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# Enzyme complex and *Saccharomyces cerevisiae* in diets for broilers in the initial phase

*Complexo enzimático e "Saccharomyces cerevisiae" em dietas para frangos de corte na fase inicial*

SOUSA, Regina Fialho de[1*]; DOURADO, Leilane Rocha Barros[1]; SANTOS, Edna Teles dos[1]; BIAGIOTTI, Daniel[1]; FARIAS, Leonardo Atta[1]; ALBUQUERQUE, Francisca das Chagas Fontenele de[1]; CARVALHO, Maria Letícia Araújo Marques de[1]; FERREIRA, Guilherme José Bolzani de Campos[1]; LOPES, João Batista[2]

[1]Universidade Federal do Piauí, Campus Professora Cinobelina Elvas, Departamento de Zootecnia, Bom Jesus, Piauí, Brasil
[2]Universidade Federal do Piauí, Campus Universitário Ministro Petrônio Portella, Centro de Ciência Animal, Teresina, Piauí, Brasil
*Endereço para correspondente: regina-so-fia@hotmail.com

## SUMMARY

This study aimed at evaluating the use of exogenous enzymes in diets with *Saccharomyces cerevisiae* and their impact on zootechnical performance, carcass yield, intestinal histomorphometry and of broiler diets in the initial phase. A completely randomized design was used in a 2x3 + 1 factorial arrangement, with two levels of enzyme complex (EC), (0 and 200g / ton), three yeast levels (0, 6 and 12%) and a control diet, making up seven treatments, with five replicates of 20 broilers per experimental unit. We evaluated the performance (feed intake, weight gain and feed conversion ratio), carcass yield and cuts, histomorphometry of the small intestine (height, circumference and width of villi, height and width of the crypt, thickness of the intestinal muscle wall and villi/crypt relationship). From 1 to 7 and 1 to 21 days, the inclusion of yeast led to reduced broiler performance. At 21 days, the addition of EC resulted in an increase of ($p < 0.05$) in the thickness of the muscular wall of the duodenum and decreased the width of the crypt in the ileum. The 12% level of yeast without the EC provided a thicker jejunum intestinal muscle wall when compared to the positive control. There was no significant effect on carcass yield and cuts between treatments. In conclusion, the inclusion of yeast reduces performance from 1-21 days. The enzyme complex and yeast does not change the performance or carcass yield, however, it does bring benefits to the intestinal mucosa.

**Keywords**: glucanase, mannanase, yeast, villus

## RESUMO

Objetivou-se avaliar o uso de enzimas exógenas em dietas com *Saccharomyces cerevisiae* sobre o desempenho zootécnico, rendimento de carcaça e histomorfometria intestinal de frangos de corte na fase inicial. Utilizou-se o delineamento inteiramente casualizado em esquema fatorial 2x3+1, sendo dois níveis de complexo enzimático (CE), (0 e 200g/ton), três níveis de levedura (0, 6 e 12%) e uma dieta controle, perfazendo sete tratamentos, cinco repetições de 20 aves por unidade experimental. Foram avaliados o desempenho (consumo de ração, ganho de peso e conversão alimentar), rendimento de carcaça e cortes e a histomorfometria do intestino delgado (altura, perímetro e largura de vilo, altura e largura de cripta, espessura da parede muscular intestinal e relação vilo/cripta). Na fase de 1 a 7 e de 1 a 21 dias, a inclusão de levedura na dieta promoveu redução no desempenho dos frangos. Aos 21 dias a adição de CE resultou em aumento ($p<0,05$) da espessura da parede muscular do duodeno, e reduziu a largura da cripta no íleo. O nível de 12% de levedura sem o CE proporcionou parede muscular intestinal do jejuno mais espessa quando comparada ao controle positivo. Não houve efeito significativo para rendimento de carcaça e cortes entre os tratamentos. Conclui-se que, a inclusão de levedura reduz o desempenho de 1 a 21 dias. A adição de complexo enzimático e levedura em dietas para frangos de corte não melhora o desempenho e rendimento de carcaça, todavia, beneficia à mucosa intestinal.

**Palavras-chave:** glucanase, mananase, levedura, vilo

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## INTRODUCTION

Sugarcane yeast and protein feed have been studied by researchers in the search for a substitute to soybean meal in poultry feed (FREITAS et al., 2013), as this is a product that is widely available on the market through its link to the processes of ethanol production (LOPES et al., 2011).

The interest in this ingredient concerning animal feed is founded principally on its high rate of protein, at around 37.20% of crude protein, however, only 21.58% of this is digestible (ROSTAGNO et al., 2011). This low digestibility of whole yeast in broilers is related to physiological limitations in broilers, as they do not have the necessary enzymatic apparatus capable of breaking down the cellular wall composed of non-starch polysaccharides (NSPs) such as glucans and mannans, and as such they are not capable of benefitting fully from the potential nutrition in yeast (FLEURI & SATO, 2007).

In animal nutrition, the use of exogenous enzymes have shown satisfactory results, mainly through their allowing for the use of alternative feeds with greater efficiency (BARBOSA et al., 2014). The collaborators Fleuri & Sato (2007) observed by means of studies *in vitro* that the use of the enzyme β-glucanase, associated or not with other enzymes, are capable of breaking down the cellular wall of yeast.

The use of exogenous enzymes has already been widely researched in terms of improving digestibility of feeds with a high rate of non-starch polysaccharides. The benefits of supplementing with polysaccharides in diets with high rates of NSPs reported in the literature refer to the capacity of partially hydrolyzing these compounds, along with reducing the viscosity of intestinal contents (WANG et al., 2005). In addition, this can improve the use of other nutrients present in the diet, such as protein through the disruption of the cell wall by means of rupture of the cell, thus resulting in nutrient absorption improvements, as well as performance (ESMAEILIPOUR et al., 2011).

Even with all the benefits that these technological advances bring, they are used more in cereals, such as rye, barley, wheat, oats and rice (TACHIBANA et al., 2010). However, reports in the literature are scarce concerning the combined use of yeast and exogenous enzymes in poultry diets.

The objective behind this study was to evaluate the use of exogenous enzymes in diets with *Saccharomyces cerevisiae,* on the zootechnical performance, carcass yield and intestinal histomorphometry of broilers in the initial phase.

## MATERIAL AND METHODS

The study was performed at the aviculture sector of the Bom Jesus- PI Technical college (Colégio Técnico de Bom Jesus, PI), in a warehouse without air-conditioning and with an average temperature of (31.24°C) and relative humidity of (48.23%). The morphometric evaluations were performed at the Animal Anatomy laboratory at the Campus "Profª. Cinobelina Elvas" of the Federal University of Piauí. The project was approved under the report number 087/2012 by the ethics committee for animal experiments - CEEA/UFPI.

The experiment was performed at the 1 to 21-day old phase, distributed using a completely randomized design in factorial scheme 2x3 + 1, consisting of two levels of enzymatic complex (0 and 200g/ton), three levels of yeast biomass inclusion (0, 6 and 12%) and a control diet. This resulted in 7 treatment procedures with five replicates and 20 broilers per experimental unit.

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The treatment procedures used were: TP1-feed reference based on corn and soya (PC); TP2-feed reference based on corn and soya with a 70 kcal reduction of metabolizable energy from the diet (NC) with 0% yeast without enzymatic complex; TP3-NC + 6% yeast without enzymatic complex; TP4-NC +12% yeast without enzymatic complex; TP5-NC +0% of yeast with enzymatic complex; TP6-NC +6% of yeast with enzymatic complex; TP7-NC+ 12% of yeast with enzymatic complex.

The diets (Tables 1 and 2) were formulated by means of nutritional requirement adjustments recommended by Rostagno et al. (2011) and the lineage manual Ross 308®.

Table 1. Composition of experimental diets for broilers in the 1 to 7-day old phase

| Ingredient | PC | NC (0%) | NC + YEAST (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 6% | 12% | 0%+CE | 6%+EC | 12%+EC |
| Corn | 52.388 | 54.003 | 51.981 | 49.965 | 54.003 | 51.981 | 49.965 |
| Soybean meal | 38.460 | 38.162 | 33.963 | 29.763 | 38.162 | 33.963 | 29.763 |
| Soybean oil | 3.354 | 2.013 | 2.167 | 2.319 | 2.013 | 2.167 | 2.319 |
| Phosphate bic. | 1.969 | 1.967 | 1.938 | 1.908 | 1.967 | 1.938 | 1.908 |
| Lime | 1.171 | 1.173 | 1.175 | 1.176 | 1.173 | 1.175 | 1.176 |
| NaCl | 0.458 | 0.457 | 0.430 | 0.403 | 0.457 | 0.430 | 0.403 |
| L-lysine HCl 79% | 0.216 | 0.222 | 0.204 | 0.187 | 0.222 | 0.204 | 0.187 |
| DL-methionine | 0.352 | 0.351 | 0.379 | 0.407 | 0.351 | 0.379 | 0.407 |
| L-threonine | 0.079 | 0.080 | 0.089 | 0.098 | 0.080 | 0.089 | 0.098 |
| L-valine | 0.151 | 0.152 | 0.167 | 0.182 | 0.152 | 0.167 | 0.182 |
| L-arginine | 0.000 | 0.000 | 0.077 | 0.147 | 0.000 | 0.077 | 0.147 |
| L-tryptophan | 0.000 | 0.000 | 0.011 | 0.021 | 0.000 | 0.011 | 0.021 |
| Suppl. Vita. Min[1] | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 |
| Inert[2] | 1.000 | 1.020 | 1.020 | 1.020 | 1000 | 1.000 | 1.000 |
| Yeast | - | - | 6.000 | 12.000 | - | 6.000 | 12.000 |
| CE[3] | - | - | - | - | 0.020 | 0.020 | 0.020 |
| Total | 100.00 | 100.00 | 100.00 | 10,.00 | 100.00 | 100.00 | 100.00 |
| Calculated composition | | | | | | | |
| PB (%) | 22.500 | 22.500 | 22.500 | 22.500 | 22.500 | 22.500 | 22.500 |
| EM (kcal/kg) | 3000 | 2930 | 2930 | 2930 | 2930 | 2930 | 2930 |
| Ca (%) | 1.050 | 1.050 | 1.050 | 1.050 | 1.050 | 1.050 | 1.050 |
| P disp. (%) | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 |
| Lysine dig. (%) | 1.270 | 1.270 | 1.270 | 1.270 | 1.270 | 1.270 | 1270 |
| Meth. dig. (%) | 0.637 | 0.637 | 0.660 | 0.682 | 0.637 | 0.660 | 0.682 |
| Meth+cist. dig. (%) | 0.940 | 0.940 | 0.940 | 0.940 | 0.940 | 0.940 | 0.940 |
| Threonine dig.(%) | 0.830 | 0.830 | 0.830 | 0.830 | 0.830 | 0.830 | 0.830 |
| Tryptophan dig. (%) | 0.249 | 0.249 | 0.249 | 0.249 | 0.249 | 0.249 | 0.249 |
| Arginine dig. (%) | 1.418 | 1.414 | 1.418 | 1.418 | 1.414 | 1.418 | 1.418 |
| Valine dig. (%) | 1.090 | 1.090 | 1.090 | 1.090 | 1.090 | 1.090 | 1.090 |
| Phenylalanine dig. (%) | 1.034 | 1.032 | 0.983 | 0.934 | 1.032 | 0.983 | 0.934 |
| Isoleucine dig. (%) | 0.876 | 0.874 | 0.850 | 0.826 | 0.874 | 0.850 | 0.826 |
| Sodium (%) | 0.200 | 0.200 | 0.200 | 0.200 | 0.200 | 0.200 | 0.200 |
| Crude Fiber (%) | 2.945 | 2.957 | 2.729 | 2.502 | 2.957 | 2.729 | 2.502 |

[1]Guarantee level per kg of feed: (Initial): folic acid – 200.00 mg; biotin-10,000mg; chloro-hydroxyquinoline -7500.00mg: zn – 17.50g ; vitamin A – 1680000.00 UI; vitamin B1 – 436.50 mg; vitamin B12 2400.00 mg; vitamin B2 – 1200.00 mg; vitamin B6 – 624.00 mg; vitamin D3 – 400000.00 UI; vitamin E 350000 mg ; vit. K 3 – 360.00 mg; niacin – 8400.00 mg; monensin sodium -25000.00mg; pantothenic acid – 3119.00 mg; choline chloride – 80.710 mg; selenium -75.00 mg; iron sulphate 11.250 mg; manganese monoxide – 18740.00 mg; copper sulphate -1996.00 mg; iodine – 187.47mg.[2] inert washed sand; [3]EC-enzymatic complex *(α- galactosidase, galactomannan, xylanase and β-glucanase).*

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Table 2. Composition of the experimental diets for broilers in the 8 to 21-day old phase

| Ingredient | PC | NC (0%) | NC + YEAST (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 6% | 12% | 0%+EC | 6%+EC | 12% +EC |
| Corn | 54.768 | 56.375 | 54.360 | 52.345 | 56.375 | 54.360 | 52.345 |
| Soybean meal | 35.467 | 35.171 | 30.970 | 26.770 | 35.171 | 30.970 | 26.770 |
| Soybean oil | 4.813 | 3.474 | 3.626 | 3.778 | 3.474 | 3.626 | 3.778 |
| Phosphate bic. | 1.739 | 1.737 | 1.708 | 1.679 | 1.737 | 1.708 | 1.679 |
| Lime | 0.947 | 0.949 | 0.951 | 0.952 | 0.949 | 0.951 | 0.952 |
| NaCl | 0.484 | 0.483 | 0.456 | 0.429 | 0.483 | 0.456 | 0.429 |
| L-lysine HCl | 0.091 | 0.097 | 0.080 | 0.062 | 0.097 | 0.080 | 0.062 |
| DL-methionine | 0.278 | 0.276 | 0.305 | 0.333 | 0.276 | 0.305 | 0.333 |
| L-threonine | 0.015 | 0.015 | 0.024 | 0.033 | 0.015 | 0.024 | 0.033 |
| L- valine | 0.000 | 0.000 | 0.015 | 0.031 | 0.000 | 0.015 | 0.031 |
| L- arginine | 0.000 | 0.003 | 0.075 | 0.147 | 0.003 | 0.075 | 0.147 |
| L-tryptophan | 0.000 | 0.000 | 0.011 | 0.021 | 0.000 | 0.011 | 0.021 |
| Suppl. Vit. Min[1] | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 |
| Inert[2] | 1.000 | 1.020 | 1.020 | 1.020 | 1.000 | 1.000 | 1.000 |
| Yeast | 0.000 | 0.000 | 6.000 | 12.000 | 0.000 | 6.000 | 12.000 |
| CE[3] | 0.000 | 0.000 | 0.000 | 0.000 | 0.020 | 0.020 | 0.020 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100,.00 |
| Calculated composition | | | | | | | |
| PB (%) | 21.00 | 21.00 | 21.00 | 21.00 | 21.00 | 21.00 | 21.00 |
| EM (kcal/kg) | 3120 | 3050 | 3050 | 3050 | 3050 | 3050 | 3050 |
| Ca (%) | 0.900 | 0.900 | 0.900 | 0.900 | 0.900 | 0.900 | 0.900 |
| P disp. (%) | 0.450 | 0.450 | 0.450 | 0.450 | 0.450 | 0.450 | 0.450 |
| Lysine dig. (%) | 1.100 | 1.100 | 1.100 | 1.100 | 1.100 | 1.100 | 1.100 |
| Methionine dig. (%) | 0.552 | 0.551 | 0.574 | 0.597 | 0.551 | 0.574 | 0.597 |
| Meth+cist. dig. (%) | 0.840 | 0.840 | 0.840 | 0.840 | 0.840 | 0.840 | 0.840 |
| Threonine dig.(%) | 0.730 | 0.730 | 0.730 | 0.730 | 0.730 | 0.730 | 0.730 |
| Tryptophan dig. (%) | 0.234 | 0.233 | 0.233 | 0.233 | 0.233 | 0.233 | 0.233 |
| Arginine dig. (%) | 1.331 | 1.330 | 1.330 | 1.330 | 1.330 | 1.330 | 1.330 |
| Valine dig. (%) | 0.893 | 0.892 | 0.892 | 0.892 | 0.892 | 0.892 | 0.892 |
| Phenylalanine dig. (%) | 0.976 | 0.975 | 0.926 | 0.876 | 0.975 | 0.926 | 0.876 |
| Isoleucine dig. (%) | 0.824 | 0.822 | 0.798 | 0.774 | 0.822 | 0.798 | 0.774 |
| Sodium (%) | 0.210 | 0.210 | 0.210 | 0.210 | 0.210 | 0.210 | 0.210 |
| Crude Fiber (%) | 2.827 | 2.839 | 2.612 | 2.384 | 2.839 | 2.612 | 2.384 |

[1]Guarantee level per kg of feed: folic acid – 200.00 mg; biotin – 10,000mg; chloro-hydroxyquinoline – 7500.00 mg; vitamin A – 1680000.00 UI; vitamin B1 – 436.50 mg; vitamin B12 – 2400.00 mcg; vitamin B2 – 1200.00 mg; vitamin B6 – 624,00 mg; vitamin D3 – 400000.00 UI; vitamin E – 3500.00 mg ; vitamin K 3 – 360.00 mg; niacin – 8400.00 mg; monensin sodium – 25000.00 mg; pantothenic acid – 3119.00 mg; choline chloride – 80.710 mg; selenium -75.00 mg; iron sulphate 11.250 mg; manganese monoxide – 18740.00 mg; copper sulphate – 1996.00 mg; iodine – 187.47mg; zinc – 17500.00 mg;[2]inert washed sand; [3]EC-enzymatic complex *(α- galactosidase, galactomannan, xylanase and β-glucanase)*.

The enzymatic complex was composed of *α-galactosidase, galactomannan, xylanase and β-glucanase,* which was added to the feed at a rate of 200g/ton.

On the first day of the experiment, the animals (male chicks from the Ross lineage) were weighed and distributed uniformly in pens, with the floor covered with rice husk, equipped with a pendulum water dispenser and tubular feeder with water and feed ad libitum, under a regime of 24 hours of light (natural + artificial), with management undertaken as set out in the manual for this lineage.

On the 7th and 21st day, the animal performance variables were evaluated (feed intake, weight gain and feed conversion ratio). At 21 days of age

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euthanasia was performed on one animal per group for the collecting of the small intestine for future histomorphometric evaluation. From these, segments were collected at 2.0 cm in length from the small intestine (duodenum, jejunum and ileum, ten centimeters from the Meckel's diverticulum). After the collection, the fragments were opened longitudinally, washed in distilled water, extended by the serous tunic and fixed in Bouin solution for 24 hours, then these were washed under running water for 12 hours and maintained in alcohol 50° Gl (BEHMER, 2003).

Posteriorly, the samples were submitted to standard histological processing with their inclusion in Histopar® (Easypath - Erviegas Ltda.) and posterior section at a thickness 4µm in a semi-automatic rotary microtome (Leica® – RM2245), the cuts were stained with hematoxylin and eosin (HU et al. 2012; SOUSA et al., 2015), the assemblage was carried out using colorless stained-glass varnish 500 (Acrilex®) (PAIVA et al., 2006).

The morphometric analyses of the histological cuts were performed using the Trinocular optical microscope (Nova Optical Systems), coupled with a digital camera TOUPCAM™ (5 Megapixels). In order to perform the measurements, the software ToupView® 3.7 was used. The variables measured were: perimeter, height, and breadth of the villus, depth and width of the crypt and thickness of muscle layer of the intestinal wall. In order to obtain these measurements, the best cut was selected from each slide, where 10 villi, 10 crypts and 10 walls were measured.

The measurements were taken in the following manner: villi (V), from the base until its apex; crypts (C), largest perpendicular diameters closest to the measured villus; thickness of intestinal wall muscle (IM) from the blade itself to the serosa (Figure 1).

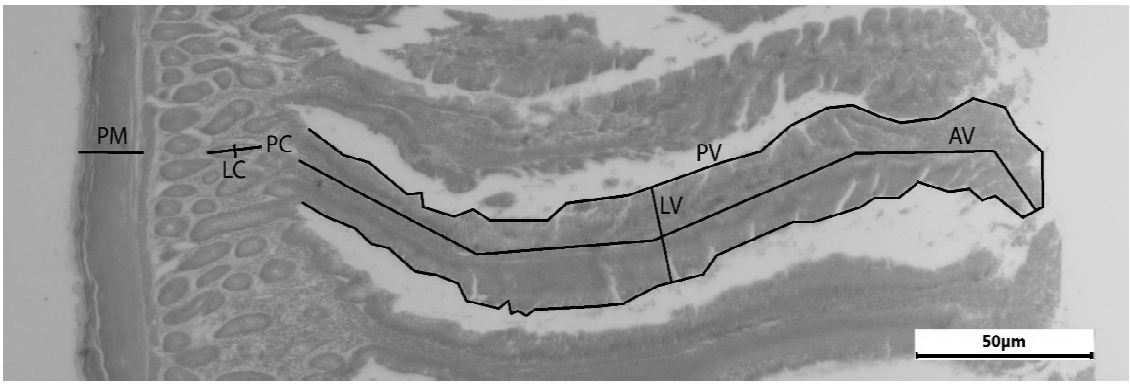


Figure 1. Photomicrography showing how the variables were measured, with: IM – Thickness of the Intestinal Wall Muscle Layer; CW – Crypt Width; CD – Crypt Depth; VW – Villus Width; VH – Villus Height; VP – Villus Perimeter. Staining HE.

In order to evaluate the carcass yield, two boilers were used in accordance with the average weight of the experimental unit, these were identified and fasted for 8 hours. After this period, they were weighed to obtain the fasting weight, then they were slaughtered, bleed, plucked and gutted. After the removal of the feet, neck and head, the cleaned carcass was weighed, and then the cuts were weighed separately. The carcass yield was determined through

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the relationship between the weight of the eviscerated carcass without feet, head or neck and the live weight of the broilers in fasting at the time of slaughter. The main cuts, breasts, thighs and drumsticks, and wings were weighed and their yields calculated in relation to weight of the eviscerated carcass.

The performance data, carcass yield and histomorphometry were submitted to variation analysis through the GLM procedure of the SAS (Statistical Analysis System, 9.0). The Dunnett test (α=0.05) was used to check for significant differences between the positive control treatment and the yeast factors and enzymatic complex. Estimations for the yeast level were established by means of linear and polynomial regression models. The means were compared through the SNK test with α=0.05.

## RESULTS AND DISCUSSION

No interaction was observed between the enzymatic complex (EC) and the yeast levels (YL), for the variables Feed Intake (FI), weight gain (WG) and feed conversion ratio (FCR) during the 1 to 7-day old phase of the broilers (Table 3).

Table 3. Effect of the yeast levels from sugarcane (*Saccharomyces cerevisiae*) with without the addition of the enzymatic complex concerning the feed intake (F weight gain (WG) and feed conversion ratio (FCR) of broilers during the 1 to7 a 1 to 21-day old phases

| Variables | PC | NC | Yeast Levels (%) | | | Mean | CV | P>F | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 6 | 12 | | (%) | EC | YL | EC*YL |
| 1 to 7 days | | | | | | | | | | |
| FI (g/bird) | 133* | Without | 131 | 143 | 142 | 137 | 6.26 | 0.117 | 0.009 | 0.807 |
| | | With | 137 | 145 | 150* | 144 | - | - | - | - |
| | | Mean[1] | 134 | 144 | 146 | - | - | - | - | - |
| WG (g/bird) | 114 | Without | 107 | 110 | 108 | 105 | 7.53 | 0.381 | 0.777 | 0.242 |
| | | With | 115 | 106 | 113 | 111 | - | - | - | - |
| | | Mean | 111 | 108 | 110 | - | - | - | - | - |
| FCR (g/g) | 1.17* | Without | 1.24 | 1.30 | 1.32 | 1.31 | 7.60 | 0.736 | 0.017 | 0.443 |
| | | With | 1.19 | 1.37* | 1.33* | 1.30 | - | - | - | - |
| | | Mean[2] | 1.21 | 1.34 | 1.33 | - | - | - | - | - |
| 1 a 21 dias | | | | | | | | | | |
| FI (g/bird) | 897 | Without | 867 | 904 | 915 | 893 | 3.14 | 0.960 | 0.003 | 0.981 |
| | | With | 870 | 902 | 916 | 896 | - | - | - | - |
| | | Mean[3] | 869 | 903 | 915 | - | - | - | - | - |
| WG (g/bird) | 659* | Without | 650 | 613* | 613* | 623 | 3.09 | 0.543 | 0012 | 0,.175 |
| | | With | 628 | 624 | 612* | 621 | - | - | - | - |
| | | Mean[4] | 639 | 618 | 612 | - | - | - | - | - |
| FCR (g/g) | 1.36* | Without | 1.34 | 1.48* | 1.49* | 1.44 | 4.05 | 0.717 | <0.0001 | 0.319 |
| | | With | 1.38 | 1.45 | 1.50* | 1.44 | - | - | - | - |
| | | Mean[5] | 1.36 | 1.46 | 1.50 | - | - | - | - | - |

*Positive control mean differs in the Dunnett test (p<0,05).
PC= positive control; NC= negative control; CV= coefficient of variation; EC= enzymatic complex; YL= yeast levels.

Feed intake and feed conversion ratio ($p<0.05$) were influenced by the level of yeast inclusion to the diet. In this case, when there were effects from yeast levels, division was performed by means of polynomial regression (Table 4). There was an increase ($p<0.05$) in feed intake (FI=135.13 + 1.036YL, $F^2$=0.87) and in feed conversion ratio (FCR=1.235+0.009YL, $F^2$=0.69) at the rate the levels of yeast increased in the diet. The increased intake of higher levels of yeast, can be attributed to the higher demand of nutrients and energy, while considering the rigidity and low digestibility of its cell wall (FREITAS et al., 2013), as the broilers did not obtain an increase in weight gain.

According to Perdomo et al. (2004), the low utilization of the yeast cell wall by the animals reduces digestibility of the nutrients from the feed, and consequently its energetic value. If one considers that energy is the main factor that controls feed intake in broilers (FREITAS et al., 2013), and that there was a reduction in the energetic density of the diets (NC), this influence on feed intake was boosted, as the animals need to find more feed to attend to their energy needs, due to the low energy extraction from the feed.

The comparison between the feed intake and feed conversion ratio data from the positive control, regarding the negative control at 12% yeast, reinforces this statement. Noteworthy here is that broilers fed with a negative control and 12% yeast with enzymatic complex present a higher feed intake ($p<0.05$). The negative control diet with 6% and 12% yeast with enzymes demonstrated higher feed conversion ratios at seven days, when compared to broilers fed with positive control. The addition of the enzymatic complex had no influence over the performance variables in the 1 to 7-day phase.

Over the total phase (1 to 21 days), there was no interaction observed between yeast levels, and the enzymatic complex in the diets for feed intake, weight gain and feed conversion ratio (Table 3). The yeast levels of (0, 6 and 12%) increased ($p<0.05$) the feed intake and feed conversion ratio, in addition to reduced weight gain. There was an increasing linear effect ($p<0.05$) for feed intake (FI=872.11+3.936NL, $F^2$=0.92) and feed conversion ratio (FCR=1.371+0.113NL, $F^2$=0.94) in broilers with yeast included in their diets, and a decreasing linear effect for weight gain (WG=636.88-2.24NL, $F^2$=0.90) in the 1 to 21-day old phase (Table 4).

Table 4. Regression equations for the weight gain, feed intake and feed conversion ratio variables of broilers, submitted to levels of sugarcane yeast inclusion into the feed at the age of 01 to 21 days

| Phase | Variable | Equation | Value of P | $R^2$ |
|---|---|---|---|---|
| 1 to 7 days | FI (g/bird) | FI =135.13 + 1.036NL | 0.004 | 0.87 |
| 1 to 7 days | FCR (g/g) | FCR=1.236+0.009NL | 0.016 | 0.69 |
| 1 to 21 days | FI (g/bird) | FI = 872.11+3.93NL | 0.001 | 0.92 |
| 1 to 21 days | WG (g/bird) | WG = 636.88-2.24NL | 0.005 | 0.90 |
| 1 to 21 days | FCR (g/g) | FCR = 1.371+0.0113NL | <.0001 | 0.94 |

YL= yeast levels.

The behavior observed in the feed conversion ratio of broilers fed with different levels of yeast can be attributed to a lower nutrient utilization from the feed, as the increase in feed intake was not followed by extra weight gain. A similar result was found by Silva et al. (2003) that stated that the inclusion of yeast in the feed, until 10%, produced losses in performance of broilers in the period of 1 to 21 days of age.

The addition of the enzymatic complex had no influence over the feed intake, weight gain and feed conversion ratio. According to Rodríguez-Peña et al. (2013), only two enzymes are essential for breaking down the yeast cell: specific lytic protease, which breaks down the external layer of the mannoprotein, and β-1,3 lytic glucanase, which breaks down the internal layer of glucan. This justifies in part the principle that the action of proteases produces an increase in porosity of the cell wall, thus allowing access for lytic activity, which is glucanase acting synergistically upon the lysis of the cell wall (FLEURI & SATO, 2010).

At 21 days old, the animals that received feed with 6% and 12% yeast without enzyme and 12% yeast with enzyme showed less weight gain and an increase in feed conversion ratio ($p<0.05$), when compared to animals that received the positive control diet.

Weight gain and feed conversion ratio of broilers at 1 to 21 days of age that were fed a diet with 6% yeast and enzymatic complex resembled those with positive control, which indicated that the complex may have acted on the yeast, thus minimizing performance losses. However, the same variables of broilers fed the diet with 6% yeast without enzyme, with 12% yeast with and without enzyme were different to the positive control, this may indicate that the dose of 200g/ton of the enzymatic complex was not sufficient, due to the increase of substrate in the diets of 12% yeast.

Note that the performance of those broilers that consumed positive control diets was similar to negative control with and without enzymatic complex, and that the addition of enzyme did not result in any difference concerning negative control. In research studies by Gonal et al. (2004) and Mourão & Pinheiro (2009) the evaluation of the use of exogenous enzymes did not demonstrate improved performance in broilers and justified the absence of a response being due to the low dose of the additive to the feed. For these authors, the addition of higher doses of enzymes can bring potential performance gains.

The relative values of carcass yield and cuts at 21 days of age, are presented on Table 5. No interaction was noted between the yeast levels and the supplement with enzymatic complex for any of the carcass yield and cut variables, which indicates that yeast can be used until a 12% inclusion rate for broilers without the addition of enzymes, as this does not cause losses in the yield of the carcass, breast, legs, thigh and wings of the broilers.

The supplementing with enzymatic complex did not show any significant effect on carcass or cuts yield (Table 5).

No significant effect was seen on the yield or cuts of animals supplied with up to 12% of yeast in the diet in the 21-day phase. Similar results were seen by Grangeiro et al. (2001) and Silva et al. (2003) who did not observe any significant effect on carcass yield when supplying increasing levels of yeast from sugarcane at a level of up to 10%.

On Table 6, the measurements obtained for the morphometric variables of the duodenum of broilers at 21 days of age are shown. No interaction was found between the two yeast levels and the enzymatic complex for the histomorphometric variables of the duodenum at 21 days of age.

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Table 5. Relative values for the carcass yield and cuts (%) of broilers fed on diets containing different levels of yeast and the addition of enzymatic complex at 21 days of age

| Variable (%) | PC | NC | Yeast Levels (%) | | | Mean | CV | P>F | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 6 | 12 | | (%) | EC | YL | EC*YL |
| CARCY | 67 | Without | 67.00 | 66.00 | 66.00 | 66.00 | 1.62 | 0.172 | 0.462 | 0.449 |
| | | With | 66.00 | 67.00 | 66.00 | 66.00 | - | - | - | - |
| | | Mean | 66.00 | 67.00 | 66.00 | - | - | - | - | - |
| BY | 32 | Without | 32.00 | 32.00 | 32.00 | 32.00 | 3.90 | 0.658 | 0.166 | 0.084 |
| | | With | 31.00 | 32.00 | 34.00 | 32.00 | - | - | - | - |
| | | Mean | 32.00 | 32.00 | 33.00 | - | - | - | - | - |
| TY | 14.71 | Without | 14.50 | 14.83 | 14.58 | 14.64 | 4.51 | 0.891 | 0.439 | 0.712 |
| | | With | 14.77 | 14.88 | 14.36 | 14.67 | - | - | - | - |
| | | Mean | 14.63 | 14.86 | 14.47 | - | - | - | - | - |
| ULY | 16.88 | Without | 15.98 | 16.22 | 15.70 | 15.97 | 5.89 | 0.599 | 0.623 | 0.801 |
| | | With | 16.05 | 15.73 | 15.58 | 15.79 | - | - | - | - |
| | | Mean | 16.02 | 15.98 | 15.64 | - | - | - | - | - |
| WINGY | 12.02 | Without | 12.73 | 12.21 | 12.58 | 12.50 | 4.83 | 0.601 | 0.053 | 0.732 |
| | | With | 12.59 | 12.59 | 11.88 | 12.36 | - | - | - | - |
| | | Mean | 12.66 | 12.40 | 12.23 | - | - | - | - | - |

CARCY= carcass yield; BY- breast yield; TY= thigh yield; ULY= upper leg yield; WINGY= wing yield; PC= positive control; NC= negative control; YL= yeast level; CV= coefficient of variation; SV= source of variation; EC= enzymatic complex.

Table 6. Effect of the yeast levels and addition of enzymatic complex on the morphometric variables of duodenal mucosa at 21 days

| Variable (µm) | PC | NC | Yeast Levels (%) | | | Mean | CV | P>F | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 6 | 12 | | (%) | YL | EC | EC*YL |
| CD | 211 | Without | 209 | 256 | 273 | 246 | 25.16 | 0.686 | 0.155 | 0.174 |
| | | With | 238 | 198 | 214 | 217 | - | - | - | - |
| | | Mean | 224 | 227 | 244 | - | - | - | - | - |
| VH | 1015 | Without | 994 | 1033 | 1000 | 1009 | 15.61 | 0.774 | 0.126 | 0.695 |
| | | With | 879 | 979 | 976 | 944 | - | - | - | - |
| | | Mean | 937 | 1006 | 988 | - | - | - | - | - |
| CW | 53 | Without | 61 | 56 | 65 | 61 | 10.98 | 0.561 | 0.438 | 0.135 |
| | | With | 59 | 63 | 63 | 62 | - | - | - | - |
| | | Mean | 60 | 60 | 64 | - | - | - | - | - |
| VW | 204 | Without | 235 | 240 | 256 | 244 | 24.70 | 0.677 | 0.075 | 0.956 |
| | | With | 201 | 191 | 218 | 203 | - | - | - | - |
| | | Mean | 218 | 216 | 237 | - | - | - | - | - |
| MW | 222 | Without | 150 | 184 | 155 | 163[b] | 22.38 | 0.669 | 0.024 | 0.593 |
| | | With | 198 | 207 | 209 | 205[a] | - | - | - | - |
| | | Mean | 174 | 196 | 182 | - | - | - | - | - |
| VP | 2216 | Without | 2270 | 2378 | 2249 | 2299 | 16.02 | 0.691 | 0.081 | 0.648 |
| | | With | 1910 | 2193 | 2176 | 2093 | - | - | - | - |
| | | Mean | 2090 | 2285 | 2213 | - | - | - | - | - |
| VH/CD | 4.95 | Without | 4.77 | 4.02 | 3.83 | 4.20 | 25.68 | 0.810 | 0.363 | 0.226 |
| | | With | 4.08 | 5.19 | 4.63 | 4.63 | - | - | - | - |
| | | Mean | 4.43 | 4.60 | 4.23 | - | - | - | - | - |

Means with the same lowercase letter in the column do not differ statistically in the SNK test ($p<0.05$). PC= Positive control; NC=Negative control; CV=Coefficient of variation; EC=Enzymatic complex; YL=yeast levels; (CD)= Crypt depth; (VH)= Villus height; (CW)=Crypt width; (VW)=Villus width; (MW)= Muscle wall (VP)= Villus perimeter; (VH/CD) = Villus height to crypt depth.

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Noteworthy here is that the intestinal muscle wall of the animals that did not receive the enzymatic complex was thinner in relation to animals that received the enzymatic mixture in the feed. As with abnormal increase, the sharp reduction in the thickness of the muscle wall, can be attributed to a physiological response to some external agent, such as microorganisms, pathogens and antinutritional substances. The collaborators Bonapaz et al. (2010) noted a reduction in the thickness of the intestinal wall within the group of broilers deliberately infected with microorganisms in relation to the control group.

The exogenous enzymes stimulate the intestinal mucosa through reducing the quantity of substrate available for bacterial growth, as a lower quantity of substrate results in a lower quantity of bacteria (OLIVEIRA et al., 2009). The broilers fed on feed without enzymatic complex had, quite possibly, a higher bacterial load in their intestine, which may have caused a reduction in the thickness of the wall.

There was no effect from the inclusion of yeast over any morphometric variable of the duodenum at 21 days of age.

The morphometric analysis of the jejunum (Table 7), did not demonstrate a significant reaction between the enzyme and yeast over the variables under consideration.

Table 7. Effect of yeast levels and addition of the enzymatic complex on the morphometric variables of jejunal mucosa at 21 days of age.

| Variable (μm) | PC | NC | Yeast Levels (%) | | | Mean | CV | P>F | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 6 | 12 | | (%) | YL | EC | EC*YL |
| CD | 187 | Without | 190 | 192 | 200 | 194 | 20.29 | 0.909 | 0.188 | 0.902 |
| | | With | 180 | 170 | 176 | 175 | - | - | - | - |
| | | Mean | 185 | 181 | 188 | - | - | - | - | - |
| VH | 724 | Without | 750 | 824 | 908 | 827 | 19.42 | 0.362 | 0.356 | 0.684 |
| | | With | 767 | 742 | 810 | 773 | - | - | - | - |
| | | Mean | 758 | 783 | 859 | - | - | - | - | - |
| CW | 59 | Without | 67 | 61 | 65 | 64 | 13.63 | 0.436 | 0.557 | 0.090 |
| | | With | 63 | 69 | 55 | 62 | - | - | - | - |
| | | Mean | 65 | 65 | 60 | - | - | - | - | - |
| VW | 185 | Without | 220 | 237 | 197 | 218 | 24.44 | 0.424 | 0.606 | 0.849 |
| | | With | 220 | 212 | 192 | 208 | - | - | - | - |
| | | Mean | 220 | 224 | 194 | - | - | - | - | - |
| MW | 144* | Without | 156 | 190 | 237* | 194 | 19.69 | 0.105 | 0.805 | 0.078 |
| | | With | 196 | 203 | 194 | 198 | - | - | - | - |
| | | Mean | 176 | 196 | 215 | - | - | - | - | - |
| VP | 1584 | Without | 1710 | 1812 | 2036 | 1853 | 17.07 | 0.310 | 0.681 | 0.689 |
| | | With | 1797 | 1733 | 1882 | 1804 | - | - | - | - |
| | | Mean | 1754 | 1773 | 1959 | - | - | - | - | - |
| VH/CD | 4.03 | Without | 4.02 | 4.43 | 4.58 | 4.34 | 26.91 | 0.586 | 0.572 | 0.988 |
| | | With | 4.34 | 4.53 | 4.94 | 4.60 | - | - | - | - |
| | | Mean | 4.18 | 4.48 | 4.76 | - | - | - | - | - |

*Differs from the mean of the positive control treatment by the Dunnett test ($p<0.05$).
PC= Positive control; NC=negative control; CV=Coefficient variation; EC=enzymatic complex; YL=yeast levels; (CD)= Crypt depth; (VH)= villus height; (CW)=crypt width; (VW)= villus width; (MW)= muscle wall; (VP)= villus perimeter; (VH/CD) = Villus height to crypt depth.

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The levels of yeast in the diet did not alter the measurements of the jejunal mucosa structures at 21 days of age. Noted here was that the broilers that belonged to the group with 12% yeast without the enzymatic complex, presented a thicker muscle wall in relation to the positive control group ($p<0.05$).

The presence, in greater part, of NSPs in the diet results in an increase in the quantity and digesta weight, thus causing an increase in the longitudinal muscle layer (BRENES et al., 2002), which is one of the layers responsible for peristalsis, justifying in this way the increase in the thickness of the wall, as a physiological manifestation of the organism to maintain digesta flow. The activation of the smooth muscle present on the muscle wall leads to a study of hypercontractility for the expulsion of digesta from the tract (BAUER, 2008).

There was no significant effect from the use of the enzymatic complex on the variables studied in this segment of the small intestine.

There was no significant interaction between the yeast and the enzymatic complex (Table 8) for the morphometric variables of the ileus at 21 days.

Table 8. Effect from yeast levels and the addition of enzymatic complex on the morphometric variables of ileal mucosa at 21 days

| Variable (µm) | PC | NC | Yeast Levels (%) | | | Mean | CV | P>F | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 6 | 12 | | (%) | YL | EC | EC*YL |
| CD | 131 | Without | 148 | 118 | 140 | 135 | 30.44 | 0.932 | 0.463 | 0.415 |
| | | With | 116 | 133 | 120 | 123 | - | - | - | - |
| | | Mean | 132 | 126 | 130 | - | - | - | - | - |
| VH | 562 | Without | 664 | 551 | 530 | 581 | 21.71 | 0.177 | 0.590 | 0.583 |
| | | With | 588 | 490 | 583 | 554 | - | - | - | - |
| | | Mean | 626 | 521 | 557 | | - | - | - | - |
| CW | 63 | Without | 64 | 68 | 68 | 67[a] | 12.27 | 0.897 | 0.028 | 0.474 |
| | | With | 62 | 60 | 56 | 59[b] | - | - | - | - |
| | | Mean | 63 | 64 | 62 | - | - | - | - | - |
| VW | 260 | Without | 206 | 224 | 255 | 228 | 23.36 | 0.466 | 0.070 | 0.619 |
| | | With | 178 | 204 | 186 | 190 | - | - | - | - |
| | | Mean | 192 | 214 | 221 | - | - | - | - | - |
| PM | 147 | Without | 153 | 133 | 155 | 147 | 25.66 | 0.098 | 0.070 | 0.365 |
| | | With | 163 | 149 | 223 | 178 | - | - | - | - |
| | | Mean | 158 | 141 | 189 | - | - | - | - | - |
| VP | 1358 | Without | 1485 | 1278 | 1280 | 1348 | 20.63 | 0.269 | 0.572 | 0.606 |
| | | With | 1329 | 1135 | 1390 | 1285 | - | - | - | - |
| | | Mean | 1407 | 1207 | 1335 | - | - | - | - | - |
| VH/CD | 4,30 | Without | 4,91 | 4,75 | 3,83 | 4,50 | 19.95 | 0.154 | 0.854 | 0.092 |
| | | With | 5,10 | 3,71 | 4,88 | 4,56 | - | - | - | - |
| | | Mean | 5,00 | 4,23 | 4,35 | - | - | - | - | -- |

Means with the same lowercase letter in the column do not differ statistically to the SNK test ($p<0.05$). PC= Positive control; NC=Negative control; CV=coefficient of variation; EC=enzymatic complex; YL=Yeast levels; (CD)= Crypt depth; (VH)= Villus height; (CW)= Crypt width; (VW)= Villus width; (MW)= Muscle wall (VP)= Villus perimeter; (VH/CD) = Villus height to crypt depth.

However, there was a significant effect on the crypt width with the addition of the enzymatic complex to the diet ($p<0.05$), even without there being any modification to the dimensions of the villus. Through the supply of the enzymatic complex, there was an observed decrease to the width in the ileus, when compared to the diets without enzymatic complex, which indicates that the exogenous enzymes were active in this region, reducing the adverse effects of the NSPs, as for example on the viscosity and proliferation of opportunist microorganisms.

The exogenous enzymes act in a way that benefits the mucosa by means of reducing the viscosity of diets rich in soluble non-starch polysaccharides, and the breakdown of substrates that would be used for the proliferation of opportunist bacteria in this segment of the intestine (OLIVEIRA et al., 2009). The collaborators Harvatovic et al. (2015) after assessing the inclusion of exogenous enzymes to diets with sunflower meal, which possesses large quantities of soluble SNP, observed that the viscosity of the digesta increased from the proximal to the distal of the intestine, and that the activity of the enzymatic complex was more effective in the reduction of viscosity in the ileus.

According to Padihari et al. (2014) a smooth crypt is an indication of the capacity of the small intestine to require less nutrients and energy for mucosa regeneration, while allowing the intestinal cells to produce digestive enzymes and improve the absorption of nutrients. In this way, the conservation of the size of the villi and the smaller width of the crypt refers to the maintenance of digestive and absorption capacities of the intestine.

The supply of yeast did not affect the intestinal mucosa structures of the ileus at the period of 21 days.

The conclusion was reached that the use of 6 and 12% of yeast in diets deteriorates the performance of broilers from 1 to 21 days. The addition of the enzymatic complex in diets reduced in 70 Kcal of the requirement and the adding of 6% yeast, maintained the performance of the broilers similar to those that receive diets based on nutritional requirement. The inclusion of yeast and enzymatic complex does not inhibit carcass yield or the intestinal morphometry of broilers. The addition of enzymatic complex is beneficial to mucosa of the ileus.

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