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Original Article

Hybrid Phytase and Carbohydrases in Corn and Soybean Meal-Based Diets for Broiler Chickens: Performance and Production Costs

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Keywords

Calcium; cost; energy; enzyme; phosphorus.

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ABSTRACT

This study aimed to evaluate the effect of using 500 FTU/kg of hybrid phytase (created from three bacteria – Natuphos E), with or without xylanase and glucanase (560 TXU/kg; 250 TGU/kg) – Natugrain TS, in corn and soybean meal-based diets, with nutritional reductions in metabolizable energy (ME), Ca, and available phosphorus (AP), on performance parameters and production costs in broiler chickens. The 1875chickens were housed in boxes and distributed among 5 treatments with 15 replicates of 25 chickens each. The experiment included a positive control (the diet of which met the nutritional requirements of the birds), two negative controls (with ME reductions of 70 and 100 kcal/kg, and fixed reductions in Ca [0.16%] and AP [0.15%]), and two treatments with identical nutritional reductions in addition to enzyme supplementation. The treatments included the following: PC= positive control (basal diet (BD) corn and soybean meal); R_70 = BD with reduction of 70 kcal/kg, Ca and AP;R_100 = BD with reduction of 100 kcal, Ca and AP; R_70 + P =BD with reduction of 70 kcal/kg, Ca and AP + phytase (500 FTU/kg); R_100 + P + XG =BD with reduction of 100 kcal/kg, Ca and AP + phytase (500 FTU/kg) + xylanase (560 TXU/kg) + glucanase (250 TGU/kg). Performance parameters, carcass yield, and production costs (USD/ton chilled carcass) were evaluated. In conclusion, the reductions of 70 kcal/kg, 0.16% Ca, and 0.15% AP did not affect performance in chickens over 42 days, if diets were supplemented with hybrid phytase (500 FTU/kg). Supplementation with hybrid phytase and carbohydrases in diets with reductions of 100 kcal/kg, 0.16% Ca, and 0.15% AP led to lower production costs.

INTRODUCTION

In recent years, the costs of feeding broiler chickens have steadily increased, making the process of production less efficient. Corn and soybean meal account for about 80% of the nutritional matrix of broilers (Zou *et al.*, 2013). Thus, fluctuations in the prices of these products greatly impact the total price of the diet. Supplementation of diets with lipids also increases feed costs, but it is often necessary to increase the energy content of the diets to meet the requirements of these birds. Moreover, diets need to be supplemented with phosphorus, owing to its low availability in plant-based foods. However, it increases feed costs and contributes to environmental pollution (Selle & Ravindran, 2007; Woyengo & Nyachoti, 2011).

Phosphorus is found in the seeds of some plants as a phytate (Maez, 2001).Poultry have a reduced ability to dephosphorylate P from the inositol ring, owing to low production of the enzyme phytase in the body (Bedford, 2000).Thus, to increase the efficacy of dietary P, use of exogenous phytase is required. Another characteristic of plant-based diets that impairs nutrient utilization is the presence of structural carbohydrates, otherwise known as non-starch polysaccharides (NSPs). Some of these NSPs increase intestinal viscosity because they are soluble and thus easily bind to water, effectively reducing the digestion rate by preventing digestive enzymes from accessing substrates (Bedford, 2000). Therefore, supplementation of poultry diets with carbohydrases would promote the cleavage of the NSP matrix found in cell walls (Meng & Slominski, 2005; Lu *et al.*, 2013), in addition to facilitating the access of other enzymes, such as phytase, to the substrate by exposing phytate (Juanpere *et al.*, 2005).

In this regard, one of the strategies used by nutritionists is to reduce the amounts of some ingredients in the nutritional matrix. This is because the enzymes improve the efficiency of nutrient utilization and prevent food from being passed whole and/or being poorly exploited by the gastrointestinal tract (Zou *et al.*, 2011; Romero *et al.*, 2014). Moreover, new enzymatic products with distinct features that can lead to greater efficiency are released every year. This is the case with hybrid bacterial phytases, which have shown to improve the utilization of phosphorus and other phytate-bound nutrients (Torrallardona *et al.*, 2017).

Although the use of phytase is relatively established, its supplementation in diets usually occurs based only on the valuations of Ca and P, without the consideration of other nutrients. It can be said that this "uncertainty" is even greater when dealing with combinations of phytase and other enzymes.

Therefore, to benefit the poultry industry, it is extremely important to conduct studies that test the products available on the market, generating evidence that will help farmers make decisions regarding the specific nutritional matrix that should be adopted. Thus, this study aimed to evaluate the efficiency of a hybrid phytase supplemented alone or in conjunction with specific carbohydrases (xylanase + glucanase) in broiler diets containing nutritional reductions in metabolizable energy, Ca, and available P, in terms of performance and production costs.

MATERIALS AND METHODS

The experiment was conducted at the Experimental Poultry Department, Embrapa Swine and Poultry - CNPSA, at Concordia, SC, Brazil. The project was approved by the Embrapa Animal Ethics Committee number 006-2017.

Animals and Treatments

A total of 1875 one-day-old *Cobb 500* male broilers were housed in pens with pine shavings, equipped with tubular feeders, nipple-type drinkers (5/box), and electric hood heating.

The broilers had an average weight of 46.9 g ± 3.7 gand were randomly distributed among 5 treatments, with 15 replicates of 25 broilers each. The broilers were housed in boxes with dimensions of 1.65m × 1.70m and a total area of 2.80 m^2 (experimental unit).

The diets had different nutritional matrices and included a positive control diet (meeting the nutritional requirements of the broilers) and diets with ME (kcal/kg) reductions of 70 (R_70) and 100 (R_100) and fixed reductions of 0.16% Ca and 0.15% AP, without or with the supplementation of a hybrid phytase [500 FTU/kg] - (R_70+P), xylanase [560 TXU/kg], and glucanase [250 TGU/kg] (R_100 + P + XG).

A previous study conducted with broiler chickens by Krabbe *et al.* (2014) tested the specific enzyme package containing xylanase and glucanase (Naturgrain TS) and found that there was a 32 kcal/kg increase in metabolizable energy in diets based on corn and soybean meal. The phytase energy matrix used in the present study (500 FTU/kg) predicts a gain of 68.9 kcal/kg ME, and this information was used to determine the energy reductions (70 kcal/kg and 100 kcal/kg). The reductions of Ca% (0.16) and AP% (0.15) applied in the diet matrix also followed the manufacturer's recommendations.

Enzymes

Phytase. The phytase used (myo-inositol-hexakisphosphate-beta-phosphohydrolase), Natuphos E® 10,000 G- [EC 3.1.3.26], BASF SA. is a granulated microbial phytase, and has a phytase activity of 10,000 FTU/g. Its activity in FTU is defined as the amount of enzyme that releases 1 µmol of inorganic phosphorus/min from 0.0051 mol/L of sodium phytate at pH 5.5 and 37 ºC.

According to the manufacturer, this new molecule was created by manufacturing a hybrid phytase from three bacteria, which confers greater resistance to the product with regards to acid pH conditions, attack by endogenous proteases, and pelleting (up to 95 ºC).

Carbohydrases. The carbohydrases used include endo-1,4-beta-xylanase and endo-1,4 beta-glucanase, Natugrain®TS - BASF SA - [EC 3.2.1.8] and [EC 3.2.1.4]. Both enzymes are produced from *Aspergillus niger* According to the manufacturer, the endoxylanase unit (TXU) is defined as the amount of enzyme that releases five micromoles of reducing sugar, measured as a xylase equivalent, per minute from a solution containing 1 g of arabinoxylan per 100 mL at pH 3.5 and 40 ºC. An endoglucanase unit (TGU) is defined as the amount of enzyme that releases one micromole of reducing sugar, measured as a glucose equivalent, per minute from a solution containing 0.714 g of beta-glucan per 100 mL at pH 3.5 and 40 ºC.

Enzymatic Activity. Enzyme activity assays were performed on the pelleted diets, which were collected and sent to CBO Analysis Laboratory, in Valinhos - São Paulo, Brazil.

For an expected dose of phytase of 500 FTU/kg, the recovery ranged from 420 to 540 FTU. For a xylanase dose of 560 TXU/kg, the recovery ranged from 517 to 579 units. For a glucanase dose of 250 TGU/kg, the recovery ranged from 265 to 381 units.

Feeding program

The following four-phase feeding program was used: pre-starter (1–7 days), starter (8–21 days), grower (22–35 days), and finishing (36–42 days). The diets were pelleted (80 ºC for 20 seconds) and supplied after grinding in the pre-starter, initial, and growth phases, and were pelletized without grinding in the final stage. The composition and nutritional profiles of the experimental diets are described in Table 1.

Variables analyzed

Performance. The following variables were evaluated: mean body weight (BW), mean daily weight gain (DWG), feed intake (FI), and feed conversion ratio (FCR). The broilers were weighed on the day they were housed (day 1) and, then, weekly throughout the experimental period (up to 42 days). The feed provided and the leftovers were weighed weekly to determine the feed intake and the feed conversion ratio. Mortality was also recorded to correct performance data.

Carcass yield and Production costs. Two 42-day-old broilers that weighed ± 5% the mean weight of each experimental unit were selected to determine carcass yield. The animals were transported to a refrigerator with municipal inspection and slaughtered according to the current legislation for this type of establishment. The animals were weighed before slaughtering to determine the live weight. After evisceration and chiller cooling, the carcass was weighed to calculate the carcass yield using the equation: CY (%) = (carcass weight × 100)/live weight.

The carcass yield was determined to calculate the cost per ton of cooled carcasses, following Miele *et al.* (2010).This methodology considers several stages and is based on the definition of the production system, the survey of technical production coefficients, and market prices (Protas, 1983; Girotto & Protas, 1994; Girotto & Santos Filho, 2000; Canever *et al.*, 1996; Santos Filho *et al.*, 1998; Girotto & Souza, 2005).

The prices of the ingredients were quoted in September 2017 and converted to USD using the Ptax rate (3,2659) on 09/14/2017 (Table 2).

Statistical analysis

The data were analyzed using analysis of variance (ANOVA), and the means were compared via Tukey's test using the LSM (least squares means) procedure. The analyses was performed at a significance level of 5% using the statistical package R (R Core Team, 2015).

RESULTS AND DISCUSSION

When considering the evaluated periods, there was no effect of the treatments on the mortality of the animals, 1 - 7 days (p = 0.29), 1 - 14 days (p = 0.75), 1 - 21 days (p = 0.14), 1 - 28 days (p = 0.18), 1 - 35 days (p = 0.17), and 1 - 42 days (0.46).

Performance

In the first week the main effect of the enzymatic supplementation was observed for FCR and showed results similar to those of treatment PC (Table 3).

A positive cumulative effect was observed for BW in animals receiving diets with enzymes from 14 days of age until the end of the experimental period (42 days). A similar pattern was observed for DWG from the second week of the experiment. In contrast, broilers receiving enzyme supplementation showed feed consumption results equivalent to those of the positive control group in all the evaluated periods (Table 3).The results of BW and DWG indicated that treatments with nutritional reductions (R_70 and R_100) worsened the zootechnical indices, which could be reversed by enzyme supplementation (R_70 + P and R_100 + P + XG)

Assuming that the improvements in BW and DWG occurred due to increased nutrient availability in the diet due to the action of the enzymes, a reduced feed intake would also be expected (Kocher *et al.*, 2003). Kocher and co-workers (2003) state that the use of carbohydrases leads to greater energy availability, resulting in a lower feed intake. However, this behavior was not observed in our study for broilers fed on the R_100 + P + XG diet between days 1 and 42 compared to the positive control.

Table 1 – Composition and nutritional profiles of the experimental diets

Ingredients (%)	Pre-starter (1–7 days)			Starter (8–21 days)			Grower (22–35 days)			Finishing (36–42 days)		
	PC	R_70/ R_70+P	R_100/ R_100+P+XG	PC	R_70/ R_70+P	R_100/ R_100+P+XG	PC	R_70/ R_70+P	R_100/ R_100+P+XG	PC	R_70/ R_70+P	R_100/ R_100+P+XG
Corn	48.440	48.440	48.440	52.380	52.380	52.380	50.727	50.727	50.727	65.590	65.590	65.590
Soybean meal (44% Crude Protein)	44.050	44.050	44.050	40.570	40.570	40.570	40.660	40.650	40.650	28.517	28.510	28.510
Soybean oil	3.240	2.450	2.105	3.385	2.565	2.236	4.456	3.663	3.321	2.750	1.963	1.607
Kaolin	0.045	1.540	1.885	0.040	1.570	1.884	1.055	2.562	1.855	0.050	1.573	1.914
Dicalcium[2] Phosphate	1.780	0.970	0.970	1.330	0.520	0.520	1.066	0.255	0.255	0.970	0.159	0.159
Limestone[3]	0.970	1.070	1.070	1.120	1.220	1.220	1.010	1.112	1.112	0.878	0.980	0.980
Salt	0.530	0.530	0.530	0.500	0.500	0.500	0.480	0.480	0.480	0.455	0.450	0.450
DL-Methionine	0.300	0.300	0.300	0.210	0.210	0.210	0.151	0.151	0.151	0.189	0.180	0.180
L-Lysine	0.170	0.170	0.170	0.060	0.060	0.060	0.000	0.000	0.000	0.195	0.190	0.190
Notox[4]	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185
L-Threonine	0.080	0.080	0.080	0.010	0.010	0.010	0.000	0.000	0.000	0.061	0.060	0.060
Vitamin premix[5]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Mineral premix[6]	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Monensin sodium (Coban)	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.000	0.000	0.000
BHT	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Phytase[7]	-	R_70+P	R_100+P+XG	-	R_70+P	R_100+P+XG	-	R_70+P	R_100+P+XG	-	R_70+P	R_100+P+XG
X+G[8]	-	-	R_100+P+XG	-	-	R_100+P+XG	-	-	R_100+P+XG	-	-	R_100+P+XG
Total	100	100	100	100	100	100	100	100	100	100	100	100
Nutritional profile - calculated												
EMAn, kcal/kg	2925[1]	2855	2825	2980[1]	2910	2880	3050[1]	2980	2950	3100[1]	3030	3000
Crude protein %	23.65	23.65	23.65	22.14	22.14	22.14	22.01	22.03	22.03	17.74	17.73	17.73
Fat, %	5.72	4.94	4.60	5.95	5.17	4.83	6.99	6.20	5.86	5.60	4.82	4.48
Crude fiber, %	3.17	3.17	3.17	3.05	3.05	3.05	3.05	3.05	3.05	2.65	2.64	2.65
Available P, %	0.47[1]	0.32	0.32	0.38[1]	0.23	0.23	0.33[1]	0.18	0.18	0.29[1]	0.14	0.14
Total P, %	0.74	0.58	0.58	0.64	0.49	0.49	0.59	0.44	0.44	0.53	0.38	0.38
Ca, %	0.92[1]	0.76	0.76	0.86[1]	0.70	0.70	0.75[1]	0.59	0.59	0.65[1]	0.49	0.49
(Dig)[9] Lys, %	1.30	1.30	1.30	1.14	1.14	1.14	1.09	1.09/1.14	1.09/1.14	0.96	0.96	0.96
Dig Met+Cys, %	0.93	0.93	0.93	0.82	0.82	0.82	0.76	0.76/0.82	0.76/0.82	0.70	0.69	0.69
Dig Met, %	0.59	0.59	0.59	0.49	0.49	0.49	0.43	0.43/0.49	0.43/0.49	0.42	0.41	0.41
Dig Threo, %	0.84	0.84	0.84	0.74	0.74	0.74	0.73	0.73/0.74	0.73/0.74	0.63	0.63	0.63
Dig Thrip, %	0.26	0.26	0.26	0.24	0.24	0.24	0.25	0.25/0.24	0.25/0.24	0.19	0.19	0.19
Nutritional profile - analyzed												
Crude Protein, %	24.8	24.7/24.4	24.4/24.8	23.3	22.7/23.0	23.3/23.5	22.6	24.0/22.9	22.9/23.6	18.7	19.0/18.7	18.4/18.9
Fat, %	8.29	5.43/5.45	5.47/5.07	6.32	5.30/5.75	5.50/5.56	7.59	6.7/6.4	6.2/6.6	5.72	4.9/5.3	4.7/4.9
Total P, %	0.69	0.52/0.55	0.52/0.51	0.60	0.50/0.43	0.51/0.43	0.50	0.37/0.35	0.34/0.39	0.47	0.31/0.32	0.30/0.32
Ca, %	0.74	0.61/0.64	0.55/0.60	0.60	0.62/0.54	0.61/0.58	0.65	0.53/0.52	0.56/0.56	0.59	0.47/0.45	0.45/0.49

PC= positive control - basal diet (BD) – based corn and soybean meal; R_70 = BD with reductions of 70 kcal/kg and 0.16% Ca and 0.15% AP; R_100 = BD with reductions of 100 kcal/kg and 0.16% Ca and 0.15% AP; R_70 + P = BD with reductions of 70 kcal/kg and 0.16% Ca and 0.15% AP + phytase (500 FTU/kg); R_100 + P + XG = BD with reductions of 100 kcal/kg and 0.16% Ca and 0.15% AP + phytase (500 FTU/kg) + xylanase (560 TXU/kg) + glucanase (250 TGU/kg);[1]According to the recommendations of the Brazilian Poultry and Swine Tables (2011) - male broilers for regular performance; [2] calcium minimum 210 g/kg, maximum 250 g/kg; phosphorus: 180 g/kg; [3] calcium minimum 33%; [4]mycotoxin sequestran – aluminosilicates, yeast wall, activated carbon; [5]Composition of the product (guarantee levels per kg of product): vit A = 11,000,000 U.I.; vit D3 = 4,000,000 U.I.; vit E = 55,000 U.I.; vit K3 = 3000 mg; vit B1 = 2,300 mg; vit B2 = 7,000 mg; pantothenic acid = 12 g; vit B6 = 4,000 mg; vit B12 = 25,000 µg; nicotinic acid = 60 g; folic Acid = 2000 mg; biotin = 250 mg; selenium = 300 mg. [6]Composition of the product (guarantee levels per kg of product): iron = 100 g; copper = 20 g; manganese = 130 g; zinc = 130 g; iodine = 2,000 mg; [7]Natuphos® 10000G – 500 FTU/kg = 0.005 kg/100 kg of diet; [8] Xylanase (560TXU/kg) + Glucanase (250 TGU/kg) = Natugrain®TS - 0.010 kg/100 kg of diet.[9] digestible.

Table 2 – Price in Brazilian Real (R$) and US Dollars (USD) per kg of the ingredients used in the experimental diets.

Ingredients	R$/kg	USD/kg
Corn	0.440	0.135
Soybean meal	0.983	0.301
Soybean oil	2.661	0.815
Kaolin	0.440	0.135
Dicalcium phosphate	2.459	0.753
Limestone	0.179	0.055
Salt	0.329	0.101
DL-Methionine	13.75	4.213
L-Lysine	6.469	1.981
Notox	23.63	7.238
L-Threonine	8.109	2.483
Vitamin premix	14.86	4.553
Mineral premix	6.828	2.091
Monensin sodium (Coban)	31.86	9.758
BHT	17.97	5.505
Phytase 500 FTU/kg	48.98	15.00
Xylanase + Glucanase	45.72	14.00

The voluntary consumption of the diet by the broilers could reflect the amount of energy in the diet. According to Ferket and Gernat (Ferket & Gernat, 2006), broilers are unable to adequately reduce food intake when there are higher energy intakes, with heavier breeds tending to maintain feed intakes regardless of the energy content in the diet. The cause of this inability is most likely associated with genetic improvement programs aimed at hyperphagia, which is associated with high muscle tissue gains in commercial broiler strains (Barbato, 1994).

For animals to reach their optimum potential, it is necessary that their diets supply the amount of nutrients required for the maintenance of the organism and a surplus for deposition of muscle tissue. In this study, diets positive control (each phase), were formulated and aimed at the growth of male broilers with regular performance according to Brazilian Tables for Poultry and Swine (2011), and it was expected that those animals had nutritional requirements and performance responses according to this category(values for the feed intake [g/day] and weight gain [g/day], respectively, per phase were: 1 to 7 days - 23.0 and 19.6; 8 to 21 days - 65.8 and 45.8; 22 to 33 days - 137 and 77.6; 34 to 42 days - 181 and 87). However, the development of the broilers in this study was greater than that reported in the Brazilian Tables for Poultry and Swine (2011) when BW, DWG, and FI were compared, with an increase of 18% in BW, 11% in DWG, and almost 2% in FI. This should be considered when evaluating the FCR results.

The supplementation of Phytase (R_70 + P) and phytase + carbohydrase (R_100 + P + XG) in diets with reduced ME, resulted in feed conversion equivalent to the positive control in the periods of 1–7, 1–28, and 1–35 days. However, in the periods of 1–14, 1–21, and 1–42 days, only the treatment R_70 + P showed a feed conversion equivalent to the positive control (Table 3). A comparison of the results found in this study with those expected for male broilers with regular performance showed that the highest growth rates were obtained at 1–14 and 1–21 days of age and that the BW, DWG, and FI values were higher than expected, reaching 21%, 13%, and 6%, respectively.

Providing energy and nutrient deficient diets in the initial growth stages has a negative impact on the growth rate and performance indices of broilers. Therefore, it can be assumed that the nutritional reductions in the experimental diets of 70 and 100 kcal/kg, 0.16% Ca, and 0.15% AP were even more restrictive, as the development of the animals was greater than expected. This fact could explain the lack of effect of supplementing phytase + carbohydrases (R_100 + P + XG) on FCR in the periods of 1–14 and 1–21 days, as this treatment had the highest restriction on metabolizable energy (100 kcal/kg).

Some studies have evaluated the use of phytase associated with carbohydrases, mostly in diets with high quantities of NSPs, such as wheat, rice, and barley-based diets (Ravindran *et al.*, 1999; Cowieson & Adeola, 2005; Olukosi & Adeola, 2008), and the effect of carboydrase had no obvious effect on either P or Ca digestibility (Cowieson & Adeola, 2005). This could be related to the composition of the main cereal ingredient which was corn, a feed ingredient with a low concentration of arabinoxylan (Choct, 1997). Therefore, the potential for xylanase to improve P digestibility was somewhat lower than would be the case in a wheat-based diet.

For some time, it was considered that diets based on corn and soybean meal did not cause digestive issues in poultry, and, thus, the use of exogenous enzymes was considered unnecessary in these cases (Zou *et al.*, 2013). However, these products contain considerable amounts of NSPs, such that using carbohydrases may have a positive impact on animal performance. Schramm *et al.* (2017) affirmed that the presence of phytase, in a complete corn/soy-based diet, has a potentiating effect for exogenous xylanase. In our study, the performance results were improved in broilers fed diets containing nutritional reductions and enzymatic supplementation with carboydrases, showed the action of this enzyme.

Table 3 – Performance of broilers fed diets with different energy, Ca, and AP content and supplemented or not with exogenous enzymes (mean ± standard deviation).

Days	Treatment[1]	BW (g)	DWG (g/broiler/day)	FI (g/broiler/day)	FCR (g/g)
1 – 7	PC	200.11±8.60 ab	21.87±0.99 ab	21.52±0.93 a	0.984±0.02 c
	R_70	192.88±6.78 b	20.85±0.64 c	21.28±0.65 a	1.021±0.02 ab
	R_100	195.40±7.95 ab	21.20±0.99 bc	21.75±0.99 a	1.025±0.02 a
	R_70+P	200.93±5.65 ab	21.99±0.74 ab	21.46±1.18 a	0.983±0.03 c
	R_100+P+XG	202.09±9.93 a	22.15±1.12 a	22.04±1.30 a	0.994±0.02 bc
	*p**	0.008	<0.001	0.323	<0.0001
1 –14	PC	563.27±14.70 a	36.87±0.87 a	42.63±0.95 ab	1.156 ±0.01 d
	R_70	528.05±27.65 b	34.36±1.81 b	41.38±1.55 b	1.205±0.02 ab
	R_100	529.13±18.79 b	34.44±1.18 b	42.06±1.35 ab	1.221±0.02 a
	R_70+P	557.56 ±18.11 a	36.47±1.14 a	42.82±1.08 ab	1.174±0.02 cd
	R_100+P+XG	561.07±25.22 a	36.72±1.64 a	43.49±1.91 a	1.184±0.01 bc
	*p**	<0.0001	<0.0001	0.001	<0.0001
1 –21	PC	1126.43±25.30 a	51.40±1.11 a	63.81±1.13 a	1.241±0.01 d
	R_70	1034.92±38.82 b	47.04±1.75 b	60.89±2.16 b	1.294± 0.02 b
	R_100	1037.69±29.00 b	47.17±1.28 b	62.05±1.68 b	1.315±0.02 a
	R_70+P	1111.24±30.15 a	50.67±1.36 a	63.81±1.36 a	1.259±0.01 cd
	R_100+P+XG	1119.12±36.56 a	51.05±1.63 a	64.91±1.97 a	1.271±0.01 c
	*p**	<0.0001	<0.0001	<0.0001	<0.0001
1 –28	PC	1796.85±38.26 a	62.49±1.34 a	82.87±1.63 ab	1.326±0.01 c
	R_70	1682.51±55.74 b	58.41±1.91 b	79.33±2.63 c	1.358±0.01 b
	R_100	1680.11±52.37 b	58.32±1.79 b	80.92±2.33 bc	1.387±0.02 a
	R_70+P	1788.60±69.71 a	62.20±2.45 a	83.09±1.91 ab	1.336±0.02 c
	R_100+P+XG	1797.91±64.48 a	62.53±2.21 a	84.06±2.54 a	1.344±0.01 bc
	*p**	<0.0001	<0.0001	<0.0001	<0.0001
1 –35	PC	2578.80±60.15 a	72.33±1.72 a	101.37±2.00 ab	1.401±0.02 b
	R_70	2440.97±79.12 b	68.40±2.20b	97.58±3.10 c	1.426±0.02 b
	R_100	2407.25±62.30 b	67.43±1.72 b	98.98±2.62 bc	1.467±0.02 a
	R_70+P	2563.38±62.30 a	71.89±1.76 a	101.86±2.00 a	1.417±0.01 b
	R_100+P+XG	2592.92±93.66 a	72.73±2.62 a	103.22±2.35 a	1.419±0.03 b
	*p**	<0.0001	<0.0001	<0.0001	<0.0001
1 –42	PC	3364.30±67.98 a	78.98±1.60 a	118.44±2.17 bc	1.499±0.03 b
	R_70	3226.84±85.92 b	75.71±2.01 b	115.12±3.18 d	1.520±0.02 ab
	R_100	3219.56±82.94 b	75.53±1.94 b	116.80±2.92 cd	1.546±0.03 a
	R_70+P	3357.18±98.86 a	78.81±2.34 a	120.05±2.89 ab	1.523±0.02 ab
	R_100+P+XG	3385.52±102.98 a	79.48±2.40 a	121.38±2.85 a	1.527±0.02 a
	*p**	<0.0001	<0.0001	<0.0001	<0.001

Body weight (BW); Daily weight gain (DWG); Feed intake (FI); Feed conversion ratio (FCR). [1] Treatments: PC= positive control - basal diet (BD) - corn and soybean meal; R_70 = BD with reductions of 70 kcal/kg and 0.16% Ca and 0.15% AP; R_100 = BD with reductions of 100 kcal/kg and 0.16% Ca and 0.15% AP; R_70 + P = BD with reductions of 70 kcal/kg and 0.16% Ca and 0.15% AP + phytase (500 FTU/kg); R_100 + P + XG = BD with reductions of 100 kcal/kg and 0.16% Ca and 0.15% AP + phytase (500 FTU/kg) xylanase (560 TXU/kg) + glucanase (250 TGU/kg); *probability; **means followed by different lowercase letters in the column differ from each other according to the Tukey's test at 5% probability.

Carcass yield and Production costs

In the evaluation of cold carcass yield, there was no significant difference between treatments (P = 0.21), and the mean values found for each treatment were:(PC) = 79.94±0.83; (R_70) = 79.05±0.89; (R_100) = 79.07±1.39; (R_70 + P) = 79.54±1.07; (R_100 + P + XG) = 79.77±0.77.

The positive control treatment came to a higher production cost (USD/ton chilled carcass), and this cost differed significantly from that of treatment R_100 + P + XG (Figure 1).

The cost analysis showed that one ton of chilled carcasses of broilers fed the positive control diet cost USD 782 to produce, whereas the production cost for broilers fed the R_100+P+XG diet was USD 752, i.e., a reduction of USD 30.0 in the production cost at the end of the process. Treatments containing nutritional reductions without enzyme supplementation (R_70 and R_100) presented intermediate production costs, on matching their means to the PC and R_100 + P + XG.

The use of hybrid phytase (500 FTU/kg) alone is effective to maintain the productive performance of

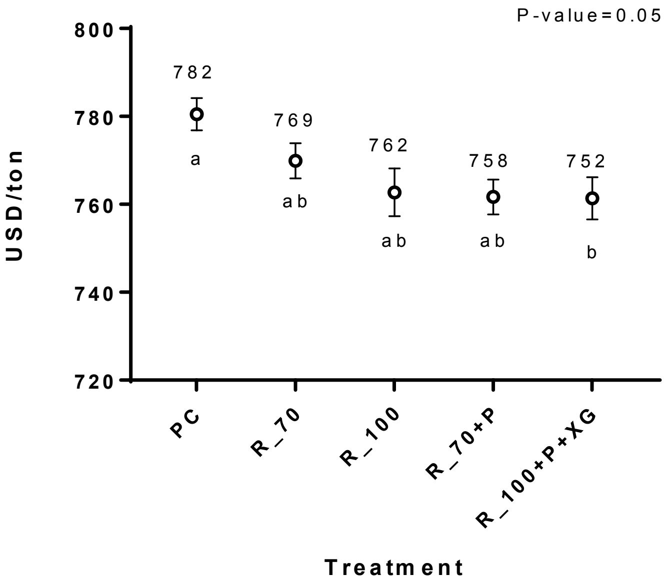

Figure 1 – Production costs of chilled carcasses of broilers fed diets with nutritional reductions supplemented or not with enzymes.

PC = positive control - basal diet (BD) - corn and soybean meal; R_70 = BD with reductions of 70 kcal/kg and 0.16% Ca, and 0.15% AP; R_100 = BD with reductions of 100 kcal/kg, 0.16% Ca and 0.15% AP; R_70+P = BD with reductions of 70 kcal/kg and 0.16% Ca, and 0.15% AP + phytase (500 FTU/kg); R_100+P+XG = BD with reductions of 100 kcal/kg, 0.16% Ca and 0.15% AP + phytase (500 FTU/kg) + xylanase (560 TXU/kg) + glucanase (250 TGU/kg). Means followed by different lowercase letters differ according to Tukey's test (p<0.05).

broilers fed diets with a reduction of up to 70 kcal/kg, 0.16% Ca, and 0.15% AP during a 42-day period. The combination of the hybrid phytase (500 FTU/kg) with xylanase (560 TXU/kg) and glucanase (250 TGU/kg) is effective to maintain the body weight and daily weight gain when there are nutritional reductions in the diet of broilers of 100 kcal/kg, 0.16% Ca, and 0.15% AP.

Broilers fed a specific enzyme diet (500 FTU/kg phytase + 560 TXU/kg xylanase + 250 TGU/kg glucanase) together with a diet with a nutritional reduction of 100 kcal/kg, 0.16% Ca, and 0.15% AP for a period of 42 days showed the lowest production cost in USD/ton chilled carcass.

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