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Impact of protease supplementation on growth performance and economic efficiency in broiler chicks

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Abstract

This study was conducted to determine the effect of protease supplementation on the growth performance and economic returns of broiler chicks fed standard and low-protein diets. A total of 396 one-day-old Ross 308 broiler chicks were randomly allotted to nine dietary treatments according to a 3 × 3 factorial arrangement that consisted of three basal rations, the standard broiler ration (SBR), a low-protein ration with 4% less protein (4% LPR), and a low-protein ratio with 8% less protein (8% LPR) each supplemented with 0, 125, and 250 mg/kg of exogenous protease enzyme. Chicks were raised over 35 days of phased feeding (pre-starter, starter, and finisher) and were evaluated for changes in body weight, weight gain, feed intake, feed conversion ratio (FCR), and economic efficiency (EEF). The results showed that protease supplementation had no effect on body weight in the early phases but enhanced ($p \leq 0.05$) in the later stages, especially in T6, which performed nearly similar to the control group (T1). Feed intake increased with enzyme supplementation in T8. The FCR was greatly enhanced by protease supplementation, as observed in T6. From the economic analysis, it transpired that treatment T6 yielded the highest EEF, the same as the control, that is, the herbal medication; however, it was statistically higher than that of the LPR group (T4). Thus, it may be inferred that supplementation of the diet with protease would help reduce crude protein levels without the growth or profit of broilers being unduly affected, hence aiding sustainable poultry nutrition. The use of protease is a means of reducing feed costs and nitrogen emissions while maintaining production efficiency.

Keywords: Protease, broiler, low-protein diet, feed conversion ratio, economic efficiency

I. Introduction

Poultry nutrition examines how to provide birds with the nutrients necessary for growth, development, and productive performance while maintaining health and preventing disease (Faraj, 2023; Khidhir, 2023). The poultry industry is a vital economic sector for supplying high-quality protein to humans, which demands the development of sustainable feeding strategies to meet rising global needs (Albashr et al., 2024; Barszcz et al., 2024). Since the mid-20th century, poultry nutrition research has evolved from traditional structural approaches to emphasize economic efficiency, environmental sustainability, and consumer preferences (Cao et al., 2024; Zampiga et al., 2021). Advances in precision breeding and targeted nutrition have introduced challenges such as metabolic disorders from strictly production-oriented strategies, lack of precise methods to assess specific nutrient requirements, and concerns over feed safety and environmental contamination (Ravindran, 2012). Contemporary poultry nutrition aims to balance growth, maintenance, and production requirements with economic considerations by employing precision feeding technologies and environmental monitoring systems (Cao et al., 2024), novel enzyme additives (particularly proteases), crystalline amino acids to enhance protein utilization efficiency (Qiu et al., 2023) and genome-editing tools and advanced sensor

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technologies to improve performance and productivity (Barszcz et al., 2024). Protein is the costliest component of poultry feed and is essential for tissue synthesis, hormone and enzyme production, and immune function (Hamma et al., 2024; Majid et al., 2020). To minimize nitrogen waste and environmental impact, feeds are formulated based on digestible amino acid requirements, often using phase-feeding strategies that tailor nutrient composition to each growth stage (Applegate & Angel, 2008). Low-protein feeding systems reduce total dietary protein by supplementing with crystalline amino acids and exogenous enzymes, resulting in lower feed costs and reduced nitrogen excretion and enhanced environmental sustainability (Jabbar et al., 2021; Mohammadigheisar & Kim, 2018). These systems also decrease proliferation of pathogenic microbes in the lower gastrointestinal tract (Beski et al., 2015). Common alternative protein sources include cottonseed meal, canola meal, corn gluten meal, and dried distillers grains with soluble (Beski et al., 2015). Proteases break down proteins into peptides and amino acids, improving protein digestion and increasing amino acid availability (Sumanasekara et al., 2020). Supplementation of protease in feed can enhance nutrient absorption, allowing for reduced crude protein levels without compromising performance, and decreasing nitrogen output in waste, thus mitigating the environmental impact (Oxenboll et al., 2011; Zheng et al., 2023). Research demonstrates improvements in weight gain, feed conversion ratio, and gut health, although efficacy varies with enzyme type, concentration, feed composition, and processing conditions (Duque-Ramírez et al., 2023). Incorporating protease into low-protein diets has been shown to reduce the production cost per kilogram of live weight while maintaining meat quality and overall performance (Oxenboll et al., 2011; Zampiga et al., 2021). It also reduces reliance on soybean meal, which is the primary protein source, and helps mitigate nitrogen pollution (Beski et al., 2015). The study objectives are to discuss strategies for enhancing crude protein digestion to achieve optimal poultry performance and to evaluate the effects of exogenous protease supplementation in standard and low-protein diets on growth metrics and economic outcomes. Therefore, the aims of this study are to assess the effect of exogenous protease enzyme supplementation to the standard and low-protein broiler diets on growth performance.

II. Materials and Methods

This study was applied at the poultry farm of the Animal Science Department/College of Agricultural Engineering Sciences/University of Sulaimani during the period from September 27th, 2024, to November 1st, 2024, to investigate the impact of protease supplementation on growth performance and economic efficiency in broiler chicks fed diets containing different levels of protein.

2.1 Birds, experimental design, and diet

A total of 396 unsexed one-day-old Ross 308 broiler chicks were raised on a poultry farm in separate floor cages, divided into nine treatment groups with four replicates each, consisting of 11 chicks per replicate. Three basal diets were formulated (table 1): a standard broiler ration (SBR), a 4% low-protein ration (4% LPR), and an 8% low-protein ration (8% LPR), each supplemented with protease enzyme at 0, 125, and 250 g per kg of diet, resulting in nine distinct groups. The protease used was JEFO, an extracellular alkaline enzyme derived from a non-GMO bacterium produced in Canada, added at 1 mg per kg of diet. The chicks were reared for 35 days under a deep litter system, following a phased feeding approach (table 2): pre-starter (1-10 days), starter (11-24 days), and finisher (25-35 days), with ad libitum access to feed and water throughout the study.

Table 1. Feeds and levels of enzyme offered for each treatment during 35-day periods of .experiment

Treatments		Protease (mg/kg)
T1 (control)	Standard broiler ration (SBR)	0
T2	Standard broiler ration (SBR)	125
T3	Standard broiler ration (SBR)	250
T4	4% Low protein ration (4% LPR)	0

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T5	4% Low protein ration (4% LPR)	125
T6	4% Low protein ration (4% LPR)	250
T7	8% Low protein ration (8% LPR)	0
T8	8% Low protein ration (8% LPR)	125
T9	8% Low protein ration (8% LPR)	250

Table 2. Ingredient Percentages and Nutrient Composition of the Diet Provided to the Broilers in the Specific Period

Ingredients	Diet								
	T1, T2, and T3			T4, T5, and T6			T7, T8, and T9		
	Pre-starter (1-10)	Starter (11-24)	Finisher (25-35)	Pre-starter (1-10)	Starter (11-24)	Finisher (25-35)	Pre-Starter (1-10)	Starter (11-24)	Finisher (25-35)
Soya bean meal	35.5	31	25	33	28.5	22.4	30.5	26.2	21
Yellow Corn	50	53	50	50	53	53	50	53	53.1
Wheat	8.4	10	18.5	11	12.6	18.5	13.6	15	20
Soy oil	1.8	2	2.7	1.7	1.9	2.3	1.6	1.8	2.1
Primex*	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Toxin binder	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Limestone	1.5	1.2	1	1.5	1.2	1	1.5	1.2	1
Total	100	100	100	100	100	100	100	100	100
	Chemical compositions								
Crude protein (%)	22	20	18	21.2	19.2	17.25	20.3	18.4	16.6
Metabolizable energy (kilocalories/kg feed consumed)	3000	3100	3150	3000	3100	3150	3000	3100	3150

* Dofamix Premix consist of :17% crud protein, 0.6% crud fiber, 0.37%crud fat, 70% crud ash, 14.4calcium, 3.65phosphorus, 7%lysin, 9%methionine, 1%thruonine. Trace elements: 2,400mg Fe (3b103 Iron (II)sulphate monohydrate); 80 mg I (3b202 Calcium iodate); 600 mg Cu (E4 Cupric (II)sulphate-pentahydrate); 2,800 mg Mn (3b502 Manganese (II)oxide); 2,800 mg Zn (3b605 Zinc sulphate monohydrate); 10 mg Se (E8 Sodium- Selenite).

2.2 Studied characteristics

The characteristicsstudied in this experiment included body weight, body weight gain, feed intake, feed conversion ,ratio and economic efficiency.

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Statistical analysis

The factorial experiment used a random design and analysed data with ANOVA and the linear model feature of the XLSTAT (2016, version 2.2) program to determine how different treatments affected various factors. Duncan's multiple comparison test was used to determine significant differences between means of traits, with a likelihood level of $p \leq 0.05$.

III. Results and discussion

Effect of protease enzyme on live body weight of broiler chicks

The results in Table 3 show the effects of protease supplementation on body weight (BW) in broiler chicks during a five-week period (P). The values of body weight from T1 to T9 at P1 and P2 were not significant ($P > 0.05$). At P1 from 1 to 7 days, the mean was higher in T1 (control), and the lower mean was in T8. At P2, from 8 to 14 days, the mean was higher in T1 (control), and the lower mean was in T9. At P3, P4, and P5, there were significant differences ($p \leq 0.05$) between treatments. The means at the P3 in age for 15-21 days indicated that maximum and significantly ($p \leq 0.05$) higher BW was observed in broilers assigned the T1 (control) diet (832.78 g), which was statistically similar to the T2, T3, T4, T5, T6, and T8 diets. It was minimal and significantly lower ($p \leq 0.05$) in T7 (719.55 g). The means at the P4 (22-28) days indicated that maximum and significantly ($p \leq 0.05$) higher BW was observed in broilers assigned the T1 (control) diet (1314.69 g), which was statistically similar to the T5, T6, and T8 diets. It was minimal and significantly lower ($p \leq 0.05$) in T4 (1144.28 g). At the P5 from 29 to 35 days, that maximum and significantly ($p \leq 0.05$) higher BW was observed in broilers assigned the T1 (control) diet (2419.50 g), which was statistically similar to the T6 diets. It was minimal and significantly lower ($p \leq 0.05$) in T7 (2167.22 g).
These results can be supported by the findings of Wealleans et al. (2024), who concluded that the birds fed diets that were reduced by 3.5% in crude protein (CP) and amino acids (AA) and supplemented with different protease enzymes showed no difference ($p > 0.05$) in body weight compared to birds assigned to the control diet. Similar results have been published by Amer et al. (2021) In their study, who discovered that different feeding regimens and protease did not significantly change the body weight of the birds; moreover, the birds' body weight was not influenced by low-protein soybean meal diets and adding protease (250 mg/kg). In addition,, al. (2020) found in his study that broilers had a negative impact on final body weight (BW) when supplemented with protease at 200 mg/kg in a diet with reduced crude protein and digestible amino acid contents at 6% compared to those fed the control diet. In addition, Rada et al. (2013) observed that the birds fed a reduced CP-level diet of 4% supplemented with a nanocomponent serine protease had a significantly lower body weight than those assigned to a standard CP diet, especially on day 17 of the rearing period, and had no significant differences with them in their final body weight. On the other hand, several studies have shown that protease can positively affect body weight in birds, including Tajudeen et al. (2022), who found that broiler chickens, which were fed a three-level protein basal diet with a 0.75% decrease in protein levels and supplemented with an alkaline exogenous protease at the recommended dose of 250 g/MT, showed improved body weight compared to those that did not receive the supplementation. Ndazigaruye et al. (2019) reported that the live body weight of the chickens significantly increased at 21 days from the rearing period of 35 days by adding In addition (1 g/kg of diet) to the low-protein diet and amino acid. Saleh et al. (2020) showed that the final body weights of broiler chickens significantly improved ($P \leq 0.05$) due to the supplementation (200 and 300 mg/kg of diet) of serine protease from Bacillus licheniformis to their diets compared to those that were not supplemented.

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Table 3: Impact of protease supplementation on body weight (g) in different growth periods (days) in broiler chicks (Mean ± S.E.).

Treatments	Periods/days				
	1-7	8-14	15-21	22-28	29-35
T1 (control)	182.59 ± 5.87^{a}	452.53 ± 2.37^{a}	832.78 ± 39.45^{a}	1314.69 ± 24.77^{a}	2419.50 ± 48.55^{a}
T2	174.89 ± 1.70^{a}	423.16 ± 4.05^{a}	759.45 ± 29.15^{ab}	1206.40 ± 21.14^{b}	2254.84±19.64b^{c}
T3	178.22 ± 3.62^{a}	423.68 ± 5.31^{a}	748.77 ± 17.02^{ab}	1210.50 ± 26.77^{b}	2199.76 ± 71.94^{c}
T4	168.33 ± 3.61^{a}	418.82 ± 4.10^{a}	734.46 ± 17.72^{ab}	1144.28 ± 38.34^{c}	2181.23 ± 41.28^{c}
T5	170.66 ± 5.84^{a}	443.67 ± 8.29^{a}	821.40 ± 34.17^{ab}	1308.86 ± 43.72^{a}	2304.80 ± 77.54^{b}
T6	171.54 ± 2.80^{a}	419.79 ± 4.04^{a}	797.39 ± 22.73^{ab}	1267.87± 53.07^{ab}	2402.43 ± 66.23^{a}
T7	167.18 ± 4.23^{a}	398.00 ± 9.10^{a}	719.55 ± 20.18^{b}	1175.68 ± 24.01^{c}	2167.22 ± 44.77^{c}
T8	166.41 ± 3.16^{a}	430.55 ± 8.21^{a}	813.46 ± 19.82^{ab}	1296.32 ± 6.79^{a}	2226.26±63.29b^{c}
T9	172.27 ± 2.73^{a}	397.09 ± 9.06^{a}	725.36 ± 17.52^{b}	1154.59 ± 20.69^{c}	2184.35 ± 43.53^{c}

a, b, c: means within columns followed by different letters are differ significantly at ($p \leq 0.05$).
* The treatments were, T_1 = (control) standard broiler ration (SBR) without protease, $T_2 = T_1$ + 125 mg /kg protease, $T_3 = T_1$ + 250 mg /kg protease, T_4 = 4% low protein ration (LPR) without protease, $T_5 = T_4$ + 125 mg /kg protease, $T_6 = T_4$ + 250 mg /kg protease, T_7 = 8% low protein ration (LPR) without protease, $T_8 = T_7$ + 125 mg /kg protease, $T_9 = T_7$ + 250 mg/kg protease.

Effect of protease enzyme on body weight gain of broiler chicks

The results of weight gains influenced by treatments and periods (P) were summarized in Table 4. No significant differences ($p \geq 0.05$) were observed between treatments (T1 to T9) in the entire age period (P1, P2, P3, and P4) except for P5 and the overall. At the P1 from (1-7) days, T1 was the maximum mean, while T8 was the minimum mean. At the P2 from 8 to 14 days, the higher mean represents T5 with T9, which has a lower mean. The highest mean was observed in T8, whereas the lowest mean was observed in T4. At the P3 from 15 to 21 days. At P4 from days 22 to 28, T5 was the maximum mean, while T4 was the minimum mean. At the P5 from 29 to 35 days, the body weight gain (BWG) was enhanced with increased enzyme supplementation; there were significant differences ($p \leq 0.05$) between the means, the highest weight gain was obtained by birds in T6 (1134.56 g), which was statistically similar to the T1, T2, and T4 diets, and the lowest weight gain was obtained by birds in T8 (929.94 g). For the overall body weight gain, there were significant decreases ($p \leq 0.05$). The best mean was 2371 g in T1 (control), which was statistically similar to the T2, T5, T6, and T8 diets. The overall body weight gain was minimal and significantly lower ($p \leq 0.05$) in the group assigned to T7, which had a weight gain of 2118.86 g.

Weight gain is shown. It is obvious that the protease enzymes have an important role in enhancing growth performance parameters. The present study is also in agreement with the study of Sumanasekara et al. (2020) reported that enzymes significantly improved weight gain. Therefore, the addition of the protease to the low-protein diets positively affected body weight gain and promoted a performance similar to that of the group fed standard protein (Law et al., 2017; Park et al., 2020). They also observed improved weight gains in broilers fed with protease supplementation.

Cardinal et al. (2019), reported in his study a significant effect on the overall weight gain of broilers with protease supplementation on their reduced crude protein (CP) diets. The low-CP diet resulted in poorer weight gain during the starter phase, which is a period of increased protein requirements due to muscle deposition. The treatments of periods

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showed no significant differences; they had poorer BWG than the standard diet. Previous studies noticed that growth performance was markedly affected by changes in dietary protein levels, accompanied by varying gastrointestinal digestive enzymes (Liu et al., 2017; Yuan et al., 2017). Enzyme action may not be very good in these periods. Another hypothesis is that the results may be associated with adaptation of the animal. Studies have shown that broilers reduce the endogenous secretion of enzymes when exogenous addition occurs. According to Yuan et al. (2017), broilers fed a diet supplemented with 80 or 160 mg/kg of diet of protease showed reduced cholecystokinin and pancreatic trypsin activity.

Table 4: Impact of protease supplementation on body weight gain (g) in different growth periods (days) in broiler chicks (mean ± S.E.).

Treatments	Periods/days					overall
	1-7	8-14	15-21	22-28	29-35	
T1(control)	134.10 ± 6.25^{a}	269.93± 1.32^{a}	380.25 ± 15.80^{a}	481.90 ± 16.03^{a}	1104.81± 40.58^{ab}	2371.00 ± 48.96^{a}
T2	126.57 ± 1.40^{a}	248.28 ± 8.63^{a}	336.29 ± 14.05^{a}	446.95 ± 17.41^{a}	1048.44 ± 8.24^{abc}	2206.52 ± 18.69^{ab}
T3	129.55 ± 3.79^{a}	245.45 ± 7.76^{a}	325.09 ± 13.48^{a}	461.73 ± 12.77^{a}	989.26 ± 37.46^{cd}	2151.08 ± 71.15^{b}
T4	119.70 ± 3.81^{a}	250.48 ± 7.51^{a}	315.63 ± 13.81^{a}	409.83 ± 15.52^{a}	1036.95± 22.91^{abc}	2132.60 ± 41.26^{b}
T5	123.57 ± 6.10^{a}	273.01± 2.18^{a}	377.73 ± 13.77^{a}	487.45 ± 14.61^{a}	995.95 ± 26.39^{cd}	2257.71 ± 77.40^{ab}
T6	122.50 ± 3.01^{a}	248.25 ± 9.81^{a}	377.59 ± 12.47^{a}	470.48 ± 18.15^{a}	1134.56 ± 27.18^{a}	2353.39 ± 66.41^{a}
T7	118.82 ± 4.04^{a}	230.82 ± 9.87^{a}	321.55 ± 11.22^{a}	456.13 ± 12.93^{a}	991.54 ± 40.71^{cd}	2118.86 ± 44.46^{b}
T8	118.37 ± 3.77^{a}	264.13 ± 5.98^{a}	382.91 ± 8.16^{a}	482.86 ± 21.01^{a}	929.94 ± 31.15^{d}	2178.21 ± 57.00^{ab}
T9	124.59 ± 3.19^{a}	224.82±10.73^{a}	328.27 ± 15.48^{a}	429.23 ± 19.05^{a}	1029.76± 30.54^{bcd}	2136.67 ± 43.77^{b}

a, b, c, d: means within columns followed by different letters are significantly different at ($p \leq 0.05$).* The treatments were, T_1 = (control) standard broiler ration (SBR) without protease, $T_2 = T_1$ + 125 mg /kg protease, $T_3 = T_1$ + 250 mg /kg protease, T_4 = 4% low protein ration (LPR) without protease, $T_5 = T_4$ + 125 mg /kg protease, $T_6 = T_4$ + 250 mg /kg protease, T_7 = 8% low protein ration (LPR) without protease, $T_8 = T_7$ + 125 mg /kg protease, $T_9 = T_7$ + 250 mg/kg protease.

Effect of protease enzyme on feed intake of broiler chicks

Table (5) shows the influenced interaction of the treatments and periods on feed intake in broiler chicks during a five-week period. Throughout the entirety of the age periods (P1, P2, P3, and P4), no statistically significant differences ($P > 0.05$) were found among their treatments except for P5 in age from 29 to 35 days and overall. At the P1 from 1 to 7 days, the best mean of feed intake was up to T2, whereas the lowest mean was in T9. At the P2 from 8 to 14 days, T2

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was the maximum mean, while T3 was the minimum mean. The higher feed intake mean was in T2, whereas the lower mean was in T8. At P3 from days 15 to 21. At the P4, from 22 to 28 days, T1 had the best mean, and T2 had the worst mean. The feed intake of birds was significantly increased ($p \leq 0.05$) with protease enzyme supplementation at P5 from days 29 to 35, showing that T8 had a maximum mean of feed intake (1587.47 g), which was statistically similar to the T1, T2, T4, and T6 diets mean, and the minimum mean of feed intake by birds in T3 was (1372.53 g), On the other hand, at the overall feed intake, there were significant differences ($p \leq 0.05$); T1 (control) obtained the highest amount of feed intake during the experiment (3422.69 g), which was statistically similar to T2 to T9 except for T3, while T3 obtained the lowest amount (3130.30 g).
Improved feed consumption for T8 at P5 (29-35) days may be due to the quantity of the levels of enzyme in the diet. The performance of birds on the lower CP diets was enhanced by the supplementation with protease; the results of adding the protease enzyme were significantly ameliorative for the feed intake parameter. A similar response was observed by Jabbar et al. (2021), who reported that feed intake was higher ($p \leq 0.05$) in broilers fed CP-17% than in the group of birds fed CP-19% supplemented with s protease.
These findings are in agreement with the results obtained by Akter et al. (2022) showed that feed intake increase with enzyme supplementation. In contrast, Ajayi (2015) demonstrated in a study that feed intake for all birds decreased significantly with the inclusion of protease in their diets. In addition, Tajudeen et al. (2022) found the broiler chickens fed the supplementation of exogenous protease in a low-CP diet had no significant difference in overall feed intake in 1-35 days. Moreover, the results of overall feed intake are in agreement with the findings of Sarica et al. (2020) who noted that broilers fed the positive control (PC) diets produced the highest final feed intake compared to the other diets, which had 6% less crude protein and were supplemented with protease at 200 mg/kg with reduced crude protein diets.

Table 5: Impact of protease supplementation on feed intake (g) in different growth periods (days) in broiler chicks (mean ± S.E.)

Treatments	Periods/days					overall
	1-7	8-14	15-21	22-28	29-35	
T1(control)	150.87 ± 4.27^{a}	357.55 ± 9.61^{a}	548.61 ± 14.95^{a}	791.77 ± 19.03^{a}	1573.88 ± 0.33^{a}	3422.69 ± 47.32^{a}
T2	153.33 ± 6.41^{a}	359.39 ± 16.63^{a}	550.23 ± 22.80^{a}	744.17 ± 4.57^{a}	1565.41 ± 67.37^{a}	3372.53 ± 51.27^{ab}
T3	145.04 ± 0.35^{a}	340.64 ± 0.27^{a}	522.18 ± 0.20^{a}	749.91 ± 0.09^{a}	1372.53 ± 33.87^{b}	3130.30 ± 34.06^{b}
T4	148.05 ± 2.72^{a}	349.34 ± 8.55^{a}	535.61 ± 13.13^{a}	768.50 ± 18.50^{a}	1520.75 ± 42.03^{a}	3322.26 ± 75.34^{ab}
T5	146.60 ± 2.80^{a}	348.94 ± 8.22^{a}	535.43 ± 13.19^{a}	768.16 ± 18.95^{a}	1429.41 ± 34.56^{b}	3228.55 ± 77.52^{ab}
T6	148.94 ± 3.55^{a}	348.69 ± 7.90^{a}	535.75 ± 13.08^{a}	787.40 ± 21.65^{a}	1550.94 ± 76.07^{a}	3371.73 ± 104.15^{ab}

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T7	144.41 ± 0.69ᵃ	340.68 ± 0.17ᵃ	522.27 ± 0.45ᵃ	767.82 ± 19.06ᵃ	1415.86 ± 39.05ᵇ	3191.05 ± 52.78ᵃᵇ
T8	145.05 ± 0.24ᵃ	340.82 ± 0.09ᵃ	522.00 ± 0.61ᵃ	748.82 ± 0.72ᵃ	1587.47 ± 64.62ᵃ	3344.15 ± 63.75ᵃᵇ
T9	141.41 ± 3.15ᵃ	340.91 ± 4.50ᵃ	522.46 ± 0.27ᵃ	749.80 ± 0.13ᵃ	1428.15 ± 35.13ᵇ	3182.72 ± 35.74ᵃᵇ

a, b: means within columns followed by different letters are differ significantly at ($p \leq 0.05$). * The treatments were, T_1 = (control) standard broiler ration (SBR) without protease, $T_2 = T_1$ + 125 mg /kg protease, $T_3 = T_1$ + 250 mg /kg protease, T_4 = 4% low protein ration (LPR) without protease, $T_5 = T_4$ + 125 mg /kg protease, $T_6 = T_4$ + 250 mg /kg protease, T_7 = 8% low protein ration (LPR) without- protease, $T_8 = T_7$ + 125 mg /kg protease, $T_9 = T_7$ + 250 mg/kg protease.

Effect of protease enzyme on feed conversion ratio of broiler chicks

Data of the feed conversion ratio (FCR) of broiler chicks is noticed in Table 6. The values of treatments at P1 and P2 were not significant (P > 0.05). At P1 from days 1 to 7, the mean was higher in T4, and the best mean was in T3. At P2 (8 -14) days, the higher mean was in T9, and the lower mean was in T5 and T8 because they were similar. There was a significant effect ($p \leq 0.05$) for the interaction between treatments and periods on the feed conversion ratio that was seen during the periods (P3, P4, P5) of the experiment due to the raised effect level of enzyme supplementation and raising of the periods of age. While the results of treatments at P3 from days 15 to 21 indicate significant differences ($P \leq 0.05$), the highest mean was observed in broilers assigned the T4 diet (1.71), and the best mean was T8 (1.37), which was statistically similar to the T5 and T6 diets. Next, the maximum mean at P4 from 22 to 28 days was T4 (1.93), and the minimum mean was in T8 (1.56), which represented the best mean and was statistically similar to T9. For P5 from (29-35) days, the worst mean was 1.81 at T8, whereas the best mean was 1.38 at T6, which was statistically similar to the T1 to T9 diets except for T2 and T8. The overall FCR of broilers did not differ significantly (P > 0.05) between experimental groups; T4 had the higher mean for FCR, and the lower mean was in T5.

Generally, at these values, the highest mean value shows the worst efficiency, while the lowest value shows the best efficiency. Feed conversion ratio (FCR) is an important tool to measure the feed efficiency of birds. This indicates the magnitude of profitability in broiler production and reflects the feeding management practices followed in broiler farms. In addition,, the results of this study showed that birds supplemented with protease enzyme had lower FCR than control birds. The decrease may be attributed to improveddigestion and agreement with Saleh et al. (2020) reported that FCR was significantly improved due to dietary 200 and 300 mg/kg protease supplementation. A study by Duque-Ramírez et al. (2023) found that the birds that consumed the reduced protein diets with 100 ppm of protease had a better ($P \leq 0.05$) FCR than the control diets during the 12 to 21 experiment days. Additionally, Fru-Nji et al. (2011); Kamel et al. (2015) reported that broilers fed with protease enzyme supplementation showed significantly ($P \leq 0.05$) improved FCR. The results of Rada et al. (2014) showed that the standard crude protein (CP) and lower crude protein (LP) level diets with or without serine protease supplementation did not have (P > 0.05) a significant effect on the final FCR of birds among dietary treatments during experimental periods in 1-35 days. However, Tripathi et al. (2020) conducted an experiment on supplementation of different levels of protease enzyme with reduced crude protein (CP) levels of the diet, and they reported that broilers fed with protease enzyme supplementation did not significantly improve FCR compared with the control.

Table 6: Impact of protease supplementation on feed conversion ratio (g) in different growth periods (days) in broiler chicks (mean ± S.E.)

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Treatments	periods/days					overall FCR
	1-7	8-14	15-21	22-28	29-35	
T1(control)	1.14 ± 0.05^{a}	1.33 ± 0.03^{a}	1.46 ± 0.04^{a}	1.66 ± 0.02^{ab}	1.44 ± 0.06^{c}	1.45 ± 0.04^{a}
T2	1.21 ± 0.03^{a}	1.45 ± 0.05^{a}	1.65 ± 0.06^{a}	1.67 ± 0.06^{ab}	1.49 ± 0.08^{b}	1.53 ± 0.06^{a}
T3	1.12 ± 0.04^{a}	1.39 ± 0.04^{a}	1.61 ± 0.07^{a}	1.63 ± 0.05^{ab}	1.39 ± 0.03^{c}	1.46 ± 0.03^{a}
T4	1.24 ± 0.05^{a}	1.40 ± 0.07^{a}	1.71 ± 0.06^{a}	1.93 ± 0.08^{a}	1.47 ± 0.02^{c}	1.56 ± 0.03^{a}
T5	1.20 ± 0.06^{a}	1.29 ± 0.06^{a}	1.44 ± 0.02^{ab}	1.58 ± 0.05^{ab}	1.45 ± 0.07^{c}	1.43 ± 0.01^{a}
T6	1.22 ± 0.02^{a}	1.41 ± 0.06^{a}	1.45 ± 0.05^{ab}	1.75 ± 0.08^{b}	1.38 ± 0.07^{c}	1.44 ± 0.05^{a}
T7	1.22 ± 0.04^{a}	1.48 ± 0.07^{a}	1.63 ± 0.06^{a}	1.70 ± 0.02^{ab}	1.43 ± 0.06^{c}	1.51 ± 0.04^{a}
T8	1.23 ± 0.04^{a}	1.29 ± 0.03^{a}	1.37 ± 0.07^{b}	1.56 ± 0.07^{c}	1.81 ± 0.08^{a}	1.55 ± 0.05^{a}
T9	1.14 ± 0.05^{a}	1.53 ± 0.07^{a}	1.60 ± 0.08^{a}	1.78± 0.09^{abc}	1.40 ± 0.06^{c}	1.49 ± 0.02^{a}

a, b, c: means within columns followed by different letters are differ significantly at ($p \leq 0.05$).
[*] The treatments were, $T_{1=}$ (control) standard broiler ration (SBR) without protease, $T_{2=}$ T_1 + 125 mg /kg protease, $T_{3=}$ T_1 + 250 mg /kg protease, $T_{4=}$ 4% low protein ration (LPR) without protease, $T_{5=}$ T_4 + 125 mg /kg protease, $T_{6=}$ T_4 + 250 mg /kg protease, $T_{7=}$ 8% low protein ration (LPR) without- protease, $T_{8=}$ T_7+ 125 mg /kg protease, $T_{9=}$ T_7 + 250 mg/kg protease.

Effect of protease enzyme on economic efficiency of broiler chicks

Table 7 shows the impact of protease in broiler diet on economic efficiency (EEF). There were significant differences ($p \leq 0.05$) between the treatments. The different amounts of protease enzyme in a low-protein broiler diet and those assigned to the control diet had a similar significant effect compared to broilers fed the low-protein diet without protease. The treatment means indicated that maximum and significantly ($p \leq 0.05$) higher EEF was observed in broilers assigned the T6 diet (1.11), which was statistically and numerically similar to the T1 (control) diet and was statistically similar to those of the T2 to T9 except the T4 diets. It was minimal and significantly ($p \leq 0.05$) lower in T4 (0.85).

These results agree with those of Chandrasekar et al. (2017), who found that coated compound (CC) protease significantly improves nutritional and economic efficiency in animal protein production. Noted: adding CC protease to the low-protein, amino acid diet elicited a closer response to the control diet. It allows partial substitution of digestible amino acids and crude protein. Abd-Elsamee et al. (2025) demonstrated that adding protease enzyme with or without amino acids to low-protein diets (2 or 4% CP) in proportions that met the birds' nutritional demands improved their relative economic efficiency as compared to the control group. Using protease enzymes or a combination of amino acids can lower broiler feed crude protein by up to 4%. In addition, it improves the financial effectiveness. Kaimkhani et al. (2025) reported that while supplementing various doses of protease enzyme to a standard diet, the birds that received a supplementation of 500 g/ton protease had improved economic performance compared to birds that received lower and higher doses. This dose significantly improved economic performance. In contrast, Pasquali et al. (2017) observed that the use of enzyme complexes composed of protease and cellulase with the joint application of nutritional matrices does not favor the economic viability of broiler chickens. to determine the economic efficiency of the dietary supplementation of protease (250 mg/kg) to different feeding regimens was investigated by Amer et al. (2021) in his study, he found no positive effect of different feeding regimens, protease supplementation, or their interaction on economic efficiency parameters. Zahid Kamran et al. (2011) found that maximum economic returns were observed in groups fed the low CP

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diets, and it clearly indicated that this approach is useful for economical broiler production. Fru-Nji et al. (2011) observed that a low protein supplemented with protease was significantly better than a low-protein diet without protease supplementation. Results showed that enzyme addition improved protein digestibility, which led to significant improvements in broiler performance. It also helped address some of the current challenges faced by the livestock industry, such as feed costs and demands for sustainable farming. Dosković et al. (2013) reported that enzyme supplementation in broiler diets had no adverse effect on cost of production.

Table 7: Impact of protease supplementation on economic efficiency of broiler chicks (mean ± S.E).)

Treatments	EEF
T1(control)	1.11 ± 0.05^{a}
T2	0.93 ± 0.04^{ab}
T3	0.92 ± 0.03^{ab}
T4	0.85 ± 0.04^{b}
T5	1.02 ± 0.02^{ab}
T6	1.11 ± 0.06^{a}
T7	0.86 ± 0.02^{ab}
T8	0.90 ± 0.07^{ab}
T9	0.89 ± 0.05^{ab}

a, b: means within column followed by different letters are significantly different at ($p \leq 0.05$).
[*] The treatments were, T_{1} = (control) standard broiler ration (SBR) without protease, T_{2} = T_{1} + 125 mg /kg protease, T_{3} = T_{1} + 250 mg /kg protease, T_{4} = 4% low protein ration (LPR) without protease, T_{5} = T_{4} + 125 mg /kg protease, T_{6} = T_{4} + 250 mg /kg protease, T_{7} = 8% low protein ration (LPR) without- protease, T_{8} = T_{7}+ 125 mg /kg protease, T_{9} = T_{7} + 250 mg/kg protease.

IV. Conclusion:

Growth performance and economic efficiency were significantly enhanced with protease supplementation, especially at 250 mg/kg in the 4% low-protein diet. Body weight gain and feed conversion ratio improved to match those of birds fed standard protein diets. The feed intake in some of the groups receiving the supplement was higher than that in the non-supplemented groups, indicating improvements in nutrient utilization. Economic efficiency in the T6 group was the highest, similar to that of the control group but better than that of the non-supplemented diets. The results showed that it is possible to reduce crude protein with protease supplementation without a decline in performance. Such supplementation would be a costly and sustainable solution for broiler production in the modern era.

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