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## Effects of enzyme supplementation on diets of medium-heavy laying hens at 28 to 40 weeks<sup>1</sup>

Efeitos da suplementação enzimática em dietas de poedeiras semipesadas de 28 a 40 semanas de idade

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**ABSTRACT** - The aim of this study was to verify the effect of the addition of an enzyme complex on performance (feed intake, egg production, egg weight and egg mass, feed conversion per egg mass, and feed conversion per dozen eggs), and on egg quality (% of shell, albumen and yolk, shell thickness, specific gravity, Haugh unit, yolk index, and albumen index), in medium-heavy laying hens at 28 to 40 weeks of age. A total of 240 Hy-Line Brown laying hens were used in a randomised block design with 10 replications of six birds per lot and four treatments: positive control (basal feed), negative control (with a reduction in metabolisable energy, crude protein, calcium and phosphorus), negative control + enzymes, and positive control + enzymes. The enzyme complex, composed of  $\beta$ -glucanases,  $\beta$ -xylanase, cellulase and phytase, was added to the feed at a ratio of 50 g t<sup>-1</sup>. The data were submitted to analysis of variance with the mean values compared by Tukey's test at 5%. There was no difference in feed intake or egg weight between treatments. However, the addition of the enzyme complex to the negative control diet gave results similar to the remaining performance variables when compared to the positive control group. For the external and internal quality of the eggs, there was no difference between treatments for the variables under evaluation, except for the albumin index. It was concluded that use of the enzyme complex in the diet of medium-heavy laying hens gives a reduction in nutritional density without compromising production performance or egg quality.

**Key words:** Exogenous enzyme. Phytase. Glucanase. Hy-line Brown. Egg quality.

**RESUMO** - Objetivou-se verificar o efeito da adição de complexo enzimático sobre o desempenho (consumo alimentar, produção, peso e massa dos ovos, conversão alimentar por massa e dúzia de ovos) e qualidade dos ovos (% de casca, albúmen e gema, espessura da casca, gravidade específica, unidade Haugh, índice de gema e de albúmen) de poedeiras semipesadas no período de 28 a 40 semanas de idade. Foram utilizadas 240 poedeiras da linhagem Hy-Line Brown, em delineamento blocos ao acaso, com 10 repetições de seis aves por parcela e quatro tratamentos: controle positivo (ração basal), controle negativo (com redução de energia metabolizável, proteína bruta, cálcio e fósforo), controle negativo + enzimas e controle positivo + enzimas. O complexo enzimático, composto por  $\beta$ -glucanases,  $\beta$ -xilanase, celulase e fitase, foi adicionado à ração na proporção de 50 g/t. Os dados foram submetidos à análise de variância com comparação de médias pelo teste Tukey a 5%. Não houve diferença sobre o consumo de ração e peso dos ovos entre os tratamentos. Entretanto, a adição do complexo enzimático na dieta controle negativo permitiu resultados semelhantes às demais variáveis de desempenho, quando comparadas ao grupo controle positivo. Quanto à qualidade externa e interna dos ovos, não houve diferença entre os tratamentos para as variáveis avaliadas, exceto para o índice de albúmen dos ovos. Conclui-se que o uso do complexo enzimático em dietas de poedeiras semipesadas permite uma redução na densidade nutricional sem comprometer o desempenho produtivo e a qualidade dos ovos.

**Palavras-chave:** Enzima exógena. Fitase. Glucanase. Hy-line Brown. Qualidade de ovos.

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## INTRODUCTION

Exogenous enzymes have mainly been used to improve the digestibility of ingredients with high levels of non-starch polysaccharides (NSP), such as rye, wheat, barley and oats. NSP are the main constituents of the cell wall of foods of plant origin that during digestion have a high water retention capacity, making enzymatic action difficult, and forming a gelatinous substance in the intestinal tract (LIMA *et al.*, 2007).

Even with the constant search for alternative foods, maize and soybean meal are respectively still the principal sources of energy and protein used in poultry diets, however, the composition of soybean meal includes 20% NSP, with almost no digestibility. Wheat bran is a good alternative in poultry diets and can supply up to 70% of the metabolisable energy of the diet, but the high levels of arabinoxylans (AX) in its composition restrict its use in feed for commercial poultry (O'NEILL; SMITH; BEDFORD, 2014).

As feed ingredients have differences in their chemical composition, and considering that enzymes are specific in their reactions, commercial products composed of several enzymes are generally effective (MURAKAMI *et al.*, 2007). According to Barbosa *et al.* (2012), enzymes can be used as nutritional strategies to generate a reduction in the nutrient level of diets and achieve a similar or better response from the birds when compared to a diet of adequate nutrient levels. This response comes from increases in the nutritional value of the ingredients due to the action of the enzyme in improving nutrient availability.

Silva, Fonseca and Guedes Filho (2000) identified the possibility of using enzyme complexes in diets based on low-viscosity cereals, such as maize, sorghum and soybean meal, with the aim of increasing the use of starch and protein. Enzymes, in addition to increasing digestibility and feed efficiency, lessen the action of growth inhibitors, especially NSP, by helping endogenous enzymes in the digestive processes.

Gálik and Horniaková (2010) found that the addition of the enzymes xylanase and glucanase in the feed of Isa Brown laying hens had a positive effect on the productivity of the birds. Similarly, Khan *et al.* (2011), evaluating performance and egg quality in laying hens supplemented with an enzyme complex in comparison to non-supplemented birds, found that addition of the enzyme to the basal diet significantly increased egg production, weight and mass, and improved feed conversion, as well as having a positive effect on the Haugh unit.

The aim of this study was to evaluate the effects of including an enzyme complex in diets based on maize,

soybean meal and wheat bran on the performance and egg quality of medium-heavy commercial laying hens.

## MATERIAL AND METHODS

The experiment was conducted in the Poultry-Farming Sector of the Goiano Federal Institute (IF Goiano), in the town of Ceres in the State of Goiás, Brazil. All experimental procedures were carried out in accordance with the ethical standards and approval of the Ethical Committee on the Use of Animals (CEUA) of IF Goiano, under Protocol 018/2012.

One hundred and twenty-five Hy-Line Brown medium-heavy laying hens from 28 to 40 weeks of age were used, housed in a shed containing galvanised wire cages measuring 25 × 45 × 40 cm, distributed laterally on two floors and equipped with a gutter trough feeders and drinking nipples.

Management of the birds was daily, when they were supplied with water and feed at will and lighting for 17 hours a day; the eggs were collected in the morning and afternoon. According to the data obtained from the Ceres Meteorological Station located on the IF Goiano campus, the mean maximum and minimum temperatures during the experimental period were 31.6 °C and 16.7 °C respectively, with a mean relative humidity of 76.6%.

The experimental design was of randomised blocks, with four treatments and ten replications of six birds per experimental unit. The treatments were distributed in two blocks, comprising opposite sides of the rows of cages, due to the greater incidence of solar radiation on one side.

The treatments consisted of four diets based on maize, soybean meal and wheat bran: positive control diet; positive control diet with the on-top addition of enzymes; negative control diet; and negative control diet with the addition of enzymes.

The positive control diet was formulated according to the recommendations of the Hy-Line Brown Performance Standards Manual (HY-LINE DO BRASIL, 2011); the negative control diet was calculated with a reduction of 79 kcal kg<sup>-1</sup> metabolisable energy, 0.49% crude protein, 0.09% calcium and 0.13% available phosphorus, as per the nutritional matrix of the multi-enzyme complex used, which was provided by the manufacturer (Table 1).

The enzyme complex, containing β-glucanases, β-xylanases, cellulose and phytase, was added on top of the feed at a dose of 50 g t<sup>-1</sup>.

**Table 1** - Percentage and calculated composition of the experimental diets

Ingredients	Positive Control	Negative Control
Maize grain	60.900	51.746
Soybean meal 45%	21.026	18.543
Wheat bran	4.000	12.595
Limestone	9.486	9.763
Soybean oil	2.038	3.509
Dicalcium phosphate	1.427	0.640
Mineral-vitamin premix <sup>1</sup>	0.400	0.400
Common Salt	0.360	0.361
DL-methionine	0.233	0.244
L-lysine HCl	0.109	0.148
L-threonine	0.021	0.046
Inert	0.000	2.000
Calculated nutritional composition		
Metabolisable energy (kcal kg <sup>-1</sup> )	2,800	2,721
Crude protein (%)	15.180	14.688
Calcium (%)	4.000	3.915
Available phosphorus (%)	0.360	0.232
Digestible lysine (%)	0.760	0.750
Methionine + digestible cystine (%)	0.660	0.653
Digestible methionine (%)	0.443	0.440
Digestible threonine (%)	0.530	0.523
Digestible tryptophan (%)	0.160	0.157
Digestible arginine (%)	0.911	0.897
Digestible isoleucine (%)	0.565	0.556
Digestible Valine (%)	0.636	0.626

<sup>1</sup>Quantity per kg of feed: Vit. A, 1,750,000 IU; Vit. D3, 500,000 IU; Vit. E, 1,250 IU; Vit. K3, 400 mg; Vit. B, 750 mg; Vit. B12, 2,000 mg; Niacin, 5,000 mg; Choline, 58,590 g; Pantothenic acid, 1,250 mg; Methionine, 247.50 g; Copper, 2,000 mg; Iron: 12,500 g; Manganese, 17,500 g; Zinc, 12,500 g; Iodine, 300 mg; Selenium, 50 mg; Bacitracin Zn, 5,000 mg

The experiment was divided into three periods of 28 days to collect data and to calculate variables of performance and egg quality.

The performance variables being analysed were: feed intake (g bird<sup>-1</sup> day<sup>-1</sup>), egg production (% bird<sup>-1</sup> day<sup>-1</sup>), egg weight (g), egg mass (g bird<sup>-1</sup> day<sup>-1</sup>), feed conversion for egg mass (g g<sup>-1</sup>) and feed conversion per dozen eggs (kg dozen<sup>-1</sup>).

To determine egg quality, three eggs per experimental unit were collected during the last three days of each period. The variables under analysis were egg percentage composition (shell, albumen and yolk), shell thickness (mm), specific gravity and internal egg quality (Haugh unit, yolk index (%) and albumen index (%)).

To obtain the percentage composition, the eggs were weighed on a semi-analytical precision balance with a readability of 0.001 g (total egg weight) and then broken to weigh the yolk and shell. The weight of the albumen was obtained from the difference between the total egg weight and the weight of the shell plus the weight of the yolk. The shell was weighed after drying at room temperature, and shell thickness was measured with a digital caliper at two points in the centre-transversal area, the mean value obtained being expressed in millimeters (mm). Specific gravity was determined by immersing the eggs in plastic buckets containing different saline solutions, with densities that varied from 1.0650 to 1.100, at intervals of 0.0025, as per the methodology of Moreng and Avens (1990). The diameter of the yolk and albumin

were measured with a caliper to determine the yolk index and albumen index, according to the following formulas: yolk index = yolk height/mean value for yolk diameter; albumen index = albumen height/mean value for albumen diameter. The Haugh unit was obtained according to the following formula (1):

$$HU = 100 \log (H + 7,57 - 1,7 W^{0,37}) \quad (1)$$

where H = albumen height (mm) and W = egg weight (g) (HARDER *et al.*, 2008).

The data were submitted to analysis of variance, and the mean values were compared by Tukey's test at 5% probability using the ASSISTAT statistical software (SILVA, 2016).

## RESULTS AND DISCUSSION

There was no difference ( $P > 0.05$ ) between the intake of birds fed the basal diet and those fed a diet of low nutritional density, with or without the addition of enzymes (Table 2). Laying hens usually respond to an energy reduction in the diet with an increase in feed intake to meet their daily energy needs. However, the reduction in energy of the low nutritional density feed was not sufficient to influence intake in the birds.

This result was similar to that found by Vieira Filho *et al.* (2015), who saw no effect on the intake of medium-heavy laying hens fed diets with basal and reduced energy levels, supplemented or not supplemented with 500 g t<sup>-1</sup> protease.

However, Gentilini *et al.* (2009) found a significant reduction in the feed intake of laying hens that received diets containing an on-top enzyme complex with no energy increase, showing that the enzymes improved the energy value of the diets, having an influence on

feed intake. Nonetheless, Geraldo *et al.* (2014) found that feed intake was higher in birds fed diets with low nutritive levels, with or without the addition of enzymes (carbohydrases and phytase), indicating that the levels of enzyme supplementation were not sufficient to meet the energy requirements of the birds.

Comparing the birds fed a low nutritional density diet, and supplemented or not supplemented with enzymes, those that received enzyme supplementation showed better results for egg production, egg mass, feed conversion per dozen eggs, and feed conversion for egg mass, being similar to the results found for birds that received the positive control diets with no enzymes. With the nutritional matrix recommended by the manufacturer, inclusion of the enzyme complex had a beneficial effect on nutrient digestibility, which can be attributed to a reduction in intestinal viscosity.

Similarly, Lázaro *et al.* (2003) found that laying hens fed diets based on rye, wheat and rice, and supplemented with an enzyme combination, produced 2.1% more eggs per housed hen. Silva *et al.* (2012) also found that a diet of reduced nutrients and no enzyme supplementation provided the worst values for feed conversion, confirming the beneficial effect of including enzymes in the diets of medium-heavy laying hens.

There was no difference in egg weight for birds submitted to the different treatments. The reduction in nutrient levels and the inclusion of exogenous enzymes did not influence egg weight, but had an effect on production, which reflected in the values for egg mass.

This result agrees with Viana *et al.*, who found no effect from diet (positive or negative control, with or without the addition of xylanase) on egg weight ( $P > 0.05$ ).

On the other hand, Murakami *et al.* (2007) found that egg weight in laying hens fed a basal diet formulated

**Table 2** - Performance of laying hens fed diets supplemented with enzyme complex, from 28 to 40 weeks of age

Variable	Treatment				P <sup>1</sup>	CV <sup>1</sup> (%)
	PC <sup>1</sup>	PC+Enzymes	NC <sup>1</sup>	NC+Enzymes		
Intake (g bird <sup>-1</sup> day <sup>-1</sup> )	102.0	104.4	114.2	102.2	0.1066	11.6
Egg production (%)	80.4 a	81.8 a	61.4 b	84.1 a	<0.001	7.4
Mean egg weight (g)	59.3	59.7	60.2	60.3	>0.050	2.5
Egg mass (g bird <sup>-1</sup> day <sup>-1</sup> )	47.7 a	49.9 a	37.0 b	50.7 a	<0.001	9.4
Feed conversion per dozen (kg/dozen)	1.54 b	1.56 b	2.29 a	1.47 b	<0.001	10.9
Feed conversion for egg mass (g/g)	2.08 b	2.09 b	3.07 a	1.95 b	<0.001	11.5

<sup>1</sup>PC = positive control; NC = negative control; P = probability; CV = coefficient of variation; a,b = mean values followed by different letters differ statistically by Tukey's test at 5% probability

with a 5% deficiency in relation to the nutritional requirements of the line, and supplemented with a multi-enzyme complex at levels of 400 or 500 ppm, was higher than that achieved by birds fed with a product-free diet. Araújo *et al.* (2008) found that the addition of an enzyme complex containing galactosidase, galactomannanase, xylanase and glucanase, improved egg weight in medium-heavy laying hens fed a diet containing different levels of wheat bran.

Birds submitted to the diet of low nutrient density without the addition of enzymes, displayed less egg mass in comparison to birds that received the same diet with the addition of the enzyme, which did not differ statistically from the Positive Control treatment and the Positive Control treatment with enzymes ( $P>0.05$ ). The addition of the enzyme complex in diets with reduced nutrient levels guaranteed the availability of nutrients and their efficient use by the birds, since there was no difference from the positive control treatment. It is therefore possible to reduce feed costs by reducing the inclusion of certain ingredients in the diet of birds, without having an effect on production performance.

Viana *et al.* (2011) also obtained similar results and found lower values for egg production and egg mass in the group fed diets with no addition of xylanase. Similarly, Fuente and Soto-Salanova (1997) found a significant increase in the mass of eggs produced daily by laying hens that received diets based on corn and soybean with the inclusion of 8% and 16% enzyme complex.

The on-top addition of the enzyme complex to the positive control diet did not improve production performance in the birds when compared to birds submitted to the positive control diet with no addition of the enzyme, or those submitted to the negative control

diet with enzymes. In this case, it was confirmed that the positive basal diet easily met the nutritional needs of the birds, with no effect from the on-top addition of the enzyme.

Similar results were found by Murakami *et al.* (2007), Viana *et al.* (2009) and Viana *et al.* (2011), who emphasised that the use of a multi-enzyme complex is efficient when added to nutrient-deficient diets, with a percentage increase in the variables of productive performance. Oba *et al.* (2013) likewise found that the on-top addition of an enzyme complex to feeds based on maize and soybean meal gave no improvements in egg performance or quality in laying hens at 60 weeks of age.

On the other hand, Gentilini *et al.* (2009) found that when the enzyme complex was added on top of the diet, it led to a reduction in intake, but maintained production and bone resistance in laying hens.

According to the analysis of variance of the data for external and internal egg quality, there was no significant difference ( $P\geq 0.05$ ) between treatments for the egg parameters percentage shell, percentage albumen, percentage yolk, shell thickness or specific gravity (Table 3).

These results demonstrate that birds that were fed the diet with a reduced nutrient content showed the same characteristics for external egg quality as birds that received the positive control diet, since the reduction in nutrient levels was not sufficient to have any effect on these parameters. Even under conditions of nutrient deficiency, the laying hens maintained the qualitative characteristics of the eggs, to the detriment of production.

**Table 3** - Parameters of egg quality in semi-heavy laying hens fed diets supplemented and not supplemented with enzyme-complex, from 28 to 40 weeks of age

Variable	Treatment				P <sup>1</sup>	CV <sup>1</sup> (%)
	PC <sup>1</sup>	PC+Enzymes	NC <sup>1</sup>	NC+Enzymes		
Shell (%)	10.31	10.26	10.16	10.32	>0.05	3.3
Albumen (%)	60.74	60.28	61.18	59.85	0.2185	24
Yolk (%)	28.78	23.3	28.49	29.11	>0.05	4.4
Shell thickness (mm)	41.08	40.96	40.33	40.86	>0.05	4.0
Specific gravity (g mL)	1.101	1.093	1.094	1.095	0.076	0.65
Haugh unit	96.29	96.20	96.29	96.13	>0.05	1.73
Yolk index	0.43	0.44	0.42	0.40	0.142	9.17
Albumen index	1.39 b	1.42 ab	1.51 a	1.41 ab	0.046	6.8

<sup>1</sup>PC = positive control; NC = negative control; P = probability; CV = coefficient of variation

Similar results were seen by Viana *et al.* (2011), who found that egg composition in commercial laying hens from 24 to 48 weeks of age was not affected ( $P>0.05$ ) by the experimental diets (positive and negative controls, with and without xylanase). Similarly, Han *et al.* (2010), in a study of the influence of enzyme supplement on performance, nutrient digestibility and egg quality in laying hens, noted that enzyme supplement in the diet had no significant effect on egg production or egg quality. Oba *et al.* (2013) also found that there was no difference in percentage yolk, albumen or eggshell in laying hens fed different levels of an enzyme complex containing amylase, phytase and protease.

In work developed by Gentilini *et al.* (2009), the variables of specific gravity, shell weight and shell thickness were not affected by the different energy values of the enzyme complex added to the diets. However, egg weight and albumen weight showed reduced values in birds that received the on-top enzyme complex, probably due to the lower feed intake of these birds.

As for the internal quality of the eggs, there was no effect from the enzyme complex on the Haugh unit or yolk index; the exception was the albumen index, where the negative control diet presented a higher index compared to the other diets. The albumin index and the yolk index correlate height with diameter as an indication of consistency. As the yolk or albumen diameter increases, there is a reduction in these indices.

Similar results were found by Geraldo *et al.* (2014), who noted that nutrient reduction and supplementation with different levels of a mixture of phytase and carbohydrases, did not affect the internal quality of the eggs (Haugh unit) in medium-heavy laying hens at 42 to 57 weeks of age.

However, in studies by Murakami *et al.* (2007), a difference ( $P<0.01$ ) was seen in mean values of the Haugh unit in the eggs of laying hens fed diets supplemented with an enzyme complex. This was similar to Khan *et al.* (2011), who found that the Haugh unit was significantly greater in a group of birds fed diets supplemented with enzymes, compared to the negative control group. However, the same authors found that the remaining parameters of internal and external egg quality showed no differences between the various treatments.

## CONCLUSION

The inclusion of an enzyme complex in the diets of medium-heavy laying hens from 28 to 40 weeks of age reduces the nutritional density of the diet without compromising production performance or egg quality.

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