

Impact of Feeding Growing Japanese Quail on Low Protein Diets Supplemented with Protease Enzyme

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Abstract

Background: The increasing cost of protein-rich feedstuffs presents a significant challenge in poultry production, especially in developing countries. Feed additives such as protease can enhance protein digestibility. This could reduce crude protein levels while maintaining poultry performance, making it a promising solution to improve feed efficiency and reducing costs.

Aim: the aim of this study to evaluate the effects of low protein diets with or without protease enzyme supplementation on the growth performance, carcass traits, immune response, antioxidant indices, and blood metabolites of growing Japanese quail.

Methods: A total of 294 one-week-old Japanese quail chicks (15.73 ± 0.18 g) were randomly assigned to seven groups each with three replicates (14 chicks per replicate). Birds were fed diets containing varying levels of crude protein (CP): G1 control, 24% CP (diet 1), G2 22.8% CP, (diet 2), G3 (diet 2 + 1.4g protease /kg diet), G4 21.6% CP (diet 3), G5 (diet 3 + 1.4g protease /kg diet), G6 20.4% CP (diet 4), and G7 (diet 4 + 1.4g protease /kg diet).

Results: A linear decline in weight and body weight gain were observed as dietary protein levels decreased, while protease enzyme supplementation numerically improved body weight and weight gain, it did not had a significant effect at the same protein levels. Higher protein levels led to increased feed intake and improved feed conversion ratio (FCR), although the effects of protease were

inconsistent. Carcass traits remained unaffected, except for increased liver weight in the lower-protein groups. Liver function markers as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were highest in the severely protein-restricted groups, indicating potential stress. Protease improved antioxidant status and reduced corticosterone levels but did not influence immune parameters. Economic efficiency was highest at 21.6% CP, particularly without protease, while the 24% CP diet exhibited the lowest efficiency.

Conclusion: From these studies we can conclude that the supplementation of low diet protein with protease enzyme enhanced antioxidant status and stress mitigation but had a limited effect on growth and immunity. The dietary CP (22.8%) maintained final body weight and weight gain similar to 24% CP, but with the advantage of 12.23% greater economic efficiency.

Keywords: grower Japanese quail, protein level, protease, growth performance, blood parameters, antioxidant, carcass traits.

1. Introduction

The increasing cost of protein-rich feedstuffs poses a significant challenge to the poultry industry, a trend that has been exacerbated in recent years (*Spring, 2013*). In intensive poultry production systems, protein costs typically account for 60-70% of total feed expenses (*Mallick et al., 2020*). Furthermore, feeding costs generally constitute 65-70% of the overall production expenses in poultry farming (*Attia et al., 2020; FAO, 2006*).

Rising protein costs necessitate the exploration of cost-effective feed alternatives and strategies to alleviate financial pressure on the poultry sector (*Vlaicu et al., 2024*). Reducing dietary protein levels, particularly in species such as the Japanese quail (*Coturnix coturnix*

japonica), while enhancing protein utilization, presents a viable solution to sustain industry growth and profitability (*Zampiga et al., 2021; Alfonso-Avila et al., 2022*).

Supplementing poultry diets with exogenous protease enzymes enhances protein digestibility and bioavailability, offering a cost-effective strategy to optimize nutrition in the face of rising feed costs (*Silva et al., 2021*). This study evaluates protease efficacy in low-protein diets, providing insights for industry application and future research.

2. Materials and Methods

The study was conducted at the Animal Education and Research Farm, Faculty of Environmental and Agricultural Sciences, Al-Arish, North Sinai, Egypt, under the

supervision of the Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Suez Canal University. The experiment was carried out from June 22 to July 28, 2023. All experimental procedures were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine at Suez Canal University (*Approval No. 2022054*).

2.1. Animals and experimental design

A total of 294, 7-day-old Japanese quail chicks with an initial mean body weight of 15.73 ± 0.18 g were sourced from a commercial veterinary center. These chicks were randomly assigned to seven experimental groups, each consisting of three replicates (14 chicks per replicate). The groups are detailed in Table 1.

The experimental diets were formulated to provide consistent energy levels (2900 kcal metabolizable energy per kg) across all treatment groups. Protein levels in the experimental diets were reduced by 5%, 10%, and 15% relative to the control diet. The diets were provided from the chicks' 1st week of age until the end of the 5-week experimental period.

2.2. Housing and Management

Quail chicks were housed in a five-tier battery system that maintained controlled temperature (35°C initially, then 31–34°C), ventilation, and humidity. The cages were equipped with central drinkers and feeders, with daily water

replacement and regular sanitation. A photoperiod of 23 hours of light and 1 hour of darkness, was maintained (*Ionita et al., 2012*).

2.3. Diet formulation and ingredient analysis

Feed ingredients were analyzed at the Regional Center for Food and Feed (RCFF) in Cairo following *AOAC (2016)* methods to ensure nutritional adequacy for Japanese quail (*NRC, 1994*). The control diet (24% CP, 2900 kcal ME/kg) was compared to experimental diets with reduced protein levels (22.8%, 21.6%, 20.4% CP) with and without protease enzyme supplementation.

2.4. Exogenous protease: The protease enzyme **Brozyme** (Prozyme-AD). It is a bacterial-derived single-enzyme protease with **25,000 IU/g activity**, obtained from Phyto Bio-Chem Company (Mit Bader Khamees, El Mansoura, Egypt; Ref No: HI-ENZ-P). Protease was incorporated at **1.4 g/kg diet**, first mixed with fine feed particles, then blended with the entire diet.

2.5. Data Collection

2.5.1 Growth performance (body weight development) and feed consumption:

Growth performance data, including live body weight and feed intake, were recorded weekly until the end of the fifth week of the experimental period. Initial body weight was measured at 7 days of age. The live body weight of each replicate was weighed using a digital balance (SF-400D, ATOM, China). Body weight

gain was calculated as the difference between successive weights (**Brady, 1968**), and feed consumption was determined by subtracting leftovers from the total.

2.5.2 Carcass traits

At the age of 42 days, after 12 hours of feed withdrawal, 42 birds (2 per replicate) were slaughtered using halal neck cutting method (**Farouk et al., 2014**). Feathers, heads, and feet were removed, and the eviscerated carcasses were cleaned, weighed, and expressed as a percentage of live body weight. Giblet weight (heart, liver, gizzard) was calculated relative to the body weight (**Esen et al., 2006**).

2.5.3 Serum biochemical indices

Blood samples were collected in plain tubes during slaughter, centrifuged at 3,000 rpm for 5 minutes, and stored at -20°C until analysis. Serum biochemistry was assessed, including protein fractions

(total protein, albumin and globulin), liver enzymes such aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and immunoglobulins (IgG, IgM) were measured using a commercial kit and automatic biochemical analyzer. Antioxidant markers Superoxide Dismutase (SOD) and Glutathione (GSH) were assessed calorimetrically with a microplate spectrophotometer using a commercial kit.

2.6 Statistical Analysis

All statistical analysis were conducted using the Statistical Package for the Social Science (SPSS 16) (**Coakes & Steed, 2009**). Differences among the means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests, as outlined by (**Snedecor and Cochran., 1989**)

Table 1: *Experimental design*

Treatments	Protein level (%)	Protease supplementation (1.4g/kg diet)
G1	24	-
G2	22.8	-
G3	22.8	+
G4	21.6	-
G5	21.6	+
G6	20.4	-
G7	20.4	+

Table 2: *ingredients and nutrient composition of the experimental diets fed to growing Japanese quails in the present study*

Dietary protein level% Ingredients	Diet 1 (24%)	Diet 2 (22.8%)	Diet 3 (21.6%)	Diet 4 (20.4%)
Ground yellow corn (7% CP) ^a	56.47	58.78	61.07	63.28
Soybean meal (46 % CP) ^a	33.24	32.55	31.79	30.39
Poultry meal (51.6 % CP) ^a	3.5	3.5	3.5	3.5
C Corn gluten (63 % CP) ^a	4.41	2.75	1.13	0.00
Di-calcium phosphate (22% Ca&19%P)	0.522	0.538	0.555	0.615
Limestone (38% Ca)	1.26	1.23	1.246	1.439
Salt	0.30	0.30	0.30	0.30
L-Lysine (Purity 99%)	0.106	0.135	0.167	0.212
DL-Methionine (Purity 99%)	0.092	0.117	0.142	0.164
Mineral & Vitamins Premix ^b	0.10	0.10	0.10	0.10
Calculated composition				
Crude protein (%)	24.00	22.80	21.60	20.41
ME (Kcal per kg)	2900.30	2900.55	2900.06	2900.83
Calcium (%)	0.81	0.80	0.80	0.89
Available phosphorus (%)	0.300	0.301	0.302	0.310

- a) Determined values
- b) Premix (vilofoss ® premix for broiler 0.1%, origin Germany, importer Interpharma Corporation IPC): Each 1kg contains the following vitamins and minerals: Vit.A12 MIU, VitD₃. 4MIU, VitE. 30000 mg, B₁1000 mg, B₂ 5000 mg, B₆ 2500 mg, B₁₂ 20000 mcg, k3 3000 mg, biotin 100000 mcg, pantothenic acid 2000 mg, niacinamide 35000 mg, folic acid 3000 mg, selenium 226 mg, copper sulfate 5000 mg, cobalt 100 mg, manganese 62000 mg, zinc 75000 mg, iodate 1.300 mg, selenium 226 mg, cobalt 100 mg. carrier calcium carbonates up to 1kg (high mix premix, patch NO. 9475, production 18.11.2022).

3. Results

3.1. Body weight and body weight gain

Table 3: Average live body weight of grower Japanese quail fed a diet containing different levels of protein with or without protease enzyme supplementation (g/bird).

Groups Weeks/age	G1	G2	G3	G4	G5	G6	G7	P values
Initial weight(g) (7 days)	15.33± 0.82 ^a	16.87± 0.43 ^a	15.73± 0.78 ^a	16.67± 0.66 ^a	15.73± 0.62 ^a	15.63± 0.38 ^a	15.15± 0.59 ^a	0.923
1 st (14 days)	47.36± 1.05 ^{ab}	46.31± 1.57 ^{ab}	47.65± 1.74 ^a	43.29± 0.80 ^{bc}	45.07± 0.84 ^{ab}	39.93± 1.91 ^c	40.50± 1.11 ^c	0.001
2 nd (21 days)	103.31 ±1.76 ^a	96.50 ±1.49 ^b	98.07 ±1.60 ^b	94.93 ±1.74 ^b	96.36 ±1.91 ^b	86.50 ±1.31 ^c	88.86 ±1.43 ^c	0.001
3 rd (28 days)	153.64± 1.06 ^a	152.71± 1.12 ^a	154.29± 1.77 ^a	147.36± 2.41 ^b	147.57± 1.51 ^b	136.43± 1.57 ^c	141.07±2.19 ^c	0.001
4 th (35 days)	198.50 ±1.12 ^a	194.36 ±1.54 ^{abc}	196.43 ±1.52 ^{ab}	191.36 ±1.74 ^{bc}	190.43 ±2.65 ^c	183.67 ±2.50 ^d	183.21 ±1.31 ^d	0.001
5 th (42 days)	237.71± 3.03 ^a	233.50± 2.98 ^{ab}	236.29± 2.55 ^a	228.15± 1.87 ^{cd}	229.21± 2.19 ^{bc}	217.43± 2.29 ^c	222.64±2.19 ^{de}	0.001

Values are expressed as mean ± standard error (SE).

Values in the same row with different superscripts are significantly different (p<0.05).

G1: control group 24% CP (diet 1), G2: 22.8% CP (diet 2), G3: (diet 2) with 1.4g protease/kg diet, G4: 21.6% CP (diet 3), G5: (diet 3) with 1.4g protease/kg G6: 20.4% CP (diet 4), G7: (diet 4) with 1.4g protease/kg

Table 4: Average body weight gain of grower Japanese quail fed diet containing different levels of protein with or without protease enzyme supplementation (g/bird/week).

Groups Period/Week	G1	G2	G3	G4	G5	G6	G7	P values
Initial weight(g) (7 days)	15.33± 0.82 ^a	16.87± 0.43 ^a	15.73± 0.78 ^a	16.67± 0.66 ^a	15.73± 0.62 ^a	15.63± 0.38 ^a	15.15± 0.59 ^a	0.923
1-2	31.98± 0.72 ^a	29.40± 1.36 ^{ab}	31.92± 1.02 ^a	26.53± 0.77 ^{bc}	29.87± 1.11 ^a	24.31 ± 1.06 ^c	25.25 ± 0.77 ^c	0.001
2-3	55.30± 0.19 ^a	50.10± 2.54 ^{bc}	50.60 ± 2.24 ^{bc}	51.73 ±0.75 ^{ab}	50.70 ± 0.75 ^{bc}	46.50 ± 0.65 ^c	48.33 ± 1.33 ^{bc}	0.022
3-4	51.16± 0.84 ^b	56.30± 1.41 ^a	56.06± 2.27 ^a	52.45± 1.45 ^{ab}	51.27± 0.45 ^b	49.98± 0.37 ^b	52.22± 1.78 ^{ab}	0.036
4-5	44.62 ± 1.31 ^a	41.72 ± 0.87 ^a	42.15 ± 2.32 ^a	43..97 ± 1.00 ^a	42.87 ± 2.26 ^a	47.25 ± 1.78 ^a	42.08 ± 2.25 ^a	0.359
5-6	39.07± 0.62 ^a	39.05± 2.12 ^a	40.17± 1.44 ^a	36.78± 1.07 ^a	38.87± 1.44 ^a	33.78± 1.69 ^a	39.55± 1.48 ^a	0.379
1-6	228.72± 1.71 ^a	216.56± 1.86 ^{bc}	220.89± 4.10 ^b	210.79± 1.88 ^d	213.57± 1.56 ^{cd}	201.82± 1.23 ^f	207.43± 1.76 ^{df}	0.001

Values are expressed as mean ± standard error (SE).

Values in the same row with different superscripts are significantly different (p<0.05).

G1: control group 24% CP (diet 1), G2: 22.8% CP (diet 2), G3: (diet 2) with 1.4g protease/kg diet, G4: 21.6% CP (diet 3), G5: (diet 3) with 1.4g protease/kg diet, G6: 20.4% CP (diet 4), G7: (diet 4) with 1.4g protease/kg diet

Table 5: Weekly feed intake of grower Japanese quail fed diet containing different levels of protein with or without protease enzyme supplementation(g/bird).

Groups Period/Week	G1	G2	G3	G4	G5	G6	G7	P values
1-2	58.33± 0.40 ^c	58.61± 1.53 ^c	63.91± 1.81 ^b	74.00± 0.45 ^a	72.82± 0.26 ^a	64.80± 1.17 ^b	58.21± 0.27 ^c	0.001
2-3	118.33± 3.13 ^a	104.84± 1.80 ^{cd}	111.43± 0.51 ^b	109.51± 1.36 ^{bc}	118.03± 1.26 ^a	110.84± 0.39 ^b	103.25± 1.60 ^d	0.001
3-4	158.86± 2.13 ^b	166.36± 3.38 ^a	138.54± 0.60 ^d	150.97± 6.36 ^c	136.94± 1.51 ^d	136.90± 2.18 ^d	158.11± 1.54 ^b	0.001
4-5	180.46± 1.04 ^b	191.09± 3.99 ^a	180.73± 1.09 ^b	154.29± 2.67 ^c	171.87± 1.86 ^c	167.2± 3.27 ^{cd}	162.05± 1.29 ^d	0.001
5-6	208.14± 4.15 ^{ab}	200.4± 5.19 ^{cd}	214.65± 5.88 ^a	184.36± 2.45 ^c	195.77± 5.77 ^d	208.92± 5.96 ^{ab}	206.24± 5.12 ^{bc}	0.001
1-6	724.12± 8.55 ^a	721.29± 10.62 ^a	709.25± 9.15 ^{ab}	673.12± 8.26 ^c	695.42± 7.42 ^b	688.57± 9.48 ^{bc}	687.84± 11.03 ^{bc}	0.001

Values are expressed as mean ± standard error (SE).

Values in the same row with different superscripts are significantly different (p<0.05).

G1: control group 24% CP (diet 1), G2: 22.8% CP (diet 2), G3: (diet 2) with 1.4g protease/kg diet, G4: 21.6% CP (diet 3), G5: (diet 3) with 1.4g protease/kg diet, G6: 20.4% CP (diet 4), G7: (diet 4) with 1.4g protease/kg diet.

Table 6: Impact of different levels of protein with or without protease enzyme supplementation on weekly feed conversion ratio of grower Japanese quail.

Groups Period/Weeks ek	G1	G2	G3	G4	G5	G6	G7	P value s
1-2	1.82±0.03 d	2.00±0.10 ^d	2.01±0.10 ^d	2.79±0.09 ^a	2.45±0.10 ^b c	2.67±0.08 ^a b	2.31±0.08 c	0.001
2-3	2.14±0.06 bc	2.10±0.04 ^c	2.21±0.10 ^a bc	2.12±0.06 ^b c	2.33±0.02 ^a b	2.38±0.04 ^a	2.14±0.05 bc	0.050
3-4	3.10±0.27 a	2.96±0.51 ^a bc	2.48±0.30 ^d	2.89±0.41 ^a bc	2.67±0.08 ^c d	2.74±0.53 ^b cd	3.04±0.05 ab	0.006
4-5	4.05±0.12 a	4.58±0.04 ^a bc	4.32±0.24 ^a bc	3.52±0.13 ^c	4.04±0.26 ^a bc	3.55±0.19 ^c	3.87±0.20 bc	0.010
5-6	4.57±0.07 b	5.16±0.29 ^b	5.45±0.56 ^a	5.11±0.08 ^b	5.05±0.20 ^b	6.21±0.32 ^a	5.22±0.11 b	0.037
Average FCR	3.17±0.04 c	3.33±0.04 ^a b	3.21±0.05 ^b c	3.19±0.07 ^b c	3.26±0.03 ^b c	3.41±0.04 ^a	3.31±0.01 ab	0.010

Values are expressed as mean ± standard error (SE).

Values in the same row with different superscripts are significantly different (p<0.05).

G1: control group 24% CP (diet 1), G2: 22.8% CP (diet 2), G3: (diet 2) with 1.4g protease/kg diet., G4: 21.6% CP (diet 3), G5: (diet 3) with 1.4g

protease/kg diet., G6: 20.4% CP (diet 4), G7: (diet 4) with 1.4g protease/kg diet

Table (7): Carcass traits of grower Japanese quail at the end of the experimental period.

Groups Parameters	G1	G2	G3	G4	G5	G6	G7	P values
Live weight	236 ± 5.47 ^a	230.8 ± 3.67 ^{ab}	234.2 ± 2.37 ^{ab}	223.60 ± 2.68 ^{bc}	226.80 ± 2.78 ^{abc}	218.80 ± 4.09 ^c	223.80 ± 3.39 ^{bc}	0.021
Dressed carcass %	72.37 ± 1.57 ^a	70.94 ± 0.90 ^a	72.07 ± 1.61 ^a	69.91 ± 2.09 ^a	71.85 ± 2.07 ^a	69.74 ± 2.74 ^a	70.17 ± 1.72 ^a	0.918
Giblets weight %	4.60 ± 0.46 ^a	4.14 ± 0.23 ^a	4.85 ± 0.34 ^a	4.12 ± 0.21 ^a	4.81 ± 0.32 ^a	5.06 ± 0.20 ^a	4.98 ± 0.25 ^a	0.161
Liver weight %	2.09 ± 0.13 ^{ab}	1.76 ± 0.09 ^{bc}	1.76 ± 0.07 ^{bc}	1.50 ± 0.13 ^c	2.55 ± 0.24 ^a	2.54 ± 0.21 ^a	2.06 ± 0.18 ^{ab}	0.001
Heart weight %	1.13 ± 0.08 ^a	1.04 ± 0.04 ^{ab}	0.92 ± 0.04 ^{bc}	0.92 ± 0.03 ^{bc}	0.98 ± 0.06 ^{abc}	0.88 ± 0.06 ^c	0.88 ± 0.05 ^c	0.010
Gizzard weight %	1.69 ± 0.13 ^{bc}	1.44 ± 0.06 ^c	1.46 ± 0.08 ^c	1.53 ± 0.06 ^c	1.69 ± 0.06 ^{bc}	2.06 ± 0.08 ^a	1.91 ± 0.06 ^{ab}	0.001

Values are expressed as mean ± standard error (SE).

Values in the same row with different superscripts are significantly different (p<0.05).

G1: control group 24% CP (diet 1), G2: 22.8% CP (diet 2), G3: (diet 2) with 1.4g protease/kg diet., G4: 21.6% CP (diet 3), G5: (diet 3) with 1.4g protease/kg diet., G6: 20.4% CP (diet 4), G7: (diet 4) with 1.4g protease/kg diet.

Table (8): Liver profile of grower Japanese quail affected by different low crude protein levels and protease enzyme supplementation.

Groups Parameters	G1	G2	G3	G4	G5	G6	G7	P value s
ALT (U/L)	15.00 ± 1.00 ^c	14.00 ± 1.53 ^c	16.20 ± 1.83 ^c	18.75 ± 2.68 ^c	36.60 ± 1.7 ^b	35.66 ± 1.76 ^b	46.96 ± 0.74 ^a	0.001
AST (U/L)	218.25 ± 9.57 ^d	248.25 ± 5.50 ^{bc}	233.50 ± 8.7 ^{cd}	236.33 ± 4.63 ^{cd}	285.75 ± 6.67 ^a	247.75 ± 3.68 ^{bc}	265.25 ± 7.3 ^{ab}	0.001
ALB (G/L)	1.49 ± 0.06 ^a	1.47 ± 0.01 ^{ab}	1.37 ± 0.04 ^{abc}	1.40 ± 0.03 ^{abc}	1.32 ± 0.01 ^{abc}	1.24 ± 0.12 ^c	1.27 ± 0.09 ^{bc}	0.073
TP (mg/dl)	3.24 ± 0.12 ^b	3.36 ± 0.24 ^b	3.30 ± 0.04 ^b	4.00 ± 0.10 ^a	2.94 ± 0.10 ^b	2.48 ± 0.15 ^c	3.02 ± 0.08 ^b	0.001
Globulin (mg/dl)	1.77 ± 0.01 ^b	1.95 ± 0.18 ^b	1.87 ± 0.01 ^b	2.50 ± 0.03 ^a	1.71 ± 0.07 ^b	1.38 ± 0.10 ^c	1.93 ± 0.18 ^b	0.001
A/G Ratio	0.85 ± 0.05 ^a	0.74 ± 0.06 ^{abc}	0.74 ± 0.03 ^{abc}	0.54 ± 0.01 ^d	0.69 ± 0.06 ^{bc}	0.83 ± 0.02 ^{ab}	0.67 ± 0.06 ^{cd}	0.001

Values are expressed as mean ± standard error (SE).

Values in the same row with different superscripts are significantly different (p<0.05).

ALT= Alanine aminotransferase, AST = Aspartate aminotransferase, ALB = Albumin, TP= Total protein

A/G ratio = Albumin/ Globulin, TP = total protein. G1: control group 24% CP (diet 1), G2: 22.8% CP (diet 2), G3: (diet 2) with 1.4g protease/kg diet., G4: 21.6% CP (diet 3), G5: (diet 3) with 1.4g protease/kg diet., G6: 20.4% CP (diet4), G7: (diet 4) with 1.4g protease/kg diet

Table (9): *Antioxidant, Corticosterone and Immunity of grower Japanese quail affected by different low crude protein levels and protease enzyme supplementation.*

Groups Parameter	G1	G2	G3	G4	G5	G6	G7	P values
GSH	127.17 ± 1.46 ^d	126.10 ± 2.16 ^d	163.81 ± 4.24 ^a	125.31 ± 2.01 ^d	148.44 ± 3.41 ^b	112.43 ± 3.58 ^c	138.83 ± 2.24 ^c	0.001
SOD	579.77 ± 8.51 ^c	579.00 ± 12.20 ^c	700.50 ± 13.05 ^a	574.37 ± 8.64 ^c	644.60 ± 12.72 ^b	540.07 ± 3.82 ^d	606.10 ± 6.44 ^c	0.001
CORT	5.88 ± 0.14 ^a	5.84 ± 0.04 ^a	4.87 ± 0.06 ^c	5.77 ± 0.08 ^a	4.84 ± 0.08 ^c	5.72 ± 0.06 ^{ab}	5.48 ± 0.06 ^b	0.001
IgM	3.51 ± 0.14 ^a	3.16 ± 0.02 ^a	3.49 ± 0.16 ^a	3.44 ± 0.12 ^a	3.54 ± 0.08 ^a	3.44 ± 0.11 ^a	3.33 ± 0.16 ^a	0.378
IgG	6.36 ± 0.09 ^a	6.60 ± 0.09 ^a	6.35 ± 0.17 ^a	6.43 ± 0.11 ^a	6.39 ± 0.14 ^a	6.40 ± 0.10 ^a	6.74 ± 0.12 ^a	0.256

Values are expressed as mean ± standard error (SE).

Values in the same row with different superscripts are significantly different (p<0.05).

GSH = Glutathione, SOD = Superoxide Dismutase, CORT = Corticosterone
IgM = Immunoglobulin M IgG = Immunoglobulin G

G1: control group 24% CP (diet 1), G2: 22.8% CP (diet 2), G3: (diet 2) with 1.4g protease/kg diet, G4: 21.6% CP (diet 3), G5: (diet 3) with 1.4g protease/kg diet, G6: 20.4% CP (diet 4), G7: (diet 4) with 1.4g protease/kg diet.

Table (10): *Economic efficiency of grower Japanese quail affected by different low crude protein levels and protease enzyme supplementation.*

Groups Parameter	G1	G2	G3	G4	G5	G6	G7
Price/chick (LE)	6.25	6.25	6.25	6.25	6.25	6.25	6.25
Final wt. (g)	231.67	235.13	236.07	226.53	229.87	225.47	222
Feed intake/chick (g)	707.22	699.64	694.54	669.65	669.09	681.18	670.68
Feed cost/chick (LE)*	18.03	17.84	17.71	17.07	17.06	17.37	17.1
Management/chick (LE)	1.55	1.55	1.55	1.55	1.55	1.55	1.55
Feed additives cost/chick	0	0	0.291	0	0.281	0	0.281
Total cost/chick (LE)	25.83	25.64	25.8	24.87	25.14	25.17	25.18
Selling price (LE)	27.5	27.5	27.5	27.5	27.5	27.5	27.5
Net revenue (LE)	1.67	1.86	1.7	2.63	2.36	2.33	2.32
Economic efficiency (EE)	6.46	7.25	6.58	10.57	9.37	9.25	9.21

*Feed cost / kg of diet =25.5 LE.

** Total cost = Price of chick + management feed cost additive cost.

***=selling price – total cost

G1: control group 24% CP (diet 1), G2: 22.8% CP (diet 2), G3: (diet 2) with 1.4g protease/kg diet, G4: 21.6% CP (diet 3), G5: (diet 3) with 1.4g protease/kg diet, G6: 20.4% CP (diet 4), G7: (diet 4) with 1.4g protease/kg diet.

4. Discussion

4.1 Growth performance

4.1.1 Body weight (BW) and body weight gain (BWG)

In (Tables 3 and 4) a linear decline in live body weight and body weight gain was observed as protein levels decreased. This finding aligns with *Hamid and Yassin (2018)* and *Emadinia et al. (2020)*, who found higher crude protein (CP) levels enhance quail growth. Similarly, *Qiu et al. (2023)* and *Niu et al. (2025)* reported reduced CP negatively impacted broiler growth. However, our findings contradict those of *Elsayed et al. (2021)* and *Omidwura et al. (2016)*, who reported that no adverse effects of reducing CP to 20% in quail diets. However, similar growth performance was recorded in groups receiving 24% CP (control) and 22.8% CP, regardless of whether protease supplementation was included.

Protease supplementation resulted in a slight improvement in body weight and body weight gain; however, it did not have a significant effect at the same protein levels. This finding is consistent with the studies conducted by *Sultan et al. (2024)*, *Song et al. (2023)*, and *Amer et al. (2021)*. In contrast, our results differ from those of *Cowieson et al. (2022)*, and *Qiu et al. (2023)*, who

reported that protease supplementation enhanced growth in broilers.

4.1.2 Feed consumption

Table 5, dietary protein levels and protease supplementation significantly affected feed intake (FI) in quail. Increased crude protein (CP) levels enhanced FI, consistent with *Jahanian & Edriss (2015)* and *Erakpotobor (2004)*, likely due to improved nutrient absorption. However, *Ndazigaruye et al. (2019)* and *El-Damarawy (2019)* found no impact of CP reduction on FI, suggesting that other factors, such as amino acid balance, may play a role in regulating intake.

Protease effects on feed intake (FI) were mixed, inconsistent, showing reductions of 22.8% and 21.6% crude protein (CP) levels, respectively but not at 20.4% CP. This supports the view that enzyme efficacy is influenced by dietary composition, protein content, and bird age (*Bao et al., 2013*). Similarly, (*Sarica et al., 2020*; *Qiu et al., 2023*) reported significant changes in FI with protease supplementation. Other studies, including those by *Lee et al. (2023)*; *Wealleans et al. (2023)*; and *Song et al. (2023)*, found no significant effect on feed intake with protease supplementation in poultry diets.

4.1.3 Feed Conversion Ratio

In (Table 6), higher protein levels improved FCR in quails. The addition of protease did not have a significant effect at the same protein level, except in G3, where it slightly enhanced the FCR compared to Group 2 (G2) with 22.8% crude protein (CP) and no protease.

These findings are consistent with the research conducted by **Jahanian & Edriss (2015)** and **Saker et al. (2015)**, which established a correlation between higher crude protein (CP) levels and improved feed conversion ratio (FCR) in quails. Similarly, **Ndazigaruye et al. (2019)**, **Rouhi et al. (2022)**, and **Niu et al. (2025)** reported that a reduction in dietary CP negatively impacted the FCR in broilers. However, our results differ from those of **Blake & Hess (2013)** and **Benahmed et al. (2023)**, who found no significant effect of CP reduction on FCR. Additionally, **Cowieson et al. (2022)** reported that the addition of protease improved FCR, whereas our study did not observe a consistent effect. **Lee et al. (2023)** observed a numerical increase in FCR with protease supplementation, although this increase was not statistically significant.

4.2. Carcass Characteristics

In (Table 7), reducing dietary protein, with or without the addition of protease, had no significant effect on dressed carcass percentage or giblet weights, which is consistent with the findings of **Reda et al. (2015)** and **Hamid & Yassin (2018)**. Similarly, protease did not alter

carcass traits, as reported by (**Sultan et al., 2024; Mahmood et al., 2017**). However, liver weight increased in lower-protein groups (21.6% CP with protease, 20.4% CP without protease), aligning with the results of **Zhang et al. (2020)**, while **Song et al. (2023)** found no significant effect. Heart weight was higher in the control group (24% CP), suggesting that protein influences cardiac development, although protease had no impact, as noted by (**Nastain et al., 2021**). Gizzard weight was highest in the lowest-protein groups (20.4% CP), supporting the findings of **Sarica et al. (2020)**, although **Emadinia et al. (2020)** reported an opposite trend.

4.3. Blood parameters

Liver function

In (Table 8), elevated ALT and AST indicate liver stress, with the highest ALT in G7 (20.4% CP with protease) and the highest AST in G5 (21.6% CP with protease) and G7. This suggests that severe protein reduction may impair liver function. In contrast, G1 (24% CP) and G2 (22.8% CP without protease) exhibited the lowest levels, indicating normal liver function. These findings are consistent with the research of **Ogunbode & Iyayi (2021)** but contradict **Serge-Olivier et al. (2021)** and **Elsayed et al. (2021)**. Higher CP levels were associated with increased ALB and TP, consistent with the findings of **Serge-Olivier et al. (2021)** and **Hernández et al. (2012)**, though **Song et al. (2023)** found no effect.

Protease did not significantly impact ALB, it did increase TP in low-protein groups (G6, G7), supporting the conclusions of *Allouche et al. (2015)*, while *Law et al. (2018)* and *Ndazigaruye et al. (2019)* reported no significant effect.

4.4 Antioxidant Status, Corticosterone (CORT) and immunity

In Table 9, the protease-supplemented groups (G3, G5, G7) exhibited significantly higher antioxidant status, as indicated by levels of glutathione (GSH) and superoxide dismutase (SOD), compared to the non-supplemented groups ($p < 0.001$), this findings suggests a protective effect against oxidative stress, aligning with *Angel et al. (2011)*. However, *Elsayed et al. (2021)* and *Arczewska et al. (2018)* associated a higher CP (24%) to increased antioxidant activity, while *Law et al. (2019)* pointed out that the effects of protease supplementation can vary depending on dietary composition and the physiology of the birds.

Corticosterone levels were highest in the control group (G1, 24% CP) and the unsupplemented groups (G2, G4, G6), while the protease-supplemented groups exhibited significantly lower levels, indicating a potential mitigation of stress. A reduction in dietary protein alone (24% to 20.4% CP) did not affect CORT. Similarly, *Law et al. (2019)* showed that lowering dietary CP (21%CP to 17% CP) did not affect CORT levels of broiler chickens.

Also, protease supplementation has been shown to alleviate stress, as evidenced by the reduced CORT levels in protease-supplemented groups (G3, G5, and G7). conversely *Law et al. (2019)*, who showed that no found significant differences were found in corticosterone levels with protease supplementation in broiler diets.

No significant differences were observed in IgM or IgG levels ($p > 0.05$), consistent with the findings of *Mohamed (2019)* and *Qiu et al. (2023)*, who found no impact of CP levels or protease on immunoglobulin levels. However, *Amer et al. (2021)* suggested a potential immune-modulatory effect of protease.

4.5 Economic evaluation

In (Table 10), economic efficiency varied significantly among the dietary protein regimens. Group 4 (G4) with 21.6% crude protein (CP) exhibited the highest efficiency, followed by group 5 (G5), which also contained 21.6% CP with protease) and G6 (20.4% CP without protease), demonstrating the best cost-to-performance ratio. In contrast, G1 (24% CP) had the lowest efficiency, indicating that the higher protein costs did not result in proportional performance gains.

These findings align with the work of *Abd-Elsamee et al. (2014)* and *Sherif et al. (2023)*, who reported optimal economic returns at moderate protein levels (22% CP). However, protease supplementation did not improve economic

efficiency. Our findings are consist with those of *Ali et al. (2019)* and *Amer et al. (2021)* who found no significant cost impact from protease enzyme supplementation. In contrast, *Aletor et al. (2000)* and *Canogullari et al. (2009)* reported that supplementary protease decreased feed costs in broiler diets.

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تأثير تغذية السمان الياباني النامي علي علائق منخفضة في نسبة البروتين مع إضافة إنزيم البروتياز

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الملخص

تبحث هذه الدراسة في تأثير خفض مستويات البروتين الخام في علف السمان الياباني وإضافة إنزيم البروتياز على الأداء الإنتاجي، وصفات الذبيحة، والمناعة، ومضادات الأكسدة. شملت التجربة 294 كوكوت سمان ياباني وزعت على سبع مجموعات بنسب بروتين مختلفة (24%-22.8%-21.6% - 20.4%) مع أو بدون إضافة إنزيم البروتياز. أظهرت النتائج أن تقليل البروتين إلى أقل من 22.8% أثر على الوزن النهائي، حيث سجلت المجموعة الضابطة (24% بروتين) ومجموعة 22.8% بروتين أعلى وزن مع تميز الثانية بزيادة في الكفاءة الاقتصادية مقدارها 12.23%. كما حققت مجموعة (21.6% بروتين) أفضل كفاءة اقتصادية. تشير النتائج إلى إمكانية خفض البروتين في علف السمان الي المستوي 22.8%. وأن إضافة إنزيم البروتياز أدى الي زيادة غير معنوية في الوزن النهائي بين المجاميع التي لها نفس المستوي من البروتين.