



Effect of protease supplementation on production performance of laying hens

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ABSTRACT. The experiment was conducted with the objective of evaluating the effect of protease enzyme supplementation on performance parameters and quality shell of laying hens. We used 240 Isa Brown commercial laying hens with 44 weeks of age. A completely randomized split-plot design (5 periods of 21 days) with four treatments and six replications (10 hens per replication) was used. The experimental diets were formulated according to the requirements of the breed. The following parameters were evaluated: egg production, feed intake, feed conversion, average egg weight, egg loss, specific gravity, percentage and shell thickness. After collection, the data were analyzed with SISVAR Statistical Package, and the means compared by SNK test at 5% probability. It was concluded that supplementation of diets low in nutrients with 500 g ton⁻¹ of protease (100 U g⁻¹), provides egg production and feed conversion rates similar to those obtained in laying hens fed diet with the nutritional level recommended for the breed. However, protease supplementation did not show effect on egg shell quality.

Keywords: laying hen farming, enzymes, grains, nutrition.

Suplementação de protease sobre o desempenho produtivo de poedeiras

RESUMO. O experimento foi conduzido com o objetivo de avaliar o efeito da suplementação da enzima protease sobre o desempenho produtivo e a qualidade de casca de poedeiras semipesadas. Utilizaram-se 240 poedeiras da linhagem comercial Isa Brown com 44 semanas de idade. Foi adotado um delineamento inteiramente casualizado em esquema de parcela subdividida no tempo (5 períodos de 21 dias cada) com quatro tratamentos e seis repetições (10 aves por repetição). Foram avaliados: produção de ovos, consumo de ração, conversão alimentar, peso médio dos ovos, perda de ovos, gravidade específica, porcentagem e espessura de casca. Após a coleta, os dados foram submetidos à análise pelo pacote estatístico SISVAR com as médias comparadas pelo teste de SNK a 5% de probabilidade. Para as variáveis de qualidade externa dos ovos, não houve diferença significativa ($p > 0,05$) dos tratamentos. Conclui-se que a suplementação de 500 g ton⁻¹ da protease (100 U g⁻¹), em dietas com redução dos níveis nutricionais, proporciona produção de ovos e conversão alimentar semelhantes aos obtido em poedeiras semipesadas, recebendo ração com níveis nutricionais indicados pelo manual da linhagem, porém não mostra efeito sobre a qualidade de casca dos ovos.

Palavras-chave: avicultura de postura, enzimas, cereais, nutrição.

Introduction

Global demand for foods has increased over the years as croplands in the world has diminished continuously. This results in increased costs of the main inputs used in poultry farming and, consequently, in higher production costs. In this scenario, it is necessary that farmers make use of techniques that enable the rational use of inputs, managing to offset the increased production costs with improved nutritional efficiencies (Oba et al., 2013).

The need to produce foods gained status of worldwide concern, leading to the development and availability of new techniques that improve the conversion of foods into products.

Significant technological development has been attained throughout the poultry production chain and fostered the development of highly specialized synthetic enzymes that have important goals in animal feed, complementing the endogenous digestive enzymes that are produced

in the digestive tract of the animals (Bertechini, 2006).

The use of synthetic enzymes, also called exogenous enzymes, has become an increasingly indispensable technique used in poultry farming because it allows higher rational formulation of diets and foods supply, with concomitant decrease in the levels of excreted nutrients, such as nitrogen and phosphorous. Exogenous enzymes increase the nutrients digestibility, especially of protein and minerals, reducing the anti-nutritional effect of complexation of amino acids and phosphorus (Costa, Clementino, Jácome, Nascimento & Pereira, 2004; Hahn-Didde & Purdum, 2014).

Other important aspect in regards to the use of synthetic enzymes is the increased use of alternative foods or by-products of processed grains to maintain the birds' productive performance and make the nutritional quality of diets that use alternative ingredients attain the quality of conventional diets that use corn and soybean meal (Al-Saffar, Attia, Mahmoud, Zewell & Bovera, 2012).

The goal of the present study was to evaluate the effects of protease supplementation to diets of commercial laying hens and its effects on the performance and egg quality.

Material and methods

The experiment was conducted in the Instituto Federal de Educação Ciência e Tecnologia IFMG (Federal Institute of Education, Science and

Technology – IFMG), campus of Bambuí, in the experimental sector of egg layers farming. A total of 240 semi-weighted Isa Brown hens aged 44 weeks were used. The birds were kept in metal cages measuring 25 x 45 x 35 cm), corresponding to a stocking density of 562.5 cm² chicken⁻¹, and fitted with trough-type feeders and nipple drinkers. The birds were fed in the morning and afternoon with food and water provided *ad libitum*.

The experimental design used was a completely randomized split-plot design (five experimental periods of 21 days for each plot), comprised of four treatments and six replications with ten birds per replication. The experimental treatments used were as follows: Treatment 1. Positive Control (PC); Treatment 2. Negative Control (NC); Treatment 3. PC + supplementation of 500 g ton⁻¹ of protease (100 U g⁻¹); Treatment 4. NC + supplementation of 500 g ton⁻¹ of protease (100 U g⁻¹).

Table 1 shows the ingredients used as well as the nutritional composition of the experimental feeds.

The diets were formulated according to the requirements proposed by the ISA (2007) breed manual, prepared fortnightly, based on corn, soybean meal, and packed immediately after being made. The meals were stored on wooden pallets in the experimental poultry house and protected from moisture and temperature.

Table 1. Ingredients used in the experimental diets formulated according to the requirements of Isa Brown breed and its respective quantities and nutritional values.

Ingredients	T1 (PC)	T2 (NC)	T3	T4
Corn	63.552	63.486	63.552	63.486
Soybean meal	19.100	15.900	19.100	15.900
Meat and bone meal	2.670	2.470	2.670	2.470
Wheat bran	5.000	8.290	5.000	8.290
Limestone	8.610	8.770	8.610	8.770
Mineral Premix ^{1,1}	0.100	0.100	0.100	0.100
Vitamin Premix ^{1,2}	0.100	0.100	0.100	0.100
Choline chloride	0.040	0.040	0.040	0.040
Dicalcium phosphate	0.200	0.190	0.200	0.190
Salt	0.310	0.310	0.310	0.310
DL-methionine (98%)	0.118	0.108	0.118	0.108
L-lysine HCl (78%)	0.000	0.036	0.000	0.036
Protease 500 g ton ⁻¹ – 100 U g ⁻¹	0.000	0.000	0.050	0.050
Kaolin	0.200	0.200	0.150	0.150
Total	100.00	100.00	100.00	100.00
Calculated nutritional composition of the diets				
Apparent Metabolizable Energy (kcal kg ⁻¹)	2730	2715	2730	2730
Crude Protein (%)	15.98	14.92	15.89	15.92
Calcium (%)	3.773	3.800	3.773	3.800
Available phosphorus (%)	0.300	0.290	0.300	0.290
Digestible methionine (%)	0.350	0.325	0.350	0.345
Methionine + Digestible cysteine (%)	0.580	0.545	0.580	0.580
Digestible lysine (%)	0.675	0.636	0.675	0.676
Digestible threonine (%)	0.509	0.469	0.509	0.497
Digestible tryptophan (%)	0.645	0.599	0.645	0.634

^{1,1}Composition of mineral Premix per kg of product: 5,000 mg manganese; 50,000 mg iron; 1,500 mg iodine, 70,000 mg zinc; 8,500 mg copper; 200 mg cobalt. ^{1,2}Composition of vitamin Premix per kg of product: vitamins: A 800,000 µ, B12 1,000 mg, D3 2,000,000 µ, E 15,000 mg, K3 2,000 mg, B2 4,000 mg, B6 1,000 mg, niacin 19,900 mg, pantothenic acid 5,350 mg, folic acid 200 mg, selenium 2,500 mg, antioxidant 100,000 mg.

The birds underwent a lighting program of 16 hours. Maximum and minimum temperatures (in Celsius degrees) in the poultry house were recorded every day with the aid of a maximum/minimum thermometer. The eggs were collected every day from 12h00 to 15h00 pm, and the number of eggs produced were recorded, including cracked, broken, abnormal, shell-less and soft-shelled eggs.

The productive characteristics that were evaluated were: eggs production, obtained by dividing the total eggs produced in each plot by the number of chickens in each plot, and the result was shown in percentage; feed consumption, obtained on a weekly basis as the ratio between the amount of feed consumed in each experimental plot and the number of birds in each plot, and shown in grams/bird/day; average eggs weight, measured once a week, with individual weighing of all eggs produced in each experimental plot and the mean value expressed in grams (g); feed conversion per eggs mass, according to the following formula:

$$FC = 100 * (FI / (LR * AEW))$$

where:

FC = feed conversion;

FI = feed intake (g hen⁻¹);

LR = egg laying rate (% of egg laying hen⁻¹ day⁻¹);

AEW = average eggs weight (g).

The shell quality characteristics were assessed at the end of each experimental period for three consecutive days. Specific gravity was assessed by immersing all eggs produced at each experimental plot into 1.060 to 1.100 g cm⁻³ of saline solutions, according to Adubados (2011). The shell ratio was determined after random collection of two perfect eggs of each experimental plot, which were then broken, and the shells washed without removing the membranes. Subsequently, the shells were dried for six days at room temperature and weighed on a precision scale (0.01 g). The value was obtained as a function of the egg weight and expressed in percentage. To assess the shell thickness (mm), it was considered the mean value found after measuring three points of the equatorial region using a caliper (0.01 mm precision).

Analysis of variance was used to analyze the data set using the resources of SISVAR statistical software (Ferreira, 2000). The means were compared by the SNK test at 5% of probability level.

Results and discussion

There was no significant interaction ($p > 0.05$) between treatments and the experimental periods regarding the variables of performance and shell quality.

Table 2 shows the results of production performance and shell quality in the experimental treatments.

Table 2. Feed intake (FI), eggs production (EP), average eggs weight (AEW), feed conversion rate (FCR), specific gravity (SG), shell ratio (SR), shell thickness (ST) of eggs of semi-weighted laying hens aged 44 to 50 weeks fed diet with enzyme supplementation.

Characteristics	Treatments				CV(%)
	T1 (PC)	T2 (NC)	T3	T4	
FI (g hen ⁻¹ day ⁻¹)	115.97	112.57	114.93	114.33	4.51
EP (% hen ⁻¹ day ⁻¹)	91.34 a	84.46 b	89.89 a	89.06 a	5.18
AEW (g)	63.74 a	63.32 ab	63.64 a	62.71 b	2.11
FCR (g g ⁻¹)	1.99 b	2.11 a	2.01 b	2.05 b	5.71
SG (g cm ⁻³)	1.091	1.091	1.091	1.091	0.14
S (%)	9.77	9.84	9.92	10.00	4.23
ST (mm)	0.554	0.558	0.565	0.558	5.60

^{a,b}Means in a row followed by different letters are not significantly different by the SNK test ($p < 0.05$).

There was no significant effect ($p > 0.05$) of the dietary enzyme supplementation on feed intake. This result corroborates the study conducted by Adubados (2011), who evaluated the addition of enzyme complex (300 U g⁻¹ xylanase, 4,000 U g⁻¹ protease and 400 U g⁻¹ α -amylase), to the diets of lightweight layers, with or without reduction of the diet energy levels, and no significant effect of the supplementation was found on feed intake. The same result was reported in a study by Wen, Wang, Zhou, Jiang & Wang (2012), where the addition of a dietary enzyme complex did not influence the feed intake of commercial laying hens.

It was observed a significant effect ($p < 0.05$) of the treatments on eggs production. The lowest rate of eggs laying shown by the Negative Control, with reduction of the nutritional levels, illustrates the impact of reduction of the levels of metabolizable energy, raw protein and amino acids, when compared to the Positive Control. Such decreased nutrient levels found in the Negative Control compromised the supply of the nutrients required to maintain eggs production, reflecting on the lower eggs laying rates found. The other treatments did not differ from one another.

The results of this study are consistent with those cited by Bedford (2000). The author points out that the use of exogenous enzymes had positive results in terms of improvements in the utilization of nutrients, reduction of moisture in the excreta and partial or total elimination of the anti-nutritional factors found in the dietary feeds commonly used in poultry farming.

The data relating to eggs laying suggests that the protease used in the experiments contributed to improve the intake of the calories and amino acids

contained in the diets, due to the fact that eggs production can be altered mainly by changing the energy levels. Such effect was observed in an experiment conducted by Araújo & Peixoto (2005) with semi-weighted layers, where they found an increased eggs production and metabolizable energy changed from 2,450 to 2,650 kcal of ME kg⁻¹.

According to the results, a decrease in nutritional levels is possible in case of enzyme supplementation at the levels used in the present study, without compromising eggs production.

A significant difference ($p < 0.05$) was observed in the variable of average eggs weight. Treatment 4 showed lower eggs weights compared to the other treatments. Such findings are consistent with those found by Gentilini et al. (2009), who assessed the effects of addition of an enzyme complex to the diets of semi-weighted layers and found that such addition yielded lower weights of eggs and albumen, but had no effect on eggs production, Haugh unit and total plasma proteins. The authors also reported that the enzyme supplementation reduced the cecal bacterial load, indicating an improved sanitary condition of the intestines. But Oba et al. (2013) studied the addition of a dietary enzyme complex based on corn and soybean meal and did not find improvement in production performance, eggs quality or in the intestinal microbiology of laying hens at the end of the production cycle.

Novak, Yakout & Remus (2008) reported that commercial laying hens fed diets with low concentration of crude protein, but supplemented with an enzyme complex based on α -amylase, protease and xylanase over a period of 60 days, showed reduction in eggs weight, but gains in weight and eggs mass.

Studies conducted by Murakami, Fernandes, Sakamoto, Souza & Furlan (2007) to evaluate the addition of a multi-enzyme complex at the levels of 400 and 500 ppm to the diets of lightweight laying hens, as well as its effects on the parameters of performance and eggs quality, showed that the addition of 400 ppm yielded higher values of feed intake and feed conversion. However, the authors did not find significant effect on eggs production.

There was a significant effect ($p > 0.05$) on feed conversion. The birds of the negative control showed a decrease in this variable as a function of eggs reduction and lower egg laying rate, factors that together contributed to this result.

According to the results found, the birds fed on protease-supplemented diets (Treatments 3 and 4) showed a similar performance to the birds fed the positive control diet, which demonstrates the

effectiveness of the enzyme supplementation. However no significant difference ($p > 0.05$) of the enzyme supplementation on the shell quality was found.

Table 3 presents the results relating to the experimental periods.

Table 3. Feed intake (FI), eggs production (EP) (% hen⁻¹ day⁻¹), feed conversion (FCR), average eggs weight (AEW), shell ratio (SR), shell thickness (ST) and specific gravity (SG) of semi-weighted laying hens per experimental period.

Period	FI (g bird ⁻¹ day ⁻¹)	PO (% hen ⁻¹ day ⁻¹)	AEW (g)	FCR (g g ⁻¹)	SG (g mL ⁻¹)	SR (%)	ST (mm)
I (w/o)	119.12 c	91.13 b	63.13	2.07 b	1.096 c	9.84 ab	0.570 b
II (w/o)	118.27 c	90.91 b	63.91	2.04 ab	-	9.92 ab	0.568 b
III (w/o)	114.46 b	89.42 b	63.47	2.02 ab	1.086 a	9.69 a	0.549 ab
IV (w/o)	107.93 a	86.47 a	63.25	1.98 a	-	10.02 b	0.562 ab
V (w/o)	112.44 b	85.51 a	63.10	2.08 b	1.091 b	9.92 ab	0.543 a
CV (%)	3.14	3.15	1.11	3.05	0.12	4.04	4.54

^{a,b} In the same column, means followed by different letters are statistically different by the SNK test ($p < 0.05$).

There was a significant effect ($p < 0.05$) of the experimental periods on feed intake, eggs production and feed conversion. Such results are consistent with Cotta (1997), who mentions that over the productive life production diminishes as a function of lower feed conversion, and increases the production costs per dozen of eggs.

There was a significant effect ($p < 0.05$) of the experimental periods on eggs production, where such variable showed a decrease in treatments IV and V. According to the results found, there was a significant decrease in feed intake ($p < 0.05$) and a lower feed conversion ($p < 0.05$) as the birds got older. The average weight of the eggs was not different ($p > 0.05$) in the experimental period.

There was a significant effect ($p < 0.05$) of the experimental periods on the eggs shell ratio, and the lowest percentages were found in experiment III. Shell thickness and specific gravity of the eggs were influenced by the experimental periods ($p < 0.05$), and treatments II and V presented the lowest shell thickness, and period III the lowest specific gravity.

The layers' age is one of the factors that most influence the eggs shell, and such deterioration results from changes in the body of the animals caused by ageing, such as a decrease in the shell weight/egg weight ratio, a lower calcium absorption in the intestines and a higher mobilization of this nutrient in the bones. Thus, all these factors together reduce the shell quality and increase the number of cracked eggs (Cotta, 1997).

Added to the nutritional benefits, the use of enzymes can help maintain the normal balance of the body, which can reflect on better conditions for higher performance and eggs production.

Conclusion

It can be concluded that the supplementation of 500 g ton⁻¹ of protease (100 U g⁻¹) to diets with low nutritional energy levels yields performance and external egg quality similar to those achieved by semi-weighted layers that are fed diets with the nutritional levels recommended by the breed manual. The main productive indicators that changed because of the dietary supplementation with protease were eggs production and feed conversion. These results were similar to the Positive Control, which proves the efficiency of enzyme supplementation. However, supplementation with protease was not able to change the indicators of eggs shell.

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