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Use of a Multi-Carbohydrase Complex with Phytase in Diets of Broiler Chickens containing Ingredients of Animal Origin

Uso de um Complexo Multi-Carboidrase com Fitase em Dietas de Frangos de Corte Contendo Ingredientes de Origem Animal

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Abstract

Currently, the use of exogenous enzymes in the diet of broilers has grown, not only in diets based on corn and soybean meal, but also in diets with proteins of animal origin. The objective of this study was to evaluate the effect of supplementing a multi-carbohydrase complex (xylanase, glucanase, other carbohydrases) with phytase (MCCP) in broiler diets containing animal ingredients on performance, litter quality, jejunum morphometry and feed cost. Three treatments were evaluated: T1 - positive control diet: reference diet; T2 - negative control diet + MCCP; T3 - negative control diet (reference ration minus the MCCP nutritional matrix), with five replicates per treatment, totaling 15 experimental units of 15 female chicks each for an experimental period of 47 days. It was found that the T1 animals showed better feed conversion when compared to the T2 and T3 in the period from one to 21 days. In addition, greater weight at 28 days was found for T1compared to T3. The greater villus height of the jejunum at seven days was verified for the T3 animals concerning the T2 and T1 treatments. There were no differences between treatments in relation to the litter quality and the cost of the feed to produce one kilogram of broiler chicken. It is concluded that the supplementation of the MCCP in broiler chicken diets with ingredients of animal origin does not improve the performance, does not modify the jejunum morphometric, litter quality, and cost parameters evaluated.

Keywords: Feed Conversion. Glucanase. Phytase. Villus Height. Xylanase.

Resumo

Atualmente, tem crescido o uso de enzimas exógenas na dieta de frangos de corte, não só em rações à base de milho e farelo de soja, mas também em rações com proteínas de origem animal. O objetivo deste estudo foi avaliar o efeito da suplementação de um complexo multi-carboidrase (xilanase, glucanase, outras carboidrase) com fitase (CMCF) em dietas de frangos de corte contendo ingredientes de origem animal sobre o desempenho, qualidade da cama, morfometria do jejuno e custo da alimentação. Foram avaliados três tratamentos: T_1 - dieta controle positivo: dieta referência; T_2 - dieta controle negativo + CMCF; T_3 - dieta controle negativo (ração referência menos a matriz nutricional CMCF), com cinco repetições por tratamento, totalizando 15 unidades experimentais de 15 pintos fêmeas cada, por um período experimental de 47 dias. Verificou-se que os animais T_1 apresentaram melhor conversão alimentar quando comparados aos T_2 e T_3 no período de um a 21 dias. Além disso, maior peso aos 28 dias foi encontrado para T_1 em relação a T_3. Maior altura de vilo do jejuno aos sete dias foi verificada para os animais T_3 em relação aos tratamentos T_2 e T_1. Não houve diferenças entre os tratamentos em relação à qualidade da cama e ao custo da ração para produzir um quilo de frango de corte. Conclui-se que a suplementação do CMCF em rações de frangos de corte com ingredientes de origem animal não melhora o desempenho, não altera os parâmetros morfométricos do jejuno, a qualidade da cama e o custo avaliado.

Palavras-chave: Altura de Vilo. Conversão Alimentar. Fitase. Glucanase. Xilanase.

1 Introduction

The production and consumption of chicken meat has increased in the last years, because of the fast production cycle and a more affordable price compared to other animal proteins on the market. In this context, Brazil stands out in the global market, being the third largest producer of chicken meat, also occupying the position of the largest exporter, allocating 32,17% of its production for export in 2021 (ABPA, 2022).

Due to the great economic importance, the poultry market is in constant technological evolution, so to maintain this activity competitive, companies have adhered to new technologies related to management, genetics, sanity, and nutrition, since the latter represents about 70% of the production cost (CARVALHO *et al.,* 2009; SARANGI *et al.*, 2016),

Currently, broiler chicken feed is formulated based on corn and soybean meal. However, these ingredients have some antinutritional factors that affect the absorption of some nutrients, such as nonstarch polysaccharides or substances that are not digested by the enzymes of the gastrointestinal tract of birds (BARBOSA FILHO *et al*., 2018). Thereby, an exogenous enzyme emerged as an alternative additive to reduce the production costs, and has been used for more than 20 years in poultry diets, to present also better digestibility and absorption of nutrients, becoming a routine practice (AMERAH *et al*., 2011; RAVINDRAN, 2013). On the other hand, the efficacy of enzymes in diets varies according to the raw material used, the antinutritional factors present, and their inclusion levels (MARTINS, 2013).

Xylanases, glucanases, pectinases, cellulases, proteases, amylases, phytases, galactosidases and lipases, they are among the main enzymes used in poultry supplementation (LUCIO *et al.,* 2021). Exogenous enzymes have some functions which are to increase the digestibility, to remove the antinutritive factors derivative from corn and soybeans and to improve the nutrient availability, in addition to a modification in the intestinal microbiota and the better protein solubility and digestibility (LIMA *et al.*, 2007; COWIESON; RAVIDRAN, 2008).

Related to phytase, Parra-Martin *et al*. (2015) says that its use contribute to the greater absorption of phosphorus in the feed, in function of increased hydrolysis of phytic acid, which in turn decreases the inclusion of dicalcium phosphate in the diet. Selle and Ravindran (2007) related that phytase in combination with carbohydrase and protease has additive effects in deficient broiler chicken diets. In the literature, several studies have been published with the use of exogenous enzymes in diets based on corn and soybean meal. Promising results were observed by Torres *et al.* (2003), Costa *et al.* (2004), Carvalho *et al.* (2009), and others which no zootechnical gains were found with the use of these enzymes (CARDOSO *et al.*, 2011; FISCHER *et al.*, 2002).

The use of ingredients of animal origin is widely used in broiler production; however, few studies evaluate the use of exogenous enzymes in this type of diet. According to Cong, Lee and Adeola (2011) β-mannanase supplementation in corn-soy bean meal diet increases growth performance and energy utilization in broilers. Therefore, the objective of this work is to evaluate the efficacy of a multi-carbohydrate complex with phytase in diets of broilers containing ingredients of animal origin in the parameters of zootechnical performance, intestinal morphometry and litter quality.

2 Material and Methods

The experiment was executed at the Experimental Broiler House in the Paranaense University - Umuarama, located in the Northwest region of Paraná state under latitude 23º46'00,19" and longitude 53º16'26.39" after approval by the Animal Experimentation Ethics Committee of the Paranaense University, under protocol 35335/2018.

Were used 225 *Cobb Slow* female chicks from a commercial hatchery located in the southwest region of the Paraná state, for 47 days of experimental period, distributed in a completely randomized delimitation with three treatments: T_1 - positive control diet: reference diet; T_2 - negative control diet plus multi carbohydrase complex with phytase (MCCP); T_3 - negative control diet (reference diet minus the nutritional matrix of MCCP - 50 kcal ME/kg, 0.017% digestible lysine (reduction of other amino acids proportional and relation with lysine), 0.076% calcium and 0.09% available phosphorus), with five replicates per treatment, totaling 15 experimental units of 15 birds each.

The MCCP used was based on xylanase (endo-1,4-b-xylanase: 2.500 units/g), glucanase (endo-1,3(4)-b-glucanase: 1.720 units/g), other carbohydrates (endo-1,4-B-glucanase (cellulase): 240 units/g, arabinofuranosidase: 9.200 units/g) and 6-phytase from *Schizosaccharomyces pombe* (1.000 units/g), being added at the dosage of 500g/ton of feed.

The diet was formulated according to the nutritional recommendations of agroindustry in the region, and the feeding program consisted of five phases: pre-initial: one to seven days; initial: eight to 14 days (Table 1); growth 1: 15 to 21 days; growth 2: 22 to 35 days (Table 2); final: 36 to 47 days of age (Table 3).

Table 1 - Percentage and calculated composition of experimental diets (pre-initial: one to seven days; initial: eight to 14 days of age)

Ingredients	Diets					
	Pre-initial T_1	Pre-initial T_2	Pre-initial T_3	Initial T_1	Initial T_2	Initial T_3
Corn	54.32	56.33	56.38	58.95	60.53	60.58
Soybean meal	35.27	35.17	35.17	31.44	31.89	31.89
Meat meal	6.10	5.01	5.01	5.40	4.00	4.00
Offal oil	2.36	1.32	1.32	2.24	1.33	1.33
DL-Methionine 99%	0.41	0.40	0.40	0.39	0.37	0.37
L-Threonine 98%	0.13	0.11	0.11	0.13	0.11	0.11
Lysine HCL	0.41	0.41	0.41	0.39	0.37	0.37
Limestone	0.10	0.28	0.28	0.24	0.52	0.52
MCCP		0.05			0.05	
Salt	0.42	0.44	0.44	0.43	0.45	0.45
Choline Chloride	0.09	0.09	0.09	0.09	0.09	0.09
Nicarbazin 25%	0.04	0.04	0.04	0.04	0.04	0.04
Premix	0.25	0.25	0.25	0.25	0.25	0.25
Adsorvent*	0.10	0.10	0.10			
Total	100	100	100	100	100	100

Ingredients	Diets					
	Pre-initial T_1	Pre-initial T_2	Pre-initial T_3	Initial T_1	Initial T_2	Initial T_3
	Nutritional composition					
ME (kcal/kg)	3.018	3.018	2.970	3.058	3.058	3.010
Crude Protein %	23.96	23.91	23.56	22.20	22.20	21.85
Dig. Lysine %	1.32	1.32	1.30	1.21	1.21	1.19
Dig. Methionine %	0.71	0.70	0.70	0.68	0.66	0.66
Dig. Cystine %	0.28	0.29	0.28	0.27	0.28	0.27
Dig. Met.+Cys.%	0.98	0.96	0.96	0.93	0.92	0.92
Dig. Threonine %	0.83	0.83	0.81	0.79	0.79	0.77
Dig.Tryptophan %	0.23	0.23	0.23	0.21	0.22	0.21
Calcium %	0.95	0.95	0.87	0.90	0.90	0.82
Phosphorus available %	0.50	0.52	0.43	0.45	0.45	0.36
Sodium %	0.22	0.22	0.22	0.21	0.21	0.21
DEB (mEq/kg)	244	244	244	225	225	225

MCCP: Multi carbohydrase complex + phytase - xylanase (endo-1,4-b-xylanase: 2.500 units/g), glucanase (endo-1,3(4)-b-glucanase: 1.720 units/g), other carbohydrates (endo-1,4-B-glucanase (cellulase): 240 units/g, arabinofuranosidase: 9.200 units/g) and 6-phytase from *Schizosaccharomyces pombe* (1.000 units/g). Premix composition (per kilogram of diet): vitamin A 5,200 IU; vitamin D_3 1,400 IU; vitamin E, 24.00 IU; vitamin K_3 2.00 mg; vitamin B_1 1.60 mg; vitamin B_2 4.40 mg; vitamin B_6 2.28 mg; vitamin B_{12} 14 mg; folic acid 1.20 mg; niacin 20.00 mg; pantothenic acid 8.0 mg; biotin 100 mg; manganese 32.00 mg; iron 20.00 mg; copper 6.00 mg; zinc 28.00 mg; selenium 120.00 mg; iode 4.80 mg; antioxidant BHT 40 g. * Mycotoxin adsorbent based on bentonite (690g/kg), yeast cell wall (300g/kg) and silymarin (500mg/kg); DEB: Diet Eletrolyte Balance.

Source: resource data.

Table 2 - Percentage and calculated composition of experimental diets (grower 1: 15 to 21 days; grower 2: 22 to 35 days)

Ingredients	Diets					
	Grower 1			Grower 2		
	T_1	T_2	T_3	T_1	T_2	T_3
Corn	64.37	65.87	65.92	71.47	73.06	73.11
Soybean meal	26.52	27.02	27.02	20.62	21.05	21.05
Meat meal	5.04	3.63	3.63	3.14	1.74	1.74
Offal oil	2.30	1.40	1.40	2.01	1.10	1.10
DL-Methionine 99%	0.34	0.32	0.32	0.24	0.22	0.22
L-Threonine 98%	1.00	0.08	0.08	0.06	0.04	0.04
Lysine HCL	0.33	0.31	0.31	0.32	0.30	0.30
Sodium bicarbonate	0.04	0.05	0.05			
Limestone	0.24	0.52	0.52	0.57	0.85	0.85
MCCP		0.05			0.05	
Feather flour				0.80	0.80	0.80
Salt	0.34	0.35	0.35	0.42	0.44	0.44
Choline Chloride	0.09	0.09	0.09	0.08	0.08	0.08
Nicarbazin	0.04	0.04	0.04			
Salinomycin				0.03	0.03	0.03
Premix	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100
	Nutritional composition					
ME (kcal/kg)	3.118	3.118	3.070	3.169	3.169	3.120
Crude Protein %	20.05	20.08	19.72	17.50	17.50	17.14
Digestible.Lysine %	1.06	1.06	1.04	0.90	0.90	0.88
Dig. Methionine %	0.61	0.59	0.59	0.48	0.47	0.46
Dig. Cystine %	0.25	0.26	0.25	0.24	0.25	0.24
Dig. Met.+Cys.%	0.84	0.83	0.83	0.71	0.70	0.70
Dig. Threonine %	0.69	0.69	0.67	0.58	0.58	0.57
Dig. Tryptophan %	0.18	0.19	0.19	0.15	0.16	0.16
Calcium %	0.84	0.84	0.76	0.84	0.84	0.76
Phosphorus available %	0.42	0.42	0.33	0.42	0.42	0.33
Sodium %	0.19	0.19	0.19	0.19	0.19	0.19
DEB (mEq/kg)	206	206	206	172	172	172

MCCP: Multi carbohydrase complex + phytase - xylanase (endo-1,4-b-xylanase: 2.500 units/g), glucanase (endo-1,3(4)-b-glucanase: 1.720 units/g), other carbohydrates (endo-1,4-B-glucanase (cellulase): 240 units/g, arabinofuranosidase: 9.200 units/g) and 6-phytase from *Schizosaccharomyces pombe* (1.000 units/g). Premix composition (per kilogram of diet): vitamin A 5,200 IU; vitamin D_3 1,400 IU; vitamin E, 24.00 IU; vitamin K_3 2.00 mg; vitamin B_1 1.60 mg; vitamin B_2 4.40 mg; vitamin B_6 2.28 mg; vitamin B_{12} 14 mg; folic acid 1.20 mg; niacin 20.00 mg; pantothenic acid 8.0 mg; biotin 100 mg; manganese 32.00 mg; iron 20.00 mg; copper 6.00 mg; zinc 28.00 mg; selenium 120.00 mg; iode 4.80 mg; antioxidant BHT 40 g. DEB: Diet Eletrolyte Balance

Source: resource data.

Table 3 - Percentage and calculated composition of experimental diets (final: 36 to 47 days of age)

Ingredients	Diets		
	Finisher		
	T_1	T_2	T_3
Corn	75.29	76.78	76.83
Soybean Meal	15.04	15.55	15.55
Meat Meal	4.13	2.72	2.72
Offal oil	1.45	0.55	0.55
DL-Methionine 99%	0.20	0.19	0.19
L-Threonine 98%	0.09	0.07	0.07
Lysine HCL	0.42	0.40	0.40
Sodium bicarbonate	0.06	0.08	0.08
Limestone	0.26	0.54	0.54
MCCP	---	0.05	---
Offal flour	1.20	1.20	1.20
Feather Flour	1.20	1.20	1.20
Salt	0.32	0.33	0.33
Choline Chloride	0.06	0.06	0.06
Salinomycin	0.02	0.02	0.02
Premix	0.25	0.25	0.25
Total	100	100	100
Nutritional composition			
ME (kcal/kg)	3.200	3.200	3.150
Crude Protein %	16.79	16.78	16.51
Dig. Lysine %	0.87	0.87	0.85
Dig.Methionine %	0.43	0.42	0.42
Dig.Cystine %	0.23	0.25	0.24
Dig. Met.+Cys.%	0.66	0.64	0.64
Dig.Threonine %	0.57	0.57	0.55
Dig. Tryptophan %	0.13	0.14	0.13
Calcium %	0.76	0.76	0.68
Phosphorus available %	0.38	0.38	0.29
Sodium %	0.18	0.18	0.18
DEB (mEq/kg)	160	160	160

MCCP: Multi carbohydrase complex + phytase - xylanase (endo-1,4-b-xylanase: 2.500 units/g), glucanase (endo-1,3(4)-b-glucanase: 1.720 units/g), other carbohydrates (endo-1,4-B-glucanase (cellulase): 240 units/g, arabinofuranosidase: 9.200 units/g) and 6-phytase from *Schizosaccharomyces pombe* (1.000 units/g). Premix composition (per kilogram of diet): vitamin A 5,200 IU; vitamin D_3 1,400 IU; vitamin E, 24.00 IU; vitamin K_3 2.00 mg; vitamin B_1 1.60 mg; vitamin B_2 4.40 mg; vitamin B_6 2.28 mg; vitamin B_{12} 14 mg; folic acid 1.20 mg; niacin 20.00 mg; pantothenic acid 8.0 mg; biotin 100 mg; manganese 32.00 mg; iron 20.00 mg; copper 6.00 mg; zinc 28.00 mg; selenium 120.00 mg; iode 4.80 mg; antioxidant BHT 40 g. DEB: Diet Eletrolyte Balance.
Source: resource data.

The performance was evaluated by determining weight gain (g), weight (g), feed intake (g), and feed conversion (g/g), and the birds were weighed weekly. The average weight gain (WG) was determined by the difference between the final and initial weights of each experimental unit for periods one to seven, seven to 21, 21 to 35, 35 to 47, and one to 47 days of age. The average feed intake (FI) was calculated by the difference between the feed provided and the leftovers in the buckets and feeders in each of the periods evaluated and mentioned above. Feed conversion (FC) was calculated by dividing feed intake by weight gain of birds, and in the calculation of FI and FC, the weight of the feed in the day of bird mortality was considered according to Sakomura and Rostagno (2007).

At seven days old and until the end of the experimental period, one bird from each experimental unit was randomly chosen to evaluate the intestinal morphometry of the jejunum. The animals were referred for euthanasia using anesthetic overdose: intramuscular xylazine (IM) - 4mg/kg and intravenous thiopental (IV) - 25 mg/kg. Then, a segment of two centimeters from jejunum (anterior to *Meckel's diverticulum)* of each bird was submitted to histological routine.

The samples were washed with 0.9% saline solution to remove all intestinal contents, fixed in neutral formaldehyde solution buffered at 10% for a minimum period of 48 hours and then dehydrated in a series of increasing concentrations of alcohols, diaphanized in xylol and included in paraffin. After were obtaining semi-serial longitudinal cuts of five micrometers thickness, which were subsequently stained with hematoxylin and eosin (HE), to measure villus height (µm), crypt depth (µm) and villus: crypt ratio (µm/(µm)). For analysis, images were obtained through a digital camera attached to a microscope Nikon Eclipse E200® (4 x and 10 x) connected to Motic® (Moticam 5MP) image analysis system for subsequent measurement of 30 villi and 30 crypts of each animal.

At the end of the experimental period, a litter sample of each boxing was collected for the determination of dry matter, pH, ash and phosphorus content (P). The methodology of analysis of dry matter and ash was described by Silva e Queiroz (2006). The pH analysis according to the methodology by Benabdeljelil e Ayachi (1996) and the phosphorus content according to Silva (2009).

To calculate the cost of feed required to produce one kilogram of broiler chicken (CKC), it was assumed that the total feed consumed by the chickens during the rearing period corresponded to 3.1% of the total feed in the pre-initial phase, 8.1% in the initial phase, 13.9% in growth phase 1, 34.6% in growth phase 2 and 40.3% in the final phase. Then, the price of the kilogram of each feed was multiplied by its percentage of participation in the total consumption, and the results were added to obtain the price of the kilogram of "combined" feed (PKR), according to the methodology described by Kamwa (1997). Then, the calculated PKR value was multiplied by the feed conversion of the period from one to 35 days of age and in the total production (one to 47 days of age), thus obtaining the CKC.

Regarding statistical analysis, the data were analysed from statistical software package Bioestat 5.0 (Mamirauá Institute, Belém, Pará, Brazil). The data were first analyzed with normality and homogeneity of the variance respectively, by *Lilliefors* and *Levene* tests. As the data presented normal distribution and homogeneity of variance, the results were submitted to variance analysis, and when the averages were relevant it was compared by *Tukey* test. For all tests, a significance level of 5% was considered.

3 Results and Discussion

There were no differences in feed intake and weight gain in periods from one to seven, seven to 21, 21 to 35, 35 to 47, and one to 47 days of age (Table 4). However, it was found that the animals of the positive control group (reference diet) presented better feed conversion (*P*<0.01) in relation to the negative control groups with MCCP and positive control in the period from one to 21 days old.

The fact that the animals in the negative control group presented feed conversion similar to the group supplemented with the MCCP may be related to the fact that nutritional restriction caused by an increase in the pancreatic activity and intestinal enzymes, according to Pinheiro *et al.* (2004) after a quantitative nutritional feed restriction there is an increase in the activity of intestinal enzymes immediately after the nutritional restriction period, and as in the present study, the nutritional restriction continued through the whole production period of this treatment, such activity may have remained elevated until the end of the growing process.

Table 4 - Mean weight gain (kg), feed intake (kg) and feed conversion (kg/kg) of broilers at different periods (one to seven, one to 21, one to 35, one to 42 and one to 47 days of age) receiving diet with animal ingredients and supplemented or not with multi carbohydrase + phytase complex (MCCP)

Treatments	Weight gain (kg)	Feed intake (kg)	Feed conversion (kg/kg)
	One to seven days old		
PC	0.145	0.151	1.043
NC + MCCP	0.142	0.149	1.049
NC	0.144	0.151	1.052
MSE	0.003	0.002	0.012
CV%	4.67	3.25	2.57
P Value	0.834	0.848	0.913
	Seven to 21 days old		
PC	0.844	1.135	1.344[b]
NC + MCCP	0.833	1.145	1.375[a]
NC	0.806	1.103	1.369[a]
MSE	0.009	0.011	0.005
CV%	2.55	2.27	0.89
P Value	0.055	0.085	<0.01
	21 to 35 days old		
PC	0.867	1.803	2.085
NC + MCCP	0.876	1.823	2.085
NC	0.800	1.693	2.121
MSE	0.031	0.039	0.034
CV%	8.34	4.98	3.63
P Value	0.254	0.121	0.693
	35 to 47 days old		
PC	1.054	2.114	2.010
NC + MCCP	0.950	1.993	2.099
NC	1.053	2.109	2.025
MSE	0.045	0.066	0.056
CV%	9.70	7.13	6.14
P Value	0.268	0.601	0.569
	One to 47 days old		
PC	2.930	5.196	1.774
NC + MCCP	2.802	5.110	1.825
NC	2.803	5.054	1.806
MSE	0.070	0.105	0.021
CV%	5.52	4.47	2.68
P Value	0.597	0.671	0.330

PC - Positive control (reference ration); NC + MCCP - Negative control + multi carbohydrase complex + phytase; NC – Negative control (reduction 50 kcal ME/kg, 0.017% digestible lysine (reduction of other amino acids proportional to its relationship with lysine), 0.076% calcium and 0.09% available phosphorus). MSE: Mean Standard Error; CV%: Coefficient of variation.
Source: resource data.

Different studies evaluated the zootechnical performance of broilers using different enzymatic complexes in diets based on corn and soybean meal (FERNANDES *et al.*, 2010; FISCHER *et al.*, 2002; KUTLU *et al.*, 2019; TORRES *et al.*, 2003). However, no beneficial effects of its supplementation were verified.

Kutlu *et al.* (2019), evaluating the effect of a multienzymes complex in diets based on corn and soybean meal, whose negative control diet presented 3% fewer nutrients compared to the standard diet (energy, protein, digestible amino acids, calcium, and available phosphorus), the authors did not verify the effect of supplementation of the multienzymes complex in relation to weight gain, feed intake and feed conversion in the period from one to 35 days.

Similarly, in diets with different levels of soybean oil in the diet with or without supplementation of an enzymatic complex composed of xylanase, amylase and protease, Pucci *et al.* (2003), verified that adding oil to the feed improved feed conversion, however, the use of enzymes did not improve in this parameter.

On the other hand, in work carried out by Rios *et al.* (2017), the effect of an enzymatic complex composed mainly of xylanase and glucanase in diets based on corn and soybean meal was evaluated, having a control diet and diets with reductions of 78 and 119 kcal of kg and 3 and 6 % of digestible amino acids with and without supplementation of the enzymatic complex, the authors verified that feed conversion in the period of one to 42 days of age of treatments with reduction of nutritional matrix and supplemented with the enzymatic complex did not differ from the control treatment, demonstrating that the enzyme was effective in maintaining the feed conversion index.

In a similar way, Cowieson and Ravindran (2008) evaluating the effects of an enzymes complex compost by xylanase, amylase and protease in diets based on corn and soybean meal, in positive control diets and negative control diets (containing 63 MJ/kg of apparent metabolizable energy (AME) and 3% less amino acids than the positive control diet) the authors concluded that the supplementation of both, the positive and negative control diets with the enzyme improved weight gain and feed efficiency compared with the unsupplemented diets. In the case of the negative control, supplemental enzyme improved performance to that of the unsupplemented positive control diet. There was no interaction between diet and enzyme for either weight gain or FCR, suggesting similar beneficial responses regardless of the nutrient density of the diet.

Malathi and Devegowda (2001), *evaluated in vitro the* action of enzymatic complexes and the levels of pentoses, celluloses, pectin, and nonstarch polysaccharides (NSP) of different raw materials used in the formulation of broiler diets, among them, corn that presented lower levels of NSP than other ingredients such as defatted rice bran, peanut meal and soybean meal, thus having little substrate for the action of enzymes, while with soybean meal the enzymes xylanase + cellulase obtained better digestibility results because it presented higher levels of NSP.

Wu *et al.* (2004), evaluating the supplementation of xylanase enzymes (1700 xylanase/g units alone and 1000 units of xylanase/g in combination with phytase) and phytase (1080 units of phytase/g alone and 500 units of phytase/g in mixture with xylanase) in wheat-based diets and soybean meal in broilers during the 21-day period, and verified improved weight gain, feed conversion and feed intake in treatments supplemented with enzymes alone or in combination, compared to the group that did not receive the enzymes.

It should be noted that in the present study the diets were formulated with corn and soybean meal. It was also used animal protein source (meat meal, offal meal, and feather meal), which may have influenced the effectiveness of the use of MCCP, whose carbohydrates mainly act on the degradation of NSP, improving nutrient absorption (SIMON, 1998) in diets of plant origin, since the NSP are present only in these types of ingredients.

The average weight of the birds during the weeks (seven to 47 days of age) did not differ between treatments (Table 5) except for the average weight at 28 days of age, where the negative control diet presented lower weight in relation to the other treatments. This difference can be explained by the fact that the negative control treatment presents a 50 kcal reduction of metabolizable energy (ME) in the composition of the diet and amino acids, calcium, and phosphorus. And the fact that it did not occur with the treatment supplemented with MCCP demonstrates a beneficial result of its supplementation.

Table 5 - Average weight at seven, 14, 21, 28, 35, 42 and 47 days of age of broilers receiving diet with ingredients of animal origin and supplemented or not with multi carbohydrase + phytase complex (MCCP)

Age (days)	Treatments			MSE	VC%	P Value
	PC	NC +MCCP	PC – MCCP matrix			
7	0.188	0.186	0.187	0.003	3.83	0.891
14	0.525	0.518	0.508	0.005	2.11	0.110
21	1.032	1.019	0.992	0.012	2.56	0.116
28	1.400[a]	1.385[ab]	1.315[b]	0.021	3.37	0.047
35	1.900	1.894	1.793	0.036	4.35	0.135
42	2.576	2.497	2.478	0.050	4.42	0.588
47	2.954	2.845	2.845	0.070	5.41	0.508

PC - Positive control (reference ration); NC + MCCP - Negative control + multi carbohydrase complex + physiasis; PC – Positive control minus the nutritional matrix of multi carbohydrase + phytase complex(50 kcal ME/kg, 0.017% digestible lysine (reduction of other amino acids proportional to its relationship with lysine), 0.076% calcium and 0.09% available phosphorus). Means followed by different letters in the line differ by the *Tukey test* (P<0.05); MSE: Mean Standard Error; VC%: Coefficient of variation %.
Source: resource data.

The results found in the present study are similar to those found by Fischer *et al.* (2002), when evaluating diets based on corn and soybean meal with and without the addition of a commercial enzymatic complex (protease, amilases, cellulases), which the negative control (diet five percent lower in energy, protein and aminoacids, without enzyme addition), it was found that at 35 days old the negative control diet presented better feed conversion than the negative diet supplemented with the enzymatic complex.

The intestinal morphometry of the jejunum, evaluated by determining the villus height, crypt depth and villus ratio: crypt is presented in Table 6. There was only a higher villus height for the animals of the negative control treatment in relation to other treatments at seven days old. However, at 47 days old, no difference (*P*>0.05) was verified between treatments for the morphometric variables evaluated.

This higher villus height for the broiler chickens of the treatment that received less nutritional level (less 50 kcal of metabolizable energy, 0.017% of digestible lysine, 0.076% of calcium, and 0.09% of phosphorus), i.e., animals that did not receive MCCP at seven days of age may be an attempt by the animal's intestine to compensate the lower nutritional level, thus increasing the villus height at a stage that the animal is not yet with the mature jejunum, since according to Uni *et al.* (1999), the villus height of the jejunum reaches its growth at 10 days of age.

Table 6 - Average villus height (μm), crypt depth (μm) and villus ratio: crypt (μm/μm) of the jejunum of broilers at seven and 47 days of age receiving diet with ingredients of animal origin and supplemented or not with multi carbohydrase + phytase complex (MCCP)

Treatments	Villus height μm	Crypt depth μm	Villus ratio: crypt μm/μm
	Seven days old		
PC	457.778[b]	79.684	5.776
NC + MCCP	461.974[b]	90.735	5.181
NC	535.318[a]	94.551	5.698
Mean Standard Error	18.994	5.033	0.334
VC%	8.35	11.95	12.70
P Value	0.039	0.212	0.568
	47 days old		
PC	1387.232	210.8411	6.538
NC + MCCP	1301.944	199.7799	6.533
NC	1332.165	221.4906	6.164
Mean Standard Error	102.766	9.891	0.507
VC%	16.97	10.26	17.74
P Value	0.861	0.549	0.847

PC - Positive control (reference ration); NC + MCCP - Negative control + multi carbohydrase complex + phytase; NC – Negative control (reduction of 50 kcal ME/kg, 0.017% digestible lysine (reduction of other amino acids proportional to its relationship with lysine), 0.076% calcium and 0.09% available phosphorus); Means followed by different letters in the column differ by the *Tukey test* (P<0.05).
Source: resource data.

In a meta-analysis by Jong *et al.* (2017), regarding the effect of feed and water restriction on several parameters, including intestinal development, the authors found that the length of the duodenum, the jejunum is ileum, is in many cases numerically smaller, but not in the long term. However, although some studies show a negative effect on the villus height, the restriction showed an increase in the villus height, corroborating the present work in many studies.

Similar results were found by Wu *et al.* (2004), which did not observe differences in villus height and crypt depth of broilers' jejunum at 21 days old receiving diets supplemented with xylanase and phytase individually or in association.

According to Kuzmuk *et al.* (2005), the parameters villus height and crypt depth are essential to evaluate the intestinal health, and the villus: crypt ratio is an indicator of digestive capacity since, according to the authors, the increase in this parameter means a greater degree of the intestine to absorb nutrients from the diet.

Different of this study, Zou *et al.* (2013) evaluating the effects of adding β-mananase and α-galactosidase enzymes, and conjoint supplementation of xylanase and β-glucanase in diets based on corn and soybean meal, the authors verified that the addition of xylanase and β-glucanase increased the length of ileum villus at 21 days old and the crypt depth at 42 days old when compared with a diet without enzymes supplementation, however, in the duodenum, it was not verified difference in the villus height and crypt depth and relation of villus: crypt ratio when compared these treatments with 21 and 42 days old.

In the literature, other authors obtained results partially similar to those found in this study as Oliveira *et al.* (2009), who found higher villus height in diets containing mannan oligosaccharides, there was no difference when the enzymatic complex composed of cellulase, protease, and amylase was added, as well as with Yasar & Forbes (2000). They did not find an increase in the villus height of the jejunum of broilers at 28 days old in diets supplemented with enzymatic complex composed of xylanase, glucanase, and protease.

There were no differences between treatments for the following variables: dry matter, ash, pH, and phosphorus from broiler litter (Table 7).

Table 7 - Mean dry matter (%), ash (%), pH and phosphorus (mg/kg) of broiler litter at 47 days of age receiving diets supplemented or not with multi carbohydrase complex + phytase (MCCP)

Treatments	Dry matter (%)	Ashes (%)	pH	Phosphorus mg/kg
PC	72.16	8.48	6.94	14.42
NC + MCCP	70.28	8.49	7.13	14.67
NC	70.46	8.09	7.21	14.16
Mean	70.97	8.36	7.09	14.42
CV%	4.54	11.20	4.31	17.65
MSE	1.44	0.42	0.14	1.13
P value	0.6196	0.5349	0.3984	0.9536

PC - Positive control (reference ration); NC + MCCP - Negative control + MCCP; NC – Negative control (reduction of 50 kcal ME/kg, 0.017% digestible lysine (reduction of other amino acids proportional to its relationship with lysine), 0.076% calcium and 0.09% available phosphorus); VC%: Coefficient of Variation; MSE: Mean Standard Error; Not significant by ANOVA.
Source: resource data.

In a study by Laurentiz *et al.* (2007), the effect of phytase enzyme (100grams/tons of feed) was evaluated in broilers with a reduction of phosphorus levels in the different growing phase. As a result, the authors did not find differences in the percentage of litter dry matter. The means varied from 69% to 71%, which is close to what was found in the present study. Although, the phosphorus contents present in the litter were lower in the groups with reductions in the levels of phosphorus available plus phytase enzyme differing statistically from the control treatment, which the phosphorus levels in the litter were higher, differing from those found in the present study.

The phytase use in diets of broiler chickens associate or not with another enzymes with the objective to improve the use of phosphorus and to reduce the excretion is very well known (DILGER *et al.*, 2004; GAUTIER *t al.*, 2018). According to Cancelli *et al.* (2017), the use of enzymes helps to reduce the excretion of chemical elements, which would bring as benefits the reduction of damage to the environment caused by the elements excreted in the litter. However, the authors did not find a reduction in the percentage of ash with the inclusion of different protease levels and inclusion of the same level of phytase. The phytase supplementation in diets with low non phytic phosphorus (NPP) increased the digestibility of phosphorus (P), that reduced the levels of phosphorus in the excretion (GAUTIER *et al*., 2018).

Lima *et al.* (2007), reported that the enzyme glucanase reduces the water content in the excretes, leading to reduced litter moisture. On the other hand, Zou *et al.* (2013), did not verified reduction of water contents in the broiler chickens excretes that received feed supplementation with xylanase and β-glucanase when compared with the animals that did not receive the enzymes. In the present study, the water content of the excreta was not evaluated, but the litter, thus, the true effect of the enzyme may have been masked by the absorptive capacity of the shaving that was used in the present study.

Regarding the cost of the feed, it was found that the feed added from the MCCP had a lower cost/kg (PKD 1-47 days US $ 0.158) when compared to the PC diet (PKD 1-47 days US $ 0.161) (Table 8), with a reduction of 1.89%. However, this did not reflect the cost of feed required to produce one kilogram of chicken. On the other hand, it should be noted that there was no difference between treatments ($P > 0.05$) in the cost of feed required to produce one kilogram of broiler chicken (Table 8). This result may be related to the lower weight gain of the animals in the treatment that received the MCCP when compared to the PC diet (Table 4).

Table 8 - Price of kilogram of "combined" diets (PKD) and average feed cost to produce one kilogram of chicken (CKC) in broilers receiving diet with ingredients of animal origin and supplemented or not with multicarbohydrate + phytase complex (MCCP)

Treatments	1 to 35 days		1 to 47 days	
	PKD U$	CKC U$*	PKD U$	CKC U$*
PC	0.094	0.155	0.161	0.286
NC +MCCP	0.093	0.157	0.158	0.288
NC	0.092	0.155	0.157	0.283
P value	---	0.5391	---	0.6460

PC - Positive control (reference ration); NC + MCCP - Negative control + MCCP; NC – Negative control (reduction of 50 kcal ME/kg, 0.017% of digestible lysine (reduction of other amino acids proportional to its relationship with lysine), 0.076% calcium and 0.09% of available phosphorus). PKD: Price of the kilogram of the "combined" rations, i.e. the proportional sum of the rations of the respective phases, supplied to the chicken during the rearing period. CKC: Feed cost to produce one kilogram of chicken. * Value expressed as means and not significant by ANOVA.
Source: resource data.

Results found by Fernandes *et al.* (2010), showed that diets with reduced energy levels without the addition of enzymes presented lower production costs. On the flip side, Toledo *et al.* (2007), when evaluating an enzymatic complex based on glucanase, xylanase, and cellulase to the broiler chicken diet, obtained a savings of 7.02% in relation to the standard diet. In the same way, diets with the addition of xylanase and β-glucanase reduce the feed cost per kg of body weight gain, increasing the profits in relation to the negative control diet (ME reduction of 100 kcal/kg) and positive control (normal ME) (ZOU *et al.*, 2013). In the present study, the lowest cost was observed for the negative control feed in 1.05% with the positive control feed.

4 Conclusions

Under the conditions which the study was made, it was concluded that the supplementation of the multi carbohydrase complex plus phytase in diets of broilers with ingredients of animal origin does not change the performance and cost to produce one kilo of chicken meat, does not modify the intestinal morphometric parameters and litter quality.

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References

ABPA - Associação Brasileira de Proteína Animal (2020) *Relatório anual* [Online]. Available at: https://abpa-br.org/wp-content/uploads/2022/05/Relatorio-Anual-ABPA-2022-vf.pdf (verified 20 november 2022).

AMERAH, A.M. *et al.* Influence of feed processing on the efficacy of exogenous enzymes in broiler diets. *World's Poult. Sci. J.*, v.67, n.1, p.29-46, 2011. doi:10.1017/S0043933911000031

BARBOSA FILHO, J.A. *et al.* Características produtivas e qualitativas de frangos de corte alimentados com diferentes complexos enzimáticos. *Bol. Ind. Animal,* v.75, p.1-9, 2018.

BENABDELJELIL K.; AYACHI, A. Evaluation of alternative litter materials for poultry. *J. Appl. Poult. Res.*, v.5, n.3, p.203-209, 1996. doi: 10.1093/japr/5.3.203

CANCELLI, T. *Protease em dietas para frango de corte.* Curitiba: Universidade Federal do Paraná, 2017.

CARDOSO, D.M. *et al.* Efeito do uso de complexo enzimático em rações para frangos de corte. *Arch. Zoot.,* v.60, n.232, p.1053-1064, 2011. doi:10.4321/S0004-05922011000400021

CARVALHO, J.C.C. *et al.* Performance and carcass characteristics of broilers fed diets based on corn and soybean meal supplemented with enzymatic complexes. *Rev. Bras. Zoot.*, v.38, p.292-298, 2009. doi: 10.1590/S1516-35982009000200011

COSTA, F.G. *et al*. Utilização de um complexo multienzimático em dietas de frangos de corte. *Ciênc. Anim. Bras.,* v.5, n.2, p.63-71, 2004.

COWIESON, A.J.; RAVINDRAN, V. Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy, minerals and amino acids. *Brit. Poult. Sci.*, v. 49, n. 1, p. 37-44, 2008. doi: 10.1080/00071660701812989.

DILGER, R.N. *et al.* Evaluation of microbial phytase in broiler diets. *Poult. Sci.*, v.83, n.6, p.962-970, 2004. doi: 10.1093/ps/83.6.962

FERNANDES, J.I.M. *et al*. Efeito da adição de enzimas em dietas a base de milho e soja para frangos de corte. *Arq. Ciênc. Vet. Zool. UNIPAR,* v.13, n.1, p.25-31, 2010.

FISCHER, G. *et al*. Performance of broilers fed diets based on corn and soybean meal, with or without the addition of enzymes. *Rev. Bras. Zoot.,* v. 31, p. 402-410, 2002.

doi: 10.1590/S1516-35982002000200015

GAUTIER, A.E.; WALK, C.L.; DILGER, R.N. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. *Poult. Sci.,* v.97, n.1, p.211-218, 2018. doi: 10.3382/ps/pex291

JONG, I.C. e*t al.* A 'meta-analysis' of effects of post-hatch food and water deprivation on development, performance and welfare of chickens. *PloS One*, v.12, n.12, p.e0189350, 2017. doi: 10.1371/journal.pone.0189350

KAMWA, E.B. Utilização de diferentes subprodutos de origem animal na alimentação de frangos de corte: avaliação do desempenho zootécnico e da viabilidade econômica. [Dissertation], Curitiba (PR): Federal University of Paraná. v.3, n.1, 1998.

KONG, C.; LEE, J.; ADEOLA, O. Supplementation of β-mannanase to starter and grower diets for broilers. *Canad. J. Anim. Sci.*, v.91, n.3, p.389-397, 2011. doi: 10.4141/cjas10066

KUTLU, H.R. *et al.* Effect of multienzyme produced by a single fungus on growth performance and some carcass parameters of broiler chicks fed on maize-soya based diets. *Kafkas* Üniv. *Vet. Fak. Derg.*, v.25, n.2, p.221-230, 2019. doi:10.9775/kvfd.2018.20765

KUZMUK, N.K. *et al.* Diet and age affect intestinal morphology and large bowel fermentative end product concentration in senior and young adult dogs. *J. Nut.*, v.135, n.8, p.1940-1945, 2005. doi: 10.1093/jn/135.8.1940

LAURENTIZ, A.C. *et al.* Efeito da adição da enzima fitase em rações para frangos de corte com redução dos níveis de fósforo nas diferentes fases de criação. *Ciênc. Anim. Bras.*, v.8, n.2, p.207-216, 2007.

LIMA, R.M. e*t al.* Enzimas exógenas na alimentação de aves. *Acta Vet. Bras.*, v.1, n.4, p.99-110, 2007.

LUCIO, B.S.V. *et al.* Exogenous enzymes as zootechnical additives in animal feed: a review. *Catalysts*, v.11, n.7, p. 1-21, 2021. doi: 10.3390/catal11070851

MARTINS, G.C.F. Uso de complexo enzimático em dietas para frangos de corte. Cruz das Almas: Universidade Federal do Recôncavo da Bahia, 2013.

MALATHI, V.; DEVEGOWDA, G. In vitro evaluation of nonstarch polysaccharide digestibility of feed ingredients by enzymes. *Poult. Sci.*, v.80, n.3, p.302-305, 2001. doi: 10.1093/ps/80.3.302.

OLIVEIRA, M.C. *et al*. Mananoligossacarídeos e complexo enzimático em dietas para frangos de corte. *Rev.Bras. Zoot.,* v.38, p.879-886, 2009.

PARRA, J.A. *et al.* Redução da exigência de fósforo disponível em 390 dietas com adição de fitase para frangos de corte machos de oito aos 21 dias de idade. *Spei Domus*, v.11, n.22, 2015. doi: 10.16925/sp.v11i22.1152

PINHEIRO, D.F. *et al.* Effect of early feed restriction and enzyme supplementation on digestive enzyme activities in broilers. *Poult. Sci.*, v.83, n.9, p.1544-1550, 2004. doi:10.1093/ps/83.9.1544

PUCCI, L.E.A. *et al.* Níveis de óleo e adição de complexo enzimático na ração de frangos de corte. *Rev.Bras. Zoot.*, v.32, p.909-917, 2003. doi: 10.1590/S1516-35982003000400017

RAVINDRAN, V. *et al.* Feed enzymes: The science, practice, and metabolic realities. *J. Appl. Poult. Res.,* v.22, n.3, p.628-636, 2013. doi:10.3382/japr.2013-00739

RIOS, H.V. *et al.* Energy and nutrient utilization of maize-soy diet supplemented with a xylanase-β-glucanase complex from Talaromyces versatilis. *Anim. Feed Sci. Technol.*, v.232, p.80-90, 2017. doi: 10.1016/j.anifeedsci.2017.08.009

SAKOMURA, N.K.; ROSTAGNO, H.S. *Métodos de pesquisa em nutrição de monogástricos*. Jaboticabal: Funep; 2007.

SARANGI, N.R. *et al.* Efeito da suplementação alimentar de prebióticos, probióticos e simbióticos no desempenho de crescimento e nas características de carcaça de frangos de corte. *Vet. World*, v.9 n.3, p.313-319, 2016. doi:10.1016/j.anifeedsci.2006.06.010

SELLE, P.H.; RAVINDRAN, V. Microbial phytase in poultry nutrition. *Anim. Feed Sci. Tech.,* v.135, n.1-2, p.1-41, 2007.

SILVA, C.F. *Manual de análises químicas de solos, plantas e fertilizantes*. Brasília: Embrapa; 2009.

SILVA, D.J.; QUEIROZ, A.C. *Análise de alimentos: métodos químicos e biológicos.* Viçosa: Universidade Federal de Viçosa; 2006.

SIMON, O. The mode of action of NSP hydrolysing enzymes in the gastrointestinal tract. *J. Anim. Feed Sci.*, v.7, n. Suppl.1, p.115-123, 1998. doi: 10.22358/jafs/69959/1998

TOLEDO, G.S.P. *et al.* Frangos de corte alimentados com dietas de diferentes densidades nutricionais suplementadas ou não com enzimas. *Ciênc. Rural*, v.37, p.518-523, 2007. doi:10.1590/S0103-84782007000200034

TORRES, D.M. *et al.* Dietas à base de milho e farelo de soja suplementadas com enzimas na alimentação de frangos de corte. *Ciênc. Agrotecnol.,* v.27, p.199-205, 2003.

UNIZ.; NOY, Y.; SKLAN, D. Posthatch development of small intestinal function in the poult. *Poult. Sci.*, v.78, n.2, p.215-222, 1999. doi: 10.1093/ps/78.2.215

WU. YB. *et al.* Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolizable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *Brit. Poult. Sci.*, v.45, n.1, p.76-84, 2004. doi: 10.1080/00071660410001668897

YASAR, S.; FORBES, J.M. Enzyme supplementation of dry and wet wheat-based feeds for broiler chickens: performance and gut responses. *Brit. J. Nut.*, v.84, n.3, p.297-307, 2000. doi:10.1017/S0007114500001574

ZOU, J. *et al*. Effects of exogenous enzymes and dietary energy on performance and digestive physiology of broilers. *J. Anim. Sci. Biotechnol.,* v.4, n 1, p.1-9, 2013.