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Prebiotic composed of yeast (*Saccharomyces cerevisiae*) cell wall improves performance in broiler diets

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ABSTRACT - This research aimed to evaluate the influence of a commercial prebiotic in different concentrations upon several parameters. To carry out the experiment, 640 male one-day-old broiler chicks were distributed in four treatments (0, 0.5, 1.0, and 1.5 kg/ton of yeast cell wall) with eight replicates of 20 birds per experimental unit, in randomized blocks. Prebiotic effects were assessed on performance, carcass yield and prime cuts, in addition to the litter quality (its content of nitrogen and phosphorus). There were significant improvements for weight gain and feed conversion ratio in experimental growth periods. However, prebiotic level at 1.0 kg/ton is enough to provide improvement in performance and similar yield parameters than the control group. Also, 1.5 kg/ton prebiotic inclusion in the diet promotes environmental benefits by reducing the phosphorus amount in the litter by 51%. Above 1.0 kg/ton prebiotic addition in broiler diets can be safely recommended, because it promotes both performance and environmental benefits.

Keywords: broiler performance, carcass yield, chicken litter, immune system, MOS, poultry feed

1. Introduction

Intestinal health is a concept of great relevance in broiler production. It is directly linked to animal nutrition. The effects of diet on intestinal health are remarkable and influence the nutrient absorption. A healthy gut must be able to metabolize and absorb nutrients efficiently, reflecting on animal performance (Landy and Kavyani, 2013; Gibson et al., 2017; Dias et al., 2023).

Functional oligosaccharides are non-digestible feed ingredients that provide several health benefits. Among these, mannan-oligosaccharides (MOS) are emerging prebiotics with potential bioactive properties and are opening up novel opportunities in the feed industry (Jana et al., 2021).

Studies reported that prebiotics additives stimulate the growth and activity of probiotics, despite inhibiting pathogen activity in chicken, thereby attenuating the inflammatory response (Pourabedin et al., 2017;

Biswas et al., 2019; Geng et al., 2023). As these substances are beneficial to intestinal bacteria, they alter the microbiota for the host, induce systemic effects (Gibson et al., 2017), and reduce environmental pollution (Chen, 2021).

Prebiotics of different sources are being successfully applied to poultry (Wang et al., 2016; Pourabedin et al., 2017; Biswas et al., 2019; Elgeddawy et al., 2020; Jana et al., 2021; Nascimento and Marostica Junior, 2021; Asif et al., 2024). Yeast cell wall has MOS, which are claimed to benefit poultry gut. Several studies in the literature investigate the use of these substances as potential additives in replacement of growth promoters in animal nutrition (Wang et al., 2016; Biswas et al., 2019; Ahmed et al., 2023; Moawad et al., 2023; Asif et al., 2024).

Yeast (*Saccharomyces cerevisiae*) cell wall from sugarcane is a prebiotic that has potential to be added in poultry feed (Pascual et al., 2020). The yeast wall has β-glucans and MOS that inhibit pathogenic bacteria (Aleris, 2023). However, the effect of this prebiotic in broiler diets depends on several factors such as the dosage used, the obtention process, their combination, the administration mode, and mainly the health challenge to which the animals are subjected (Gao et al., 2008).

Scientific research about prebiotic incorporation in broiler diets can direct to more efficient and environmentally friendly poultry production systems by decreasing the dependence on antibiotics and improving broiler health. The positive outcomes may help the industry to adopt sustainable strategies to enhance animal rearing, at the same time improving animal welfare and environmental stewardship.

In virtue of the facts mentioned above, this research aimed to provide valuable guidance for the commercial broiler production system by evaluating a commercial prebiotic additive composed of yeast (*Saccharomyces cerevisiae*) cell wall in the diets of broilers from 1 to 42 days of age by assessing performance and environmental parameters.

2. Material and Methods

2.1. Animals, management, and experimental design

Research on animals was conducted according to the institutional committee on animal use (01/2016), in accordance with current Brazilian legislation.

An experiment consisting of 640 one-day-old male Cobb-500 broiler chicks, with an initial weight of 40 g, was carried out in Viçosa, Minas Gerais, Brazil (20°45'57.19" S, 42°51'35.42" W, and 682 m altitude).

The animals were distributed in a randomized block design with four treatments and eight replications of 20 birds each. The animals were housed in facilities of 3 m high, with a roof covered with asbestos cement tiles, 0.5 m low walls, and lateral insulation with a half-inch screen (1/2''), adapted for the experiment. The animal boxes out of concrete floor represented the experimental units, measuring 1.3 × 1.7 m, featuring an area of 2.21 m^2/box. As a challenge, no prior disinfection of the facility was carried out, and the birds were housed in reused shavings litter. During the first 24 h, the animals were subjected to water and feed fasting. Until the birds were 21 days old, water contaminated with excreta from a laying hen farm was provided twice a week. In each 20 L of water, 1 kg of litter was added; the mixture was stirred for 3 min and then given to the chickens for 24 h.

The diets were formulated to meet the nutritional requirements of broilers (regular performance) at different rearing stages, according to the Brazilian Tables of Poultry and Swine (Rostagno et al., 2017) (Table 1). The diets differed according to the rearing phases: pre-starter (1–7 days), starter (8–21 days), and grower/finisher (22–42 days) phases.

The treatments consisted of different levels of prebiotic inclusion (0, 0.5, 1.0, and 1.5 kg/ton) by replacing the feed inert (kaolin) in the diet. The prebiotic Maximos®, composed of MOS and β-glucans, was supplied by the company Aleris (2023).

Table 1 - Composition of diets for three broiler phases

Ingredient (%)	Pre-starter (1–7 days)	Starter (8–21 days)	Grower/Finisher (22–42 days)
Corn	51.3	56.2	59.4
Soybean meal (45%)	37.1	36.4	32.7
Corn gluten meal (60%)	5.00	-	-
Soybean oil	2.00	3.30	4.17
Limestone	0.92	0.91	0.86
Dicalcium phosphate	1.91	1.52	1.28
Salt	0.51	0.48	0.45
Lysine HCl (79%)	0.28	0.17	0.16
DL-Methionine (99%)	0.27	0.28	0.25
L-Threonine (98%)	0.04	0.04	0.03
Choline chloride (60%)	0.10	0.10	0.10
Vitamin supplement (UFV[1])	0.13	0.11	0.10
Mineral supplement (UFV[2])	0.13	0.11	0.10
Anticoccidial (Salinomycin 12%)	0.05	0.05	0.05
Butylhydroxytoluene (BHT) (3%)	0.01	0.01	0.01
Inert (kaolin)	0.30	0.30	0.30
Total	100	100	100
Calculated values (%)			
Crude protein	24.4	21.2	19.7
Metabolizable energy (kcal/kg)	2,950	3,050	3,150
Calcium	0.92	0.82	0.73
Available phosphorus	0.47	0.39	0.34
Sodium	0.22	0.21	0.20
Digestible lysine	1.31	1.17	1.08
Digestible methionine + cystine	0.94	0.85	0.79
Digestible methionine	0.61	0.58	0.51
Digestible threonine	0.85	0.76	0.70
Digestible tryptophan	0.26	0.24	0.22
Digestible valine	1.03	0.90	0.84
Digestible isoleucine	0.97	0.83	0.77
Digestible arginine	1.44	1.35	1.24
Digestible glycine + serotonin	1.98	1.78	1.65

[1] Vitamin supplement – guaranteed levels per kg of product: Vit. A, 8250 IU; Vit. D3, 2090 IU; Vit. E, 31.0 IU; Vit. B1, 2.20 mg; Vit. B2, 5.50 mg; Vit. B6, 3.08 mg; Vit. B12, 0.013 mg; pantothenic acid, 11.0 g; biotin, 0.077 mg; Vit. K3, 1.65 mg; folic acid, 0.77 mg; nicotinic acid, 33.0 mg; selenium, 0.330 mg;
[2] Mineral supplement – guaranteed levels per kg of feed: iron, 55.0 mg; copper, 11.0 mg; manganese, 77.0 mg; zinc, 71.5 mg; iodine, 1.10 mg.

2.2. Animal performance

To evaluate performance, the animals were weighed, as well as the feed and leftovers, at the beginning and end of each phase. Mortality was also verified. With these data, it was possible to calculate feed intake (FI, g/bird), weight gain (WG, g/bird), feed conversion ratio (FCR), livability (LIV, %) of the animals in all growing phases (from 1 to 7, 1 to 21, 1 to 35, and 1 to 42 days of age) to compare the prebiotic inclusion levels. In the total period of the experiment, from 1 to 42 days, the productive efficiency index (PEI) was determined through the calculation (equation 1) adapted from Saiyed et al. (2015):

$$PEI = \frac{WG \times LIV}{SA \times FCR} \times 100 \quad (1)$$

in which WG = average weight gain (kg), LIV = livability (%), and SA = slaughter age.

2.3. Carcass yield

To evaluate carcass yield and prime cuts, two animals with a weight 10% higher or lower than the average weight of the experimental unit were selected and identified. These animals remained fasting for 10 h, and then they were weighed again and then slaughtered. After slaughter, the carcasses, breasts, thighs, and drumsticks were weighed. Carcass yield and cuts yield were determined using the methodology described by Falaki et al. (2010). The cut yields evaluated were: breast, thigh, and drumstick yields.

2.4. Broiler litter evaluation

For litter evaluation, moisture (%), pH, and nitrogen and phosphorus contents of chicken litter were taken into account. The samples were collected at three different places in chicken litter (initial, middle, and final third) of each experimental unit, before the animals were housed and at the end of the experiment (42 days). We avoided to collect samples at places close to the feeder and drinker.

The pH was obtained by weighing 20 g of the sample and diluting it in deionized water; after resting, the reading was performed with a pH meter, as described by Pope and Cherry (2000). Moisture, N, and P contents were determined by official methods 934.01 (AOAC, 1990), 955.04 (AOAC, 1990), 958.01 (AOAC, 1990), respectively. The results of N and P are the difference obtained between the final and initial evaluation of the litter, thus providing the result of accumulation of these minerals during the 42 days of the experiment.

2.5. Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) using the PROC GLM procedure of SAS (Statistical Analysis System, version 9.4). ANOVA was performed considering the experimental design in blocks, described according to the statistical model (equation 2):

$$yijk = \mu + Ti + \beta j + \varepsilon ijk \quad (2)$$

in which $yijk$ = observation k at level i (i = 1,...,a) of treatment T and block j (j = 1,...,b), μ = overall average, Ti = effect of treatment i, βj = fixed effect of block j, and εijk = random error.

To evaluate the prebiotic inclusion levels, regression analysis was performed and contrast evaluation procedure of orthogonal polynomials for each variable dependent at the 5% probability level. When a quadratic effect was found, it was possible to estimate the optimal level of product supplementation by deriving the second-degree equation.

3. Results

3.1. Animal performance

The inclusion of prebiotics in broiler diets improved performance during the production cycle (Table 2). For instance, animal WG and FCR resulted in an increase in the PEI and did not influence the carcass parameters (Table 3). However, the amount of phosphorus decreased in the litter at 42 days of age (Table 4).

In the phase from 1 to 7 days of broiler age, the inclusion of prebiotics provided a linear increase ($P<0.05$) in WG, being 8.5% greater in the treatment with the highest prebiotic inclusion (1.5 kg/ton). A quadratic effect was also obtained ($P<0.05$), with the calculated optimal level of 1.01 kg/ton, which provided an improvement of 5.7% in FCR compared with that obtained with the control diet, free of prebiotics. There was no significant difference ($P<0.05$) for FI and LIV.

In the phase from 1 to 21 days of broiler age, with the inclusion of the prebiotic, a quadratic effect (P<0.05) was obtained for WG and FCR. The use of the optimal inclusion level, estimated at 1.02 kg/ton of prebiotic in the diet, provided WG of 802 g and FCR of 1.31, which were 6.58 and 8.39% better than the results without the use of prebiotic in the diet, respectively. There was no significant difference (P<0.05) for FI and LIV.

In the phase from 1 to 35 days of age, the inclusion of the prebiotic provided a quadratic effect (P<0.05) for WG and FCR. The use of the estimated optimal level of 1.23 kg/ton of prebiotic in the diet provided WG of 1,952 g and FCR of 1.50, which were 8.54 and 8.93% better than the results obtained with the control group, respectively. There was no significant difference (P<0.05) for FI and LIV.

Table 2 - Performance of broilers fed different prebiotic levels

	Parameter				
Treatment[1]	WG (g/bird)	FI (g/bird)	FCR[2]	LIV (%)	PEI
			1–7 days		
Control	110	144	1.3	100	-
0.5 kg/ton	115	143	1.2	99.6	-
1.0 kg/ton	116	144	1.2	99.6	-
1.5 kg/ton	117	147	1.2	99.6	-
P-linear	<0.01	0.452	0.030	0.552	-
P-quadratic	0.121	0.352	0.043	0.633	
SEM	0.914	1.953	0.015	0.002	-
	WG (g/bird)[3]	FI (g/bird)	FCR[4]	LIV (%)	PEI
			1–21 days		
Control	753	1,077	1.4	97.5	-
0.5 kg/ton	797	1,063	1.3	98.1	-
1.0 kg/ton	793	1,045	1.3	98.7	-
1.5 kg/ton	800	1,079	1.4	98.7	-
P-linear	0.0005	0.870	0.030	0.243	-
P-quadratic	0.0142	0.188	0.010	0.686	
SEM	6.714	16.06	0.021	0.060	-
	WG (g/bird)[5]	FI (g/bird)	FCR[6]	LIV (%)	PEI
			1–35 days		
Control	1,799	2,974	1.7	96.2	-
0.5 kg/ton	1,902	2,955	1.6	97.5	-
1.0 kg/ton	1,937	2,923	1.5	98.7	-
1.5 kg/ton	1,952	2,964	1.5	98.4	-
P-linear	<0.010	0.727	<0.010	0.119	-
P-quadratic	0.003	0.421	0.009	0.449	-
SEM	12.37	32.75	0.018	0.007	-
	WG (g/bird)	FI (g/bird)	FCR	LIV (%)	PEI
			1–42 days		
Control	2,337	4,016	1.7	96.2	319
0.5 kg/ton	2,467	4,010	1.6	97.5	361
1.0 kg/ton	2,460	3,932	1.6	99.4	373
1.5 kg/ton	2,463	3,924	1.6	97.5	368
P-linear	0.0180	0.5705	0.0265	0.2673	0.0152
P-quadratic	0.0937	0.8314	0.1506	0.3722	0.0947
SEM	30.73	50.48	0.028	0.033	10.07

SEM - standard error of the mean; WG - weight gain; FI - feed intake; FCR - feed conversion ratio; LIV - livability; PEI - productive efficiency index.
[1] Control (basal diet with no growth promoter and prebiotic); control diet plus 0.5, 1.0, and 1.5 kg/ton prebiotic, respectively.
[2] Regression equation: $y = 1.3064 - 0.1449x + 0.0718x^2$ ($R^2 = 0.98$; X optimum = 1.01 kg/ton).
[3] Regression equation $y = 755.75 + 84.246x - 38.112x^2$ ($R^2 = 0.88$; X optimum = 1.10 kg/ton).
[4] Regression equation $y = 1.4285 - 0.241x + 0.1267x^2$ ($R^2 = 0.99$; X optimum = 0.95 kg/ton).
[5] Regression equation $y = 1801.3 + 230.82x - 88.114x^2$ ($R^2 = 0.99$; X optimum = 1.31 kg/ton).
[6] Regression equation $y = 1.6541 - 0.2541x + 0.1095x^2$ ($R^2 = 1.00$; X optimum = 1.16 kg/ton).

Table 3 - Carcass and cuts yield of broilers fed different levels of prebiotics at the complete cycle (1–42 days)

Treatment[1]	Yield (%)			
	Eviscerated weight	Breast	Thigh	Drumstick
Control	82.1	34.5	12.6	14.4
0.5 kg/ton	82.3	34.9	12.5	14.3
1.0 kg/ton	82.1	34.1	12.4	14.6
1.5 kg/ton	82.3	34.4	12.5	14.8
P-linear	0.553	0.570	0.367	0.127
P-quadratic	0.894	0.899	0.316	0.291
SEM	0.004	0.005	0.002	0.003

SEM - standard error of the mean.
[1] Control (basal diet with no growth promoter and prebiotic); control diet plus 0.5, 1.0, and 1.5 kg/ton prebiotic, respectively.

Table 4 - Litter quality parameters of broilers at 1–42 days fed different prebiotic levels

Treatment[1]	Parameter			
	Moisture (%)	pH[2]	Nitrogen (%)	Phosphorus (%)
Control	24.9	7.6	1.40	13.1
0.5 kg/ton	30.5	7.6	1.16	9.2
1.0 kg/ton	21.7	7.5	1.06	6.9
1.5 kg/ton	30.2	7.6	0.86	6.4
P-linear	0.515	0.709	0.066	0.0007
P-quadratic	0.550	0.552	0.926	0.2150
SEM	0.085	0.025	0.121	0.0784

SEM - standard error of the mean.
[1] Control (basal diet with no growth promoter and prebiotic); control diet plus 0.5, 1.0, and 1.5 kg/ton prebiotic, respectively.
[2] pH in the litter at 41 days of housing.

In the complete production cycle, we observed a positive effect of prebiotic inclusion in the diets on WG, FCR, and PEI. There was a 5.36% increase in WG (2,337 g to 2,463 g) and 7.3% in FCR. This positive effect on WG and FCR provided 16.9% higher PEI compared with the control diet (319 to 373). There was no significant difference ($P<0.05$) for FI and LIV. Carcass yield parameters did not show significant differences ($P<0.05$) from prebiotic levels in the diets.

3.2. Broiler litter evaluation

Regarding phosphorus content in the litter, the broilers that consumed the diet with 1.5 kg/ton of prebiotic reduced the phosphorus amount in the litter ($P<0.05$) by 51%. There was a trend ($P = 0.06$) in nitrogen content reduction in the litter in 39% when comparing the birds fed prebiotic with the control group. No significant differences ($P<0.05$) were observed regarding pH and moisture.

4. Discussion

Overall, the inclusion of prebiotics in broiler diets in any amount improved performance during the production cycle. The results obtained are due to the prebiotic effect of MOS and β-glucans. Mono-oligosaccharides are complex carbohydrates with the ability to act as a non-pathogenic antigen, increasing the production of IgG and IgA immunoglobulins (Swanson et al., 2002; Asadpoor et al., 2020). Still, MOS stimulate the development of the systemic immunity of the animal, once they bind to certain gut microbiota due to mannan component and, thus, prevent attachment of microbiota to the intestinal cells (Yamamoto and Uenishi, 2010). They also provide greater absorption of nutrients by the intestinal mucosa, due to the positive effect on villus height and crypt depth both in jejunum and ileum (Asif et al., 2024).

Additionally, MOS are used as a substrate to stimulate the growth and/or metabolism of beneficial bacteria. These compounds also act as a ligand for bacteria that have type 1 fimbriae, such as the pathogenic bacteria *Salmonella* spp. and *E. coli* that affect animal production (Ferket et al., 2002). Once attached to the MOS, these bacteria are not able to bind to the specific sites of the enterocytes and are prevented from colonizing the gastrointestinal tract, being eliminated with the fecal cake (Oyofo et al., 1989).

β-glucans when digested, are absorbed through the intestinal mucosa and are recognized by the defense cells of the animal organism, being Dectin-1 one of them. Activation of the receptor of this cell induces various stimulating effects on the immune system (Stier et al., 2014), resulting in increased production of macrophages, monocytes, and cytokines (Seljelid et al., 1987; Guo et al., 2003; Cox et al., 2010). As a result, β-glucan increases resistance against infection of microorganisms and reduces mortality (Moon et al., 2016). Improvement of the intestinal health of animals fed this compound has been proven, promoting the reduction of *Clostridium perfrigens* and the increase of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* (Tian et al., 2016).

Asif et al. (2024) evaluated from 200 to 800 g/ton prebiotic composed of MOS in broiler diets. The highest prebiotic concentration provided WG (1.89 kg) and FI (2.99 kg) in the last week of experiment and FCR (1.58) similar to the 400 g/ton prebiotic inclusion group (WG = 1.88 kg; FI = 3.02 kg; FCR = 1.61). These mentioned FCR had also no difference from the positive control group that received antibiotic (FCR = 1.61). Other prebiotic concentration levels (200 and 600 g/ton) did not differ from the control negative group for the performance parameters. Therefore, the authors suggested that MOS could replace antibiotic growth promoters. Ahmed et al. (2023) evaluated performance parameters in broilers fed 1 g/kg prebiotic (MOS enriched with β-glucan) and observed higher WG (1.9 kg), less FI (3.05 kg), similar FCR (1.61), and survivability (100%), when compared with the control and positive (antibiotic) group. They suggested that antibiotic growth promoters could be replaced by prebiotic. Wang et al. (2016) also tested prebiotics containing MOS (170 g/ton) and β-glucan (250 g/ton); however, in these concentrations, no difference was observed regarding FCR, FI, and WG in any phase of broilers' life, including the complete cycle comparison.

In our study, LIV in all prebiotic treatments led to high values (> 97.5%); thus, mortality was unaltered with the use of increasing prebiotic levels, which also reflected in good PEI values. The PEI values found herein were all above 361 and better than the control group. Saiyed et al. (2015) also evaluated different prebiotic/probiotic inclusion levels in broiler diets, in which the highest concentration evaluated was 500 g/ton prebiotic combined or not with 50-100 g/ton probiotic. They observed that all treatments that used prebiotic and/or probiotic alone or combined had better results (PEI = 261–285) than the control (231) group.

Despite the good results observed by prebiotic inclusion, one must consider many factors. For instance, alterations can be found varying the concentration, brand, rearing system, and administration mode (*in ovo*, in feed, or in water). Also, the mixture of prebiotic with other compounds (for instance fiber, probiotic, or some antibacterial agent) must be considered. All these factors can provide different benefits or no benefits at all and must be carefully studied. As stated elsewhere (Moawad et al., 2023), it is crucial to consider the economic feasibility and cost-effectiveness of these interventions in a broader commercial broiler production.

With respect to carcass yield, no differences among treatments were observed. An average of 82% in eviscerated weight, 34% in breast yield, 12% thigh, and 14% drumstick was found. Similar breast yield (33%) was observed by Moawad et al. (2023) in their recent research conducted with 0.1% commercial prebiotic via water. Contrariwise, Biswas et al. (2019) showed that the weight of breast (18%), thigh (10%), back (19%), drumstick (11%), bursa of Fabricius (0.39%), and thymus (0.58%) were higher in the birds given 0.2% MOS.

In the present study, the litter quality parameters showed no difference regarding pH, moisture, and nitrogen excretion. However, phosphorus content decreased with the use of prebiotics in the diets in 30, 47, and 51% when, respectively, 0.5, 1.0, and 1.5 kg/ton of prebiotics were added in the diet. This

effect can be attributed to the increased expression of the enzyme alkaline phosphatase at the brush border of the jejunum, provided by MOS (Iji et al., 2001). All these parameters are important to be measured in broiler litter. For instance, ammonia is a problem for poultry, because it volatilizes as pH values increases and excess ammonia (> 100 ppm), in addition to impairing bird welfare, negatively impacts performance (Moore Jr. et al., 1996).

The tendency of nitrogen reduction observed in the litter can be explained by the effect of the prebiotic components (MOS and β-glucans) that decrease the colonization of ammonia-producing bacteria in the gastrointestinal tract, reducing the amount of non-protein nitrogen, and consequently the nitrogen content in the litter (Chang and Chen, 2003).

5. Conclusions

The use of a commercial prebiotic composed of yeast (*Saccharomyces cerevisiae*) cell wall in diets improves growth performance in broiler chickens. The inclusion of prebiotic for broilers promotes environmental benefits, due to the reduction of phosphorus present in the litter. Overall, the doses are safely recommended in broiler diets.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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