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Effects of a monocomponent protease from *Bacillus licheniformis* on broiler performance, digestibility, and carcass yield

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ABSTRACT. Two experiments were carried out to evaluate the effects of protease addition to the diet of broilers at a higher level (1× or 2×) than the nutritional value proposed for the enzyme. The first experiment, 1280 day-old chicks (Cobb500®) were randomly allocated (randomized block design, 2×2+1 factorial arrangement), five treatments, eight replicates containing 32 birds/replicate. Treatments consisted: control diet without protease (CD); CD + 1× nutritional value of the enzyme (CDM1); CD + 2× nutritional value of the enzyme (CDM2); CDM1 + protease; and CDM2 + protease. The experimental period was 42 days. The mean weight (AFW), feed intake (FI), weight gain (WG), feed conversion, and carcass yield were evaluated. Significant differences were observed for AFW, WG, FI, abdominal fat yield, and feet percentage in the carcass. In the second experiment, 120 Cobb500® chicks at 14 days of age were allotted in a completely randomized design, 2×2+1 factorial arrangement, five treatments, six replicates with four birds/replicate. The treatments were consistent with the first experiment. Significant improvements in the nitrogen balance were observed for the broilers that received protease. The use of the enzyme tested is recommended with the recommended nutritional matrix, improving the zootechnical indices of broilers.

Keywords: additive; exogenous enzyme; nutrition; poultry.

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Introduction

Brazil is the world's largest exporter and third largest producer of chicken meat and much of this achievement is due to the evolution in management, facilities, genetics, and most importantly, nutrition (Associação Brasileira de Proteína Animal [ABPA], 2021). Advances in chicken nutrition were achieved through the support of nutritionists. These advances included economic aspects of diets and the environmental consequences of modern feed formulation.

The concentration and composition of dietetic nutrients supplied to birds directly relate to the amount and composition of excreta produced. In order to improve animal performance and reduce environmental pollution, protein intake was reduced to decrease nitrogen loss (from around 30 to 40%) and increase the availability of energy for tissue deposition (Vasconcellos et al., 2011). Concurrently, the inclusion of exogenous enzymes in animal diets has been an important nutritional strategy. These additives help to reduce antinutritional factors, improve digestion and absorption, and increase nutrient availability to make production more efficient with less pollution (Leite et al., 2011). The suggested method to minimize these emissions is to reduce the required amount of protein while adding specific enzymes to the diet to improve the protein absorbed (Leinonen & Williams, 2015).

Among the exogenous enzymes, proteases increase protein digestibility of the ingredients used in the feed, hydrolyzing them into peptides and amino acids, thereby facilitating their absorption (Ribeiro, Fassani, Makiyama, & Clemente, 2015). Corroborating research by Oxenboll, Pontopiddan and Fru-Nji (2011), where the authors attested that the benefits of the use of proteases in broilers resulted in improved animal performance and reduced nitrogen emissions, a significant benefit for the environment.

It is possible that both bird performance and yield of commercial cuts will be improved with protease use by increasing nutrient digestibility. This saves energy expenditure, allowing energy to be directed to nutrient

deposition in muscle tissues which improves production and reduces expenses. Matias et al. (2015) confirmed that the use of exogenous enzymes could reduce production costs by improving feed efficiency.

Based on previous research findings, this experiment was carried out to evaluate the effects of adding protease (at 0.05% of monocomponent protease obtained from *Bacillus licheniformis*) in addition to the enzyme (1× or 2× the recommended rate) on the performance, nutrient metabolizability, and carcass yield of broilers from 1 to 42 days.

Material and methods

Two experiments were carried out at the Department of Animal Science of the Veterinary and Animal Science College of the Federal University of Goiás (EVZ/UFG), Goiânia, Goiás, Brazil. The research project was approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Goiás (protocol nº 026/16).

The first experiment was conducted at the Commercial Broiler Facilities of EVZ/UFG. A total of 1280 male Cobb 500® day-old chicks with an average weight (of 42 ± 2 g) were distributed in a randomized block design and 2×2+1 factorial arrangement, totaling five treatments, with eight replicates containing 32 birds in each.

The five treatments were: control diet without protease (CD); CD + 1 × nutritional value of the enzyme (CDM1); CD + 2 × nutritional value of the enzyme (CDM2); CDM1 + protease; and CDM2 + protease. The experimental period was 42 days with four phases: pre-starter (1 to 7 days), starter (8 to 21 days), growth (22 to 35 days), and final (36 to 42 days). The corn and soybean meal experimental diets were formulated according to the nutritional levels proposed by São Salvador Alimentos S/A. The nutritional composition and percentages of diets for all phases are presented in Tables 1, 2, 3, 4 and 5.

The protease enzyme used was Cibenza DP 100® and added to the feed mixture in a ratio of 0.05%. The nutritional matrix for the enzyme is provided in Table 6.

The birds were housed in 40 experimental boxes with dimensions of 1.80 x 1.60 m and equipped with nipple drinkers, tubular feeders, and rice husk litter. The boxes were inside an industrial masonry shed covered with thermic clays and concrete floors with screened walls. The facility contains a negative ventilation system, diesel heater, and nebulizers.

Food and water were supplied *ad libitum* throughout the experiment. Heating was monitored by assessing the air temperature and relative humidity. Lighting was artificial (fluorescent lamps) and constant.

Performance was evaluated on the 7^{th}, 14^{th}, 21^{st}, 28^{th}, 35^{th}, and 42^{nd} day using the variables of: feed intake (g), mean weight (g), weight gain (g) and feed conversion (kg kg^{-1}). Feed intake (g) was calculated by the difference in weight between the feed provided and the leftovers on the 7^{th}, 14^{th}, 21^{st}, 28^{th}, 35^{th}, and 42^{nd} day; the weight was calculated per experimental unit. The mean weight was calculated by the total weight of the broilers divided by the number of birds per plot. Weight gain was obtained by calculating the difference between the initial and final average weights of the birds for each period. Feed conversion was calculated by analyzing the relationship between weight gain and feed intake. The performance variables were calculated using the mortality rate, which was recorded daily.

Carcass yield, breast yield, thighs yield, wings yield, and abdominal fat were calculated using an average sized bird for each replicated plot was measured on day 41 (at 42 days old). This individual was used to represent the average weight of the plot. Birds were fasted for six hours prior to being euthanized (involving electronarcosis). Feathers were removed and the carcass was weighed again, eviscerated and the commercial cuts (breast, thighs, and wings) were sampled. The abdominal fat was collected from the cavity and the bursa; these were weighed individually on a precision scale. Carcass yield (CR) was calculated using the live weight before slaughter and expressed as a percentage. The yield of each carcass part, breast, and abdominal fat were expressed as a function of carcass weight (with the head and feet). The weights of the heart, liver, and gizzard were also expressed as a percentage of the carcass weight (including the head and feet).

The second experiment, a metabolism trial, was carried out in the Experimental Aviary of EVZ/UFG. A total of 120 Cobb 500® 14-day-old male chicks with a mean weight of 497.5±5 g were distributed in a completely randomized design and a 2×2+1 factorial arrangement with five treatments, each with six replicates containing four animals in each replicate.

Table 1. Percentual and nutritional calculated composition for pre-starter basal diet (1-7 days) for control treatment, valued once and valued 2 times according to the nutritional matrix of enzyme.

Ingredients	Control	Valuated 1 time	Valuated 2 times
Ground corn	52.34	54.85	58.31
Soybean meal	37.02	35.36	32.67
Limestone	0.80	0.80	0.80
Common salt	0.41	0.41	0.37
Sodium bicarbonate	0.09	0.09	0.15
DL-Methionine	0.41	0.40	0.39
Choline chloride	0.06	0.07	0.07
Antimicrobial	0.02	0.02	0.02
Poultry fat	3.00	2.13	1.13
Meat and bone meal	3.67	3.67	3.60
Poultry offal meal	1.53	1.53	1.80
Copper sulfate	0.03	0.03	0.03
L-lysine	0.27	0.29	0.32
L-threonine	0.10	0.09	0.09
L-valine	0.03	0.03	0.04
Phytase	0.01	0.01	0.01
Prebiotic	0.04	0.04	0.04
Antibiotic	0.01	0.01	0.01
Anticoccidial	0.04	0.04	0.04
Vitamin supplement[1]	0.05	0.05	0.05
Mineral supplement[2]	0.06	0.06	0.06
Protease[3]	-	0.03	0.03
Total	100	100	100
Calculated nutritional composition			
Metabolizable energy(kcal kg^{-1})	3000	3000	3000
Crude protein (%)	24.42	24.50	24.50
Digestible threonine(%)	0.86	0.86	0.86
Digestible methionine + cystine (%)	1.01	1.01	1.01
Digestible methionine (%)	0.72	0.71	0.70
Digestible lysine (%)	1.35	1.35	1.35
Calcium (%)	0.98	0.98	0.98
Available phosphorus(%)	0.49	0.49	0.49
Sodium(%)	0.23	0.23	0.23

[1]Vitamin supplement – Inclusion per kg of the diet: Vitamin A 400 IU, Vitamin D3 100 IU, Vitamin E 1 mg, Vitamin K3 0.08 mg, Vitamin B1 0.1 mg, Vitamin B2 0.26 mg, Vitamin B6 0.14 mg, Vitamin B12 0.72 mcg, Pantothenic acid 0.6 mg, niacin 1.68 mg, Folic acid 48 mcg, selenium 12 mcg, biotin 3.2 mcg; [2]Mineral supplement – Inclusion per kg of the diet: copper 0.325 mg, iron 2 mg, manganese 3 mg, iodine 0.04 mg, zinc 2.5 mg; [3]Protease – Cibenza DP 100®.

The following treatments were used: control diet without protease (CD); CD + 1× nutritional value of the enzyme (CDM1); CD + 2 × nutritional value of the enzyme (CDM2); CDM1 + protease; and CDM2 + protease. The experimental period consisted of seven days; this involved three days of adaptation to the experimental conditions and four days of collection. The trial involved total excreta collection, following the procedures designed by Sakomura and Rostagno (2016). The experimental diets were based on corn and soybean meal, vitamin and mineral supplements were formulated according to the nutritional requirements of São Salvador Alimentos S/A. The protease enzyme Cibenza DP 100® was added to the diet in a ratio of 0.05%. The compositions (nutrition with percentage) of the diets for the starter phase are presented in Table 2.

Chicks were housed in 30 experimental cages of galvanized steel with the dimensions of 0.40 × 0.50 m, equipped with drinkers and feeders and trays lined with plastic were used for excreta collection. The cages were placed inside an experimental masonry shed with curtain ventilation.

Water and feed were provided *ad libitum* throughout the experimental period. The maximum and minimum temperature and relative humidity were monitored daily. Incandescent lamps were placed within each experimental unit to provide constant lighting.

The metabolism trial involved excreta collection on day 17 to 21 (Sakomura & Rostagno, 2016). Collections were performed twice a day, and frozen in clearly labelled plastic bags. To perform the bromatological analyses, the frozen samples were pre-dried in a rectilinear forced ventilation oven at 55 ± 5ºC. Subsequently, the pre-dried samples were ground in a Willey mill and analyzed according to the methodology designed by Silva and Queiroz (2002). Nutritional balances were calculated, as described by Matterson, Potter, Stutz and Singsen (1965), and the metabolizability coefficients were calculated using the methodology described by Batal and Parsons (2002) and Noy and Sklan (2002). The metabolizability coefficient was calculated as the

percentage of the retained quantities (the amount ingested subtracted from the excreted) and the amount ingested of nutrients and energy, according to Sakomura and Rostagno (2016).

Table 2. Percentual and nutritional calculated composition for starter basal diet (8-21 days) for control treatment, valued once and valued 2 times according to the nutritional matrix of enzyme.

Ingredients	Control	Valuated 1 time	Valuated 2 times
Ground corn	57.14	59.90	62.68
Soybean meal	31.53	29.60	27.67
Limestone	0.87	0.87	0.87
Common salt	0.37	0.37	0.37
Sodium bicarbonate	0.05	0.05	0.05
DL-Methionine	0.37	0.37	0.37
Choline chloride	0.07	0.08	0.08
Antimicrobial	0.01	0.01	0.01
Poultry fat	3.80	2.87	2.00
Meat and bone meal	3.13	3.20	3.20
Poultry offal meal	2.00	2.00	2.00
Copper sulfate	0.03	0.03	0.03
L-lysine	0.27	0.29	0.32
L-threonine	0.10	0.09	0.09
L-valine	0.02	0.03	0.03
Phytase	0.01	0.01	0.01
Prebiotic	0.04	0.04	0.04
Antibiotic	0.01	0.01	0.01
Anticoccidial	0.06	0.06	0.06
Vitamin supplement[1]	0.05	0.05	0.05
Mineral supplement[2]	0.05	0.05	0.05
Protease[3]		0.03	0.03
Total	100	100	100
Calculated nutritional composition			
Metabolizable energy(kcal/kg)	3000	3100	3100
Crude protein (%)	22.50	22.50	22.50
Digestible threonine(%)	0.80	0.80	0.80
Digestible methionine + cystine (%)	0.93	0.93	0.93
Digestible methionine (%)	0.66	0.65	0.65
Digestible lysine (%)	1.23	1.23	1.23
Calcium (%)	0.92	0.92	0.92
Available phosphorus(%)	0.45	0.45	0.45
Sodium(%)	0.20	0.20	0.20

[1]Vitamin supplement – Inclusion per kg of the diet: Vitamin A 400 IU, Vitamin D3 100 IU, Vitamin E 1 mg, Vitamin K3 0.08 mg, Vitamin B1 0.1 mg, Vitamin B2 0.26 mg, Vitamin B6 0.14 mg, Vitamin B12 0.72 mcg, Pantothenic acid 0.6 mg, niacin 1.68 mg, Folic acid 48 mcg, selenium 12 mcg, biotin 3.2 mcg; [2]Mineral supplement – Inclusion per kg of the diet: copper 0.325 mg, iron 2 mg, manganese 3 mg, iodine 0.04 mg, zinc 2.5 mg; [3]Protease – Cibenza DP 100®.

Performance, carcass yield and digestibility data were evaluated by ANOVA, and significant results ($p \leqslant 0.05$) were compared using a Tukey post hoc test. The computational package R was used for the analyses. The statistical model was:

$$Y_{ijvk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \lambda_v + \varepsilon_{ijvk}$$

and

$$y_{vh} = \mu + \tau + \lambda_v + \varepsilon_{vh}$$

in which:

Y_{ijvk}: is the response variable related to the i-th level of the first factor (i = 1, 2, ..., a) with the j-th level of the second factor (j = 1, 2, ..., b) in the v-th block (v = 1, 2, ..., w) in the k-th repetition (k = 1, 2, ..., r);

μ: is the general mean;

α_i: it is the effect of the i-th level of the first factor;

β_j: it is the effect of the j-th level of the second factor;

γ_{ij}: it is the effect of the interaction of the i-th level of the first factor with the j-th level of the second factor;

λ_v: it is the effect of the v-th block;

ε_{ijvk}: it is the experimental error associated with the observation Y_{ijk} and it is assumed that $\varepsilon_{ijk} \sim N(0, \sigma^2)$ and independent;

y_{vh}: it is the response variable related to the v-th block of the h-th repetition of the additional treatment (h = 1, 2, ..., m);
τ: is the effect of additional treatment;
ε_{vh}: it is the experimental error associated with additional treatment and it is assumed that $\varepsilon_{vh} \sim N(0, \sigma^2)$ and independent.

Table 3. Percentual and nutritional calculated composition for growing basal diet 1 (22-28 days) for control treatment, valued once and valued 2 times according to the nutritional matrix of enzyme.

Ingredients	Control	Valuated 1 time	Valuated 2 times
Ground corn	57.57	60.58	62.97
Soybean meal	30.56	27.90	26.18
Limestone	0.90	0.93	0.93
Common salt	0.28	0.27	0.27
Sodium bicarbonate	0.10	0.10	0.10
DL-Methionine	0.32	0.30	0.29
Choline chloride	0.05	0.05	0.05
Antimicrobial	0.01	0.01	0.01
Poultry fat	4.74	3.73	3.00
Meat and bone meal	2.47	2.13	2.13
Poultry offal meal	2.53	3.47	3.53
Copper sulfate	0.03	0.03	0.03
L-lysine	0.19	0.21	0.22
L-threonine	0.06	0.06	0.05
Phytase	0.01	0.01	0.01
Prebiotic	0.02	0.02	0.02
Antibiotic	0.01	0.01	0.01
Anticoccidial	0.06	0.06	0.06
Vitamin supplement[1]	0.05	0.05	0.05
Mineral supplement[2]	0.05	0.05	0.05
Protease[3]	-	0.03	0.03
Total	100	100	100
Calculated nutritional composition			
Metabolizable energy(kcal kg^{-1})	3240	3240	3240
Crude protein (%)	21.74	21.81	21.88
Digestible threonine(%)	0.75	0.75	0.75
Digestible methionine + cystine (%)	0.87	0.87	0.87
Digestible methionine (%)	0.60	0.59	0.59
Digestible lysine (%)	1.15	1.15	1.15
Calcium (%)	0.88	0.88	0.88
Available phosphorus(%)	0.42	0.42	0.42
Sodium(%)	0.18	0.18	0.18

[1]Vitamin supplement – Inclusion per kg of the diet: Vitamin A 320 IU, Vitamin D3 76 IU, Vitamin E 0.8 mg, Vitamin K3 0.072 mg, Vitamin B1 0.072 mg, Vitamin B2 0.22 mg, Vitamin B6 0.104 mg, Vitamin B12 0.6 mcg, Pantothenic acid 0.52 mg, niacin 1.4 mg, Folic acid 36 mcg, selenium 12 mcg, biotin 2 mcg; [2]Mineral supplement – Inclusion per kg of the diet: copper 0.325 mg, iron 2 mg, manganese 3 mg, iodine 0.04 mg, zinc 2.5 mg; [3]Protease – Cibenza DP 100®.

Results

Performance data of broilers was evaluated from days 1 to 7, 1 to 21 and 1 to 42 (Table 7). Significant differences ($p < 0.05$) were observed between treatments with or without protease addition, for the average final weight (AFW) and weight gain (WG) from day 1 to 7. Birds fed diets containing protease showed higher AFW and WG. In the same period, there were differences ($p < 0.05$) between treatments that received protease and the control diet (negative control) for feed intake (FI). In the period from 1 to 21 days, differences were observed ($p < 0.05$) between treatments that received protease and the negative control, both for FI and for feed conversion (FC). No differences were observed ($p > 0.05$) for any of the variables over the entire period from 1 to 42 days of age.

The carcass yield of broilers at 42 days of age (Table 8) treated with two levels of protease showed significant differences ($p < 0.05$) in abdominal fat (AF). Birds that received diets with 2× the nutritional value of the enzyme obtained a higher deposition of AF. There was a significant difference in feet yield between protease treatments to the negative control ($p < 0.05$). Birds that did not receive protease in their diet obtained a higher feet yield. For all other variables, no differences were found ($p > 0.05$).

For the metabolizability coefficients (Table 9), there were significant differences in nitrogen balance (NB) between the treatments that received protease and the negative control ($p < 0.05$).

Table 4. Percentual and nutritional calculated composition for growing basal diet 2 (29-35 days) for control treatment, valued once and valued 2 times according to the nutritional matrix of enzyme.

Ingredients	Control	Valuated 1 time	Valuated 2 times
Ground corn	62.76	65.46	68.22
Soybean meal	26.17	24.24	22.11
Limestone	0.90	0.90	0.93
Common salt	0.26	0.26	0.26
Sodium bicarbonate	0.13	0.13	0.13
DL-Methionine	0.29	0.27	0.26
Choline chloride	0.05	0.06	0.06
Antimicrobial	0.02	0.02	0.02
Poultry fat	4.07	3.27	2.40
Meat and bone meal	2.33	2.33	2.27
Poultry offal meal	2.53	2.53	2.80
Copper sulfate	0.03	0.03	0.03
L-lysine	0.21	0.22	0.23
L-threonine	0.06	0.05	0.05
Anticoccidial	0.06	0.06	0.06
Phytase	0.01	0.01	0.01
Prebiotic	0.02	0.02	0.02
Antibiotic	0.01	0.01	0.01
Vitamin supplement[1]	0.05	0.05	0.05
Mineral supplement[2]	0.05	0.05	0.05
Protease[3]	-	0.03	0.03
Total	100	100	100
Calculated nutritional composition			
Metabolizable energy(kcal kg^{-1})	3250	3250	3250
Crude protein (%)	19.99	19.99	19.97
Digestible threonine(%)	0.68	0.68	0.68
Digestible methionine + cystine (%)	0.81	0.80	0.80
Digestible methionine (%)	0.55	0.54	0.53
Digestible lysine (%)	1.05	1.05	1.04
Calcium (%)	0.85	0.85	0.85
Available phosphorus(%)	0.41	0.41	0.41
Sodium(%)	0.18	0.18	0.18

[1]Vitamin supplement – Inclusion per kg of the diet: Vitamin A 320 IU, Vitamin D3 76 IU, Vitamin E 0.8 mg, Vitamin K3 0.072 mg, Vitamin B1 0.072 mg, Vitamin B2 0.22 mg, Vitamin B6 0.104 mg, Vitamin B12 0.6 mcg, Pantothenic acid 0.52 mg, niacin 1.4 mg, Folic acid 36 mcg, selenium 12 mcg, biotin 2 mcg; [2]Mineral supplement – Inclusion per kg of the diet: copper 0.325 mg, iron 2 mg, manganese 3 mg, iodine 0.04 mg, zinc 2.5 mg; [3]Protease – Cibenza DP 100®.

Discussion

Many studies assessing different nutritional levels and enzyme supplementation corroborate this study. Similarly, Miranda, Goulart, Leite, Batista and Lima (2017) evaluated the use of enzymatic supplementation in cottonseed meal for broiler diets and observed that regardless of the level of enzymatic supplementation of a protease in the pre-starter phase, the birds presented higher AFW and WG, when compared to those fed the control diet; however, feed conversion was not influenced. Corroborating the results of this experiment, Angel, Saylor, Vieira and Ward (2011) obtained positive results when broiler diets were supplemented with protease enzyme with a reduction of crude protein (9% less) and amino acids (10% less), and obtained better performance results (WG and FC) when compared to broilers not provided the enzyme, similar to some results observed in this experiment. Carvalho et al. (2020) stated that the use of protease during the starter rearing period is recommended mainly for vegetable-based diets.

From 1 to 21 days, our results are similar to those observed by Angel et al. (2011) who evaluated the effect of a monocomponent protease in broiler diets with different nutritional levels and observed lower FC in the group supplemented with any protease at any amount (100 to 800 mg kg^{-1} of enzyme supplementation) when compared with the control diet. Similarly, Cardinal et al. (2019) tested protease supplementation in protein and amino acid-deficient diets on broiler performance and intestinal health and observed no effect on performance in the pre-starter and starter phases. Lourenco et al. (2020) also found that when protease was included in broilers on protein-deficient diets, there was no improvement in performance and less WG and FC values.

Table 5. Percentual and nutritional calculated composition for final basal diet (36-42 days) for control treatment, valued once and valued 2 times according to the nutritional matrix of enzyme.

Ingredients	Control	Valuated 1 time	Valuated 2 times
Ground corn	66.13	68.09	70.49
Soybean meal	23.39	22.17	20.50
Limestone	0.90	0.90	0.90
Common salt	0.28	0.28	0.28
Sodium bicarbonate	0.15	0.15	0.15
DL-Methionine	0.27	0.26	0.25
Choline chloride	0.04	0.05	0.05
Antimicrobial	0.01	0.01	0.01
Poultry fat	3.87	3.27	2.47
Meat and bone meal	1.60	1.80	1.87
Poultry offal meal	3.00	2.53	2.53
Copper sulfate	0.03	0.03	0.03
L-lysine	0.22	0.23	0.24
L-threonine	0.07	0.06	0.05
Phytase	0.01	0.01	0.01
Prebiotic	0.02	0.02	0.02
Antibiotic	0.01	0.01	0.01
Vitamin supplement[1]	0.05	0.05	0.05
Mineral supplement[2]	0.05	0.05	0.05
Protease[3]	-	0.03	0.03
Total	100	100	100
Calculated nutritional composition			
Metabolizable energy(kcal kg^{-1})	3000	3000	3000
Crude protein (%)	24.42	24.50	24.50
Digestible threonine(%)	0.86	0.86	0.86
Digestible methionine + cystine (%)	1.01	1.01	1.01
Digestible methionine (%)	0.72	0.71	0.70
Digestible lysine (%)	1.35	1.35	1.35
Calcium (%)	0.98	0.98	0.98
Available phosphorus(%)	0.49	0.49	0.49
Sodium(%)	0.23	0.23	0.23

[1]Vitamin supplement – Inclusion per kg of the diet: Vitamin A 160 IU, Vitamin D3 32 IU, Vitamin E 0.32 mg, Vitamin K3 0.04 mg, Vitamin B1 0.04 mg, Vitamin B2 0.12 mg, Vitamin B6 0.06 mg, Vitamin B12 0.28 mcg, Pantothenic acid 0.36 mg, niacin 1 mg, Folic acid 18 mcg, selenium 8 mcg, biotin 0.8 mcg; [2]Mineral supplement – Inclusion per kg of the diet: copper 0.325 mg, iron 2 mg, manganese 3 mg, iodine 0.04 mg, zinc 2.5 mg; [3]Protease – Cibenza DP 100®.

Table 6. Nutritional matrix of protease enzyme used to enhance diets.

Nutrients	Nutritional matrix
Crude protein (%)	2672
Metabolizable energy (kcal kg^{-1})	97293
Lysine (%)	133
Methionine (%)	36
Methionine + Cystine (%)	88
Threonine (%)	113
Arginine (%)	184
Valine (%)	103
Thryptophan (%)	40
Isoleucine (%)	103
Leucine (%)	215

Source: Cibenza DP 100® Ficha Técnica

We expected an improvement in broiler performance with the addition of protease in the broiler diets. It is well known that when the digestibility of proteins and amino acids are increased the efficiency of endogenous enzymes also increases. However, the results we obtained also corroborate those of Yuan, Wang, Wang, Zhu and Huang (2015) who found that including protease in broiler diets could negatively affected performance, especially in the growth phase. Similarly, Walk, Juntunen, Paloheimo and Ledoux (2019) tested the dose-response effects of protease in the diets of broilers and observed that higher doses of enzyme supplementation reduced performance.

Leite et al. (2011) suggests the negative effect on performance at high doses is related to the enzyme specificity, and therefore related to ingredient quality and composition. Thus, the variation in chemical composition of feeds combined with an enzyme and/or enzyme complex not specific for that chemical combination may not be able to

improve the degradation, digestion, and absorption of the nutrients. Moura et al. (2019), provides recommendations based on the enzyme nutritional matrix of corn and soybean meal feeds. For precise results, it is essential to adjust the enzyme recommendations for the ingredients in the diet.

The results observed for carcass yield (except AF deposition and feet yield) are similar to those found by Freitas, Vieira, Angel, Favero and Maiorka (2011) who did not observe any effect of protease addition to broiler diets in carcass yield and commercial cuts.

Table 7. Average Final Weight (AFW), Weight Gain (WG), Feed Intake (FI) and Feed Conversion (FC) of broilers, in the periods from 1 to 7 days, 1 to 21 days and 1 to 42 days of age, considering the interaction enzyme X nutritional valuation.

Period	Variable	Valuation		P*	Enzyme		P**	Control	Treatments	P***	P****
		V1	V2		+	-					
1 a 7	AFW (g)	175	172	0.212	177 a	170 b	0.010	175	173	0.365	1
	WG (g)	133	130	0.181	134 a	128 b	0.012	132	131	0.202	0.941
	FI (g)	153	152	0.880	155	149	0.941	153 a	152 b	0.003	0.672
	FC	1.144	1.178	0.278	1.150	1.172	0.202	1.153	1.161	0.183	0.627
1 a 21	AFW (g)	946	941	0.589	938	949	0.280	964	944	0.140	0.625
	WG (g)	904	899	0.587	896	907	0.272	921	901	0.141	0.661
	FI (g)	1.332	1.343	0.530	1.337	1.338	0.947	1.327 b	1.338 a	0.047	0.820
	FC	1.369	1.349	0.141	1.383	1.381	0.907	1.342 b	1.382 a	0.009	0.419
1 a 42	AFW (g)	3.065	3.069	0.933	3.087	3.048	0.345	3.048	3.067	0.501	0.678
	WG (g)	3.023	3.026	0.932	3.044	3.006	0.346	3.048	3.025	0.473	0.672
	FI (g)	4.627	4.677	0.269	4.644	4.660	0.736	4.608	4.652	0.213	0.811
	FC	1.483	1.501	0.205	1.482	1.503	0.153	1.480	1.492	0.110	0.506

Valuation of nutritional valuation in 1 time, V2: Valuation of nutritional valuation in 2 times. *Probability value of the F test for the isolated effect of valuation. **Probability value for the effect of Enzyme. ***Probability value for Control VS Treatments average. ****Probability value for Interaction.

Table 8. Carcass cuts and abdominal fat percentual results in relation to live weight at 42 days of age.

Variable (%)	Valuation		P*	Enzyme		P**	Test.	Trat.	P***	P****
	V1	V2		+	-					
Carcass	83.07	82.13	0.226	82.98	82.23	0.334	81.35	82.60	0.194	0.924
Heart	0.46	0.45	0.770	0.45	0.46	0.827	0.45	0.45	0.122	0.810
Liver	1.71	1.74	0.665	1.71	1.73	0.801	1.82	1.72	1	0.186
Gizzard	1.17	1.18	0.875	1.18	1.16	0.650	1.15	1.17	1	0.086
Feet	3.65	3.69	0.590	3.67	3.67	0.943	3.81 a	3.67 b	0.044	0.299
Head + Neck	6.79	6.75	0.861	6.69	6.86	0.410	6.68	6.77	0.489	0.376
Breast fillet	22.33	21.84	0.346	22.35	21.81	0.295	21.81	22.08	0.391	0.887
Legs	25.68	25.37	0.371	25.56	25.49	0.836	25.23	25.53	0.193	0.661
Wings	8.59	8.46	0.459	8.47	8.56	0.534	8.44	8.51	0.488	0.718
Back	16.56	16.29	0.533	16.69	16.15	0.207	16.85	16.42	0.109	0.614
Abdominal fat	1.54 b	1.88 a	0.025	1.68	1.73	0.722	1.60	1.71	1	0.412

V1: Valuation of nutritional matrix in 1 time, V2: Valuation of nutritional matrix in 2 times. *Probability value of the F test for the isolated effect of valuation. **Probability value for the effect of Enzyme. ***Probability value for Control VS Treatments average. ****Probability value for Interaction.

Table 9. Dry matter balance (DMB), nitrogen balance (NB), dry matter retention (DMR), nitrogen retention (NR), dry matter metabolizability (DMM), nitrogen metabolizability (NM) for broiler diets fed diets supplemented with protease, valued 1 and 2 times, from 17 to 21 days of age.

Variable	Valuation		P*	Enzyme		P**	Test.	Trat.	P***	P****
	V1	V2		+	-					
DMB (g)	661.0	668.7	0.80	656.8	672.8	0.60	683.9	664.8	0.14	0.47
NB (g)	7.2	7.1	0.80	7.2	7.1	0.34	7.2 a	7.2 b	0.04	0.12
DMR (mg/g)	193.6	189.8	0.66	184.8	198.6	0.11	188.6	191.7	0.99	0.75
NR (mg/g)	4.6	5.1	0.53	5.5	4.2	0.13	4.2	4.9	0.99	0.16
DMM (%)	53.5	55.0	0.47	55.0	53.5	0.49	54.0	54.3	0.99	0.50
NM (%)	29.4	31.9	0.61	34.0	27.3	0.17	27.3	30.7	0.99	0.29

V1: Valuation of nutritional matrix in 1 time, V2: Valuation of nutritional matrix in 2 times. *Probability value of the F test for the isolated effect of valuation. **Probability value for the effect of Enzyme. ***Probability value for Control VS Treatments average. ****Probability value for Interaction.

This result is also consistent with that found by Cardoso et al. (2011), who evaluated enzymatic complexes in broiler diets, and did not identify any effect of enzymatic supplementation with a protease on carcass yield. Dalólio et al. (2016) corroborated these findings of protease enzyme supplements in the broiler diet did not affect carcass yield and commercial cuts. Law, Zulkifli, Soleimani, Liang and Awad (2018) also observed that protease supplementation had no effect on the yield of commercial cuts.

Conversely, Dosković et al. (2012) stated that the reduction in crude protein level (of 4 and 6%), with protease supplementation (at 0.2 and 0.3%) resulted in a significant effect on carcass yield, and commercial cuts.

The increase in AF deposition, results of this experiment are in accordance with those observed by Law et al. (2018) he found a higher AF yield in broilers fed protein-deficient diets supplemented with protease. Similar results were also found by Gomide, Rodrigues, Freitas and Fialho (2007), who observed an increase in AF content in birds provided diets with reduced crude protein levels supplemented with amino acids.

The highest percentage of abdominal fat observed in the birds in the treatment with twice the protease matrix valuations can be considered normal. According to Sklan and Noy (2004), the catabolic process of excess amino acids (AAs) accompanies energy expenditure. Thus, rations with amino acid levels close to an ideal profile promote lower energy expenditure to catabolize excess amino acids. Therefore, more abdominal fat will be synthesized due to excess energy. According to Law et al. (2018), this is due to the higher proportion of calories to proteins in low protein diets. The excess energy available, in addition to the amount required for protein deposition, is converted to abdominal fat synthesis.

Apparently, since the diets are not deficient in nitrogen (N) and requirements for protein are supplied, the body protein catabolism reduces, resulting in a positive N balance (NB) (Toghyani, Swick, & Barekatain, 2017). When the NB is positive, it indicates N retention, and the values of the corrected apparent metabolizable energy for NB (AMEn) are lower than the values of apparent metabolizable energy (AME), this indicates increased N retention and protein deposition. However, when this index is negative, the AMEn values are higher than the AME values, indicating protein degradation (Scotta et al., 2016). This suggests that the broilers were able to retain more N by consuming less and increase their protein deposition.

The findings of this study corroborate the results of Oxenboll et al. (2011) who observed that protease in broiler diets offers significant environmental benefits of reduced nitrogen compounds in water and air pollution (i.e., eutrophication, acidification). This leads to a reduction in health risks caused by NH_3 emissions in the poultry litter, supporting a reduction in N emissions from livestock production. This hypothesis was confirmed by Leinonen and Williams (2015), the use of protease in diets reduced the environmental impacts of broiler production, mainly as a result of the reduction in the protein content of the diet and subsequent emissions of nitrogen and NH_3, bringing substantial benefits to the poultry industry.

Conclusion

Diet supplementation of a monocomponent protease obtained from *Bacillus licheniformis* is recommended for the pre-starter and starter phases of broiler development but did not affect performance at the later stages.

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