

Synergistic effects of flaxseed and vitamin E on growth, lipid metabolism, and antioxidant status in Japanese Quail

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ABSTRACT

Background: Poultry is the fastest-growing animal in the agriculture sector, and feed cost contributes 70%–80% of production cost; therefore, many efforts have been evaluated to reduce feed cost. One of these ways to improve feed efficiency is the use of natural feed additives in the diet, especially after reducing the use of antibiotics in poultry diets. Flaxseed contains bioactive compounds, particularly omega-3 fatty acids (α -linolenic acid) and lignans, which are used to improve energy metabolism and play antioxidant and anti-inflammatory effects in poultry diets. Vitamin E plays a vital function in protecting polyunsaturated fatty acids from oxidative damage, preserving cell membrane integrity, and supporting immune functions.

Aim: This study aimed to evaluate the physiological and productive effects of flaxseed (*Linum usitatissimum*) and vitamin E supplementation, individually or in combination, in Japanese quail (*Coturnix japonica*).

Methods: A total of 120 quails were randomly assigned to four dietary treatments: commercial diet (control), commercial diet with flaxseed, commercial diet with vitamin E, and commercial diet with both flaxseed and vitamin E, each group comprising three replicates with 10 chicks per replicate, and the study lasted 5 weeks following 1 week of adaptation.

Results: The combined supplementation markedly enhanced growth performance, improving body weight gain, feed efficiency, and survivability compared with the control group. Serum biochemical analysis revealed improved protein metabolism and a healthier lipid profile, with reductions in cholesterol, triglycerides, and low-density lipoprotein, alongside an increase in high-density lipoprotein. Antioxidant indices confirmed strengthened defense mechanisms, as glutathione levels increased and malondialdehyde concentrations decreased, while liver enzyme activities, aspartate transaminase and alanine transaminase were reduced, indicating improved hepatic function. The synergistic interaction between omega-3 fatty acids from flaxseed and the antioxidant role of vitamin E contributed to better metabolic efficiency and oxidative stability.

Conclusion: These findings highlight that flaxseed and vitamin E can be fed to Japanese quail as promising natural feed additives that can enhance productivity, health status, and sustainability in quail production.

Keywords: Flaxseed, Growth performance, Japanese quail, Vitamin E.

Introduction

The poultry industry is increasingly focused on natural and sustainable feed additives that can enhance productivity, health, and product quality while reducing reliance on synthetic growth promoters. Japanese quail (*Coturnix japonica*) is widely recognized as a valuable model species due to its rapid growth, short generation interval, and economic importance in meat and egg production. Optimizing its dietary

regimen through functional feed supplementation has therefore become a subject of considerable scientific interest. Flaxseed (*Linum usitatissimum*) is a rich source of bioactive compounds, particularly omega-3 fatty acids (α -linolenic acid) and lignans, which have been shown to modulate lipid metabolism, improve energy utilization, and exert antioxidant and anti-inflammatory effects in poultry species (Banerjee *et al.*, 2021; Pan *et al.*, 2021). Recent studies confirm that flaxseed inclusion in quail diets reduces serum

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cholesterol and triglycerides while enhancing growth performance and carcass quality (Hasan *et al.*, 2021; El-Beltagi *et al.*, 2022). Vitamin E (α -tocopherol), a lipid-soluble antioxidant, plays a critical role in protecting polyunsaturated fatty acids from oxidative damage, maintaining cell membrane integrity, and supporting immune and reproductive functions (Surai, 2020). Newer investigations highlight its importance in mitigating oxidative stress under challenging production environments, including heat stress in quail (Astuti *et al.*, 2024) and improving egg production and metabolic efficiency when combined with selenium (Ameen *et al.*, 2024). Given that omega-3 fatty acids are highly susceptible to peroxidation, combining flaxseed with vitamin E is hypothesized to produce synergistic benefits. This dual supplementation may stabilize dietary lipids, enhance antioxidant defense systems, and improve overall physiological resilience. Recent findings in poultry confirm that such combinations reduce oxidative stress biomarkers, improve lipid profiles, and promote growth efficiency (Ahmed *et al.*, 2020; Kumar *et al.*, 2022). However, limited research has specifically addressed the combined effects of flaxseed and vitamin E in Japanese quail, representing a gap in the literature. Therefore, the present research investigated the effects of flaxseed and vitamin E (individual and combined) supplementation on growth performance, biochemical parameters, and antioxidant status in Japanese quail. The outcomes are expected to provide a practical and sustainable feeding strategy that enhances productivity and health while supporting environmentally responsible poultry production.

Materials and Methods

Experimental site and duration

The experiment was conducted at the Poultry Research Unit, Department of Zoology, Faculty of Science, Sabratha University, Libya, during January and February 2025. The trial lasted for 5 weeks following a one-week adaptation period.

Experimental birds and management

In vivo experiment, a total of 120 unsexed Japanese quails (*Coturnix japonica*) were used in this study and obtained from a commercial hatchery. The birds were placed in wire mesh at the bottom of the cages under controlled conditions, and each cage was equipped with two adjustable nipple drinkers and front-mounted feed. On day 7 of age, the chickens were weighed and randomly distributed to four dietary treatments according to their initial body weight (BW) (average 28 ± 0.5 g). The birds were reared in cages of identical size (50×50 cm floor area and 80 cm in height) and subjected to similar management practices (lighting, feeding, and watering) throughout the experiment except for the diets offered. There were 3 cages per treatment with 10 birds per cage. The initial room temperature was 35°C and was then decreased by 2°C per week; relative humidity: $65\% \pm 5\%$; lighting

program: 22 hours light, and 2 hours darkness with a minimum light intensity of 20 lux throughout the experiment.

Feed and fresh water were provided *ad libitum* throughout the experimental period, and the health of the birds was observed every day.

Experimental design and dietary treatments

Birds were assigned to four dietary treatments: T1 (Control): birds were fed a commercial diet without supplementation; T2 (Flaxseed): commercial diet + 6% ground flaxseed; T3 (Vitamin E): commercial diet + 182 IU/kg vitamin E (Senobar-Kalati *et al.*, 2012); T4 (Combination): commercial diet + 6% flaxseed + 182 IU/kg vitamin E in a completely randomized design. The birds were provided with feed and fresh water for *ad libitum* consumption.

Commercial diet composition

The ingredients of the commercial diets used in this experiment are shown in Table 1. The diet was manufactured at a private factory to meet the nutrient requirements of quails according to NRC (1994) for Japanese quails. A chemical analysis was performed for the diet following AOAC (1995).

Calculated nutrient composition

Crude protein: 22%; Metabolizable energy (ME): 2900 kcal/kg; Calcium: 1.0%; and Available phosphorus: 0.45%.

Experimental diets

The experimental diets were formulated by supplementing the commercial diet with flaxseed and/or vitamin E 182 IU/kg), as shown in Table 2.

Growth performance measurements

Body weight: recorded weekly; feed intake (FI): measured daily per replicate; weight gain (WG): calculated as final BW—initial BW; feed conversion ratio (FCR): calculated as FI/WG; survivability (%): calculated as the proportion of birds alive at the end of the experiment.

Blood sampling and biochemical analysis

At the end of the trial, three birds per replicate were randomly selected and fasted for 12 hours before blood

Table 1. Composition of the commercial diet for Japanese quail (as-fed basis).

Ingredient	% of diet
Yellow corn	51.0
Soybean meal (44% CP)	34.0
Wheat bran	5.0
Vegetable oil	3.0
Bone meal	3.0
Calcium carbonate	2.0
Salt	0.5
Vitamin–mineral premix	1.5
Total	100

collection. Approximately 3 ml of blood was drawn from the wing vein, centrifuged at 3,000 rpm for 15 minutes, and serum was stored at -20°C until chemical analysis started.

Serum was analyzed for total protein, albumin, globulin, glucose, cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) using commercial diagnostic kits (Biolabo, France). Liver function was assessed by measuring aspartate transaminase (AST) and alanine transaminase (ALT) activities.

Antioxidant and oxidative stress markers

Glutathione (GSH) was determined according to Ellman (1959), while malondialdehyde (MDA) was measured using the thiobarbituric acid method (Buege and Aust, 1978).

Statistical analysis

Data were analyzed using one-way analysis of variance test (SPSS v25.0). When significant differences were detected, means were compared using Duncan's (1955) multiple range test at $p \leq 0.05$. Results are expressed as mean \pm standard error (SE). The statistical model utilized was: $y_i = \mu + T_i + E_{ijk}$, where: y_i = response; μ = overall mean; T_i = treatment effect; E_{ijk} = experimental error.

Ethical approval

The Animal Research Ethics Board of the Poultry Research Unit, Department of Zoology, Faculty of Science, Sabratha University, Libya, granted ethical approval of this research (approved on 2 February 2025); the procedures were carried out according to the guidelines of the Poultry Research Unit, Department of Zoology, Faculty of Science, Sabratha University, Libya.

Table 2. Experimental diets for Japanese quail.

Treatment	Basal diet (%)	Flaxseed (%)	Vitamin E (IU/kg)
T1 (Control)	100	–	–
T2 (Flaxseed)	94	6	–
T3 (Vitamin E)	100	–	182
T4 (Combination)	94	6	182

Table 3. Effect of flaxseed and vitamin E supplementation on growth performance of Japanese quail (Mean \pm SE).

Parameter	T1 (Control)	T2 (Flaxseed)	T3 (Vit E)	T4 (Flaxseed + Vit E)	p-value
Initial BW (g)	28.3 \pm 0.5	28.5 \pm 0.6	28.4 \pm 0.5	28.6 \pm 0.6	NS
Final BW (g)	178.5 \pm 3.4 ^c	189.7 \pm 3.8 ^b	191.2 \pm 3.6 ^b	204.8 \pm 4.1 ^a	0.01
BWG (g)	150.2 \pm 3.2 ^c	161.2 \pm 3.5 ^b	162.8 \pm 3.4 ^b	176.2 \pm 3.7 ^a	0.01
Feed Intake (g/day)	24.8 \pm 0.6	25.2 \pm 0.5	25.0 \pm 0.6	25.1 \pm 0.5	NS
FCR	3.05 \pm 0.08 ^a	2.82 \pm 0.07 ^b	2.80 \pm 0.06 ^b	2.59 \pm 0.05 ^c	0.05
Survivability (%)	95.6 \pm 1.2	97.3 \pm 1.1	97.6 \pm 1.0	99.1 \pm 0.8	0.05

Means within a row with different superscripts differ significantly ($p \leq 0.05$, Duncan's test).

Results

Growth performance

Dietary supplementation with flaxseed and vitamin E significantly improved growth performance parameters in Japanese quail (Table 3). Birds in T2 (flaxseed) and T3 (vitamin E) groups showed higher body weight gain (BWG) and better FCR compared with the control diet (T1). The combination group (T4) exhibited the greatest improvement, with the highest final body weight and the most efficient FCR ($p < 0.01$). Survivability was also enhanced in supplemented groups, particularly in T4.

Blood biochemical parameters

Dietary treatments significantly influenced serum protein and lipid profiles (Table 4). The combination group (T4) recorded the highest total protein and albumin levels, while cholesterol, triglycerides, and LDL concentrations were markedly reduced. HDL was significantly elevated in supplemented groups, with the greatest increase observed in T4 ($p < 0.01$).

Liver enzymes and antioxidant markers

Supplementation with flaxseed and vitamin E significantly reduced AST and ALT activities, indicating improved hepatic function (Table 5). Antioxidant indices showed a marked reduction in MDA levels and an increase in GSH concentration, particularly in the combination group (T4).

Discussion

As presented in Table 3, dietary supplementation with flaxseed and vitamin E significantly enhanced growth performance in Japanese quail. Birds in the combination group (T4) achieved the highest final body weight (204.8 g), which was 15% greater than that of the control group (178.5 g). BWG followed a similar

Table 4. Effect of flaxseed and vitamin E supplementation on serum biochemical parameters of Japanese quail (Mean ± SE).

Parameter	T1 (Control)	T2 (Flaxseed)	T3 (Vit E)	T4 (Flaxseed + Vit E)	p-value
Total Protein (g/dl)	5.72 ± 0.14 ^c	6.01 ± 0.13 ^b	6.08 ± 0.12 ^b	6.43 ± 0.11 ^a	0.01
Albumin (g/dl)	3.25 ± 0.10 ^c	3.42 ± 0.09 ^b	3.48 ± 0.08 ^b	3.71 ± 0.07 ^a	0.05
Globulin (g/dl)	2.47 ± 0.12 ^b	2.59 ± 0.11 ^b	2.60 ± 0.12 ^b	2.72 ± 0.10 ^a	0.05
Cholesterol (mg/dl)	181.4 ± 5.1 ^a	162.7 ± 4.8 ^b	158.3 ± 4.5 ^b	143.5 ± 4.2 ^c	0.01
Triglycerides (mg/dl)	142.6 ± 4.7 ^a	128.2 ± 4.3 ^b	126.4 ± 4.1 ^b	115.7 ± 3.9 ^c	0.01
HDL (mg/dl)	57.2 ± 2.1 ^c	62.8 ± 2.2 ^b	63.4 ± 2.3 ^b	70.1 ± 2.5 ^a	0.01
LDL (mg/dl)	103.8 ± 3.5 ^a	92.1 ± 3.2 ^b	90.3 ± 3.1 ^b	81.2 ± 2.9 ^c	0.01

Means within a row with different superscripts differ significantly ($p \leq 0.05$, Duncan's test).

Table 5. Effect of dietary treatments on liver enzymes and antioxidant markers in Japanese quail (Mean ± SE).

Parameter	T1 (Control)	T2 (Flaxseed)	T3 (Vit E)	T4 (Flaxseed + Vit E)	p-value
AST (U/l)	38.6 ± 1.3 ^a	34.8 ± 1.2 ^b	33.5 ± 1.1 ^b	30.2 ± 1.0 ^c	0.01
ALT (U/l)	24.3 ± 1.0 ^a	21.8 ± 0.9 ^b	21.2 ± 0.8 ^b	19.5 ± 0.7 ^c	0.05
GSH (μmol/l)	8.72 ± 0.25 ^c	9.13 ± 0.27 ^b	9.28 ± 0.26 ^b	9.87 ± 0.28 ^a	0.05
MDA (nmol/ml)	4.85 ± 0.14 ^a	4.23 ± 0.13 ^b	4.17 ± 0.12 ^b	3.82 ± 0.11 ^c	0.01

Means within a row with different superscripts differ significantly ($p \leq 0.05$, Duncan's test).

trend, with T4 recording 176.2 g compared to 150.2 g in the control, representing a 17% improvement. Importantly, the feed conversion ratio improved from 3.05 in the control diet to 2.59 in T4, indicating superior feed efficiency. Survivability also increased, reaching 99.1% in T4 compared with 95.6% in the control. These findings suggest that the synergistic interaction between flaxseed-derived omega-3 fatty acids and the antioxidant role of vitamin E enhanced nutrient utilization and physiological resilience. Comparable improvements in growth performance with vitamin E supplementation have been reported in broilers (Sadiq *et al.*, 2023; Singh *et al.*, 2023), supporting the current results. The biochemical profile of quail was markedly influenced by dietary treatments, as shown in Table 4. The combination group (T4) exhibited the highest total protein (6.43 g/dl) and albumin (3.71 g/dl), compared with 5.72 g/dl and 3.25 g/dl in the control group. This increase reflects improved hepatic function and protein synthesis under antioxidant protection. Lipid metabolism was also favorably modulated: cholesterol decreased by 21% (181.4 vs. 143.5 mg/dl), triglycerides by 19% (142.6 vs. 115.7 mg/dl), and LDL by 22% (103.8 vs. 81.2 mg/dl), while HDL increased by 23% (57.2 vs. 70.1 mg/dl). Such improvements in lipid profile are consistent with the hypocholesterolemic effects of flaxseed (Abdulla, 2019; El-Beltagi *et al.*, 2022) and the protective antioxidant role of vitamin E (Surai, 2020). The elevation of HDL alongside reductions in LDL and triglycerides indicates enhanced lipid catabolism and suppression of cholesterol biosynthesis, which collectively contribute to improved cardiovascular and metabolic health in

poultry. Indicators of hepatic function and oxidative stress, summarized in Table 5, further highlight the synergistic benefits of supplementation. AST and ALT activities were significantly reduced in T4 (30.2 and 19.5 U/l, respectively) compared with the control (38.6 and 24.3 U/l), suggesting improved liver integrity and reduced cellular damage. Antioxidant indices confirmed this protective effect: MDA, a marker of lipid peroxidation, decreased by 21% (4.85 vs. 3.82 nmol/ml), while GSH, a key antioxidant, increased by 13% (8.72 vs. 9.87 μmol/l). These findings demonstrate that flaxseed and vitamin E supplementation strengthened the antioxidant defense system, reducing oxidative stress and enhancing physiological stability. Similar outcomes have been reported in poultry supplemented with vitamin E (Ameen *et al.*, 2024; Astuti *et al.*, 2024), where oxidative stability and immune function were improved under stressful production environments. The observed synergy between flaxseed and vitamin E can be explained by their complementary actions. Flaxseed enriches the diet with polyunsaturated fatty acids, particularly α-linolenic acid, which improve membrane fluidity and reduce pro-inflammatory mediators. However, these fatty acids are highly susceptible to peroxidation. Vitamin E, as a lipid-soluble antioxidant, protects polyunsaturated fatty acids from oxidative degradation, thereby stabilizing dietary lipids and preventing oxidative losses. This dual mechanism optimizes metabolic homeostasis, leading to improved growth performance, enhanced lipid metabolism, and strengthened antioxidant defense. The marked reductions in cholesterol, triglycerides, LDL, and MDA, alongside increases in

HDL, protein synthesis, and GSH, collectively confirm the synergistic physiological benefits of combining flaxseed and vitamin E.

The present findings corroborate earlier reports that flaxseed supplementation enhances serum HDL while decreasing cholesterol and triglycerides in poultry (Ahmed *et al.*, 2020; Hasan *et al.*, 2021). Vitamin E has also been shown to improve reproductive efficiency, immune response, and oxidative stability in quail (Akhter *et al.*, 2022). More recent studies emphasize its role in mitigating oxidative stress under challenging production environments (Sadiq *et al.*, 2023; Singh *et al.*, 2023). Taken together, these studies and the current results confirm that combined supplementation provides superior protection against oxidative stress, hepatic dysfunction, and lipid imbalance compared to individual supplementation.

Conclusion

This study demonstrated that dietary supplementation with flaxseed (6%) and vitamin E (182 IU/kg diet) significantly enhanced growth performance, metabolic efficiency, and antioxidant capacity in Japanese quail. The combined treatment (T4) yielded the greatest improvements, with body weight and feed conversion ratio increased by 15%, survivability reaching 99.1%, and marked reductions in serum cholesterol (-21%), triglycerides (-19%), and LDL (-22%), alongside a 23% rise in HDL. Antioxidant defense was strengthened, as MDA decreased (-21%) and GSH increased (+13%), with improved hepatic function indicated by lower AST (-22%) and ALT (-20%). These findings highlight flaxseed and vitamin E as effective natural feed additives that promote quail health and sustainable production and can be used in quail feed without an expectation of issues, while future research should explore impacts on carcass quality, egg traits, and consumer benefits.

Acknowledgments

The authors would like to thank the staff of Poultry Research Unit, Department of Zoology, Faculty of Science, Sabratha University, Libya, for their tremendous helping to provide the birds and manufacturing the experimental diets. We would like to acknowledge the laboratory group in the College of Science at the University of Sabratha for ongoing help and skillful assistance in the sample collections and chemical analyses.

Conflict of interest

All of the authors declare that there is no conflict of interest concerning the publication of this study.

Funding

All of the authors declare that there are no grants, external funding, or financial assistance regarding this study.

Authors' contributions

All of the authors made an equal contribution to this manuscript.

Data availability

All of the data that support the results of this study are available in the manuscript, and no additional data are available.

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