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Research article

Effects of dietary digestible amino acids and a novel exogenous sfericase protease on growth performance of broilers

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A R T I C L E I N F O

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A B S T R A C T

Two experiments (Exp.) were conducted to evaluate the effects of a novel exogenous sfericase protease on growth performance and ileal digestibility of broiler chickens until day 35 of age. In Exp. 1, 1350 one-day-old male chicks (Cobb 500) were allocated in 54 floor pens and fed one of the three dietary treatments, with 18 replicates of 25 birds each in a completely randomized design. Diets consisted of positive control [PC; commercially relevant ME and balanced amino acids (AA)]; negative control (NC; with reduction of 6% dig. Lys and proportional reductions for adjacent AA compared to the PC), and NC supplemented with sfericase protease [30,000 New Feed Protease units (NFP)/kg]. On day 35, ileal digesta was collected to determine apparent ileal digestibility of dry matter and nitrogen (N). In Exp. 2, 1620 one-day-old male chicks (Cobb 500) were allocated in 54 floor pens having three treatments and 18 replicates of 30 birds each in a completely randomized design from day 1–35. Broilers were fed a control basal diet (Control); Control supplemented with sfericase at 30,000 NFP/kg and at 60,000 NFP/kg. In Exp. 1, from day 1–35, body weight gain (BW gain) and feed conversion ratio (FCR) of broilers improved 3.4 and 2.5% when diets were supplemented with sfericase, respectively, whereas the digestibility of N increased by 2.7% compared to the NC. In Exp. 2, diets with usual protein and AA levels and supplemented with 30,000 NFP/kg had 2.3 and 1.75% improved BW gain and FCR from day 1–35, respectively. When diets were supplemented with 60,000 NFP/kg, BW gain and FCR were enhanced by 3.9 and 3.2%, respectively compared to the Control. In conclusion, these results demonstrate that the novel sfericase protease could be successfully used in corn-soy diets with protein and AA reductions or in feed formulations with usual digestible AA levels to enhance growth performance of broilers.

1. Introduction

New generations of enzymes have been developed due to their already discovered benefits as improved performance and nutrient digestibility, allowing cost reductions of feed formulations for birds. Studies investigating exogenous enzymes have been intensified with the global increases in the prices of ingredients and feeds required for meat production. This scenario has increased the

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opportunity to use additives as mono-component enzymes or their combination, thereby providing the opportunity for feed cost reductions or to increase productivity.

Mono-component exogenous proteases have been used with a specific nutritional matrix or with reduced levels of protein and amino acids (AA) in diets, without harming growth performance of birds [1]. Moreover, this strategy allows increasing nutrient digestibility, improving feed conversion, and reducing moisture and nutrients in the excreta. Reduced excretion of nitrogen to the environment and higher efficiency of production have also been goals achieved by proteases in the sustainability of poultry production systems.

Protein digestibility also varies according to the quality of the protein, type of feed ingredients, and composition of ingredients, as well as the birds' age and nutritional levels used in feed formulations [2]. In addition to protease supplementation, other alternatives have been used to reduce costs of feed formulations for birds, which involve the reduction of dietary crude protein, formulation of diets with digestible (dig.) AA, and improved knowledge on the AA limitation order that varies according to ingredients and their quality [3]. However, there are more benefits obtained through protease supplementation than costs, because protease reduces the amount of indigestible protein in the gastrointestinal tract of chickens, results in less undesirable fermentations, controls pH and the proliferation of pathogenic microorganisms, and reduces endogenous nutrient losses, which are also related to the improved intestinal health of birds [4–6].

Ravindran et al. [8] estimated that around 20% of dietary protein escapes digestion by the host and this can vary appreciably due to various diet and bird-related factors. As exogenous proteases enhance the digestibility of raw materials and diets with a low inherent digestibility to a greater extent than for diets with a high inherent digestibility [6], there is a concomitant reduction in heterogeneity. Formulating feeds with a more stable nutrient delivery to birds is a key objective for commercial nutritionists and this can be challenging, especially when volatility in raw material pricing and availability may influence formulation constraints. Thus, the objective of the 2 experiments (Exp.) reported herein was to evaluate the effects of a novel sfericase protease added to corn-soy diets on ileal digestibility and growth performance of broiler chickens until day 35. In Exp. 1, protease was supplemented in a control diet that had

Table 1
Ingredient and nutrient composition of the experimental diets (as-is basis), Exp. 1.

Item	Pre-starter (D 1 to 7)		Starter (D 7 to 21)		Grower (D 21 to 35)	
	PC[a]	NC[b]	PC	NC	PC	NC
Ingredient, %						
Corn	54.08	57.70	57.71	60.84	59.11	62.62
Soybean meal, 46% CP	39.40	36.40	35.40	32.70	31.70	28.70
Soybean oil	2.80	2.30	3.50	3.10	5.10	4.60
Limestone	1.08	1.00	0.98	0.99	0.88	0.89
Dicalcium phosphate	1.10	1.12	0.98	1.00	0.86	0.88
Salt	0.52	0.52	0.47	0.47	0.44	0.44
Vit. and min. premix[c]	0.20	0.20	0.18	0.18	0.15	0.15
DL-methionine, 99%	0.39	0.39	0.36	0.36	0.34	0.34
L-lysine HCl 78%	0.25	0.24	0.25	0.24	0.24	0.25
L-threonine, 98.5%	0.12	0.07	0.11	0.07	0.12	0.08
Choline chloride, 60%	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[d]	0.005	0.005	0.005	0.005	0.005	0.005
Celite[e]	0.00	0.00	0.00	0.00	1.00	1.00
Calculated composition, % or as shown[f]						
ME, kcal/kg	2980	2980	3070	3070	3170	3170
Crude protein	23.59 (23.1)	22.17 (22.0)	21.86 (22.1)	20.55 (20.9)	20.13 (20.3)	18.92 (19.0)
Ca	1.00 (1.02)	1.00 (1.03)	0.92 (0.94)	0.92 (0.93)	0.84 (0.85)	0.84 (0.85)
Av. P	0.48 (0.48)	0.48 (0.48)	0.45 (0.46)	0.45 (0.47)	0.42 (0.43)	0.42 (0.43)
Na	0.22	0.22	0.20	0.20	0.19	0.19
Cl	0.38	0.38	0.35	0.35	0.33	0.33
Dig. Lys	1.35	1.27	1.25	1.18	1.15	1.08
Dig. Met + Cys	0.99	0.96	0.93	0.90	0.87	0.84
Dig. Thr	0.88	0.79	0.81	0.73	0.76	0.68
Dig. Val	0.96	0.90	0.89	0.84	0.82	0.77
Dig. Arg	1.49	1.40	1.37	1.29	1.25	1.18
Dig Trp	0.25	0.24	0.23	0.22	0.21	0.20
Dig. Ile	0.89	0.84	0.83	0.78	0.75	0.71
Dig. Leu	1.71	1.61	1.61	1.51	1.50	1.41

[a] PC = positive control with usual ME and digestible amino acids.
[b] NC = negative control with usual ME and reduction on digestible amino acids.
[c] Composition per kg of feed: vitamin A, 9000 UI; vitamin D_3, 2500 UI; vitamin E, 20 UI; vitamin K_3, 2,5 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 3.8 mg; cyanocobalamin, 0.015 mg, pantothenic acid, 12 mg; niacin, 35 mg; folic acid, 1,5 mg; biotin, 0.1 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.25 mg.
[d] Ronozyme HiPhos (GT) with 20,000 fungal phytase units/g, using available P and total Ca (g/kg) matrix values of 29,900 and 34,445, respectively (DSM Nutritional Products AG, Kaiseraugst, Switzerland).
[e] Indigestible marker (Celite, Celite Corp., Lompoc, CA).
[f] Analyzed crude protein, calcium and phosphorus from two-pooled sample from each feed mix batch.

been reduced in protein and digestible AA concentration, whereas in Exp. 2, the enzyme was supplemented in a common basal diet with standard protein and AA levels.

2. Materials and methods

2.1. Ethical permit

All procedures used in the current study were approved by the Ethics and Research Committee of the Federal University of Santa Maria (AN 6814110319).

2.2. Birds and management

A total of 2970 one-day-old male chicks (Cobb 500) vaccinated for Marek's disease were purchased from a commercial hatchery (Grupo Agrodanieli, Tapejara, RS, Brazil). Chicks were obtained from the same broiler breeder parent flock, selected at placement, and distributed by body weight (43 g $\pm$ 1 g average BW in both Exp.). In both Exp., broilers were allocated in a conventional poultry house from day 1–35 post-hatch and placed into 54 experimental floor pens (3 m^2). Each experimental pen contained one drinker and one 18 kg tubular feeder. New wood shavings were used as litter.

Temperature was controlled to maintain bird comfort throughout the experimental period using incandescent lamps, fans, and fog nozzles when needed. The average temperature was controlled to provide comfort throughout the study [9]. A continuous lighting schedule was used until day 14, whereas an 16L:8D cycle with constant intensity was used after that. General health status, weight, mortality, and estimated cause of death were also recorded.

A total of 1350 male broilers were allocated in Exp. 1, whereas 1620 birds were used in Exp. 2. Birds had *ad libitum* access to water and mash diets. The experiments consisted of 3 treatments, where broilers were fed control diets not supplemented or supplemented with the sfericase protease, having 18 replicates and 25 birds (Exp. 1) or 30 birds each (Exp. 2), distributed in a completely randomized design.

Table 2
Ingredient and nutrient composition of the control basal diet (as-is basis), Exp.2.

Item	Starter (D 1 to 14)	Grower (D 14 to 28)	Finisher (D 28 to 35)
Ingredient, %			
Corn	54.86	59.36	61.34
Soybean meal, 46% CP	37.90	33.10	31.40
Soybean oil	3.38	4.09	4.39
Limestone	0.76	0.69	0.61
Dicalcium phosphate	1.82	1.59	1.34
Salt	0.47	0.42	0.40
Vit. and min. premix[a]	0.18	0.15	0.15
DL-methionine, 99%	0.31	0.27	0.20
L-lysine HCl 78%	0.17	0.18	0.07
L-threonine, 98.5%	0.09	0.10	0.05
Choline chloride, 60%	0.05	0.05	0.05
Phytase[b]	0.005	0.005	0.005
Nutrient and energy composition, % or as shown[c]			
ME, kcal/kg	3050	3150	3200
Crude protein	21.70 (22.5)	19.80 (20.2)	19.00 (19.6)
Ca	0.90 (0.92)	0.80 (0.83)	0.70 (0.71)
Av. P	0.50 (0.51)	0.40 (0.42)	0.40 (0.41)
Na	0.20	0.20	0.20
Dig. Lys	1.20	1.10	1.00
Dig. Met + Cys	0.60	0.50	0.50
Dig. Thr	0.90	0.80	0.70
Dig. Val	0.80	0.70	0.60
Dig. Arg	1.30	1.20	1.10
Dig Trp	0.20	0.20	0.20
Dig. Ile	0.80	0.72	0.70
Dig. Leu	1.59	1.48	1.44

[a] Composition per kg of feed: vitamin A, 9000 UI; vitamin D_3, 2500 UI; vitamin E, 20 UI; vitamin K_3, 2,5 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 3.8 mg; cyanocobalamin, 0.015 mg, pantothenic acid, 12 mg; niacin, 35 mg; folic acid, 1,5 mg; biotin, 0.1 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.25 mg.

[b] Ronozyme HiPhos (GT) with 20,000 fungal phytase units/g, using available P and total Ca (g/kg) matrix values of 29,900 and 34,445, respectively (DSM Nutritional Products AG, Kaiseraugst, Switzerland).

[c] Analyzed crude protein, calcium and phosphorus from two-pooled sample from each feed mix batch.

2.3. Experimental diets

Broilers were fed corn-soybean meal basal diets formulated with commercial nutrient and energy levels in a three-phase feeding program. In Exp. 1, pre-starter (day 1–7), starter (day 7–21), and finisher (day 21–35) feeds were formulated (Table 1). Broilers were fed a positive control (PC; commercially used ME and ideally balanced AA); negative control (NC; with reductions of 6% dig. Lys and proportional reductions for adjacent AA compared to the PC), and NC supplemented with a mono-component exogenous sfericase protease (NC + sfericase) [30,000 NFP (New Feed Protease units)/kg of diet]. All finisher feeds in the Exp. 1 had 1% Celite as indigestible marker.

In Exp. 2, the feeding program had starter (day 1–14), grower (day 14–28), and finisher (day 28–35) feeds (Table 2). Treatments consisted of a control basal diet (Control; with commercially used ME and ideally balanced AA), Control supplemented with sfericase at 30,000 NFP/kg (Control + 30,000 NFP/kg), and at 60,000 NFP/kg (Control + 60,000 NFP/kg).

The sfericase was a commercial protease product (ProAct 360™, DSM Nutritional Products AG, Kaiseraugst, Switzerland), manufactured from the fermentation of *Bacillus licheniformis.* The test product is a microbial serine sfericase endopeptidase, expressed by *Bacillus licheniformis*. The protease activity is defined in NFP (New Feed Protease units), which is a measure of the enzyme amount required to hydrolyze a fixed amount of the substrate Suc-Ala-Ala-Pro-Phe-pNA, releasing the chromogen *para*-nitroaniline (pNA). The amount of released yellow pNA is proportional to the protease activity of the enzyme and is measured photometrically at a wavelength of 405 nm. One NFP unit is defined as the amount of enzyme that releases approximately 1 μmol of *p*-nitroaniline from 1 mM substrate (N-Succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide) per minute at pH 9.0 and 37 °C. More details of the product are reported by Cupi et al. [10]. All feeds were formulated with 1000 phytase units (FYT)/kg [Ronozyme HiPhos (GT), DSM Nutritional Products AG, Kaiseraugst, Switzerland].

Feeds were formulated as usual in the Brazilian broiler industry and following the genetics broiler management guide [9]. Ingredient and nutrient composition of the experimental diets are presented in Table 1 (Exp. 1) and Table 2 (Exp. 2). All the ingredients were analyzed prior feed formulation and the analyzed crude protein, calcium, and phosphorus contents of the experimental feeds are presented in these tables.

2.4. Experimental procedures

2.4.1. Experiment 1

In Exp. 1, chicks were individually weighed into groups of 25 birds per pen before placement. Bird weights, averaged by pen were recorded on 1, 7, 21, and 35 days of age. Body weight gain (BW gain), feed intake (FI), and mortality-corrected feed conversion ratio (FCR) were evaluated per feeding phase, and from day 1–21 and day 1–35.

On day 35, ileal digesta was collected from 4 birds per experimental unit (with the average BW of the experimental unit) to determine apparent ileal digestibility of dry matter (DM) and nitrogen (N). Contents from the 2/3 distal ileum were collected by flushing the digesta with distilled water into containers. Ileal digesta was subsequently pooled by cage, immediately stored at −18 °C, and samples were freeze-dried. Feeds and dried samples of ileal digesta were ground to pass a 0.5-mm screen in a grinder. Diets and ileal digesta DM analysis was performed after oven drying the samples at 105 °C for 16 h (method 934.01; [11]). Nitrogen of diets and ileal digesta was determined using the combustion method (Thermo-Finnigan Flash EA 1112, Waltham, MA, US). There is further description on calculations of ileal digestibility of DM and N in the publication by Stefanello et al. [12].

2.4.2. Experiment 2

In Exp. 2, chicks were allocated at the same poultry house used in Exp. 1 and individually weighed into groups of 30 birds per pen before placement. Handling and weighing procedures were the same in both experiments. Feed and bird weights, averaged by pen were recorded at 1, 14, 28, and 35 days of age. Growth performance (BW gain, FI, and mortality-corrected FCR) was evaluated per feeding phase and from day 1–35.

2.5. Statistical analysis

In Exp. 1 and 2, normality was tested to all data set prior to any further analysis. Data were submitted to one-way analysis of variance using the MIXED procedure of SAS [13]. Means were compared by the Tukey test when effects of dietary treatments were significant at $P \leq 0.05$. Tendencies for differences among treatment means were declared when $0.0501 \leq P \leq 0.10$.

3. Results and discussion

The analyzed gross energy, N, calcium, and available phosphorus composition of the experimental diets were similar to the formulated values. In-feed analysis of the sfericase protease agrees with the expected values in Exp. 1 and 2. Diets not supplemented with sfericase did not have declared protease activities. In Exp. 1, diets supplemented with sfericase had 33,200, 32,400, and 32,100 NFP/kg of diet in starter, grower, and finisher feeds, respectively, whereas in Exp. 2, the Control +30,000 NFP/kg had declared protease activities in starter, grower, and finisher feeds at 36,000, 34,497, and 35,397 NFP/kg of diet, respectively. In the Control +60,000 NFP/kg diets, sfericase recovery was 63,200, 60,873, and 61,305 NFP/kg in the starter, grower, and finisher feeds, respectively.

Although Exp. 1 and 2 were conducted independently, interpretation of the relative effects of the novel sfericase requires

Table 3
Growth performance and apparent ileal digestibility of dry matter and nitrogen of broilers fed diets supplemented with a sfericase protease, Exp. 1.

Item	Body weight gain/bird, g					Feed conversion ratio					Feed intake/bird, g		Ileal digestibility, D 35	
	D 1 to 7	D 7 to 21	D 21 to 35	D 1 to 21	D 1 to 35	D 1 to 7	D 7 to 21	D 21 to 35	D 1 to 21	D 1 to 35	D 1 to 21	D 1 to 35	DM, %	N, %
PC[a]	170^{xy}	943^{a}	$1,364^{a}$	$1,114^{a}$	$2,478^{a}$	1.158^{xy}	1.308^{c}	1.642^{b}	1.287^{c}	1.482^{c}	1434	3675	73.9^{a}	85.4^{a}
NC[b]	169^{y}	909^{b}	$1,297^{b}$	$1,078^{b}$	$2,375^{b}$	1.195^{x}	1.376^{a}	1.719^{a}	1.348^{a}	1.545^{a}	1454	3670	69.9^{b}	81.3^{b}
NC + Sfericase[c]	173^{x}	927^{ab}	$1,355^{a}$	$1,101^{a}$	$2,456^{a}$	1.156^{y}	1.338^{b}	1.665^{b}	1.312^{b}	1.506^{b}	1445	3700	71.5^{ab}	84.0^{a}
SEM	0.74	3.61	7.13	3.69	8.87	0.008	0.005	0.005	0.005	0.005	4.01	9.77	0.468	0.489
P value	0.0842	0.0002	0.0001	0.0001	0.0001	0.0918	0.0001	0.0002	0.0001	0.0001	0.1384	0.4230	0.0012	0.0013

^{a,b} Means with different superscript letters differ ($P < 0.05$) based on Tukey's honest significant difference test.
^{x,y} Means with different superscripts were considered tendencies ($0.0501 \le P \le 0.10$).
[a] PC = positive control with usual ME and digestible amino acids.
[b] NC = negative control with usual ME and reduction on digestible amino acids.
[c] Supplemental mono-component exogenous sfericase protease was 30,000 New Feed Protease (NFP) units/kg of diet.

contextualization because the control diets in the two experiments differed, as did the performance of the birds in the control cohorts. Comparatively, the birds fed the NC in Exp. 1 had around 6.3% lower terminal body weight compared to those in Exp. 2, although FCR in both cases was similar. The control diet in Exp. 1 had lower metabolizable energy and added fat, but higher crude protein, limestone, calcium and digestible AA than Exp. 2, this could be the cause of the different BW gain and FI between the two experimental cohorts.

The response of the sfericase at equivalent dose was similar in both experiments over the whole trial period, with a slightly greater performance in Exp. 1 (3.4% increase in BW, 3.9 points reduction in FCR) compared with Exp. 2 (2.3% increase in BW and 2.7 points reduction in FCR). This slightly greater effect of the sfericase in Exp. 1 may be due in part to the lower BW of the cohort per se or possibly the composition of the diets as previously reported in the review by Cowieson & Roos [6]. Diet composition such as the level of limestone and added fat as well as the inherent performance of the cohort are important explanatory terms for the efficacy of exogenous protease. Diets with lower concentrations of limestone and higher added fat levels may create conditions in the intestine that are favorable for gastric digestion. When gastric digestion of feed is optimized, higher concentrations of soluble, dissociated, proteins and oligopeptides are generated, and these represent suitable substrates for sfericase in the small intestine. Further work is needed to explore the mechanisms involved and the extent to which exogenous protease effect can be increased via changes in diet design.

3.1. Experiment 1

There were no statistical differences on mortality among the dietary treatments in Exp. 1 (grand mean = 2.8%). The effect of the reduction in protein and digestible AA and the supplementation of exogenous sfericase protease is presented in Table 3. Feed intake was not affected ($P > 0.05$) by reductions in AA in the control feeds or by protease supplementation. From day 1–7, BW gain and FCR of broilers were not affected by dietary treatments ($P < 0.05$).

Broilers fed the PC diet (with usual digestible AA and CP levels) had higher BW gain and lower FCR ($P < 0.05$) than broilers fed the NC diet from day 1–21 and day 1–35. In the current study, from day 7–21 the PC and NC diets were formulated with 1.25 and 1.18% dig. Lys, respectively. Broilers fed the PC diet had higher BW gain and better FCR compared to broilers fed the NC diet ($P < 0.05$). Contrarily, Truelock et al. [14] evaluated the effects of dietary amino acid density and exogenous protease inclusion on performance and AA digestibility in poultry and did not observe response in broiler BW gain to increasing AA density up to 1.21% dig. Lys. Increasing dig. Lys from 1.18 to 1.21% also resulted in no differences in FCR and AA digestibility in 20-day-old broiler chicks not consuming a protease.

The NC + sfericase diet resulted in higher BW gain from day 7–21, day 21–35 d, day 1–21, and day 1–35 than the NC diet, probably due to the protease effect. The NC + sfericase also had similar BW gain per phase and in the overall period when compared to the PC diet ($P < 0.05$). Intermediate FCR was observed in broilers fed NC + sfericase where protease supplementation resulted in reductions on FCR from day 7–21, day 1–21, and day 1–35 compared to the NC diet. Broilers fed the PC diet also had higher apparent ileal digestibility of DM and N compared to the NC diet ($P < 0.05$). There were no differences between ileal digestibility of DM and N in 35-day-old broilers fed the PC diet or the NC + sfericase diet ($P > 0.05$).

Significant improvements on weight gain, FCR, and AA digestibility associated with exogenous protease addition to the broiler diets have been reported previously [1–3,15]. In the current study, from day 1–35, BW gain and FCR of broilers were improved at 3.4 and 2.5% when diets were supplemented with sfericase protease, respectively; whereas the BW gain increased 2.3% from day 1 do 21. These results agree with findings by Cowieson et al. [6] where 35-day-old broilers fed diets supplemented with 15,000 protease units (PROT; protease units from the first generation of mono-component exogenous protease)/kg returned higher BW gain (+7.3%) and lower FCR (−4%) than the NC diet. Results from Cowieson et al. [2] supported the beneficial effect of exogenous protease (15,000 PROT/kg) on performance of 24-day-old broilers with an increase in BW gain and a reduction in FCR of 4–5% compared with the birds fed the control diet.

In the present study, broilers fed diets that had been reduced in protein and dig. AA concentration and supplemented with 30,000 NFP/kg had 2.7% increased N digestibility on day 35. Freitas et al. [1] found an increase of 1.8% in CP digestibility when 15,000 PROT/kg was added to a high-protein diet (+7% the usual CP level), whereas an improvement of 1% was seen in the low-protein diet (−12% dig. Lys). Angel et al. [15] also reported improvements on CP and AA digestibility in corn-soybean-based diets supplemented with increased levels of exogenous protease ranging from 7500 to 60,000 PROT/kg in 22-day-old broilers.

Exogenous protease improved broiler performance when supplemented in a corn-soy diet having reduction of 6% digestible AA. Sfericase supplementation increased digestibility of DM and N, which is interpreted to be associated with reduced FCR. In this study, only performance trials were conducted having different diet formulations to generate the first data in the evaluation of this novel sfericase protease. Further studies should be conducted to confirm the mode of action of this enzyme. In the literature, the benefits of protease supplementation on broilers performance have been associated to the improved ileal DM, N, and AA digestibility and the increased expression of nutrient transporter proteins as well as the reduced endogenous losses and maintained intestinal barrier function [6,7]. Additional benefits have been associated to the so-called ‘extra-proteinaceous’ effects, which include enteric resilience, litter management, environmental benefits, uniformity and microbial stability [6].

The magnitude of response to a given protease can be variable considering the digestibility of the undigested nutrient fraction. It has been widely reported that protein and AA digestibility are variable according to ingredients, quality of protein in feedstuffs from vegetable origin and diet composition as well as the substrate composition, birds’ age, and nutritional AA levels [2,3,6,16]. The digestibility of CP and AA in poultry feeds has indicated that valuable amounts of protein pass through the gastrointestinal tract without being completely digested [17], which has been the main opportunity to exogenous proteases. The undigested protein fraction also can decrease nutrient digestibility due to the undesirable gut bacterial fermentation, insufficient hydrolysis of proteinaceous antinutrients and increased nutrient excretion to the environment [5].

3.2. Experiment 2

In Exp. 2, there were no effects of dietary treatments on mortality (grand mean = 0.62%). Growth performance of broilers fed the Control diet or Control supplemented with sfericase protease at 30,000 NFP/kg or 60,000 NFP/kg is shown in Table 4. The FI was similar among the experimental diets ($P > 0.05$). Dietary treatments did not affect ($P > 0.05$) BW gain from day 14–28 and day 28–35 as well as the FCR from day 1–14, day 14–28 and day 28–35.

From day 1–14, the BW gain was higher ($P < 0.05$) in broilers fed the Control + 30,000 NFP/kg or Control + 60,000 NFP/kg diets compared to the non-supplemented Control diet. In the overall period, broilers fed Control +60,000 NFP/kg had higher BW gain and lower FCR that the Control ($P < 0.05$), whereas Control + 30,000 NFP/kg resulted in similar performance when compared to Control or Control + 60,000 NFP/kg diets ($P > 0.05$). The increased FI that results in higher ingested substrates for protease in older broilers compared to young birds can be one reason to the enhanced effect of protease supplementation in later phases. Higher doses of protease also can be needed to improve performance in older broilers. In Exp. 2, diets with standard protein and AA levels and supplemented with 60,000 NFP/kg, BW gain and FCR were enhanced by 3.9 and 3.2%, respectively. These results demonstrate that protease can be successfully used in feeds with protein and AA reductions as demonstrated in Exp. 1 or feed formulations with standard digestible AA levels as Exp. 2.

Some recent studies have been focused on supplementation with mono-component proteases in diets formulated with xylanase and high doses of phytase, because nutrients in the feed ingredients are present in a complex matrix involving starch and non-starch carbohydrates, protein, lipid and various minerals and vitamins [2,6,7]. These authors reported an increased interest in evaluating the beneficial effects of protease that extend beyond improvements in ileal digestibility of AA to substantial effects on the ME, net energy, and ileal digestibility of starch. In fact, part of the complexity is associated with the extended nutrient release values for different enzymes, which may be additive when these enzymes are added to a diet simultaneously.

As nutritionists increasingly require precision in feed formulations, it is indispensable that enzyme suppliers provide enzyme products of quality and recommendations for enzyme matrices according to the substrates in the diet composition as well as the objective of the production systems. Considering the recent scenarios of increasing costs of ingredients and diets for poultry, exogenous protease can be considered as an enzyme choice in feed formulation systems and it may contribute significantly to enhance the efficiency and sustainability of poultry operations.

4. Conclusion

In conclusion, a novel sfericase protease resulted in improved body weight gain, feed conversion ratio, and protein digestibility when supplemented in corn-soy diets formulated with 6% reduction of protein and digestible amino acids. Diets having reduced protein and amino acid levels negatively affected growth performance of broilers until 35 days of age; however, the sfericase supplementation was able to recover the performance results. Additionally, the supplementation of sfericase in corn-soy diets with standard protein and amino acid concentration also resulted in improved growth performance. Supplementing the novel sfericase protease (30,000 or 60,000 NFP/kg) provided an increase on broiler performance, indicating that substrate concentration was not a rate limiting factor for protease effect on bird growth.

Data availability statement

Data will be made available on request.

Table 4
Growth performance of broilers fed diets supplemented with increasing levels of sfericase protease, Exp. 2.

Item	Body weight gain/bird, g				Feed intake/bird, g				Feed conversion ratio			
	D 1 to 14	D 14 to 28	D 28 to 35	D 1 to 35	D 1 to 14	D 14 to 28	D 28 to 35	D 1 to 35	D 1 to 14	D 14 to 28	D 28 to 35	D 1 to 35
Control	540[b]	1352	632	2525[b]	671	1992	1208	3871	1.244	1.474[x]	1.952	1.536[a]
Control +30,000 NFP/kg[a]	557[a]	1370	657	2585[ab]	675	2004	1219	3899	1.213	1.463[xy]	1.874	1.509[ab]
Control +60,000 NFP/kg	562[a]	1381	684	2628[a]	677	1994	1235	3906	1.204	1.444[y]	1.814	1.486[b]
SEM	2.95	6.43	12.45	16.02	3.90	8.97	12.85	17.23	0.009	0.005	0.030	0.008
P value	0.0058	0.1795	0.2393	0.0287	0.8330	0.8363	0.6970	0.6992	0.1773	0.0818	0.1908	0.0416

[a,b] Means with different superscript letters differ ($P < 0.05$) based on Tukey's honest significant difference test.
[x,y] Means with different superscripts were considered tendencies ($0.0501 \leq P \leq 0.10$).
[a] Supplemental mono-component exogenous sfericase protease was 30,000 New Feed Protease (NFP) units/kg of diet or 60,000 NFP/kg of diet.

Declaration of interest's statement

The authors declare no conflict of interest.

CRediT authorship contribution statement

Catarina Stefanello: Writing – original draft, Resources, Methodology. **Yuri K. Dalmoro:** Resources, Methodology. **Daniele P. Rosa:** Resources, Methodology. **Levy Teixeira:** Supervision, Project administration, Conceptualization. **Jose-Otavio B. Sorbara:** Resources, Project administration. **Aaron J. Cowieson:** Writing – review & editing, Methodology. **Murtala Umar Faruk:** Writing – review & editing, Writing – original draft, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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