RESEARCH ARTICLE

Physiological and Metabolic Response of Peking Ducks Supplemented with Graded Levels of Hesperidin: Focus on Lipid Metabolism, Oxidative Balance, and Thyroid Axis Activity

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ABSTRACT

Research has identified the high incidence of deposition in abdominal fat as one of the economic practical challenges for improving carcass quality, especially among well-known Pekin duck strains. This makes it necessary to find natural, safe alternatives for synthetic stimulants. Thus, this study was designed to assess the influence of different concentrations of hesperidin on lipid profile and oxidative status as well as thyroid hormones activation in Pekin ducks. The experiment was conducted in the fields and laboratories of the College of Agriculture at the University of Kerbala using 160 one-day-old birds. The results of the study showed a substantial linear improvement in the lipid profile at the dose of 100 mg/kg. Total cholesterol decreased from 190.50 to 155.50 mg/ dL, triglycerides from 95.10 to 55.86 mg/dL, and low-density lipoproteins (LDL) from 65.26 to 38.72 mg/dL. In contrast, HDL levels increased to 78.87 mg/dL. Regarding the oxidative status, the activity of the enzyme (GPX) increased from 35.20 to 63.40 units/mL, and the lipid stress indicator (MDA) decreased from 3.60 to 1.62 nmol/dL. This reflects superior protection of the cellular membranes. Metabolic activity was also enhanced through the increase in the level of the hormone (T3) from 1.60 to 2.42 units/L and the hormone (T4) from 7.10 to 9.25 units/L. This increased their conversion ratio (T3/T4) from 0.23 to 0.26. It is concluded from this that hesperidin acts as an effective dual-function metabolic regulator. It improves lipid metabolism and strengthens the bird's defensive system efficiently. We recommend using hesperidin at a dose of 100 mg/kg as a natural feed additive to enhance health and productivity in duck-rearing projects.

Keywords: *Pekin ducks, Hesperidin, Lipid metabolism, Oxidative balance, Thyroid hormones*

INTRODUCTION

Poultry farming is one of the building blocks of the International food security section. Raising Pekin ducks is becoming popular due to their fast growth and high production rate. Nevertheless, the industry brings in the most serious issue "abdominal fat deposition". A decrease in carcass quality is caused by the

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deposition of excess fat. Such meat consumes is also avoided by health-associated consumers. Also, it is a waste of energy which has formed from the digestion of feed for meat. As a result, it causes project feed conversion efficiency to be low and economic losses (Chen *et al*., 2023).

Chemical substances termed "natural feed additives" have been undergone investigations to ensure their safety and efficacy in response to recent global movements aimed at restricting the use of synthetic stimulants and antibiotics in feed. Hesperidin is a citrus bioactive compound that comes from citrus peel. This compound has strong antioxidant, anti-inflammatory and lipid metabolism regulating activities.

As a result, hesperidin enhances the general health condition of the bird. Alam and colleagues (2022)

Moreover, recent research shows some feed additives play an important role in meat quality. According to Tang and his colleagues' latest research from 2023, the incorporation of certain micronutrients into dietary feed enhances the fatty acid composition and antioxidant capacity of Pekin duck breast muscles. The inclusion of hesperidin in broiler diets led to significantly enhanced blood parameters and subsequently oxidative status (Abdel-Wareth & Lohakare 2021). In this context, the effects also passed to other species, such as reported by El-Sayed *et al*. (2023), who claimed hesperidin improved lipid profile and markers of oxidative stress in Japanese quail.

Moreover, the basal metabolic rate and spatial distribution of energy in a bird are regulated by the thyroid hormones (T3 and T4). In recent investigation, Zhang *et al*. (2025) has correlated hesperidin with the modulation of thyroid axis activity. This supports the argument that stimulation of hormone secretion and/or activity improves lipid and protein metabolism. Although the role of hesperidin has been evaluated in broiler studies, no comprehensive data are available on its normalizing efforts for integrated "Pekin ducks" on lipid metabolism, oxidative balance, and action of the thyroid gland simultaneously.

The present study was conducted to investigate the effects of graded levels of performance margarine (25, 50 and 100 mg/kg) on the lipid profile (T. Cholesterol, TG, LDL & HDL), assessment of antioxidant system efficiency (GPX, SOD & MDA), quantification of hormonal activity from the thyroid gland (T3 & T4) and their metabolic conversion rate in Pekinducks to determine the recommended dose that gave maximum physiological and productive performance..

MATERIALS AND METHODS

Sample Collection and Processing

The applied part of the study was carried out in the poultry fields belonging to the College of Agriculture at the University of Karbala. At the end of the experimental period (day 42), blood samples were collected from birds randomly selected across all experimental replicates, at a rate of 6 birds per treatment, to ensure adequate statistical representation. Venipuncture was used to collect samples, with 2 mL of blood drawn from each bird via the brachial vein using sterile medical syringes. This method was adopted because it is a standard, internationally approved method in poultry physiology, owing to its low side effects on the bird and the ease of obtaining the required blood volume (Hassan *et al.*, 2022).

After the collection process, the blood samples were placed in special test tubes (anticoagulant-free) and left at room temperatu(25°C) for a sufficient period to allow natural clotting and serum separation. After that, samples were transferred to the college's central laboratory to complete the separation process, with full adherence to cold chain standards and to ensure samples were not exposed to sudden freezing temperatures that could cause hemolysis. This ensures preservation of the integrity of biochemical components and hormonal indicators to be measured (Parasuraman & Raveendran, 2022).

To obtain pure serum, the samples were centrifuged at 3000 rpm for 15 minutes. The clear serum was withdrawn using a micropipette and distributed into numbered Eppendorf tubes. The samples were then preserved in a deep freezer at -20°C until biochemical analyses of the lipid profile, oxidative stress indicators, and thyroid hormone activity.

Experimental design and dietary treatments

The experiment was conducted using 160 male Pekin ducklings at one day of age. They were randomly distributed according to a completely randomized design (CRD) among four dietary treatments. Each treatment included four replicates, with 10 birds for each replicate. The cage was considered the independent experimental unit. Thus, the total number of birds in the study was 160, distributed among 16 group cages. An additional reserve group (cage No. 161) was also allocated for the purpose of compensating for possible mortality cases during the first days of the experiment, to ensure the stability of the numerical density. The experiment continued for 42 days. During this period, the birds were reared under controlled environmental conditions for temperature, humidity, and lighting to ensure uniformity of external influences. The dietary treatments were divided as follows:

1. The first treatment (T1): represented the control group and depended on the basal diet only, without any additives.
2. The second treatment (T2): the basal diet supplemented with the compound hesperidin at a concentration of 25 mg/kg feed.
3. The third treatment (T3): the basal diet supplemented with the compound hesperidin at a concentration of 50 mg/kg feed.
4. The fourth treatment (T4): the basal diet supplemented with the compound hesperidin at a concentration of 100 mg/kg feed.

The compound hesperidin was selected as a bioactive feed additive because it is a natural flavonoid extracted from citrus fruits. It has distinguished physiological properties, as it is a natural growth promoter and a strong antioxidant. The scientific importance of adding it lies in its ability to reduce oxidative stress and enhance metabolic efficiency. This positively reflects on the general productive performance of birds (Hanna *et al.*, 2024). Feed and water were provided to the birds freely (ad libitum) throughout the experimental period, while observing the precise technical and medical standards in flock management.

Physiological measurements and biochemical analyses

At the age of 42 days of experimentation, blood was taken from six birds randomly selected from each experimental unit. Each bird's brachial vein yielded roughly 2 mL of blood. Following the "continuous cold chain" preservation protocol to maintain the chemical stability and biological and enzymatic components against decomposition, all samples were transferred directly into sterile laboratory tubes without anticoagulant. We obtained blood serum by centrifugation at 3000 rpm for 15 minutes. Afterward, serum separation was performed; the separated serum was stored in Eppendorf tube at -20°C until analysis.

Laboratory tests were conducted for the analysis of the activity of antioxidant defense system enzymes, using ready-made kits containing colorimetric methods, superoxide dismutase, SOD, catalase (CAT), and glutathione peroxidase (GPx) [8]. The study also assessed the metabolic efficiency of the birds by estimating the levels of thyroid hormones (thyroxine: T4; triiodothyronine: T3). The complete lipid panel was also assessed. The scientists measure the total cholesterol, triglycerides as well as the high-density lipoproteins (HDL) as well as low-density lipoproteins (LDL) to look at the exact physiologic and biochemical effect of the compound hesperidin through a proper methodology. (Sultanayeva *et al.*, 2023).

The laboratory tests analyzed the activity of antioxidant defense system enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [8], using ready-made kits based on colorimetric methods. Thyroid hormone levels (thyroxine: T4; triiodothyronine: T3) were also quantified to assess the birds' metabolic efficiency. Additionally, the full lipid panel was analyzed. This involved the determination of total cholesterol, triglycerides, and high-density lipoproteins (HDL) and low-density lipoproteins (LDL) to assess the precise physiologic and biochemical effects of the compound hesperidin using appropriate scientific methods (Sultanayeva *et al.*, 2023).

Biochemical analysis of blood serum

Protocol of sample collection and serum preparation

Upon completion of the experimental stage on the forty-second day, random blood samples of 2 mL for each bird were withdrawn from the brachial vein, while observing the ethical standards for dealing with birds. To prevent the enzymatic decomposition of the biological constituents and ensure the good metabolic stability, the samples were transferred to the laboratory using a cold chain. Centrifugation at 3000 rpm for 15 minutes was done for serum separation process. After that, aliquots of the samples were prepared in sterile micrometric tubes and stored at -20°C to preserve the biomarkers until analysis.

Lipid profile characterization

To evaluate the regulatory

role of hesperidin, one of the active phenolic compounds, in lipid metabolism, the main components of the blood serum lipid profile were quantified. These included total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Enzymatic colorimetric methods were adopted in these measurements. These methods are characterized by high accuracy, as these indicators are considered reliable vital evidence for detecting changes in lipid metabolism pathways resulting from dietary treatments (Yang *et al.*, 2023).

Evaluation of oxidative stress biomarkers

To evaluate how well the birds' defence system copes with free radicals, oxidative balance would be assessed based on the activity of the superoxide dismutase (SOD) and the glutathione peroxidase (GPx) enzymes. A measure of malondialdehyde (MDA), the final secondary product of lipid peroxidation, was established as a precise chemical marker for the level of oxidative damage in cellular membranes. The increase in SOD and GPx activities, along with a reduction in MDA levels shows that the addition of the diet improves reduces the total antioxidant power, which is an established physiological criterion. (El-Ghareeb *et al.*, 2023).

Hormonal analysis of the thyroid gland

Scientists used serum concentration of triiodothyronine (T3) and thyroxine (T4) as evaluation factors because thyroid hormones are important in FCR efficiency and growth rates. Pekin duck's useful performance is linked by the physiological responses of the duck's useful hormones that indicate the metabolic state and basal metabolic rate (BMR).

Quality control and chemical analysis

All biochemical tests were performed with commercial kits validated according to the manufacturer's precise technical recommendations. For each analytical run, standard calibration curves were set up to guarantee accuracy and reproducibility. Laboratory analyses based on international quality control standards allow to guarantee that the data is reliable and appropriate for scientific interpretation. (Gao & Xu, 2025).

Statistical analysis

The experiment's data were analysed using the Statistical Package for the Social Sciences (SPSS, Version 26.0). Preliminary tests to check the basic assumptions of parametric models were performed to ensure the accuracy of statistical inference. Data distribution normality was assessed by using Shapiro–Wilk test. Homogeneity of variance among experimental groups was confirmed using Levene's test.

Once these conditions were verified, one-way ANOVA was used to estimate the intrinsic effects of added hesperidin doses relative to the control group. In cases where ANOVA revealed significant differences, Duncan's multiple range test was used as a post-hoc test for multiple comparisons. This was done to precisely determine the significance of differences in the arithmetic means of the experimental treatments.

All results were expressed as mean ± standard error of the mean (Mean ± SEM). The globally recognized statistical confidence levels in biological studies were adopted. Differences were considered significant at the probability level ($P < 0.05$) and highly significant when the probability value was ($P < 0.01$), based on standard statistical methodologies used in similar research (Janczyk, 2023; Laerd, 2025).

RESULTS AND DISCUSSION

Effect of adding hesperidin as a dual-function metabolic modulator on the lipid profile in Pekin ducks

The experimental data presented in the current study showed the presence of a substantial physiological response associated with the dose (dose-dependent response) in the metabolic lipid profile of Pekin ducks as a result of dietary supplementation with hesperidin. Based on the analytical data presented in Table 1, a highly significant linear improvement was observed in all indicators of the lipid profile in conjunction with the increasing levels of dietary incorporation of the flavonoid. Dietary intervention with hesperidin led to a clear hypocholesterolaemia effect, as total cholesterol levels decreased from 190.50 mg/dL in the control group to a lowest value of 155.50 mg/dL with a dose of 100 mg/kg. Physiologically, this effect is attributed to the specific role of hesperidin as a biological inhibitor of the activity of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which is the rate-limiting enzyme in endogenous cholesterol synthesis, in addition to its ability to regulate the molecular pathways of hepatic transcription factors such as SREBP-1c and peroxisome proliferator-activated receptors (PPARα), which are responsible for the balance of lipid metabolism.

These conclusions are consistent with findings from Abdel-Wareth and Lohakare (2021) in their study on poultry, which confirmed that hesperidin acts as a genetic modulator, inhibiting lipid synthesis and improving the bird's overall metabolic efficiency.

Under the same metabolic context, results showed a very steep and highly significant drop (P = 0.01) in triglyceride concentration, 95.10 mg/dL for maximum-level treatment to 55.86 mg/dL. The drop results from the effectiveness of hesperidin in fatty acid oxidation (β-oxidation), causing a reduction in fat in the blood, thus ultimately providing direct protection to birds against fatty liver syndrome which is common in ducks. The findings of El-Sayed *et al*. (2023) for quail where flavonoids triggered pathways responsible for burning energy corroborate with these data. Research by Hager-Theodorides *et al*. (2021) indicates that the combined effect of flavonoids is due to the reprogramming of genes responsible for fatty acid metabolism in domestic birds.

At lipoprotein balance level, low-density lipoprotein (LDL) decreases significantly in a gradual manner (P = 0.02) with the number of 38.72 mg/dL compared to control. Hesperidin is believed to be a biological indicator which protects the vascular system against lipid oxidation damage. Conversely, the study observed a remarkable rise of high-density lipoprotein (HDL) levels to 78.87 mg/dL, showing improved reverse cholesterol transport from peripheral tissues to the liver. As noted by Kumar *et al*. (2023), hesperidin can have potentially lipid-lowering effects and prevent vascular inflammation associated with hyperlipidaemia.

Hesperidin has a dual function as metabolic modulators and production of active substances. Its parallel action can reduce harmful lipids fractions and increase beneficial lipids. It became a functional feed additive, which can improve carcass quality and reduce abdominal fat. Global trends focus on the use of bioactive plant additives to improve the metabolic and productive health of the poultry sector.

Effect of dietary supplementation with hesperidin on the oxidative balance status and stress indicators in Pekin ducks

The experimental results showed a significant, and a dynamic improvement in the antioxidative balance of Pekin ducks when receiving graded dietary levels of hesperidin. Laboratory analyses revealed a gradual, notable enhancement of defensive enzymatic system activity, with glutathione peroxidase (GPX) presenting the greatest increase from 35.20 units/mL recorded in the control rat group to reach its peak at 63.40 units/mL for the higher dose treated rats (100 mg/kg), statistically significant P=0.02).

Concomitantly, the activity of superoxide dismutase (SOD) exhibited a continuous dose-dependent increase ranging from 152.10 units/mL in the control to 185.90 units/mL at the highest supplementation level (P=0.03). The increased activity of these enzymes also demonstrates the role of hesperidin as a stimulatory compound in genetic pathways involved in endogenous antioxidant production, thereby improving birds' ability to scavenge free radicals effectively.

With rise in the enzymatic defence levels, there was a rapid and highly significant (P= 0.01) reduction in malondialdehyde (MDA) concentration, the most important biochemical marker for lipid peroxidation and destruction of cellular membrane. The MDA concentrations were decreased from the control's 3.60 nmol/dL to 1.62 nmol/dL with 100 mg/kg of hesperidin addition with more than 50% reduction in oxidative degradation. The reason is that hesperidin can scavenge hydroxyl radicals and singlet oxygen better, and thus it can protect relatively better the unsaturated fatty acids in cell wall from oxidative degradation. The effect of the compound is twofold, increasing the intracellular strength of cells as well as lowering stress by-products in blood.

These results are consistent with previous findings. Research conducted on broiler chickens has shown that flavonoid compounds like hesperidin activate the Nrf2 pathway that promotes the expression of antioxidant genes (Abdel-Wareth and Lohakare, 2021). This information also agrees with the results of a study conducted in Japanese quail. It shown that hesperidin intervention greatly reduces oxidative stress induced by environmental factors (El-Sayed and El-Sheikh, 2023). In addition, the apparent reduction in MDA levels has been confirmed in animal studies with metabolic disorders indicating that hesperidin functions like a biological defence shield preventing tissue injury (Kumar *et al.,* 2023). Recent studies show that hesperidin has unique abilities in regulating oxidative balance and protection against stress-induced programmed cell death which can positively affect general health status and productive efficiency of Pekin ducks (El-Ghareeb *et al*., 2023; Ileriturk *et al*., 2023).

Effect of dietary intervention with hesperidin on the hormonal response of the thyroid gland axis and metabolic efficiency

concentration of 100 mg/kg recorded the highest value of 2.42 units/L compared to control of 1.60 units/L (P = 0.03). Increasing amounts of hesperidin displayed increased levels of thyroxine (T4) in the body. Its level increased from 7.10 units/L in the control to 9.25 units/L in the highest treatment (P = 0.02). The control group's physiological state allows us to have a high reliability as it falls in the reference range of Pekin ducks. (For the control group T3: 1.5-2.0 U/L and T4: 6.5-8.0 U/L). In other words, the stimulating effect of hesperidin did not drive the birds to the far boundary of safe physiology but rather to the upper limit of secretory efficiency.

T3/T4 ratio was significantly higher (P=0.04) during third treatment phases (control at 0.23) and reached a ratio value (0.26) at the level of efficiency of hormonal conversion. Prior evidence indicates the striated (muscle) tissues markedly upsurge T3 as well as oxidative metabolism in these instances. Higher activity of hormones means an increase in basal metabolic rate and protein biosynthesis. One of a bird's high growth and production efficiency. Scientific data shows flavonoid compounds may directly influence the thyroid–pituitary axis, as seen in these findings. The gland's functional performance evidence can be regulated and improved by flavonoid compounds (Zhang *et al*., 2025).

Hesperidin displays both agonist and antagonist properties, making it a unique biomodulator, as scientific explanation of these results shows. It reduces lipid peroxidation (MDA) and enhances the efficacy of defensive enzymes (GPX and SOD). Thus, on one hand, it acts as an antoxidant. It prevents oxidative damage of the thyroid gland cell. It also acts as a hormonal modulator by modulating the thyroid–pituitary–metabolic axis for homeostasis. The ability of hesperidin to increase T3/T4 ratio in Pekin ducks is in agreement with the result observed in Japanese quail, which hesperidin is a metabolic stimulant for rapid growth (El-Sayed and El-Sheikh, 2023). Studies with hyperactive rats, on the other hand, show that it can decrease such ratios thus establishing its functional versatility to restore equilibrium in accordance to the body's physiological condition. Cite the works of Abdel-Wareth and Lohakare and Kumar. According to a study conducted by El-Sayed and El-Sheikh in 2023 as well as Hager et al. in 2021, when hesperidin is added to the diet at 100 mg/kg , it is effective in improving metabolic and production performance by a natural and safe modulation of the thyroid gland hormonal response.given food additives hesperidin. Triiodothyronine hormone levels T3 gradually and significantly increased with the increase in the added dose, where treatment at a concentration of 100 mg/kg recorded the highest value of 2.42 units/L compared to control of 1.60 units/L (P = 0.03). A similar linear increase in thyroxine (T4) was observed with increasing levels of hesperidin. Its level increased from 7.10 units/L in the control to 9.25 units/L in the highest treatment (P = 0.02). Due to the physiological state of the control group, whose values fall in the reference range of Pekin ducks (T3: 1.5-2.0 U/L and T4: 6.5-8.0 U/L), high reliability may be taken. It means that the stimulatory effect of hesperidin didn't push the birds to afar boundary of safe physiology, but close to the upper limit of secretory efficiency.

The T3/T4 ratio (control at 0.23) was significantly higher (P=0.04) throughout the third treatment and reached a ratio value (0.26) at the level of hormonal conversion efficiency. Prior evidence suggests that striated (muscle) tissues see a marked upsurge of T3 along with increased oxidative metabolism in such cases. An improvement of hormone activity indicates a direct enhancement of basal metabolic rate and protein biosynthesis. This demonstrates a bird's great growth rate and productive efficiency. These findings are in agreement with scientific data revealing that flavonoid compounds may directly affect the thyroid–pituitary axis. This provides evidence that flavonoid compounds can regulate and improve the functional performance of the gland (Zhang *et al*., 2025).

The scientific explanation of these results is that hesperidin has both agonist and antagonist properties, making it a unique biomodulator. On one hand, it acts as an antioxidant that prevents oxidative damage of the thyroid gland cell finally reducing lipid peroxidation (MDA) and enhancing the efficiency of defensive enzymes (GPX and SOD). It also works as a hormonal modulator by regulating the thyroid–pituitary–metabolic axis for homeostasis. Hesperidin's capacity to enhance the T3/T4 ratio in Pekin ducks is in keeping with the outcome noted in Japanese quail, where the compound acted as a metabolic stimulant to promote fast growth (El-Sayed and El-Sheikh, 2023). On the other hand, in other experiments such as rats with hyperactivity, it can reduce these ratios demonstrating its functional versatility to restore equilibrium in tune with the physiological condition of the body. Abdel-Wareth and Lohakare (2021) and Kumar *et al*. (2023). The dietary addition of hesperidin at a dose of 100 mg/kg is effective in improving metabolic and productive performance via the natural and safe modulation of the hormonal response of the thyroid gland (El-Sayed and El-Sheikh, 2023; Hager *et al*., 2021).

CONCLUSIONS

The present study presents a holistic perspective on the essential and intermediate role of hesperidin as a natural feed additive for reprogramming metabolic and physiological pathways in Pekin ducks. From experimental data, this study confirmed that hesperidin proved to be an effective lipid metabolism modulator (potent lipid regulator) at a level of supplementation, namely 100 mg/kg, which greatly improved the serum lipid profile by significantly lowering total cholesterol, triglycerides, and low-density lipoproteins (LDL), unlike HDL levels observed with significant increases. This effect is not only beneficial in enhancing avian cardiovascular health but also an ideal approach to diminishing potential additive deposition of abdominal fat, which alone improves carcass yield and profitability.

The outcomes also showed that hesperidin has a strong ability to fortify the defense system against oxidative stress. A significant increase in GPX and SOD activities, as well as a highly significant decrease in MDA levels, was observed due to the dietary intervention. This improvement illustrates the function of hesperidin as a ubiquitous antioxidant that protects cellular membranes from damage, a key point in fast-growing duck strains subjected to high metabolic stress. Dietary supplementation in this study was also shown to be tightly linked to activation of the thyroid–pituitary axis. This well-proportioned increment in T3 and T4 hormones and their conversion ratio, contributing to improved basal metabolic rates and influencing the bird's general metabolic activity, is clearly reflected in the improvements recorded in productive performance as well as in health performance.

We conclude that the present study advocates using hesperidin, a safe and cost-effective nutritional supplement at a 100 mg/kg dose, as an effective alternative for synthetic stimulants in poultry production. This input is used not only to achieve higher quality of the end product, which is meat, but also for the maintenance and sustainable preservation of health conditions in a herd, with combined antioxidant, lipid-regulating, and metabolism-stimulating effects. These findings elucidate the future perspectives for employing this compound to gain deep insight into its underlying molecular impacts on gene expression related to meat quality in ducks.

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Ethics Statement

All procedures of experimental including animals in this study conducted in strict accordance with the ethical standards and guidelines of the College of Agriculture, University of Kerbala. The research protocol reviewed and approved by the institutional committee responsible for animal welfare.

The welfare of the ducks prioritized during the experiment, Birds housed under optimal environmental conditions which provided with balanced nutrition and monitored daily to discomfort and minimize stress ,No invasive or harmful procedures performed beside standard husbandry practices.

The study adhered to internationally recognized principles of animal research ethics. all procedures refined to maximize animal being well,including the 3Rs framework (Replacement, Reduction, and Refinement), ensuring that the number of animals used was the minimum necessary to achieve reliable scientific results.

Originality and Plagiarism

The authors confirm that this manuscript is an original work ,and performed in the University of Kerbala, College of Agriculture. the research team do all the works such as ,data collection, study design, analysis, and interpretation conducted independently without duplication or reproduction of prior published work. The manuscript has not been submitted or published in any other repository, journal or conference.

All sources of information, references and data used in this manuscript have been properly cited to acknowledge the contributions of previous researchers.

Consent for Publication

confirm the authors that all participants involved in this study, including the institution where the research conducted, their full consent provided for the publication of this manuscript. The College of Agriculture, University of Kerbala has authorized the dissemination of the results , the authors collectively agree to the submission and publication of the work in scientific outlets.

this manuscript does not have part of infringes upon the rights of any individual or institution . all data presented generated and analysed by the research team with transparency and integrity. Have no competing interests related to this study, The research conducted independently at the College of Agriculture, University of Kerbala, without any commercial, financial or personal relationships that could be construed as a potential conflict of interest.

All results and interpretations presented in this manuscript based solely onobjective analysis and scientific evidence .there is No external influence has affected execution, the design, or reporting of this artical.

Data Availability

the corresponding author generated and analyzed of The datasets during the current study The datasets are available upon reasonable request. All raw data were collected and archived at the College of Agriculture, University of Kerbala including lipid profile values, oxidative stress indicators,biochemical measurements, and thyroid hormone levels .Any request for data access will be considered in line with ethical standards and the protection of research integrity.Due to institutional policies. the data are not publicly deposited in an open repository; however, the authors ensure that the full dataset supporting the conclusions of this research can be provided to qualified researchers for academic purposes.

Author Contributions

The first Author Conceived the study idea, designed the experimental framework and supervised the overall project. The second Author Managed the experimental birds, conducted fieldwork and recorded primary observations. Third Author Performed biochemical assays, including oxidative stress indicators, lipid profile measurements and thyroid hormone anayses. Fourth Author Analysed the results, performed statistical evaluations, and interpreted the metabolic and physiological outcomes. Fifth Author Drafted the manuscript, check out the text for academic lucidity and coordinated submission to the journal.

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