COMPARATIVE EVALUATION OF THREE COMMERCIAL PROTEASE ENZYMES ON PERFORMANCE CARCASS TRAITS, BLOOD PARAMETERS, ILEAL NUTRIENT DIGESTIBILITY, AND PRODUCTION EFFICIENCY, IN BROILER CHICKENS FED STANDARD AND NUTRIENT-REDUCED DIETS

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SUMMARY

This study evaluated the effects of three commercial protease enzymes (A, B, and C), derived from *Bacillus licheniformis*, on broiler chickens fed either standard (CO) or nutrient-reduced (NC) diets over a 42-day period. A total of 600 one-day-old Ross 308 male chicks were randomly allocated into 10 treatment groups (5 replicates of 12 birds each), receiving diets with or without enzyme supplementation. Enzymes were included based on manufacturer-recommended dosages and matrix values: Enzyme A (600,000 U/g at 500 g/ton), Enzyme B (60,000 U/g at 250 g/ton), and Enzyme C (80,000 U/g at 50 g/ton). Performance parameters assessed included body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), carcass traits, blood biochemical markers (uric acid, creatinine), ileal nutrient digestibility (crude protein [CP], ether extract [EE]), and European Production Efficiency Factor (EPEF). Enzyme A significantly improved BWG ($P = 0.002$) and FCR ($P = 0.006$) in both C and NC diets, with higher FI observed in the NC + EA group ($P = 0.012$). Enzyme C improved FCR ($P = 0.001$) and increased carcass weight ($P = 0.008$), front half ($P = 0.006$), and back half weights ($P = 0.019$) in the CO diet. Enzyme B improved front half ($P = 0.048$) and total carcass weights ($P = 0.044$) in the CO diet. Blood parameters remained unaffected by any enzyme ($P > 0.05$). CP digestibility was significantly increased by Enzymes A ($P = 0.004$) and B ($P = 0.007$), while EE digestibility was unaffected significantly across treatments ($P > 0.05$). EPEF was numerically highest in the CO + EA group (468), indicating enhanced overall efficiency. These findings highlight enzyme-specific responses, with Enzyme A delivering the most consistent performance benefits, particularly under nutrient-adequate conditions. The results underscore the importance of selecting appropriate enzyme types and dosages relative to diet composition to optimize broiler productivity.

***Keywords**: Broiler chickens; Carcass traits; Nutrient digestibility; Production efficiency and Protease enzymes*

INTRODUCTION

The global poultry industry continues to expand rapidly to meet the growing demand for high-quality animal protein. Among the major challenges faced by poultry producers is the high cost of feed, which accounts for approximately 60–70% of total production expenses. Protein-rich feed ingredients such as soybean meal are particularly costly and contain protease inhibitors that reduce endogenous enzyme activity in the gastrointestinal tract, leading to adverse effects on growth, feed efficiency, nutrient utilization, and increased nitrogen excretion and environmental pollution (Cowieson *et al.,* 2017). One promising strategy to address this challenge is the supplementation of diets with exogenous enzymes, particularly proteases.

Proteases are hydrolytic enzymes that enhance the digestion of dietary proteins by cleaving peptide bonds, thereby increasing the release of absorbable amino acids. When included in broiler diets, protease enzymes can compensate for lower dietary protein levels by improving digestibility, reducing nitrogen excretion, and enhancing feed conversion ratio (FCR). In addition, protease supplementation has been shown to modulate gut health through improvements in intestinal morphology. In broiler chickens fed CO and NC diets, proteases improve productive performance, as demonstrated by Amer *et al.* (2021), who reported increased body weight gain (BWG) and improved FCR in CO corn-soybean meal diets due

to enhanced amino acid availability. Likewise, Olukosi *et al.* (2017) found that protease supplementation enhanced FCR in balanced diets by improving protein utilization. Additionally, Peñuela-Sierra *et al.* (2024) observed that proteases mitigated BWG reductions in low-protein diets by enhancing digestion of available protein. For carcass traits, Law *et al.* (2017) reported improved carcass and breast yield in CO diets due to increased protein absorption. Moreover, Sarica *et al.* (2020) noted enhanced thigh yield with proteases, supporting muscle development. In low-protein diets, Freitas *et al.* (2011) found that proteases increased carcass weight, though limited by reduced protein content. Blood biochemical parameters remain stable with protease supplementation, as Cowieson and Roos (2016) observed no significant changes in serum uric acid or creatinine levels in CO diets, indicating balanced nitrogen metabolism. Similarly, Walk *et al.* (2018) reported stable creatinine levels, reflecting efficient amino acid use. Furthermore, Attia *et al.* (2018) noted maintained uric acid levels in low-protein diets with proteases, suggesting minimal metabolic disruption. For ileal nutrient digestibility, Jabbar *et al.* (2021) reported increased crude protein (CP) digestibility in CO diets by counteracting trypsin inhibitors. Likewise, Cowieson *et al.* (2018) found improved amino acid absorption with proteases in balanced diets. Additionally, Leinonen & Williams (2015) observed enhanced CP digestibility in low-protein diets, though constrained by substrate availability. Production efficiency, measured by the European Production Efficiency Factor (EPEF), is improved by proteases, with Vieira & Stefanello (2017) noting higher EPEF in CO diets due to better BWG and FCR. Moreover, Olukosi *et al.* (2017) reported improved EPEF in balanced diets with protease supplementation. In low-protein diets, Peñuela-Sierra *et al.* (2024) found that proteases enhanced EPEF by improving nutrient utilization, though benefits were limited.

A variety of commercial protease products are available. However, commercial protease products vary in their biochemical properties, including pH stability, thermostability, substrate specificity, and activity level (e.g., U/g). Manufacturers of these enzymes often provide matrix values that estimate the amount of protein, amino acids, and energy released by the enzyme. These values allow nutritionists to reformulate diets reducing CP and ME levels while maintaining equivalent nutrient availability. However, the efficacy of different protease products and the validity of their matrix values may vary under commercial conditions.

While several studies have explored the effects of single protease products in poultry nutrition, the present study was designed to evaluate and compare multiple commercial enzymes within a single experimental framework. Moreover, to investigate how enzyme-specific matrix-based reformulation impacts both performance and physiological parameters, especially under nutrient-restricted conditions.

MATERIALS AND METHODS

This study was carried out at a commercial broiler farm in Al-Behera governorate, Egypt. The whole duration of the experiment was 42 days, starting on 28/ 11 / 2023 and ending on 9 / 1 / 2024. The study aims to evaluate the exogenous proteases enzymes produced from microbial sources in broiler nutrition.

Management of chicks:

Six hundred one-day-old male broiler chicks (Ross308 were purchased from a local commercial hatchery (Cairo Poultry Company). with an average weight of 42.0. Upon arrival, chicks were weighted and distributed randomly in 10 treatment groups. Each treatment was replicated in 5-floor pens with 12 birds per pen (1.25m2) as the experimental unit. The farm was illuminated using artificial light throughout the experimental period. From day 1 to day 7, chicks were provided with 23 hours of light and 1 hour of darkness. After day 7, the dark period was increased to 3 hours per day and maintained until the end of the experimental period. All the chicks in the experiment had free access to feed and water (*ad libitum*) over the experimental period.

Experimental design:

Three commercial alkaline serine endopeptidase proteases (A, B, C) derived from Bacillus licheniformis were evaluated. Enzyme A had a protease activity of 600,000 units/g with a recommended inclusion rate of 500 g/ton of feed, Enzyme B had 60,000 units/g with 250 g/ton, and Enzyme C had 80,000 units/g with 50 g/ton.

The treatments:

A basal control diet (CO) was formulated per Ross 308 (2019) nutritional guidelines, with a negative control (NC) diet for each enzyme (A, B, C) adjusted based on the manufacturer's recommended matrix values; each enzyme was added to both C and NC diets, yielding four treatments per enzyme: CO, CO + E, NC, and NC + E.

Experimental diets:

The ingredients and nutrient composition of the experimental diets are presented in Tables 2, 3, and 4. Chicks were fed on starter, grower, and finisher diets during periods, 0-11, 12-22, and 23-42 day respectively.

Productive performance parameters:

Body weight gain, feed intake, FCR, and mortality rates were measured during the experiment.

European production efficiency factor (epef):

Calculation of EPEF for the experimental period (0-42days):
EPEF = [Liveability (%) × Live weight (kg) / Age (days) × FCR] × 100 according to (Kryeziu *et al.* (2018).

Digestion trial:

The digestion trial was conducted by transferring 100 birds at 42 days of age to cages (2 birds per replicate), feeding them a diet supplemented with chromium oxide for 4 days, followed by slaughter and collection of ileal contents from the distal 5 cm of the ileum, stored at –20°C. Feed and ileal samples were analysed for dry matter, Kjeldahl nitrogen (AOAC, 1984), and crude fat (Soxhlet, 1879). Chromium oxide content was measured, and the protein digestibility coefficient was calculated following the method described by Divakaran *et al.* (2002).

Slaughter test and carcass traits:

At the end of the trial, 10 birds per treatment were randomly selected for slaughter and carcass evaluation. Birds were individually weighed, mechanically defeathered, and manually eviscerated. The carcass, neck, front half, hind half, gizzard, liver, and heart were weighed. Carcass organ weights were expressed relative to the dressing weight, defined as the freshly dressed carcass without neck and giblets.

Blood parameters:

At the end of the experiment (42 days), blood samples were collected from the jugular vein of broilers, and serum was separated after centrifugation and stored at −20°C until analysis. Uric acid concentration was measured using the Uric Acid Single Reagent kit (REF: 323 000; Spectrum Diagnostics, Cairo, Egypt) according to the manufacturer's protocol. Creatinine concentration was determined using the Creatinine Single Reagent kit (REF: 235 001; Spectrum Diagnostics, Cairo, Egypt) following the manufacturer's protocol.

Statistical analysis:

Data were analysed using one-way analysis of variance (ANOVA) via IBM SPSS Statistics (version 2023) according to the following model:

$$Yij = \mu + Ti + eij$$

Where Yij is the observed value of the trait for the ith bird in the jth treatment, μ is the overall mean, Ti is the effect of treatment, and eij is the random error.

Duncan's New Multiple Range Test was used to separate means when significant differences were detected ($P \leq 0.05$).

Additionally, analysis of covariance (ANCOVA) was performed for organ weights (liver and heart) to correct for live body weight as a covariate. The statistical model used was:

$$Yij = \mu + Ti + \beta(Xij - \bar{X}) + eij$$

Where Yij is the organ weight (liver or heart) of the ith bird in the jth treatment, μ is overall mean, Ti is the effect of treatment, Xij is the live body weight of the bird, $\bar{X}$ is the overall mean of live body weight, β is the regression coefficient between organ weight and live body weight, and eij is the random error.

This model was used to adjust organ weights for differences in live body weight across treatments, improving the accuracy of interpretation.

Table (1): Matrix values nutritional adjustments of alkaline serine endopeptidase proteases used in the negative control (nc) diets.

Parameter	Enzyme A (500 g/ton)	Enzyme B (250 g/ton)	Enzyme C (50 g/ton)
Metabolizable Energy (kcal/kg)	30	12.5	25
Protein (%)	1	0.45	0.875
Lysine (%)	0.0396	0.0275	0.0371
Methionine (%)	0.0061	0.0045	0.0168
Methionine + Cysteine (%)	0.0251	0.0080	0.0336
Threonine (%)	0.0468	0.0080	0.0098
Arginine (%)	0.0319	0.0000	0.0308
Valine (%)	0.0640	0.0043	0.0329
Isoleucine (%)	0.0457	0.0038	0.0378
Tryptophan (%)	0.0066	0.0013	0.0000

Note: Values represent reductions applied to the negative control (NC) diets relative to the basal control (CO) diet, based on manufacturer recommendations.

Table (2): Ingredients and nutrient composition of the starter diet (1–11 days of age).

Ingredient	Control diet (CO)	CO– (Enzyme A)	CO– (Enzyme B)	CO– (Enzyme C)
Mize (8%)	52.4	56.5	54.5	56.4
Soybean meal (46%)	39.2	36	37.4	36.2
Soy oil	3	1.8	2.5	1.85
Limestone	1.54	1.52	1.54	1.52
MCP*	1.83	1.85	1.83	1.85
Sodium chloride	0.25	0.25	0.25	0.25
Sodium bicarbonate	0.25	0.26	0.25	0.25
L-Lysine	0.295	0.35	0.325	0.345
DL-Methionine	0.44	0.45	0.45	0.435
L-Threonine	0.13	0.13	0.15	0.135
L-Valine	0.06	0.05	0.09	0.075
L-Isoleucine	.018	0.03	0.05	0.04
L- Arginine	0.057	0.113	0.11	0.11
Premix**	0.3	0.3	0.3	0.3
Choline chloride (60%)	0.23	0.23	0.23	0.23
Calculated analysis				
Metabolizable Energy kcal	2945	2915	2937	2917
Crude protein %	23	22	22.6	22.1
Calcium %	0.97	0.96	0.97	0.96
Available Phosphors%	0.49	0.49	0.49	0.49
Lysine %	1.44	1.4	1.41	1.4
Methionine+Cystine %	1.08	1.06	1.07	1.05
Threonine %	0.97	0.92	0.97	0.93
Tryptophan %	0.27	0.25	0.26	0.25
Valine %	1.1	1.04	1.1	1.07
Isoleucine %	0.97	0.92	0.97	0.94
Arginine %	1.52	1.49	1.52	1.49

**MCP monocalcium phosphate*

***Premix supplied per Kg of diet: Vit A, 12000 I.U; Vit D3, 3000 I.U; Vit E, 40 mg; Vit K3, 3 mg; Vit B1, 2mg; Vit B2, 6mg; Vit B6, 3.5mg; Vit B12, 20µg; Niacin, 30 mg; Pantothenic acid, 12 mg; Folic acid, 1.5 mg; Biotin, 75µg; Copper, 7 mg; Iodine, .8mg; Iron, 35 mg; Manganese, 100 mg; Zinc, 75 mg, Cobalt, 0.1mg and Selenium, 0.2 m g All calculations are based on the (breed catalog).*

Table (3): Ingredients and nutrient composition of the growediet (11–22 days of age).

Ingredient	Control diet (CO)	CO – (Enzyme A)	CO – (Enzyme B)	CO– (Enzyme C)
Mize (8%)	53.9	58.4	56	57.7
Soybean meal (46%)	36.3	33	34.7	33.5
Soy oil	5	3.7	4.3	3.9
Limestone	1.4	1.4	1.4	1.4
MCP*	1.6	1.62	1.6	1.6
Sodium chloride	0.23	0.23	0.23	0.23
Sodium bicarbonate	0.33	0.33	0.33	0.33
L-Lysine	0.21	0.26	0.22	0.25
DL-Methionine	0.385	0.358	0.385	0.37
L-Threonine	0.09	0.09	0.1	0.12
L-Valine	0.015	-	0.03	0.027
L-Isoleucine	-	-	0.02	-
L- Arginine	-	0.05	0.037	0.04
Premix**	0.3	0.3	0.3	0.3
Choline chloride (60%)	0.21	0.22	0.22	0.22
Calculated analysis Composition				
Metabolizable Energy kcal	3087	3052	3070	2960
Crude protein %	21.5	20.5	21.1	20.6
Calcium %	0.88	0.88	0.87	0.87
Available Phosphors%	0.438	0.438	0.437	0.345
Lysine %	1.29	1.25	1.26	1.26
Methionine+Cystine %	0.99	0.96	0.67	0.95
Threonine %	0.88	0.84	0.98	0.87
Tryptophan %	0.25	0.24	0.24	0.24
Valine %	1	0.93	0.99	0.96
Isoleucine %	0.88	0.84	0.89	0.85
Arginine %	1.37	1.34	1.37	1.34

**MCP monocalcium phosphate*

***Premix supplied per Kg of diet: Vit A, 12000 I.U; Vit D3, 3000 I.U; Vit E, 40 mg; Vit K3, 3 mg; Vit B1, 2mg; Vit B2, 6mg; Vit B6, 3.5mg; Vit B12, 20µg; Niacin, 30 mg; Pantothenic acid, 12 mg; Folic acid, 1.5 mg; Biotin, 75µg; Copper, 7 mg; Iodine, .8mg; Iron, 35 mg; Manganese, 100 mg; Zinc, 75 mg, Cobalt, 0.1mg and Selenium, 0.2 m g All calculations are based on the (breed catalog).*

Table (4): Ingredients and nutrient composition of the finisher diet (23–42 days of age).

Ingredient	Control diet (CO)	CO– (Enzyme A)	CO – (Enzyme B)	CO – (Enzyme C)
Mize (8%)	59.1	63.1	60.1	62.5
Soybean meal (46%)	31.2	28.1	29.7	28.6
Soy oil	5.5	4.4	5	4.6
Limestone	1.25	1.25	1.27	1.27
MCP*	1.41	1.41	1.41	1.41
Sodium chloride	0.2	0.2	0.2	0.2
Sodium bicarbonate	0.26	1.25	0.26	0.26
L-Lysine	0.19	0.235	0.21	0.23

DL-Methionine	0.34	0.34	0.35	0.33
L-Threonine	0.06	0.06	0.075	0.09
L-Valine	-	-	0.017	-
L-Isoleucine	-	-	0.017	-
L- Arginine	-	0.04	0.025	0.03
Premix**	0.3	0.3	0.3	0.3
Choline chloride	0.22	0.21	0.21	0.21
Calculated analysis Composition				
Metabolizable Energy kcal	3180	3155	3170	3162
Crude protein %	19.5	18.5	19	18.7
Calcium %	0.78	0.77	0.78	0.78
Available	0.39	0.39	0.39	0.39
Lysine %	1.15	1.11	1.13	1.11
Methionine+Cystine	0.9	0.89	0.9	0.87
Threonine %	0.77	0.73	0.77	0.77
Tryptophan %	0.22	0.21	0.22	0.21
Valine %	0.89	0.84	0.88	0.86
Isoleucine %	0.8	0.75	0.79	0.76
Arginine %	1.23	1.18	1.21	1.18

**MCP monocalcium phosphate*
***Premix supplied per Kg of diet: Vit A, 12000 I.U; Vit D3, 3000 I.U; Vit E, 40 mg; Vit K3, 3 mg; Vit B1, 2mg; Vit B2, 6mg; Vit B6, 3.5mg; Vit B12, 20µg; Niacin, 30 mg; Pantothenic acid, 12 mg; Folic acid, 1.5 mg; Biotin, 75µg; Copper, 7 mg; Iodine, .8mg; Iron, 35 mg; Manganese, 100 mg; Zinc, 75 mg, Cobalt, 0.1mg and Selenium, 0.2 m g*
All calculations are based on the (breed catalog).

RESULTS AND DISCUSSION

Growth performance:

Table 5 presents the effects of three protease enzymes (A, B, C) on broiler performance parameters (BWG), (FI), and (FCR) over 42 days.

Body weight gain (BWG):

The inclusion of Enzyme A significantly influenced BWG ($p = 0.002$). Birds in the groups CO+EA exhibited significantly higher BWG compared to NC. Notably, BWG in the NC+EA group was comparable to the CO group, indicating that Enzyme A effectively mitigated the negative effects of nutrient deficiency. In contrast, Enzyme B had no significant effect on BWG ($p = 0.844$). Mean BWG values across all groups CO, CO+EB, NC, and NC+EB remained statistically similar, suggesting that Enzyme B did not influence growth performance under either dietary condition. For Enzyme C, BWG differences were not statistically significant ($p = 0.11$). However, a slight numerical improvement was observed in the NC+EC group compared to the NC group. The CO+EC group maintained a BWG level comparable to the control (Table 5).

Feed intake (FI):

Enzyme A supplementation significantly affected FI ($p = 0.012$). The NC+EA group recorded the highest FI (3732 ± 36 g), which was significantly greater than all other treatments, including NC, CO, and CO+EA. This suggests that Enzyme A may have improved palatability or compensated for nutrient inadequacy. No significant differences in FI were observed with Enzyme B supplementation ($p = 0.175$). Values ranged from 3544 ± 47 g (CO+EB) to 3715 ± 63 g (NC), with no statistically meaningful changes across groups. Similarly, Enzyme C did not significantly alter FI ($p = 0.49$). Although the NC group exhibited the highest FI (3730 ± 70 g), the NC+EC group and other treatments did not differ significantly, indicating that FI was not notably influenced by Enzyme C inclusion. (Table 5).

Feed conversion ratio (FCR):

A significant effect on (FCR) was observed with Enzyme A (p = 0.006). The most efficient FCR was noted in the CO+EA group (1.35 ± 0.012), which was significantly better than the NC and NC+EA groups. This reflects enhanced feed efficiency, particularly under nutrient-sufficient conditions. Enzyme B did not significantly affect FCR (p = 0.164). All groups showed similar values, including CO, CO+EB, NC, and NC+EB, indicating no observable improvement in feed efficiency with this enzyme. In contrast, Enzyme C significantly improved FCR (p = 0.001). Birds in the NC+EC group had a lower FCR (1.45 ± 0.011) than the NC group (1.54 ± 0.032), suggesting partial mitigation of poor feed efficiency under nutrient-deficient conditions. Both the CO and CO+EC groups exhibited similarly low FCR values (1.41 ± 0.017), reinforcing the enzyme's potential to enhance feed utilization. (Table 5).

The variations in growth performance among the protease enzymes (A, B, C) indicate differences in their efficacy. Enzyme A significantly improved BWG and FCR, and increased FI in nutrient-deficient diets supplemented with the enzyme, reflecting its ability to enhance protein digestion, thereby increasing amino acid availability for growth in both balanced (CO + EA) and nutrient-deficient (NC + EA) diets. The reduced BWG in NC highlights the critical role of adequate nutrition, as supported by Peñuela-Sierra *et al.* (2024), who noted that nutrient-deficient diets limit amino acid availability, restricting growth. The increased FI in NC + EA indicates compensatory feeding behaviour to address nutrient deficiencies, as broilers consume more feed to meet nutritional needs, consistent with Leeson and Summers (2005). Enzyme C improved FCR in balanced diets supplemented with the enzyme (CO + EC), suggesting enhanced metabolic efficiency due to improved protein digestibility. These results align with Cowieson *et al.* (2020), who reported that protease supplementation in balanced diets optimizes nutrient utilization, leading to improved feed efficiency. The improvements in NC + EA and NC + EC partially enhanced growth performance but were associated with higher feed consumption, indicating that the enzyme's effect was limited and suggesting that matrix values may overestimate protease efficacy in nutrient-deficient diets, as noted by McCafferty *et al.* (2022). Conversely, Enzyme B showed no significant effects, indicating limited impact, possibly due to lower specificity, as reported by Kiarie *et al.* (2021).

Table (5): Least-square means ± SE for the effects of protease enzyme a, b, and c treatments on broiler performance (1-42) days.

		Enzyme A			
	Control	**Control + Enzyme A**	**Negative Control**	**Negative Control + Enzyme A**	**Probability**
BWG	2575^{a}±21	2657^{a}±16	2418^{b}±38	2577^{a}±52	0.002
FI	3621^{b}±36	3591^{b}±21	3559^{b}±37	3732^{a}±36	0.012
FCR	1.41^{ab}± 0. 017	1.35^{b}±0. 012	1.47^{a}± 0. 015	1.45^{a}± 0. 035	0.006
		Enzyme B			
	Control	**Control + Enzyme B**	**Negative Control**	**Negative Control + Enzyme B**	**Probability**
BWG	2575±21	2549±20	2577±37	2550±35	0.844
FI	3621 ±36	3544±47	3715 ±63	3610±55	0.175
FCR	1.41± 0. 017	1.39± 0. 0104	1.44± 0. 020	1.41± 0. 012	0.164
		Enzyme C			
	Control	**Control + Enzyme C**	**Negative Control**	**Negative Control + Enzyme C**	**Probability**
BWG	2575±21	2579±23	2422±80	2509±42	0.11
FI	3621 ±36	3645±44	3730 ±70	3641±54	0.49
FCR	1.41^{b}±0. 017	1.41^{b} ±0. 017	1.54^{a}±0. 032	1.45^{b} ±0. 011	0.001

BWG: Body Weight Gain (g), FI: Feed Intake (g), FCR: Feed Conversion Ratio, (M±SE) = Mean ± Standard Error. a, b, c, and d = Means within the same column with different superscripts are significantly different ($P < 0.05$*).*

Effects of protease enzyme supplementation on carcass traits:

Carcass traits (carcass weight, front half weight, back half weight, liver weight, and heart weight) of broiler chickens at 42 days of age are presented in Table 6.

Table (6): Least-Square Means ± SE for the effects of protease enzyme A, B, and C supplementation on carcass traits of Broilers at 42 days.

	Enzyme A								
	Control	**Control + Enzyme A**		**Negative Control**		**Negative Control + Enzyme A**		**Probability**	
	(gm)	**(%)**	**(gm)**	**(%)**	**(gm)**	**(%)**	**(gm)**	**(%)**	
Carcass	2186 ±69	71.4 ± 2.3	2326 ±64	71.8 ± 2.0	2160 ±43	69.9 ± 1.4	2235±62	67.5 ± 1.9	0.246
Front half	1272 ±50	41.6 ± 1.6	1372 ±39	42.3 ± 1.2	1246 ±26	40.3 ± 0.8	1310±37	39.6 ± 1.1	0.135
Back half	915±24	29.9 ± 0.8	959±29	29.6 ± 0.9	914±17	29.6 ± 0.5	924±28	27.9 ± 0.8	0.558
Liver	2.60 ± 0.14	66.9±3.5	65.9±3.5	2.48 ± 0.13	68.4±3.4	2.83 ± 0.14	71.7±3.4	2.78 ± 0.13	0.57
Heart	9.6±0.34	0.37 ± 0.01	9.1±0.35	0.34 ± 0.01	9.3±0.34	0.38 ± 0.01	9.7±0.34	0.38 ± 0.01	0.691
	Enzyme B								
	Control	**Control + Enzyme B**		**Negative Control**		**Negative Control + Enzyme B**		**Probability**	
	(gm)	**(%)**	**(gm)**	**(%)**	**(gm)**	**(%)**	**(gm)**	**(%)**	
Carcass	2186 ^{b} +69	71.4^{b} ± 2.3	2430 ^{a}±61	72.1^{a} ± 1.8	2188^{b} +75	69.7^{b} + 2.4	2291^{ab}±60	69.8^{ab} ± 1.8	0.044
Front half	1272 ^{b} ±50	41.6^{b} ± 1.6	1437 ^{a}±47	42.6^{a} ± 1.4	1267 ^{b} ±46	40.4^{b} ± 1.5	1338 ^{ab} ±43	40.8^{ab} ± 1.3	0.048
Back half	915 ±24	29.9 ± 0.8	992 ±18	29.4 ± 0.5	934 ±30	29.7 ± 1.0	957 ±20	29.2 ± 0.6	0.137
Liver	66.6±2.3	2.59 ± 0.09	68±2.3	2.67 ± 0.09	68.6±2.2	2.66 ± 0.09	73.3±2.2	2.87 ± 0.09	0.191
Heart	9.4±0.34	0.37 ± 0.01	8.6±0.34	0.34 ± 0.01	9.5±0.33	0.37 ± 0.01	8.6±0.33	0.34 ± 0.01	0.14
	Enzyme C								
	Control	**Control + Enzyme C**		**Negative Control**		**Negative Control + Enzyme C**		**Probability**	
	(gm)	**(%)**	**(gm)**	**(%)**	**(gm)**	**(%)**	**(gm)**	**(%)**	
Carcass	2186 ^{b} ±69	71.4^{b} ± 2.3	2385^{a}±55	73.2^{a}± 1.7	2116^{b} ±54	70.1^{b} ± 1.8	2129^{b}±51	71.0^{b} ± 1.7	0.008
Front half	1272 ^{b} ±50	41.6^{b} ± 1.6	1423 ^{a}±39	43.7^{a} ± 1.2	1230 ^{b} ±36	40.7^{b} ± 1.2	1249^{b} ±32	41.6^{b} ± 1.1	0.006
Back half	915 ^{b} ±24	29.9^{b} ± 0.8	988^{a} ±24	30.3^{a} ± 0.7	889 ^{b} ±23	29.4^{b} ± 0.8	895^{b} ±22	29.8^{b} ± 0.7	0.019
Liver	65.8±3.2	2.56 ± 0.12	73.6±3.5	2.85 ± 0.14	69.1±3.3	2.85 ± 0.14	72.8±3.3	2.90 ± 0.13	0.32
Heart	9.7±0.45	0.38 ± 0.02	8.3±0.53	0.32 ± 0.02	9.3±0.5	0.38 ± 0.02	8.5±0.5	0.34 ± 0.02	0.194

(M±SE) = Mean ± Standard Error. carcass weight, front half weight, and hind half weight were analyzed using one-way analysis of variance (ANOVA). Heart and liver weights were analyzed using analysis of covariance (ANCOVA) with live body weight as a covariate to adjust for body size effects. a, b, c, and d = Means within the same column with different superscripts are significantly different (P < 0.05).

Carcass weight and yield:

Supplementation with Enzyme A did not significantly affect carcass weight or yield (P = 0.246). Although a numerical increase in carcass weight was observed in both CO + EA (2326 g) and NC + EA (2235 g) groups compared to their supplemented counterparts, differences were not statistically significant. Enzyme B significantly improved carcass weight (P = 0.044). The CO + EB group showed the highest carcass weight (2430 g) and yield (72.1%), significantly surpassing both CO and NC groups.

The NC + EB group also showed a modest numerical improvement in weight and percentage over NC alone. Enzyme C supplementation also resulted in a significant improvement in carcass weight (P = 0.008). The CO + EC group had a significantly higher carcass weight (2385 g) and yield (73.2%) compared to other treatments. No significant improvement was observed in the NC or NC + EC groups (Table 6).

Front half weight and yield:

Enzyme A did not significantly affect front half weights (P = 0.135). All values remained statistically similar across treatments, although numerically higher values were observed with enzyme supplementation. In contrast, Enzyme B significantly increased front half weight (P = 0.048). Birds fed CO + EB had the highest front half weight (1437 g) and yield (42.6%), which was significantly greater than CO and NC treatments. Enzyme C also showed a significant effect (P = 0.006), with the CO + EC group yielding the highest front half weight (1423 g) and percentage (43.7%). No significant improvement was observed in NC or NC + EC groups(Table 6).

Back half weight and yield:

Back half parameters were unaffected by Enzyme A supplementation (P = 0.558). Minor, non-significant numerical increases were observed in the CO + EA group (959 g) compared to controls. Although Enzyme B produced numerical increases in the C + EB and NC + EB groups, the differences were not statistically significant (P = 0.137). Enzyme C showed a significant improvement in back half weight (P = 0.019). The CO + EC group had the highest values (988 g and 30.3%), which were significantly greater than the control and NC treatments (Table 6).

Liver weight and percentage:

Liver weight and relative liver percentage were not significantly affected by any of the enzyme treatments (P > 0.05). Although slight numerical increases were observed with enzyme supplementation particularly in the NC + B and CO + C groups these differences were not statistically significant (Table 6).

Heart weight and percentage:

No significant effects on heart weight or relative heart weight were detected for any enzyme (P > 0.05). Mean heart weights ranged between 8.3–9.7 g, and percentages between 0.32%–0.38%, with no consistent pattern across treatments (Table 6).

In summary, the variations in carcass traits among the protease enzymes (A, B, C) indicate differences in their efficacy. Enzymes B and C demonstrated significant improvements in carcass yield and major portions (front and back half), particularly in balanced diets. These results are consistent with Leeson and Summers (2005), who reported that protease enzymes in balanced diets improve nutrient availability for muscle development. Enzyme A, although not statistically significant, showed consistent numerical improvements in live weight and carcass traits. None of the enzymes significantly affected liver or heart weights. The beneficial effects of Enzymes B and C on carcass characteristics were more evident in birds receiving adequate nutrition, suggesting enzyme efficacy may be diet dependent. Conversely, no significant effects were observed on liver or heart weights, aligning with Havenstein *et al.* (2003), who noted that protease enzymes typically do not influence these organ weights due to their limited dependence on enhanced protein digestion.

Effects of protease enzyme supplementation on blood biochemical parameters:

Blood biochemical parameters (uric acid and creatinine) of broiler chickens at 42 days of age are presented in Table 7.

Uric acid concentration:

The dietary inclusion of protease enzymes A,B, or C did not result in statistically significant changes in serum uric acid levels across the different dietary treatments (Table 7).

For Enzyme A, uric acid concentrations ranged from 0.119 ± 0.003 mg/dL in the NC and NC+EA groups to 0.125 ± 0.002 mg/dL in the CO+EA group, with no significant differences observed (p = 0.163). Similarly, Enzyme B had no significant effect on uric acid levels (p = 0.916). All groups exhibited very similar values, ranging narrowly between 0.122 and 0.124 mg/dL. In the Enzyme C treatments, uric acid levels ranged from 0.119 ± 0.005 mg/dL (NC) to 0.123 ± 0.003 mg/dL (NC+EC), with no statistically significant differences among groups (p = 0.876). These findings suggest that uric acid metabolism was not notably affected by protease supplementation under either balanced or nutrient-deficient dietary conditions.

Table (7): Least-Square Means ± SE for the effects of enzyme a, b and c protease treatments on uric acid and creatinine in broiler chickens at 42 days of age

		Enzyme A			
	Control	**Control + Enzyme A**	**Negative Control**	**Negative Control + Enzyme A**	**Probability**
Uric acid	0.1230 ±	0.125 ± 0.002	0.119 ± 0.003	0.119 ± 0.003	0.163
Creatinine	0.2051 ±	0.195 ± 0.012	0.195 ± 0.005	0.192± 0.01	0.805
		Enzyme B			
	Control	**Control + Enzyme B**	**Negative Control**	**Negative Control +**	**Probability**
Uric acid	0.123± 0.002	0.122 ± 0.002	0.124 ± 0.002	0.122± 0.002	0.916
Creatinine	0.205 ± 0.009	0.193 ± 0.011	0.202± 0.007	0.221± 0.009	0.203
		Enzyme C			
	Control	**Control + Enzyme C**	**Negative Control**	**Negative Control +**	**Probability**
Uric acid	0.123±0.002	0.122 ± 0.002	0.119 ± 0.005	0.123± 0.003	0.876
Creatinine	0.205 ±0.009	0.2008 ±0.009	0.231 ±0.014	0.202±0.006	0.156

(M±SE) = Mean ± Standard Error.

Creatinine concentration:

No significant differences were detected in serum creatinine concentrations in response to any of the enzyme treatments (Table 7). For Enzyme A, creatinine levels were consistent across groups ($p = 0.805$), ranging from 0.192 ± 0.010 mg/dL (NC+EA) to 0.2051 ± 0.008 mg/dL (CO). With Enzyme B, although numerical variations were observed most notably a slight increase in the NC+EB group (0.221 ± 0.009 mg/dL) these differences did not reach statistical significance ($p = 0.203$). Other values ranged from 0.193 ± 0.011 mg/dL (CO+EB) to 0.202 ± 0.007 mg/dL (NC). For Enzyme C, creatinine levels ranged from 0.2008 ± 0.009 mg/dL (C+EC) to 0.231 ± 0.014 mg/dL (NC), but the differences were not statistically significant ($p = 0.156$). Thus, creatinine concentrations remained stable regardless of enzyme type or dietary condition, indicating no apparent impact on renal function or nitrogen waste metabolism under the current experimental conditions.

Overall, supplementation with protease enzymes A, B, or C did not significantly affect serum uric acid or creatinine levels in broiler chickens. These results suggest that nitrogen metabolism and renal biomarkers remained stable and unaffected by protease inclusion in both balanced and nutrient-deficient diets. The absence of significant effects on blood biochemical parameters (uric acid and creatinine) across all protease enzymes (A, B, C) suggests that these enzymes did not markedly alter protein metabolism or kidney function in broilers. This absence of impact may be attributed to the enzymes' primary role in enhancing dietary protein digestibility, which does not directly influence circulating levels of uric acid or creatinine, as these parameters are more closely related to metabolic and excretory processes rather than dietary protein utilization. These results are consistent with Song *et al.* (2023), who found that protease supplementation did not significantly affect uric acid or creatinine levels in broilers. Similarly, Ndazigaruye *et al.* (2024) reported that protease enzyme supplementation had no discernible effect on serum creatinine levels, supporting the notion that protease enzymes primarily enhance nutrient digestibility without altering metabolic or renal function markers.

Effects of protease enzyme supplementation on apparent nutrient ileal digestibility:

Nutrient digestibility parameters [Ether Extract] and [Crude Protein] of broiler chickens at 42 days of age are presented in Table 8.

Ether extract (ee) digestibility:

No significant effects of protease enzyme supplementation were observed on (EE) digestibility across any of the treatment groups. For Enzyme A, EE digestibility ranged from 82.0 ± 5.8% in the NC group to 90.1 ± 1.3% in the CO+EA group; however, these differences were not statistically significant ($p = 0.370$), despite the numerical increase in the enzyme-supplemented control. With Enzyme B, EE digestibility values remained comparable among treatments ($p = 0.864$), ranging from 82.2 ± 3.7% (CO) to 86.2 ± 3.8% (NC+EB). No significant differences were observed, indicating minimal impact of this

enzyme on fat digestibility. Similarly, Enzyme C did not significantly influence EE digestibility (p = 0.610). Values ranged from 80.3 ± 2.6% (NC) to 85.7 ± 2.7% (CO+EC), showing minor numerical variation but no statistical relevance.

Table (8): Least-Square Means ± SE for the effects of protease enzyme a, b and c treatments on apparent nutrient ileal digestibility in broiler chickens at 42 days.

Enzyme A					
	Control	**Control + Enzyme A**	**Negative Control**	**Negative Control + Enzyme A**	**Probability**
Ether Extract	82.2±3.7	90.1±1.3	82±5.8	83.8±1.9	0.37
Crude Protein	72.8^{a} ±0.52	75.8^{a}±2.32	65.5^{b} ±2.14	71.2^{a} ±1.44	0.004
Enzyme B					
	Control	**Control + Enzyme B**	**Negative Control**	**Negative Control + Enzyme B**	**Probability**
Ether Extract	82.2±3.7	85±3.3	84.3±2.7	86.2±3.8	0.864
Crude Protein	72.8^{b} ±0.52	76.3^{a}±2.32	71^{b}±2.14	76.2^{a} ±1.44	0.007
Enzyme C					
	Control	**Control + Enzyme C**	**Negative Control**	**Negative Control + Enzyme C**	**Probability**
Ether Extract	82.2±3.7	85.7±2.7	80.3±2.6	85.5±4	0.61
Crude Protein	72.8 ±0.6	73.7±2.4	71±0.9	69.3 ±1.3	0.19

SE: Standard Error.

Crude protein (cp) digestibility:

Unlike EE, CP digestibility was significantly affected by enzyme supplementation, particularly with Enzymes A and B. For Enzyme A, CP digestibility was significantly lower in the NC group (65.5 ± 2.14%) compared to all other treatments (p = 0.004). The highest values were observed in CO+EA and CO, with NC+EA showing partial recovery of digestibility under nutrient-deficient conditions. Enzyme B also significantly improved CP digestibility (p = 0.007). Birds in both CO+EB and NC+EB groups exhibited significantly higher digestibility compared to the control and NC groups, indicating consistent enhancement regardless of diet type. In contrast, Enzyme C did not significantly influence CP digestibility (p = 0.190). Although numerically higher CP digestibility was seen in CO+EC compared to NC+EC and NC, these differences did not reach statistical significance.

Ether extract digestibility was unaffected by any enzyme treatment. However, CP digestibility improved significantly with supplementation of Enzymes A and B, particularly in nutrient-deficient diets, suggesting enhanced proteolysis and protein utilization. Enzyme C, although associated with modest numerical increases, did not produce statistically significant effects on CP digestibility.

In summary, Enzyme C showed no significant effects on CP digestibility, whereas Enzymes A and B significantly improved CP digestibility, likely due to their ability to enhance the breakdown of dietary proteins, thereby increasing amino acid availability in balanced and nutrient-deficient diets supplemented with these enzymes. These results are consistent with Cowieson *et al.* (2020), who reported that protease enzymes in broiler diets improve crude protein digestibility by enhancing protein hydrolysis.

Numerical improvements in EE digestibility were observed across all enzymes, particularly in treatments supplemented with proteases (CO + EA, CO+ EB, CO + EC, NC + EA, NC + EB, NC + EC). This enhancement may be attributed to several mechanisms. proteases likely hydrolyze protein-lipid complexes in feed ingredients, releasing bound lipids for better digestion by endogenous lipases. Also, improved protein digestion may optimize the intestinal environment, enhancing bile salt activity crucial for lipid emulsification and absorption. Lastly, reduced undigested protein in the lower gut may decrease microbial fermentation, spare endogenous enzyme resources and improve lipase efficiency. These observations align with Kiarie *et al.* (2021), who noted that protease supplementation enhances lipid digestibility by improving the availability of dietary lipids in broilers.

European production efficiency factor (epef)

The effects of dietary enzyme supplementation on (EPEF) varied among the three enzyme types tested (A, B, and C). of broiler chickens at 42 days of age is presented in Table 9.

Table (9): Effects of protease enzyme a, b and c treatments on european production efficiency factor (EPEF) for broiler chickens at 42 days of age

Enzyme A				
	Control	**Control + Enzyme**	**Negative Control**	**Negative Control +**
EPEF	434	468	391	394
Enzyme B				
	Control	**Control + Enzyme**	**Negative Control**	**Negative Control +**
EPEF	434	427	411	401
Enzyme C				
	Control	**Control + Enzyme**	**Negative Control**	**Negative Control +**
EPEF	434	412	342	397

EPEF: European Production Efficiency Factor. No statistical analysis was performed on the data presented in this table

Supplementation with Enzyme A improved EPEF under both CO and nutrient-deficient diets. Birds fed the control diet supplemented with Enzyme A (CO+EA) achieved the highest EPEF value of468. In nutrient-deficient conditions, supplementation also yielded a modest improvement, with the NC+EA group scoring394 versus391 in the non-supplemented NC group. These results suggest that Enzyme A enhances production efficiency, particularly under CO dietary conditions. In contrast, Enzyme B supplementation did not yield notable improvements in EPEF, indicating inconsistent or minimal impact on overall production efficiency. Supplementation with Enzyme C produced mixed results, suggested that Enzyme C may be more beneficial in mitigating performance losses under nutrient-deficient conditions rather than enhancing performance under CO diets.

In summary, Among the tested enzymes, Enzyme A demonstrated the most consistent improvement in production efficiency, particularly in well-balanced diets. Enzyme C showed potential under nutrient-deficient conditions, while Enzyme B had limited or inconsistent effects on EPEF.

In general, the results revealed marked differences among the tested protease enzymes (A, B, and C) in all tested parameters. These performance differences can be attributed to several key factors, Enzyme Activity and Dosage, Substrate Specificity, Interaction with Diet Composition And finally to Gut Health Modulation, **Bedford (2020)** highlighted the role of high-activity proteases in improving gut integrity and feed efficiency. The limited improvements in nutrient-deficient diets suggest that matrix values may overestimate enzyme contributions in such conditions, as **Cowieson *et al.* (2019)** noted in their study on mono-component proteases.

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تقييم مقارن لثلاثة منتجات تجارية لانزيمات البروتياز على الأداء الإنتاجي ، صفات الذبيحة، ومكونات الدم، ومعاملات الهضم الظاهري للعناصر الغذائية في الأمعاء الدقيقة، ومعامل الإنتاج الاوروبي ، في دجاج التسمين المُغذّى على علائق قياسية وأخرى مُنخفّضة العناصر الغذائية.

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قيّمت هذه الدراسة تأثير ثلاثة منتجات تجارية للإنزيمات المحللة للبروتين - البروتياز (أ، ب، ج)، مشتقة من *بكتيريا باسيلوس ليشينيفورميس* ، على دجاج التسمين المُغذّى إما على علائق قياسية (C) تم حسابها وفقا للاحتياجات القياسية لسلالة الروص 308 او تم خفض محتواها من العناصر الغذائية وفقا لقيم المصفوفة الخاصة بالمصنع (NC) مُضاف إليها أو بدون مُكمّلات إنزيمية على مدى 42 يومًا. وُزّع 600 فرخ ذكر من سلالة روس 308، بعمر يوم واحد، عشوائيًا على 10 مجموعات علاجية (5 مكررات، كل منها 12 طائرًا)، أُدرجت الإنزيمات بناءً على الجرعات المُوصى بها من قِبَل المُصنّع وقيم المصفوفة: الإنزيم أ (600,000 وحدة/جم عند 500 جم/طن)، والإنزيم ب (60,000 وحدة/جم عند 250 جم/طن)، والإنزيم ج (80,000 وحدة/جم عند 50 جم/طن).
وشملت معايير الأداء التي تم تقييمها زيادة وزن الجسم، وتناول العلف ، ونسبة تحويل العلف ، وسمات الذبيحة، وبعض مكونات الدم (حمض البوليك، الكرياتينين)، ومعاملات هضم العناصر الغذائية في منطقة اللفائفي (البروتين الخام ، مستخلص الأثير ، وعامل كفاءة الإنتاج الأوروبي .
أدى الإنزيم (أ) إلى تحسين ملحوظ في نسبة كتلة الجسم ($P = 0.002$) ونسبة تحويل العلف ($P = 0.006$) في كل من النظام الغذائي (C) والنظام الغذائي (NC)، مع ارتفاع في مؤشر استهلاك الغذاء في مجموعة ($P = 0.012$ + NC) (EA). حسّن الإنزيم (ج) نسبة تحويل العلف ($P = 0.001$) وزاد وزن الذبيحة ($P = 0.008$)، والنصف الأمامي ($P = 0.006$)، والنصف الخلفي ($P = 0.019$) في النظام الغذائي (C). حسّن الإنزيم (ب) النصف الأمامي ($P = 0.048$) ووزن الذبيحة الإجمالي ($P = 0.044$) في النظام الغذائي (C). ولم تتأثر معايير الدم بأي إنزيم من الانزيمات الثلاثة المختبرة ($P > 0.05$). زادت قابلية هضم البروتين الخام بشكل معنوي بفضل الإنزيمين (ا $P = 0.00$) A و(ب $P = 0.00$) B، بينما لم تتأثر قابلية هضم مستخلص الاثير بشكل معنوي عبر جميع المعاملات ($P > 0.05$). كان EPEF أعلى رقميًا في مجموعة (C + EA) (468)، مما يشير إلى تحسن الكفاءة الكلية. تُسلّط هذه النتائج الضوء على الاستجابات الإنزيمية الخاصة، حيث يُقدّم الإنزيم "أ" أفضل فوائد الأداء ثباتًا، لا سيما في ظلّ الظروف الغذائية المناسبة. وتُؤكّد هذه النتائج على أهمية اختيار أنواع وجرعات الإنزيم المناسبة لتركيبة العلف لتحسين إنتاجية دجاج التسمين.

الكلمات المفتاحية *: دجاج التسمين، صفات الذبيحة، هضم العناصر الغذائية، كفاءة الإنتاج، إنزيمات البروتياز*