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Sfericase protease, phytase, and xylanase combination improves body weight, feed conversion rate, ileal digestibility, and gut morphology in broilers

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Introduction: This study aimed to evaluate the effects of a novel sfericase protease—an endopeptidase from the serine protease family, subtilisin subfamily A (MEROPS ID S08.113)—combined with phytase and xylanase on broiler performance, gut morphology, litter quality, and ileal digestibility.

Methods: A total of 1,800 Cobb 500 male chickens were randomly allocated into 36 pens with four dietary treatments and nine replicates per treatment (50 birds per replicate) until 42 days of age. The treatments included: (1) a positive control (PC) following adapted Cobb nutritional recommendations; (2) a negative control (NC) with crude protein and amino acid reductions (0.500% crude protein, 0.041% digestible lysine, 0.040% digestible methionine + cysteine, 0.049% digestible threonine, and 0.032% digestible arginine) compared to PC; (3) NC supplemented with 10,000 new feed protease units (NFP)/kg feed; and (4) NC supplemented with 30,000 NFP/kg feed. Diets, provided in mash form, consisted of corn, soybean meal, canola meal, distiller's dried grains with solubles, meat and bone meal, corn gluten meal, and soybean oil.

Results: At 42 days of age, broilers fed the NC diet had significantly lower body weight than those in the PC and protease-supplemented groups ($p < 0.05$). Feed conversion ratio (FCR) was poorest in the NC group, significantly differing from PC and NC + 10,000 NFP/kg ($p < 0.05$). Gut morphology analysis revealed significant differences in villus length and number, crypt depth, and surface area among treatments ($p < 0.05$), with the NC exhibiting the lowest surface area per μm^2. Energy digestibility was lowest in the PC group, whereas protease-

supplemented groups (NC + 10,000 and 30,000 NFP/kg) had the highest digestibility values ($p < 0.05$). Improvements in energy and nitrogen digestibility correlated significantly with body weight, FCR, and gut morphology ($p < 0.05$).

Discussion: These findings demonstrate that the inclusion of sfericase protease, in combination with phytase and xylanase, positively influences broiler performance, gut morphology, and nutrient digestibility. Optimizing enzyme inclusion based on diet composition and ingredient quality provides practical benefits in commercial broiler production.

KEYWORDS

broilers, protease, Sfericase, digestibility, gut morphology, performance, enzymes combination

1 Introduction

Commercial feed enzymes were first introduced in 1984 to improve the nutritional quality of barley-based rations (Bedford and Partridge, 2001). In the last 15 years, the use of enzymes increased in intensive animal farm production because of their proven effects in reducing antinutritional factors in feeds and improving feed efficiency, digestibility, and bioavailability of nutrients. This has resulted in significant economic and environmental impacts (Dida, 2016). Recently, there has been a growing interest in the effect of enzymes on gastrointestinal functionality, specifically, by reducing substrates for putrefactive microorganisms and increasing substrates for beneficial fermentative organisms, especially with the removal of antibiotics as growth promoters (Cowieson and Kluenter, 2019). In poultry production the use of several types of enzymes, such as phytases, carbohydrases and proteases in feed is common and is based on the diet composition.

Globally phytases are the most widely used feed enzymes and are present in approximately 90% of poultry diets. The use of phytase in feed increases P utilization by hydrolyzing phytate and influences feed conversion, weight gain, egg production, egg traits, mineral availability, and the digestibility of amino acids and energy (Mohamed et al., 2018). Currently, non-starch polysaccharide enzymes in poultry are used worldwide, with a penetration of approximately 70% in poultry diets (Aftab and Bedford, 2018). Xylanases degrade complex non-starch polysaccharides and increase the availability of nutrients, such as starch, protein, and oil, by decreasing digesta viscosity and consequently increasing nutrient absorption (Zhou et al., 2009; Saleh et al., 2018; Raza et al., 2019).

Proteases are also frequently used in the poultry industry and reduce the need for amino acids and energy by improving protein hydrolysis in the presence of anti-nutritional factors such as lectins or trypsin inhibitors (Huo et al., 1993; Ghazi et al., 2002; Cowieson and Roos, 2014). Moreover, proteases may improve ingredient quality by reducing ingredient variability (Cowieson et al., 2016). Since proteases are added to feed to enhance protein hydrolysis and improve nitrogen utilization, there is potential to reduce dietary protein, which can subsequently lower nitrogen levels in manure as observed by Ndazigaruye et al. (2019), when low protein diets where used. Ammonia (NH_3) is the primary gas produced in poultry houses as a result of the chemical decomposition of uric acid (the main form of nitrogen excretion in birds) by bacteria present in the litter. The rate of ammonia volatilization depends on factors such as litter pH, humidity, ventilation rate, air velocity, manure nitrogen content and temperature. High ammonia levels have been reported to adversely affect productivity in poultry (>25 ppm) (Swelum et al., 2021). Potentially, if protease supplementation improves nitrogen digestibility, it could lead to reduce uric acid levels in manure, thereby decreasing ammonia emission, as the emissions are directly related to manure nitrogen content. Considering the complex structure of poultry diets and their substrates, proteases are added to poultry diets as part of enzyme mixtures. Since the introduction of the first commercial proteases in the 1990s, their use has grown significantly due to their proven effects (Angel et al., 2011; Cowieson et al., 2019). Extensive research has focused on developing new protease molecules with greater and faster efficiency in protein hydrolysis, enhanced degradation of antinutritional factors, improved stability, and the ability to act on a wide range of raw materials.

After a screening process, a sfericase protease, which is an endopeptidase from the serine protease subtilisin subfamily (MEROPS ID S08.113), was found to be safe and suitable for use in animal production (Cupi et al., 2022). The screening process started with 4,000,000 possible candidates that were narrowed down by exposing the proteases to different tests, including biochemical characterization of feed-relevant parameters (pH, substrate activity, and thermostability), *in vitro* screening, *in vivo* digestibility screening, and *in vivo* extensive performance studies (Cupi et al., 2022). Considering the characteristics of this novel protease observed in this screening process, which are high stability in feed production conditions, a suitable pH profile and a broad specificity that could provide nutritional advantages in practical diets, the objective of this study was to evaluate the efficacy of this newly identified sfericase protease used in combination with a phytase and xylanase, with their respective matrices, in broilers. The

combination's effects on performance, gut health, litter quality, and ileal digestibility were evaluated.

2 Materials and methods

2.1 Facilities and care of experimental animals

The experiment was conducted at Integración y Desarrollo Agropecuario SA de CV experimental poultry farm, Tarimbaro Michoacán, Mexico (19° 48' N, 101° 10' W). The location is 1,860 m above sea level with an average temperature of 17.7°C (min, –2.4°C; max, 37.5°C) and has an annual rainfall of 609 mm. Experiments were conducted in accordance with the Official Mexican Norm Guide for Animal welfare; NOM-033SAG/ZOO-2014.

2.2 Experimental design and animal management

One-day-old Cobb 500 male broiler chickens (n = 1,800) were allocated to 36 experimental pens in a completely randomized design with four treatments and nine replicates of 50 chickens each. The birds were housed in an open-sided house with a natural environment and thermal insulation. Each pen (1.8 × 2.5 m) was equipped with two manual 1-L drinkers, a plastic feeding tray, and a mini hopper until seven days of age. From 8–42 days of age, two small feeders and an automatic bell-shaped drinker were used, and the birds were provided feed *ad libitum*. The diets were provided in mash form and were based on corn, soybean meal, canola meal, distiller's dried grains with solubles, meat and bone meal, corn gluten meal, and soybean oil. The temperature and lighting programs were based on the Cobb 500 Broiler Management Guide (Cobb-Vantress, 2018). The trial was conducted up to 42 days of age utilizing four feeding phases: Pre-starter (0–7 days), Starter (8–21 days), Grower (22–35 days), and Finisher (36–42 days). For this study, the Cobb nutritional recommendations (Cobb-Vantress, 2022) were adjusted to align with Intensive Commercial Broiler Production Systems (ICBPS) scenarios. The feeding phases were specifically adjusted to optimize productive performance under conditions typically observed on ICBPS. These adaptations represent a common practice in the global poultry industry, where producers tailor guide recommendations to address specific factors, including environmental conditions, management practices, infrastructure, and feed input costs.

The experimental design was as follows:

A positive control (PC) consisted of a diet formulated to meet 100% of an adaptation of Cobb nutritional recommendations (Cobb-Vantress, 2022), specifically aligned with ICBPS scenarios.

A negative control (NC) consisted of a diet with a reduction in crude protein and amino acids compared to the positive control (Table 1). The criteria for reducing crude protein and aminoacids were based on manufacturer recommendations, which consider the inclusion levels of ingredients and the improvements in standardized ileal digestibility observed during *in vivo* studies (DSM, 2021).

Additional treatments included the NC diet supplemented with sfericase protease at a concentration of 10,000 New Feed Protease units (NFP)/kg feed, and another treatment in which 30,000 NFP/kg feed was added to the NC diet. NFP measures the enzyme amount required to hydrolyze 1 mmol of para-nitroaniline (pNA) from 1 M 128 substrate Suc-Ala-Ala-Pro-Phe-pNA (Cupi et al., 2022).

The used test protease was granulated and contained 600,000 NFP per gram (Proact 360, DSM Nutritional Products Ltd). The additions of 10,000 and 30,000 NFP/kg feed were made at the expense of silica (Sipernat D17, Evonik Industries). All diets included 1,000 phytase units/kg feed (HiPhos GT 20000, DSM Nutritional Products Ltd) and 200 fungal xylanase units/kg feed (Ronozyme WX, DSM Nutritional Products Ltd), with its matrix values for all feeding phases (Table 2). The positive and negative control diets are shown in Tables 3 and 4. Table 5 presents the protease enzyme activity (NFP) analyzed in the finished feeds of treatments supplemented with protease.

TABLE 1 Reduction of crude protein and digestible amino acids in the negative control compared to the positive control.

Nutrient	Pre-starter	Starter	Grower	Finisher
CP (%)	−0.630	−0.570	−0.370	−0.430
Dig Lys (%)	−0.047	−0.043	−0.040	−0.034
Dig Met + Cys (%)	−0.046	−0.041	−0.039	−0.033
Dig Thr (%)	−0.056	−0.051	−0.047	−0.041
Dig Arg (%)	−0.040	−0.036	−0.023	−0.029

CP, crude protein; Dig Lys, digestible lysine; Dig Met + Cys, digestible methionine + cysteine; Dig Thr, digestible threonine; Dig Arg, digestible arginine.

TABLE 2 Nutritional matrix values for nutrient contribution of phytase and xylanase combination.

Parameter	Phytase + xylanase matrix values
ME (kcal)	100
CP %	0.72
AvP %	0.15
Ca %	0.172
Dig Lys %	0.028
Dig Met %	0.002
Dig Met + Cys %	0.028
Dig Trp %	0.004
Dig Thr %	0.038
Dig Arg %	0.020
Dig Val %	0.032
Dig Ile %	0.029
Dig Leu %	0.037

ME, metabolizable energy; CP, crude protein; AvP, available phosphorous; Ca, calcium; Dig, digestible; Lys, lysine; Met, methionine; Cys, Cysteine; Trp, tryptophan; Thr, threonine; Arg, arginine; Val, valine; Ile, isoleucine; Leu, leucine.

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TABLE 3 Composition of the positive and negative control for the pre-starter and starter diets.

Ingredients (kg)	Pre-starter		Starter	
	Positive control	Negative control	Positive control	Negative control
Corn	544.000	561.000	595.000	611.000
Soybean meal	310.000	296.000	232.000	219.000
Canola meal	40.000	40.000	50.000	50.000
DDGS	30.000	30.000	40.000	40.000
Meat and bone meal	20.000	20.000	20.000	20.000
Corn gluten meal	20.000	20.000	30.000	30.000
Soy oil	8.000	5.000	3.000	0.000
Limestone 38%	8.721	9.495	8.963	9.495
Titanium dioxide	0.000	0.000	5.000	5.000
Monodicalcium phosphate phosphate 21	4.040	4.160	2.810	2.920
DL-Methionine 99%	3.092	2.725	2.179	1.853
Vit-Min Premix[1]	3.000	3.000	3.000	3.000
L-Lysine HCl 78%	2.757	2.575	2.933	2.777
Salt	2.723	2.716	1.886	1.880
Sodium bicarbonate	1.500	1.500	1.500	1.500
L-Threonine 98%	1.074	0.679	0.779	0.425
Nicarbazin 25%	0.500	0.500	0.500	0.500
Blend of BA and EO[2]	0.300	0.300	0.300	0.300
Silica[3]	0.000	0.200	0.000	0.200
L-Valine 96.5%	0.143	0.000	0.000	0.000
Xylanase[4]	0.100	0.100	0.100	0.100
Phytase[5]	0.050	0.050	0.050	0.050
Estimated nutrient values based on analyzed ingredient composition				
Poultry ME (kcal/kg)	3.000	3.000	3.050	3.050
Crude protein %	24.58	23.95	22.47	21.90
Total lysine %	1.426	1.374	1.260	1.212
Dig lysine %	1.300	1.253	1.150	1.107
Total Met + Cys %	1.045	0.966	0.927	0.884
Dig Met + Cys %	0.962	0.916	0.851	0.810
Total threonine %	0.976	0.915	0.864	0.809
Dig threonine %	0.858	0.802	0.759	0.708
Total tryptophane %	0.268	0.260	0.233	0.226
Dig tryptophane %	0.235	0.232	0.202	0.199
Total arginine %	1.519	1.476	1.322	1.282
Dig arginine %	1.392	1.352	1.207	1.171
Total isoleucine %	0.976	0.949	0.868	0.843
Dig isoleucine %	0.881	0.887	0.788	0.792

(Continued)

TABLE 3 Continued

Ingredients (kg)	Pre-starter		Starter	
	Positive control	Negative control	Positive control	Negative control
Estimated nutrient values based on analyzed ingredient composition				
Total valine %	1.114	1.074	1.003	0.979
Dig valine %	1.002	1.003	0.903	0.916
Av. phosphorus %	0.480	0.480	0.450	0.450
Calcium %	0.932	0.959	0.904	0.922

[1]Premix provided per kg of diet: 13,000 IU vitamin A, 0.069 mg 25-OH-D3, 4,000 IU vitamin D, 50 mg vitamin E, 3 mg vitamin K3, 3 mg vitamin B1, 10 mg vitamin B2, 4 mg vitamin B6, 0.025 mg vitamin B12, 15 mg pantothenic acid, 60 mg niacin, 2 mg folic acid, 0.25 mg biotin, 461 mg choline, 15 mg copper, 50 mg iron, 100 mg manganese, 100 mg zinc, 1.5 mg iodine, 0.3 mg selenium, 228 mg calcium carbonate and 228 mg of rice hulls as carrier.
[2]Blend of benzoic acid and essential oils (thymol, eugeniol and piperine)
[3]Silica. For the treatments supplemented with sfericase protease at 10,000 and 30,000 NFP/kg feed, the supplementation was carried out at the expense of silica.
[4]200 fungal xylanase units (FXU)/kg feed.
[5]1,000 phytase units (FYT)/kg feed.
DDGS, distiller's dried grains with soluble; ME, metabolizable energy; Dig, digestible; Av., available.

TABLE 4 Composition of the positive and negative control for the grower and finisher diets.

Ingredients (kg)	Grower		Finisher	
	Positive control	Negative control	Positive control	Negative control
Corn	635.000	646.000	677.000	690.000
Soybean meal	217.000	209.000	172.000	162.000
Canola meal	20.000	20.000	20.000	20.000
DDGS	50.000	50.000	60.000	60.000
Meat and bone meal	20.000	20.000	15.000	15.000
Corn gluten meal	30.000	30.000	30.000	30.000
Soy oil	2.000	0.000	2.000	0.000
Limestone 38%	9.166	8.877	8.160	7.572
Monodicalcium phosphate	2.410	2.480	1.130	1.220
DL-Methionine 99%	1.804	1.474	1.363	1.098
Vit-Min Premix[1]	3.000	3.000	3.000	3.000
L-Lysine HCl 78%	2.638	2.378	2.796	2.670
Salt	1.838	1.833	1.865	1.861
Yellow xanthophylls[6]	2.020	2.015	2.649	2.643
Sodium bicarbonate	1.500	1.500	1.500	1.500
L-Threonine 98%	0.544	0.163	0.447	0.146
Salinomycin 12%	0.600	0.600	0.600	0.600
Blend of BA and EO[2]	0.300	0.300	0.300	0.300
Silica[3]	0.000	0.200	0.000	0.200
Xylanase[4]	0.100	0.100	0.100	0.100
Phytase[5]	0.050	0.050	0.050	0.050
Canthaxanthin 10%[7]	0.030	0.030	0.0400	0.040

(Continued)

TABLE 4 Continued

Ingredients (kg)	Grower		Finisher	
	Positive control	Negative control	Positive control	Negative control
Estimated nutrient values based on analyzed ingredient composition				
Poultry ME (kcal/kg)	3.100	3.100	3.150	3.150
Crude protein %	21.07	20.70	19.22	18.79
Total lysine %	1.142	1.104	1.029	0.993
Dig lysine %	1.050	1.010	0.949	0.915
Total Met + Cys %	0.841	0.804	0.760	0.725
Dig Met + Cys %	0.777	0.738	0.703	0.670
Total threonine %	0.781	0.734	0.704	0.659
Dig threonine %	0.693	0.646	0.626	0.585
Total tryptophane %	0.207	0.208	0.187	0.182
Dig tryptophane %	0.185	0.185	0.163	0.160
Total arginine %	1.194	1.201	1.078	1.048
Dig arginine %	1.121	1.098	0.987	0.958
Total isoleucine %	0.788	0.793	0.721	0.702
Dig isoleucine %	0.739	0.750	0.666	0.671
Total valine %	0.918	0.923	0.852	0.834
Dig valine %	0.849	0.868	0.777	0.790
Av. phosphorus %	0.435	0.435	0.380	0.380
Calcium %	0.880	0.868	0.180	0.180

[1]Premix provided per kg of diet: 13,000 IU vitamin A, 0.069 mg 25-OH-D3, 4,000 IU vitamin D, 50 mg vitamin E, 3 mg vitamin K3, 3 mg vitamin B1, 10 mg vitamin B2, 4 mg vitamin B6, 0.025 mg vitamin B12, 15 mg pantothenic acid, 60 mg niacin, 2 mg folic acid, 0.25 mg biotin, 461 mg choline, 15 mg copper, 50 mg iron, 100 mg manganese, 100 mg zinc, 1.5 mg iodine, 0.3 mg selenium, 228 mg calcium carbonate and 228 mg of rice hulls as carrier.
[2]Blend of benzoic acid and essential oils (thymol, eugeniol and piperine)
[3]Silica. For the treatments supplemented with sfericase protease at 10,000 and 30,000 NFP/kg feed, the supplementation was carried out at the expense of silica.
[4]200 fungal xylanase units (FXU)/kg feed.
[5]1,000 phytase units (FYT)/kg feed.
[6]Xanthophylls extracted from Marigold flowers 3%.
[7]Canthaxanthin 10%.
DDGS, distiller's dried grains with soluble; ME, metabolizable energy; Dig, digestible; Av., available.

Prior to formulation, representative samples of corn, soybean meal, canola meal, distiller's dried grains with solubles, meat and bone meal and corn gluten meal were analyzed to determine their composition. The analysis included dry matter, crude protein, ether extract, crude fiber, ash, starch, acid detergent fiber, neutral detergent fiber, phosphorus, phytic phosphorus, and amino acids (Met, Cys, Met+Cys, Lys, Thr, Trp, Arg, Ile, Leu and Val), using near infrared spectroscopy (AMINONIR, Evonik Industries). Aminoacids coefficients of digestibility where according AMINODAT 6.0 (Evonik Industries).

Representative feed samples of approximately 1 kg were collected from each phase for both PC and NC to undergo proximal chemical analysis. The analyses were conducted as follows: crude protein (CP) was determined using the combustion method (AOAC International, 2012), moisture content was assessed by oven drying at 105°C (AOAC International, 1990a), crude fiber was analyzed using standard procedures (AOAC International, 1990b), and ash content was measured by incineration at 550°C (AOAC International, 1990c). The results obtained are presented in Table 6.

2.3 Productive performance

Body weight, weight gain, and feed intake were measured weekly. Feed conversion was adjusted for mortality, and the index was calculated per week. Mortality was recorded on daily, including the cause of death.

2.4 Ileal digestibility

Ileal digestibility was measured using titanium dioxide as an indigestible marker. On day 22, three birds per replicate were

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TABLE 5 Recovery analysis of protease enzyme activity in treatments with Sfericase protease addition.

Treatments	Feeding phase	Expected (NPF/kg)	Results (NFP/kg)
NC + Protease (10,000 NFP)	Prestarter	10,000	10,645
	Starter	10,000	11,645
	Grower	10,000	6,324
	Finisher	10,000	9,646
		Average	9,565
NC + Protease (30,000 NFP)	Prestarter	30,000	37,285
	Starter	30,000	35,960
	Grower	30,000	27,980
	Finisher	30,000	19,950
		Average	30,294

[1]Análisis for the determination of the protease enzymatic activity were carried out by Biopract GmbH.

humanely euthanized in compliance with NOM-033SAG/ZOO-2014 to collect ileal digesta samples from the section between Meckel's diverticulum and 2 cm before the ileocecal junction. Samples from each replicate were mixed to obtain one sample per replicate, immediately frozen on dry ice, and maintained frozen at −20°C until lyophilization prior to analysis. The ileal digesta samples were analyzed for dry matter (lyophilized), titanium (Myers et al., 2004), nitrogen (AOAC International, 2012), and energy (by adiabatic bomb calorimetry). Three 500g feed samples per treatment of the starter feed were collected and analyzed for titanium dioxide, dry matter, nitrogen, and energy. The average of the feed results per treatment was used to calculate digestibility.

The ileal digestibility of nitrogen and energy was obtained using the digestibility index described by Kong and Adeola (2014), as follows:

$$\text{Digestibility}\ (\%) = 100 - \left[\left(\frac{CI_{input} \times CC_{output}}{CI_{output} \times CC_{input}}\right) \times 100\right]$$

In which CI_{input} and CI_{output} are the concentrations of the index compound (TiO_2) in the feed and feces, respectively, and CI_{input} and CI_{output} are the concentrations of the components (energy or nitrogen) in the feed and digesta, respectively.

2.5 Evaluation of gut morphology

Gut morphology was evaluated as described by Serrano (Serrano Gamboa, 2024). At 22 days of age, a 1.5-cm section of the ascending duodenal was obtained from one bird per replicate and preserved in 10% formaldehyde with sterile green dye for subsequent image analysis using an Optisum Industrial Digital Camera Model 9.0 MP 1/2.4. The measurements were performed using Motic Image 2.0. Duodenum gut morphology was evaluated by measuring villi length (μm), villi width (μm), Lieberkühn crypt depth (μm), number of villi on a surface of 1,000,000 μm^2, and the ratio between villi length and crypt depth. Additionally, the nutrient contact zone surface area was calculated as follows:

$$\text{Area} = [(\text{length} \times \text{width} \times \pi) \times \text{villus number}]/\text{measurement area}\ (1,000,000\ \mu m^2)$$

2.6 Litter dry matter, pH and total ammoniacal nitrogen and environmental ammonia

Litter samples were collected at 41 days of age. Samples were collected by taking five 100 g samples per pen from five different areas: four samples from the corners and one from the center. The five samples per pen were thoroughly mixed into a composite sample and preserved with dry ice during transportation for further analysis. For determining dry matter, 100 g per sample were dried at 55°C for 48 h and the weight was determined using a precision scale as described by Brauer-Vigoderis et al. (2014).

The pH was determined by placing 10 g of each sample in beakers with 100 mL of distilled water. After shaking and allowing to stand for 30 min, the pH value was obtained using a pH-meter. Total nitrogen was analyzed according to AOAC, 2001.11 (AOAC International, 2012), and ammoniacal nitrogen was analyzed using distillation with MgO according to AOAC 1980 (modified) (AOAC International, 1980). At 41 days of age, ammonia levels were

TABLE 6 Proximal chemical analysis results across different feeding phases for the NC and PC diets.

	Prestarter		Starter		Grower		Finisher	
Nutrient (%)	PC	NC	PC	NC	PC	NC	PC	NC
Moisture Content	8.75	9.02	9.01	9.62	9.45	9.45	9.08	9.18
Ether Extract	4.58	4.33	4.08	3.89	4.33	4.58	3.39	2.52
Crude protein	23.19	22.51	22.51	22.07	20.54	19.45	18.46	18.03
Crude fiber	3.12	3.2	2.92	3.11	3.11	3.16	2.66	2.5
Ash	5.38	5.31	5.23	5.31	5.01	5.07	4.32	4.42

PC, positive control diet formulated to meet 100% of an adaptation of the Cobb-Vantress (2022) nutritional recommendations, specifically aligned with Commercial Broiler Production Systems. NC, negative control diet with a reduction in crude protein and amino acids.

TABLE 7 Effects of treatments on broiler performance at 42 days of age.

Treatments	Body weight (g)	Feed consumption (g)	Adj. FCR (g/g)	% Mortality
PC	3.110 a	4.682	1.510 c	4.6
NC	2.968 d	4.611	1.570 a	3.0
NC + Protease (10,000 NFP)	3.067 b	4.654	1.532 bc	3.4
NC + Protease (30,000 NFP)	3.012 c	4.625	1.556 ab	3.0
Average	3.039	4.643	1.542	3.5
p value	<0.0001	0.1906	<0.0001	0.3926
SEM	0.0108	0.0243	0.0069	0.7290

PC, positive control diet formulated to meet 100% of an adaptation of the Cobb-Vantress (2022) nutritional recommendations, specifically aligned with Commercial Broiler Production Systems; NC, negative control diet with a reduction in crude protein and amino acids; NFP, new feed protease; Adj., adjusted; FCR, feed conversion rate; SEM, standard error of the mean. Two sfericase protease concentrations (10,000 and 30,000 NFP units/kg feed) were used. The absence of lowercase letters between the means of each column indicates that there were no significant differences ($p > 0.05$). Analysis of variance was performed, and in case of significant differences, Tukey's test was performed ($\alpha = 0.05$). Differences are indicated with different lowercase letters $p \leq 0.05$.

measured in each pen using a previously calibrated GasAlert Extreme device (NH3 0–400 ppm) (BW Technologies, Honeywell, Schaumburg, IL, USA). The device was placed directly on the litter, 1 meter away from the bell-shaped drinker in each pen.

2.7 Statistical analysis

To evaluate differences among treatments, results were analyzed using analysis of variance (ANOVA) based on General Linear Model (GLM) procedure. When statistically significant differences were detected ($p < 0.05$), Tukey's test was applied at a significance of $\alpha = 0.05$. Weekly mortality, ileal digestibility for energy and nitrogen, litter dry matter, and litter nitrogen percentages were transformed using the arcsine function to ensure normality.

A multivariate correlation analyses were performed to evaluate the linear relationships between the analyzed variables, including body weight, adjusted feed conversion, villus length, and digestibility metrics. Pearson's correlation coefficient was used to assess linear relationships, with significant correlations identified at $p < 0.05$.

Additional linear regression analyses were conducted to evaluate the influence of ileal digestibility of energy and nitrogen on body weight and adjusted feed conversion. The analysis included data from the negative control (NC) and treatments supplemented with 10,000 and 30,000 NFP/kg of feed to assess the effect of improved digestibility on performance. The models' coefficients of determination (R^2) were used to assess the strength of these relationships, with significance determined at $p < 0.05$.

All statistical analyses were performed using JMP® software version 18.1.

TABLE 8 Effects of treatments on energy and nitrogen digestibility of broilers at 22 days of age.

Treatments	Energy digestibility (%)	Nitrogen digestibility (%)
PC	71.0 b	83.9
NC	73.1 ab	85.0
NC + Protease (10,000 NFP)	77.2 a	87.1
NC + Protease (30,000 NFP)	75.9 ab	85.8
Average	74.3	85.4
p value	<0.03	0.24
SEM	1.490	1.095

PC, positive control diet formulated to meet 100% of an adaptation of the Cobb-Vantress (2022) nutritional recommendations, specifically aligned with Commercial Broiler Production Systems; NC, negative control diet with a reduction in crude protein and amino acids; NFP, new feed protease; Adj., adjusted; FCR, feed conversion rate; SEM, standard error of the mean. Two sfericase protease concentrations (10,000 and 30,000 NFP units/kg feed) were used. The absence of lowercase letters between the means of each column indicates that there were no significant differences ($p > 0.05$). Analysis of variance was performed, and in case of significant differences, Tukey's test was performed ($\alpha = 0.05$). Differences are indicated with different lowercase letters $p \leq 0.05$.

3 Results

3.1 Productive performance

Table 7 shows the effects of the diets on broilers performance at 42 days of age, with or without the addition of two sfericase protease concentrations. Performance results indicated a significant effect ($p < 0.05$) on body weight at 42 days. The negative control group showed lower body weight compared to the positive control and the groups supplemented with sfericase protease (10,000 or 30,000 NFP units/kg feed). The highest body weight was observed in the positive control ($p < 0.05$). The negative control exhibited the worst adjusted feed conversion rate, which was significantly different from that of the positive control and the group supplemented with 10,000 NFP units/kg feed ($p < 0.05$). No significant differences were observed between the groups supplemented with 10,000 and 30,000 NFP units/kg feed. Feed intake and mortality did not differ among treatments ($p > 0.05$).

3.2 Ileal digestibility

Table 8 shows results of ileal digestibility for energy and nitrogen at 22 days of age, measured by using titanium dioxide as

indigestible biomarker. The positive control showed the lowest energy digestibility among the treatments (71.0%), while the higher digestibility value was observed in treatment supplemented with protease at 10,000 (77.2%) ($p < 0.05$). No significant differences were observed in nitrogen digestibility among treatments ($p = 0.20$). Additionally, it was notably that the energy and nitrogen digestibility percentages followed the same trend.

3.3 Evaluation of gut morphology

Table 9 presents results of the intestinal villi morphology at 22 days. Significant differences in villus length ($p < 0.05$) were observed, with the negative control showing the shortest villi length. No significant differences were found between the positive control and the diets supplemented with protease. No differences were observed in villus width ($p > 0.05$). The Lieberkühn crypt was significantly different among treatments ($p < 0.05$). The positive control exhibited the shallowest crypt depth, while no differences were observed between the negative control and the diets supplemented with protease. Villi number (1,000,000 μm²) was significantly different among treatments ($p < 0.05$). The positive control showed the highest number of villi, while the addition of 30,000 NFP/kg feed resulted in the lowest count. The villus surface area per μm^2 revealed significant differences among treatments ($p < 0.05$). The positive control was significantly different from the negative control and the group supplemented with of 10,000 NFP/kg feed; however, no differences were observed between the positive control and the group supplemented with 30,000 NFP/kg feed.

3.4 Litter dry matter, pH, total nitrogen, ammoniacal nitrogen and environmental ammonia

Litter was kept in an excellent–good condition on day 22 and maintained throughout the experimental period (dry and crumbly). The trial was conducted during season characterized by average temperatures exceeding 19°C and relative humidity below 46%, occurring between March and April. These conditions allowed excellent ventilation and low environmental moisture. The average, maximum and minimum temperatures within the broiler house were 25.2°C, 31.9°C and 19.2°C, respectively, while relative humidity values were 43.1%, 62.3% and 21.8, respectively, throughout the experimental period.

Table 10 shows results for litter dry matter, pH, total nitrogen, ammoniacal nitrogen and environmental ammonia. No significant differences were observed in litter pH, litter nitrogen, or ammoniacal nitrogen ($p > 0.05$). However, the positive control showed the highest values for litter pH, nitrogen, and ammoniacal nitrogen. Litter dry matter differed among treatments, with the negative control showing a higher value compared to the treatment supplemented with 10,000 NFP/kg feed protease. Environmental ammonia levels did not differ significantly among treatments($p > 0.05$).

3.5 Multivariate correlation analysis of the variables

Table 11 presents results of P-values and correlations coefficients, obtained in the multivariate correlation analysis. The multivariate analysis included data from the PC, NC, NC + Protease (10,000 NFP) and NC + Protease (30,000 NFP). The variables analyzed were energy digestibility, nitrogen digestibility, body weight at 42 days, adjusted feed conversion, villi length, villi width, crypt depth, villi number/1,000,000 μm² and villi surface area/μm².

Significant correlations ($p < 0.05$) were as follows:

- Energy digestibility with nitrogen digestibility ($r = 0.9761$) and crypt depth ($r = 0.4705$).
- Nitrogen digestibility with crypt depth ($r = 0.4460$).
- Body weight with adjusted feed conversion ($r = -0.6545$)
- Villi length with crypt depth ($r = 0.5811$) and villi surface area/μm² ($r = 0.6384$).
- Villi number/1,000,000 μm² with villi surface area/μm² ($r = 0.6470$).

TABLE 9 Effect of treatments on gut morphology in broilers at 22 days of age.

Treatments	Villi length (μm)	Villi width (μm)	Crypt depth (μm)	Villi number/ 1,000,000 μm²	Villi surface area per μm²
PC	1030.8 a	80.5	79.8 b	40.7 a	10.6 a
NC	932.7 b	82.3	85.9 ab	37.4 ab	9.0 b
NC + Protease (10,000 NFP)	987.5 ab	79.2	96.9 a	37.3 ab	9.1 b
NC + Protease (30,000 NFP)	1048.3 a	79.7	95.7 a	36.5 b	9.4 ab
Average	999.8	80.4	89.6	38.0	9.5
p value	0.0024	0.1967	0.0002	0.0105	0.0028
SEM	22.78	1.08	3.06	0.94	0.32

PC, positive control diet formulated to meet 100% of an adaptation of the Cobb-Vantress (2022) nutritional recommendations, specifically aligned with Commercial Broiler Production Systems; NC, negative control diet with a reduction in crude protein and amino acids; NFP, new feed protease; Adj., adjusted; FCR, feed conversion rate; SEM, standard error of the mean. Two sfericase protease concentrations (10,000 and 30,000 NFP units/kg feed) were used. The absence of lowercase letters between the means of each column indicates that there were no significant differences ($p > 0.05$). Analysis of variance was performed, and in case of significant differences, Tukey's test was performed ($\alpha = 0.05$). Differences are indicated with different lowercase letters $p \leq 0.05$.

TABLE 10 Effect of treatments on litter dry matter, pH, total nitrogen, ammoniacal nitrogen and environmental ammonia at 41 days of age.

Treatments	Litter dry matter	Litter pH	Litter nitrogen % (dry basis)	Ammoniacal nitrogen mg/g (dry basis)	NH^3 (ppm)
PC	83.2 ab	6.71	4.26	3.11	2.6
NC	84.6 a	6.52	4.13	2.67	0.8
NC + Protease (10,000 NFP)	81.8 b	6.67	4.18	2.63	1.3
NC + Protease (30,000 NFP)	83.0 ab	6.66	4.17	2.97	2.9
Mean	83.2	6.64	4.18	2.84	1.9
Probability*	0.037	0.619	0.696	0.592	0.583
SEM**	0.643	0.103	0.075	0.287	1.228

PC, positive control diet formulated to meet 100% of an adaptation of the Cobb-Vantress (2022) nutritional recommendations, specifically aligned with Commercial Broiler Production Systems; NC, negative control diet with a reduction in crude protein and amino acids; NFP, new feed protease; Adj., adjusted; FCR, feed conversion rate; SEM, standard error of the mean. Two sfericase protease concentrations (10,000 and 30,000 NFP units/kg feed) were used. The absence of lowercase letters between the means of each column indicates that there were no significant differences ($p > 0.05$). Analysis of variance was performed, and in case of significant differences, Tukey's test was performed ($\alpha = 0.05$). Differences are indicated with different lowercase letters $p \leq 0.05$.

3.6 Relationship between digestibility metrics and performance variables

The detailed regression results are presented in Table 12. which summarizes the variability explained (R^2) and statistical significance of the models. Models were considered significant if $p < 0.05$.

For body weight at 42 days, the model with digestible energy explained 61.3% of the variability ($R^2 = 0.613$, $p < 0.001$), indicating that 1%increase in digestible energy corresponded to a 0.0228 kg increase in body weight. Similarly, the model using digestible nitrogen explained 65.8% of the variability ($R^2 = 0.658$, $p < 0.001$), with 1% increase in digestible nitrogen leading to a 0.0467 kg increase in body weight.

For adjusted feed conversion, the model with digestible energy explained 30.3% of the variability ($R^2 = 0.303$, $p = 0.003$), where a 1% increase in digestible energy resulted in a reduction of 0.0088 units. In comparison, the model with digestible nitrogen explained 34.4% of the variability ($R^2 = 0.344$, $p = 0.001$), with a 1% increase in digestible nitrogen corresponding to a reduction of 0.0185 units in adjusted feed conversion.

These results suggest that nitrogen digestibility has a slightly stronger influence on both performance variables compared to energy digestibility.

4 Discussion

4.1 Performance parameters

In the present study, differences were observed in broiler body weight and feed conversion at 42 days of age ($p < 0.05$). The use of sfericase protease resulted in a positive effect on performance as evidenced by the lower efficiency exhibited in the treatment with reduced amino acid and crude protein content without protease supplementation. The response obtained for sfericase protease was observed in the presence of a phytase and xylanase in the diets, with their matrix value considered. The composition of diets and nutrient concentrations play key roles in feed enzyme responses. Thanabalan et al. (2021) and Doskovič et al. (2013) used multienzyme complexes that included carbohydrases, phytases, and proteases and produced variable results. These discrepancies may be attributable to confounding effects resulting from the presence of several enzyme activities. Moreover, these trials did not consider the separate effects of the phytase-carbohydrase and the protease, specifically the effects of proteases in each ingredient of the diet. Therefore, a partitioning of the value for the phytase-carbohydrase and protease combination should be considered. Several studies have reported beneficial effects of monocomponent proteases on feed efficiency (Freitas et al., 2011; Vieira et al., 2013; Ding et al., 2016), body weight gain, and feed intake (Angel et al., 2011). Lee et al. (2023) evaluated the effect of the sfericase protease using similar protease matrix values than in the current experiment and found no differences in BW, BWG, FI, or FCR at 35 days of age. In Lee et al. (2023) experiment, supplementation with exogenous sfericase protease numerically improved these parameters in the same manner as in the present experiment, in which differences were observed; nevertheless, the diet composition was different. In this experiment, ingredients such as canola meal, meat and bone meal, and corn gluten meal were used to calculate the protease matrix value, considering the level of inclusion of each (DSM, 2021). In another trial, Walk et al. (2019) did not find differences in the use of different proteases; nevertheless, in that case, the protease was added to a nutrient-adequate diet and its matrix value according to ingredient inclusion was not considered. As mentioned by Walk et al. (2019), the lack of effects could be associated with the nutrient adequacy of the diet and the digestibility of the adequate diet.

4.2 Ileal digestibility

In previous studies, Cowieson and Roos (2014) showed that the effect on increasing the apparent ileal digestibility on poultry diets of a first-generation monocomponent protease was +4.5% on average

TABLE 11 Correlation P-values and correlation coefficients obtained in the multivariate analysis for energy digestibility (%), nitrogen digestibility (%), body weight at 42 days (g), adjusted feed conversion (g/g), villi length (μm), villi width (μm), crypt depth (μm), villi number/1,000,000 μm and villi surface area per μm.

	Digestibility of energy (%)	Digestibility of nitrogen (%)	Body weight 42d (g)	Adjusted feed conversion (g/g)	Villi length (μm)	Villi width (μm)	Crypt depth (μm)	Villi number/ 1,000,000 μm^2	Villi surface area per μm^2
Digestibility of energy (%)	P <.0001 r = 1.000	P <.0001 r = 0.9761	P = 0.2793 r = −0.1853	P = 0.2012 r = 0.2181	P = 0.9214 r = 0.0170	P = 0.3573 r = −0.1580	r = 0.0038 r = 0.4705	P = 0.1006 r = −0.2780	P = 0.1152 r = −0.2672
Digestibility of nitrogen (%)	<.0001 r = 0.9761	<.0001 r = 1.000	P = 0.4078 r = −0.1423	P = 0.3100 r = 0.1740	P = 0.8291 r = −0.0373	P = 0.3939 r = −0.1465	0.0064 r = 0.4460	P = 0.1335 r = −0.2549	P = 0.1030 r = −0.2762
Body weight 42d (g)	P = 0.2793 r = −0.1853	P = 0.4078 r = −0.1423	P <.0001 r = 1.000	P <.0001 r = −0.6545	P = 0.4738 r = 0.1233	P = 0.2848 r = −0.1832	P = 0.1947 r = −0.2212	P = 0.0844 r = 0.2916	P = 0.1625 r = 0.2378
Adjusted feed conversion (g/g)	P = 0.2012 r = 0.2181	P = 0.3100 r = 0.1740	P <.0001 r = −0.6545	P <.0001 r = 1.000	P = 0.4626 r = −0.1264	P = 0.9445 r = −0.0120	P = 0.0880 r = 0.2884	P = 0.2513 r = −0.1963	P = 0.1334 r = −0.2550
Villi length (μm)	P = 0.9214 r = 0.0170	P = 0.8291 r = −0.0373	P = 0.4738 r = 0.1233	P = 0.4626 r = −0.1264	P <.0001 r = 1.0001	P = 0.6643 r = −0.0749	P = 0.0002 r = 0.5811	P = 0.7629 r = −0.0521	P <.0001 r = 0.6384
Villi width (μm)	P = 0.3573 r = −0.1580	P = 0.3939 r = −0.1465	P = 0.2848 r = −0.1832	P = 0.9445 r = −0.0120	P = 0.6643 r = −0.0749	<.0001 r = 1.000	P = 0.6227 r = 0.0848	P = 0.0521 r = −0.3262	P = 0.7797 r = 0.0483
Crypt depth (μm)	P = 0.0038 r = 0.4705	P = 0.0064 r = 0.4460	P = 0.1947 r = −0.2212	P = 0.0880 r = 0.2884	P = 0.0002 r = 0.5811	P = 0.6227 r = −0.0848	P <.0001 r = 1.000	P = 0.2654 r = −0.1906	P = 0.1486 r = 0.2457
Villi number/ 1,000,000 μm^2	P = 0.1006 r = −0.2780	P = 0.1335 r = −0.2549	P = 0.0844 r = 0.2916	P = 0.2513 r = −0.1963	P = 0.7629 r = −0.0521	P = 0.0521 r = −0.3262	P = 0.2654 r = −0.1906	<.0001 r = 1.000	<.0001 r = 0.6471
Villi surface area per μm^2	P = 0.1152 r = −0.2672	P = 0.1030 r = −0.2762	P = 0.1625 r = 0.2378	P = 0.1334 r = −0.2550	<.0001 r = 0.6384	P = 0.7797 r = 0.0483	P = 0.1486 r = 0.2457	<.0001 r = 0.6470	<.0001 r = 1.000

P, Correlation P values; r, correlation coefficients. Significant correlations were considered at $P < 0.05$.

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TABLE 12 Effects of digestibility metrics on broiler performance at 42 days of age.

Digestibility	Response variable	Equation	R^2	P-value	Notes
Energy	Body weight (42 days)	1.2961 + 0.0228 × Energy	0.613	< 0.001	Significant positive effect
Energy	Feed conversion	2.2197 − 0.0088 × Energy	0.358	0.001	Significant negative effect
Nitrogen	Body weight (42 days)	−0.9989 + 0.0467 × Nitrogen	0.658	< 0.001	Significant positive effect
Nitrogen	Feed conversion	3.0695 − 0.0176 × Nitrogen	0.369	0.001	Significant negative effect

Regression analyses were performed separately for energy and nitrogen digestibilities to evaluate their individual effects on performance variables. The results indicate that both energy and nitrogen digestibilities have significant effects on body weight and feed conversion at 42 days of age, with positive effects on weight and negative effects on feed conversion. Models were considered significant if $p < 0.05$.
The analysis included data from the negative control (NC) and treatments supplemented with 10,000 and 30,000 NFP/kg of feed to assess the effect of improved digestibility on performance.

for the most critical amino acids (lysine, cysteine, methionine, and threonine) and was dependent on the inherent amino acid digestibility of the control diet. In the current study, as with performance parameters, energy digestibility showed differences among treatments ($p < 0.05$), even though no differences were observed for nitrogen digestibility ($p > 0.05$). In a previous study, Lee et al. (2023) reported significant improvements in the apparent total digestibility of protein, arginine, threonine, and glycine with the addition of sfericase protease when compared with that of the negative control. An important consideration regarding the efficiency of enzyme use is the amount of substrate required to improve digestibility, and proteases are known to differ in their efficacy due to inherent characteristics such as stability, activity at specific pH levels, and substrate specificity.

4.3 Gut morphology

The gastrointestinal system digests and absorbs ingested nutrients and excretes waste products from digestion. Most nutrients are ingested as chemical structures that are too complex to absorb. In the gastrointestinal tract, many of these substances are solubilized and further degraded by enzymes to simple molecules absorbed by the mucosal epithelium (Hornbuckle et al., 2008). The morphology of the villi is associated with the absorption of nutrients (Yamauchi et al., 1996; Awad et al., 2009; Zulkifli et al., 2009; Rysman et al., 2023); long villi provide a large absorption area, which is expected to result in improved broiler performance (Laudadio et al., 2012), whereas deeper crypts and shorter villi result in smaller absorption areas, which are expected to result in poor performance (Awad et al., 2009). The use of monocomponent enzymes or combinations of multi-enzymes has been shown to have some effect on gut morphology by increasing villus length (Ayoola et al., 2015; Kim et al., 2021; Lee et al., 2023; Vasanthakumari et al., 2023). The proposed mechanisms underlying the effects of enzymes on gut morphology include a reduction in digesta viscosity, which may stimulate cell division and tissue renewal of intestinal cells, this process can result in shorter villus height or deeper crypt depth, additionally enzymes have shown to increase the production of short fatty acids (Iji et al., 2001; Lee et al., 2017; Kim et al., 2021). Previous studies have shown beneficial effects on gut morphology with the use of proteases (Tajudeen et al., 2022; Lee et al., 2023).

4.4 Nitrogen environmental impact

In the current study, no differences were observed in total or ammoniacal nitrogen or pH. Interestingly, higher values for total and ammoniacal nitrogen were recorded in the positive control. This suggest that the positive control may not have had an excess of nitrogen, leading to significant higher nitrogen output to the litter via feces. Ammonia is a product of the microbial decomposition of uric acid excreted by poultry. Numerous factors affect ammonia emissions from broiler litter, including temperature, humidity, air exchange rate, pH, moisture content, nitrogen content, and litter type (Liu et al., 2006). The present study was conducted in an open house, during season characterized by average temperatures exceeding 19°C and relative humidity below 46%, which allowed for adequate ventilation. These conditions may have favored the control of ammonia production. Additionally, no differences in pH were observed among treatments, with values ranging between 6.5 and 6.71. Reece et al. (1979) demonstrated that ammonia release from the litter was negligible when litter pH was below 7. In the current study, the pH remained threshold. As previously mentioned, the ammonia concentrations measured on day 41 were very low, which could be attributed to the consistent lower levels of the main factors involved in ammonia production through the entire period. Ammonia concentration measured in all treatments were below the maximum recommendations for poultry, which are less than 10 ppm and should not exceed 25 ppm (Ross-Aviagen, 2015; Cobb-Vantress, 2018; Bist et al., 2023).

4.5 Multivariate analysis and correlations

In the present experiment, multivariate analysis was employed to assess responses of multiple variables simultaneously, enabling a deeper understanding of their interrelationships. Significant correlations were observed between energy digestibility and nitrogen digestibility ($r = 0.9761$), as well as between these metrics and crypt depth ($r = 0.4705$ and $r = 0.4460$, respectively).

Additionally, villi length was positively correlated with crypt depth (r = 0.5811) and villi surface area (r = 0.6384), supporting the hypothesis that improvements in digestibility metrics are associated with positive changes in intestinal morphology. Regression analysis further demonstrated that nitrogen digestibility had a slightly stronger influence on body weight (R^2 = 0.658) and feed conversion (R^2 = 0.344) compared to energy digestibility (R^2 = 0.613 and R^2 = 0.303, respectively). A 1% increase in nitrogen digestibility corresponded to an increase of 0.0467 kg in body weight and a reduction of 0.0185 units in feed conversion.

4.6 Study limitations and future directions

This study had a few limitations. Poultry diets used worldwide exhibit significant variability in composition, quality and nutritional profiles. It is essential to consider these differences, including diet compositions (substrate), ingredient quality, potential improvement on digestibility, and partitioning nutrient values for each enzyme used. Further, more extensive research is required to assess the effects of this sfericase protease under diverse production conditions, across different diet types, and in combination with other enzymes, including detailed evaluations of digestibility of individual amino acids.

Additionally, further studies are necessary to elucidate the mechanism by which proteases could exert their beneficial effects on gut morphology, overall health, nutrient absorption processes, and the microbiome. Regarding environmental ammonia emissions, it is crucial to acknowledge the numerous factors influencing these emissions. Measuring blood biomarkers related to nitrogen metabolism, such as uric acid and NH3, could serve as more sensitive indicators of nitrogen metabolism and environmental ammonia emissions.

5 Conclusion

The objective of this study was to evaluate the efficacy of a newly identified sfericase protease, used in combination with phytase and xylanase, in broiler diets. The results showed that this protease enhanced broiler performance, improved ileal nutrient digestibility, and positively influenced gut morphology. Furthermore, litter quality parameters remained unaffected, indicating no adverse environmental impacts. These findings confirm the efficacy of this enzyme in improving broiler productivity and optimizing nutrient utilization.

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 experiment was carried out in accordance with Official Mexican Norm (NOM-033-SAG/ZOO-2014) guidelines for animal welfare, and experimental protocols were approved by the Institutional Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

FR-S: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. CL-C: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. EA-G: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. JA-M: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SC: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review & editing. VF: Supervision, Writing – review & editing, Conceptualization, Funding acquisition, Investigation, Methodology.

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Conflict of interest

The 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2025.1453735/full#supplementary-material

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