

## The effect of exogenous enzymes on the performance and digestibility of nutrients in broiler

### O efeito de enzimas exógenas no desempenho e digestibilidade de nutrientes em frangos de corte

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#### Abstract

Here we tested how feeding with exogenous enzymes affects the performance, carcass yield, and ileal digestibility in broilers from 21- to 42-days-old. The study included 1008 male broilers randomly distributed into six diets: positive control (PC), corn and soybean meal containing all the nutritional requirements; negative control (NC), with a 120 kcal.kg<sup>-1</sup> reduction in metabolizable energy relative to the PC; enzyme complex one (NC + 100 ppm of endo-1,4-beta-xylanase); enzyme complex two (NC + 200 ppm of xylanase, amylase and protease); enzyme blend one (NC + 100 FXU kg<sup>-1</sup> of xylanase and 200 FXU kg<sup>-1</sup> of amylase); and enzyme blend two (NC + 100 FXU kg<sup>-1</sup> of xylanase and 300 FXU kg<sup>-1</sup> of amylase). The enzyme complex two (CN + 100 FXU.kg de xilanase e 300 FXU.kg de amilase) and enzyme blend two (NC + 100 FXU kg<sup>-1</sup> of xylanase and 300 FXU kg<sup>-1</sup> of amylase) had greater weight gain, feed conversion, carcass yield, and digestibility of nutrients than the NC (P < 0,05). We found that enzyme complex two and enzyme blend two valued their nutritional matrix promoting better performance and digestibility of broilers.

**Key words:** Amylase. Soybean meal. Corn. Protease. Xylanase.

#### Resumo

O trabalho foi realizado para avaliar o uso de enzimas sobre o desempenho, rendimento de carcaça e digestibilidade ileal em frangos de corte no período de 21 a 42 dias de idade. Foram utilizados 1008 frangos de corte, machos, distribuídos em seis dietas: controle positivo (CP) a base de milho e farelo de soja, contendo todas as exigências nutricionais, controle negativo (CN) com redução de 120 kcal.kg<sup>-1</sup> de energia metabolizável, complexo enzimático um (CN + 100 ppm de endo-1,4-beta-xilanase); complexo enzimático dois (CN + 200 ppm de xilanase, amilase e protease); blend enzimático um (CN + 100 FXU.kg de xilanase e 200 FXU.kg de amilase e blend enzimático dois (CN + 100 FXU.kg de xilanase e 300 FXU.kg de amilase). O complexo enzimático dois (CN + 200 ppm de xilanase, amilase e protease) e blend enzimático dois (CN + 100 FXU.kg de xilanase e 300 FXU.kg de amilase) proporcionaram ganho de peso, conversão alimentar, rendimento de carcaça e digestibilidade de nutrientes superior ao controle negativo (P < 0,05). Concluiu-se que o complexo enzimático dois e blend enzimático dois valorizaram sua matriz nutricional promovendo melhor desempenho e digestibilidade em frangos de corte.

**Palavras-chave:** Amilase. Farelo de soja. Milho. Protease. Xilanase.

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## Introduction

The reduction of feed ingredient prices for poultry is a constant challenge (CARVALHO et al., 2009; SLOMINSKI, 2011), with feed accounting for approximately 65% of the total cost of production (YAN et al., 2017). Therefore, alternative products are needed to reduce the cost, but also to maintain the adequate levels of nutrition for the birds (KACZMAREK et al., 2014).

The use of exogenous enzymes in the diets allows greater energy release, decreasing digest viscosity in the gastrointestinal tract, and thus reducing feed costs (COWIESON, 2010; STEFANELLO et al., 2016). However, much of the available scientific information on the use of exogenous enzymes in poultry diets is related to high viscosity grains, including wheat and barley, which contain high levels of non-starch polysaccharides (CARDOSO et al., 2011). In corn and soybean meal based diets, enzyme supplementation might improve energy availability and inhibit anti-nutritional agents (DOSKOVIC et al., 2013; LATHAM et al., 2016).

Several studies have detected an improvement in performance of broilers with enzyme supplementation of fed corn and soybean-based diets (KIARIE et al., 2014; FLORES et al., 2016). However, the existing knowledge about the role of the enzymes remains limited, controversial or inconsistent, demonstrating that further studies are needed (CARDOSO et al., 2011; KACZMAREK et al., 2014; AMERAH et al., 2016). Here we test the effects of exogenous enzymes on performance, ileal digestibility, and carcass yield and cuts of broilers.

## Material and Methods

The trial was conducted at the Research Center of the Western Paraná State University (UNIOESTE), *Campus* Marechal Candido Rondon, Parana, and approved by the Animal Experimentation Ethics Committee under the protocol no. 63/2016.

For the study, 1008 male broilers from the Cobb 500 lineage (21-days-old) were distributed in a completely randomized design, with six treatments and eight replications, for a total of 48 experimental units with 21 birds each. Two diets were formulated: a positive control (PC) based on corn and soybean meal without enzyme complex, meeting all the nutritional requirements of the birds, and a negative control (NC) diet, with a 120 kcal kg<sup>-1</sup> reduction in metabolizable energy (Table 1). The other diets were based on the NC plus enzymes: the enzyme complex 1 fed was supplemented with 100 ppm endo-1,4-beta-xylanase; enzyme complex 2 fed was supplemented with 200 ppm xylanase, amylase, and protease; enzyme blend 1 fed was supplemented with 100 FXU kg<sup>-1</sup> xylanase and 200 FXU kg<sup>-1</sup> amylase; and enzyme blend 2 fed was supplemented with 100 FXU kg<sup>-1</sup> xylanase and 300 FXU kg<sup>-1</sup> amylase.

The metabolizable energy values of corn and soybean meal were determined using the crude energy value as described by Rostagno et al. (2011). For the digestible amino acid values of corn and soybean meal, aminograms were performed using near-infrared reflectance spectroscopy (NIRS).

At the end of the experimental period (when broilers were 42-days-old), the broilers and the leftovers were weighed to evaluate feed intake, weight gain, and feed conversion. Simultaneously, two broilers per plot were slaughtered by cervical dislocation and bleeding to determine the carcass yield. After scalding and manual defeathering, the carcasses were eviscerated, washed, weighed, and cut into pieces. Subsequently, the carcass, cuts, pancreas, liver, gizzard, and abdominal fat were weighed.

The carcass yield was calculated considering the dressed carcass weight in relation to the body weight of the broiler, while the drumstick, thigh, chest, and wing yields were considered in relation to the weight of the eviscerated carcass. The viscera

and abdominal fat were considered in relation to the live weight of the broiler. The abdominal fat was constituted by the adipose tissue present around the cloaca.

**Table 1.** Formulation and proximate composition of experimental diets (% dry matter).

Ingredients	Positive control	Negative control
Corn	62,436	64,883
Soybean meal	29,812	29,686
Soy oil	4,192	1,864
Dicalcium phosphate	1,293	1,291
Limestone	0,862	0,865
Salt	0,458	0,456
DL-Methionine 99%	0,273	0,270
L-Lysine HCL 78%	0,230	0,240
L-Threonine 98%	0,069	0,070
Choline chloride 60%	0,060	0,060
Vitamin premix <sup>1</sup>	0,100	0,100
Mineral premix <sup>2</sup>	0,050	0,050
Avilamycin	0,005	0,005
Salinomycin	0,050	0,050
Antioxidant	0,010	0,010
Sand	0,050	0,050
Chromium oxide	0,050	0,050
Proximate composition		
EM (kcal kg <sup>-1</sup> )	3150,00	3030,00
Crude protein (g kg <sup>-1</sup> )	193,00	193,00
Digestible lysine (g kg <sup>-1</sup> )	10,70	10,70
Digestible arginine (g kg <sup>-1</sup> )	11,37	11,31
Digestible threonine (g kg <sup>-1</sup> )	6,70	6,70
Digestible tryptophan (g kg <sup>-1</sup> )	1,91	1,89
Digestible valine (g kg <sup>-1</sup> )	7,57	7,57
Digestible isoleucine (g kg <sup>-1</sup> )	6,87	6,84
Digestible Methionine (g kg <sup>-1</sup> )	5,29	5,27
Calcium (g kg <sup>-1</sup> )	7,32	7,30
Potassium (g kg <sup>-1</sup> )	7,25	7,25
Sodium(g kg <sup>-1</sup> )	2,00	2,00
Phosphorus available (g kg <sup>-1</sup> )	3,42	3,42

<sup>1</sup> Vitamin premix for poultry (lot BR0119Y025), assurance levels per kilo, vitamin A (min) 9000000,00 IU, vitamin D3 (min) UI 2500000,00, vitamin E (min) 20000,00 UI, vitamin K3 (min) 0,0025 kg, vitamin B1 (min) 0,0015 kg, vitamin B2 (min) 0,006 kg, vitamin B6 (min) 0,003 kg, pantothenic acid (min) 0,012 kg, niacin (min) 0,025 kg, folic acid (min) 0,008 kg, selenium (min) 0,025 kg.

<sup>2</sup> Mineral premix for poultry (lot BR0112B375), assurance levels per kilogram of product: copper (min) 0,020 kg, iron (min) 0,100 kg, manganese (min) 0,200 kg, zinc (min) 0,100 kg.

To determine the ileal digestibility of nutrients, chromium oxide ( $\text{Cr}_2\text{O}_3$ ) added to the diets at 36- to 42-days-old was used as an indicator of indigestibility at 0,5%. The ileal content (from the Meckel's diverticulum, up to 4 cm from the ileocecal junction) of four broilers per experimental unit was analyzed for dry matter, chromium (nitric-perchloric digestion), crude protein (KJELDHAL), and crude energy (bomb calorimeter), according to methodologies described by Silva and Queiroz (2005).

For the determination of the digestibility coefficients of dry matter, crude energy, and crude protein and determination of the digestible dry matter, digestible crude protein and digestible energy, the equations described by Sakomura and

Rostagno (2016) were used. The data were submitted to the exploratory factor analysis to remove outliers (when necessary) and, later, a variance analysis and a Tukey test were performed at 5% probability level.

## Results and Discussion

Feed intake (Table 2) did not differ between diets ( $P>0,05$ ), indicating that the reduction of 120 kcal of metabolizable energy was not sufficient to alter the feed intake. Carvalho et al. (2009) and Shaw et al. (2010), studying enzymatic supplementation in broiler diets, also detected no difference in feed intake and attributed this result to a reduction in metabolizable energy.

**Table 2.** Performance of broiler fed with complex and enzyme blends in the period of 21 to 42 days of age <sup>(1)</sup>

Diets	Feed intake <sup>ns(2)</sup> (g)	Weight gain (g)	Feed conversion (g g)
Positive control	3193,11	1805,77 <sup>a</sup>	1,768 <sup>a</sup>
Negative control	3138,45	1697,45 <sup>b</sup>	1,849 <sup>b</sup>
Enzyme complex 1	3151,10	1720,90 <sup>b</sup>	1,831 <sup>b</sup>
Enzyme complex 2	3186,63	1743,05 <sup>ab</sup>	1,801 <sup>ab</sup>
Enzyme blend 1	3144,63	1720,87 <sup>b</sup>	1,827 <sup>b</sup>
Enzyme blend 2	3144,42	1745,11 <sup>ab</sup>	1,802 <sup>ab</sup>
MSE <sup>(3)</sup>	10,517	8,304	0,006
CV <sup>(4)</sup>	2,326	2,812	1,758

<sup>(1)</sup> Means followed by different letters in the column differ from each other by the Tukey test at the 5% level <sup>(2)</sup> ns: not significant.

<sup>(3)</sup> MSE: mean standard error. <sup>(4)</sup> CV: coefficient of variation.

Weight gain and feed conversion of broilers fed enzyme complex 2 and enzyme blend 2 were similar to that of the positive control ( $P<0,05$ ) and but higher than the negative control. Possibly, the difference in energy intake during this period was ~500 kcal, which accounts for the additional weight gain. Therefore, the use of enzyme complexes and blends, regardless of level, was able to metabolically sensitize the broilers and equalize daily energy consumption (LATHAM et al., 2016).

In the study conducted by Cowieson (2010), enzyme supplementation enhanced broiler performance, although such effects may be minor and difficult to detect. Zhou et al. (2009) reported that enzymes improved the feed efficiency using a diet based on corn and soybean meal with lower levels of metabolizable energy (2758 and 2818 kcal). Amerah et al. (2016) observed a synergistic influence of the enzymes on the weight gain of broilers from 1- to 42-days-old.

In this context, the results obtained in this study, as well as others cited above, might be related to the reduction of the undigested residue that enters the large intestine, providing better use of the nutrients in the small intestine (KIARIE et al., 2014). Also, the enzymes might have maximized the performance of the starch molecules from the feed and, at the same time, altered the architecture of the cell walls of the corn, providing better contact between the enzymes and their substrates inside the grain (FLORES et al., 2016).

The carcass yield (Table 3) of broilers fed enzyme diets was similar to the positive control

( $P < 0,05$ ) and the increase in the feed digestibility might have contributed to this result. In the studies of Nagata et al. (2011) and Williams et al. (2013), the carcass results were attributed to the enzyme's influence on weight gain and feed conversion.

Leg, breast, and wing yields, and abdominal fat (Table 3) did not differ between diets ( $P > 0,05$ ). Carvalho et al. (2009) and Cardoso et al. (2011), using enzyme complexes in diets for broilers, also observed no change in breast and leg yields and abdominal fat. The same authors attributed these results to a possible action of the enzymes on the nutrients of corn and soybean meal.

**Table 3.** Carcass yield (%), breast yield (%), wing yield (%), leg yield (thigh and drumstick) (%), abdominal fat and relative liver weight (%) of broiler chickens slaughtered at 42 days of age <sup>(1)</sup>.

Diets	Carcass	Yield <sup>ns(2)</sup>	Wing <sup>ns</sup>	Leg <sup>ns</sup>	Abdominal fat <sup>n.s</sup>	Liver
Positive control	73,63 <sup>a</sup>	37,27	10,41	27,65	2,08	2,74 <sup>ab</sup>
Negative control	71,31 <sup>b</sup>	37,32	10,37	26,76	1,75	2,61 <sup>ab</sup>
Enzyme complex 1	74,01 <sup>a</sup>	35,90	10,55	27,43	2,20	2,94 <sup>a</sup>
Enzyme complex 2	73,89 <sup>a</sup>	37,40	10,09	26,85	2,13	2,78 <sup>ab</sup>
Enzyme blend 1	72,46 <sup>ab</sup>	37,75	10,65	27,52	1,90	2,57 <sup>b</sup>
Enzyme blend 2	73,67 <sup>a</sup>	37,03	10,79	28,05	2,25	2,90 <sup>ab</sup>
MSE <sup>(3)</sup>	0,236	0,203	0,082	0,165	0,056	0,035
CV <sup>(4)</sup>	2,937	5,130	7,441	5,786	26,571	12,265

<sup>(1)</sup> Means followed by different letters in the column differ from each other by the Tukey test at the 5% level. <sup>(2)</sup> ns: not significant.

<sup>(3)</sup> MSE: mean standard error. <sup>(4)</sup> CV: coefficient of variation.

The liver weight (Table 3) of broilers fed enzyme diets was similar to that of the positive control ( $P < 0,05$ ) a greater release of nutrients in the digestion of enzyme-fed broilers compared to the negative control, which resulted in greater absorption and action of the liver. Yan et al. (2017) also detected an increase in relative liver weight when using enzymes for broilers at 42-days-old.

The digestibility coefficient of dry matter and crude protein (Table 4) of broilers fed enzyme complex 2 and enzyme blend 2 were similar to the positive control ( $P < 0,05$ ), suggesting that the enzymes promoted enhanced nutrient digestibility and energy content of the feeds (AMERAH et al., 2016). Also, xylanase along with other enzymes may have increased the free sugars content, releasing arabinose and xylose from corn, in both the jejunum and ileum of broilers (STEFANELLO et al., 2016).

**Table 4.** Dry matter digestibility coefficient (DMDC), crude protein (CDCP) and digestible energy (DE) (kcal.kg<sup>-1</sup>) <sup>(1)</sup>

Diets	DMDC	CPDC	DE
Positive control	69,87 <sup>ab</sup>	76,73 <sup>ab</sup>	3249 <sup>a</sup>
Negative control	69,12 <sup>bc</sup>	77,65 <sup>a</sup>	2902 <sup>bc</sup>
Enzyme complex 1	70,22 <sup>ab</sup>	78,89 <sup>a</sup>	2878 <sup>c</sup>
Enzyme complex 2	70,70 <sup>ab</sup>	78,85 <sup>a</sup>	2967 <sup>bc</sup>
Enzyme blend 1	67,90 <sup>c</sup>	74,83 <sup>b</sup>	2960 <sup>bc</sup>
Enzyme blend 2	70,98 <sup>a</sup>	77,85 <sup>a</sup>	3051 <sup>b</sup>
MSE <sup>(2)</sup>	0,178	0,293	14,997
CV <sup>(3)</sup>	1,765	2,622	3,462

<sup>(1)</sup> Means followed by different letters in the column differ from each other by the Tukey test at the 5% level.

<sup>(2)</sup> MSE: mean standard error. <sup>(3)</sup> CV: coefficient of variation.

The digestible energy (DE) was influenced by the enzyme diets ( $P < 0,05$ ). It is possible that the broilers saved energy by reducing the production of endogenous enzymes, and that some nutrients (such as starch, fat, and proteins) were absorbed more efficiently in the intestinal tract, leading to higher DE values. Zhou et al. (2009) reported a 3,55% increase in DE for 42-day-old broilers. Also, Santos et al. (2008) reported that the addition of enzymes improved the energy availability of feeds.

Therefore, enzyme supplementation can improve poultry performance, acting as an important tool to reduce costs and improve nutrient absorption. However, further studies of the balance of the microbiota and the immune system associated with the gut should be conducted. In the present study the use of exogenous enzymes reduces the amount of undigested residue that enters the large intestine, as a consequence of better use of nutrients in the small intestine, reducing the microbial population in the terminal ileum

## Conclusion

The use of enzymatic complexes containing xylanase and amylase and an enzymatic blend composed of 100 FXU kg<sup>-1</sup> xylanase and 300

FXU kg<sup>-1</sup> amylase in a corn and soybean-based diet improves the performance and digestibility of nutrients in broilers.

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