probiotic supplementation observed here resemble the results of Agboola et al. (2015), who investigated the effects of probiotic and carbohydrase supplementation on broilers fed wheat-based diets.

In this sense, considering the results found in relation to the use of probiotics, one must infer on two important points: the enzymatic complex of bacteria and the improvement of the intestinal environment. First, it is possible that some commensal microbes, such as certain *Lactobacillus*, *Bacillus*, and *Bifidobacterium* species, are capable of producing enzymes that hydrolyze NSP. In fact, when evaluating the potential of *Lactobacillus reuteri* Pg4 as a multifunctional probiotic in barley-based broiler diets, Yu et al. (2008) observed that *L. reuteri* supplementation reduced intestinal viscosity of 21- and 37-day-old animals; the authors reported that the strain used had an effective action on dietary glucan. Similarly, Latorre et al. (2015) observed that supplementing poultry diets with *Bacillus*-DFM (*in vitro*) significantly reduced digestion viscosity and *Clostridium perfringens* proliferation when compared with the control diet. Second, it is necessary to understand the intestinal environment as a whole and recognize that all interactions that occur in the local microbiome are extremely dynamic and multi-dependent. Thus, the use of probiotics may help improve the intestinal environment by increasing host disease resistance and by partially ameliorating the negative growth effects associated with coccidiosis (Lee et al., 2007).

Probiotic supplementation (alone or with xylanase) reduced the pH of the ileum and cecum. This effect can be attributed to the increased abundance of organic acid-producing bacteria. Indeed, the increase of such acid synthesis, along with subsequent reductions in intestinal pH, is one of the main factors associated with the exclusion of gastrointestinal pathogens (Hinton Jr. et al., 1990).

Co-supplementation also affected pH, as noted above. However, the effect was not additive. It is possible that the more complete digestion of nutrients, owing to the enzymatic activity of the xylanase, reduced the amount of substrate available to support larger populations of microorganisms at the end of the gut (Bao and Choct, 2010).

In regards to the microbiota, supplementation with xylanase and probiotics, alone or in combination, provided divergent microbial modulation of the control diet, thereby demonstrating the importance of their inclusion in broiler diets. In the non-metric multidimensional scale, it is possible to verify a proximity between the probiotic and xylanase + probiotic treatments, highly influenced by the concentration of *Lactobacillus* bacteria. The predominance of the *Lactobacillales* is important since the order contains a variety of microorganisms with probiotic potential. The results of the present study support those of Wang et al. (2018), who reported that the *Lactobacillales* were the most predominant order in the ileal mucosa samples, whereas the *Clostridiales* were the most predominant in cecal digesta samples. *Lactobacillus aviaries* were most prevalent in the supplemented group, accounting for 57.16, 70.52, and 36.10% of intestinal microbiota sequences from the xylanase, probiotic, and co-supplemented groups, respectively.

Many actions are associated with the antimicrobial activity of these bacteria under various mechanisms, one of which is their fermentative activity. According to Turnbaugh et al. (2006), the fermentation of NSP results in the production of short-chain fatty acids that are absorbed and catabolized by the host, thereby contributing to animal nutrition, inhibition of acid-sensitive pathogens, and production of hydrogen peroxides, which will influence the survival of pathogenic microorganisms. Indeed, Heravi et al. (2011) reported that H_2O_2 was produced by all *Lactobacillus* strains (except *L. reuteri* strain) that were isolated from the broiler digestive tract and that strong H_2O_2 production was exhibited by *L. johnsonii*, *L. ingluviei*, *and L. agilis*.

The treatment with xylanase alone presented proximity with *Estaphylococcus*, *Shigella*, and *Lactococus* among other genera, while higher values of *Clostridium* and *Sphingomonas* were close for the control treatment.

Considering the observed frequency, the predominance of certain phyla agree with the observations of Wei et al. (2013), who performed a bacterial census of the intestinal microbiome of chickens and found that the *Firmicutes* was the most predominant phylum, representing almost 70% of all bacterial sequences, followed by *Bacteroidetes* (12.3%) and *Proteobacteria* (9.3%). According to Allen and Stanton (2014), bacteria belonging to the *Firmicutes* and *Bacteroidetes* phyla are involved in the decomposition of indigestible polysaccharides by the host enzyme system, such as resistant starch and cellulose. About this, Jumpertz et al. (2011) demonstrated that the filament *Firmicutes* has a positive relationship with the ability to collect energy from the diet.

The observed effects of xylanase and probiotic supplementation on the ileal microbiota provides insight into the intestinal environment and into its reflexes in performance, since studies have shown that the microbial composition of broiler ilea affect intestinal function, digestion, and nutrient absorption.

5. Conclusions

The present study demonstrates benefits of the xylanase and probiotic supplementation in broiler diets, such as the modulation of the ileal microbiota with a higher frequency of *Lactobacillales* bacteria and a lower intestinal viscosity value, with a positive effect on broiler weight gain, indicating that it can increase growth performance and contribute to broiler productivity.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: N.J.B. Machado, F. Dilelis, D.V. Quaresma, L. W. Freitas and C.A.R. Lima. Data curation: N.J.B. Machado, R.J.M. Brasil and J.P.F. Rufino. Formal analysis: N.J.B. Machado. Funding acquisition: N.J.B. Machado, F.G.G. Cruz and C.A.R. Lima. Investigation: N.J.B. Machado, R.J.M. Brasil and J.P.F. Rufino. Methodology: N.J.B. Machado. Project administration: N.J.B. Machado and C.A.R. Lima. Resources: F.G.G. Cruz and C.A.R. Lima.

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