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Synbiotic supplements as antibiotic alternatives in broiler diets

Suplemento simbiótico como alternativa aos antibióticos em dietas para frangos de corte

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Highlights

The tested synbiotic is a viable option for broiler diets at all life stages.
A dosage of 1kg/t is recommended for broilers aged 1 to 21 days.
BD + 1kg/t synbiotic significantly improves feed conversion compared to the basal diet.

Abstract

Antibiotics can contribute to bacterial resistance, posing a significant public health concern. Synbiotics represent an effective alternative to antibiotics, promoting the balance of intestinal microbiota and creating a conducive environment for beneficial bacteria growth. This study aimed to assess the utility of the synbiotic supplement (+Poultry) as a substitute for antibiotics in broiler diets from 1 to 42 days of age. We evaluated its impact on performance during two stages: 1 to 21 days and 1 to 42 days, digestibility coefficients, metabolizable energy, duodenum and jejunum morphometry, carcass yield, and hematological parameters. A total of 440 Ross 308 strain broiler chicks, both males and females, were accommodated in 2m² cages from day 1 to day 42. The birds were randomly assigned to four treatments,

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with each treatment having five replications consisting of 22 birds, thus forming 20 experimental plots. The treatment groups were as follows: Basal diet (BD) without the addition of antibiotics or synbiotics, BD with 150ppm zinc bacitracin (BCZ), BD with 0.5kg/t of synbiotic, and BD with 1kg/t of synbiotic. The synbiotic supplement (+Poultry) used in this experiment, provided by Nutri+, contained amino acids, minerals, probiotic culture, prebiotic additives, and flavoring additives. Throughout the experiment, all birds were subjected to a weekly sanitary challenge, which involved using reused litter and water contaminated with litter. Treatment effects were observed on feed conversion (P = 0.0100) of broilers aged 1 to 21 days, indicating improved feed conversion in broilers consuming BD + 1kg/t synbiotic compared to BD alone. Consequently, we recommend the incorporation of a synbiotic supplement at a rate of 1kg/t as a viable antibiotic replacement for chickens aged 1 to 21 days. The tested synbiotic supplement shows promise as an antibiotic alternative in broiler diets at all life stages, maintaining both performance and bird health.
Key words: Animal nutrition. Growth promoters. Poultry.

Resumo

Os antibióticos podem possibilitar a resistência bacteriana, podendo gerar problema de saúde pública. Uma das alternativas eficazes aos antibióticos são os simbióticos. Os simbióticos favorecem o equilíbrio da microbiota intestinal e possibilitam um meio favorável para o crescimento das bactérias benéficas. O objetivo deste trabalho foi avaliar o uso do suplemento simbiótico (+Poultry) como alternativa aos antibióticos em dietas para frangos de corte de 1 a 42 dias de idade, sobre o desempenho nas fases de 1 a 21 e de 1 a 42 dias de idade, coeficientes de digestibilidade, energia metabolizável, morfometria do duodeno e jejuno, rendimento de carcaça e parâmetros hematológicos. Foram utilizados 440 pintos de corte, machos e fêmeas da linhagem Ross308 alojados em boxes de 2m2, no período de 1 a 42 dias de idade. As aves foram distribuídas em um delineamento inteiramente casualizado, com cinco tratamentos, quatro repetições de 22 aves cada, constituindo 20 parcelas experimentais. Os tratamentos propostos foram: Dieta basal (DB), sem adição de antibiótico ou simbiótico; DB + 150ppm de bacitracina de zinco (BCZ); DB + 0,5kg/t de simbiótico; DB + 1kg/t de simbiótico. A composição do suplemento simbiótico comercial (+ Aves) da empresa Nutri + utilizado neste experimento possui aminoácidos, minerais, cultura probiótica, aditivo prebiótico, aditivo aromatizante. Neste experimento, o desafio sanitário foi realizado em todas as aves uma vez por semana, que consistiu na utilização de cama reaproveitada mais ingestão de água contaminada com cama. Houve efeito dos tratamentos na conversão alimentar (P = 0.0100) de frangos de corte de 1 a 21 dias de idade, indicando melhor conversão alimentar para frangos de corte consumindo a RB (Ração Basal) + 1kg/t de simbiótico em relação a RB. Recomenda-se a adição de suplemento simbiótico na proporção de 1kg/t em substituição aos antibióticos para frangos de 1 a 21 dias de idade. O suplemento simbiótico testado pode ser utilizado como alternativa aos antibióticos em rações para frangos de corte em todas as fases da vida, pois mantém o desempenho e a saúde das aves.
Palavras-chave: Aves. Nutrição animal. Promotores de crescimento.

Introduction

The yeast cell wall of *Saccharomyces cerevisiae* (SCC), known as a prebiotic, has garnered significant interest for its potential use in poultry feed. The poultry industry has seen continuous growth driven by advancements in breeding, nutrition, and health practices, resulting in enhanced productivity and the production of high-quality, nutritious food for consumers (Valentim et al., 2018). In broiler farming, the historical inclusion of growth-promoting antibiotics in bird diets, now referred to as performance enhancers following IN 013 of 2004 by the Ministry of Agriculture, Livestock, and Supply (MAPA) in Brazil (BRASIL, 2004), aimed to counteract health issues arising from production systems and genetic characteristics.

However, the widespread and indiscriminate use of these performance enhancers in the past has led to the development of resistance among numerous bacterial strains. This resistance phenomenon has been exacerbated by the capacity of bacteria to transfer resistance genes, even across different genera and species (Álvarez-Martínez et al., 2020). Many of these antibiotics or their residues can persist in animal tissues intended for human consumption, such as meat and eggs (Blajman et al., 2015), posing a significant risk of transmitting multidrug-resistant strains between humans and animals.

Synbiotics, as defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP), represent a combination of live microorganisms (probiotics) selectively used by beneficial host microorganisms that confer health improvements to the host (Swanson et al., 2020), along with a substrate (prebiotics) that is selectively utilized by host microorganisms, also conferring health benefits (Gibson et al., 2017).

Synbiotics function by favoring the balance of the intestinal microbiota through their components that lower the luminal pH and create an environment conducive to the growth of beneficial bacterial strains. These strains, in turn, stimulate the production of bacteriocins, aiding in the inhibition of pathogenic bacteria growth (Alavi et al., 2012). Additionally, synbiotics promote the activity of pancreatic enzymes, enhancing nutrient utilization in the diet, which supports improved animal performance and health (Al-Khalaifah, 2018; Forte et al., 2018; Kuritza et al., 2014).

Certain synbiotics incorporate proteins, amino acids, and minerals into their composition, further enhancing animal performance. They may also contain beneficial bacteria (e.g., *Bifidobacterium bifidum*) that produce acids (lactic and acetic acids) to lower the pH of the large intestine, delaying the colonization of undesirable bacteria. Moreover, synbiotics may include yeasts, such as *Saccharomyces cerevisiae,* which possess glucans and mannans in their cell walls. Yeasts are rich in enzymes, fatty acids, vitamins, and peptides, all of which contribute to improved feed palatability, heightened immunological resistance, and reduced stress in animals (Machado, 1997).

Grigoletti et al. (2002) emphasized the yeast cell wall's ability to prevent the establishment of pathogenic bacterial strains in the intestine, primarily due to the presence of complex carbohydrate molecules, such

as mannan oligosaccharides (MOS) and oligosaccharides. Oligosaccharides modify the bacterial ecosystem by increasing the populations of beneficial Bifidobacterium and Lactobacillus (Sun, 2004) while reducing the production of toxic fermentation products from putrefactive bacteria, such as ammonia, amines, and nitrosamines (Flickinger et al., 2003).

Given the myriad possible combinations of probiotics and prebiotics, the application of synbiotics to modulate the intestinal microbiota in animals holds great promise. Therefore, our primary goal was to assess the effectiveness of the synbiotic supplement (+Poultry) as a substitute for antibiotics in broiler diets, spanning from 1 to 42 days of age.

Materials and Methods

Animal research in this study adhered to institutional guidelines set by the Committee on Animal Use (protocol 568/19). The experiment was conducted in Bom Jesus, Piauí, Brazil, at coordinates 09º 04' 28" S and 44º 21' 31" W, with an altitude of 277 meters.

A total of 440 Ross 308 broiler chicks, including both males and females, were housed in 2m² cages, spanning from 1 to 42 days of age. The birds were allocated using a completely randomized design, comprising four treatments, each with five replications consisting of 22 birds, thereby forming 20 experimental plots. The proposed treatments included: Basal diet (BD) without the addition of antibiotics or synbiotics; BD + 150ppm zinc bacitracin (BCZ); BD + 0.5kg/t synbiotic; BD + 1kg/t synbiotic.

The diets (as detailed in Table 1) were formulated using corn and soybean meal to meet the nutritional requirements of broilers with a focus on medium performance, following Rostagno et al. (2017) guidelines. Table 2 lists the composition of the commercial synbiotic supplement (+Poultry).

Throughout the experiment, a weekly sanitary challenge was administered to all birds. This challenge involved the use of reused litter and water contaminated with litter (a mixture of one kilogram of litter in four liters of water). The litter-water mixture was mixed and filtered after a two-hour water fasting period, following the protocol of Barbosa et al. (2011).

Daily temperature and relative humidity readings, as well as maximum and minimum temperatures, were recorded from day 1 to day 42 of age in the morning using a thermo-hygrometer placed at the center of the shed, at the height of the birds' backs. The birds were exposed to continuous lighting, comprising natural light supplemented with artificial light, while both feed and water were made available *ad libitum.*

Table 1
Composition and calculated values

Ingredients, %	Age, days			
	1-7	8-21	22-33	34-42
Corn grain	57.332	58.770	60.807	66.991
Soybean meal	35.191	33.461	30.791	26.631
Soy oil	2.322	3.096	4.183	2.827
Dicalcium phosphate	1.955	1.731	1.486	1.263
Calcitic Limestone	0.957	0.871	0.825	0.670
Salt	0.534	0.517	0.493	0.466
Supplement Nucleopar[1]	0.500	0.400	0.350	0.300
DL- Methionine	0.407	0.384	0.349	0.270
L- Lysine HCL	0.390	0.377	0.356	0.319
L- threonine	0.161	1.496	0.131	0.088
L- arginine	0.133	0.127	0.114	0.059
Inert[2]	0.115	0.115	0.115	0.115
Total	100.00	100.00	100.00	100.00
Nutrients				
Crude Protein, %	21.469	20.751	19.618	18.409
Metabolizable energy, Kcal/kg	2.9750	3.0500	3.1500	3.2000
Methionine, %	0.686	0.656	0.609	0.528
Methionine + Digestible Cystine, %	0.967	0.929	0.870	0.787
Digestible lysine, %	1.307	1.256	1.175	1.064
Digestible arginine, %	1.398	1.344	1.257	1.138
Linoleic acid, %	2.553	2.975	3.569	2.897
Calcium, %	0.971	0.878	0.792	0.666
Phosphorus Available, %	0.463	0.419	0.370	0.311
Potassium, %	0.827	0.800	0.758	0.702
Sodium, %	0.225	0.218	0.208	0.197

[1]Composition per kg of product: vitamin A, 3000.000 IU; vitamin E, 9.500 IU; vitamin B1, 588 mg; vitamin B2, 1.160 mg; vitamin B6, 792 mg; vitamin B12, 4.150 mcg; vitamin K3, 520 mg; vitamin D3, 800 IU; calcium pantothenate, 3.230 mg; niacin, 9.800 mg; folic acid, 200; biotin, 20 mg; zinc, 13 g; iron, 13 g; manganese, 15 g copper, 3.120 mg; iodine, 254 mg; cobalt, 48 mg; selenium, 88 mg; ethoxyquin, 52 mg; Butylated hydroxyanisole (BHA), 40 mg; Vehicle qsp, 1.000 mg;
[2]washed sand

Table 2
Composition of the commercial synbiotic supplement (+ Poultry) from the company Nutri +

Crude Protein (minimum)	132 g/kg
lysine (minimum)[a]	3.9 g/kg
Methionine (minimum)[a]	4.9 g/kg
Calcium (minimum/maximum)[b]	85/112 g/kg
Phosphorus (minimum)[b]	4.4 g/kg
Saccharomyces cerevisiae (minimum)[c]	$2.0x10^{11}$ cfu/kg
Bifidobacterium bifidum (minimum)[c]	$2.0x10^{11}$cfu/kg
Bacillus subtilis (minimum)[c]	$2.88x10^{11}$ cfu/kg
Enterococcus faecium (minimum)[c]	$2.08x10^{11}$cfu/kg
Lactobacillus acidophilus (minimum)[c]	$1.04x10^{11}$cfu/kg
Glucans (minimum)[d]	52 g/kg
Mananos (minimum)[d]	28 g/kg
Vanilla (minimum)[e]	2.5 g/kg
Moisture (maximum)	28 g/kg
Ethereal Extract (minimum)	1.0 g/kg
Raw fiber (maximum)	18 g/kg
Mineral matter (maximum)	377,5 g/kg

a- Aminoacids;
b- minerals;
c- probiotic culture;
d- prebiotic additive;
e- flavoring additive.

Performance parameters were assessed during two phases: from 1 to 21 days and from 1 to 42 days of age. These parameters included: 1) feed intake (FI), calculated as the difference between the amount of feed provided and the remaining experimental diet; 2) weight gain (WG), determined by the difference in bird weight at the beginning and end of each phase; 3) feed conversion (FC), computed from data on feed intake and weight gain in each phase; and 4) uniformity, which was expressed as the ratio of the number of birds within a 10% weight variation range to the total number of birds, multiplied by 100. Performance parameters were adjusted for mortality following the method described by Sakomura and Rostagno (2016).

To evaluate diet digestibility, the total excreta collection method was employed, following Sakomura and Rostagno (2016). This process involved a completely randomized design with five treatments, each having five replications of two birds. The birds were weighed and sorted by average weight into metabolic cages measuring 1 x 1 x 0.5 meters. To prevent potential losses, the cages were lined with plastic trays.

The experimental period included four days for diet adaptation (from the 14th to the 17th day of age) and four days for total excreta collection (from the 18th to the 21st day of age). To mark the beginning and end of the collection period, 1% ferric oxide was added to the diets. This allowed for the exclusion of unmarked excreta during the initial collection and marked excreta during the final collection.

Excreta collection procedures were conducted once daily in the morning for four days. Following collection, the excreta were weighed, labeled, and placed in plastic bags for identification according to the experimental plots. Subsequently, they were frozen, and, at the end of the total collection period, the excreta were thawed, homogenized, and samples of approximately 200g per experimental unit were taken for drying in a lyophilizer at -40°C for 72 hours. These dried samples were analyzed for dry matter, gross energy, nitrogen, fat, and ash using methods number 925.10, 65.17, 974.24, and 992.16 as per Association of Official Analytical Chemists [AOAC] (2000) guidelines.

Based on the laboratory results, the apparent metabolizable energy (AME) and the apparent digestibility coefficient of dry matter (CDMS), crude protein (CDPB), and ether extract (CDAEE) were calculated using the equations described by Sakomura and Rostagno (2016).

At 21 days of age, intestinal morphometric analysis was conducted. Two birds per experimental unit were euthanized for the collection of 2 cm-long segments from the duodenum and jejunum. These segments were carefully collected, washed in distilled water, and fixed in a 10% neutral formalin buffer (37- 40% formalin, distilled water, monobasic sodium phosphate, dibasic sodium phosphate) for 24 hours.

Following the fixation period, the segments were subjected to a series of procedures, including dehydration using ascending concentrations of alcohol (70%, 80%, 90%, and 100% I, 100% II, 100% III), clearing with xylene (I and II), embedding in histological paraffin, and placement into paraffin blocks (Prophet et al., 1992). Subsequently, the blocks were sectioned using a rotary microtome (LUPETEC™MRP09) to obtain histological sections with a thickness of 4 µm. Each animal yielded one slide, and on each slide, up to three semi-serial sections were placed, with the exclusion of 10 sections between one section and the next. These sections were then stained with hematoxylin-eosin and mounted between glass slides and coverslips using 500™ colorless glass varnish (Paiva et al., 2006).

Histomorphometric analysis, encompassing measurements of perimeter, height, and width of the villi, as well as the height and width of the crypts, and internal and external muscle measurements, was conducted using a trinocular optical microscope (Leica DM250) equipped with a Leica digital color camera (DFC7000T), 1,920 x 1,440 resolution (2.8 Pixel), 4.54 µm x 4.54 µm pixel size, for photographic record of images. Measurements were taken using the Leica LAS Interactive Measurement Module.

In each intestinal region, 10 villi and 10 crypts per animal were selected and measured for length in a straight line (µm). Villus height measurements were taken from the upper base of the crypt to the apex of the villus, while crypts were measured from

the lower base to the upper base of the crypt (Fukayama et al., 2005; Lopes et al., 2005).

Carcass and cut yields were evaluated at 42 days of age. Two birds were selected based on the average plot weight and slaughtered after a six-hour fasting period. The birds were individually weighed to determine live weight, followed by cervical dislocation, bleeding, and plucking. Carcass yield was determined as the ratio of the weight of the eviscerated carcass to the weight of the fasting bird, considering the weight of the bird slaughtered on an empty stomach, devoid of feathers, viscera, head, neck, and feet. Subsequently, the carcasses were dissected, and the weight of the breast, thigh, drumstick, wings, tulips, heart, gizzard, liver, abdominal fat, and bursa of Fabricius were recorded. The yield of each cut was expressed relative to the weight of the eviscerated carcass, while the percentage of abdominal fat was calculated based on the fat found around the gizzard, cloaca, and abdominal muscles.

Hematological parameters were analyzed at 42 days of age. One bird from each replication was randomly selected, manually immobilized, and subjected to local asepsis with 70% isopropyl alcohol. Two milliliters of blood samples were drawn from the ulnar vein, using 10% EDTA as an anticoagulant. The following parameters were evaluated: 1) globular volume (GV), representing the percentage of red blood cells in the blood; 2) plasma concentration, determined after analyzing the hematocrit or VG, to measure plasma protein content, the plasma column in the microhematocrit tube was used; 3) erythrocyte and leukocyte counts, employing toluidine blue solution. A 20 µL blood sample was diluted in 2 mL of 0.01% toluidine blue solution, homogenized, and loaded into a Neubauer chamber, left to rest a few minutes, and cells in the 5. The result was obtained by multiplying the cell count in the central squares of the Neubauer chamber, the result was multiplied by 5000. For the leukocyte and thrombocyte count, all cells in the central squares were counted and the results were multiplied by 1000.

For leukocyte differential count, blood smears were prepared on glass slides, fixed with methyl alcohol for 5 minutes, stained with hematoxylin-eosin, washed, dried, and observed under an optical microscope with an immersion objective. Leukocytes were categorized as granular (heterophils, eosinophils, and basophils) and non-granular (lymphocytes and monocytes).

Data for all variables were screened for outliers and assessed for normality (Cramer-von Mises test) and variance homogeneity (Levene test). After confirming the assumptions, the data underwent analysis of variance and were compared using the SNK test at a 0.05% significance level, employing the GLM procedure in SAS™ software (Statistical Analysis Systems [SAS], 2002).

The mathematical model used was:

$$Yij = \mu + Ti + eij,$$

Where:

Yij = response variable;

µ = overall mean;

Ti = effect of treatment; and

e = residual error associated with each observation.

Results and Discussion

Throughout the study, the temperature was monitored at different intervals, showing values between 27.22°C and 25.72°C from 1 to 7 days, 25.87°C to 24.13°C from 7 to 14 days, 25.57°C to 26.27°C from 14 to 21 days, and 34.3°C at 23.2°C from 21 to 42 days of broiler life. Relative humidity of the air ranged from 63.33% to 24.67% from 1 to 7 days, 60.14% to 24.29% from 7 to 14 days, 55% to 22.4% from 14 to 21 days, and 47.97% to 19.11% from 21 to 42 days.

The recommended temperature range for broilers according to the management manual for the Ross 308 strain is 30°C to 27°C from 1 to 7 days, 27°C to 24°C from 7 to 14 days, 24°C to 22°C from 14 to 21 days, and 21°C to 20°C from 21 to 42 days. Consequently, the birds experienced periods outside the recommended thermal comfort zone from the third week of life.

As for relative air humidity (RH), Menegali et al. (2013) indicated an ideal range between 60 and 70%, with values below 50% RH resulting in bird dehydration. Therefore, monitoring temperature and air relative humidity is crucial for animal production, as they could induce animal stress, affecting behavior, and well-being, and lead to problems such as decreased feed intake, respiratory diseases, and increased mortality.

Regarding mortality, we observed a mortality rate of 11.82% over the 1 to 42-day experimental period, exceeding the accepted standard of 3% for modern poultry farming. High mortality (11.82%) was influenced by high temperatures and low humidity, rather than the treatment effects.

There were no significant treatment effects observed for average weight, feed intake, weight gain, and uniformity of broilers during the 1 to 21 and 1 to 42-day phases. However, there was a significant effect on feed conversion ($P = 0.0100$) from 1 to 21 days of age (Table 3). Broilers in the BD + 1kg/t synbiotic treatment demonstrated improved feed conversion compared to the basal diet (BD).

Results in Table 3 align with those reported by Leite et al. (2020), who subjected birds to a challenge involving reused litter without treatment, infrequent drinker cleaning, and consumption of untreated water. They evaluated diets with antibiotics (zinc bacitracin) (positive control), no antibiotics, no probiotic/synbiotic (negative control), and with probiotics and with synbiotics. These authors found no significant dietary effects on broiler performance and concluded that alternative additives did not differ in terms of bird performance up to 42 days of age.

Table 3
Effect of treatments on broilers performance

Treatments	1 - 21 days				
	AW, g	FI, g	WG, g	FC, g/g	UNF, %
BD	786.1	959.0	752.4	1.274 a	65.6
BD+ZBC	798.3	952.8	764.5	1.246 b	63.9
BD + S0,5	786.7	949.2	752.9	1.261 ab	60.9
BD +S1	821.1	972.6	787.4	1.235 b	60.1
Probability	0.1438	0.6606	0.1449	0.0100	0.8323
C.V., %	3.10	3.26	3.25	1.23	17.27
Treatments	1 - 42 days				
	AW, g	FI, g	WG, g	FC, g/g	UNF, %
BD	2364.8	3631.8	2336.6	1.554	57.5
BD+ZBC	2380.5	3726.9	2347.0	1.588	62.0
BD + S0,5	2375.3	3650.9	2341.6	1.559	46.2
BD +S1	2447.3	3788.5	2427.9	1.560	51.0
Probability	0.5781	0.5433	0.4170	0.7047	0.3508
C.V., %	4.24	5.06	4.09	3.17	26.49

Average Weight (AW); Feed Intake (FI); Weight Gain (WG); Feed Conversion (FC); Uniformity (UNF);
Basal diet (BD); BD + 150 ppm zinc bacitracin (BD + ZBC); BD + 0.5 kg/t of symbiotic (BD + S0.5); BD + 1kg/t of symbiotic (BD + S1);
Means with similar letters in the columns do not differ statistically by the SNK test (P>0.05);
C.V.: Coefficient of variation.

Other studies (Cheng et al., 2017; Kridtayopas et al., 2019; Naghi Shokri et al., 2017; Rehman et al., 2020) showed positive outcomes with synbiotic use on broiler performance. Nevertheless, these studies have adopted different experimental conditions from ours. According to Clavijo and Flórez (2018) and Otutumi et al. (2012), variability in results may be attributed to factors such as probiotic and prebiotic composition, microbial strains, administration methods, age, immunological status, breed, and environmental conditions, all influencing responses to additive supplementation.

The improvement in feed conversion observed in the BD + S1 treatment from 1 to 21 days of age suggests that this synbiotic treatment positively influenced the balance of the intestinal microbiota in broilers, allowing for better adaptation to the sanitary challenge applied during the experiment from 1 to 42 days of age. This result is indicative of the effectiveness of the synbiotic used in the study and supports its potential use as a substitute for antibiotics in broiler diets up to 42 days of age.

However, it is worth noting that there were no significant effects of the treatments on the histomorphometry of the duodenum and jejunum of broilers at 21 days of age (Table 4). Additionally, no significant differences were observed in the parameters of nutrient

digestibility and apparent metabolizable energy (Table 5), carcass traits of broilers at 42 days of age (Table 6), or hematological parameters (Table 7).

These findings suggest that the sanitary challenge applied during the study may not have been severe enough to induce significant changes in these parameters. The absence of treatment effects on intestinal histomorphometry of the duodenum and jejunum of broilers (Table 4), and nutrient digestibility indicates that the probiotic strains (*Saccharomyces cerevisiae, Bifidobacterium bifidum, Bacillus subtilis, Enterococcus faecium* and *Lactobacillus acidophilus*) and prebiotic additives (glucans and mannans) can be effective in maintaining gut health and nutrient utilization, similar to the antibiotic zinc bacitracin.

Table 4
Effect of treatments on villi perimeter (VP), villus height (VH), villus width (VW), crypt height (CH), crypt width (CW), villus crypt ratio (VCR) and width muscle (WM) of the duodenum and jejunum of broilers

Treatments	Duodenum (µm)						
	VP	VH	VW	CH	CW	VCR	WM
BD	3639.4	1738.6	209.0	269.4	30.75	6.65	170.6
BD+ZBC	3614.0	1750.4	237.8	250.4	31.00	7.10	166.6
BD + S0,5	3651.2	1748.2	241.2	248.4	27.20	7.08	154.0
BD +S1	3654.4	1753.4	217.8	260.6	29.40	6.77	156.2
Probability	0.9975	0.9994	0.2984	0.8396	0.4749	0.8756	0.7674
C.V., %	9.37	10.85	13.29	16.00	13.85	15.20	17.91
Treatments	Jejunum (µm)						
	VP	VH	VW	CH	CW	VCR	WM
BD	3030.3	1467.0	173.0	300.7	24.40	4.95	191.8
BD+ZBC	2992.3	1377.2	216.0	299.6	29.50	4.63	201.2
BD + S0,5	3112.0	1394.8	182.4	268.8	26.25	5.18	212.8
BD +S1	2984.0	1450.3	188.4	320.0	26.60	4.69	203.0
Probability	0.7797	0.7666	0.2469	0.3903	0.2470	0.6238	0.6414
C.V., %	6.21	10.13	15.81	14.02	13.36	14.56	12.58

Basal diet (BD); BD + 150 ppm zinc bacitracin (BD + ZBC); BD + 0.5 kg/t of symbiotic (BD + S0.5); BD + 1kg/t of symbiotic (BD + S1);
C.V.: Coefficient of variation.

Table 5
Effect of treatments on energy and digestibility coefficient in broilers

Treatments	AMEDM, kcal/kg	AMENM, kcal/kg	DCDM, %	DCCP, %	DCEE, %
BD	3226.20	2947.20	68.158	61.480 b	73.950
BD+ZBC	3184.25	2916.50	67.805	62.267 b	74.180
BD + $S_{0,5}$	3317.75	3030.50	69.370	67.900 a	76.425
BD +S_1	3314.20	3040.80	70.254	64.040 ab	74.150
Probability	0.3092	0.2893	0.4353	0.0225	0.6594
C.V., %	3.68	3.67	3.57	4.29	4.27

Apparent Metabolizable Energy of Dry Matter (AMEDM); Natural Matter (AMENM); Digestibility Coefficient of Dry Matter (DCDM); Digestibility coefficient of Crude Protein (DCCP); Digestibility coefficient of Ether Extract (DCEE);
Basal diet (BD); BD + 150 ppm zinc bacitracin (BD + ZBC); BD + 0.5 kg/t of symbiotic (BD + $S_{0.5}$); BD + 1kg/t of symbiotic (BD + S_1);
C.V.: Coefficient of variation.

Table 6
Effect of treatments on carcass yield and cuts of broilers

Treatments	Carcass yield and cuts, %					
	CY	BY	TY	DY	WY	TY
BD	77.9	35.9	13.7	15.6	4.9	5.7
BD+ZBC	76.8	36.1	13.7	15.5	4.8	5.8
BD + $S_{0,5}$	77.6	37.5	13.3	15.6	4.6	5.8
BD +S_1	77.4	36.1	13.9	15.4	4.9	5.8
Probability	0.0913	0.5912	0.2723	0.9710	0.0654	0.8955
C.V., %	0.74	5.24	3.83	4.15	3.52	5.53
Treatments	Relative weight of organs, %					
	H	G	L	AF	BF	
BD	0.54	2.3	2.3	1.4	1.15	
BD+ZBC	0.62	2.3	2.2	1.5	0.15	
BD + $S_{0,5}$	0.58	2.1	2.2	1.4	0.13	
BD +S_1	0.59	2.1	2.3	1.6	0.17	
Probability	0.1379	0.5645	0.7998	0.2510	0.6281	
C.V., %	8.72	12.69	9.46	14.47	26.96	

Carcass Yield (CY); Breast Yield (BY); Thigh Yield (TY); Drumstick Yield (DY); Wing Yield (WY); Tulip Yield (TY); relative heart weight (H); relative gizzard weight (G); relative liver weight (L); relative abdominal fat weight (AF); relative bursa of fabric weight (BF);
Basal diet (BD); BD + 150 ppm zinc bacitracin (BD + ZBC); BD + 0.5 kg/t of symbiotic (BD + S0.5); BD + 1kg/t of symbiotic (BD + S1);
C.V.: Coefficient of variation.

Table 7
Effect of treatments on hematological parameters of broilers

Treatments	BD	BD+ZBC	BD + S0,5	BD +S1	Prob.	CV, %
Ht, %	25.2	26.8	26.4	27.0	0.5350	7.86
TPP, g/dL	3.48	3.48	3.64	3.56	0.8830	10.38
Er, 10^6/µ L	1.386	1.243	1.263	1.445	0.5336	18.70
Th, /µL	13200	5000	4800	10800	0.7886	73.35
Leu, /µ L	33500	33000	23700	37000	0.4922	43.60
Het, /µ L	14051	14491	8096	16482	0.0981	38.44
Lym, /µ L	13778	12366	11909	12890	0.9869	54.18
Eos, /µ L	2746	2110	1422	4041	0.0937	59.39
Mon, /µ L	2925	3946	1961	3501	0.6430	80.50

hematocrit (Ht); total plasma protein (TPP); erythrocyte (Er); thrombocytes (Th); leukocytes (Leu); heterophils (Het); lymphocytes (Lym); eosinophils (Eos); monocytes (Mon);
Basal diet (BD); BD + 150 ppm zinc bacitracin (BD + ZBC); BD + 0.5 kg/t of symbiotic (BD + $S_{0.5}$); BD + 1kg/t of symbiotic (BD + S_1);
CV: Coefficient of variation;
Prob.: Probability.

The capacity of the digestive tract in chickens during the first week of life can be considered a limiting factor for food consumption, digestion, and the absorption of nutrients for growth. Therefore, substances that exert a trophic action on the intestinal mucosa, enhancing its functional capacity, may improve the performance of birds. This is due to their greater ability to digest and absorb nutrients from the diet (Maiorka, 2002).

The animal gastrointestinal tract serves as an environment for a vast number of microorganisms and also plays a significant immunological role. It constitutes the most critical barrier that protects the host from toxins, pathogens, and the consequences of their actions, including inflammation. Currently, available data on the effects of synbiotics on animal health are insufficient and require further studies. Nonetheless, these data do indicate the effective synergistic action of probiotics, prebiotics, and synbiotics in reducing populations of bacterial gastrointestinal pathogens (Markowiak & Śliżewska, 2018).

However, replacing performance-enhancing antibiotics with synbiotics has shown varying effects on bird performance and intestinal microbiota. Several factors can influence the responses to additives used, including product composition, survival of microorganisms, dosage, type of sanitary challenge, and stress conditions to which birds are exposed (Chen & Yu, 2020; Reis & Vieites, 2019; Shanmugasundaram et al., 2019). Therefore, the lack of a significant effect of treatments on intestinal morphometry may have been due to the conditions of broiler rearing, as the low microbiological

contamination of the environment and the challenge imposed on the animals may not have been sufficient to induce changes in intestinal health.

Treatments affected the protein digestibility coefficient of broiler chickens (Table 5). The BD+ 0.5kg/t synbiotic treatment showed better digestibility (67.900%) compared to the control treatment (61.480%) and zinc bacitracin treatment (62.267%).

According to Mangisah et al. (2021), adding synbiotics to broiler feeds reduces gastrointestinal pH and pathogenic bacteria, increasing non-pathogenic ones. Balance of intestinal bacteria leads to improved digestion and absorption, and the greater the absorption of nutrients, the more nutrients are available for the formation of meat tissue. Therefore, based on our findings for carcass quality and performance, we can consider the use of synbiotics as an alternative to antibiotics in broiler feeding.

According to Bozkurt et al. (2014), the microbial ecosystem in the intestine of broilers plays a crucial role in the digestion of ingested food. Imbalances in the composition of the microbiota can lead to disruptions in performance and the ability to utilize nutrients.

In this context, the digestibility of nutrients can be influenced by the balance of the gastrointestinal microbiota, which can enhance the absorption of nutrients from the diet. However, results from studies on the use of probiotics or synbiotics in broiler production have been contradictory, with many satisfactory outcomes being intricately linked to the level of biological challenge in the environment.

For instance, Sampath et al. (2021) observed no effect of 0.10% *L. plantarum* on nutrient digestibility in broilers. The authors suggested that variations in results may be attributed to different probiotic strains, indicating the need for further research to elucidate the impact of *L. plantarum* on nutrient digestibility in broilers, potentially through altering the supplementation level in the experimental diet.

Santos et al. (2012) evaluated the effects of probiotics (Enterococcus faecium-1010 CFU/g: 30g/t) and enzymes (xylanase: 100 g/t and amylase + β-glucanase: 400g/t) in diets with two energy levels for broilers aged 28 to 35 days. Their study found no significant impact on apparent metabolizable energy and metabolizability of dry matter, crude protein, and gross energy for diets containing probiotics. According to the authors, the probiotic used did not directly affect the metabolizability of the studied substrates but may have had an indirect effect associated with activities promoted in the microbial community of the digestive tract.

The influence of nutrient digestibility (utilization of nutrients from the diet) also had a similar effect on the carcass traits evaluated at the end of this study. Cheng et al. (2017) reported that the best results in carcass traits were related to the improved efficiency of nutrient utilization due to the presence of the synbiotic in the diet. This synbiotic consisted of probiotics (*Bacillus subtilis, Bacillus licheniformis* and *Clostridium butyricum*) and prebiotics (yeast cell wall and xylooligosaccharide) and was tested in Avian broilers aged 1 to 42 days.

The carcass yield in this study ranged from 76.6% to 77.9%, which was higher than what Sarangi et al. (2016) observed in

Vencobb broilers at 42 days of age (73.77% to 76.04%). Sarangi et al. (2016) found no differences in carcass and cut yields when testing treatments with a prebiotic derived from yeast cell wall extracts, a probiotic containing various strains of bacteria (*L. bulgaricus, L. plantarum, S. faecium, B. bifidus* and *S. cerevisiae*), and a synbiotic combination of both. The authors suggested that the results could be explained by the low doses used in the study (0.4; 0.1; and 0.5 kg/t).

Following this study, Abdel-Hafeez et al. (2017) observed no significant effect on total protein levels with the addition of probiotics and synbiotics. However, these authors did find that birds fed diets supplemented with probiotics, prebiotics, and synbiotics (with and without feed restriction) showed increased hematocrit levels in treatments with additives and with feed restriction at the end of the experiment.

Al-Baadani et al. (2018) evaluated the effect of an antimicrobial growth promoter (AGP), probiotic, prebiotic, and their combination (synbiotic) on the blood biochemistry of broilers challenged with Clostridium perfringens. They found that the ratio of heterophils to lymphocytes (H/L) was significantly ($P \leq 0.001$) lower, and lymphocyte counts were significantly ($P \leq 0.001$) higher in all groups compared to the positive control. The synergistic effect of *Bacillus subtilis* was superior to AGP in improving the blood biochemical profile of chickens challenged with *Clostridium perfringens.*

It is worth noting that the analysis of hematological parameters is essential in veterinary practice. However, it is important to highlight that several factors can influence hematological values, such as the breed and strain of the analyzed birds, type of food, ambient temperature, altitude, venipuncture site, technique, and the laboratory kit used. These factors may explain the differences between the values found in the literature and in the present study. According to the findings, it can be emphasized that the animals in this study did not have infectious diseases, and despite being challenged weekly, they maintained good immunological conditions.

Conclusions

In summary, using a synbiotic supplement (+Poultry) at 1 kg/t is a practical substitute for antibiotics in the diets of broilers aged 1 to 21 days, particularly when facing sanitary challenges. This supplement effectively preserves the birds' performance and health, making it a valuable and sustainable alternative to antibiotics across different stages of broiler farming.

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