RESEARCH ARTICLE AGROBIOLOGICAL RECORDS
ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

EVALUATING GROWTH PERFORMANCE AND PHENOTYPIC CORRELATIONS OF PRODUCTIVE TRAITS IN BROILERS FED VARYING LEVELS OF PROTEASE

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ABSTRACT

Exogenous proteases are widely used in broiler nutrition to enhance protein digestibility and nutrient utilization; however, information regarding their dose-dependent effects and the relationships between protease supplementation and productive performance remains limited. This study evaluated the effects of graded levels of dietary protease supplementation on growth performance and the relationships among productive traits in Ross 308 broiler chickens. A total of 120 one-day-old chicks were randomly allocated to four dietary treatments containing 0, 200, 300, or 400 g of a commercial protease per ton of feed and were reared for 35 days. Growth performance data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test, while Pearson correlation, graphical dose-response, and relative improvement analyses were performed to further characterize the productive responses to protease supplementation. Initial body weight did not differ among treatments ($P = 0.1954$), confirming uniform group allocation at the start of the experiment. Dietary protease supplementation significantly increased final body weight ($P = 0.0058$), whereas average daily feed intake was not significantly affected ($P = 0.6166$). Feed conversion ratio showed a consistent numerical improvement in all protease-supplemented groups compared with the control group. Graphical dose-response analysis demonstrated progressive improvements in productive performance with increasing dietary protease inclusion, while relative improvement analysis indicated that supplementation at 300–400 g/ton elicited the greatest productive response. Pearson correlation analysis revealed stronger positive associations between feed intake and final body weight, as well as stronger negative associations between final body weight and feed conversion ratio, at higher levels of protease supplementation, suggesting enhanced nutrient-utilization efficiency. In conclusion, dietary protease supplementation improved the productive performance of broiler chickens in a dose-dependent manner. Under the conditions of the present study, supplementation at 300–400 g/ton produced the most favorable productive responses, supporting the use of exogenous protease as an effective nutritional strategy to improve broiler production efficiency.

***Keywords*:** Broiler chickens, Protease, Growth performance, FCR, Nutrient utilization, Dose-response, Pearson correlation.

Article History: ABR-26-223 || Received: 11-Jun-2026 || Revised: 05-Aug-2026 || Accepted: 13-Aug-2026 || Published Online: 21-Aug-2026

1. INTRODUCTION

The poultry industry has experienced substantial progress over the past decades through continuous advancements in genetic selection, nutritional management, disease prevention strategies, and husbandry practices. These developments have markedly improved growth rates, feed efficiency and meat yield in modern broiler strains. Nevertheless, the productive performance of broiler chickens remains highly dependent on the interaction between genetic potential and environmental factors, particularly nutritional management, which plays a pivotal role in maximizing growth and production efficiency under commercial conditions (El-Tahawy et al., 2017; Gado et al., 2019; Zhao et al., 2026).

Corn–soybean meal (CSBM)-based diets remain the primary feeding system for broiler chickens because of their high energy content, balanced amino acid profile and widespread availability. Despite their generally high digestibility, these diets contain several anti-nutritional factors (ANFs), including arabinoxylans, cellulose, galactomannans, and heat-stable protein fractions, which may impair nutrient utilization by increasing intestinal viscosity, limiting nutrient accessibility and reducing protein digestibility (Erdaw et al., 2017; Lee et al., 2023). Consequently, nutritional strategies designed to enhance nutrient digestibility and utilization have become increasingly important in modern poultry production systems.

Citation: Al-Mudyir, H., Alharbi, F., Alyousef, Y., & Rayan, G. (2026). Evaluating growth performance and phenotypic correlations of productive traits in broilers fed varying levels of protease. Agrobiological Records, 25: 275-283. https://doi.org/10.47278/journal.abr/2026.062

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Feed additives have emerged as essential tools for enhancing nutrient utilization, productive performance, immune competence, and overall health status in broiler chickens. Various additives, including probiotics, phytogenic compounds, organic acids, and exogenous enzymes, have been successfully incorporated into poultry diets to improve production efficiency and support sustainable poultry farming (Alagawany et al., 2018; Arif et al., 2019; Arif et al., 2020; Ashour et al., 2020). Among these additives, exogenous enzymes have received considerable attention because they can compensate for the limited digestive capacity of young birds and improve nutrient availability. Young broiler chickens often exhibit insufficient endogenous enzyme activity, which can restrict the complete digestion and utilization of dietary nutrients. Therefore, enzyme supplementation has become an effective nutritional strategy for enhancing nutrient digestibility and growth performance (Alagawany et al., 2018; Azizah et al., 2026).

Exogenous enzymes such as xylanase and protease are widely used to overcome the anti-nutritional effects associated with plant-based feed ingredients. Xylanase hydrolyzes arabinoxylan polymers, thereby reducing digesta viscosity and improving nutrient release, metabolizable energy utilization, and overall bird performance (Wang et al., 2024a, 2024b; Longhini et al., 2025). Protease facilitates the hydrolysis of complex dietary proteins into small peptides and free amino acids, enhancing protein digestion and nutrient utilization efficiency. Furthermore, protease supplementation can reduce endogenous nitrogen losses and mitigate the negative effects of anti-nutritional protein fractions present in feed ingredients, ultimately increasing amino acid availability (Cowieson & Roos, 2016; Lee et al., 2023; Nuraini et al., 2026).

Beyond its effects on nutrient digestion, protease may contribute to skeletal development by increasing the availability of amino acids required for collagen synthesis, which constitutes the structural framework of bone matrix and supports proper mineralization and mechanical strength (Siegert et al., 2019; Lee et al., 2023; da Nobrega et al., 2025). Recent studies have also demonstrated that dietary protease supplementation can improve gut health, carcass characteristics, and the balance of the cecal microbiota, particularly when alternative protein sources are incorporated into broiler diets (Zafar et al., 2025).

Several investigations have reported beneficial effects of protease supplementation on broiler performance. Cho et al. (2020) observed significant improvements in nutrient digestibility and growth performance in broilers fed amino acid-deficient diets supplemented with a multi-protease enzyme. Similarly, Amer et al. (2021) reported enhanced growth performance, amino acid digestibility, economic efficiency, blood biochemical parameters, and intestinal histomorphology following dietary protease supplementation. In addition, Park et al. (2020) demonstrated positive effects of protease on nutrient utilization and the expression of genes involved in growth regulation and amino acid transport.

Improved protein digestion and amino acid availability resulting from protease supplementation may enhance feed conversion efficiency, increase body weight gain, and reduce nitrogen excretion into the environment. These improvements are economically important because they allow the formulation of more cost-effective diets while maintaining optimal productive performance (Alagawany et al., 2016; Amer et al., 2021). Supporting this concept, Nath et al. (2025) demonstrated that matrix-dosed protease supplementation significantly improved growth performance, nutrient utilization, and economic returns in broiler chickens, highlighting the practical value of protease supplementation in commercial poultry production.

Despite the well-documented benefits of protease supplementation, its effectiveness may vary depending on enzyme source, dietary composition, bird genotype, management conditions, and supplementation level. Consequently, identifying the optimal inclusion rate remains critical for maximizing both biological performance and economic efficiency (Cowieson & Roos, 2016; Amer et al., 2021). Furthermore, recent evidence suggests that enzyme efficacy may be influenced by interactions among dietary ingredients, enzyme combinations, and the physiological status of the birds, emphasizing the need for dose-optimization studies under practical production conditions (Onbaşılar et al., 2026).

Therefore, the present study evaluated the effects of graded levels of dietary supplementation with a commercial protease enzyme (0, 200, 300, and 400 g/ton) on the growth performance of Ross 308 broiler chickens during a 35-day rearing period. In addition to evaluating growth performance, the study aimed to investigate the relationship between protease inclusion level and productive performance traits using correlation and relative improvement analyses in order to identify the most effective supplementation strategy for commercial broiler production.

2. MATERIALS AND METHODS

2.1. Experimental Design and Husbandry

The present study was conducted at the Experimental Animal Research Station, King Faisal University. A total of 120 one-day-old Ross 308 broiler chicks were used in a 35-day feeding trial. Upon arrival, the initial body weight of each chick was recorded, and the birds were randomly allocated to one of four dietary treatments. Each treatment consisted of 30 chicks, distributed into three replicates of 10 birds each.

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Environmental conditions were carefully controlled throughout the experimental period to ensure optimal bird performance. During the first week, the ambient temperature was maintained at 32–34°C, and was subsequently reduced by approximately 2–3°C per week until it reached 22–24°C, where it was maintained until the end of the experiment. Relative humidity was maintained between 60% and 70% throughout the study. The lighting program consisted of 23 h of light and 1 h of darkness during the first week, followed by 20 h of light and 4 h of darkness for the remainder of the experimental period. All birds were vaccinated according to the standard vaccination program recommended for commercial broiler production.

The commercial protease preparation was thoroughly premixed with a small quantity of the basal feed before being incorporated into the complete diet to ensure uniform distribution of the enzyme throughout the feed. A standard basal diet was formulated as the control diet, and a commercial protease enzyme preparation was supplemented at inclusion levels of 0, 200, 300 and 400 g/ton of feed, based on inclusion rates commonly used in previous broiler nutrition studies (Dalólio et al., 2016; Salman et al., 2017). To achieve complete homogeneity, the enzyme powder was carefully mixed into the feed during diet preparation.

All experimental groups were maintained under identical management, environmental, and hygienic conditions. The chicks were housed in well-ventilated floor pens, with each pen providing a floor area of 1.49 m². Wood shavings were used as litter material, and the birds had *ad libitum* access to both feed and water throughout the 35-day experimental period.

2.2. Growth Performance Traits

Live body weight (BW) of each chick was measured to the nearest gram at weekly intervals. Body weight gain (BWG) was calculated as the difference between the initial and final body weights for each corresponding growth period. Feed intake (FI) was determined weekly by collecting and weighing the residual feed remaining in the feeders and subtracting this amount from the total feed offered. The feed conversion ratio (FCR) was subsequently calculated as the ratio of FI to BWG.

2.3. Relative Improvement Analysis

To facilitate the biological interpretation of the effects of dietary protease supplementation, the relative improvement (%) in productive performance traits was calculated for each protease-supplemented treatment relative to the control group according to the following equation:

$$\text{Relative improvement (\%)} = [(\text{Mean of treatment} - \text{Mean of control}) / \text{Mean of control}] \times 100$$

This analysis was performed for final body weight and feed conversion ratio to illustrate the practical magnitude of the productive responses obtained with increasing dietary protease supplementation levels.

2.4. Graphical Presentation

Graphical visualization of the productive performance data was performed to illustrate the dose-response relationship between dietary protease supplementation and growth performance traits. Bar charts with standard error bars (SEM) were prepared to compare treatment means, whereas dose-response plots were constructed to demonstrate the biological response associated with increasing protease supplementation levels.

2.5. Statistical Analysis and Correlations

The collected data were subjected to a one-way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS software (version 2004), evaluating the dietary treatment considered as the primary fixed effect. The statistical model was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

- Y_{ij} represents the observed response variable.
- μ is the overall population mean.
- T_i denotes the specific effect of the dietary protease supplementation level.
- e_{ij} signifies the random experimental error.

Data are expressed as treatment means ± standard error of the mean (SEM). When significant treatment effects were detected, Duncan's multiple range test was used for mean separation ($P < 0.05$). Furthermore, Pearson's phenotypic correlations between the graded levels of dietary protease supplementation and growth performance traits (initial body weight, final body weight, daily feed intake, and feed conversion ratio) at 5 weeks of age were analyzed using the CORR procedure of SAS.

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3. RESULTS

3.1. Growth Performance

The effects of dietary protease supplementation on the growth performance of broiler chickens are presented in Table 1. Initial body weight (IBW) did not differ significantly among the experimental treatments (P = 0.1954), indicating that the birds were uniformly allocated among the treatment groups at the beginning of the experiment.

Dietary supplementation with protease significantly affected final body weight (FBW) at 35 days of age (P = 0.0058). Birds fed the diet supplemented with 400 g of protease/ton exhibited the highest FBW, followed by those receiving 300 and 200 g/ton, whereas the control group had the lowest FBW. These findings indicate a progressive improvement in growth performance with increasing levels of dietary protease supplementation.

Daily feed intake (DFI) was not significantly influenced by dietary protease supplementation (P = 0.6166), although birds receiving the highest protease supplementation level showed numerically higher feed intake than those in the control group.

Feed conversion ratio (FCR) showed a clear numerical improvement in all protease-supplemented groups compared with the control group. The lowest FCR was observed in birds receiving 400 g of protease/ton, while the control group showed the highest value. Although the overall treatment effect was not statistically significant (P = 0.0548), it approached statistical significance, and the numerical trend suggests improved feed utilization efficiency in response to dietary protease supplementation.

Table 1: Effect of different levels of protease on initial body weight, final body weight, daily feed intake and feed Conversion of broilers at 5 weeks of age

Trait	Treatments (Protease level)				Probability
	200 g/ton	300 g/ton	400 g/ton	0 g/ton Control	
Initial body weight (IBW), g	43.66 ± 0.33	43.00 ± 0.57	42.33 ± 0.33	41.33 ± 1.20	0.1954
Final body weight (FBW), g	1902.7 ± 11.57b	1939.3 ± 21.6ab	1980.7 ± 4.63a	1750.0 ± 61.6c	0.0058
Daily feed intake (DFI), g at 5 wks	117.9 ± 4.72	121.1 ± 8.25	124.1 ± 2.07	113.8 ± 5.43	0.6166
Feed Conversion (FCR) at 5 wks	1.58 ± 0.01b	1.57 ± 0.06b	1.54 ± 0.02b	1.70 ± 0.03a	0.0548

Values (mean ± SD) bearing different alphabets within a row differ significantly (P<0.05).

3.2. Dose–response Relationship of Dietary Protease Supplementation

As illustrated in Fig. 1, dietary protease supplementation resulted in a progressive increase in final body weight (FBW) compared with the control group. Birds receiving 200, 300, and 400 g of protease/ton exhibited higher FBW values than the control group. The greatest FBW was observed in birds supplemented with 400 g /ton, indicating that increasing levels of dietary protease supplementation enhanced growth performance under the present experimental conditions.

Fig. 2 demonstrates a gradual decline in feed conversion ratio as the dietary protease supplementation level increased. Compared with the control group, all protease-supplemented treatments exhibited improved feed efficiency, with the lowest FCR observed in birds receiving 400 g protease/ton. These findings indicate improved efficiency of nutrient utilization following enzyme supplementation.

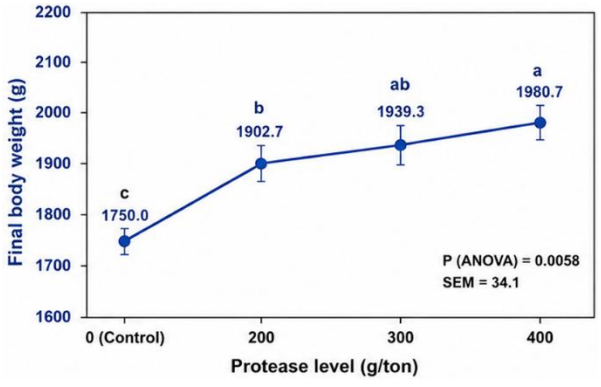

Fig. 1: Dose–response relationship between dietary protease supplementation and final body weight.

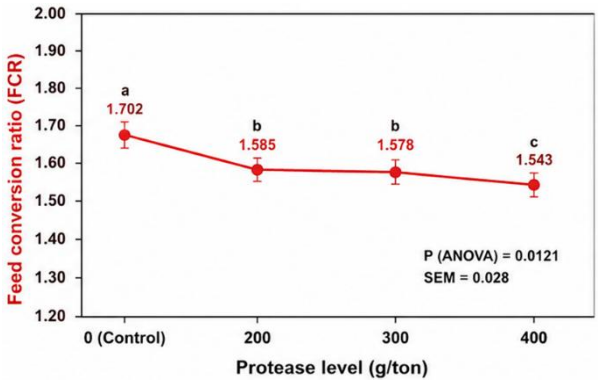

Fig. 2: Dose–response relationship between dietary protease supplementation and feed conversion ratio.

Relative improvement analysis (Fig. 3) revealed that protease supplementation produced progressive enhancements in productive performance. Final body weight increased by approximately 8.7, 10.8, and 13.2% in birds receiving 200, 300, and 400 g of protease/ton, respectively. Likewise, feed conversion ratio improved by approximately 6.9, 7.3, and 9.3% compared with the control treatment.

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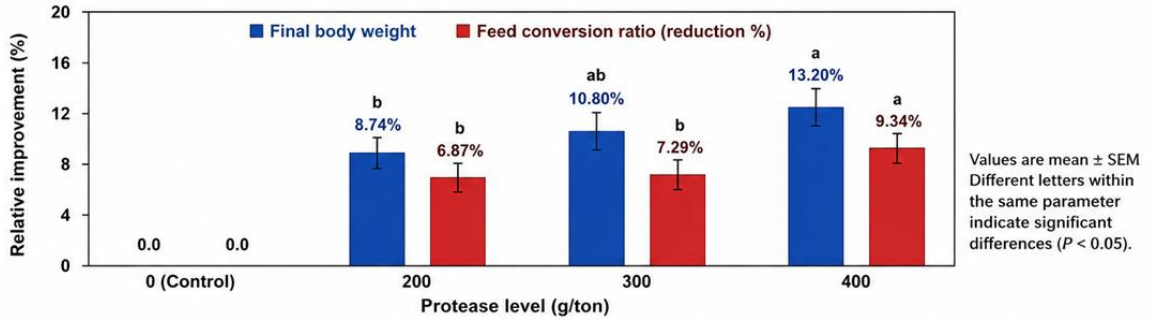

Fig. 3: Relative improvement (%) in final body weight and feed conversion ratio (reduction) compared with the control treatment.

Graphical dose-response evaluation demonstrated progressive improvements in productive performance with increasing dietary protease supplementation, whereas relative improvement analysis indicated that supplementation at 300–400 g/ton produced the greatest productive response under the conditions of the present study. Furthermore, Pearson correlation analysis revealed stronger positive associations between feed intake and final body weight, together with stronger negative associations between final body weight and feed conversion ratio, particularly in birds receiving the higher levels of protease supplementation.

3.3. Pearson Correlation Coefficients

The Pearson correlation matrices among the evaluated parameters revealed different correlation patterns across the dietary protease supplementation levels (Table 2).

3.3.1. 200 g/ton Protease (T1): A strong positive correlation was observed between IBW and DFI (r = 0.98070). In addition, FBW showed a negative correlation with FC (r = −0.66285); however, this relationship was not statistically significant, suggesting that birds with higher final body weight tended to exhibit lower (improved) feed conversion ratios.

3.3.2. 300 g/ton Protease (T2): A highly significant negative correlation was observed between IBW and FBW (r = −0.99997; P = 0.0049). In addition, DFI showed a strong positive correlation with FC (r = 0.95330); however, this relationship did not reach statistical significance (P = 0.1953).

3.3.3. 400 g/ton Protease (T3): At the highest level of protease supplementation, IBW showed a strong positive correlation with FBW (r = 0.89977). Furthermore, FBW showed a negative correlation with FC (r = -0.82779, P = 0.3792) and a positive correlation with DFI (r = 0.88161, P = 0.3129). Although these correlations were not statistically significant, they indicated a tendency for birds with greater feed intake to achieve higher final body weight while maintaining lower feed conversion ratios.

Table 2: Pearson's correlation coefficients among growth performance indicators of broilers fed graded levels of dietary protease

Traits	IBW	FBW	DFI	FC
		T1 (200 g/ton) Protease		
IBW	1	0.31701	0.98070	0.50000
		0.7946	0.1253	0.6667
FBW		1	0.49634	-0.66285
			0.6694	0.5387
DFI			1	0.32102
				0.7919
FC				1
	T2 (300 g/ton) Protease			
IBW	1	-0.89997*	0.11195	0.40686
		0.0049	0.9286	0.7333
FBW		1	-0.10429	-0.39981
			0.9335	0.7382
DFI			1	0.95330
				0.1953
FC				1
	T3 (400 g/ton) Protease			
IBW	1	0.89977	-0.03206	-0.50000
		0.2875	0.9796	0.6667
FBW		1	-0.46499	-0.82779
			0.6921	0.3792
DF			1	0.88161
				0.3129
FC				1

* P<0.05; IBW = Initial body weight, FBW = Final body weight, DFI = Daily feed intake, FC = Feed conversion.

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4. DISCUSSION

Exogenous proteases are widely used in broiler nutrition to enhance protein utilization by improving the hydrolysis of dietary proteins and increasing the release of small peptides and amino acids. In addition, protease supplementation may reduce the adverse effects of protein-related anti-nutritional factors in plant-based feed ingredients, thereby improving nutrient availability and overall feed efficiency (Cowieson & Roos, 2016; Park et al., 2020).

The lack of significant differences in initial body weight (IBW) among the experimental groups indicates that the observed differences in performance parameters were mainly attributed to dietary protease supplementation rather than to initial variation in chick quality or body weight distribution. This finding strengthens the validity of the observed treatment effects on growth performance.

The improvement in final body weight (FBW) and feed conversion ratio (FCR) observed in the protease-supplemented groups suggest enhanced efficiency of nutrient utilization. This response is consistent with previous studies demonstrating that dietary protease improves protein digestibility and growth performance in broilers (Cho et al., 2020; Park et al., 2020; Amer et al., 2021). These improvements are primarily attributed to the enzymatic hydrolysis of complex dietary proteins into smaller peptides and free amino acids, thereby enhancing amino acid absorption, protein deposition, and muscle growth.

The numerically higher daily feed intake (DFI) observed in the protease-supplemented groups suggests that dietary protease supplementation did not adversely affect feed palatability or feed acceptance. Although the increase in DFI was not statistically significant, the numerically greater feed intake observed in some supplemented groups may be associated with the increased metabolic demand resulting from enhanced growth and protein deposition, a response that has been reported when nutrient utilization efficiency is improved.

The present study demonstrated a dose-dependent improvement in broiler growth performance following dietary protease supplementation, as evidenced by the progressive increase in final body weight and the concomitant reduction in feed conversion ratio. These findings suggest that exogenous protease enhanced dietary protein utilization by hydrolyzing complex proteins into smaller peptides and free amino acids, thereby improving protein digestibility and increasing amino acid availability for protein deposition, muscle growth, and tissue development. Similar improvements in growth performance, feed efficiency, and nutrient digestibility have been reported in recent studies evaluating exogenous protease supplementation in broiler diets under different nutritional strategies (Stefanello et al., 2016; Moss et al., 2017; Lee et al., 2018; Jabbar et al., 2021; McCafferty et al., 2022; Vieira et al., 2023; Nath et al., 2025).

The reduction in feed conversion ratio observed in the present study further indicates that dietary protease enhanced feed utilization efficiency. Improved hydrolysis of dietary proteins increases the supply of digestible amino acids available for growth and metabolic processes, enabling birds to achieve greater body weight gain without a proportional increase in feed consumption. Moreover, enhanced protein digestion reduces the quantity of undigested protein reaching the hindgut, thereby reducing substrate availability for undesirable microbial fermentation and contributing to a more favorable intestinal environment for nutrient absorption (Qaisrani et al., 2015). These physiological responses may explain the superior feed efficiency observed in protease-supplemented birds.

To facilitate biological interpretation of the productive responses, relative improvement analysis was performed by comparing each protease-supplemented treatment with the control the group. The progressive increase in final body weight, together with the continuous reduction in feed conversion ratio, indicates that dietary protease improved production efficiency rather than simply stimulating feed intake. Furthermore, the gradual enhancement in productive performance with increasing protease inclusion supports a biological dose-response relationship, whereby increasing enzyme availability enhances the hydrolysis of dietary proteins, improves amino acid digestibility and nutrient utilization, and consequently promotes growth performance. However, as enzyme supplementation increases, the magnitude of the response may gradually diminish because enzyme efficacy ultimately becomes constrained by substrate availability and the birds' physiological digestive capacity, resulting in a plateau in performance responses. Similar dose-dependent improvements in growth performance, feed efficiency, and nutrient digestibility following protease supplementation have been documented in recent broiler studies (Vieira et al., 2023; Nath et al., 2025).

A comparable dose-dependent response to protease supplementation has also been reported by Ding et al. (2016), who observed progressive improvements in growth performance and nutrient utilization in broiler chickens receiving increasing dietary protease levels.

To better understand the relationship between enzyme inclusion levels and productive traits, correlation analysis was performed. The negative relationship between FBW and FCR indicates that improved body weight gain was associated with better feed efficiency, which is consistent with the biological relationship between these two productive traits in broiler chickens. However, the observed correlation coefficients should be interpreted cautiously, as they represent statistical associations rather than direct causal relationships.

The moderate correlations observed between initial body weight and final performance parameters suggest that although early body weight contributes to final performance, its influence may be modified by dietary interventions

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ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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such as enzyme supplementation. This aligns with previous findings indicating that exogenous proteases can partially overcome variability in nutrient utilization efficiency among birds (Amer et al., 2021). At higher supplementation levels, performance improvements were more evident, suggesting a dose-dependent response up to an optimal inclusion level. The highest protease inclusion level resulted in the most efficient conversion of feed into body mass, indicating enhanced protein digestion and increased amino acid availability for growth. Similar improvements in growth performance and nutrient digestibility at higher protease inclusion levels have been reported by Cho et al. (2020) and Park et al. (2020), who observed enhanced feed efficiency and nutrient utilization in broilers supplemented with exogenous protease enzymes.

Overall, the present findings indicate that dietary protease supplementation improved broiler productive performance, which may be attributed to enhanced nutrient utilization and protein digestibility. The observed dose-dependent response, together with the improvements in final body weight and feed conversion ratio, supports the biological efficacy of exogenous protease as a nutritional strategy for improving broiler productivity. Although the current study focused on productive performance, the observed responses are consistent with previous reports describing improvements in amino acid digestibility, metabolizable energy utilization, and intestinal functionality following dietary protease supplementation.

Study Limitations and Future Perspectives

The present study focused primarily on productive performance responses to graded dietary protease supplementation. Therefore, evaluating nutrient digestibility, metabolizable energy, intestinal morphology, gut microbiota, immune function, carcass characteristics, organ development, and blood biochemical parameters was beyond the scope of the current experiment. Future studies should integrate these physiological and nutritional parameters with productive performance and correlation-based analyses would provide a more comprehensive understanding of the mechanisms underlying the beneficial effects of exogenous protease in broiler nutrition.

5. CONCLUSION

The present findings demonstrate that dietary protease supplementation improved broiler growth performance in a dose-dependent manner. Graphical dose-response analysis further confirmed progressive increases in final body weight, along with consistent improvements in feed conversion ratio, as dietary protease level increased. Relative improvement analysis indicated that supplementation at 300–400 g/ton yielded the greatest productive response under the conditions of the present experiment. These findings support the use of dietary protease as an effective nutritional strategy to improve broiler production efficiency.

Declarations

Funding: This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFU263507].

Acknowledgement: The authors acknowledge the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia, for supporting the work.

Conflicts of Interest: No conflicts of interest were encountered in the paper.

Data Availability: The data that supports the findings of this study are available in the material of this article. There is no other supporting data.

Ethics Statement: All animal-conducted investigational processes were permitted by the Research Ethics Committee of King Faisal University, Saudi Arabia (KFU-2026- ETHICS4350).

Author's Contributions: All authors contributed equally to the investigation and preparation of the present study. All authors discussed the results and commented on the manuscript.

Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Citation: Al-Mudyir, H., Alharbi, F., Alyousef, Y., & Rayan, G. (2026). Evaluating growth performance and phenotypic correlations of productive traits in broilers fed varying levels of protease. Agrobiological Records, 25: 275-283. https://doi.org/10.47278/journal.abr/2026.062