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Protease and sugarcane yeast in diets for broiler chicks

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ABSTRACT. The objective of this study was to evaluate the inclusion of protease and sugarcane yeast in poultry diets on performance, organ weight, intestinal biometry, chemical composition and deposition of body nutrients in the 1 to 7-day old phase. The experimental design consisted of a randomized complete block design, in a factorial arrangement of 2 (without and with the enzyme protease) x 3 (yeast levels: 0, 6 and 12%) + 1 (positive control diet), totaling seven treatments with five replicates, birds per experimental unit. The treatments used consisted of a positive control diet and the others were the negative control, with reductions in nutritional levels of protein and amino acids by 4% requirement. In the negative control diets, protease enzyme was included in three levels of sugarcane yeast. In isolation, treatments with or without inclusion of protease influenced the chemical composition of the birds. The levels of yeast from sugarcane yeast presented effects for weight gain, feed conversion, moisture and crude protein in the nutrient deposition. The inclusion of protease in diets for broiler chicks cannot remedy the poor performance provided by the use of sugarcane yeast, in addition to presenting lower levels of deposition of body nutrients.

Keywords: poultry farm; enzyme; nutrition; by-product.

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Introduction

Corn and soybean meal are ingredients that most contribute to increase poultry production costs, due to price seasonality (Costa et al., 2013). In this context, there is an increased demand for alternative foods, especially agro-industrial by-products, which are ingredients easily obtained at certain times of the year (Freitas, Lima, Silva, Sucupira, & Bezerra, 2013).

Among the various dietary alternatives, sugarcane yeast (*Saccharomyces cerevisiae*) has good characteristics for use in animal feed mainly in terms of availability, since it is produced on a large scale mainly in the Central-South region of Brazil (Araújo, Dias, Brito, & Oliveira Junior, 2009). Its chemical composition contains 37.2% crude protein (Rostagno et al., 2011), which potentiates it as an ingredient to be used in broiler diets and can replace part of soybean meal protein (Freitas et al., 2013). In addition, it has amino acid diversity, especially lysine and B-complex vitamins, enzymes, nucleotides and minerals (Machado et al., 2010).

The substances composing sugarcane yeast include the cell wall components: proteins and polysaccharides (80 to 85%), mainly glycans, mannan and chitin, which are resistant to enzymatic digestion and have low digestibility, which consequently reduces nutrient availability and energy value (Lopes et al., 2011). Thus, the use of exogenous enzymes that enable increased digestibility may improve nutrient utilization by birds with positive effects on performance, organ biometrics, intestinal morphology, health and immunity (Law, Zulkifli, Soleimani, Hossain, & Liang, 2015).

Supplementation with mono-component proteases in broiler diets improves nutritional value through the hydrolysis of certain types of proteins that resist digestion in poultry. It can also promote the best use of proteins, with the release of peptides and amino acids, which enables a reduction in the inclusion levels of ingredients, sources of these nutrients and a decrease in nitrogen excretion (Leinonen & Williams, 2015).

Therefore, the present study aimed to evaluate the inclusion of protease associated with sugarcane yeast in diets for broiler chicks and its influence on animal performance, organ weight, intestinal biometry, chemical composition and deposition of body nutrients in the phase of 1 to 7 days of age.

Material and methods

The experiment was performed at the Poultry Industry Performance Shed of the Center for Agricultural Sciences of the Federal University of Piauí (UFPI), in the city of Teresina, State of Piauí, in June 2014. Chemical analyses were performed at the Animal Nutrition Laboratory (LANA) of the Department of Animal Sciences. The procedures were approved by the Animal Experimentation Ethics Committee/CEEAA/UFPI, under protocol number 087/12.

A total of 700 male Ross 308 broiler chicks from 1 day to 7 days of age, with an average initial weight of 42.8 ± 0.23 g were vaccinated in the hatchery against marek and gumboro diseases. The experiment was a randomized block design, according to the layout of the sheds, in a factorial arrangement 2 (with and without enzyme) \times 3 (yeast levels: 0, 6 and 12%) +1 (positive control diet), totaling seven treatments with five replications, 20 birds per experimental unit distributed in 35 boxes measuring 2.70 m² with a density of 7.4 birds m⁻².

The birds were housed in a conventional masonry shed built in the East-West direction, 15 m long and 10 m wide, covered with ceramic tiles, containing lanternin, cemented floor, 2.6 m ceiling height, the boxes are divided by plain wire mesh. Litter used on the boxes was rice husk, approximately 5 cm thick. Tubular feeders and suspended drinkers were used to supply rations and water at will.

To control the entry of sunlight and air currents, blue curtains were used on the sides of the shed. When the temperature exceeded the thermoneutrality zone of the birds, between 32-35°C as proposed by Silva et al. (2009), fans were turned on to minimize heat stress to animals.

Temperature and relative humidity were monitored by means of a digital thermohygrometer, dry bulb and wet bulb, and a black globe, at the center of the shed at the height of the birds' back, taking daily readings. Temperatures were subsequently converted to Black-Globe Temperature-Humidity Index (BGTHI), according to the equation proposed by Buffington, Collazo-Arocho, Canton, and Pitt (1981): $BGTHI = 0.72 (T_{bu} + T_{gn}) + 40.6$ (where: T_{bu} = wet bulb temperature in °C; T_{gn} = black globe temperature in °C). The continuous light program was adopted with 24 hours of natural + artificial light, the latter using 60-watt incandescent lamps.

The treatments (Table 1) consisted of a positive control diet to meet the nutritional requirements during the rearing phase according to Rostagno et al. (2011), formulated with corn and soybean meal, supplemented with minerals and vitamins, and the other diets were the negative control, with reductions in protein and amino acid nutritional levels by 4% of the requirement, according to the nutritional matrix indicated for the evaluated protease enzyme. The negative control diets were included or not with protease with three levels of sugarcane yeast, composing the following treatments: positive control (CP); negative control (CN) with 0% yeast from sugarcane without protease (SE); CN with 6% yeast from sugarcane without protease (SE); CN + 12% sugarcane yeast without protease (SE); CN with 0% sugarcane yeast and with protease (CE); CN with 6% sugarcane yeast and with protease (CE); CN with 12% sugarcane yeast and with enzyme protease (CE).

Mono-component protease commercially available as Ronozyme ProAct (DSM, Brazil) was added to the diets according to the manufacturer's recommendations (200g ton⁻¹ feed). The sugarcane yeast used was the inactive whole yeast obtained commercially and according to the analyses performed at LANA, this by-product has on average 85.37% dry matter and 34.35% crude protein on a natural matter basis. For the calculation of the rations, these data were considered along with the other values of chemical composition and metabolizable energy proposed by Rostagno et al. (2011), as well as for the other dietary ingredients.

Feed and poultry weights were measured at the beginning and end of the rearing phase in order to evaluate performance (feed intake, weight gain and feed conversion). Feed intake and feed conversion were corrected for mortality, according to the methodology described by Sakomura and Rostagno (2007).

At 7 days of age, a bird with average weight close to the experimental plot, totaling five birds per treatment, was slaughtered by cervical dislocation after fasting for six hours. The lymphoid (spleen and

cloacal sac) and digestive (liver, pancreas, gizzard + proventriculus and intestine) organs were removed to obtain their relative weights, which was obtained considering the body weight of the fasting birds. The small intestine was removed and its segments (duodenum: from the pylorus to the distal portion of the duodenal loop; jejunum: from the distal portion of the duodenal loop to the Meckel diverticulum; ileum: between the Meckel diverticulum and the opening of the cecum) were measured with a measuring tape.

Table 1. Proximate and calculated composition of experimental diets for broilers chicks at the pre-starter phase (1-7 days of age)⁽¹⁾.

Ingredient	CP	CN(0%lev)	CN+6% lev	CN+12% Lev	CN(0% lev)+CE	CN+6% lev+CE	CN+12% lev+CE
Corn	63.48	66.11	64.10	62.10	66.11	64.10	62.10
Soybean meal	30.26	28.02	23.82	19.61	28.02	23.82	19.61
Soybean oil	0.41	0.00	0.08	0.15	0.00	0.08	0.15
Dicalcium phosphate	1.94	1.96	1.94	1.91	1.96	1.94	1.91
Calcitic limestone	0.88	0.88	0.89	0.90	0.88	0.89	0.90
NaCl	0.51	0.51	0.48	0.45	0.51	0.48	0.45
L-lysine HCl 79%	0.40	0.40	0.41	0.42	0.40	0.41	0.42
DL-methionine 99%	0.35	0.33	0.36	0.40	0.33	0.36	0.40
L-threonine 98.5%	0.13	0.12	0.14	0.16	0.12	0.14	0.16
L-tryptophan 98%	0.00	0.00	0.01	0.02	0.00	0.01	0.02
L- valine 98.5%	0.11	0.10	0.13	0.16	0.10	0.13	0.16
L- arginine 99 %	0.13	0.14	0.22	0.30	0.14	0.22	0.30
Min/vit Premix ⁽²⁾	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Kaolin	1.00	1.02	1.02	1.02	1.00	1.00	1.00
Inactive Yeast	-	-	6.00	12.00	-	6.00	12.00
Protease Enzyme	-	-	-	-	0.02	0.02	0.02
TOTAL	100	100	100	100	100	100	100
Calculated composition							
Dig. Arginine (%) ⁽³⁾	1.41	1.35	1.35	1.35	1.35	1.35	1.35
Calcium (%)	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Chlorine (%)	0.36	0.36	0.34	0.32	0.36	0.34	0.32
ME (Mcal/kg) ⁽⁴⁾	2925	2925	2925	2925	2925	2925	2925
Available phosphorus (%) ⁽⁵⁾	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Dig. Lysine (%) ⁽³⁾	1.30	1.25	1.25	1.25	1.25	1.25	1.25
Dig. Met+Cyst (%) ⁽³⁾	0.94	0.90	0.90	0.90	0.90	0.90	0.90
Potassium (%)	0.82	0.78	0.76	0.73	0.78	0.76	0.73
Crude protein (%)	22.00	21.12	21.12	21.12	21.12	21.12	21.12
Sodium (%)	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Dig. Threonine (%) ⁽³⁾	0.85	0.81	0.81	0.81	0.81	0.81	0.81
Available Tryptophan (%) ⁽⁵⁾	0.23	0.22	0.22	0.21	0.22	0.22	0.21
Available Valine (%) ⁽⁵⁾	1.00	0.96	0.96	0.96	0.96	0.96	0.96

⁽¹⁾CP: positive control; CN(0%lev): negative control with 0% yeast; CN+6%lev: negative control with 6% yeast; CN+12%lev: negative control with 12% yeast; CN(0%lev)+CE: negative control with 0% yeast with enzyme; CN(6%lev)+CE: negative control with 6% yeast with enzyme; CN(12%lev)+CE: negative control with 12% yeast with enzyme; ⁽²⁾Mineral and vitamin Premix: Guarantee levels per kg product- folic acid: 0.796 mg; pantothenic acid: 0.012mg; biotin: 0.04 mg; chlorohydroxyquinoline: 30.00 mg; copper: 7.984 mg; choline: 0.322g; iron: 0.045 g; iodine: 0.748 mg; manganese: 0.074 g; niacin: 33.596 mg; nicarbazin: 0.1 g; selenium: 0.3 mg; vitamin A: 672,000 IU; vitamin B1: 1.746 mg; vitamin B12: 9.6 mcg; vitamin B2: 4.8 mg; vitamin B6: 2.496 mg; vitamin D3: 1,600 IU; vitamin E: 14.00 UI; vitamin K3: 1.44 mg; zinc: 0.07 mg ⁽³⁾available ⁽⁴⁾ME: metabolizable energy ⁽⁵⁾Available

At the end of the pre-starter phase, after 24-hour fasting for solids (for complete emptying of the digestive tract), one bird from each repetition was slaughtered for analysis of chemical composition and deposition of body nutrients, which were taken to the freezer for freezing. Subsequently, whole frozen carcasses (including feathers, blood and viscera) were cut into pieces on the bandsaw and processed in a meat mill to obtain a homogeneous material. Then the carcasses were weighed, frozen again and dried by vacuum freeze drying at -50°C for 72 hours to obtain the pre-dry matter. Then, the samples were weighed and re-ground and sent to the laboratory for analysis of moisture, crude protein, lipid and ash, according to the methodologies described by Silva and Queiroz (2002). This procedure was performed on 10 chicks on the first day of age to analyze the deposition of body nutrients of birds.

Temperature, relative humidity and ITGU were presented as mean and standard deviation. The other parameters were tested by analysis of variance, and when significant, the inclusion or not of the enzyme were compared by the Newman-Keuls-Student's Test (SNK) and for the yeast levels, the regression analysis was applied. In comparing the positive control treatment with the others, the Dunnett's test was applied according to the GLM procedures of Statistical Analysis System (SAS, 2013). $\alpha = 0.05$ was adopted.

Results and discussion

The mean values of temperature, relative humidity and ITGU recorded inside the shed during the period from 1 to 7 days of age of the birds were: $28.0 \pm 0.60^\circ\text{C}$; $78 \pm 0.92\%$ and 79.5 ± 0.46 , respectively. These results suggest that the study was conducted in a comfortable environment for animals, since according to the Line manual (Aviagen Ross, 2009), chicks require temperatures of $31.54 \pm 2.58^\circ\text{C}$ for the pre-starter phase. Similarly, Menegali et al. (2009) admit that ITGU values ranging from 77 to 81.3, for the phase from 1 to 7 days of age, corresponding to the warming phase for broilers, indicate comfortable conditions.

When comparing the treatments with the positive control diet, no differences ($p > 0.05$) were detected for the variables: feed intake, weight gain and feed conversion. Similarly, there was no interaction ($p > 0.05$) between the factors, with or without protease inclusion and sugarcane yeast levels, for the performance (Table 2) in the pre-starter phase of broilers.

Table 2. Performance of broiler chicks, from 1 to 7 days of age, fed diets containing protease and sugarcane yeast.

Item ¹	CP	CN	Yeast levels (%)			Mean	CV (%)	P ² value		
			0	6	12			EM	NL	EN*NL
CR (g bird ⁻¹)	159.8	SEN	159.2	158.5	156.1	157.9	3.6	0.2471	0.6013	0.4495
		CEN	159.7	162.1	159.4	160.4				
		Mean	159.5	160.3	157.8					
GP (g bird ⁻¹)	144.5	SEN	140.0	135.2	128.8	134.7	3.9	0.4268	0.0017	0.3836
		CEN	138.0	139.7	131.1	136.2				
		Mean	139.0	137.4	129.9					
ER for GP _{NL}			$\hat{y} = 139.96 - 0.7523X$. $r^2 = 0.87$; $P = 0.0008$.							
CA	1.11	SEN	1.14	1.17	1.21	1.17	2.85	0.7632	0.0006	0.5299
		CEN	1.16	1.16	1.22	1.18				
		Mean	1.15	1.17	1.21					
ER for CA _{NL}			$\hat{y} = 1.435 + 0.0055X$. $r^2 = 0.94$; $P = 0.0002$.							

¹CR = feed intake; GP = weight gain; CA = feed conversion; CV = coefficient of variation; CP = positive control; CN = negative control; SEN = without enzyme; CEN = with enzyme; ER = Regression equation; r^2 = coefficient of determination. ²Statistical probability for: EN = enzyme; NL = yeast levels; EN*NL interaction enzyme*yeast levels.

In isolation, the inclusion of protease did not influence ($p > 0.05$) feed intake, weight gain and feed conversion of birds (Table 2). In contrast, Angel, Saylor, Vieira, and Ward (2011) state that protease supplementation in broiler diets, regardless of the concentration used, promotes an increase in weight gain and an improvement in feed conversion of poultry similar to those fed with control group (no protease supplementation).

Sugarcane yeast levels had effects ($p < 0.05$) on weight gain and feed conversion of broiler chicks (Table 2). For the weight gain, there was a linear decrease ($p < 0.05$), according to the equation $\hat{y} = 139.96 - 0.7523X$, $r^2 = 0.87$, in which there was a decrease in weight gain with increasing levels of sugarcane yeast in the diets. There was an increasing linear effect ($p < 0.05$) for feed conversion, according to the equation $\hat{y} = 1.435 + 0.0055X$, $r^2 = 0.94$.

Similarly to this research, for the weight gain, Freitas et al. (2013) replaced soybean meal protein with sugarcane yeast in diets for broiler chicks, and found that 12.08% sugarcane yeast in the starter phase resulted in an increase in feed intake and decreases in weight gain, but feed conversion was not affected.

The use of protease in diets was not sufficiently able to access the protein contained in sugarcane yeast, probably due to the yeast wall that is composed mainly of dietary fiber represented by carbohydrates such as mannans and glucans, which encapsulated the nutrients needed for animal use. Protease does not act on carbohydrate and for sugarcane yeast to be degraded by birds, a more complete enzymatic apparatus would be required with specific activities not only for protein but also for carbohydrates (Cowieson, 2010).

Some studies (Abdelrahman, 2013; Barroso et al., 2013; Sousa et al., 2018) were conducted to determine the best level of inclusion of sugarcane yeast in broiler diets, however the results are variable. These differences have been attributed to the fact that this by-product is subject to variations in its nutritional value, due to changes in chemical composition, which depend on the method of obtaining, which in turn varies as to substrate, microorganism and drying method used (Freitas et al., 2013). According to the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011), the maximum level of inclusion of sugarcane yeast in diets for broilers in the starter phase is 3%.

The highest level of inclusion of this by-product in diets for broiler chicks possibly reduced nutrient and energy digestibility (Lopes et al., 2011), because the sugarcane yeast cell wall is thick and resistant to enzymatic digestion, making the intracellular content unavailable. In order for feed nutrients to be efficiently degraded and absorbed, they must become available in metabolic processes.

When comparing the treatments with the positive control diet, no differences ($p > 0.05$) were detected for weights of spleen, cloacal sac, liver, pancreas, gizzard + proventriculus, intestine and length of duodenum, jejunum and ileum of the birds. There was no interaction between the factors, with or without inclusion of protease and sugarcane yeast levels ($p > 0.05$) on the studied variables at the pre-starter phase of the birds (Table 3).

In isolation, the inclusion of protease and sugarcane yeast levels had no effect ($p > 0.05$) on the analyzed variables (Table 3).

Table 3. Organ weight and intestine biometry of 7-day-old broiler chicks fed diets containing protease and sugarcane yeast.

Item ¹	CP	CN	Yeast levels (%)			Mean	CV (%)	P ² value		
			0	6	12			EN	NL	EN*NL
Organ weight										
Spleen	0.09	SEN	0.07	0.08	0.07	0.07	37.7	0.5363	0.4093	0.9324
		CEN	0.08	0.09	0.08	0.08				
		Mean	0.07	0.09	0.07					
Sac	0.25	SEN	0.18	0.22	0.20	0.20	25.7	0.7436	0.6665	0.7429
		CEN	0.19	0.19	0.20	0.19				
		Mean	0.19	0.21	0.20					
Liver	4.46	SEN	4.14	4.40	4.03	4.19	11.9	0.5866	0.2630	0.9070
		CEN	4.36	4.45	4.07	4.29				
		Mean	4.25	4.43	4.05					
Pancreas	0.44	SEN	0.44	0.40	0.40	0.41	14.9	0.0909	0.6939	0.7523
		CEN	0.46	0.46	0.45	0.46				
		Mean	0.45	0.43	0.42					
Gizzard + pro	7.46	SEN	6.89	7.03	6.94	6.95	12.1	0.7521	0.7338	0.7917
		CEN	6.79	7.03	7.33	7.05				
		Mean	6.84	7.03	7.14					
Intestine	7.31	SEN	7.47	7.09	7.32	7.29	18.1	0.5579	0.8707	0.7590
		CEN	7.24	7.59	7.93	7.59				
		Mean	7.35	7.34	7.62					
Intestine biometry										
Duodenum	15.4	SEN	15.90	15.60	15.60	15.70	16.3	0.6475	0.6704	0.6204
		CEN	15.00	16.40	14.50	15.30				
		Mean	15.45	16.00	15.05					
Jejunum	36.1	SEN	38.20	37.40	36.30	37.30	9.8	0.1578	0.7814	0.7784
		CEN	35.80	34.70	35.70	35.40				
		Mean	37.00	36.05	36.00					
Ileum	29.2	SEN	32.70	31.00	31.20	31.63	11.8	0.1189	0.6342	0.3359
		CEN	28.60	31.60	28.30	29.50				
		Mean	30.65	31.30	29.75					
Total length	83.8	SEN	93.30	86.50	85.60	88.47	7.7	0.0692	0.5215	0.2040
		CEN	82.40	85.90	83.20	83.83				
		Mean	87.85	86.20	84.40					

¹Gizzard + pro = gizzard and proventriculus; CV = coefficient of variation; CP = positive control; CN = negative control; SEN = without enzyme; CEN = with enzyme; ER = Regression equation; r^2 = coefficient of determination. ²Statistical probability for: EN = enzyme; NL = yeast levels; EN*NL interaction enzyme*yeast levels.

The study of organ growth may lead to a better understanding of metabolic diseases resulting from the high growth rate of broilers and differences in organ growth may alter the physiology of broilers (Marcato et al., 2010). In line with the results of this study, Ferreira et al. (2009) found no effect ($p > 0.05$) on lymphoid organ weight with increasing levels of sugarcane yeast cell wall in broiler diets. Thus, the authors inferred that the use of yeast wall in broiler diets still needs further studies, including, for example, the purification of the yeast wall as for its main components (mannan oligosaccharides and β -glucan).

From a nutritional point of view, the size of the intestine can affect the rate of food passage through the digestive tract and thus influence the efficiency of digestion and absorption of dietary nutrients. Similar results were reported by Nunes et al. (2011), because they did not find effects ($p > 0.05$) on organ allometric and intestinal biometric variables when using sweet potato meal, with or without supplementation of

enzymatic complex for broilers at the starter phase.

Considering the chemical composition and body nutrient deposition, when comparing the treatments with the positive control diet, no differences ($p > 0.05$) were detected for the analyzed variables. Likewise, there was no interaction between the factors, with or without inclusion of protease and sugarcane yeast levels ($p > 0.05$) (Table 4).

In isolation, it was observed that the inclusion of protease in the diets caused a decrease ($p < 0.05$) for the crude protein and ash regarding the body chemical composition of broiler chickens, but sugarcane yeast levels were not influenced ($p > 0.05$). Sugarcane yeast levels linearly influenced ($p < 0.05$) the deposition of body nutrients for the variables moisture and crude protein, represented by equations $\hat{y} = 28.6 - 0.1383X$; $r^2 = 0.87$ and $\hat{y} = 18.3 - 0.1092X$; $r^2 = 0.71$, respectively (Table 4).

Table 4. Chemical composition and body nutrient deposition of broiler chicks, from 1 to 7 days of age, fed diets containing protease and sugarcane yeast, expressed on a natural matter basis.

Item ¹	CP	CN	Yeast levels (%)			Mean	CV (%)	P ² value		
			0	6	12			EN	NL	EN*NL
Chemical composition										
Moisture	77.1	SEN	77.5	77.5	77.8	77.6	1.8	0.3699	0.0591	0.2071
		CEN	77.6	77.7	77.1	77.5				
		Mean	77.5	77.6	77.4					
Crude protein	13.7	SEN	13.7	13.9	13.3	13.6A	3.2	0.2717	0.0861	0.8749
		CEN	13.1	13.2	13.4	13.2B				
		Mean	13.4	13.5	13.3					
Lipids	6.3	SEN	6.6	5.9	6.2	6.2	18.9	0.1376	0.6314	0.5604
		CEN	6.8	7.1	6.7	6.9				
		Mean	6.7	6.5	6.5					
Ash	1.8	SEN	2.0	2.2	2.1	2.1A	14.0	0.0847	0.9424	0.3799
		CEN	2.0	1.7	2.0	1.9B				
		Mean	2.0	2.0	2.0					
Nutrient deposition										
Moisture	29.3	SEN	28.9	27.7	26.1	27.6	5.5	0.3698	0.0592	0.2071
		CEN	28.0	28.7	27.5	28.1				
		Mean	28.5	28.2	26.8					
ER for Moisture _{NL}						$\hat{y} = 28.6 - 0.1383X$; $r^2 = 0.87$; $P = 0.0221$				
Crude protein	19.3	SEN	18.8	18.4	16.4	17.9	5.9	0.2074	0.0104	0.1202
		CEN	17.3	17.8	17.0	17.4				
		Mean	18.0	18.1	16.7					
ER for Crude protein _{NL}						$\hat{y} = 18.3 - 0.1092X$; $r^2 = 0.71$; $P = 0.0096$				
Lipids	9.9	SEN	10.1	8.69	8.79	9.19	22.0	0.1376	0.6314	0.5604
		CEN	10.3	11.0	9.81	10.4				
		Mean	10.2	9.86	9.30					
Ash	27.5	SEN	29.9	32.6	29.3	30.6	18.6	0.0849	0.9426	0.3803
		CEN	28.6	25.2	27.5	27.1				
		Mean	29.3	28.9	28.4					

Means followed by different upper case letters, in the same column, for the same variable, are significantly different by SNK test ($P > 0.05$). ¹CV = coefficient of variation; CP = positive control; CN = negative control; SEN = without enzyme; CEN = with enzyme; ER = regression equation; r^2 = coefficient of determination. ²Statistical probability for: EN = enzyme; NL = yeast levels; EN * NL enzyme interaction * yeast levels.

Values for deposition of moisture and protein were similar; this demonstrates that water deposition is closely linked to body protein in birds, that is, broiler chicks retain less water with increasing levels of sugarcane yeast, due to the lower protein synthesis (Mansano, Stéfani, Pereira, & Macente, 2013).

Furlan, Farias Filho, Rosa, and Macari (2004) reported that protein deposition in the carcass is predetermined by the bird, according to its genetic information, i.e. there is a limit to daily protein deposition, which cannot be compensated for by diet. Excess protein can cause kidney and liver problems because it increases the amount of nitrogen eliminated by the kidneys, which overloads the kidneys and can compromise their functioning.

Conclusion

The inclusion of protease in diets for broiler chicks from 1 to 7 days of age cannot remedy the poor performance provided by the use of sugarcane yeast, besides presenting lower levels of body nutrient deposition.

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