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Effects of Dietary Gum Arabic Supplementation on Serum Biochemical Indices and Endocrine Profile of Older Laying Hens

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A B S T R A C T

Gum Arabic is a natural polysaccharide with potential physiological benefits as a functional feed additive in poultry, but information on its effects on serum biochemical and hormonal parameters in older laying hens remains limited. In the present study, the effects of dietary supplementation with Gum Arabic on serum biochemical indices, liver enzyme activities, and thyroid and reproductive hormone profiles in older laying hens were evaluated. A 140-day feeding trial was conducted using 105 Lohmann Brown laying hens aged 65 weeks. Birds were randomly assigned to five dietary treatments with three replicates of seven hens each: a control diet (T1) and diets supplemented with 15 (T2), 20 (T3), 25 (T4), or 30 g/kg (T5) Gum Arabic powder. Serum biochemical parameters, liver enzymes (AST, ALT, and ALP), thyroid hormones (T3 and T4), and reproductive hormones (estrogen, progesterone, LH, and FSH) were determined during the final experimental period (81–84 weeks of age). Gum Arabic supplementation significantly increased serum total protein in all supplemented groups compared with the control group. Albumin was significantly higher in T4 and T5, while globulin generally improved in supplemented groups. Serum AST and ALT activities decreased, whereas ALP activity increased following supplementation. Birds receiving 25 and 30 g/kg Gum Arabic showed higher serum T3 and T4 concentrations than controls. Reproductive hormones, including estrogen, progesterone, LH, and FSH, were also improved, particularly at higher supplementation levels. These findings indicate that dietary Gum Arabic positively influenced selected serum biochemical, hepatic enzyme, and endocrine indicators in older laying hens under the conditions of this study. Further research is needed to clarify the underlying mechanisms and evaluate effects on productive performance and other physiological traits.

Keywords: *Biochemical profile; Gum Arabic; Hormonal profile; laying hens; Liver enzymes*

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1 Introduction

The productive lifespan of commercial laying hens has substantially increased due to advances in genetics, nutrition, and flock management. However, as hens age, progressive physiological changes may affect metabolic activity, liver function, endocrine regulation, and nutrient utilization. These changes can alter serum biochemical and hormonal profiles, which are widely recognized as valuable indicators of the physiological and health status of laying hens. Therefore, maintaining normal metabolic and endocrine functions in older laying hens has become an important objective in modern poultry nutrition, particularly through safe and effective dietary interventions (Al-Salhi, 2026b; Oke et al., 2024).

Natural feed additives have received increasing attention as sustainable alternatives to synthetic growth-promoting substances due to their potential to support normal physiological functions and improve animal health. Among these additives, Gum Arabic, a natural water-soluble polysaccharide derived primarily from Acacia senegal, has attracted considerable interest owing to its high soluble fiber content and bioactive compounds, including arabinogalactans, glycoproteins, minerals, and small quantities of phenolic constituents (Mariod, 2018). Previous studies have reported that Gum Arabic possesses several biological properties, including prebiotic, antioxidant, and anti-inflammatory activities under different experimental conditions (Ahmed et al., 2024; Calame et al., 2008). Although these biological activities have been described in the literature, their physiological significance may vary across animal species, ages, dietary inclusion levels, and experimental conditions.

Several studies have evaluated the effects of Gum Arabic supplementation in poultry. Dietary inclusion of Gum Arabic has been associated with improvements in selected physiological and biochemical traits, including serum protein fractions and liver enzyme activities, while beneficial effects on intestinal morphology and general health status have also been reported in broiler chickens and laying hens (Ismail et al., 2016; Khan et al., 2022; Saad et al., 2022). Nevertheless, the available information remains limited, particularly regarding its influence on the endocrine profile of older laying hens. Thyroid hormones play essential roles in regulating metabolic activity and energy metabolism, whereas reproductive hormones are closely associated with the maintenance of reproductive physiological functions. Likewise, serum biochemical indices and liver enzyme activities provide valuable information regarding protein metabolism and hepatic function. Comprehensive evaluation of these physiological indicators may therefore contribute to a better understanding of the biological responses of aged laying hens to dietary supplementation with Gum Arabic.

Despite growing interest in the use of Gum Arabic in poultry nutrition, few studies have comprehensively evaluated its effects on serum biochemical indices, thyroid hormone profiles, and reproductive hormone profiles in older laying hens maintained under long-term feeding conditions. Therefore, the present study was conducted to evaluate the effects of dietary Gum Arabic supplementation at different inclusion levels on selected serum biochemical parameters, liver enzyme activities, and thyroid and reproductive hormone concentrations in older Lohmann Brown laying hens. The findings are expected to contribute to the current understanding of the physiological responses of aged laying hens to dietary Gum Arabic and provide useful information for future investigations on its application as a functional feed additive in poultry nutrition.

2 Materials and Methods

2.1 Gum Arabic Source and Characterization

Gum Arabic powder used in this study was obtained from the local market in Hilla, Babylon Province, Iraq, and incorporated into the experimental diets at inclusion levels of 15, 20, 25, and 30 g/kg feed. Gum Arabic is a natural water-soluble polysaccharide derived mainly from *Acacia senegal* and consists primarily of arabinogalactan, arabinogalactan–protein complexes, and glycoproteins. It is characterized by a high content of soluble dietary fiber and contains naturally occurring sugars together with minor amounts of bioactive constituents, including phenolic compounds and flavonoids (Calame et al., 2008; Mariod, 2018). The general chemical characteristics of Gum Arabic reported in previous studies are summarized in Table 1. The chemical characteristics presented in Table 1 represent literature-based general characteristics of Gum Arabic and were not obtained from laboratory analysis of the specific Gum Arabic batch used in the present experiment.

Table 1. General chemical characteristics of Gum Arabic reported in the literature

Parameter	Description/Value
Source	Local market, Hilla, Babylon Province, Iraq
Botanical origin	*Acacia senegal*
Major constituents	Arabinogalactan, arabinogalactan–protein complexes, glycoproteins
Dietary fiber	High soluble dietary fiber
Dry matter (g/kg)	870–877.9
Crude protein (g/kg)	25–37.1
Crude fiber (g/kg)	73.2–79.8
Ether extract (g/kg)	1.8–4.3
Nitrogen-free extract (g/kg)	851.5–868
Ash (g/kg)	24.7–27.3
Major sugars	Galactose, Arabinose, Glucuronic acid, Rhamnose
Bioactive constituents	Phenolic compounds, flavonoids
References	Calame et al. (2008); Mariod (2018)

2.2 Experimental hens and Management

The feeding trial was conducted in Babylon Province, Iraq, over 140 days, from 26 October 2024 to 14 March 2025, to evaluate the effects of dietary supplementation with Gum Arabic powder on serum biochemical and hormonal responses in older laying hens. A total of 105 Lohmann Brown laying hens, 65 weeks of age at the beginning of the experiment, were used.

Birds were randomly allocated to five dietary treatments, each consisting of three replicates with seven hens per replicate (21 hens per treatment). The experimental treatments included a control diet without Gum Arabic supplementation (T1) and four diets supplemented with 15 g (T2), 20 g (T3), 25 g (T4), and 30 g (T5) Gum Arabic powder/kg diet. The experimental unit for statistical analysis was the replicate (cage). The ingredient composition and chemical composition of the experimental diets are presented in Table 2. Before data collection, all birds were acclimated to the experimental conditions for 2 weeks under identical management. The hens were housed in a battery cage system (2×2 m² units), with each unit containing six batteries, and each battery consisting of five cages (40×40 cm²). Each cage was equipped with two automatic nipple drinkers, and feed was manually offered through longitudinal feeders. Feed was provided at a rate of 115 g/hen/day. A 16 h light/8 h dark lighting program was maintained throughout the experiment, with an intensity of approximately 4 W/m². Ambient temperature was monitored twice daily (morning and evening) using thermometers placed at four locations within the poultry house, and electric heaters were used when necessary to maintain optimal environmental conditions.

2.3 Blood Sampling and Biochemical and Hormonal Analysis

Blood samples were collected only during the final experimental period (81–84 weeks of age), which was selected because it represented the stabilized physiological response following long-term dietary supplementation with Gum Arabic. Blood samples were obtained from all experimental birds (n=7 birds per replicate) via the brachial vein using sterile disposable syringes and transferred into plain anticoagulant-free tubes. The samples were allowed to clot at room temperature, and serum was then separated by centrifugation at 3000 rpm for 15 min. The collected serum was stored under appropriate laboratory conditions until biochemical and hormonal analyses were performed.

For each replicate (cage), serum biochemical and hormonal measurements obtained from the seven individual birds were averaged to produce a single replicate mean. These replicate means were subsequently used for statistical analysis, consistent with the replicate being the experimental unit.

Serum biochemical traits, including total protein, albumin, globulin, and liver enzyme activities (aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)), were determined using commercial diagnostic kits (BIOLABO, France). Analyses were conducted using a spectrophotometer following the manufacturer's instructions and standard validated procedures for poultry serum analysis (Al-Salhi, 2025).

Serum concentrations of thyroid hormones (triiodothyronine (T3) and thyroxine (T4)) and reproductive hormones (follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, and progesterone) were measured

using chicken-specific ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China). All hormonal assays were performed according to the manufacturer's protocols, and absorbance was recorded using a microplate reader. All samples were analyzed under identical laboratory conditions to ensure analytical consistency and reliability.

2.4 Experimental Design and Statistical Analysis

The present study was conducted using a completely randomized design (CRD) involving 105 Lohmann Brown laying hens (65 weeks of age at the start of the experiment). Birds were randomly allocated to five dietary treatments, each with three replicates per treatment (7 hens per replicate; 21 hens per treatment). The experimental treatments consisted of a control group (T1) and four groups supplemented with 15, 20, 25, and 30 g/kg of Gum Arabic powder (T2–T5).

All hens were maintained under identical environmental and management conditions throughout the experimental period. The replicate (cage) was considered the experimental unit for all statistical analyses. Blood biochemical and hormonal data were analyzed using one-way analysis of variance (ANOVA) to determine the effect of dietary treatments. Prior to analysis, data were tested for normality and homogeneity of variance. When significant differences among treatment means were detected, means were separated using Tukey's Post Hoc test. Differences were statistically considered significant at $p \leq 0.05$.

Table 2. Composition and Chemical Analysis of the Experimental Diet Used During the Study

Dietary component	(%)
Yellow Corn	55
Bran	5.5
Soybean Meal (46% Protein)	24
Protein Premix 2.5%	2.5
Vegetable Oil	1.3
Limestone	10.85
Dicalcium phosphate (DCP)	0.5
Methionine %	0.1
Lysine %	0.05
Salt	0.1
Antitoxin	0.1
Total	100
Calculated chemical composition*	
Metabolizable energy (kilocalorie/kg feed)	2723
Crude protein (%)	16
Calcium (%)	4
Available phosphorus (%)	0.37
Methionine (%)	0.39
Lysine (%)	0.9
Threonine (%)	0.54
Salt (%)	0.31
Fiber (%)	3.8

Protein premix from the Dutch company Provim, each kg contains: (5.9%) crude protein, (3600) kcal metabolized energy/kg, (6.4%) calcium, (5.7%) phosphorus, (6.5%) sodium,(4000) mg/kg iron, (2800) mg/kg zinc, (600) mg/kg copper, (8.35) mg cobalt, (60) mg/kg iodine, (10) mg/kg selenium, (5.9%) methionine, (1.5%) lysine, (5.9%) methionine with cysteine, (1200) mg/kg niacin, (400,000) IU vitamin A, (140,000) IU D3, (2000) mg/kg E, (100) K, (90) mg/kg Thiamine, (160) ppb Riboflavin, (200) mg/kg Pyridoxine and (1000) meg/kg Vitamin Cobalamin.

*Calculated chemical composition based on (National Research Council, 1994).

3 Results and Discussion

Effects of dietary Gum Arabic supplementation on serum total protein, albumin, and globulin concentrations are presented in Table 3. Dietary supplementation with Gum Arabic significantly affected all measured biochemical parameters ($p \leq 0.05$).

Total protein concentration was significantly higher in all supplemented groups (T2–T5) than in the control group (T1), with the highest value observed in T5 (5.92 g/100 ml). Albumin concentration was significantly higher in hens receiving 25 and 30 g/kg of Gum Arabic (T4 and T5),

whereas intermediate values were observed in T2 and T3, which did not significantly differ from either the control or the higher supplementation groups. Similarly, serum globulin concentration was significantly influenced by dietary treatment, with all supplemented groups generally exhibiting higher values than the control group.

Table 3. Effects of dietary Gum Arabic supplementation on serum total protein, albumin, and globulin concentrations in older laying hens during 81–84 weeks of age*

Treatment	Total protein (g/100 ml)	Albumin (g/100 ml)	Globulin (g/100 ml)
T1	4.18±0.12^{b}	2.16±0.08^{b}	2.02±0.14^{b}
T2	5.43±0.27^{a}	2.52±0.17^{ab}	2.91±0.07^{a}
T3	5.57±0.23^{a}	2.73±0.31^{ab}	2.84±0.36^{ab}
T4	5.75±0.14^{a}	3.38±0.36^{a}	2.37±0.27^{ab}
T5	5.92±0.19^{a}	3.23±0.37^{a}	2.69±0.39^{ab}

*Data are presented as Mean±SE based on three replicate means per treatment (n=3). The heterogeneous superscript letters within the same column indicate significant differences at $p \leq 0.05$.

The first treatment (T1) served as the control group and received no gum Arabic supplementation. The second, third, fourth, and fifth treatments (T2, T3, T4, and T5, respectively) were supplemented with gum Arabic powder at inclusion levels of 15, 20, 25, and 30 g/kg of feed, respectively.

The significant increase in serum total protein observed in all Gum Arabic-supplemented groups may indicate an improvement in the physiological and metabolic status of older laying hens. Serum total protein is widely recognized as an important biochemical indicator of protein metabolism and nutritional status, and values within the normal physiological range are generally associated with normal liver synthetic function and adequate protein turnover (Nelson & Cox, 2004). Likewise, albumin, the major circulating serum protein, plays an essential role in maintaining osmotic pressure and transporting various endogenous and exogenous substances, including fatty acids, vitamins, and minerals (Al-Salhi, 2026a).

The present findings are in agreement with previous reports indicating that dietary supplementation with Gum Arabic or other functional feed additives can improve serum biochemical profiles in laying hens. For instance, Ismail et al. (2016) reported increases in serum protein fractions following Gum Arabic supplementation (Ismail et al., 2016), while Abaas et al. (2026) and Al-Salhi and Al-Shatty (2023) observed improvements in physiological and biochemical indices in laying hens receiving functional dietary supplements (Abaas et al., 2026; Al-Salhi & Al-Shatty, 2023). The consistency between the present study and previous findings supports the potential beneficial role of Gum Arabic in modulating serum protein metabolism in older laying hens.

Although Gum Arabic has been reported to possess antioxidant and prebiotic properties in previous studies (Ali et al., 2020; Calame et al., 2008; Jaafar et al., 2016; Nakov et al., 2016), the present study did not assess oxidative stress biomarkers, intestinal microbiota, or nutrient digestibility. Therefore, the underlying mechanisms responsible for the observed changes in serum total protein and albumin cannot be fully elucidated from the current data and warrant further investigation.

Table 4. Effect of adding Gum Arabic powder at different levels to the feed of older laying hens on the activity of liver enzymes and thyroid hormones for the period from 81–84 weeks*

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	T3 (ng/ml)	T4 (ng/ml)
T1	32.79±1.08^{a}	19.86±0.26^{a}	53.86±0.45^{d}	1.49±0.13^{b}	8.16±0.04^{c}
T2	26.17±0.17^{b}	15.87±0.25^{b}	59.18±0.25^{c}	1.57±0.21^{b}	9.60±0.15^{b}
T3	25.73±0.25^{b}	15.20±0.43^{bc}	61.83±0.17^{b}	1.99±0.28^{ab}	10.22±0.13^{b}
T4	24.92±0.38^{b}	14.87±0.39^{bc}	61.64±0.55^{b}	2.30±0.15^{a}	12.31±0.16^{a}
T5	24.74±0.41^{b}	14.28±0.30^{c}	64.29±0.50^{a}	2.32±0.11^{a}	13.05±0.24^{a}

*Data are presented as Mean±SE based on three replicate means per treatment (n=3). The heterogeneous superscript letters within the same column indicate significant differences at $p \leq 0.05$.

The first treatment (T1) served as the control group and received no gum Arabic supplementation. The second, third, fourth, and fifth treatments (T2, T3, T4, and T5, respectively) were supplemented with gum Arabic powder at inclusion levels of 15, 20, 25, and 30 g/kg of feed, respectively.

The activities of serum liver enzymes and thyroid hormones in older laying hens receiving different dietary levels of Gum Arabic are presented in Table 4. Serum AST and ALT activities are widely recognized as sensitive biochemical indicators of liver function, whereas ALP activity is associated with both hepatic and bone metabolism. In the present study, dietary supplementation with Gum Arabic reduced AST and ALT activities and increased ALP activity compared with the control group, indicating potential improvements in physiological status under the experimental conditions.

These findings are in agreement with previous reports describing beneficial effects of Gum Arabic supplementation on liver-related biochemical parameters (Ismail et al., 2016). Gum Arabic contains several bioactive constituents, including minerals such as copper and zinc, vitamins (C, A, and E), and phenolic compounds and flavonoids, which have been reported to exhibit antioxidant properties (Abdul Basit et al., 2020; Mariod, 2018). These compounds may contribute to the maintenance of liver function and cellular integrity through antioxidant-related mechanisms and protection against oxidative damage (Ismail et al., 2016). However, since oxidative stress biomarkers, antioxidant enzyme activities, and liver histopathology were not evaluated in the present study, these mechanisms remain speculative.

Gum Arabic has been reported to contain vitamins and bioactive compounds that may support mineral metabolism (Mariod, 2018). However, parameters related to calcium metabolism, bone turnover, and eggshell quality were not assessed in this study; therefore, mechanistic interpretations regarding ALP activity should be made with caution.

Triiodothyronine (T3) and thyroxine (T4) are the principal thyroid hormones that regulate metabolic activity, energy utilization, growth, and physiological homeostasis in poultry (Al-Rekabi et al., 2020; Rippamonti & Dzialowski, 2023). In the present study, birds receiving higher levels of Gum Arabic supplementation (T4 and T5) exhibited significantly higher serum T3 concentrations, whereas all supplemented groups showed higher T4 concentrations than the control group.

Previous studies have suggested that flavonoids, vitamins, and trace minerals present in Gum Arabic may influence thyroid function through multiple physiological pathways, including antioxidant protection of thyroid tissue, modulation of deiodinase activity, regulation of the hypothalamic–pituitary–thyroid axis, and improved nutrient availability required for hormone synthesis (Ali et al., 2021; Cinar et al., 2014; Kohrle, 2023). In addition, the prebiotic properties of Gum Arabic may support beneficial gut microbiota, which could indirectly enhance nutrient utilization (Kohrle, 2023). However, thyroid-stimulating hormone, oxidative stress markers, intestinal microbiota, nutrient absorption, and deiodinase activity were not measured in the present study. Therefore, these mechanisms remain hypothetical and are inferred from previous literature rather than directly demonstrated by the current experiment. Overall, the observed increases in T3 and T4 suggest an association between dietary Gum Arabic supplementation and improved thyroid hormone profiles in older laying hens, while the underlying mechanisms require further research.

Table 5. Effect of adding gum arabic powder at different levels to the diet of older laying hens on serum progesterone, estrogen, LH, and FSH concentrations (pg/ml) for the period of 81-84 weeks*

Treatment	Estrogen (pg/mL)	Progesterone (pg/mL)	LH (pg/mL)	FSH (pg/mL)
T1	269.17±42.68^{b}	5.01±0.39^{c}	8.38±0.15^{b}	12.96±0.15^{c}
T2	285.66±37.59^{b}	6.14±0.44^{c}	9.19±0.83^{ab}	14.25±0.34^{b}
T3	332.11±36.02^{ab}	8.04±0.23^{b}	9.83±0.48^{a}	14.96±0.28^{ab}
T4	350.95±12.73^{a}	8.16±0.34^{b}	9.74±0.56^{a}	15.48±0.39^{a}
T5	366.10±18.68^{a}	9.56±0.28^{a}	10.02±0.72^{a}	16.81±0.81^{a}

*Data are presented as Mean±SE based on three replicate means per treatment (n=3). The heterogeneous superscript letters within the same column indicate significant differences at $p \leq 0.05$.

The first treatment (T1) served as the control group and received no gum Arabic supplementation. The second, third, fourth, and fifth treatments (T2, T3, T4, and T5, respectively) were supplemented with gum Arabic powder at inclusion levels of 15, 20, 25, and 30 g/kg of feed, respectively.

The effects of dietary Gum Arabic supplementation on serum reproductive hormone concentrations are presented in Table 5. In the present study, hens receiving higher levels of Gum Arabic supplementation showed significantly higher serum concentrations of estrogen, progesterone, LH, and FSH than those in the control group, suggesting a beneficial effect on the endocrine profile of older laying hens under the experimental conditions.

The observed increases in reproductive hormone levels are consistent with previous studies reporting physiological benefits of biologically active compounds present in Gum Arabic. The powder contains various bioactive constituents, including phenolic compounds, flavonoids, carotenoids, terpenes, polysaccharides, and vitamins, which have been suggested to influence reproductive physiology in poultry and other animal species (Abdel-Farid et al., 2014). Estrogen plays a key role in oviduct development, tubular gland activity, and yolk precursor protein synthesis, whereas progesterone is involved in regulating reproductive processes (Ali et al., 2019). Likewise, vitamin E has been reported to influence gonadotropin secretion and reproductive endocrine regulation in poultry (Yin et al., 2019).

In addition, carotenoids and other phytochemicals present in Gum Arabic may contribute to reproductive performance through pathways associated with vitamin A metabolism, ovarian follicular development, antioxidant defense, and reproductive tissue function (Abdel-Farid et al., 2014). Improved antioxidant status has also been linked to enhanced physiological and immune responses in poultry (Al-Salhi, 2026a).

However, the present study did not assess hypothalamic–pituitary–gonadal (HPG) axis activity, ovarian morphology, follicular development, ovulation rate, oxidative stress biomarkers, antioxidant enzyme activities, or immune parameters. Therefore, these mechanisms cannot be confirmed by the current data and should be interpreted as hypotheses derived from prior literature rather than as direct findings of the present experiment. Overall, the observed hormonal changes suggest an association between dietary Gum Arabic supplementation and improved reproductive hormone profiles in older laying hens, while the underlying biological mechanisms require further research.

4 Conclusion

Dietary supplementation with Gum Arabic powder had a positive effect on several serum biochemical and endocrine parameters in older laying hens under the conditions of the present study. Compared with the control group, Gum Arabic supplementation improved serum total protein and albumin concentrations, modulated liver enzyme activities (AST, ALT, and ALP), and enhanced thyroid (T3 and T4) and reproductive hormone profiles (estrogen, progesterone, LH, and FSH).

These results suggest that Gum Arabic may serve as a functional feed additive to improve selected physiological and endocrine responses in older laying hens. However, the underlying biological mechanisms were not assessed in the present study. Therefore, further research is required to elucidate its mode of action. Future studies should focus on evaluating oxidative stress biomarkers, intestinal health, reproductive physiology, and long-term productive performance to better elucidate the physiological and molecular mechanisms associated with Gum Arabic supplementation.

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Use of Artificial Intelligence

AI-assisted language editing tools were solely used to enhance the linguistic quality and readability of this manuscript. All scientific aspects of the work, including the study design, methodology, data analysis, interpretation of results, and conclusions, were developed and critically evaluated by the authors. The authors assume full responsibility for the final content of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

EHY conceived the study, conducted the experimental work, collected the data, and prepared the original draft of the manuscript. NALA contributed to the study design, supervised the experimental work, performed the statistical analysis, interpreted the data, and reviewed the manuscript. AAAS critically revised and edited the manuscript, supervised the final revisions, handled the manuscript submission and all correspondence with the journal as the corresponding author. All authors reviewed the manuscript. All authors checked and approved the final draft of the manuscript.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Considerations

All procedures involving live laying hens were reviewed and approved by the Scientific Committee of the College of Agriculture, Al-Qasim Green University, Babylon, Iraq, the institutional committee responsible for reviewing and approving animal research protocols. The study was approved under Approval No. 53 on 15 October 2024. All animal handling, husbandry, and blood sampling procedures were conducted in accordance with institutional animal welfare requirements and internationally accepted guidelines for the care and use of animals in research.

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