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Abstract

This study investigated the effects of dietary supplementation with protease and xylanase, individually or in combination, on broilers fed diets with reduced energy and essential amino acids (lysine, methionine + cysteine, and threonine) levels. A total of 280 male Ross 308 broilers were randomly assigned to five treatments: a positive control (PC) with adequate nutrients; a negative control (NC) with 100 kcal/kg reduced energy and 4% lower amino acids; NC supplemented with protease (NCP); NC with xylanase (NCX); and NC with both protease and xylanase (NCPX). Statistical analyses were conducted using the general linear model (GLM) procedure for one-way analysis of variance (ANOVA), with significance declared at $p < 0.05$. Broilers in the NCP group showed higher ($p < 0.01$) body weight and average daily gain (ADG) compared to the NC group during overall (days 11-35) period. Feed conversion ratio (FCR) was improved in all enzyme-supplemented groups relative to NC ($p < 0.001$), with no differences in feed intake ($p > 0.05$) during all phases. Apparent ileal and jejunal digestibility of crude protein and crude fat were increased in the NCP, NCX, and NCPX groups compared to NC ($p < 0.001$). Additionally, NCPX supplementation resulted in improved villus height and villus height-to-crypt depth ratio at day 21 ($p < 0.05$), indicating enhanced intestinal structure and absorptive potential. Carcass traits, including dressing percentage, breast and leg yields, and abdominal fat, did not differ significantly among treatments ($p > 0.05$). In conclusion, the combined supplementation of protease and xylanase effectively mitigated the adverse effects of nutrient reduction by enhancing growth performance, nutrient digestibility, and gut morphology. These findings suggest that xylanase and protease supplementation, either individually or in combination, can help improve feed efficiency and nutrient utilization in broilers fed diets with reduced energy and amino acid levels.

Keywords: broiler, digestibility, reduced-nutrient diet, protease, xylanase

Introduction

Feed accounts for approximately 65-70% of total poultry production costs in intensive poultry systems, with ingredients such as corn and soybean meal contributing substantially to this cost [1, 2]. The fluctuating prices and quality of these feed raw materials are attributed to global demand for food and feed, climatic variability, and the diversion of grains for biofuel production [3, 4]. In response, nutritional strategies aimed at enhancing feed efficiency and reducing costs have gained attention, particularly through the use of exogenous enzymes such as xylanase and protease [5].

Traditional poultry diets, including ingredients such as corn, wheat, and soybean meal, contain approximately 10-22.7% non-starch polysaccharides (NSPs) [6]. NSPs are known to increase digesta viscosity, encapsulate nutrients, and hinder the access of digestive enzymes to substrates, ultimately impairing nutrient digestibility and energy utilization [7-9]. In broilers fed corn-soybean meal-based diets, this class of anti-nutritional compounds has been shown to reduce digestible energy by as much as 400-450 kcal/kg [10]. Xylanase, a glycosyl hydrolase targeting arabinoxylans, can break down these fiber structures, thereby improving apparent metabolizable energy (AME), lowering digesta viscosity, and enhancing feed conversion ratio (FCR) in broilers [11-13].

Protease supplementation improves nutrient digestibility by hydrolyzing dietary proteins and mitigating the negative effects of anti-nutritional factors such as trypsin inhibitors and lectins, which are abundant in soybean meal [14, 15]. It also enhances nitrogen retention, net energy, and amino acid absorption while reducing nitrogen excretion, ultimately improving both performance and environmental sustainability [16-18]. In addition, protease supplementation has been linked to improved intestinal morphology and reduced feed costs, even in nutrient-restricted diets [4].

Although the individual effects of xylanase and protease supplementation in poultry diets are well-documented, particularly regarding improvements in growth performance, nutrient digestibility, and gut morphology under single nutrient deficiencies [4, 8, 9, 19, 20], the combined use of these enzymes is biologically plausible because they target different substrate fractions in plant-based feed ingredients. Amerah et al. [21] compared the individual and combined effects of xylanase, amylase, and protease in corn–soybean diets and reported improvements in nutrient digestibility and feed efficiency. In addition, previous studies have evaluated enzyme supplementation in broilers fed diets with reduced metabolizable energy or crude protein/amino acid levels [2, 22, 23]. However, limited information is available regarding the effects of xylanase and protease, supplied individually or in combination, in broilers fed diets with simultaneous reductions in metabolizable energy and selected essential amino acids, including lysine, methionine plus cysteine, and threonine. Therefore, the objective of this study was to determine the effect of diets with reduced energy and amino acid levels supplemented with protease and xylanase, as single or combined activities, on growth performance, carcass traits, nutrient utilization, and intestinal morphology in broilers from 11 to 35 days of age. We hypothesized that protease and xylanase supplementation, either individually or in combination, would partially alleviate the negative effects of reduced dietary energy and amino acid levels by improving nutrient digestibility and intestinal morphology.

Materials and Methods

Animal Ethics Statement

The experimental procedures and protocol were reviewed and approved by the Animal ethics Committee of Chungnam National University (Protocol Number: 202410A-CNU-217).

Birds and housing

A total of 280 one-day-old male Ross 308 chickens were obtained from a local hatchery (Yangji hatchery, Pyeongtaek, Gyeonggi-do, Republic of Korea). The birds were raised in battery cage (76 × 60 × 40 cm^3) according to the general guidelines of 308 broiler management until 10 day of age [24]. All chicks were fed a crumbled commercial pre-starter broiler diet (ME: 3000 kcal/kg; CP: 22%) before the start of the experiment. On day 11, broiler chicks with similar body weights (275.49 ± 2.174

g) were selected and randomly divided into 5 treatment groups, each with 7 replicates of 8 birds. Each cage was fitted with two nipple drinkers and a metal trough to ensure efficient provision of water and feed. Birds were provided experimental diets *ad libitum* and had unrestricted access to fresh water via nipple drinkers throughout the experimental period. Vaccinations against infectious bronchitis and Newcastle disease were administered at the hatchery. The ambient temperature was controlled at 30 ± 1 ℃ from days 1 to 3, gradually reduced to 25 ± 1 ℃ by day 14, and then maintained at 24 ± 1 ℃ for the remainder of the experiment in accordance with the Ross 308 broiler management guide [24]. The average relative humidity during the study was 56 ± 14%. For the first 7 days the birds were adapted to a 23: 1 light to dark cycle which was then adjusted to 18:6 light to dark cycle until day 35.

Experimental design and diets

A total of five dietary treatments were assigned to birds in a completely randomized design, with seven replicate pens per treatment. The treatments consisted of: (1) a positive control (PC) diet meeting all nutritional requirements; (2) a negative control (NC) diet with a 100 kcal/kg reduction in metabolizable energy (ME) and a 4.0% reduction in key amino acids (lysine, methionine, and threonine) relative to PC; (3) NC supplemented with 300 g/MT protease (NCP); (4) NC supplemented with 15 g/MT xylanase (NCX); and (5) NC supplemented with both 300 g/MT protease and 15 g/MT xylanase (NCPX). The basal PC diet was formulated to meet or exceed the nutrient requirements for broilers [25]. The ingredient composition, calculated nutrient levels, and analyzed data for the grower (days 11-21) and finisher (days 22-35) phases are shown in Tables 1 and 2. All diets were provided in mash form and contained phytase (Phygest™ HT Dry, 1,000 FTU/g) with full matrix values applied according to the manufacturer's recommendations. Chromium oxide (Cr_2O_3; >99.95% purity, Sigma-Aldrich, St. Louis, MO, USA) was added to all diets at 0.3% as an indigestible marker for digestibility determination. The xylanase used was Xygest™ HT (Kemin, Asia Pacific, Singapore), which provides 3,000,000 U/g of activity and exhibits optimal activity within a pH range of 5-7, retaining more than 65% of its maximum activity under physiological temperature conditions (40-43℃; [19]). One unit of xylanase activity corresponds to the amount of enzyme that releases 1 μg of reducing sugars per minute from a beechwood xylan (Megazyme P-XYLNBE) at 50 °C and pH 5.3. The protease (Kemzyme™ Protease, Kemin, Asia Pacific, Singapore) was included at 300 g/MT of feed, equivalent to 2,400 U/kg of activity [20]. One unit of protease activity represents the amount of enzyme that solubilizes 1 μg of azo-casein substrate (Megazyme S-AZCAS) per minute at 37 °C and pH 7.0. This multiprotease blend contains acid (pepsin-type), neutral (metallo-endopeptidase), and alkaline (serine endopeptidase) proteases derived from *Aspergillus niger*, *Bacillus subtilis*, and *Bacillus licheniformis*, respectively. The enzyme inclusion levels followed the manufacturer's recommendations.

Growth performance measurements

On days 21 and 35, the body weight (BW) of all birds and the remaining feed in each cage were recorded to determine BW and average daily feed intake (ADFI). Average daily gain (ADG) was calculated as the difference in BW between two weighing periods divided by the number of days between them. Feed intake was determined by subtracting the residual feed from the total feed offered. Mortality-adjusted ADFI and feed conversion ratio (FCR) were subsequently calculated for each cage.

Post-mortem procedure and sample collection

On days 21 and 35, seven birds per treatment (one bird per cage) with body weights closest to the group mean were selected and individually weighed to record live body weight. The selected birds were euthanized by carbon dioxide asphyxiation for sample collection. The dressing percentage, including giblets (heart, gizzard, and liver), was calculated as a proportion of live body weight. Breast and leg muscles were separated and weighed to determine their relative percentages of the total carcass weight. The abdominal fat pad was also excised and expressed as a percentage of carcass weight. Following euthanasia, abdominal incisions were made, and the ileum was isolated from the gastrointestinal tract. The ileum was defined as the section of the small intestine between Meckel's diverticulum and the ileocecal junction [26]. A 3 cm segment of the ileum was collected from each bird, rinsed with phosphate-buffered saline (pH 7.4), and preserved in 10% formaldehyde for histological analysis. For digesta collection, one bird per cage with a body weight close to the cage mean was selected. Jejunal and ileal digesta were obtained from these birds, placed into labeled containers, and stored at -80°C until further analysis of nutrient digestibility.

Nutrient digestibility

To verify the analyzed composition of the experimental diets, representative diet samples were analyzed for dry matter, crude protein, crude fat, gross energy, and amino acid concentrations (Table 2). Digesta samples collected from the jejunum and ileum were dried at 55 °C for 24 h, ground, and passed through a 0.75-mm sieve (ZM 200 Ultra-Centrifugal Mill, Retsch GmbH & Co., KG, Haan, Germany) following the procedure described by [27] for nutrient digestibility analysis. Dry matter (DM) was analyzed according to the AOAC method 930.15 [28]. Chromic oxide concentration was determined following the procedure of [29]. Diet and digesta samples were analyzed for crude protein (AOAC method 984.13) and crude fat (AOAC method 920.39), while amino acid content was analyzed only in the diets using AOAC method 982.30 E (a, b). Gross energy in both digesta and diets was measured with an adiabatic bomb calorimeter (IKA Calorimeter System C 6000; IKA Works, Wilmington, NC, USA). The apparent jejunal and ileal digestibility of DM, energy, crude protein, and crude fat were calculated as follows;

$$\text{Digestibility, \%} = 100 - [100 \times \frac{M_{diet} \times N_{digest}}{M_{digest} \times N_{diet}}]$$

M_{diet} is the marker concentration in the diet whereas N_{digest} is the nutrient concentration in jejunal or ileal digesta whereas M_{digest} is the marker concentration in jejunal or ileal digesta, and N_{diet} is the nutrient concentration in the diet.

Intestinal morphology

Ileal morphometric analysis was conducted following the procedure described by [30]. Ring-shaped ileal tissue samples were sectioned diagonally (4-6 µm thickness), dehydrated, and embedded in paraffin wax. Ten well-oriented villi and their corresponding crypts were examined using an inverted microscope (Eclipse TE2000, Nikon Instruments Inc., Melville, NY, USA). Villi height and width, as well as crypt depth, were measured from histological images using the NIS-Elements Viewer software (Version 4.20; Nikon, USA). Villus height was defined as the distance from the tip to the base, and villus width was measured at the midpoint of the villus height. Crypt depth was defined as the distance from the top of the crypt to the muscularis mucosa [31]. The villus absorptive surface area was calculated using the following equation based on [32]:

$$\text{Villus absorptive surface area} = 2\pi \times (\text{villus width} \div 2) \times \text{villus height}$$

Statistical analysis

All data were analyzed using the general linear model (GLM) procedure for one-way ANOVA in SPSS software (Version 28; Armonk, NY, USA). The pen served as the experimental unit for growth performance measurements, while individual birds were considered the experimental unit for carcass traits, nutrient digestibility, and intestinal morphology. Statistical significance was set at $p < 0.05$, and when treatment effects were significant, means were separated using Tukey's multiple range test in SPSS.

Results

Growth performance

As summarized in Table 3, broilers fed the PC and NCP diets exhibited greater BW on day 35 compared to those receiving the NC diet ($p < 0.01$). During the overall period (days 11-35), the PC, NCP, and NCX groups demonstrated higher ADG than the NC group ($p < 0.01$). No significant differences ($p > 0.05$) in ADFI were observed among treatments during the grower (days 11-21), finisher (days 22-35), and overall (days 11-35) phases. FCR was consistently higher in the NC group relative to all other treatments ($p < 0.001$).

Carcass traits

As presented in Table 4, there were no significant differences ($p > 0.05$) among treatments in the dressing percentage or in the relative weights of the breast, leg, and abdominal fat on both days 21 and 35.

Nutrient digestibility

On day 21, apparent jejunal crude protein digestibility was greater in the NCX and NCPX groups compared to the NC group ($p < 0.01$), with the NCPX group also showing improvement over the PC group (Table 5). By day 35, all enzyme-supplemented groups (NCP, NCX, NCPX) exhibited enhanced jejunal crude protein digestibility relative to the NC group ($p < 0.001$). Additionally, the NCX and NCPX groups had higher jejunal crude protein digestibility than the PC group ($p < 0.001$). Broilers fed the PC diet exhibited lower jejunal crude fat digestibility compared to other treatments on day 35 ($p < 0.001$).

In the ileum, crude protein digestibility was increased on day 21 in all enzyme-supplemented groups compared to the NC group ($p < 0.001$), and the NCX and NCPX groups also outperformed the PC group (Table 6). Crude fat digestibility was higher in the PC group than in the NC group on day 21 ($p = 0.018$). On day 35, broilers in the NCPX group exhibited superior ileal digestibility of dry matter, energy, crude protein, and crude fat compared to those in the NC group ($p < 0.05$). Additionally, the digestibility of crude protein and crude fat was improved in the NCX and NCP groups compared to the NC group ($p < 0.001$).

Intestinal morphology

On day 21, the NCPX group exhibited a reduced villus width ($p < 0.001$) and an increased villus height to crypt depth (VH:CD) ratio ($p < 0.05$) compared to the NC and NCX groups (Table 7, Fig 1).

Discussion

In this study, supplementation of enzymes in diets with reduced energy and amino acid levels led to noticeable improvements in body weight and feed efficiency. At day 35, broilers fed the NC diet supplemented with protease (NCP), xylanase (NCX), or both enzymes (NCPX) exhibited numerically increased body weights by 8.37%, 7.98%, and 6.55%, respectively, compared to the NC group. Additionally, over the entire experimental period (day 11-35), FCR was significantly improved by 11.88% in the NCP group, 12.50% in the NCX group, and 11.25% in the NCPX group relative to the NC group. These findings indicate that supplementing protease and xylanase into diets with reduced energy and amino acid levels can partially or fully restore growth performance in broilers, depending on the specific enzyme and combination applied. The performance recovery observed in the NCP and NCX groups suggests that each enzyme can independently alleviate distinct metabolic constraints imposed by amino acid or energy deficiency, respectively. Xylanase enhances growth primarily by depolymerizing arabinoxylans and other NSPs, which reduces intestinal viscosity [7, 13] and improves the diffusion of nutrients and endogenous enzymes toward the absorptive mucosa [33-35]. This mechanism facilitates more efficient digestion and absorption of available nutrients even under energy-limited conditions. Protease, on the other hand, improves amino acid digestibility by hydrolyzing resistant dietary proteins and anti-nutritional factors such as lectins and trypsin inhibitors [4, 36, 37], thereby expanding the pool of absorbable amino acids and reducing the burden on endogenous enzyme systems. Previous studies reported reductions in pancreatic amylase and protease activities following exogenous enzyme supplementation, suggesting a feedback-related response in endogenous enzyme secretion [38, 39]. Interestingly, although the NCPX group, which received both enzymes, showed a statistical significant improvement in BW compared with the NC, it was not significantly different from that of protease supplemented group. Also, FCR was significantly improved in all the enzyme-treated groups in comparison to NC, indicating enhanced feed efficiency despite lacking a statistically significant change between different enzyme treatments. The magnitude of growth response to enzyme supplementation can be influenced by several factors, including the level and the physiological stage of the birds. Therefore, the present results should be interpreted as enzyme-specific or potentially complementary responses rather than clear evidence of synergy between xylanase and protease. Moreover, previous studies [40, 41] noted that enzyme combinations did not consistently outperform single enzyme supplementation, suggesting limited synergy under certain conditions rather than a proportional increase in benefit. These findings indicate that the response to combined enzyme supplementation can vary depending on diet composition, substrate availability, and enzyme inclusion level. This idea is further supported by [42], who reported a quadratic response in FCR to increasing multienzyme levels, indicating that enzyme supplementation beyond a certain point may lead to diminishing returns. Thus, the observed performance patterns not only reflect the functional specificity of each enzyme but also underscore the importance of synchronizing enzyme activity with the animal's physiological status and substrate availability.

Despite improvements in nutrient digestibility and feed efficiency in the enzyme-supplemented groups, carcass traits, including dressing percentage, breast and leg yields, and abdominal fat content, did not differ significantly among treatments. The lack of difference in carcass yield can be explained by the proportional development of carcass components relative to body weight. In this study, the NC group showed numerically lower body weight due to reductions in metabolizable energy (100 kcal/kg) and essential amino acids (4.0%), yet the proportional yields of carcass parts remained unchanged. This suggests that as overall growth declined, carcass components such as breast and leg muscles developed in proportion, maintaining consistent yield ratios across treatments. Additionally, under nutrient-restricted conditions, the improved digestibility provided by enzyme supplementation may have supported essential physiological functions such as gut epithelial maintenance, immune regulation, or basal metabolism rather than promoting carcass deposition. Studies have shown that in stressful metabolic conditions, including nutrient limitation, organisms prioritize survival and homeostasis over growth [43]. Previous study reported that exogenous enzyme supplementation can improve nutrient digestibility and support gut or skeletal development without consistently altering carcass composition [42]. Collectively, these findings suggest that carcass traits are influenced not only by nutrient utilization but also by the absolute nutrient supply, physiological priorities, and the localization of enzymatic effects.

In this study, supplementation of broiler diets with xylanase (NCX), protease (NCP), or their combination (NCPX) improved selected jejunal and ileal nutrient digestibility parameters in broilers fed diets with reduced energy and amino acid levels. These improvements were generally consistent with the enhanced FCR observed in the enzyme-supplemented groups. However, improved digestibility did not always translate into proportional improvements in BW or ADG. For example, although the enzyme-supplemented groups showed increased ileal crude protein digestibility on day 21, their ADG during days 11 to 21 did not differ significantly from that of the NC group. Similarly, NCPX improved several ileal digestibility parameters on day 35, but its overall ADG was not significantly different from that of the NC group. Therefore, improved nutrient digestibility may have partially contributed to better feed efficiency, but it does not fully explain the growth performance responses observed in this study. The improvements in nutrient digestibility observed in the NCX and NCP groups may be attributed to the specific enzymatic actions of xylanase and protease. Xylanase hydrolyzes arabinoxylans and other NSPs present in plant cell walls, which reduces digesta viscosity and releases encapsulated nutrients, thereby enhancing nutrient availability and absorption [19]. Protease, on the other hand, breaks down anti-nutritional proteins and cleaves complex protein structures into absorbable peptides and amino acids, improving the efficiency of protein digestion and absorption [44, 45]. These enzymatic actions likely contributed to the improved digestibility of crude protein and crude fat in the jejunum and ileum, increasing the pool of absorbable nutrients available for metabolic processes and growth in birds receiving the NCX and NCP diets.

Our findings are consistent with those of [46], who reported that a combination of Sfericase protease, phytase, and xylanase improved ileal digestibility of energy (but not nitrogen) in broilers fed a diet deficient in crude protein and amino acids, measured at 22 days of age. However, other studies such as Yi et al. [42] did not observe significant changes in protein digestibility, although they did report improved AME and neutral detergent fiber digestibility. Such variability across studies may be due to differences in diet formulation and enzyme spectrum. Interestingly, ileal digestibility values were consistently higher than those in the jejunum across all treatments. This may reflect functional and anatomical differences between intestinal segments. The jejunum, being an early site of digestion, is subject to rapid digesta flow and pH variation, which can limit enzymatic activity [47]. In contrast, the ileum provides a more stable environment for prolonged enzyme-substrate interactions. Van Hoeck et al. [19] also noted that xylanase supplementation particularly enhanced nutrient uptake and gut morphology in the ileum, supporting our interpretation.

In this study, enzyme supplementation had minimal effects on intestinal morphology by day 35. However, at day 21, birds in the NCPX group exhibited a significantly reduced villus width and an increased VH:CD ratio compared to the NC and NCX groups, indicating potential enhancement in the intestinal absorptive surface and mucosal maturation. These effects may be attributable to the combinatorial action of xylanase and protease, which modulate gut microenvironment and reduce the inflammatory burden, thereby supporting epithelial development. Xylanase has been shown to improve gut morphology by lowering digesta viscosity, facilitating nutrient diffusion, and promoting epithelial integrity [19, 48]. Similarly, protease contributes to protein digestion by degrading undigested proteins and proteinaceous anti-nutritional factors, which can reduce the amount of undigested protein entering the lower gastrointestinal tract [4]. Although only partial improvements were observed in villus parameters, the trend toward enhanced VH:CD ratio suggests that multienzyme supplementation could support intestinal structure and function, particularly during the early growth phase. The absence of morphological differences at day 35 may reflect the compensatory adaptation of the intestinal epithelium over time, as birds stabilize their digestive efficiency. This is consistent with findings by

[42], who also noted no significant morphological changes in later phases of broiler growth despite improvements in efficiency of feed utilization and nutrient transporter gene expression.

Conclusion

This study demonstrated that the combined supplementation of protease and xylanase in broiler diets with reduced metabolizable energy and selected essential amino acids significantly improved growth performance and nutrient digestibility, particularly crude protein and ether extract, compared to the negative control group. The improvements in digestibility were notably evident in the jejunum and ileum. Furthermore, birds receiving the enzyme combination exhibited enhanced villus height and villus-to-crypt ratio in both intestinal segments, indicating improved absorptive capacity. Despite these positive effects on digestive function and nutrient utilization, carcass traits were not significantly influenced. These results suggest that protease and xylanase supplementation can partially alleviate the adverse effects of reduced dietary energy and amino acid levels by improving feed efficiency and nutrient utilization, providing a promising strategy to reduce feed costs without compromising broiler productivity.

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Table 1. Composition (g/kg, as-fed basis) of the experimental diets

Items	Experimental diets[1]			
	Grower (Day 11-21)		Finisher (Day 22-35)	
	PC	NC	PC	NC
Corn	55.51	53.20	56.43	59.38
Soybean meal, 44%	23.79	30.88	26.54	27.54
Wheat	3.00	3.00	3.00	3.00
Corn DDGS	7.00	7.00	7.00	5.95
Corn gluten meal	5.00	0.86	0.31	-
Crude palm oil	1.78	1.50	3.77	1.22
Limestone	1.14	1.12	0.93	0.90
Mono-calcium phosphate	0.90	0.87	0.44	0.59
Salt	0.14	0.21	0.15	0.15
L-Lysine sulfate, 70%	0.83	0.48	0.50	0.38
DL-Methionine, 98%	0.27	0.27	0.29	0.27
L-Threonine, 98%	0.03	-	0.03	0.01
Phytase[2]	0.01	0.01	0.01	0.01
Vitamin-mineral premix[3]	0.30	0.30	0.30	0.30
Cr_2O_3	0.30	0.30	0.30	0.30
Calculated value				
Metabolizable energy, kcal/kg	3,050	2,950	3,100	3,000
Crude protein, %	21.50	21.50	19.50	19.50
Crude Fat, %	4.41	4.04	6.33	3.83
Calcium, %	0.84	0.84	0.72	0.72
Available phosphorus, %	0.42	0.42	0.36	0.36
SID lysine,%	1.30	1.25	1.16	1.11
SID methionine, %	0.63	0.60	0.59	0.57
SID methionine + cystine, %	1.03	0.99	0.98	0.94
SID threonine, %	0.79	0.76	0.75	0.72

[1]PC, Positive control diet; NC, negative control diet with 100 kcal/kg metabolizable energy reduction and reducing amino acids by 4.0%

[2]Phytase: product name, 1000 FTU/g (100 g/metric ton)

[3]Provided per kilogram of diet: vitamin A (trans-retinyl acetate), 6,400 IU; vitamin D3 (cholecalciferol), 1,760 IU; vitamin E, 12,000 IU; vitamin K3, 1,760 mg; biotin, 80 mg; thiamin, 1,600 mg; riboflavin, 3,520 mg; pyridoxine, 2,400 mg; vitamin B12, 8 mg; niacin, 28,000 mg; pantothenic acid, 10,000 mg; folic acid 480 mg; Fe (from iron sulfate), 20,000 mg; Cu (from copper sulfate), 81,000 mg; Zn (from zinc oxide), 32,500 mg; Mn (from manganese oxide), 43,800 mg; I (from potassium iodide), 750 mg; Se (from sodium selenite), 50 mg.

Abbreviations: DDGS, distillers dried grains with soluble; SID, standardized ileal digestible.

Table 2. Analyzed composition of the experimental diets

Items	Experimental diets[1]			
	Grower (Day 11-21)		Finisher (Day 22-35)	
	PC	NC	PC	NC
Dry matter, %	91.23	91.24	91.23	91.24
Crude protein (Nitrogen × 6.25), %	21.23	21.99	19.38	20.14
Gross energy, kcal/kg	4123.12	4001.24	4225.24	4103.36
Crude fat, %	4.51	3.98	6.21	4.01
Amino acids				
Lysine, %	1.41	1.19	1.29	1.08
Methionine, %	0.65	0.61	0.62	0.56
Cystine, %	0.59	0.51	0.50	0.44
Threonine, %	0.92	0.75	0.79	0.71
Arginine, %	1.44	1.43	1.24	1.22
Histidine, %	0.48	0.48	0.41	0.41
Isoleucine, %	0.93	0.91	0.80	0.78
Leucine, %	1.56	1.55	1.35	1.33
Phenylalanine, %	0.84	0.71	0.72	0.71
Tryptophan, %	0.21	0.20	0.18	0.18
Valine, %	1.09	1.07	0.93	0.91
Alanine, %	1.05	0.99	0.90	0.88
Aspartic acid, %	1.67	1.65	1.44	1.42
Glutamic acid, %	3.30	3.21	2.84	2.73
Glycine, %	1.06	0.98	0.91	0.89
Proline, %	1.34	1.32	1.15	1.14
Serine,%	0.91	0.90	0.79	0.77
Tyrosine, %	0.56	0.55	0.48	0.47

Table 3. Effect of exogenous protease and xylanase as single or combined activities on growth performance in broilers fed diets with reduced energy and amino acid levels[1]

Items	Dietary treatment[2]					SEM[3]	P-value
	PC	NC	NCP	NCX	NCPX		
BW, g							
Day 11	278.18	274.77	276.59	272.70	275.21	2.174	0.948
Day 21	865.47	794.84	842.75	829.45	826.85	7.535	0.078
Day 35	1960.03[b]	1737.14[a]	1882.62[b]	1875.72[ab]	1850.90[ab]	15.288	0.002
ADG, g/day							
Day 11-21	53.39[b]	47.28[a]	51.47[ab]	50.61[ab]	50.15[ab]	0.574	0.034
Day 22-35	78.18[b]	67.31[a]	74.28[ab]	74.73[ab]	73.15[ab]	1.001	0.029
Day 11-35	67.27[b]	58.49[a]	64.24[b]	64.12[b]	63.03[ab]	0.597	0.002
ADFI, g/day							
Day 11-21	69.87	71.95	67.58	65.63	66.77	0.802	0.120
Day 22-35	111.39	110.34	108.15	108.71	107.99	1.372	0.916
Day 11-35	93.12	93.45	90.30	89.75	89.86	0.917	0.536
FCR, g/g							
Day 11-21	1.31[a]	1.53[b]	1.31[a]	1.30[a]	1.33[a]	0.015	<0.001
Day 22-35	1.43[a]	1.64[b]	1.46[a]	1.45[a]	1.48[a]	0.009	<0.001
Day 11-35	1.39[a]	1.60[b]	1.41[a]	1.40[a]	1.42[a]	0.007	<0.001

[1] Values are the mean of seven replicates per treatment

[2] PC, positive control diet; NC, negative control diet with a 100 kcal/kg metabolizable energy (ME) reduction and reducing amino acids by 4.0%; NCP, NC + KEMZYME™ Protease 300 g/MT; NCX, NC + Xygest™ HT 15 g/MT; NCPX, NC + KEMZYME™ Protease 300 g/MT + Xygest™ HT 15 g/MT

[3] Pooled standard error of the mean

[a,b] Values in a row with different superscripts differ significantly ($P < 0.05$)

Abbreviations: BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, Feed conversion ratio.

Table 4. Effect of exogenous protease and xylanase as single or combined activities on carcass traits in broilers fed diets with reduced energy and amino acid levels[1]

Items	Dietary treatment[2]					SEM[3]	P-value
	PC	NC	NCP	NCX	NCPX		
Day 21							
Dressing ratio, %	90.46	90.93	89.75	90.28	90.95	0.189	0.261
Breast, %	22.40	23.30	22.71	22.76	24.44	0.235	0.077
Leg, %	9.61	9.30	9.28	9.43	9.30	0.074	0.584
Fat pad, %	1.10	1.03	0.96	0.88	0.86	0.043	0.391
Day 35							
Dressing ratio, %	90.94	89.96	90.77	90.47	91.70	0.270	0.358
Breast, %	28.25	28.37	28.80	29.25	28.77	0.214	0.606
Leg, %	10.26	10.12	10.33	10.24	11.05	0.114	0.106
Fat pad, %	1.31	1.29	1.10	1.13	0.95	0.055	0.242

[1] Values are the mean of seven replicates per treatment

[2] PC, positive control diet; NC, negative control diet with a 100 kcal/kg metabolizable energy (ME) reduction and reducing amino acids by 4.0%; NCP, NC + KEMZYME™ Protease 300 g/MT; NCX, NC + Xygest™ HT 15 g/MT; NCPX, NC + KEMZYME™ Protease 300 g/MT + Xygest™ HT 15 g/MT

[3] Pooled standard error of the mean

Table 5. Effect of exogenous protease and xylanase as single or combined activities on jejunal nutrient digestibility in broilers fed diets with reduced energy and amino acid levels[1]

Items	Dietary treatment[2]					SEM[3]	P-value
	PC	NC	NCP	NCX	NCPX		
Day 21							
Dry matter	67.52	69.26	69.12	68.88	69.71	0.272	0.146
Energy	62.15	62.10	62.24	62.40	63.05	0.230	0.687
Crude protein	55.17^{ab}	54.49^{a}	57.15^{abc}	57.90^{bc}	58.04^{c}	0.304	0.002
Crude fat	51.05	48.72	49.30	48.86	50.24	0.305	0.105
Day 35							
Dry matter	69.19	70.04	70.71	70.87	71.18	0.257	0.134
Energy	65.78	64.06	65.37	65.98	66.02	0.268	0.145
Crude protein	55.55^{ab}	53.65^{a}	57.61^{bc}	58.95^{c}	58.38^{c}	0.265	<0.001
Crude fat	48.24^{a}	51.39^{b}	53.34^{b}	51.71^{b}	52.43^{b}	0.267	<0.001

[1] Values are the mean of seven replicates per treatment

[2] PC, positive control diet; NC, negative control diet with a 100 kcal/kg metabolizable energy (ME) reduction and reducing amino acids by 4.0%; NCP, NC + KEMZYME™ Protease 300 g/MT; NCX, NC + Xygest™ HT 15 g/MT; NCPX, NC + KEMZYME™ Protease 300 g/MT + Xygest™ HT 15 g/MT

[3] Pooled standard error of the mean

[a-c] Values in a row with different superscripts differ significantly ($P < 0.05$)

Table 6. Effect of exogenous protease and xylanase as single or combined activities on ileal nutrient digestibility in broilers fed diets with reduced energy and amino acid levels[1]

Items	Dietary treatment[2]					SEM[3]	P-value
	PC	NC	NCP	NCX	NCPX		
Day 21							
Dry matter	73.91	75.31	75.19	74.99	75.70	0.198	0.082
Energy	75.87	75.17	76.09	76.16	76.64	0.158	0.082
Crude protein	67.49[ab]	66.69[a]	68.86[bc]	69.40[c]	69.53[c]	0.190	<0.001
Crude fat	62.37[b]	60.48[a]	61.06[ab]	60.71[ab]	61.85[ab]	0.189	0.018
Day 35							
Dry matter	73.80[ab]	73.50[a]	75.08[ab]	75.19[ab]	75.52[b]	0.211	0.015
Energy	76.45[ab]	75.96[a]	76.68[ab]	77.03[ab]	77.14[b]	0.120	0.029
Crude protein	65.88[b]	62.59[a]	67.38[bc]	68.35[c]	67.98[c]	0.205	<0.001
Crude fat	52.73[a]	58.88[b]	61.53[c]	60.07[bc]	60.83[c]	0.201	<0.001

[1] Values are the mean of seven replicates per treatment

[2] PC, positive control diet; NC, negative control diet with a 100 kcal/kg metabolizable energy (ME) reduction and reducing amino acids by 4.0%; NCP, NC + KEMZYME™ Protease 300 g/MT; NCX, NC + Xygest™ HT 15 g/MT; NCPX, NC + KEMZYME™ Protease 300 g/MT + Xygest™ HT 15 g/MT

[3] Pooled standard error of the mean

[a-c] Values in a row with different superscripts differ significantly ($P < 0.05$)

Table 7. Effect of exogenous protease and xylanase as single or combined activities on ileal morphology in broilers fed diets with reduced energy and amino acid levels[1]

Items	Dietary treatment[2]					SEM[3]	P-value
	PC	NC	NCP	NCX	NCPX		
Day 21							
Villus height, µm	828.88	739.38	839.44	838.41	898.12	15.800	0.056
Villus width, µm	98.76[c]	99.02[c]	97.68[bc]	97.01[b]	95.24[a]	0.179	<0.001
Crypt depth, µm	147.47	139.03	145.73	152.45	130.60	3.773	0.420
Villus height: Crypt depth ratio	5.68[ab]	5.56[a]	5.79[ab]	5.52[a]	6.89[b]	0.135	0.018
Villus absorptive surface area, mm^2	0.26	0.23	0.26	0.26	0.27	0.005	0.173
Day 35							
Villus height, µm	874.49	920.87	883.63	966.84	926.36	34.176	0.917
Villus width, µm	114.68	107.91	108.17	103.83	110.78	1.979	0.524
Crypt depth, µm	112.07	126.74	144.02	142.03	122.65	3.847	0.067
Villus height: Crypt depth ratio	7.90	7.46	6.39	6.74	7.72	0.296	0.442
Villus absorptive surface area, mm^2	0.32	0.31	0.30	0.31	0.32	0.013	0.983

[1] Values are the mean of seven replicates per treatment

[2] PC, positive control diet; NC, negative control diet with a 100 kcal/kg metabolizable energy (ME) reduction and reducing amino acids by 4.0%; NCP, NC + KEMZYME™ Protease 300 g/MT; NCX, NC + Xygest™ HT 15 g/MT; NCPX, NC + KEMZYME™ Protease 300 g/MT + Xygest™ HT 15 g/MT

[3] Pooled standard error of the mean

[a,b] Values in a row with different superscripts differ significantly ($P < 0.05$)

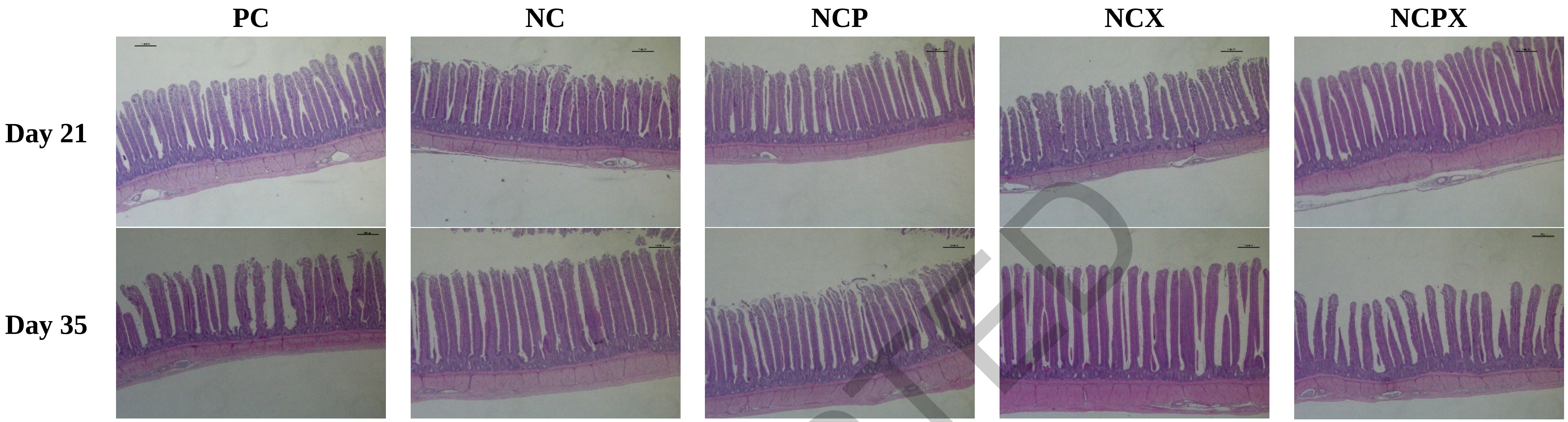

Fig 1. Effect of exogenous protease and xylanase as single or combined activities on ileal morphology in broilers fed diets with reduced energy and amino acid levels. PC, positive control diet; NC, negative control diet with a 100 kcal/kg metabolizable energy (ME) reduction and reducing amino acids by 4.0%; NCP, NC + KEMZYME^{TM} Protease 300 g/MT; NCX, NC + Xygest^{TM} HT 15 g/MT; NCPX, NC + KEMZYME^{TM} Protease 300 g/MT + Xygest^{TM} HT 15 g/MT. The scale bar is 100 µm.