R. Bras. Zootec., 53:e20230083, 2024
https://doi.org/10.37496/rbz5320230083

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Effect of the use of prebiotics, alone and in combination with antibiotics, in broiler diets

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Received: May 16, 2023
Accepted: January 30, 2024

How to cite: Martins, A. P. F.; Mello, H. H. C.; Mascarenhas, A. G.; Sales-Campos, H.; Pires, M. F. and Café, M. B. 2024. Effect of the use of prebiotics, alone and in combination with antibiotics, in broiler diets. Revista Brasileira de Zootecnia 53:e20230083. https://doi.org/10.37496/rbz5320230083

Editors:
Mateus Pies Gionbelli
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ABSTRACT - The objective of this study was to evaluate the effects of prebiotics, alone and in combination with antibiotics, on broiler performance, carcass yield, intestinal permeability, and intestinal morphometry. A total of 1440 day-old male Cobb 500 chicks were distributed in a completely randomized design, with four treatments and 10 replications per treatment. The treatments used were a basal diet without prebiotics and antibiotics (BD), a basal diet with antibiotics (AB), a basal diet with prebiotics (PRE), and a basal diet with antibiotics and prebiotics (AB+PRE). To perform the treatment, the antibiotic zinc bacitracin and a prebiotic of *S. cerevisiae*-derived mannan-oligosaccharides and beta-glucans were used. The treatments did not affect the performance of birds from 1 to 7 and 1 to 21 days of age. From 1 to 35 and 1 to 42 days, birds fed the AB+PRE treatment had higher feed intake than those fed the AB treatment. From 1 to 35 days, birds fed the AB+PRE treatment showed a worse feed conversion ratio. However, there was no difference in carcass yield between treatments. A higher number of goblet cells was observed in the duodenum and ileum of birds fed AB+PRE, but lower counts were obtained in birds fed AB. Intestinal histomorphometry displayed similar responses between both ages regarding antibiotic and prebiotic treatments, except in the ileum at 42 days, in which the antibiotic elicited a better response. Intestinal permeability analysis showed no significant difference between diets. The use of prebiotics, alone or in combination with antibiotics, does not improve the performance of broilers.

Keywords: additives, goblet cells, growth promoters, intestinal permeability, poultry

1. Introduction

In poultry nutrition, antibiotics are used to improve the feed conversion ratio (FCR), enhance body weight gain (BWG), and prevent diseases. However, this issue has been revisited because antibiotics have been associated with bacterial resistance and residues in animal products (Han et al., 2020). Antibiotic-resistant bacteria and antibiotic-resistance genes in food animals are currently considered emerging contaminants, which are a serious threat to public health globally (Xu et al., 2022). According to Han et al. (2020), drug-resistant bacteria in animals and the environment proliferate during the feeding cycle, leading to the widespread distribution of drug-resistance genes and an increase in the overall resistance of bacteria. In recent years, research on food additives, such as prebiotics, probiotics, and organic acids, has intensified. These substances have the potential to replace antibiotics as growth promoters in animal feed (Nunes et al., 2012; Cruz-Polycarpo et al., 2020). The aim of these alternatives

is to maintain a low mortality rate and a good level of animal yield while preserving the environment and consumer health (Mehdi et al., 2018).

Prebiotics are compounds that influence the metabolism of resident microorganisms; any medicinal component or feed ingredient beneficial to the intestinal microecosystem can be considered a prebiotic (Yaqoob et al., 2021). In recent years, the search for antibiotic alternatives in animal nutrition has gained momentum. Studies, such as that of Gois et al. (2023), explored the effects of replacing antibiotics with prebiotics, specifically β-glucans, glucomannans, and mannan-oligosaccharides (MOS), on weaned piglets. Results suggested potential benefits for commercial farming conditions, highlighting the role of prebiotics in maintaining performance and microbiota balance. Additionally, Pinzón-Osorio et al. (2023) investigated the use of *Ganoderma* spp. as a potential substitute for growth-promoting antibiotics in broiler diets. Their findings indicated that Ganoderma supplementation, particularly at 150 ppm, led to higher body weight gain and improved indicators of intestinal morphometry. This suggests the viability of *Ganoderma* spp. as an alternative to traditional antibiotics in broiler production.

It is known that *Saccharomyces cerevisiae* cell walls have prebiotic action because they contain MOS and short-chain oligosaccharides, together with beta-glucans, in their structure (Xue et al., 2017; Gloria-Trujillo et al., 2022). Mannan-oligosaccharides are mannose-rich carbohydrates present in the outermost layer of yeast cell walls and are responsible for cell-to-cell and cell-to-environment interactions. They occur as alpha (α)- and beta (β)-MOS based on the glycosidic linkage present in the parent mannan polymer. Hydrolysis of the α-(1 → 6) bond present in the mannan of yeast cell walls produces α-MOS, whereas plant mannans linked by β-(1 → 4)-glycosidic bonds are broken down to release mainly β-MOS (Ayimbila and Keawsompong, 2022). This compound favors beneficial bacteria selectively since most pathogens cannot use it as an energy source (Fomentini et al., 2016). Furthermore, MOS agglutinate gram-negative bacteria that have type 1 fimbriae in their cell walls, preventing them from attaching to the intestinal mucosa and initiating proliferation; consequently, they are eliminated in the excreta (Spring et al., 2000). Xiao et al. (2012) verified that genes with diverse biological functions, including energy production, cell death, and protein translation, were differentially expressed in the jejunum by MOS supplementation.

Beta-glucans from *S. cerevisiae* are glucose polymers that have 1,3 (linear chain) and 1,6 (lateral branches) beta bonds between glucose units. These polysaccharides are present in the cell wall and, together with chitin, harden and shape the cell walls. They are associated with antitumor, antimutagenic, anticholesterolemic, and hypoglycemic effects (Magnani and Castro-Gómez, 2008). Beta-glucans act on the natural activation of the immune system in the intestines of broilers. This activation occurs because intestinal mucosal macrophages identify the 1,3 and 1,6 bonds of beta-glucans and react with chain activation of the innate immune system, in an immediate and non-specific way, increasing immunity and the response to infectious agents (Magnani and Castro-Gómez, 2008).

Nevertheless, the impact of yeast cell wall-based prebiotics on broiler health and performance remains a subject of debate. Some researchers have reported positive effects on performance and gut health (Pascual et al., 2020; Teng et al., 2021; Asif et al., 2024), while others found no discernible benefits (Munyaka et al., 2012; Kiros et al., 2019). Therefore, this study aims to assess the effects of using prebiotics, specifically *S. cerevisiae* yeast wall, both independently and in conjunction with antibiotics, on broiler performance, carcass yield, intestinal permeability, and intestinal morphometry.

2. Material and Methods

Research on animals was conducted according to the institutional committee on animal use (case number 003/21). The experiment was carried out in Goiânia, Goiás, Brazil (16°35'48.3" S and 49°17'08.8" W).

The study was performed in an industrial shed with an area of 1,500 m^2 (12 × 125 m), 0.40-m masonry side walls, 2.80-m high wire mesh, and a ceiling height of 3.20 m. For the study, we used 1,440 one-day-old male Cobb 500 chicks, with an average weight of 42±2.1 g. The experimental design used was completely randomized, with four treatments and 10 replications of 36 birds per treatment.

The experiment lasted 42 days and was divided into four rearing stages: pre-starter (1 to 7 days), starter (8 to 21 days), grower (22 to 35 days), and finisher (36 to 42 days).

The treatments consisted of a basal diet without antibiotics and prebiotics (BD), a basal diet with antibiotics (AB), a basal diet with prebiotics (PRE), and a basal diet with antibiotics plus prebiotics (AB+PRE). To this end, 55 g/ton of antibiotic (zinc bacitracin) and 250 g/ton of a commercial prebiotic (Safmannan®, a source of *S. cerevisiae*-derived MOS and beta-glucans) were used. Antibiotics and/or prebiotics were included to replace the inert component (kaolin) in the diets. The feeds provided were isonutritive and formulated according to the nutritional levels recommended by Rostagno et al. (2017) (Table 1).

The birds were housed in 40 experimental pens measuring 1.80 × 1.60 m, made of plastic mesh and PVC pipes, and placed inside an industrial shed to mimic industrial poultry-rearing conditions. Boxes contained drinkers, feeders, and first-use rice husk litter. Water and feed were made available *ad libitum* throughout the experimental period. The internal heating of the shed was monitored by measuring air temperature and relative humidity. Constant lighting was provided by fluorescent lamps. The light program was carried out as follows: on the first day, a total of 24 h of light were provided; from the second to the seventh day, 23 h of light; from the eighth to the 21st day, 19 h of light; from the 22nd to the 28th day, 20 h of light; from the 29th to the 35th day, 21 h of light; and from the 36th to the 42nd day, 22 h of light. The temperature and humidity of the shed followed the Broiler Management Manual (São Salvador Alimentos) (Table 2).

Table 1 - Composition and nutritional levels of basal diets

Ingredient (g/kg)	Pre-starter	Starter	Grower	Finisher
Corn	503.44	539.81	617.25	647.73
Soybean meal 45%	389.48	353.48	261.46	236.55
Meat and bone meal	0.00	0.00	0.00	10.00
Limestone	7.77	7.39	7.21	6.54
Salt	4.53	4.02	3.63	3.60
Poultry fat	35.49	39.18	46.81	46.99
Poultry meal	35.00	35.00	45.00	35.00
Dicalcium phosphate	15.20	13.92	11.02	8.09
Plant choline	0.20	0.19	0.25	0.21
DL-methionine	3.41	2.97	2.17	1.63
Lysine sulfate	2.62	2.33	2.70	2.37
L-threonine	0.86	0.63	0.50	0.30
Inert ingredient	1.00	1.00	1.00	1.00
Vitamin premix[1]	0.50	0.50	0.50	0.50
Mineral premix[2]	0.50	0.50	0.50	0.50
Total	1000.00	1000.00	1000.00	1000.00
Nutritional levels (g/kg)				
Metabolizable energy (kcal/kg)	3,010	3,080	3,239	3,268
Crude protein	238.38	224.4	193.75	183.02
Digestible lysine	13.28	12.28	10.57	9.72
Digestible methionine	6.62	6.04	4.96	4.29
Digestible threonine	8.77	8.11	6.98	6.42
Calcium	9.60	9.02	8.34	8.00
Available phosphorus	4.68	4.40	3.97	3.72
Sodium	2.20	2.00	1.90	1.90

[1] Vitamin supplement (PX Vitamin Frango SSA), guarantee levels: Starter - vitamin A, 220,000,000 IU/kg; vitamin D3, 88,000,000 UI/kg; vitamin E, 80,000 UI/kg; vitamin K3, 6000 mg/kg; vitamin B1, 5000 mg/kg; vitamin B2, 15 g/kg; vitamin B6, 8000 mg/kg; vitamin B12, 40,000 mcg/kg; vitamin B5, 32 g/kg; vitamin B3, 100 g/kg; folic acid, 3200 mg/kg; biotin, 300 mg/kg; selenium, 1000 mg/kg. Grower: vitamin A, 100,000,000 UI/kg; vitamin D3, 40,000,000 UI/kg; vitamin E, 50,000 UI/kg; vitamin K3, 6000 mg/kg; vitamin B1, 4000 mg/kg; vitamin B2, 12 g/kg; vitamin B6, 8000 mg/kg; vitamin B12, 40,000 mcg/kg; vitamin B5, 37 g/kg; vitamin B3, 36 g/kg; folic acid, 2000 mg/kg; biotin, 200 mg/kg; selenium, 800 mg/kg. Finisher: vitamin A, 100,000,000 UI/kg; vitamin D3, 40,000,000 UI/kg; vitamin E, 20,000 UI/kg; vitamin K3, 4000 mg/kg; vitamin B1, 3000 mg/kg; vitamin B2, 8000 mg/kg; vitamin B6, 4000 mg/kg; vitamin B12, 20,000 mcg/kg; vitamin B5, 19 g/kg; vitamin B3, 40 g/kg; folic acid 1400 mg/kg; biotin, 150 mg/kg; selenium, 500 mg/kg.

[2] Mineral supplement (PX Micro mineral Frango SSA), guarantee levels: manganese, 150 g/kg; zinc, 140 g/kg; iron, 100 g/kg; copper, 20 g/kg; iodine, 2000 mg/kg.

Table 2 - Temperature and humidity in the shed during the experimental period according to age of broilers

Age (days)	Temperature (°C)	Humidity (%)
0-3	32-33	30-50
4-7	30-32	40-60
8-14	29-30	50-60
15-21	27-28	50-60
22-28	24-26	50-65
29-25	21-23	50-70
36-42	19-21	50-70

Bird performance was evaluated at 7, 21, 35, and 42 days of age. Feed intake (FI; kg/bird) was calculated as the difference between the amount of feed supplied and the leftovers in the feeder. Chickens were weighed (kg) individually. Body weight gain (kg) was obtained as the difference between the initial and final body weight in each phase. The FCR was estimated from the ratio between FI and BWG. Data were corrected for mortality. Viability was evaluated as the number of live birds expressed as a percentage of the initial number of birds.

At 42 days of age, six birds per treatment were slaughtered after electronarcosis. The birds represented the average weight of each replication, with a standard deviation of 5%. Each bird was weighed and identified. During evisceration, abdominal (retroperitoneal) fat was removed. After evisceration, the feet, head, and neck were removed from the carcass, which was then weighed. Afterward, commercial cuts (breast, wings, thigh, and drumstick) were sectioned and weighed. Finally, cut yields were calculated as the ratio between the cut weight and live-bird weight, multiplied by 100.

Eight chickens at 28 days of age and five chickens at 42 days of age were randomly selected per treatment for intestinal permeability testing. The birds were inoculated orally, directly into the esophagus, with fluorescein isothiocyanate-dextran (FITC-d, 3-5 kDa; Sigma Aldrich Co™) diluted in saline at 4.16 mg/kg, with the aid of a feeding tube. Two hours after inoculation, 2.0 mL of blood was collected from each bird through an occipital venous sinus puncture. The blood was placed in collection tubes without coagulants and protected from light. The serum was extracted by centrifugation and placed in microcentrifuge tubes. We diluted FITC-d in saline in a 96-well plate to determine a FITC-d standard curve from a concentration of 50 µg/mL. Serum levels of FITC-d were measured at excitation and emission wavelengths of 485 nm and 528 nm, respectively, using a spectrofluorometer (Cary Eclipse; FL1011M010). Then, fluorescence was plotted against the standard curve function to obtain FITC-d concentrations (Vicuña et al., 2015).

For the goblet cell count and histomorphometry of the intestinal mucosa, six birds per treatment were euthanized by cervical dislocation at 21 and 42 days of age for the collection of intestinal fragments. Five-centimeter samples were taken from the small intestinal segments (duodenum, jejunum, and ileum). For slide preparation, these segments were fixed in a 10% buffered formaldehyde solution for 24 hours. After fixation, they were stored in 70% alcohol, processed according to Luna (1968), stained with hematoxylin-eosin (HE) for histomorphometry, and stained with Alcian Blue for goblet cell counting. Images for reading were acquired with a 2.5X and a 20X objective using a Leica DM 4000B optical microscope coupled to a microcomputer. The images were analyzed using ImageJ software, where the villus height (VH), crypt depth (CD), and villus height:crypt depth ratio (VH:CD) were measured. For goblet cell counting, an area of 7 mm^2 per fragment was considered, a technique adapted from Smirnov et al. (2006).

Data from analysis of performance, carcass yield, intestinal histomorphometry, goblet cell count, and intestinal permeability were subjected to analysis of variance, with means compared by the Tukey test at the 5% probability level. The SAS (Statistical Analysis System, version 9.1) computer software was used for the analysis.

The statistical model used was:

$$y_{ik} = m + a_i + e_{ik},$$

in which y_{ik} = an observation in level i of factor a (i = 1,2,3,4) and repetition k (k = 1,2, ... ,10), m = the overall mean, a_i = fixed effect of factor a (i = 1,2,3,4), and e_{ik} = the random error with mean 0 and variance σ^2.

3. Results

The use of a prebiotic, alone or in combination with an antibiotic, in diets did not influence the final body weight (FBW), BWG, FI, or FCR of birds up to 21 days of age (P>0.05; Table 3). At 35 days of age, broilers fed the AB+PRE diet showed a higher FI (P = 0.0304) and a worse FCR (P = 0.0278). At 42 days of age, the FI was affected by the diets; broilers fed the AB+PRE diet showed the highest FI (P = 0.0266; Table 3).

Table 3 - Performance of broilers fed diets containing antibiotics and/or prebiotics in different rearing periods

Parameter	Treatment				ANOVA	
	BD	AB	PRE	AB+PRE	SE	P-value
			1 to 7 days			
FBW (g)	195.4	193.8	197.5	197.2	0.002	0.3763
BWG (g)	152.9	151.3	155.1	155.0	3.18	0.2511
FI (g)	161.9	154.0	159.6	157.7	0.003	0.2253
FCR (g/g)	1.06	1.02	1.03	1.02	0.014	0.1355
			1 to 21 days			
FBW (g)	1,053	1,041	1,055	1,054	0.007	0.4416
BWG (g)	1,010.9	998.0	1,012.9	1,011.5	0.007	0.5558
FI (g)	1,282	1,248	1,268	1,277	0.015	0.4224
FCR (g/g)	1.27	1.25	1.25	1.26	0.012	0.6841
			1 to 35 days			
FBW (g)	2,482.8	2,433.7	2,467.9	2,462.1	0.014	0.1143
BWG (g)	2,440.3	2,391.2	2,425.5	2,419.9	0.014	0.1089
FI (g)	3,547ab	3,465b	3,511ab	3,574a	0.026	0.0304
FCR (g/g)	1.45b	1.45b	1.45b	1.48a	0.007	0.0278
			1 to 42 days			
FBW (g)	3,306.9	3,284.6	3,269.1	3,307.6	0.023	0.5907
BWG (g)	3,264.4	3,242.1	3,226.7	3,265.4	0.023	0.5890
FI (g)	5,052ab	4,950b	4,967ab	5,091a	0.036	0.0266
FCR (g/g)	1.55	1.53	1.54	1.56	0.010	0.1391
Viability (%)	99.17	99.17	98.61	97.78	0.591	0.3119

BD - basal diet; AB - basal diet + antibiotic; PRE - basal diet + prebiotic; AB+PRE - basal diet + antibiotic + prebiotic; FBW - final body weight; BWG - body weight gain; FI - feed intake; FCR - feed conversion ratio; SE - standard error.
Means followed by different letters in the row differ from each other by Tukey's test at 5% probability.

Carcass and cut yield and the percentage of abdominal fat of broilers at 42 days of age did not differ among treatments with or without prebiotics and/or antibiotics in diets (P>0.05; Table 4).

The intestinal morphometry and goblet cell count were affected by the treatments. Regarding the intestinal histomorphometry analysis, at 21 days of age, we observed a deeper CD (P<0.0001) and lower VH:CD (P = 0.0003) in the duodenum of broilers fed the BD (Table 5). Broilers fed the PRE diet had shorter CD (P<0.0001). In the jejunum and ileum, antibiotic treatment resulted in similar VH, CD, and VH:CD to those of broilers fed prebiotics. Moreover, feeding diets with a combination of antibiotics and prebiotics improved the VH:CD in the duodenum, jejunum, and ileum (Table 5).

Table 4 - Carcass parameters of broilers at 42 days of age fed diets containing antibiotics and/or prebiotics

Parameter	Treatment				ANOVA	
	BD	AB	PRE	AB+PRE	SE	P-value
Carcass yield (%)	71.8	73.8	71.5	71.6	1.171	0.4736
Chest (%)	23.5	24.6	23.6	23.2	1.054	0.8049
Drumstick + thigh (%)	24.5	23.6	23.5	24.0	0.402	0.2596
Wings (%)	8.6	9.3	8.6	8.7	0.323	0.4027
Abdominal fat (%)	1.54	1.31	1.36	1.28	0.170	0.7094

BD - basal diet; AB - basal diet + antibiotic; PRE - basal diet + prebiotic; AB+PRE - basal diet + antibiotic + prebiotic; SE - standard error.

Table 5 - Intestinal morphometry and goblet cell count (no./7 mm^2) of broilers at 21 and 42 days of age fed diets containing antibiotics and/or prebiotics

Parameter	Treatment				ANOVA	
	BD	AB	PRE	AB+PRE	SE	P-value
				21 days		
				Duodenum		
VH (µm)	1491	1535	1497	1493	30.192	0.7067
CD (µm)	445a	397b	361c	404b	9.088	<0.0001
VH:CD	3.42b	3.96a	4.21a	3.96a	0.129	0.0003
Goblet cell	36.67	31.00	30.83	34.17	3.129	0.5104
				Jejunum		
VH (µm)	1241ab	1308ab	1194b	1320a	32.155	0.0191
CD (µm)	351a	373a	358a	376b	8.693	<0.0001
VH:CD	3.73b	3.67b	3.43b	4.93a	0.143	<0.0001
Goblet cell	34.50	28.83	37.67	33.33	3.102	0.2748
				Ileum		
VH (µm)	951a	818b	786b	826b	16.861	<0.0001
CD (µm)	250a	249a	247a	215b	5.134	<0.0001
VH:CD	3.88a	3.36b	3.28b	3.95a	0.099	<0.0001
Goblet cell	34.17	32.50	34.00	39.67	2.759	0.3020
				42 days		
				Duodenum		
VH (µm)	1505a	1363ab	1360b	1426ab	33.788	0.0031
CD (µm)	349a	289b	314b	356a	9.820	<0.0001
VH:CD	4.43ab	4.79a	4.47ab	4.08b	0.135	0.0088
Goblet cell	30.67ab	30.00b	36.50ab	37.83a	1.852	0.0127
				Jejunum		
VH (µm)	983b	1021b	1064b	1309a	34.862	<0.0001
CD (µm)	245b	288a	252ab	268ab	8.628	0.0164
VH:CD	4.06b	3.62b	4.33b	4.99a	0.176	<0.0001
Goblet cell	40.33	36.67	38.83	37.33	2.495	0.7340
				Ileum		
VH (µm)	648b	1067a	678b	484c	16.459	<0.0001
CD (µm)	239a	208b	190bc	185c	5.596	<0.0001
VH:CD	2.79c	5.26a	3.67b	2.69c	0.113	<0.0001
Goblet cell	48.50	38.67	46.00	48.17	2.144	0.0136

BD - basal diet; AB - basal diet + antibiotic; PRE - basal diet + prebiotic; AB+PRE - basal diet + antibiotic + prebiotic; VH - villus height; CD - crypt depth; VH:CD - villus height:crypt depth ratio; SE - standard error.
Means followed by different letters in the row differ from each other by Tukey's test at 5% probability.

At 42 days of age, birds receiving the PRE diet had a lower villus height in the duodenum (P = 0.0031; Table 5) and, in the jejunum and ileum, birds fed the PRE diet and BD had lower VH (P<0.0001). The prebiotics had a similar effect to that of antibiotics in the duodenum and jejunum for VH, CD, and VH:CD (P<0.0001). However, in the ileum, the use of antibiotics resulted in a higher VH and VH:CD (P<0.0001). The combination of AB and PRE in the diet resulted in lower VH, lower CD, and a lower VH:CD (P<0.0001; Table 5). At 21 days of age, the goblet cell count in the duodenum, jejunum, or ileum was not affected by the treatments (P>0.05). At 42 days of age, it was observed that broilers fed AB diet had lower goblet cell counts in the duodenum (P = 0.0127; Table 5).

Regarding intestinal permeability, serum concentrations of FITC-d were not significantly different among treatments at 28 days of age (P = 0.2263) and 42 days of age (P = 0.0545; Table 6).

Table 6 - Serum concentrations of FITC-d (μg/mL) of broilers fed or not diets with antibiotics and/or prebiotics at 28 and 42 days of age

	Treatment				ANOVA	
	BD	AB	PRE	AB+PRE	MSE	P-value
28 days	0.473	0.295	0.294	0.513	0.092	0.2263
42 days	0.327	0.304	0.261	0.328	0.018	0.0545

BD - basal diet; AB - basal diet + antibiotic; PRE - basal diet + prebiotic; AB+PRE - basal diet + antibiotic + prebiotic; MSE - mean squared error.

4. Discussion

The experimental diets did not affect broiler performance up to 21 days of age. These results agree with those of Kamran et al. (2021), who compared the use of MOS to basal diet and basal diet plus different antibiotics and found no differences in broiler performance from 1 to 35 days.

From 1 to 35 and 1 to 42 days of age, birds fed a diet in combination with antibiotics plus prebiotics showed higher FI when compared with birds fed only antibiotics in the diet. Similarly, Froebel et al. (2019) concluded that broilers fed a prebiotic derived from *Saccharomyces cerevisiae* at a high dose (100 g t^{-1}) showed an increase in FI. Also, between 1 and 35 days, birds that received a diet containing antibiotics plus prebiotics showed worse feed conversion compared with birds from the other treatments. Therefore, a combination of prebiotics and antibiotic impairs the FCR. The deleterious effect of the combination of additives on feed conversion has been reported in other studies (Murshed and Abudados, 2015). According to Yaqoob et al. (2021), it is useful to evaluate combinations of prebiotics with other dietary supplements to identify potential synergism. However, in the present study, the combination of these additives was not beneficial to broilers. According to van der Aar et al. (2017), the improvement of FI as a result of prebiotics is an indirect effect of improvement of the health status of the animals. Colonization of the gut by pathogens may be used as an indicator of intestinal health. In this context, a prebiotic derived from the *Saccharomyces cerevisiae* cell wall is effective at reducing the *Clostridium perfringens* count in broiler litter (Silva et al., 2023), improving the intestinal health and FI.

Broilers fed AB+PRE presented a worse FCR at 35 days of age. Franco et al. (2005) evaluated different levels of yeast in association with antibiotics, observing a decreasing trend in weight, and believed that antibiotics may have interfered with yeast molecules. There is a correlation between the cecal microbiota composition and the efficiency of the host at extracting energy from the diet and depositing that energy into an improved FCR (Rinttilä and Apajalahti, 2013). Baurhoo et al. (2009a) concluded that MOS increased intestinal concentrations of Bifidobacteria and Lactobacilli in broilers, and Gilani et al. (2021) reported a correlation between the cecal *Lactobacillus* spp. population and FCR. Currently, the association between different additives in feed has been studied to maximize broiler performance, and many interactions between the molecules are not known.

The use of prebiotics, alone or in combination with antibiotics, did not result in differences in carcass yield or the percentage of abdominal fat in broilers. According to Yaqoob et al. (2021), the nutritional benefits to the host of prebiotic supplementation are correlated with their fermentation into short-chain fatty acids (SCFA) in the lower gut (propionic, butyric, acetic, and lactic acids). The SCFA improve protein and mineral availability because they decrease the intestinal pH and promote nutrient solubility (Yaqoob et al., 2021). Therefore, we hypothesized that PRE, alone or in combination with AB, in the diet could improve carcass yields and that SCFA, particularly acetic acid, could result in higher abdominal fat production. Li et al. (2020) verified that abdominal fat could increase in broilers fed AB and AB+PRE, since the antibiotics induced fat deposition in adipose tissues of broilers by increasing the expression levels of the peroxisome proliferator-activated receptor γ (PPARγ), diacylglycerol acyltransferase 2 (DGAT2), lipoprotein lipase (LPL), and fatty acid-binding protein (FABP4) genes. However, Kamran et al. (2021) also found no differences in carcass traits, including carcass yield, breast meat yield, and abdominal fat of broilers fed basal diets, with MOS or different antibiotics. Baurhoo et al. (2009b) explained that the lack of differences in carcass yields might be a consequence of similarity in BWG responses among the treatments studied. These authors also did not verify the influence of dietary MOS on carcass yield in broilers.

Intestinal development was affected by the treatments. At 21 days of age, the AB+PRE diet resulted in a higher VH:CD, although at 42 days of age, the PRE diet resulted in a higher VH:CD. Teng and Kim (2018) related that MOS supplementation resulted in increased VH and surface area, decreased CD, increased numbers of goblet cells, and upregulated the expression of *MUC* gene, which is related to mucin secretion that is produced by goblet cells. In fact, we observed that the association of a prebiotic and an antibiotic resulted in a higher number of goblet cells. Pascual et al. (2020) observed an increase in the number of goblet cells in treatments with prebiotics and suggested that the yeast cell wall induces the proliferation of goblet cells. The continuous process of mucin secretion coupled with peristaltic movement allows excretion of trapped pathogens from the intestines; therefore, increased mucin secretion due to MOS would contribute to greater elimination of intestinal pathogens (Baurhoo et al., 2009a). Reducing colonization of the intestine by pathogens reduces competition for nutrients with the host and, therefore, improves bird performance. In the present study, there was no improvement in BWG in broilers fed the PRE diet. It has been reported that the effectiveness of the prebiotic depends on the challenge to which the birds are exposed (Lourenço et al., 2016).

Serum concentrations of FITC-d are an indicator of intestinal permeability. The serum FITC-d concentration can be measured and used as an indicator of paracellular permeability and the extent and severity of intestinal mucosal barrier dysfunction (Liu et al., 2021); therefore, lower serum concentrations of FITC-d indicate better intestinal integrity. Cheng et al. (2019) observed an improvement in the intestinal barrier of chickens supplemented with prebiotics and subjected to heat stress. These authors demonstrated that the use of MOS increases the mRNA expression of occludin and claudin-3, important proteins for tight junctions. In the present study, no improvement in intestinal permeability was observed with the use of a prebiotic in the diet; however, the P-value obtained (0.0545) may be considered significant by some researchers, indicating that the prebiotic promotes better intestinal permeability.

5. Conclusions

The prebiotic source of *S. cerevisiae*-derived mannan-oligosaccharides and beta-glucans increase the goblet cell count and show a tendency to reduce the intestinal permeability of broilers, without improving their performance and carcass yield.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Martins, A. P. F.; Mello, H. H. C. and Café, M. B. **Data curation:** Martins, A. P. F. **Funding acquisition:** Café, M. B. **Investigation:** Martins, A. P. F.; Mello, H. H. C. and Sales-Campos, H. **Methodology:** Martins, A. P. F.; Mello, H. H. C.; Mascarenhas, A. G.; Sales-Campos, H. and Café, M. B. **Project administration:** Mello, H. H. C. and Café, M. B. **Resources:** Pires, M. F. **Software:** Martins, A. P. F. **Supervision:** Mello, H. H. C. **Validation:** Sales-Campos, H. **Visualization:** Mascarenhas, A. G. **Writing – original draft:** Martins, A. P. F.; Mello, H. H. C. and Pires, M. F. **Writing – review & editing:** Mello, H. H. C.

Acknowledgments

The authors would like to thank Phileo and São Salvador Alimentos.

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