Journal of Animal Research: v.12 n.05, p. 613-621. October 2022

**DOI:** 10.30954/2277-940X.05.2022.2

# Impact of Exogenous Protease on Performance, Nutrient Digestibility, and Excreta Odor Emission in Broiler Chickens Fed Corn-Soybean-Based Diet

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**Received:** 02 Sept., 2022 **Revised:** 26 Sept., 2022 **Accepted:** 29 Sept., 2022

**ABSTRACT**

This experiment was conducted to evaluate dietary protease supplementation in broilers fed corn-soybean meal-based diets on performance, apparent ileal digestibility of nutrients and amino acids, ileal digesta viscosity, blood profile, and excreta odor content. A total of 2,000 1-day-old male broilers (Ross 308, 43.34±1.12 g) were allotted randomly to five dietary treatments on the basis of initial body weight (BW). Each treatment had 10 replicate pens with 40 birds per pen. The 4 dietary treatments were corn-soybean meal diets supplemented with 0, 3,750, 5,000, and 6,250 PCU/kg feed protease. Experimental diets were fed for 42 days in four phases (Pre Starter: d 0-11; Starter: d 12-21; Grower: d 22-32 and Finisher: d 33-42) in pellet form. During d 1 to 21, d 22-42, and the overall study period (d 0-42), with increasing dietary protease levels from 0 to 6, 250 PCU/kg feed, the BWG was improved (linear, $P$ <0.05), whereas the FCR was decreased (linear, $P$ <0.05). There was a linear ($P$ <0.05), improvement in ATTD of CP and GE and most amino acids (with exception of arginine, phenylalanine, and tyrosine) as dietary protease level increased. Blood profiles including blood urea nitrogen and creatinine concentrations were not affected by dietary treatments, similarly,odor emission in excreta of broilers including ammonia, hydrogen sulfide, and total mercaptan was not affected with dietary protease. In conclusion, dietary protease supplementation in broilers corn-soybean meal-based diets had beneficial effects on growth performance, and apparent total tract digestibility of nutrients and amino acids.

**HIGHLIGHTS**

- Protease supplementation linearly increased growth performance and feed conversion ratio.
- Dietary protease at 2.5 g kg−1 dose improved crude protein, gross energy,lysine, methionine, cysteine, threonine, tryptophan, isoleucine, leucine, and histidine.

**Keywords**: Broilers, protease, performance, nutrient digestibility, excreta odor content

Rapidly increasing demand for meat and skyrocketing prices of feed ingredients insert pressure on livestock producers as well as nutritionists to the inclusion of alternative low-quality feed in poultry diet. Soybean protein is the major source in poultry diet meals, which contains the trypsin inhibitors like glycinin and ß-conglycinin and allergic proteins like lectin (Boudry *et al.*, 2004, Erdaw *et al.*, 2007; Kaczmarek *et al.*, 2014). These anti-nutritional factors cause allergic reactions to disrupt the small intestinal structure which lower nutrient absorption, often accompanied by suboptimal growth performance and higher morbidity and mortality, causing large economic losses to the livestock industry (Boudry *et al.*, 2004).

The lack of adequate levels of endogenous protease in monogastric animals to digest proteins in the diet leads to the flow of undigested protein in the hindgut (Lee *et al.*, 2018; Song *et al.*, 2022). Fermentation of undigested

**How to cite this article:** Lokhande, A., Hosseindoust, A., Lee, S. and Kim, J.S. (2022). Impact of Exogenous Protease on Performance, Nutrient Digestibility, and Excreta Odor Emission in Broiler Chickens Fed Corn-Soybean-Based Diet. *J. Anim. Res.,* **12**(05): 613-621.

**Source of Support:** None; **Conflict of Interest:** None

protein in hind guts leads to the growth of pathogenic bacteria like *E.coli, Clostridium sp., Salmonella sp., and Campylobacter sp.* resulting in toxicity, intestinal legions, inflammations and production of noxious metabolites such as indoles, skatoles, biogenic amines, branched-chain fatty acids and ammonia which are detrimental to host health (Jeong *et al.*, 2019; Amiri *et al.*, 2021; Hosseindoust *et al.*, 2022).The inclusion of exogenous feed enzymes in animal diets has been considered an effective strategy to improve feed efficiency and reduce feed costs in animal production (Adeola and Cowieson, 2011; Kim *et al.*, 2021;). Exogenous protease has the potential to hydrolyze commonly used indigestible proteins in pig and poultry diets (Zuo *et al.*, 2015; Walk *et al.*, 2019; Mohammadigheisar *et al.*, 2021). It is also receiving more attention as an effective tool to increase amino acid digestion, improve growth performance, and reduce feed cost by allowing lower-quality protein alternatives to be used in the diet, especially in young birds where endogenous protease levels may be limiting (Angel *et al.*, 2011; Mohammadi *et al.*, 2011). Recent studies have shown that protease was able to improve growth performance, nutrient absorption efficiency, intestinal development, and health status of weaned pigs and broilers (Zuo *et al.*, 2015; Park *et al.*, 2020). Previous reports showed protease supplementation improved animal performance by decreasing digesta viscosity, improving endogenous enzyme activity, and decreasing pancreas weight (Erdaw *et al.*, 2017). However, the scientific evidence regarding the influence of using a blend of acid, alkaline, and neutral protease in broiler chickens' diets is limited. Therefore, the objective of this experiment was to evaluate the influence of exogenous protease blend on growth performance, nutrients and amino acid digestion, blood profiles, and excreta noxious gas emissions in broilers fed corn-soybean meal-based diets.

## MATERIAL AND METHODS

All experimental protocols describing the management and care of animals were reviewed and approved by the Institution of Animal Care and Use Committee, Kangwon National University (Ethical code: KW-210503-6).

### Enzyme preparation

The enzyme used in our experiment was produced by Advanced Enzymes Technologies Ltd., Louiswadi, Thane, India. In short, protease preparation (25,000,000 PCU/kg) used was acidic, alkaline, and neutral protease blend produced by controlled fermentation of a selected strain of *Bacillus licheniformis*. One unit of protease is the amount of enzyme, which liberates 1µg of phenolic compound (tyrosine equivalent) from casein substrate per minute at 7.0 pH and 37$^{o}$C.

### Animals and experimental design

A total of 2,000 male broilers (Ross 308, 43.34±1.12 g, 1 day old) were allotted randomly to five dietary treatments on the basis of initial body weight (BW). Each treatment had 10 replicate pens with 40 birds per pen. The 4 dietary treatments were corn-soybean meal diets supplemented with 0, 3,750, 5000, and 6,250 PCU/kg feed protease. The formula and chemical composition of experimental diets has presented in Table 1. Experimental diets were fed for 42 days in four phases (Pre starter: d 0-11; Starter: d 12-21; Grower: d 22-32 and Finisher: d 33-42). All diets met or exceeded the nutrient requirements recommended by Aviagen (2019) for Ross 308.

**Table 1:** Composition and calculated nutrient content diets (as-fed basis)

| Ingredients, kg | Pre-starter 0-12 | Starter 13-21 | Grower 22-35 | Finisher 36-42 |
|---|---|---|---|---|
| Maize | 555.97 | 559.34 | 573.77 | 586.45 |
| HPS | 282.17 | 271.78 | 45.55 | 0 |
| Soymeal | 0 | 0 | 203.31 | 243.7 |
| Broken rice | 50 | 50 | 50 | 50 |
| MBM 44% | 40 | 40 | 40 | 40 |
| Maggot-meal | 40 | 40 | 40 | 23.72 |
| Berga fat | 4.54 | 13.95 | 24.99 | 35.22 |
| DCP | 10.56 | 7.78 | 6.34 | 6.53 |
| Lysine sulphate | 4.84 | 4.25 | 4.62 | 4.3 |
| Methionine | 3.24 | 2.9 | 2.68 | 2.63 |
| Salt | 2.65 | 2.66 | 2.4 | 2.1 |
| Threonine | 1.18 | 0.94 | 0.99 | 1 |
| Soda bicarb | 1 | 1 | 1 | 1 |
| Toxin binder | 1 | 1 | 1 | 1 |
| Trace minerals | 1 | 1 | 1 | 1 |
| Pelex | 1 | 1 | 1 | 0 |
| Vitamins | 0.5 | 0.5 | 0.5 | 0.5 |

| | | | | |
|---|---|---|---|---|
| Calcite | 0 | 1.55 | 0 | 0 |
| Ligosa fat | 0.1 | 0.1 | 0.1 | 0.1 |
| Salinomycin | 0 | 0 | 0.5 | 0.5 |
| DigeGrain Pro 6 or corn | 0.25 | 0.25 | 0.25 | 0.25 |
| **Chemical composition** | 1000 | 1000 | 1000 | 1000 |
| ME (kcal/kg) | 3030 | 3090 | 3150 | 3200 |
| CP (%) | 23.42 | 22.86 | 21.03 | 19.8 |
| Ca (%) | 0.8 | 0.8 | 0.7 | 0.7 |
| Available P (%) | 0.5 | 0.45 | 0.42 | 0.42 |
| Dig. M + C(%) | 0.94 | 0.9 | 0.84 | 0.79 |
| Dig. Met (%) | 0.64 | 0.6 | 0.56 | 0.53 |
| Dig. Lys (%) | 1.26 | 1.2 | 1.12 | 1.06 |
| Dig. Thr (%) | 0.81 | 0.78 | 0.72 | 0.68 |
| Chloride, % | 0.22 | 0.22 | 0.21 | 0.19 |
| Sodium, % | 0.18 | 0.18 | 0.17 | 0.16 |
| Potasium, % | 0.8 | 0.78 | 0.74 | 0.73 |
| Linolic acid | 1.22 | 1.22 | 1.25 | 1.25 |

* Diets were formulated using Brill formulations software and meet and exceed nutrients requirements of Cobb 430 broiler chickens; HPS, high protein soya; MBM, meat and bone meal; DCP, di-calcium phosphate.

### Experimental procedure and sampling

The feeding trial was conducted in floor pens with rice hulls used as litter. Temperature and humidity were controlled by an automatic ventilation system according to the Ross Broiler Management Handbook (Aviagen, 2019). The house temperature was maintained at 34° for the first 5 d and was then gradually reduced according to normal management practices until a temperature of 23° was achieved The lighting program was started with 20 h light (15 lux) according to Ross 2018, then gradually decreased to 19 h at d 7. Feed and water were provided *ad libitum* with each pen having 2 feeders and 2 nipple drinkers.

Dietary Ca, P, and amino acids (lysine) were analyzed according to the procedures described by the AOAC (2007) official methods of analysis. Dietary Ca was assayed by atomic absorption spectrophotometry after wet ash procedures. P was determined by colorimetry. Lysine and methionine were measured using an amino acid analyzer (Beckman 6300; Beckman Coulter Inc.) after 24 h of 6 N-HCl hydrolyses at 110°C. There were 10 replicate pens per treatment with 15 broilers per pen.

### Growth performance

The birds were individually weighed at the start of the trial (d 0) and at end of starter (d 22) and finisher (d 42) phases. Feed that was not consumed was weighed at end of each phase and feed intake was calculated The feed conversion ratio was calculated by dividing feed intake by the BWG of each phase. The number of dead birds was recorded daily and pooled for calculating the mortality during the whole 42 d feeding trials. Body weight (BW) gain, feed intake, and feed conversion ratio (FCR) were corrected for the weight of dead birds during each phase.

### Nutrient digestibility

At the end of the experiment, nutrient digestibility of dry matter (DM), CP, ether extracts (EE), and AA was determined by apparent total tract digestibility (ATTD), using chromic oxide as an indicator. Concisely, all broilers were fed diets mixed with 0.25% $Cr_2O_3$ for five days before excreta collection at 42 d. All fecal samples were collected and pooled within the pen, and a representative sample was stored in a freezer at −20°C until analysis. Before chemical analysis, the excreta samples were thawed and dried at 75°C for 72 h in a forced-air oven (model FC-610, Advantec, Toyo Seisakusho Co. Ltd., Tokyo, Japan). After drying, they were finely ground to a size that could pass through a 1-mm screen. The analysis of CP was then carried out using the CP method, 990.03 (AOAC, 2007), DM was analyzed using the DM method 930.15 (AOAC, 2007), EE was analyzed using the EE Method 2003.03 (AOAC, 2007), and ash using the method 942.05 (AOAC, 2007). The Gross energy of feeds, excreta, and digest a samples was analyzed using a 6400 model bomb calorimetry (Parr Instruments, Moline, IL, USA) and the AA composition of feed samples was analyzed using high-performance liquid chromatography according to Soleimani *et al.* (2010). Chromium concentration was determined with an automated spectrophotometer (Jasco V-650; Jasco Corp., Tokyo, Japan) according to (Jeong *et al.*, 2019). The ATTD of nutrients was calculated using the method by (Mohammadi *et al.*, 2015) below,

$$\text{Digestibility (\%)} = 1 - \left[\frac{Nf \times Cd}{Nd \times Cf}\right] \times 100$$

*Nf* is the concentration of nutrients in feces (percentage of DM), *Nd* is the concentration of nutrients in the diet, *Cd*

means the concentration of chromium in the diet, and Cf is the concentration of chromium in the feces.

### Ileum digesta viscosity

On d 42, ileum digesta samples of 2 birds per pen (20 birds per treatment) were collected. The viscosity of the liquid fraction of digest a was measured immediately after centrifugation at 12000 × g for 10 min. The supernatant was then withdrawn and viscosity (in centipoise, *cp* = 1/100 dyne sec×cm$^{-2}$) was determined in a Brockfield Digital DV-II cone/plate viscometer[2] maintained at 40°C and shear rates of 1.15-450 s$^{-1}$. Absolute viscosity (cps) was presented at shear rate 45 s$^{-1}$ in the following. When measurable viscosity was not in the range of 45 s$^{-1}$, data were plotted as log (shear rate) vs. log (absolute viscosity) giving a straight line from which the line could be extrapolated to 45 s$^{-1}$.

### Blood Profiles

On d 28, blood samples of 1 bird per pen (10 birds per treatment) were collected from the wing vein. Approximately 2.5 ml of blood was collected in vacuum tubes containing $K_3$EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ). To obtain the serum sample, blood samples were centrifuged at 3000 × g for 15 min at 4°C, and then stored at -20°C until assay. The blood urea nitrogen (BUN) concentration and serum creatinine concentration were analyzed using the Abbott Spectrum urea nitrogen test (Series, Abbot Laboratories, Dallas, TX).

### Noxious gas emission

On d 42 of the experiment, feces samples were collected from each cage, for the analysis of noxious gas emissions. The feces samples were stored in 2.5-L plastic boxes, in triplicates. Each box had a small hole in the middle of one side wall, which was sealed with adhesive plaster. The samples were permitted to ferment for a period of 120 hrs at room temperature (25°C). After the fermentation period, the Gastec (model GV-100) gas sampling pump was used for gas detection (Gastec Corp., Kanagawa, Japan) for Ammonia ($NH_3$), Hydrogen Sulfide ($H_2S$) and used detecting tube for Total mercaptan (R.SH) (No. 3L, No. 4LT and No. 70L, Gastec, Japan). In these measurements, the adhesive plaster was punctured and 100 mL of headspace air was sampled approximately 2.5 cm above the feces. After air sampling, each box has again covered with adhesive plaster. Headspace measurements were again performed every 24 h. The gas contents were averaged by 3 measurements from the same box.

## STATISTICAL ANALYSIS

Data were statistically analyzed with analysis of variance (ANOVA) using general linear model procedure of the SAS program (SAS Institute, NC) with a completely randomized design. Data were presented as mean values and standard errors of means. To determine the significance between treatments for growth performance and nutrient digestibility, data were analyzed with the general linear model (GLM) in SAS. Differences among all treatments were separated by Duncan's multiple-range tests. In addition, orthogonal comparisons were conducted using polynomial regression to measure the linear and quadratic effects of increasing the dietary supplementation of acid protease. A P<0.05 was considered significant, and a level of 0.1 was considered a trend.

## RESULTS

### Growth performance

As described in Table 2, during d 1 to 21, with increasing dietary protease levels from 0 to 6, 250 PCU/kg feed, the BWG was improved (linear, $P$ <0.05), whereas the FCR was decreased (linear, $P$ <0.05). During the 22–42 d phase, with increasing dietary protease levels from 0 to 6, 250/ton feed, the BWG was improved (linear, $P$ <0.05), whereas the FCR was decreased (linear, $P$ <0.05). During the overall study period (0-42 d), there was a linear increase in BWG ($P$ <0.05) and a linear decrease in FCR ($P$ <0.01) with increasing protease supplementation. Protease supplementation did not affect the feed intake of birds during any phases of feeding or during the overall study period.

### Nutrient digestibility and ileal digesta viscosity

The effects of dietary protease on ATTD of nutrients and amino acids are presented in Table 3 and Table 4, respectively. There was a linear ($P$ <0.05), improvement in ATTD of CP and GE ($P$ <0.01) as dietary protease level

**Table 2:** Effect of dietary supplementation of DigeGrain Pro 6 on performance of broilers

| Treatment | Dietary protease level (PCU/kg) | | | | SEM[1] | P - value | |
|---|---|---|---|---|---|---|---|
| | 0 | 3,750 | 5,000 | 6,250 | | Linear | Quadratic |
| **d 0-21 (Starter)** | | | | | | | |
| BWG, g/bird | 964 | 968 | 1011 | 1025 | 12.44 | 0.002 | 0.574 |
| Feed Intake, g/bird | 1238 | 1235 | 1235 | 1283 | 18.95 | 0.633 | 0.218 |
| FCR | 1.29 | 1.28 | 1.22 | 1.25 | 0.020 | 0.005 | 0.334 |
| **d 22-42 (Grower-finisher)** | | | | | | | |
| BWG, g/bird | 1461 | 1479 | 1493 | 1518 | 20.30 | 0.014 | 0.250 |
| Feed Intake, g/bird | 2823 | 2734 | 2784 | 2727 | 21.10 | 0.209 | 0.200 |
| FCR | 1.94 | 1.85 | 1.87 | 1.80 | 0.030 | 0.007 | 0.020 |
| **Overall (d 0-42)** | | | | | | | |
| BWG, g/bird | 2425 | 2447 | 2504 | 2543 | 22.70 | 0.003 | 0.184 |
| Feed Intake, g/bird | 4061 | 3969 | 4018 | 4010 | 30.60 | 0.561 | 0.141 |
| FCR | 1.68 | 1.62 | 1.61 | 1.58 | 0.020 | 0.005 | 0.144 |

[1]Standard error of means.

**Table 3:** Effect of dietary supplementation of protease on nutrient digestibility and ileal viscosity in broilers

| Treatment | Dietary protease level (PCU/kg) | | | | SEM[1] | P - value | |
|---|---|---|---|---|---|---|---|
| | 0 | 3,750 | 5,000 | 6,250 | | Linear | Quadratic |
| Dry matter | 70.88 | 71.23 | 71.63 | 71.84 | 0.37 | 0.567 | 0.891 |
| Crude protein | 75.53$^{c}$ | 76.87$^{b}$ | 78.31$^{a}$ | 78.51$^{a}$ | 0.42 | 0.010 | 0.821 |
| Gross energy | 73.78$^{b}$ | 74.82$^{ab}$ | 75.28$^{a}$ | 75.58$^{a}$ | 0.48 | 0.024 | 0.416 |
| Viscosity | 4.78 | 4.81 | 4.74 | 4.71 | 0.27 | 0.331 | 0.877 |

[1]Standard error of means; $^{a,b,c}$Means in the same row with different superscripts differ (P<0.05).

**Table 4:** Effect of dietary supplementation of protease on amino acid digestibility

| Treatment | Dietary protease level (PCU/kg) | | | | SEM | P - value | |
|---|---|---|---|---|---|---|---|
| | | | | | | Linear | Quadratic |
| Lysine | 82.48 | 85.37 | 86.96 | 87.52 | 0.79 | 0.002 | 0.472 |
| Methionine | 85.47 | 88.38 | 90.09 | 91.18 | 0.86 | 0.002 | 0.589 |
| Cysteine | 79.51 | 80.11 | 81.02 | 82.22 | 0.53 | 0.032 | 0.376 |
| Threonine | 78.89 | 80.79 | 82.62 | 83.43 | 0.74 | 0.004 | 0.832 |
| Arginine | 84.08 | 84.56 | 84.97 | 85.37 | 0.73 | 0.145 | 0.467 |
| Tryptophan | 78.71 | 80.3 | 81.19 | 81.52 | 0.54 | 0.028 | 0.438 |
| Isoleucine | 76.31 | 78.5 | 80.28 | 82.42 | 0.87 | 0.002 | 0.694 |
| Leucine | 75.47 | 76.59 | 78.29 | 79.19 | 0.78 | 0.002 | 0.568 |
| Phenylalanine | 80.28 | 80.96 | 81.37 | 81.27 | 0.42 | 0.376 | 0.421 |
| Tyrosine | 81.86 | 82.59 | 83.07 | 83.59 | 0.39 | 0.115 | 0.564 |
| Histidine | 82.06 | 83.87 | 86.40 | 86.46 | 0.75 | 0.001 | 0.828 |

[1]Standard error of means.

increased. Moreover, increasing protease supplementation resulted in a greater (linear, $P < 0.05$) ATTD of amino acids like lysine, methionine, cysteine, threonine, tryptophan, leucine, isoleucine, and histidine. Dietary increasing levels of protease supplementation did not ($P > 0.05$) affect ATTD of dry matter, arginine, phenylalanine, and tyrosine. Supplementation of acid protease to the diets of broilers had no significant effect ($P > .05$) on ileum digesta viscosity.

### Blood profiles

Effects of dietary increasing levels of protease on the blood profile of broiler chickens are shown in Table 5. There were no significant differences ($P > .05$) in blood urea nitrogen and creatinine concentrations among any dietary treatments.

### Excreta odor contents

The results of the effects of protease level on the odor emission in excreta of broiler chickens are presented in Table 6. These results showed that NH3, H2S, and R.SH levels were not affected ($p > .05$) by dietary supplementation of increasing levels of protease.

## DISCUSSION

There is a clear understanding that the presence of anti-nutritional factors like trypsin inhibitors and allergic proteins in soybean meal causes an allergic reaction that disrupts the small intestinal structure, microflora, and lower nutrient absorption, which results in suboptimal growth performance causing large economic losses to the livestock industry (Boudry *et al.*, 2004; Adeola and Cowieson., 2011; Kaczmarek *et al.*, 2014). Supplementation of exogenous protease may degrade the allergenic proteins and trypsin inhibitors in poultry gut and then attenuate the allergic reaction to improve the integrity of intestinal epithelium, but this requires further investigation. Exogenous protease has the potential to hydrolyze commonly used indigestible proteins in pig and poultry diets (Zuo *et al.*, 2015; Park *et al.*, 2020; Song *et al.*, 2022). Moreover, the inclusion of exogenous protease in animal diets was considered an effective strategy to improve feed efficiency and reduce feed costs in animal production (Adeola and Cowieson, 2011). It is well documented that supplementation of exogenous proteases in broiler chickens diet can improve growth performance (Tajudeen *et al.*, 2022; Mohammadigheisar *et al.*, 2021; Amiri *et al.*, 2021). Similar to these reports, the results of the present study showed that dietary supplementation of protease linearly improved the BW gain and decreased the FCR during d 0-21, d 23-42, and during the overall study period. Park et al, (2020) also reported an increase in BWG and FCR of broiler chickens supplemented with protease. Such results might be due to the beneficial effect of protease on the breakdown of indigestible protein and improvement of nutrients absorption (Angel *et al.*, 2011; Walk *et al.*,

**Table 5:** Effect of dietary supplementation of protease on noxious gas emission in excreta of broiler chickens.

| Treatment | Dietary protease level (PCU/kg) | | | | SEM[1] | P - value | |
|---|---|---|---|---|---|---|---|
| | 0 | 3,750 | 5,000 | 6,250 | | Linear | Quadratic |
| Ammonia | 36.24 | 34.32 | 34.21 | 34.78 | 1.16 | 0.116 | 0.129 |
| Hydrogen sulphide | 2.63 | 2.58 | 2.67 | 2.66 | 0.34 | 0.487 | 0.156 |
| Total mercaptan | 2.150 | 2.070 | 2.020 | 2.100 | 0.34 | 0.365 | 0.219 |

[1]Standard error of means.

**Table 6:** Effect of dietary supplementation of protease on blood profile of broiler chickens.

| Treatment | Dietary protease level (PCU/kg) | | | | SEM[1] | P - value | |
|---|---|---|---|---|---|---|---|
| | 0 | 3,750 | 5,000 | 6,250 | | Linear | Quadratic |
| Blood urea nitrogen | 3.67 | 3.53 | 3.47 | 3.57 | 0.38 | 0.376 | 0.189 |
| Creatinine | 0.23 | 0.25 | 0.24 | 0.27 | 0.16 | 0.341 | 0.231 |

[1]Standard error of means.

2019) and more effective in ameliorating the anti-nutritive trypsin inhibitors or lectins present in SBM. Previous reports showed protease supplementation improved animal performance by decreasing digesta viscosity, improving endogenous enzyme activity, and decreasing pancreas weight (Erdaw *et al.*, 2017). However, there have also been reports of a lack of positive effects of exogenous protease on BW gain (Ghazi *et al.*, 2002; Freitas *et al.*, 2011). The same results were also found by Kaczmarek *et al.* (2014), who reported that protease supplementation had no effect on growth performance when added to corn-soybean meal-based diets. Such variations in results might be due to variations in environmental conditions, types of raw material used in feed, sources or dosages of protease used or growth phases broilers.

Due to high ingredient prices and the presence of anti-nutritional factors in commonly used ingredients like soybean meal, the use of exogenous protease has become a common practice in poultry diets and has been well-documented to improve protein and amino acid digestibility and growth performance in previous studies. Supplementing exogenous protease helps monogastric animals that lack an adequate level of endogenous proteases to digest proteins in diets, which reduced the flow of indigestible proteins and other anti-nutritional factors entering the large intestine. These indigestible proteins serve as fermentation substrates for pathogenic bacteria such as *E. coli*, *Clostridium* sp., *Salmonella*, and *Campylobacter* in the hindgut (Jeong *et al.*, 2019; Hosseindoust *et al.*, 2022). These pathogenic bacteria can produce bacteriotoxins and harmful metabolites like biogenic amines, ammonia, and volatile sulfur compounds. Supplementing poultry diets with exogenous proteases may be a complementary strategy to improve the protein and amino digestibility in pigs and poultry (Ghazi *et al.*, 2002; Erdaw *et al.* 2017) and reduce adverse effects caused due to hindgut fermentation. In the present study dietary supplementation of protease improved ATTD of CP, GE, and amino acids like lysine, methionine, cysteine, threonine, tryptophan, leucine, isoleucine, and histidine. In agreement with the present study,significant linear improvements in crude protein, energy, and amino acids within broilers are supplied with increasing levels of protease in broiler diets (Park *et al.*, 2020). Similarly, exogenous protease supplemented to diets increased the apparent AA digestibility of arginine, threonine, isoleucine, aspartate, lysine, histidine, serine, and cysteine thereby improving BWG and FCR in broiler chickens (Angel *et al.*, 2011). Furthermore, a recent study in the authors lab reported dietary supplementation of protease increased the digestibility of CP and amino acids like lysine, tryptophan, cysteine, glutamine, and proline in the protease-supplemented diets deficient in protein (Tajudeen *et al.*, 2022). An increase in digestibility in the present study might be due to the breakdown of anti-nutritional factors like trypsin inhibitors and lectins and improving intestinal health.

Microbial fermentation of undigested proteins and amino acids in the hindgut produces $NH_3$ and contributes to the $NH_3$ output in the excreta (Hosseindoust *et al.*, 2022). Ammonia emissions can be reduced by feeding broilers with a low CP diet supplemented with crystalline amino acids (Kim *et al.*, 2021). Although lower emissions of ammonia may not necessarily correlate with the reduced odor emission rates (Jeong *et al.*, 2019), the simultaneous reduction of phenol, nitrogen and sulphur-containing odorants along with ammonia by feeding a low CP diet as observed in the study of Tajudeen *et al.* (2022). Exogenous proteases can also degrade anti-nutritional factors in feed, reduce nitrogen and phosphorus emissions from animal waste, and reduce environmental pollution from the source. The present study dietary supplementation of protease had no effects on blood profiles including BUN and creatinine concentrations and the excreta of noxious gas emission including ammonia, $H_2S$, and R.SH. In agreement with the present findings, Hosseindoust *et al.* (2022) reported no effects of dietary protease on the blood BUN, creatinine concentrations, and excreta odor emission in excreta of broilers including ammonia, hydrogen sulfide, and total mercaptans. Nevertheless, our current results partially contradict the study by Tajudeen *et al.* (2022).

## CONCLUSION

In conclusion, this study demonstrated that dietary supplementation of a protease product to broilers corn-SBM-based diets improved growth performance and apparent total tract digestibility of crude protein, amino acids, and gross energy. However, dietary supplementation of protease had no effects on blood BUN, creatinine concentrations, and excreta noxious gas emission.

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