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Sugarcane yeast (*Saccharomyces cerevisiae*) and β-mannanase enzyme in broilers' diets

Levedura de cana-de-açúcar (*Saccharomyces cerevisiae*) e enzima β-mananase em dietas para frangos de corte

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Highlights

7% sugarcane yeast, without β-mannanase showed a loss of 6.08% in feed conversion.
Shallower crypts occurred on YD+ β-mannanase (120 g/t) compared to the basal.
7% sugarcane yeast, with or without β-mannanase, worsens economic viability.

Abstract

Our objective was to evaluate the effect of supplementing β-mannanase enzyme, with and without sugarcane yeast (*Saccharomyces cerevisiae*), on broiler chickens aged 1-21 days. The study used 720 one-day-old male Cobb chicks in a randomized design, with six treatments and six replications of 20 birds each. The treatments were: basal diet (BD), BD + β-mannanase (100 g/t), 7% sugarcane yeast (SY), SY + β-mannanase (80 g/t), SY + β-mannanase (100 g/t), and SY + β-mannanase (120 g/t). Zootechnical results (animal performance) were evaluated during the pre-starter (1 to 7 day-old) and starter (1 to 21 day-old) phases, as well as small intestine morphometry (duodenum, jejunum, and ileum) and diets' economic viability. Data were subjected

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to variance analysis using the SAS software, and the means were compared by the Student Newman Keuls (SNK) test. In the pre-starter phase, treatments with YD + β-mannanase (80, 100, and 120 g/t) showed the best feed conversion averages. In the starter phase, chickens consuming the basal diet (BD) and BD + β-mannanase showed better average weights, weight gains, and feed conversion rates. For intestinal morphometry, shallower ileal crypts were observed in the treatment with YD + β-mannanase $^{(120\ g/t)}$ compared to the basal diet, and wider ileal villi were observed in the treatment with YD + β-mannanase $^{(100\ g/t)}$ compared to the diet with YD + β-mannanase $^{(80\ g/t)}$. The thickness of the muscular wall in the duodenum was lower in chickens consuming BD compared to BD + β-mannanase $^{(100\ g/t)}$, higher in YD and supplementation with 100 g/t compared to 80 and 120 g/t in the jejunum, and higher in diets with β-mannanase supplementation compared to BD and YD in the ileum. For economic viability, adding 7% sugar cane yeast, with or without enzyme, increased the average feed cost and cost index, and reduced the economic efficiency index. Based on the zootechnical results, YD + β-mannanase $^{(120\ g/t)}$ is recommended for the pre-starter and starter phases. However, using sugar cane yeast with or without β-mannanase enzyme supplementation is not economically viable in the 1- to 21-day period.
Key words: Antinutritional factors. Exogenous enzymes. Intestinal mucosa. Poultry performance.

Resumo

Objetivou-se avaliar o efeito da suplementação da enzima β-mananase com e sem levedura de cana de açúcar (*Saccharomyces cerevisiae*) para frangos de corte 1 a 21 dias de idade. Utilizou-se 720 pintos de corte de um dia de idade, machos, da linhagem Cobb, distribuídos em delineamento inteiramente casualizado com seis tratamentos e, seis repetições de 20 aves cada. Os tratamentos foram: Dieta basal (DB); DB + β-mananase $^{(100\ g/t)}$; DB + 7% de levedura de cana-de-açúcar (DL); DL + β-mananase $^{(80\ g/t)}$; DL + β-mananase $^{(100\ g/t)}$; DL + β-mananase $^{(120\ g/t)}$. Avaliou-se o desempenho zootécnico na fase pré-inicial (1 a 7) e na fase inicial (1 a 21 dias de idade), a morfometria do intestino delgado (duodeno, jejuno e íleo) e, a viabilidade econômica da ração. Os dados foram submetidos à análise da variância do programa SAS e as médias foram comparadas pelo teste de Student Newmann Keuls (SNK). Na fase pré-inicial, os tratamentos com DL + β-mananase (80, 100 e 120 g/t) apresentaram as melhores médias de conversão alimentar. Na fase inicial, aos frangos que consumiram à dieta basal (DB) e DB + β-mananase apresentaram peso médio, ganho de peso e conversão alimentar melhores. Para a morfometria intestinal, criptas ileais mais rasas foram observadas no tratamento com DL+ β-mananase $^{(120\ g/t)}$ em relação a dieta basal e vilos ileais mais largos foram observados no tratamento com DL+ β-mananase $^{(100\ g/t)}$ em relação a dieta com DL+ β-mananase $^{(80\ g/t)}$. A espessura da parede muscular, no duodeno, foi menor nos frangos que consumiram a DB em relação a DB + β-mananase $^{(100\ g/t)}$, no jejuno, foi maior na DL e na suplementação com 100 g/t em relação a 80 e 120 g/t e no íleo, foi maior nas dietas com suplementação de β-mananase em relação a DB e DL. Para a viabilidade econômica, a adição de 7% de levedura de cana-de-açúcar, com ou sem enzima proporcionaram aumento do custo médio de ração e índice de custo, e redução do índice de eficiência econômica. Com base nos resultados zootécnicos recomenda-se DL + β-mananase $^{(120g/t)}$ para a fase pré-inicial e inicial. Contudo, o uso de levedura de cana-de-açúcar com e sem suplementação da enzima β-mananase não é economicamente viável no período de 1 a 21 dias.
Palavras-chave: Desempenho. Enzimas exógenas. Fatores antinutricionais. Mucosa intestinal.

Introduction

Due to the seasonality of production and the variation in prices of corn and soybean meal, many researchers have evaluated agro-industrial byproducts that can be used as alternative ingredients for broiler chicken feed. Among these ingredients, sugarcane yeast (Saccharomyces cerevisiae) stands out as a high-quality protein source with components that act on the intestinal mucosa, improving nutrient absorption and, consequently, the performance of broiler chickens (Lopes et al., 2017).

Sugarcane yeast (Saccharomyces cerevisiae) is often used in animal diets due to its high nutritional value: 37.2% crude protein, good levels of essential amino acids (lysine 2.99% and threonine 2.14%), 2526 kcal/kg of metabolizable energy for birds, and 0.82% total phosphorus. However, only 21.6% of this is digestible (Rostagno et al., 2017).

Sugarcane yeast has nutritional qualities, but its cell wall contains components that contribute to its rigidity. Specifically, the outer layer is composed of complex mannan-protein polysaccharides (35 to 40%), while the inner layer contains β-glucans (55 to 60%) and chitin (1 to 2%) (Aquino et al., 2012; Barroso et al., 2013).

Due to the high amount of non-starch polysaccharides (NSPs) in its composition, the consumption of sugarcane yeast can cause physiological limitations in birds, as they lack vital endogenous enzymes needed to digest these NSPs, thus increasing intestinal viscosity and delaying nutrient migration and absorption (Raza et al., 2019). Among the NSPs, β-mannans have negative impacts such as the ability to bind to large amounts of water, resulting in increased digesta viscosity, decreased diffusion of digestive enzymes, and stimulation of bacteria proliferation in the gastrointestinal tract (V. R. S. M. Barros et al., 2015).

The low digestibility of nutrients and high intestinal viscosity negatively affect the immune response, microbial proliferation, performance, and carcass characteristics of birds. Exogenous enzymes such as protease, phytase, β-mannanase, and β-glucanase can be added to bird diets to help digest fibers and reduce their negative impact on productivity and bird health (Alagawany & Attia, 2015; Alagawany et al., 2017).

Freitas et al. (2013) evaluated the effects of replacing soybean meal protein with sugarcane yeast in diets for broiler chickens up to 21 days of age containing cashew nut meal and found that replacing levels of up to 20% did not impair performance or alter carcass characteristics compared to the control diet. In the economic study, the substitution of soybean meal protein with yeast was viable up to the 20% level.

These zootechnical and economic performance characteristics could be enhanced with the supplementation of exogenous enzymes in the diet, as they can maximize digestibility and make nutrients such as amino acids, minerals, and vitamins available, as well as contribute to the metabolizable energy of the feed (Romero et al., 2013).

This study aims to evaluate the effect of β-mannanase enzyme supplementation in diets with and without sugarcane yeast

(Saccharomyces cerevisiae) for broiler chickens aged 1 to 21 days on performance, intestinal morphometry, and economic viability.

Materials and Methods

The study was carried out in the Poultry Sector of the Technical College of the Federal University of Piauí, at the Professora Cinobelina Elvas Campus, in the municipality of Bom Jesus-PI, under the approval of the Ethics Committee for Animal Studies, protocol number: 078/12-CEEA/UFPI. A total of 720 one-day-old male Cobb chicks were used in a completely randomized design with six treatments and six replicates of 20 birds each. The treatments consisted of a corn and soybean meal-based diet (BD), BD with 100 g/t β-mannanase enzyme, a diet with 7% sugarcane yeast (YD), YD with 80 g/t β-mannanase enzyme, YD with 100 g/t β-mannanase enzyme, and YD with 120 g/t β-mannanase enzyme.

The bromatological composition of the sugar cane yeast consisted of 96.80% dry matter, 34.41% crude protein, 0.01% ether extract, 7.80% ash, and 4189 kcal/kg of gross energy. The β-mannanase enzyme used was an imported product from the fermentation of Bacillus lentus (CGMCC 4323) with 90% corn starch in its composition. The enzyme was developed to release 1 micromole of reducing sugar per minute from mannan, at pH 5.5 and 37ºC, with a guaranteed level of 10,000,0000 µg, and could be used at a recommended rate of 80 to 120 g/ton in the feed.

The experimental diets (Table 1) were formulated based on corn and soybean meal to meet the nutritional requirements of male broilers with average performance, according to Rostagno et al. (2011), except for the energy of the basal diet (BD), which was reduced by 75 kcal of requirement, considering the matrix of the enzyme and yeast.

The birds arrived from the hatchery with an average weight of 42 g and were housed in experimental boxes with an area of 2m2, installed in a conventional masonry shed, with a ceramic tile roof and a floor covered with rice husk litter. During the experimental period, the average, maximum, and minimum air relative humidity and temperature were recorded for each experimental period (1 to 7 and 8 to 21 days of age). The lighting program used from 1 to 21 days was 24 hours of light (natural and artificial) according to the Cobb lineage manual. Feed and water were provided ad libitum.

Table 1
Percentage composition and calculated values of experimental diets for broilers in the period from 1 to 21 days of age

Ingredients, %	1 - 7 days		8 - 21 days	
	DB[a]	DB[a] + 7% SY[b]	DB	DB[a] + 7% SY[b]
Corn grain, 7,88	56.094	54.679	59.103	57.665
Soybean meal, 45,8	37.870	32.356	34.699	29.218
Sugarcane yeast	0.000	7.000	0.000	7.000
Dicalcium phosphate	1.908	1.876	1.509	1.477
Soy oil	1.163	1.099	1.501	1.444
Supplement Vitamínic c	1.000	1.000	1.000	1.000
Calcitic Limestone	0.904	0.909	0.914	0.919
Inert d	0.020	0.020	0.500	0.500
β-mannanase enzyme	0.000	0.000	0.000	0.000
Salt	0.507	0.475	0.482	0.450
L- Lysine HCL, 98%	0.276	0.274	0.200	0.198
DL- Methionine, 99%	0.163	0.201	0.091	0.128
L-Threonine, 98,5%	0.095	0.112	0.000	0.000
L-Tryptophan, 99,5%	0.000	0.000	0.000	0.000
Total	100.00	100.00	100.00	100.00
Nutritional Requirements				
Metabolizable energy, Mcal/kg	2.88	2.88	2.93	2.93
Crude Protein, %	22.20	22.20	20.80	20.80
Crude fiber, %	2.98	2.70	2.86	2.58
Calcium, %	0.92	0.92	0.82	0.82
Phosphorus available, %	0.47	0.47	0.39	0.39
Digestible lysine, %	1.31	1.31	1.17	1.17
Digestible methionine, %	0.65	0.67	0.56	0.59
Digestible methionine + cystine, %	0.94	0.94	0.85	0.85
Digestible threonine, %	0.85	0.85	0.72	0.70
Digestible arginine, %	1.41	1.31	1.32	1.22
Digestible valine, %	0.94	0.91	0.89	0.85
Digestible tryptophan, %	0.25	0.23	0.23	0.22
Sodium, %	0.22	0.22	0.21	0.21
Chlorine, %	0.36	0.33	0.34	0.32
Potassium, %	0.97	0.93	0.91	0.87
Linoleic acid, %	2.02	1.91	2.23	2.12

[a] Basal diet;
[b] Yeast: dry matter - 96.80%; crude protein - 34.41%; ether extract - 0.01%; mineral matter -7.80%; gross energy - 4189 kcal/kg;
[c] Vitamin supplement per kg of feed: folic acid - 55mg; biotin - 6mg; Virginiamycin - 2.000mg; vit. A - 750.000UI; vit. B1 - 100mg; vit. B12 - 1.400mcg; vit. B2 - 550mg; vit. B6 - 180mg; vit. D3 - 250.000 IU; vit. E - 1.500 IU; vit. K3 - 100mg; niacin - 4.000mg; nicarbazine -12.5g: Calcium pantothenate - 1.000mg; Choline chloride - 32g; Methionine - 190g; Se - 25mg; Fe - 3.500mg; Mn - 7.200mg; Cu - 8000 mg; I - 140mg; Zn - 5.000mg;
[d] Inert: kaolin.

Zootechnical performance (feed intake, average weight, weight gain, and feed conversion) was evaluated during the periods from 1 to 7 and 8 to 21 days of age. Throughout the experimental period, mortality dates were recorded to correct feed intake according to Sakomura and Rostagno (2016).

For intestinal morphometry analysis, one bird per experimental unit was selected for intestine removal at 21 days of age. The birds were euthanized for collection of intestinal segments (duodenum, jejunum, and ileum) of 2 cm in length. These were collected, washed in distilled water, and fixed in a 10% buffered formalin solution for 24 hours (Cunha et al., 2016).

After this period, the segments were dehydrated in alcohols (70, 80, 90, 100 I, 100 II, 100% III), cleared in xylene (I and II), impregnated in histological paraffin, and embedded in paraffin blocks. Tissue sections of 3.5µm thickness were obtained using a semi-automatic rotary microtome and stained with hematoxylin-eosin (D. C. Sousa et al., 2015). All sections were analyzed using a Trinocular optical microscope (Nova Optical Systems) with a TOUPCAM™ digital camera (4 Megapixels) attached. Ten measurements were taken for each variable, and the mean values were used. The variables measured were: villus height and width, crypt depth and width, intestinal muscular wall thickness, and villus: crypt ratio.

The cost-effectiveness analysis considered the prices of ingredients and the price of live chicken (3.09 R$/kg) in the national market in December 2016, updated to December 2022 using a USD: BRL exchange rate of 5.27. Moreover, to assess the economic feasibility of including yeast and β-mannanase enzyme in diets, the average cost of feed per kilogram of live weight (Yi) was calculated using the equation of Bellaver et al. (1985):

$$Yi = (Qi \times Pi) / Gi$$, wherein:

Yi: cost of feed per kilogram of live weight gain in the i^{-th} treatment;

Pi: price per kilogram of feed used in the i^{-th} treatment;

Qi: quantity of feed intake in the i^{-th} treatment;

Gi: weight gain of the i^{-th} treatment.

The economic efficiency index (EEI) and cost index (CI) were calculated using the equations described by Barbosa et al. (1992) as follows:

$$EEI = (LCei / TCei) \times 100$$, wherein:

LCei: the lowest cost of feed per kilogram gain observed among the treatments;

TCei: cost of the i^{-th} treatment considered".

All analyzed variables were checked for outliers. Data were subjected to analysis of variance using the General Linear Model (GLM) procedure of the SAS® software (Statistical Analysis System [SAS], 2002). When treatment effects were found, means were compared by the Student-Newman-Keuls (SNK) test at 5 and 10% probability levels for performance and intestinal morphometry, respectively.

Results and Discussion

The maximum and minimum average temperatures in the first week of age of the birds (1 to 7 days) were 35 and 25°C, and the maximum and minimum average relative humidity values were 64.4 and

37.4%, respectively. In the second and third weeks of age (8 to 21 days), the maximum and minimum average temperatures were 36.3 and 22.6°C, and the maximum and minimum average humidity values were 62.4 and 26.3%, respectively. Cassuce et al. (2013) found that the best parameters for productive performance occurred at temperatures of 30, 27, and 24°C in the first, second, and third weeks of age of the birds, respectively. Menegali et al. (2013) indicated an ideal relative humidity (RH) between 60 to 70%, where values below 50% RH can result in dehydration of the birds. According to our temperature results, birds aged 8 to 21 days experienced moments outside the thermal comfort zone.

There was no effect of treatments ($P>0.05$) on the average weight, weight gain, and feed intake in the 1- to 7-day-old birds. However, there was an effect of treatments ($P<0.05$) on feed conversion (Table 2). The best feed conversions were observed for the basal diet (BD) and for treatments with YD + β-mannanase (80, 100, and 120 g/t), and did not differ from each other according to the SNK mean test at 5% probability.

Table 2
Effect of β-mannanase enzyme supplementation in diets with and without sugarcane yeast for broilers from 1 to 7 days of age

Treatments	AW, g	WG, g	FI, g	FC, g/g
Basal Diet (BD)	133.78	91.39	112.13	1.227B
BD+ β-mannanase (100g/t)	132.44	89.49	115.11	1.287A
Yeast Diet (YD)	131.62	89.63	116.33	1.299A
YD+ β-mannanase (80g/t)	136.73	94.01	116.76	1.242B
YD+ β-mannanase (100g/t)	136.52	93.44	114.78	1.230B
YD+ β-mannanase (120g/t)	136.61	95.29	117.67	1.235B
MSE[a]	4.94	4.05	4.28	0.03
Probability	0.3776	0.1348	0.4023	0.0051
CV[b], %	3.98	4.41	2.88	2.80

[a] Mean standard error; [b] Coefficient of variation;
AW, average weight; WG, weight gain; FI, feed intake; FC, feed conversion;
Means with different letters in the columns differ statistically by the SNK test ($p<0.05$).

Based on this result, 7% of yeast above the percentage recommended in the Brazilian table of food composition and nutritional requirements for birds can be included if supplemented with exogenous enzyme β-mannanase, from 80 to 120 g/t from 1 to 7 days of age. According to Ferreira et al. (2016), β-mannanase improves feed conversion in broiler chickens, due to a series of benefits caused by enzyme supplementation, since it can promote the hydrolysis of a class of mannan, considered one of the main anti-nutritional factors for broiler chickens, reducing the size of the

molecules and allowing for improved nutrient digestibility (Cho & Kim, 2013).

The worsening of feed conversion observed in birds fed the basal diet with yeast (*Saccharomyces cerevisiae*) and without the β-mannanase enzyme can be justified by the low production of endogenous enzymes by the birds in the first days of life (Fortes et al., 2012), which act on the non-starch polysaccharides of the yeast, as they have high rigidity and, consequently, low digestibility of the cell wall (Freitas et al., 2013).

During the 1-21-day phase, treatment effects were observed (P<0.05) for average weight, weight gain, and feed conversion, but not for feed intake (Table 3). Birds fed the basal diet (BD) and BD + β-mannanase (100 g/t) achieved the best performance. These results may be associated with the physical characteristics of the yeast cell wall, which reduced nutrient availability in the YD and YD + β-mannanase (80 g/t) treatments. When supplemented with 100 and 120 g/t of β-mannanase in yeast-containing diets, conversion rates improved due to the presence of the enzyme that breaks down the yeast cell wall and releases nutrients.

Latham et al. (2018) investigated the impact of β-mannanase inclusion on performance, viscosity, and energy utilization in broilers aged 1-42 days fed diets with different galactomannan concentrations. According to the authors, β-mannanase inclusion can provide beneficial impacts through improvements in performance and nutrient utilization. However, the response depends on the combination of β-mannanase with galactomannan concentrations required to achieve maximum broiler performance.

Table 3
Effect of β-mannanase enzyme supplementation in diets with and without sugarcane yeast for broilers from 1 to 21 days of age

Treatments	AW, g	WG, g	FI, g	FC, g/g
Basal Diet (BD)	780.7A	738.3A	1059.4	1.436B
BD+ β-mannanase (100g/t)	769.0AB	726.0AB	1042.2	1.436B
Yeast Diet (YD)	732.4C	690.0C	1054.9	1.529A
YD+ β-mannanase (80g/t)	742.6BC	699.6BC	1068.4	1.527A
YD+ β-mannanase (100g/t)	729.2C	686.1C	1035.5	1.511AB
YD+ β-mannanase (120g/t)	747.5BC	703.8BC	1035.1	1.471AB
MSE[a]	22.45	21.85	29.80	0.05
Probability	0.0020	0.0013	0.3373	0.0038
CV[b], %	3.00	3.10	2.93	3.40

[a] Mean standard error; [b] Coefficient of variation;
AW, average weight; WG, weight gain; FI, feed intake; FC, feed conversion;
Means with different letters in the columns differ statistically by the SNK test (p<0.05).

The 7% yeast treatment without β-mannanase resulted in losses of 6.18%, 6.54%, and 6.08% in average weight, weight gain, and feed conversion of birds compared to the basal diet, respectively. According to Freitas et al. (2013), this effect may be attributed to the physical characteristics of the yeast cell wall, which contribute to low digestibility and nutrient availability for birds, despite no interference with feed intake. In addition, according to Rodríguez-Peña et al. (2013), only two enzymes are essential to break down the yeast cell wall: a specific lytic protease that acts on the outer layer of mannoprotein, and lytic β-1,3 glucanase that acts on the inner layer of glucan.

Treatment effects were observed (P>0.10) for muscular wall thickness in the duodenum, jejunum, and ileum, as well as villus width and crypt depth in the ileal segment (Table 4). Duodenal muscular wall thickness was lower in birds consuming the basal diet compared to BD + β-mannanase $^{(100\ g/t)}$ but did not differ from those fed diets with yeast (YD) or supplemented with β-mannanase (80 and 100 g/t). Therefore, supplementation of β-mannanase $^{(100\ g/t)}$ in the basal diet increased the muscular wall thickness of the duodenum in birds.

R. F. Sousa et al. (2018) evaluated the use of exogenous enzymes in diets with Saccharomyces cerevisiae in broiler chickens aged 1-21 days and found that the intestinal muscular wall of birds that did not receive the enzymatic complex was thinner than that of birds that received the enzymatic mixture in their feed. Just like abnormal thickening, a pronounced reduction in muscular wall thickness can be attributed to a physiological response to certain external agents, such as microorganisms, pathogens, and antinutritional substances.

From a histological point of view, the three segments of the small intestine (duodenum, jejunum, and ileum) present specific distinctive characteristics regarding the structure and morphology of their mucosa and submucosa villi, as well as different thicknesses of their walls, due to the differences in villi height and thickness of the muscular tunic, especially the inner circular muscle. Thus, the wall of the duodenum is thicker than that of the jejunum, and that of the jejunum is thicker than that of the ileum (Nasciutti et al., 2016). Therefore, these differences may promote diverse results regarding the presence of pathogens or anti-nutritional factors in the muscular wall of the segments.

Our findings showed treatment effects (P>0.10) on crypt depth and villus width in the ileal segment. Shallower ileal crypts were observed in the YD+ β-mannanase $^{(120\ g/t)}$ treatment compared to the basal diet. Wider villi were observed in the YD+ β-mananase $^{(100\ g/t)}$ treatment compared to the YD+ β-mananase $^{(80\ g/t)}$ diet. According to Padihari et al. (2014), a shallower crypt may indicate a better capacity of the small intestine as it requires fewer nutrients for regeneration and subsequently allows intestinal cells to produce more digestive enzymes and improve nutrient absorption. Apperson and Cherian (2017) also noted that villus width is closely related to the absorptive capacity of the intestine, such that wider villi denote improvements in nutrient absorption. However, no difference (P<0.10) was observed in the morphometric analysis of the duodenum and jejunum treatment for crypt depth and villus width, indicating that the addition of enzymes or yeast to the diet of broiler chickens did not alter the dimensions of these components of the intestinal mucosa in these segments.

Table 4
Effect of β-mannanase enzyme supplementation in diets with and without sugarcane yeast on the intestinal morphology of the duodenum, jejunum and ileum of broiler chickens at 21 days of age

Treatments	Variables, µm					
	VH	VW	CD	CW	MWT	VCR
			Duodenum			
Basal Diet (BD)	740.18	182.12	136.78	54.64	140.00C	5.41
BD+ β-mannanase (100g/t)	852.76	180.25	139.06	49.45	222.12A	6.13
Yeast Diet (YD)	688.53	170.70	136.80	54.14	172.10BC	5.03
YD+ β-mannanase (80g/t)	797.60	184.98	134.16	49.58	172.00BC	5.95
YD+ β-mannanase (100g/t)	782.25	177.58	131.25	49.82	156.88BC	6.96
YD+ β-mannanase (120g/t)	715.35	167.18	123.18	51.30	180.23B	5.81
MSE[a]	41.44	8.55	6.23	1.71	7.04	6.84
Probability	0.2066	0.7407	0.8803	0.3594	0.0002	0.8215
CV[b], %	13.08	11.19	14.96	7.51	11.68	19.49
			Jejunum			
Basal Diet (BD)	701.34	190.80	119.98	51.62	146.48AB	5.85
BD+ β-mannanase (100g/t)	717.23	154.15	136.28	50.71	152.54AB	5.26
Yeast Diet (YD)	696.76	184.64	132.46	48.02	143.10B	5.26
YD+ β-mannanase (80g/t)	666.23	169.35	136.00	49.81	178.00A	4.90
YD+ β-mannanase (100g/t)	753.36	155.84	133.52	49.56	142.65B	5.64
YD+ β-mannanase (120g/t)	640.90	154.15	122.96	52.56	172.12A	5.21
MSE[a]	44.47	9.40	6.52	2.31	8.59	5.08
Probability	0.8408	0.3516	0.7143	0.9383	0.0638	0.9459
CV[b], %	20.14	18.42	16.10	14.47	11.49	19.88
			Ileus			
Basal Diet (BD)	572.13	170.18AB	162.18A	53.01	303.05A	3.53
BD+ β-mannanase (100g/t)	544.32	167.95AB	156.10AB	53.16	256.20AB	3.49
Yeast Diet (YD)	528.02	175.46AB	136.56AB	51.62	320.46A	3.87
YD+ β-mannanase (80g/t)	450.30	141.40B	138.46AB	51.20	274.96AB	3.25
YD+ β-mannanase (100g/t)	613.18	177.48A	153.96AB	56.10	272.32AB	3.98
YD+ β-mannanase (120g/t)	503.80	153.00AB	130.67B	49.56	250.58B	3.86
MSE[a]	33.51	6.98	6.85	1.30	13.37	4.72
Probability	0.2156	0.0832	0.0820	0.1599	0.0945	0.4555
CV[b], %	19.28	13.38	13.94	7.45	15.58	16.42

[a] Mean standard error; b Coefficient of variation;
VH, villus height; VW, villous width; CD, crypt depth; CW, crypt width; MWT, muscle wall thickness; VCR, villus crypt ratio (VH/CD);
Means with different letters in the columns differ statistically by the SNK test (p<0.10).

According to D. C. Sousa et al. (2015), the difference between one intestinal portion and another is that the villi become thinner and shorter towards the ileum. In this segment, there are many goblet cells (which secrete mucus and other products, lubricating and protecting the gastrointestinal epithelium), the depth of the crypts decreases and the villi become wider as they approach the end of the digestive tract. Harvatovic et al. (2015), after evaluating the inclusion of exogenous enzymes in diets with sunflower meal, which has a large number of soluble PNAs, observed that digesta viscosity increased from the proximal to the distal portion of the intestine and that the activity of the enzyme complex was more effective in reducing viscosity in the ileum.

The modifications in villi width and crypt depth observed in this experiment may have occurred due to the functional needs of the intestinal mucosa regarding the presence of enzymes in the diet. Enzymes can promote improvements in intestinal health (Ayoola et al., 2015) by stimulating the mucosa to carry out digestive activities in the presence of food in the intestinal tract. Therefore, the treatment with YD+ β-mananase (120 g/t) presented better morphological results in the ileal segment compared to the basal diet, as it provided shallower crypts and a thinner intestinal muscular wall, as well as wider villi.

According to Ravidran (2013), the use of exogenous enzymes in poultry diets is becoming a norm to overcome the adverse effects of antinutritional factors and improve the digestion of diet components and bird performance. However, the responses to enzyme supplementation are variable, and the reasons that contribute to the variability are numerous (nutrients in the diet, animal health status, environmental temperature, electrolyte balance, management, physical form, and thermal processing of feed). Although there are opportunities to improve nutrient utilization and absorption with enzyme supplementation, there will be physiological limits to animal responses imposed by pH and retention time of digesta in the digestive tract. In this sense, Dourado et al. (2014) reported that studies where enzyme use had a positive effect on nutrient digestibility are commonly found, but it did not improve performance, or vice versa.

There was an effect of the treatments ($P<0.10$) on the economic viability variables in the period from 1 to 21 days of age (Table 5). The addition of 7% sugarcane yeast with and without β-mannanase enzyme in the diets increased the average feed cost (CRM) and cost index (IC), with a decrease in the economic efficiency index (EEI) compared to the basal diet with and without β-mannanase enzyme during the period from 1 to 21 days of age.

According to V. R. S. M. Barros et al. (2015), who evaluated the use of β-mannanase and mannan oligosaccharides (MOS) as substitutes for growth promoters in broiler diets and observed that the treatment containing the basal diet without growth promoter + MOS was superior to the other groups in terms of gross revenue, average yield, and profitability index of broilers at 42 days of age.

The use of enzymes can be quite attractive as they are more effective. They may increase nutrient use efficiency by reducing viscosity and endogenous amino

acid loss. However, using sugarcane yeast (*Saccharomyces cerevisiae*), with and without β-mannanase enzyme, was not economically feasible in feeding broilers up to 21 days of age. In this sense, the relative prices of birds and feed must be analyzed, as well as the fluctuation of dollar exchange rates, to define an appropriate replacement, promoting greater profitability in production.

Table 5
Economic viability in diets with sugarcane yeast and β-mannanase enzyme in periods from 1 to 21 days

Treatments	Variables		
	ACF/kg, $	EEI, %	CI, %
Basal Diet (BD)	7.54B	91.25A	109.86B
BD+ β-mannanase (100g/t)	7.54B	93.73A	107.44B
Yeast Diet (YD)	8.36A	84.52B	118.39A
YD+ β-mannanase (80g/t)	8.49A	83.21B	120.20A
YD+ β-mannanase (100g/t)	8.49A	83.07B	117.58A
YD+ β-mannanase (120g/t)	8.29A	85.36B	117.39A
MSE[a]	0.05	2.64	3.36
Probability	<.0001	<.0001	0.0003
CV[b], %	4.28	4.00	3.78

[a] Mean standard error; b Coefficient of variation;
American Dollar in the period at December 2022 = 5.27 R$;
ACF/Kg, Average cost of feed per kg of live weight produced; EEI, Economic Efficiency Index; CI, cost index;
Means with different letters in the columns differ statistically by the SNK test (p<0.10).

Conclusions

Based on the zootechnical results, the use of YD + β-mananase (120 g/t) is recommended for the pre-starter and starter phases. However, sugar cane yeast, with or without β-mananase enzyme supplementation, is not economically viable in the period from 1 to 21 days.

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