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Growth performance and nutrient digestibility of broiler chickens as affected by a novel protease

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Two experiments evaluated the addition of an exogenous sfericase protease in broiler diets. Experiments were run (Exp1 and Exp2) with 1,848 and 2,100 one-day-old male chicks being allocated into 84 floor pens with 14 replicates of 22 and 25 birds each, respectively. The studies were conducted in completely randomized designs. In Exp1, Standard diets were formulated with energy and AA at marginally lower levels than usual by the Brazilian integration such that broilers were expected to grow at comparatively reduced rates to the industry whereas in Exp2, the Standard diets were formulated using energy and AA as usual by the Brazilian integrations such that broilers were expected to grow comparable to industry rates. Standard diets had ideally balanced amino acids (AA). Matrix diets, in contrast, had reductions of 6% digestible lysine and of 20 kcal AME/kg compared to the Standard. Matrix diets were supplemented with an sfericase protease at 0, 10,000, and 30,000 New Feed Protease units (NFP)/kg. Outcomes showed no interaction between diet and protease in any of the experiments. However, broilers fed Standard diets had higher cumulative body weight gain (BWG) to 35 and 42 d when compared to Matrix fed birds whereas FCR were worse for birds fed the Matrix diets at 35 d in EXP1 and at 35 and 42 d in EXP2. Improvements in FCR were observed when the sfericase protease was added throughout all ages in EXP1 with a beneficial trend ($P<0.067$) observed in the cumulative FCR at 42 d in EXP2. The ileal digestible crude protein (IDCP) was significantly higher for birds fed Standard feeds in EXP1 with no other differences in digestibility found in any of the experiments. Protease addition led to improvements in ileal digestibility of dry matter (IDM) and IDCP ($P < 0.05$) compared to no protease addition in EXP1 as well as in ileal digestibility of energy (IDE) when 30,000 protease units were added. The present report demonstrates that the novel sfericase protease was successful in compensate broiler performance when reductions of 6% digestible Lys and 20 kcal/kg AME were imposed. This compensation, however, seemed more notable when birds were fed diets formulated to support moderate rather than maximum growth and having animal protein in the feed formula.

KEYWORDS
broiler, digestibility, enzyme, protein, sfericase protease

Introduction

Protein has always been a costly nutrient in poultry feeds (Beski et al., 2015). The quest for the reduction in feed costs has evolved through a sequential implementation of technologies that improve feed protein utilization. Major reductions in feed costs have first occurred with the use of synthetic amino acids in poultry feeds with further increases in the efficiency of protein utilization resulting from the implementation of the ideal protein concept (Baker, 1994; Lemme, 2003). Supplementation of proteases has lastly emerged as a tool to improve dietary protein utilization (Stefanello et al., 2016). Apart from animal performance, supplemental proteases have demonstrated to reduce feed costs as well as nitrogen excretion (Leinonen and Williams, 2015; McCafferty et al., 2022).

Decreasing supplies of protein feedstuffs have been pressuring the existing growing demands of protein to feed humans worldwide (OECD/FAO, 2022). Soy, regardless of its commercially available presentation as a feedstuff (whole bean heat treated, soybean meals with variable CP contents, soybean meal extracted with the use of polar solvents, etc.), remains the primary protein source in animal feeds (Erdaw et al., 2016). The soybean market price has increased 90% in average worldwide in the last 5 years (USDA, 2022). All other protein sources, either from plant or animal origin, have followed up in parallel (FAO, 2021). The increase in protein market price is expected to continue a path of increase since protein daily intake in the world has been steadily increasing (OECD/FAO, 2021). Simultaneously, increased public concerns regarding the environment and sustainability have been pressuring for the reduction of the waste generated by animal production, which includes nitrogen in excreta. Therefore, technologies that potentially improve protein retention of animals grown to feed humans are welcome.

Digestion of protein by birds is dependent on proteases endogenously released in the gastrointestinal tract, which largely degrade protein down to peptides and amino acids that are further absorbed (Sklan and Hurwitz, 1980; Recoules et al., 2017). However, it has been demonstrated that significant amounts of dietary protein present in commercial feeds are not thoroughly digested (Parsons et al., 1997; Adedokun et al., 2008; Bryan et al., 2019). Research on the use of exogenous proteases has been ongoing for years and many commercially available products are currently utilized in poultry feeds. Reports on the effects of supplemental proteases have been related to benefits in performance (Ghazi et al., 2002; Angel et al., 2011; Moss et al., 2017; McCafferty et al., 2022), nutrient digestibility (Freitas et al., 2011; Liu et al., 2013; Stefanello et al., 2016), intestinal health (Xu et al., 2017; Cowieson et al., 2018), and immune competence of broilers (Peek et al., 2009; Cowieson et al., 2017).

Exogenous proteases that can compete and succeed commercially are required to function at corresponding pH's in the gastrointestinal tract, but they also must sustain digestive capabilities after being heat treated at temperatures regularly used in feed processing mills (Ravindran, 2013). Broad substrate specificity of some exogenous proteases, as well as their higher concentrations at the entry of digestive tracts, may produce benefits as effective catalyzers, enabling them to break peptides down faster when compared to feeds not supplemented with proteases (Ravindran, 2013; Cupi et al., 2022).

The objective of the present research was to evaluate the effects of a novel exogenous sfericase protease when added to commercial broiler feeds. Evaluations were conducted on growth performance and extended to ileal digestibility of broilers fed varying AA and energy concentrations. We hypothesized that the protease enzyme increases protein digestibility and, therefore, live performance. It was also hypothesized that protease responses are more evident in diets having reduced AA and energy contents than their counterparts formulated to maximize growth.

Materials and methods

Two experiments were carried out at Santa Livia Research Farm, Farroupilha, Rio Grande do Sul, Brazil. All procedures conducted in the present study were approved by the local Ethics Committee on Animal Use.

Bird husbandry and experimental design

One-day-old slow feathering Cobb x Cobb 500 male broiler chicks, vaccinated for Marek's disease, were obtained from a local hatchery (Carrer Alimentos Ltda., Encantado, RS, Brazil). In both experiments chicks were allocated to one of 6 feeding treatments. Corn-soybean meal feeds were provided in a four-phases program having pre-starter (1 to 7 d), starter (8 to 21 d), grower (22 to 35 d) and finisher (35 to 42 d) feeds (Tables 1, 2). Experimental designs were completely randomized 2 *x* 3 factorials with chicks placed in 84 replicate floor pens (1.65 x 1.65 m). The number of chicks per pen was 22 in experiment 1 and 25 in experiment 2, with the total chick number at the beginning being 1,848 and 2,100 in the first and second experiments. The variation in bird density was due to the availability of healthy chicks at the moment of placement.

Supplemental protease

The tested protease was a novel subtilisin protease from *Bacillus* sp. produced in *Bacillus licheniformis* (Cupi et al., 2022). This is a sfericase protease, an endopeptidase from the serine protease family, subtilisin subfamily A, MEROPS ID S08.113 (Rawlings et al., 2014). Activity for this protease is defined in New Feed Protease units (NFP), which measures the enzyme amount required to hydrolyze 1 μmol of para-nitroaniline (**pNA**) from 1 M substrate Suc-Ala-Ala-Pro-Phe-pNA (Cupi et al., 2022). Enzyme activity in the feeds of the present study were conducted in an Infinite M200 Pro microtiter plate reader (Tecan Lifesciences, Switzerland), with the amount of released yellow pNA being proportional to the protease activity of the enzyme measured photometrically at a wavelength of 405 nm. The utilized protease product was granulated and had 600,000 NFP per g (ProAct 360®, DSM Nutritional Products AG, Kaiseraugst, Switzerland).

Dietary treatments

Both experiments were 2 *x* 3 factorials having two dietaries formulations (Standard and Matrix) and three levels of protease feeds supplementations (0, 10,000 and 30,000 NFPs per kg feed).

TABLE 1 Composition of feeds formulated in experiment 1[1].

Ingredients, kg	Pre-starter, 0 to 7 d		Starter, 8 to 21 d		Grower, 22 to 35 d		Finisher, 36 to 42 d	
	Standard	Matrix	Standard	Matrix	Standard	Matrix	Standard	Matrix
Corn	584.45	600.80	619.15	634.50	652.30	667.65	669.50	683.70
Soybean meal 46%	351.00	337.45	307.00	294.45	264.00	251.45	237.00	225.45
Meat and bone meal	25.00	25.00	30.00	30.00	35.00	35.00	27.00	27.00
Soybean oil	14.00	12.00	23.00	21.00	32.00	30.00	41.00	39.00
Dicalcium phosphate	4.00	4.00	1.10	1.20	–	–	–	–
Limestone	6.00	6.00	5.40	5.40	3.50	3.50	3.80	3.80
Salt	4.90	4.90	4.40	4.40	4.00	4.00	3.90	3.90
DL-Methionine 99%	3.65	3.45	3.25	2.95	2.95	2.65	2.55	2.30
L-Lysine HCl 78%	3.15	3.00	3.05	2.90	2.95	2.85	2.65	2.55
L-Threonine 98.5%	1.15	0.80	1.05	0.70	1.00	0.70	0.80	0.50
L-Valine 98%	0.30	0.20	0.20	0.10	0.20	0.10	–	–
Choline chloride 60%	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral & vitamin premix[2]	1.85	1.85	1.85	1.85	1.55	1.55	1.25	1.25
Indigestible marker[3]	–	–	–	–	–	–	10.00	10.00
Protease[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Nutrient composition, % or as follow								
AME, kcal/kg	2,950	2,930	3,050	3,030	3,150	3,130	3,200	3,180
Crude protein	22.0	20.7	20.5	19.3	19.0	17.9	18.2	17.1
Digestible Lys	1.28	1.20	1.18	1.11	1.08	1.02	0.98	0.92
Digestible TSAA	0.94	0.88	0.87	0.82	0.81	0.76	0.74	0.70
Digestible Thr	0.83	0.78	0.76	0.71	0.71	0.67	0.64	0.60
Digestible Val	0.96	0.90	0.88	0.83	0.82	0.77	0.75	0.71
Av. P	0.48	0.48	0.45	0.45	0.45	0.45	0.40	0.40
Total Ca	0.92	0.92	0.90	0.90	0.86	0.86	0.76	0.76
Na	0.23	0.23	0.21	0.21	0.19	0.19	0.17	0.17

[1]Standard diets were formulated with energy and AA at marginally lower contents than usual by the Brazilian integration such that broilers were expected to grow at comparatively reduced rates to the industry.
[2]Composition per kg feed: iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.40 mg: vitamin A, 8,000 IU; vitamin D3, 2,000 IU; vitamin E, 30 IU; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; phytase at 1,000 FYT/kg (Ronozyme HiPhos GT 20000®, DSM Nutritional Products, Switzerland, provided 1.5 kg/ton Av.P and total Ca); Salinomycin at 60 mg/kg (Coxifarm, Farmabase Animal Health, Jaguariúna, Brazil).
[3]Celite®, Celite Corp., Lompoc, CA.
[4]ProAct360®, DSM Nutritional Products, Switzerland; 500 g/ton corn diluted premixes had 0, 17 or 50 of the protease corresponding to 0, 10,000 and 30,000 NFP units.

Standard diets were formulated to contain energy and nutrients to provide animal well-being without limitations for health. However, since the modern broiler responds widely to protein and energy with continuous improvements in feed conversion ratios (FCR), the studies were based in different set ups for growth. In experiment 1 (EXP1), Standard diets were formulated with energy and AA at marginally lower concentrations than usual by the Brazilian integrations expecting broilers to grow at comparatively reduced rates when compared to the average industry (Table 1). In experiment 2 (EXP2), Standard diets were formulated using energy and AA as usual by the Brazilian integrations projecting birds to grow at average rates comparable to obtained by the industry (Table 2). Meat and bone meal was included in the diets of EXP1 whereas all vegetable corn-soy diets were used in the EXP2.

In comparison to Standard, Matrix diets were formulated with reductions in AME (20 kcal AME/kg) and 6% digestible Lys (Tables 1, 2), with all other nutrients being similar and resembling levels presently used commercially. An indigestible marker (Celite Corp., Lompoc, CA) was added at 10 g/kg in the finisher and starter diets, respectively in EXP1 and EXP2 to allow digestibility analyses.

TABLE 2 Composition of feeds formulated in experiment 2[1].

Ingredients, kg	Pre-starter, 0 to 7 d		Starter, 8 to 21 d		Grower, 22 to 35 d		Finisher, 36 to42 d	
	Standard	Matrix	Standard	Matrix	Standard	Matrix	Standard	Matrix
Corn	540.90	556.85	557.50	573.40	630.30	644.00	647.00	659.55
Soybean meal 46%	399.00	386.00	353.00	341.00	294.00	283.00	275.00	265.00
Soybean oil	21.00	19.00	42.00	39.00	41.00	39.00	48.00	46.00
Dicalcium phosphate	11.50	11.50	11.40	11.40	10.70	10.80	8.20	8.30
Limestone	10.40	10.40	10.20	10.20	9.80	9.80	8.60	8.60
Salt	5.30	5.30	4.80	4.80	4.50	4.50	4.20	4.20
DL-Methionine 99%	4.35	4.00	3.95	3.60	3.30	3.00	3.05	2.80
L-Lysine HCl 78%	3.15	2.95	2.95	2.75	2.75	2.60	2.70	2.55
L-Threonine 98.5%	1.50	1.10	1.30	0.95	1.05	0.75	0.95	0.70
Choline chloride 60%	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral & vitamin premix[2]	2.35	2.35	2.35	2.35	2.05	2.00	1.75	1.75
Indigestible marker[3]	–	–	10.00	10.00	–	–	–	–
Protease[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total	1,000							
Nutrient composition, % or as follow								
AME	2,950	2,930	3,080	3,060	3,180	3,160	3,250	3,230
Crude protein	23.4	22.0	21.3	20.0	19.3	18.1	18.4	17.3
Digestible Lys	1.38	1.30	1.25	1.18	1.1	1.03	1.05	0.99
Digestible TSAA	1.05	0.99	0.96	0.90	0.86	0.81	0.82	0.77
Digestible Thr	0.91	0.86	0.83	0.78	0.73	0.69	0.69	0.65
Digestible Val	0.98	0.92	0.9	0.85	0.82	0.77	0.78	0.73
Av. P	0.48	0.48	0.47	0.47	0.45	0.45	0.40	0.40
Total Ca	0.92	0.92	0.90	0.90	0.86	0.86	0.76	0.76
Na	0.23	0.23	0.21	0.21	0.19	0.19	0.17	0.17

[1]Standard diets were formulated using energy and AA as usual by the Brazilian integration levels such that broilers were expected to grow comparable to industry rates.
[2]Composition per kg of feed: iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg: vitamin A, 8,000 IU; vitamin D3, 2,000 IU; vitamin E, 30 IU; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; Phytase at 1,000 FYT/kg (Ronozyme HiPhos GT 20000®, DSM Nutritional Products, Switzerland provided 1.5 kg/ton Av.P and total Ca); Salinomycin at 60 mg/kg (Coxifarm, Farmabase Animal Health, Jaguariúna, Brazil).
[3]Celite®, Celite Corp., Lompoc, CA.
[4]ProAct360®, DSM Nutritional Products, Switzerland; 500 g/ton corn diluted premixes had 0, 17 or 50 of the protease corresponding to 0, 10,000 and 30,000 NFP units.

Feeds were prepared in 400 kg batches, mixed for 3.5 minutes. The feed mixer was thoroughly cleaned between the mixing of sequential treatments to avoid residual enzymes from the feed previously prepared. Samples (1 kg) were taken from each mixing batch for further analysis.

Data collection

Chick body weight from each pen was individually obtained at placement (49.5 ± 0.85 and 48.4 ± 1.29, respectively in EXP1 and EXP2) whereas pen totals were taken and averaged at 7, 21, 35 and 42 d. Feed intake, body weight gain (BWG) and FCR corrected for the weight of dead birds were calculated between feed changes and at the end of the studies. In both studies, 5 birds were randomly taken from each pen and euthanized by cervical dislocation at 42 and 21 d, respectively in EXP 1 and 2. Contents from the Meckel's diverticulum to approximately 3 cm cranial to the ileo-cecal junction were collected, flushed with distilled water into plastic containers, pooled by pen, frozen immediately in liquid nitrogen, and stored in a freezer at -20°C. Diet and ileal contents were freeze-dried and ground for dry matter analysis. Feed and ileal digesta samples were then analyzed for gross energy (GE) using a calorimeter (IKA Werke, Parr Instruments, Staufen, Germany). Nitrogen analysis was done using Kjeldahl (1883). Estimations of ileal digestibility were calculated for dry matter (IDM), energy (IDE), and crude protein (IDCP) as described by Kong and Adeola (2014). Acid insoluble ash concentration in the diets and ileum samples were determined using the method described by Vogtmann et al. (1975) and Choct and Annison (1992).

Statistical analyses

Collected data from the two experiments were analyzed independently using SAS 9.4 (SAS, 2013). Data was first subjected to the Levene and Shapiro Wilkinson normality tests for the homogeneity of variances (Levene, 1960; Shapiro and Wilk, 1965). Normally distributed data were then subjected to ANOVA using the general linear model (GLM) procedure in a completely randomized 2 *x* 3 factorials design with 14 replicates per treatment in each study. The Tukey-Kramer test was used for means comparison with differences being considered significant at $P < 0.05$, whereas $0.05 < P < 0.10$ was considered to indicate a trend towards significant effects (Tukey, 1991).

Results

Analysis of the protease added to the experimental diets showed similar activities with the declared values in both studies (Table 3). Feed intake was not affected by the treatments in any of the experiments (P>0.05). Overall mortality was low and not affected by the treatments either (Grand means were of 2.87% and 2.62% in EXP1 and EXP2, respectively). Growth performance of broilers are presented in Tables 4, 5, respectively for EXP1 and EXP2. There were no interaction effects between diet and protease on performance variables (P > 0.05), therefore data in the tables are presented as main factor means.

Growth in both experiments was affected by diet formulation at 35 and 42 d. Significant reductions in BWG were observed for birds fed the Matrix diets (reductions ranged from as low as 1.4% at 42 d in EXP1 to as high as 1.9% in EXP2). Standard diets presented better FCR at 35 d in EXP1 and at 35 and 42 d in EXP2 (P<0.01). A trend for improvement at 42 d was observed in EXP1 (P<0.078).

TABLE 3 Declared and analyzed activities of the protease supplemented feeds in experiments 1 and 2, NFP/kg[1].

	Experiment 1		Experiment 2	
	Declared	Analyzed	Declared	Analyzed
Pre-starter				
Standard	0 10,000 30,000	< LOD[2] 8,912 48,778	0 10,000 30,000	< LOD 10,862 29,722
Matrix	0 10,000 30,000	< LOD 17,070 45,453	0 10,000 30,000	< LOD 14,771 27,774
Starter				
Standard	0 10,000 30,000	< LOD 18,349 41,970	0 10,000 30,000	< LOD 7,552 32,368
Matrix	0 10,000 30,000	< LOD 20,113 44,699	0 10,000 30,000	<LOD 23,052 50,423
Grower				
Standard	0 10,000 30,000	< LOD 6,582 39,082	0 10,000 30,000	< LOD 10,237 37,895
Matrix	0 10,000 30,000	< LOD 9,665 30,570	0 10,000 30,000	< LOD 7,919 29,696
Finisher				
Standard	0 10,000 30,000	< LOD 7,813 27,408	0 10,000 30,000	< LOD 7,157 32,297
Matrix	0 10,000 30,000	< LOD 14,139 38,306	0 10,000 30,000	< LOD 7,774 27,871

[1] New feed protease units.
[2] Limit of detection.

TABLE 4 Growth performance of broilers fed diets added with a sfericase protease in experiment 1[1].

	Feed intake, g			Body weight gain, g			Feed conversion ratio		
Feed	1 to 21 d	1 to 35 d	1 to 42 d	1 to 21 d	1 to 35 d	1 to 42 d	1 to 21d	1 to 35d	1 to 42d
Standard	1,133	3,446	5,176	818	2,203	3,161	1.386	1.564	1.636
Matrix	1,139	3,422	5,127	818	2,164	3,113	1.392	1.581	1.647
Protease, NFP/kg[2]									
0	1,142	3,440	5,170	812	2,169	3,121	1.408[b]	1.586[b]	1.656[b]
10,000	1,129	3,416	5,134	815	2,178	3,137	1.386[a]	1.568[a]	1.636[a]
30,000	1,138	3,446	5,149	828	2,204	3,154	1.374[a]	1.563[a]	1.632[a]
SEM	3.42	12.17	14.08	2.92	8.54	7.92	0.004	0.003	0.003
Probability									
Diet	0.454	0.329	0.091	0.975	0.022	0.002	0.366	0.004	0.078
Protease	0.256	0.572	0.563	0.052	0.209	0.202	<0.001	0.004	0.002
Diet x Protease	0.344	0.319	0.324	0.636	0.454	0.262	0.864	0.934	0.987

[a>b]Means with different letters in the same column indicate significant differences ($P < 0.05$).
[1]Standard feeds formulated with energy and AA at marginally lower levels than usual by the Brazilian integration such that broilers were expected to grow at comparatively reduced rates to the industry.
[2]ProAct360®, DSM Nutritional Products, Switzerland; 50 g/ton corn diluted premixes had 0, 17 or 50 of the protease corresponding to 0, 10,000 and 30,000 New Feed Protease (NFP) units.

The protease added to the diets showed no significant effects on BWG (P>0.05) in both experiments. A trend to increase BWG (P<0.052) was observed at 21 d in EXP1. On the other hand, the protease led to significant improvements in FCR in all cumulative age measurements in EXP1 regardless of the protease level added. A trend for improvement (P<0.067) was observed in the cumulative FCR at 42 d in EXP2.

Digestibility responses obtained in both experiments are presented in Table 6. No interaction was observed between the diet and protease (P>0.05). Except for an increase IDCP for birds fed the Standard diet in EXP1 (P<0.038), there were no effects of diet in the digestibility variables. Protease addition led to improvements in IDM and IDCP ($P < 0.05$) when compared to no protease addition in EXP1; however, IDE was only increased when 30,000 units were added. No effects of protease were observed in EXP2 (P>0.05).

Discussion

An impressive number of studies with poultry fed diets supplemented with exogenous proteases have been reported in the last few years. Outcomes from these studies vary from absence of

TABLE 5 Growth performance of broilers fed diets added with a sfericase protease in experiment 2[1].

	Feed intake, g			Body weight gain, g			Feed conversion ratio		
Feed	1 to 21 d	1 to 35 d	1 to 42 d	1 to 21 d	1 to 35 d	1 to 42 d	1 to 21d	1 to 35d	1 to 42d
Standard	1,286	3,715	5,009	990	2,381	3,109	1.299	1.559	1.610
Matrix	1,294	3,681	4,963	988	2,335	3,050	1.310	1.576	1.627
Protease, NFP/kg[2]									
0	1,291	3,705	4,997	984	2,349	3,063	1.312	1.577	1.631
10,000	1,287	3,682	4,971	991	2,356	3,080	1.299	1.563	1.613
30,000	1,293	3,707	4,991	993	2,370	3,096	1.302	1.564	1.612
SEM	5.14	14.45	16.28	4.19	8.92	9.05	0.005	0.004	0.003
P-value									
Diet	0.465	0.257	0.166	0.708	0.009	<0.001	0.334	0.048	0.024
Protease	0.921	0.749	0.799	0.919	0.614	0.308	0.617	0.326	0.067
Diet x Protease	0.604	0.903	0.675	0.814	0.978	0.680	0.843	0.773	0.919

[a>b] Means with different letters in the same column indicate significant differences Tukey.
[1]Standard feeds were formulated using energy and AA as usual by the Brazilian integration levels such that broilers were expected to grow comparable to industry rates.
[2]ProAct360®, DSM Nutritional Products, Switzerland; 50 g/ton corn diluted premixes had 0, 17 or 50 of the protease corresponding to 0, 10,000 and 30,000 New Feed Protease (NFP) units.

TABLE 6 Dry matter digestibility, ileal energy digestibility, and crude protein digestibility of broilers in experiments 1 and 2[1].

	Experiment 1			Experiment 2		
	IDM[2]	IDE	IDCP	IDM2	IDE	IDCP
Standard	77.0	3,748	86.7	64.5	3,239	80.7
Matrix	77.8	3,711	85.7	66.4	3,324	81.0
Protease, NFP/kg[3]						
0	75.5[b]	3,670[b]	85.0[b]	64.6	3,239	80.3
10,000	77.9[a]	3,751[ab]	86.9[a]	65.9	3,280	80.1
30,000	78.9[a]	3,766[a]	86.8[a]	66.0	3,325	82.3
P-value						
SEM	0.36	14.97	0.24	0.65	29.85	0.56
Diet	0.213	0.21	0.038	0.153	0.15	0.735
Protease	0.001	0.017	0.001	0.60	0.487	0.188
Diet x Protease	0.067	0.56	0.074	0.793	0.302	0.143

[a>b] Means with different letters in the same column indicate significant differences (P < 0.05).
[1] Standard feeds formulated using AA and energy marginally lower (Exp1) or as usual (Exp2) by the Brazilian integrations whereas Matrix feeds had a reduction in AA as well as of 20 kcal/kg corresponding to the expected improvements by the protease; [2] IDM=ileal dry matter digestibility, IDE=ileal digestible energy, IDCP=ileal digestible crude protein.
[3] ProAct360®, DSM Nutritional Products, Switzerland; 50 g/ton corn diluted premixes had 0, 17 or 50 of the protease corresponding to 0, 10,000 and 30,000 New Feed Protease (NFP) units.

effects (Kaczmarek et al., 2014; Yuan et al., 2017; Walk et al., 2019) to significant improvements in growth performance of broilers (Freitas et al., 2011; Lee et al., 2018; Jabbar et al., 2021; McCafferty et al., 2022). Synergy between proteases with other in feed supplemental enzymes, such as phytases, xylanases and amylases have also been reported (Cowieson and Adeola, 2005; Amerah et al., 2017; Cowieson et al., 2019). In the present study, phytase was added to all feeds since this is economically mandatory in presently commercial feeds.

Enzymes only interact with one type or a group of similar substrates such that the enzyme-substrate reaction products are yielded. In the case of supplemental proteases, expected yields of dietary protein degradation resulting from supplemental proteases are absorbable short peptides or amino acids (Gilbert et al., 2014). Broiler responses to increased intestinal contents of available amino acids depend on their amount and degree of essentiality. The modern broiler has demonstrated to respond to increasing dietary intake of protein from amino acids balanced feeds with improved growth, feed conversion as well as meat yields in a wide range of contents (Lemme et al., 2006; Dozier et al., 2008; Cruz et al., 2017).

Expected benefits from supplemental proteases are dependent on the presence of protein that can function as a reactive substrate to the specific enzyme added (Vieira et al., 2014). In the present study, Matrix diets were formulated with a fair reduction in protein but a small decrease in energy when compared to the Standard diets in both experiments. These differences were expected to allow for the detection in performance and digestibility when birds were fed them and, therefore, benefits due to the protease in the Matrix diets should be able to provide a compensation for their lower AA and AME contents. Performance of broilers was negatively affected by birds fed the Matrix diets at 35 and 42 d (P<0.078 for FCR at 42 d in EXP1). However, diets with the added protease provided significant improvements in FCR in EXP1 throughout all phases, regardless of the diet and the dose added. In EXP2 the protease numerical improvements in FCR at 42 d had a P<0.067. On the other hand, protease improvements were more noticeable in FCR and could be explained by the parallel increased digestibility (IDM, IDE and IDCP) in EXP1 (which did not occur in EXP2).

An important difference in the protein profile existed between diets formulated in both experiments. Meat and bone meal, included in EXP1 but not in EXP2, have reduced AA digestibility when compared to SBM (Vieira et al., 2014). It seems that, the presence of a mix of more complex proteins in EXP1 has provided a more adequate substrate for the presently used protease.

In conclusion, commercial diets supplemented with 10,000 NFP/kg or 30,000 NFP/kg lead to improvements in FCR regardless of AA density utilized.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Comite de Etica no Uso de Animais Granja Santa Livia Producoes e Pesquisa Agropecuaria.

Author contributions

SV, main researcher; CF, analysis and text; AF, researcher; LK, researcher; JS, planning and analyses; MU-F, planning and analyses. All authors contributed to the article and approved the submitted version.

Conflict of interest

Authors JS and MU-F were employed by DSM Nutritional Products Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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