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Effects of supplemental exogenous protease enzyme on growth performance of broiler chickens in reduced protein-energy diet

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Abstract - Feed costs constitute a major portion of poultry production expenses, prompting interest in feed additives that enhance nutrient utilization. Proteases, as exogenous enzymes, have shown potential to improve protein digestibility and support the immature digestive systems of chick's birds. This study aimed to evaluate the effects of protease enzyme supplementation in broiler chicken diets, particularly in the context of Escherichia coli (E. coli) K88 challenge.

This study investigated the effects of supplemental exogenous protease enzyme on growth performance and gut microbiota of broiler chickens under a reduced protein-energy diet. A total of 200 broiler chicks one day age was allocated into four groups (T1–T4), each with 50 birds, and reared for 35 days. The experimental groups were fed different diets, T1 control group, T2 reduce protein – energy diets, T3 control with add 250g/ton of protease enzyme and T4 add 250 g/ton of protease enzyme supplementation to reduce protein – energy diets. Growth parameters including body weight (BW), weight gain (WG), feed intake (FI), and feed conversion ratio (FCR) were monitored, along with microbial analysis of drop E. coli and Lactobacillus counts.

The results indicated that protease supplementation of (T3 and T4) significantly improved final body weight (2169.02 g and 2186.81 g, respectively) and feed conversion ratio (1.45 and 1.46) compared to control (T1: 1895.7 g, FCR: 1.69). Feed intake was moderately increased in supplemented groups, reflecting improved nutrient utilization. Microbiological analysis showed a significant reduction in E. coli count and a highly significant increase in Lactobacillus populations in T4 compared to other groups ($p < 0.001$), indicating enhanced gut health.

In conclusion, dietary supplementation with exogenous protease enzyme can effectively improve growth performance and gut microbial balance in broilers fed low protein-energy diets, suggesting its beneficial role in sustainable poultry nutrition.

Key word: Protease enzyme, Growth performance, Reduced protein diet, Exogenous enzymes

INTRODUCTION

THE chicken's industry, particularly in recent years, the escalation of feed ingredient prices, especially those contributing energy, which constitutes around 70% of diet costs, has prompted the investigation of techniques to mitigate feed production expenses. Since they are the most expensive components of the diet, one tactic is to reduce the amounts of metabolizable energy (ME) and crude protein (CP). As a result, several experiments were conducted to improve the development in performance of broiler chicken and to reduce costs by reducing the proportion of specific energy components (1). Feed costs are estimated to contribute between 70% and 80% of the total cost of raising poultry. As a result, there is great interest in feed additives that can improve nutrient availability in feeds. The use of supplemental enzymes added to feed represent one means of improving nutrient availability. Exogenous enzymes are included in rations to improve production efficiency and growth performance and have been estimated to save the global feed market 3 to 5 billion USD a year. Currently, the feed enzyme business has over 1 billion USD in sales annually, with most of this accounted for by phytases, proteases, and carbohydrases (2).

The digestibility of CP and amino acids (AA) in poultry diets can be impaired by the presence of antinutritional factors (ANF) in soybean meal (3). Protein digestibility values of food ingredients reported in the literature suggest that there are significant amounts of undigested protein that pass through the gastrointestinal tract (GIT) (4). In view of the continuous increase in the cost of protein ingredients, this part of undigested protein can represent significant economic losses. In addition, fractions of undigested proteins for animal feed can

reach the hindgut, serving as substrates for the proliferation of pathogens, such as Clostridium perfringens (5). Exogenous protease supplementation has been shown to improve the digestibility of CP and AA in poultry diets, helping to reduce feed costs, environmental impact, and alterations to the chicken gut ecosystems. The supplementation of chicken diets with combinations of xylanases and proteases has been extensively investigated (6,7). Proteases have the potential to improve growth performance in poultry because the chicken's pancreatic protease activity is low at hatching and increases up to 21 days of age. Due to the immaturity of chicks' digestive (2).

Proteolytic enzymes (proteases) facilitate the breakdown of proteins into amino acids through hydrolytic reactions. These enzymes are classified based on the pH range in which they exhibit peak activity. Acidic proteases are most effective in a pH range of 2.5 to 3.5 and function primarily in the proventriculus and gizzard. Neutral proteases perform optimally at pH levels between 6.5 and 7, being active in the duodenum and jejunum. In contrast, alkaline proteases show maximum activity within a pH range of 7.2 to 7.8 and are primarily active in the ileum (7).

It has been demonstrated that exogenous enzymes can change gut morphology, pancreatic enzyme production and secretion, the microbial populations along the GIT, and the short chain fatty acid profile in the digesta. Previous work has shown that the chicken gastrointestinal microbiome plays an important role in intestinal development and can have a significant influence on bird's health and growth performance. Exogenous proteases could change the extent that feed substrates are degraded and modified in the chicken digestive system, and potentially change the nutrients used by the chicken microbiota and the chicken microbial population itself (8). Colibacillosis caused by enterotoxigenic E. coli results in economic losses in the poultry industry. Antibiotics are usually used to control colibacillosis, however, E. coli has varying degrees of resistance to different antibiotics (8) Incorporating protease into poultry feed has gained attention due to its potential to enhance the digestibility of proteins and amino acids, while also mitigating anti-nutritional compounds like trypsin inhibitors, β-conglycinin, and glycinin. This approach can be especially beneficial for young chicks, whose endogenous protease activity may not yet be fully developed (9). Research into protease has been mostly focused on supplementation with other enzymes, while very little research has been done on protease supplementation alone. found that protease supplementation improved the gain to feed ratio. The addition of protease improved growth performance and apparent metabolizable energy in broilers. found that when protease was added to lower CP diets in broilers, growth performance, intestinal health, and carcass traits improved (2). The current study will be aimed to evaluate the effects of protease enzyme that will be supplemented to broiler chicken's diet Productive traits: (body weight, weight gain, feed intake, feed conversation ratio) Bacterial count of drop (E. coli form and lactobacillus).

Materials and Methods

This study was conducted to evaluate the effect of protease enzyme on reduced protein - energy diet of broilers. Two hundred broiler chickens 1-day-old 308 Ross divide into 4 equal groups (10):

T1: control group, T2: reduced protein- energy diet (100 Kcal /kg and 1% CP) (11), T3: control group with adding 250g/ton protease enzymes (12), T4: adding 250g/ton protease enzyme in reduced protein- energy diet (100 Kcal and 1% CP). The feed and water were provided ad libitum (12,11), The environment and lightning were check daily (13), the performance traits were recorded weekly

According to the ethical code number UOK.VET. HE.2025.121 from the Scientific Council of the Department of Public health, College of Veterinary Medicine, University of Karbala, Iraq.

RESULT & DISCUSSION

Table 1: This table shows the final body weight of broilers at the end of the 35-day trial. The enzyme-supplemented groups, especially T3 and T4, recorded significantly higher body weights compared to the control group (T1), reflecting improved growth performance.Impact of protease in reduced energy diet on the BW (g) of broiler chickens (Mean ± SD)

Groups. Weeks	T.1	T.2	T.3	T.4
Day1.	**42.4 ± 2.99 a**	**42.1 ± 2.76 a**	**41.4 ±2.74 a**	**42.06 ±2.79a**
Week 1	**172.1 ±3.00 b**	**158.3 ±3.69 c**	**173.7 ±3.12 a**	**173.1 ±3.40 ab**
Week2	**425.5 ±6.67 c**	**405.4 ±4.03 d**	**440.4 ±4.29 b**	**447.3 ±2.69a**
Week3	**953.7 ±4.91 c**	**844.06 ±3.43 d**	**990.3 ±3.71 b**	**1019.6 ±30.78 a**
Week 4	**1404.2±8.88 b**	**1208.7±7.00 c**	**1497.3±8.80 a**	**1504 ±8.96 a**
Week 5	**1938.2 ±16.67 b**	**1768.4±16.47 c**	**2169.02±32.55 a**	**2186.8 ±41.92 a**

Table 2: This table illustrates the effect of protease supplementation on weekly and cumulative weight gain in broilers during the experimental period. Groups T3 and T4 demonstrated clear superiority in weight gain (WG), highlighting the enzyme's role in offsetting the effects of reduced dietary protein and energy. Effect of protease in a low protein-energy diet on WG (g) of broiler chickens

Groups. Weeks.	T.1	T.2	T.3	T.4
Week1.	129.6±2.50 b	116.1±1.11 c	132.3±1.53 a	131.08±3.67 a
Week 2	253.4±6.26 c	247.1±4.45 d	266.6±3.71 b	274.2±2.70 a
Week 3	450.5±8.94 b	364.6±10.09 c	506.9±11.55 a	484.4±34.24 a
Week 4	528.2±7.46 b	438.6±5.06 c	549.9±6.10 a	572.2±30.79 a

Week 5	622.9±25.49 b	566.6 ±23.39 b	671.7±25.73 a	682.8±45.45 a
WG	1895.7 ±25.10 b	1733.2±29.40 b	2127.6 ±33.19 a	2144.7±42.49 a

Table 3: This table displays the average feed intake across different dietary treatments under reduced-energy conditions, with or without protease supplementation. A slight increase in feed intake was observed in the treated groups, suggesting a positive influence of the enzyme on feed palatability and/or digestibility.

Impact of protease in a reduced protein-energy diet on feed intake (FI) (g) in "broiler chickens" (Mean±SD)

Groups. / Weeks	T.1	T.2	T.3	T.4
Week1.	**167.83 ±0.63 c**	**169.30 ±0.47 b**	**169.30 ±0.47 b**	**171.80 ±0.09 a**
Week 2	**349 ±6.28 a**	**338.62 ±1.63 c**	**341.58 ±4.40 bc**	**344.4 ±0.04 ab**
Week 3	**801.22 ±1.28 a**	**722.26 ±2.49 c**	**743.46 ±12.43 b**	**745.80 ±0.07 b**
Week 4	**898.54 ±16.56 a**	**832.90 ±0.06 b**	**839.42 ±16.81 b**	**842.54 ±20.40 b**
Week 5	**1056.71 ±9.40 a**	**975.90 ±0.05 d**	**999.96 ±1.11 c**	**1025.98 ±32.04 b**
FI	**3273.3040 ±12.94 a**	**3038.98 ±3.43 d**	**3094.22 ±17.14 c**	**3130.52 ±41.57 b**

Table 4: This table presents the effect of adding protease enzyme to a reduced protein-energy diet on the feed conversion ratio (FCR) of broiler chickens. Four dietary treatments were compared. The results showed a notable improvement in feed efficiency in the enzyme-supplemented groups, particularly T3, indicating enhanced nutrient utilization.

Effect of protease in a reduced protein- energy diet on the FCR of broiler chickens (Mean ± SD)

Groups / Weeks	T.1	T.2	T.3	T.4
Week1.	1 .29 ± 0.01 c	1.45 ± 0.01a	1.27 ± 0.11 d	1.31 ± 0.06 b
Week 2	1.37 ±0.04 a	1.37 ±0.02 a	1.28 ±0.03 b	1.25 ±0.01 b
Week 3	1.77 ±0.02 b	1.98 ±0.02 a	1.46 ±0.03 c	1.53 ±0.06 c
Week 4	1.70 ±0.04 b	1.89 ±0.06 a	1.52 ±0.04 c	1.74 ±0.10 b
Week 5	1.69 ±0.01 a	1.75 ±0.01 a	1.45 ±0.02 b	1.46 ±0.02 b

E. coli

Table 5: This table presents the differences in E. coli colony counts among the treatment groups after 30 days of feeding. A significant reduction in E. coli populations was observed in the protease-treated groups, particularly T3 and T4, compared to the untreated group T2, which showed the highest bacterial load.[14]

Comparison	Difference (log CFU/ml)	Lower CI	Upper CI	p-value	Significance
G2 - G1	**41.33**	**30.00**	**52.66**	**<0.001**	***
G1. – G3.	**1.67**	**12.00**	**8.66**	**0.950**	**Ns**
G1 – G4	**8.33**	**18.66**	**2.00**	**0.130**	**Ns**
G2 – G3	**43.00**	**53.33**	**32.66**	**<0.001**	***
G2 – G4	**49.66**	**60.00**	**39.33**	**<0.001**	***
G3 – G4	**6.66**	**17.00**	**3.66**	**0.250**	**Ns**

CI= confidence interval
CFU= colony forming unit

- ns: "Not significant" ($p > 0.05$)
- *: "Significant" ($p < 0.05$)
- **: "Highly significant" ($p < 0.01$)
- *******: "Very highly significant" ($p < 0.001$)

Lactobacillus

Table6: This table focuses on the count of Lactobacillus in broiler droppings after 30 days. Protease supplementation, especially in group T4, significantly increased Lactobacillus levels, indicating a positive shift in gut microbiota toward beneficial bacterial populations. [15]

Comparison	Difference (log CFU/mL)	Lower CI	Upper CI	p-value	Significance
G2 - G1	**0.17**	**0.10**	**0.24**	**< 0.001**	***
G1 - G3	**0.04**	**0.11**	**0.03**	**0.400**	**Ns**
G4 - G1	**1.29**	**1.22**	**1.36**	**< 0.001**	***
G2 – G3	**0.21**	**0.28**	**0.14**	**< 0.001**	***
G4 - G2	**1.12**	**1.05**	**1.19**	**< 0.001**	***
G4 - G3	**1.33**	**1.26**	**1.40**	**< 0.001**	***

CI= confidence interval
CFU= colony forming unit

- **ns**: "Not significant" ($p > 0.05$)
- *: "Significant" ($p < 0.05$)
- **: "Highly significant" ($p < 0.01$)
- *******: "Very highly significant" ($p < 0.001$)

The current study investigated the effects of supplemental exogenous protease enzyme on the growth performance of broiler chickens fed a reduced protein-energy diet over a 5-week period. The results clearly demonstrated that dietary supplementation with protease improved feed conversion ratio (FCR), feed intake (FI), and body weight gain (WG), particularly under reduced nutrient conditions.

Group 2, which was fed a diet with reduced protein and energy without enzyme supplementation, showed significantly lower weight gain and poorer FCR compared to the control (Group 1). This finding aligns with previous

studies indicating that protein and energy reduction negatively impacts broiler growth performance due to insufficient nutrient availability for optimal metabolism and growth (16 ,17).

Interestingly, Group 4 (reduced protein-energy diet + protease enzyme) exhibited significant improvements in body weight gain and FCR compared to Group 2. This suggests that protease supplementation can effectively compensate for the nutrient deficiency by enhancing the digestibility and availability of dietary proteins(18). These results are consistent with the findings of (11), who reported that exogenous protease improves amino acid digestibility and nitrogen retention in broilers.

Furthermore, Group 3 (control + protease) also recorded performance enhancements compared to the control group alone, indicating that even under normal nutrient conditions, protease supplementation offers additional benefits, likely through improved nutrient utilization efficiency (19).

The improved feed conversion ratio observed in Groups 3 and 4 indicates more efficient use of feed for body mass development, which is particularly valuable in commercial poultry production aiming for cost-effectiveness.

In addition to growth performance, the current study also assessed the impact of exogenous protease supplementation on intestinal microbiota, specifically E. coli and Lactobacillus counts after 30 days. The results indicated a significant modulation of gut microbial populations.

Group 2 recorded a significant increase in E. coli colony counts compared to the control, suggesting that nutrient deficiency may compromise gut microbial balance and allow pathogenic bacteria to proliferate. Conversely, both enzyme-supplemented groups showed reduced E. coli counts, with Group 4 exhibiting the most pronounced decrease when compared to Group 2. These findings are consistent with previous research indicating that protease supplementation may improve gut health by reducing undigested protein substrate, which otherwise promotes the growth of pathogenic bacteria such as E. coli (20).

On the other hand, Lactobacillus counts were significantly higher in Group 4 compared to all other groups. This suggests a beneficial effect of protease on promoting beneficial gut flora, likely due to improved protein digestion and a more favorable gut environment (17).

Overall, these microbial findings support the performance data, suggesting that protease not only improves nutrient utilization but also contributes to gut health by reducing pathogenic bacteria and enhancing beneficial ones. This dual effect may explain the superior growth observed in protease-supplemented groups, particularly under nutrient-deficient conditions.

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