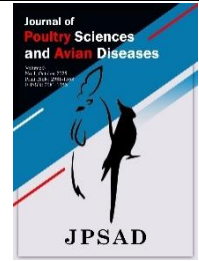


Journal of Poultry Sciences and Avian Diseases

Journal homepage: www.jpsad.com



Association of Body Weight Categories with Reproductive Hormones, Serum Biochemical Profile, and Productive Performance in ISA Brown Laying Hens during the Mid-Laying Period



Ahmed A. Al-Salhi^{1*}

1. Department of Pharmaceutical Sciences, College of Pharmacy, University of Thi-Qar, 64001, Iraq

* Corresponding author email address: ahmed-alsalhi@utq.edu.iq

Article Info

Article type:

Original Research

How to cite this article:

Al-Salhi, A. A. (2026). Association of Body Weight Categories with Reproductive Hormones, Serum Biochemical Profile, and Productive Performance in ISA Brown Laying Hens during the Mid-Laying Period. *Journal of Poultry Sciences and Avian Diseases*, 4(4), 1-15.

<http://dx.doi.org/10.61838/kman.jpsad.211>



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ABSTRACT

The aim of this study was to evaluate the physiological relationship between body weight, selected biochemical and hormonal serum parameters, and their association with productive performance in ISA Brown laying hens during the mid-laying period (42–50 weeks of age). A total of 135 ISA Brown laying hens were divided into three experimental groups according to body weight category: light, medium, and heavy body weight, with 45 hens per treatment. Productive performance traits, including hen-day egg production, egg weight, and egg mass, were recorded. In addition, serum concentrations of reproductive hormones (FSH, LH, and estradiol) and selected biochemical parameters (glucose, total protein, albumin, cholesterol, and triglycerides) were determined. The results demonstrated that medium-weight hens showed significantly higher ($p \leq 0.05$) hen-day egg production and higher concentrations of reproductive hormones, total protein, and albumin than the other groups, indicating a more favorable physiological and metabolic balance associated with improved reproductive efficiency and laying persistency. In contrast, heavy-weight hens recorded significantly higher ($p \leq 0.05$) egg weight, glucose, cholesterol, and triglyceride concentrations, reflecting increased metabolic activity and fat deposition associated with greater body weight. Pearson correlation analysis within body weight categories revealed significant positive correlations ($p \leq 0.05$) between reproductive hormones and productive performance traits, particularly in medium-weight hens, whereas elevated cholesterol and triglyceride levels were negatively associated with some productive parameters. In conclusion, maintaining a moderate body weight in ISA Brown laying hens improves physiological balance and enhances productive performance during the mid-laying period.

Keywords: ISA Brown Strain; laying Hens; Body Weight Categories; Reproductive Hormones; Serum Biochemical Profile; Egg Production; Metabolic Profile

Article history:

Received 28 April 2025

Revised 13 July 2025

Accepted 10 August 2026

Published online 01 October 2026

1 Introduction

The poultry industry is one of the fastest-growing animal production sectors worldwide, driven by increasing demand for high-quality animal protein. Table egg production represents a major economic component of this industry due to the high nutritional value of eggs and their content of highly digestible proteins, vitamins, and essential minerals required for human nutrition (Al-Salhi, 2026). Advances in genetic selection, nutritional programs, and flock management have contributed to the development of highly efficient commercial laying strains (Al-Salhi & Al-Shatty, 2023). Among these, ISA Brown hens are widely used in modern egg production systems because of their high laying persistency, efficient feed utilization, and relative adaptability to varying environmental conditions. Despite substantial improvements in productive performance, maintaining physiological and productive stability within commercial flocks remains a major challenge, particularly during the mid- and late-laying periods. Productive efficiency is influenced by several interacting factors, including age, nutrition, health condition, and metabolic status, while body weight is among the most important determinants of productive and physiological performance in laying hens (Al-Salhi et al., 2026; Muir et al., 2022).

Body weight uniformity within laying hen flocks is an important indicator of successful flock management, as variation in body weight can affect feed intake, metabolic efficiency, and reproductive development, which are directly reflected in egg production, egg mass, and egg quality. Previous studies have shown that underweight hens often exhibit lower productive performance because of inadequate energy reserves and insufficient reproductive development, whereas excessive body weight is associated with increased fat deposition and metabolic disturbances that may negatively affect ovarian function and egg production persistence (Anene et al., 2020). In ISA Brown hens, previous studies reported variation in body weight and productive traits compared with other commercial layer strains (Islam et al., 2016). More recent findings indicated that body weight, feed intake, and growth rate are directly associated with sexual maturity and productive efficiency, reflecting complex physiological relationships that influence laying performance throughout different production stages (Jesuyon, 2017).

Biochemical and hormonal blood parameters are widely used as physiological indicators of the metabolic and reproductive status of laying hens because of their

association with egg production efficiency. Serum concentrations of glucose, total protein, albumin, cholesterol, triglycerides, and calcium reflect metabolic activity and energy balance within the bird, whereas reproductive hormones such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) are important indicators of ovarian activity, follicular development, and ovulation processes. Serum biochemical profiles reflect the physiological and nutritional status of laying hens and are closely associated with metabolic activity, health status, and productive performance (Brelaz et al., 2021). In addition, Al-Jaryan et al. (2021) reported significant associations between body weight, selected biochemical indicators, and productive traits in laying hens, suggesting that metabolic and hormonal balance plays an essential role in determining productive efficiency in commercial strains (Al-Jaryan et al., 2021). Body weight variation is associated with metabolic and hormonal changes that may influence reproductive function and egg production efficiency in laying hens (Yenilmez & Atay, 2023). Despite previous studies examining productive traits and egg quality in laying hens, limited information is available on the combined relationships among body weight, reproductive hormones, and biochemical blood parameters during the mid-laying period. Few studies have specifically evaluated the interaction between body weight, reproductive hormones, biochemical blood parameters, and productive performance in ISA Brown laying hens under commercial production conditions. The novelty of the present study lies in the integrated evaluation of body weight categories, reproductive hormones, serum biochemical parameters, and productive performance traits within the same ISA Brown laying hen population during the mid-laying period. This approach provides a more comprehensive understanding of the physiological and productive implications of body-weight variation and may help improve body-weight management strategies in commercial laying flocks.

It was hypothesized that maintaining moderate body weight in ISA Brown laying hens would promote a more favorable physiological and metabolic condition associated with improved reproductive hormonal activity and enhanced productive performance compared with light- and heavy-weight hens. Therefore, the present study was conducted to evaluate the relationship between body weight, selected biochemical and hormonal serum parameters, and productive performance traits in ISA Brown laying hens during the mid-laying period. The study also aimed to clarify the physiological mechanisms underlying production

efficiency and to contribute to the improvement of productive management strategies in commercial laying hen flocks.

2 Materials and Methods

2.1 Management of ISA Brown Hens and Experimental Conditions

This experiment was conducted using 135 laying hens of the ISA Brown chicken strain at 42 weeks of age. Each group consisted of 45 hens, distributed across three replicates with 15 hens per replicate. Within the rearing system, the housing organization, hens in each replicate were further distributed into three equal cages, with five hens per cage. The replicate was considered the experimental unit for statistical analysis. At 42 weeks of age, all hens were individually weighed and classified into three body-weight categories according to their actual body weight measurements. Birds were assigned to light-, medium-, and heavy-weight groups with mean body weights of 1835.4 ± 16.8 g, 2018.7 ± 20.5 g, and 2196.9 ± 28.6 g, respectively. The classification was based on the observed body weight distribution of the flock, ensuring clear separation among groups without overlap and within the normal physiological range recommended for ISA Brown laying hens. A schematic illustration of the experimental design, body weight classification, and the studied biochemical, hormonal, and productive performance parameters in ISA Brown laying hens is presented in Figure 1.

Hens were housed in standard laying cages under uniform environmental and managerial conditions. Stocking density, ventilation, ambient temperature, relative humidity, and a daily lighting schedule of 16 h light were carefully controlled to maintain appropriate husbandry conditions and ensure environmental stability throughout the experimental period (Al-Salhi et al., 2026). Body weight classification, housing management, and husbandry practices were implemented according to the recommendations provided in the ISA Brown Management Guide (Hendrix, 2023).

The same diet was provided to all experimental groups throughout the study period, and no dietary differences were applied among body-weight categories. The diet contained 2850 kcal/kg metabolizable energy and 17% crude protein, in addition to adequate levels of calcium, phosphorus, and essential amino acids necessary to support egg production and improve eggshell quality. Feed was offered at 110 g/hen/day, divided equally into two meals (55 g in the morning and 55 g in the evening), while clean drinking water was provided ad libitum throughout the experimental period. The experiment lasted for eight weeks, from 42 to 50 weeks of age. Eggs were collected twice daily at 10:00 a.m. and 4:00 p.m., and egg number and egg weight were daily recorded for the calculation of productive performance traits. Body weight measurements were obtained at the beginning (week 42), midpoint (week 46), and end (week 50) of the experiment. Prior to weighing, hens were subjected to a feed withdrawal period of 6–8 h to minimize the influence of gastrointestinal tract contents on body weight accuracy, while water remained available during the fasting period.

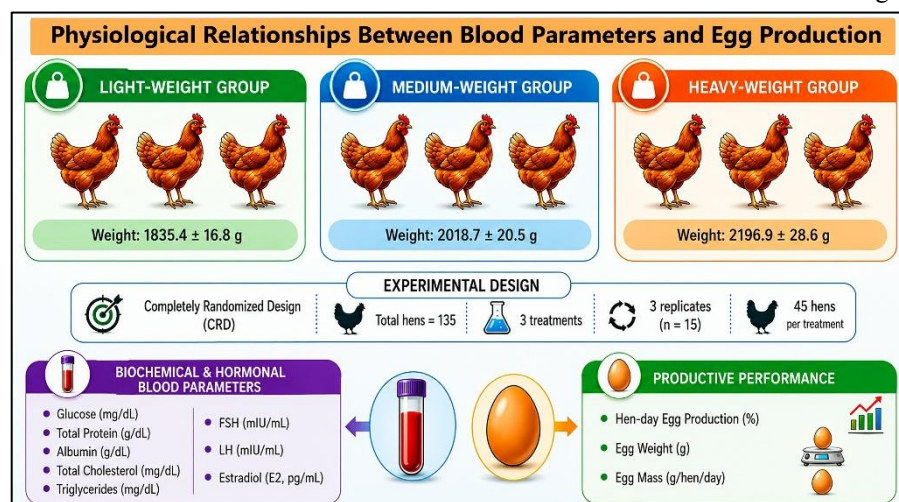


Figure 1. The experimental design and physiological and productive parameters studied

2.2 Productive Performance Measurements

Productive performance traits of ISA Brown laying hens were evaluated every 14 days throughout the experimental period (42 to 50 weeks of age). The measured parameters included hen-day egg production (HD %), egg weight (EW), and egg mass (EM). Body weight was measured three times during the experiment, including at the beginning of the trial (42 weeks of age), mid-experiment (46 weeks of age), and at the end of the experiment (50 weeks of age).

The number of eggs produced was daily recorded for each replicate, and hen-day egg production percentage, egg weight, and egg mass were calculated according to the equations described below, following the procedures reported by Duran Narinc et al. (2014) and Anna Wolc et al. (2020) (Narinc et al., 2014; Wolc et al., 2020).

Hen – day egg production (%)

$$= \frac{\text{Number of eggs produced}}{\text{Number of live hens}} \times 100$$

Egg weight was determined by dividing the total egg weight by the number of eggs produced during each recording period.

Egg mass was calculated using the following equation:

Egg mass (g/hen/day)

$$= \frac{\text{Hen – day egg production (\%)} \times \text{Egg weight (g)}}{100}$$

These traits were used to evaluate the physiological relationships among body weight, blood biochemical and hormonal parameters, and laying performance.

2.3 Blood Sampling and Biochemical and Hormonal Analysis

Blood samples were collected from ISA Brown laying hens at three consecutive time points during the experimental period, including the beginning of the experiment (42 weeks of age), mid-experiment (46 weeks of age), and the end of the experiment (50 weeks of age), simultaneously with body weight and productive performance measurements.

At each sampling time, blood samples were drawn from the brachial vein of all hens included in the experiment (45 hens per body-weight group; n=135) using sterile syringes and collected into anticoagulant-free tubes. Serum obtained from these samples was used for biochemical and hormonal analyses. The samples were allowed to clot at room

temperature, and serum was separated by centrifugation at 3000 rpm for 15 min. Biochemical analyses were performed using a spectrophotometer and commercial diagnostic kits from BIOLABO (France), following the methodological considerations for poultry serum analysis described by Al-Salhi (2025) (Al-Salhi, 2025). The measured parameters included glucose, total protein, albumin, total cholesterol, and triglycerides.

In addition, serum concentrations of reproductive hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2), were determined using commercial chicken-specific ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China). Assays were performed according to the manufacturer's instructions, and absorbance values were measured using a microplate reader. All samples were analyzed under identical laboratory conditions to ensure accurate and reliable hormone quantification.

2.4 Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS). Hens were classified into three naturally occurring body-weight categories (light, medium, and heavy), and the experiment was analyzed using a Completely Randomized Design (CRD), with the replicate as the experimental unit. Prior to analysis, data were tested for normality and homogeneity of variance using Levene's test. Differences among body-weight categories were evaluated using one-way ANOVA, and significant differences among means were separated using Duncan's Multiple Range test at $p \leq 0.05$. Productive performance data were analyzed for each experimental period and for the overall study period. Pearson correlation coefficients were separately calculated within each body-weight category using individual bird data obtained from sampled hens to determine relationships between physiological blood parameters and productive performance traits. Correlation analyses were based on measurements collected at the end of the experimental period, and each bird served as the unit of analysis. All statistical analyses followed the procedures described by Detry and Ma (2016) (Detry & Ma, 2016).

3 Results and Discussion

3.1 Body Weight

Table 1 shows the body weight changes of ISA Brown laying hens during the experimental period. At 42 weeks of

age, the heavy-weight group recorded significantly higher body weight than the light-weight group, whereas the medium-weight group showed intermediate values without significant differences from either group.

At 46 weeks of age, a similar trend was observed, where the heavy-weight hens maintained higher body weight values, while the medium-weight group remained

statistically intermediate between the heavy- and light-weight groups.

By 50 weeks of age, body weight continued to increase across all treatments, and clearer significant differences ($p \leq 0.05$) emerged among the three experimental groups, with the heavy-weight group recording the highest values and the light-weight group the lowest.

Table 1. Body weight (g) of laying hens during the experimental period (Mean $\pm$ SE)

Weight Groups	42 weeks	46 weeks	50 weeks
Light weight	1835.4 $\pm$ 16.8 ^b	1878.6 $\pm$ 18.4 ^b	1915.3 $\pm$ 20.1 ^c
Medium weight	2018.7 $\pm$ 20.5 ^{ab}	2064.8 $\pm$ 22.7 ^{ab}	2110.6 $\pm$ 24.5 ^b
Heavy weight	2196.9 $\pm$ 28.6 ^a	2258.4 $\pm$ 30.2 ^a	2316.7 $\pm$ 32.4 ^a
Significance	*	*	*

*The heterogenous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$)

3.2 Body Weight Gain

Table 2 shows the body weight gain of ISA Brown hens during the experimental period. During the period from 42 to 46 weeks of age, no significant differences were observed among the experimental groups in body weight gain, although the heavy-weight group recorded the highest value compared with the medium- and light-weight groups. During the period from 46 to 50 weeks of age, significant

differences ($p \leq 0.05$) were observed among treatments. The heavy-weight group recorded the highest body weight gain, whereas the light-weight group showed the lowest, and the medium-weight group occupied an intermediate position. Regarding total body weight gain over the entire experimental period (42–50 weeks), the heavy-weight group recorded significantly greater total gain than the light-weight group, while the medium-weight group remained intermediate between the two.

Table 2. Body weight gain (g) of laying hens during the experimental period (Mean $\pm$ SE)

Weight Groups	42–46 weeks	46–50 weeks	Total weight gain 42–50 weeks
Light weight	43.2 $\pm$ 4.6	36.7 $\pm$ 4.1 ^b	79.9 $\pm$ 6.2 ^b
Medium weight	46.1 $\pm$ 5.1	45.8 $\pm$ 4.8 ^{ab}	91.9 $\pm$ 7.4 ^{ab}
Heavy weight	61.5 $\pm$ 6.3	58.3 $\pm$ 5.6 ^a	119.8 $\pm$ 8.1 ^a
Significance	NS	*	*

*The heterogenous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$).

NS=Non-significant differences.

3.3 Effect of Body Weight Variation on Growth Dynamics in ISA Brown Laying Hens

Differences in body weight among ISA Brown laying hens may reflect variations in metabolic efficiency, nutrient partitioning, and the ability to utilize dietary energy for both productive functions and body tissue deposition. Heavier hens are generally characterized by a greater capacity for feed utilization and nutrient assimilation, which may enhance anabolic processes and promote increased energy storage in the form of body tissues and adipose reserves. This physiological response is likely associated with shifts in energy partitioning during the mid-laying period, where nutrients are distributed between maintenance requirements, egg production, and body tissue deposition. Similar

observations were reported by Muir et al. (2022) (Muir et al., 2022), who highlighted a close relationship between body weight, nutritional efficiency, and productive performance in ISA Brown laying hens. In addition, Yenilmez and Atay (2023) demonstrated that advancing age in laying hens is associated with progressive metabolic alterations and increased lipid accumulation, reflecting long-term energy balance changes during sustained egg production (Yenilmez & Atay, 2023).

In contrast, the observed differences in body weight gain during the later phase of the experiment indicate clear physiological and metabolic variability among body weight categories. Heavier hens may exhibit more efficient energy utilization, allowing excess dietary nutrients to be redirected

toward tissue growth and fat deposition rather than solely supporting maintenance and egg production. This pattern may also be influenced by endocrine regulation of growth and energy metabolism, which plays a key role in controlling nutrient partitioning during the laying period. Furthermore, the progressive increase in body weight in heavier hens may reflect a positive energy balance resulting from the interaction between production demands and maintenance requirements. These findings are consistent with Jesuyon (2023), who reported a direct association between body weight, feed intake, growth rate, and productive efficiency in ISA Brown chickens (Jesuyon, 2017).

3.4 Effect of Body Weight Variation on Hen-Day Egg Production in ISA Brown Laying Hens

The results presented in Table 3 demonstrated significant differences ($p \leq 0.05$) in hen-day egg production percentage among the different body weight categories of ISA Brown laying hens during the experimental period. The medium-weight group consistently recorded the highest hen-day egg production values compared with the light- and heavy-weight groups, whereas the light-weight hens showed the lowest productive performance. The heavy-weight group occupied an intermediate position and did not differ significantly from the medium-weight group in some recording periods.

Table 3. Hen-day egg production (%) of laying hens during the experimental period (Mean $\pm$ SE)

Weight Groups	42–46 weeks	46–50 weeks	Overall mean
Light weight	85.9 $\pm$ 1.7 ^b	86.8 $\pm$ 1.6 ^b	86.4 $\pm$ 1.5 ^b
Medium weight	91.5 $\pm$ 1.4 ^a	91.9 $\pm$ 1.3 ^a	91.7 $\pm$ 1.2 ^a
Heavy weight	88.1 $\pm$ 1.5 ^{ab}	88.4 $\pm$ 1.4 ^{ab}	88.2 $\pm$ 1.3 ^{ab}
Significance	*	*	*

*The heterogeneous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$)

3.5 Effect of Body Weight Variation on Egg Weight in ISA Brown Laying Hens

The results presented in Table 4 revealed significant differences ($p \leq 0.05$) in egg weight among the different body weight categories of ISA Brown laying hens throughout the

experimental period. The heavy-weight group consistently produced significantly heavier eggs compared with the medium- and light-weight groups, whereas the light-weight hens recorded the lowest egg weight values. The medium-weight group showed intermediate values between both treatments.

Table 4. Egg weight (g) of ISA Brown laying hens during the experimental period (Mean $\pm$ SE)

Weight Groups	42–46 weeks	46–50 weeks	Overall mean
Light weight	59.6 $\pm$ 0.7 ^c	60.1 $\pm$ 0.8 ^c	59.8 $\pm$ 0.6 ^c
Medium weight	62.3 $\pm$ 0.8 ^b	62.9 $\pm$ 0.7 ^b	62.6 $\pm$ 0.7 ^b
Heavy weight	64.8 $\pm$ 0.9 ^a	65.4 $\pm$ 0.8 ^a	65.1 $\pm$ 0.8 ^a
Significance	*	*	*

*The heterogeneous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$)

3.6 Effect of Body Weight Variation on Egg Mass in ISA Brown Laying Hens

The results presented in Table 5 showed significant differences ($p \leq 0.05$) in egg mass among the different body

weight categories of ISA Brown laying hens during the experimental period. Both the medium- and heavy-weight groups recorded significantly higher egg mass values compared with the light-weight group, whereas no significant differences were observed between the medium- and heavy-weight hens.

Table 5. Egg mass (g/hen/day) of laying hens during the experimental period (Mean±SE)

Weight Groups	42–46 weeks	46–50 weeks	Overall mean
Light weight	51.2±1.1 ^b	52.0±1.2 ^b	51.6±1.0 ^b
Medium weight	57.0±1.2 ^a	57.8±1.1 ^a	57.4±1.1 ^a
Heavy weight	57.4±1.3 ^a	58.3±1.2 ^a	57.9±1.2 ^a
Significance	*	*	*

*The heterogeneous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$)

The superior productive performance observed in medium-weight hens may indicate an optimal physiological and metabolic balance that supports ovarian activity, improves nutrient partitioning, and enhances laying persistency during the mid-laying period. Hens within the moderate body weight range are likely to allocate dietary nutrients more efficiently between maintenance requirements and egg production, resulting in improved reproductive efficiency and more stable egg output. In contrast, insufficient body reserves in light-weight hens may limit the availability of metabolic energy and nutrients required to sustain continuous follicular development, ovulation, and egg formation.

Although heavy-weight hens exhibited relatively high productive output, excessive body weight may be associated with increased adiposity and metabolic imbalance, which can negatively affect ovarian function and laying persistency. Excessive fat deposition has been reported to impair follicular development and reduce ovulatory efficiency in commercial laying hens, thereby influencing reproductive performance. Similarly, Muir et al. (2022) emphasized that maintaining optimal body weight is essential for maximizing productive efficiency in ISA Brown hens (Muir et al., 2022).

The increase in egg weight observed in heavy-weight hens may be attributed to greater body energy reserves, enhanced feed intake capacity, and increased availability of metabolic substrates required for yolk deposition and albumen synthesis. Larger body size is generally associated with enhanced hepatic activity and increased synthesis of lipoproteins and yolk precursors, which contribute to the production of larger eggs and higher egg mass. In contrast, lower egg weight in light-weight hens may reflect limited nutrient reserves and reduced metabolic capacity for sustained synthesis of egg components. Medium-weight hens maintained moderate egg weight values while simultaneously showing superior laying persistency and hen-day egg production, indicating a more balanced physiological state between egg size and egg number.

These findings are consistent with Muir et al. (2022), who reported a positive association between body weight, egg weight, and egg mass in ISA Brown hens (Muir et al., 2022). Likewise, Yenilmez and Atay (2023) demonstrated that increases in body weight and metabolic activity are frequently accompanied by gradual improvements in egg size and related production traits during the laying cycle (Yenilmez & Atay, 2023).

The higher egg mass observed in medium-weight hens may be explained by their superior laying persistency and higher hen-day egg production, which compensated for their moderate egg size compared with heavy-weight hens. In contrast, heavy-weight hens achieved comparable egg mass primarily due to increased egg weight rather than sustained egg number. These results indicate that egg mass is determined by the integrated interaction between egg number and egg weight rather than either trait independently. Medium-weight hens appear to maintain a more favorable physiological balance that supports both efficient nutrient utilization and sustained reproductive performance. Conversely, although heavy-weight hens produce larger eggs, excessive body weight may compromise reproductive efficiency due to increased fat deposition and metabolic load.

The lower egg mass observed in light-weight hens may reflect insufficient body reserves and reduced metabolic capacity to support both egg production rate and egg size simultaneously. Limited nutrient availability may negatively affect yolk deposition, albumen synthesis, and ovulatory activity, thereby reducing total egg output. These findings are consistent with Muir et al. (2022), who demonstrated that body weight and nutrient utilization efficiency are closely associated with egg mass production in ISA Brown hens (Muir et al., 2022).

3.7 Effect of Body Weight Variation on Serum FSH Concentration in ISA Brown Laying Hens

The results presented in Table 6 revealed significant differences ($p \leq 0.05$) in serum follicle-stimulating hormone

(FSH) concentrations among the different body weight categories of ISA Brown laying hens during the experimental period. The medium-weight hens consistently recorded the highest FSH concentrations compared with the

light- and heavy-weight groups, whereas the light-weight hens showed the lowest hormonal values. The heavyweight group exhibited intermediate values between the two treatments.

Table 6. Serum follicle-stimulating hormone (FSH, mIU/mL) levels of laying hens during the experimental period (Mean±SE)

Weight Groups	42 weeks	46 weeks	50 weeks
Light weight	4.82±0.21 ^b	4.95±0.24 ^b	4.71±0.20 ^b
Medium weight	6.14±0.27 ^a	6.32±0.25 ^a	6.08±0.23 ^a
Heavy weight	5.41±0.24 ^{ab}	5.56±0.26 ^{ab}	5.29±0.22 ^{ab}
Significance	*	*	*

*The heterogenous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$)

3.8 Effect of Body Weight Variation on Serum LH Concentration in ISA Brown Laying Hens

The results in Table 7 demonstrated significant differences ($p \leq 0.05$) in serum luteinizing hormone (LH)

concentrations across body weight categories throughout the experimental period. The medium-weight hens recorded significantly higher LH concentrations compared with the light-weight group, while the heavy-weight hens showed intermediate values.

Table 7. Serum luteinizing hormone (LH, mIU/mL) levels in laying hens during the experimental period (Mean±SE)

Weight Groups	42 weeks	46 weeks	50 weeks
Light weight	3.26±0.18 ^b	3.39±0.19 ^b	3.11±0.17 ^b
Medium weight	4.58±0.22 ^a	4.73±0.24 ^a	4.49±0.21 ^a
Heavy weight	3.94±0.20 ^{ab}	4.08±0.21 ^{ab}	3.86±0.18 ^{ab}
Significance	*	*	*

*The heterogenous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$).

3.9 Effect of Body Weight Variation on Serum Estradiol Concentration in ISA Brown Laying Hens

The results presented in Table 8 showed significant differences ($p \leq 0.05$) in serum estradiol (E2) concentrations among the different body weight categories of ISA Brown

laying hens during the experimental period. The medium-weight hens consistently exhibited the highest estradiol concentrations, whereas the light-weight hens recorded the lowest values. Heavy-weight hens showed intermediate concentrations between both groups.

Table 8. Serum estradiol (E2, pg/mL) levels of laying hens during the experimental period (Mean±SE)

Weight Groups	42 weeks	46 weeks	50 weeks
Light weight	118.4±4.6 ^b	121.7±4.9 ^b	115.3±4.4 ^b
Medium weight	146.8±5.3 ^a	150.6±5.5 ^a	144.2±5.1 ^a
Heavy weight	132.5±5.0 ^{ab}	135.8±5.2 ^{ab}	129.4±4.8 ^{ab}
Significance	*	*	*

*The heterogenous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$).

The observed differences in reproductive hormone profiles (FSH, LH, and estradiol) among body weight categories suggest a coordinated regulation of the hypothalamic–pituitary–gonadal (HPG) axis, which plays a central role in controlling follicular development, ovulation, and egg production in laying hens. Medium-weight hens exhibited a more favorable endocrine profile characterized by relatively higher and more balanced concentrations of

reproductive hormones, indicating improved hypothalamic and ovarian responsiveness. This hormonal balance likely supports enhanced follicular recruitment, accelerated maturation, and increased ovulatory efficiency, which is reflected in their superior hen-day egg production and laying persistency.

In contrast, lower concentrations of FSH, LH, and estradiol observed in light-weight hens may indicate

insufficient metabolic reserves and reduced endocrine activity, leading to impaired follicular development and limited reproductive efficiency. Energy deficiency is known to suppress HPG axis activity, thereby reducing gonadotropin secretion and ovarian steroidogenesis in poultry. On the other hand, although heavy-weight hens maintained moderate hormone levels, excessive body fat deposition may negatively affect endocrine signaling pathways by altering metabolic feedback mechanisms, reducing insulin sensitivity, and impairing ovarian responsiveness, ultimately limiting optimal reproductive efficiency despite their larger body size.

Estradiol plays a key role in regulating yolk precursor synthesis, hepatic lipoprotein production, and oviduct development; therefore, its higher concentration in medium-weight hens further indicates enhanced hepatic–ovarian coordination and improved reproductive performance. Similarly, LH is directly involved in final follicular maturation and ovulation, while FSH regulates early follicular recruitment, highlighting the integrated role of gonadotropins in sustaining laying performance. Collectively, these findings demonstrate that maintaining

moderate body weight provides an optimal physiological condition that supports endocrine homeostasis and maximizes reproductive efficiency in ISA Brown laying hens.

These results are consistent with recent studies reporting that reproductive hormonal activity in laying hens is strongly influenced by body weight, metabolic status, and energy balance, where moderate physiological condition enhances HPG axis function and productive performance (Anene et al., 2020; Jiang et al., 2022; Van Eck et al., 2023).

3.10 Effect of Body Weight Variation on Serum Glucose Concentration in ISA Brown Laying Hens

The results presented in Table 9 demonstrated significant differences ($p \leq 0.05$) in serum glucose concentration among the different body weight categories of ISA Brown laying hens during the experimental period. The heavy-weight hens consistently recorded the highest glucose concentrations compared with the light-weight group, whereas the medium-weight hens showed intermediate values.

Table 9. Serum glucose concentration (mg/dL) of laying hens during the experimental period (Mean $\pm$ SE)

Weight Groups	42 weeks	46 weeks	50 weeks
Light weight	208.4 $\pm$ 5.7 ^b	212.6 $\pm$ 6.1 ^b	215.3 $\pm$ 5.9 ^b
Medium weight	221.8 $\pm$ 6.2 ^{ab}	226.5 $\pm$ 6.4 ^{ab}	229.1 $\pm$ 6.1 ^{ab}
Heavy weight	236.7 $\pm$ 6.5 ^a	241.4 $\pm$ 6.8 ^a	245.6 $\pm$ 6.4 ^a
Significance	*	*	*

*The heterogenous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$)

The elevated glucose concentration observed in heavy-weight hens may reflect increased feed intake, enhanced hepatic gluconeogenesis, and greater metabolic activity associated with larger body size and higher nutrient consumption. Increased body weight in laying hens is frequently associated with intensified carbohydrate metabolism and elevated hepatic glucose output to meet the high energy demands required for maintenance, ovarian activity, and egg formation. Moreover, excessive body weight may impair metabolic efficiency and disrupt glucose homeostasis through increased adiposity and metabolic overload, particularly during the later stages of the laying cycle. These findings are consistent with the observations of Van Eck et al. (2023), who emphasized the importance of body composition and metabolic regulation in maintaining physiological performance in laying hens (Van Eck et al., 2023).

In contrast, the lower glucose concentrations observed in light-weight hens may indicate reduced nutrient reserves, limited hepatic metabolic activity, and decreased energy availability resulting from lower feed utilization capacity. Medium-weight hens maintained moderate glucose concentrations, suggesting a more balanced physiological and metabolic status that supports efficient nutrient partitioning and productive performance without excessive metabolic stress. Stable glucose homeostasis is essential for sustaining reproductive activity, follicular development, and persistent egg production in commercial laying hens. These findings are consistent with previous reports indicating that blood glucose concentration in laying hens is closely associated with body weight, feed intake, metabolic status, and endocrine regulation of energy balance. Therefore, maintaining moderate body weight may contribute to

improved metabolic stability, insulin sensitivity, and overall productive efficiency in ISA Brown laying hens.

3.11 Effect of Body Weight Variation on Serum Total Protein Concentration in ISA Brown Laying Hens

The results in Table 10 showed significant differences ($p \leq 0.05$) in serum total protein concentration among the

experimental groups. Medium-weight hens recorded significantly higher total protein concentrations compared with the light-weight hens, whereas the heavy-weight group showed intermediate values.

Table 10. Serum total protein concentration (g/dL) of laying hens during the experimental period (Mean $\pm$ SE)

Weight Groups	42 weeks	46 weeks	50 weeks
Light weight	4.21 $\pm$ 0.14 ^b	4.28 $\pm$ 0.15 ^b	4.17 $\pm$ 0.13 ^b
Medium weight	5.03 $\pm$ 0.17 ^a	5.11 $\pm$ 0.18 ^a	5.02 $\pm$ 0.16 ^a
Heavy weight	4.62 $\pm$ 0.16 ^{ab}	4.71 $\pm$ 0.17 ^{ab}	4.59 $\pm$ 0.15 ^{ab}
Significance	*	*	*

*The heterogenous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$)

3.12 Effect of Body Weight Variation on Serum Albumin Concentration in ISA Brown Laying Hens

The results presented in Table 11 demonstrated significant differences ($p \leq 0.05$) in serum albumin concentration among the different body weight categories of

ISA Brown laying hens during the experimental period. The medium-weight hens consistently recorded significantly higher albumin concentrations compared with the light-weight hens, whereas the heavy-weight group showed intermediate values.

Table 11. Serum albumin concentration (g/dL) of ISA Brown laying hens during the experimental period (Mean $\pm$ SE)

Weight Groups	42 weeks	46 weeks	50 weeks
Light weight	1.82 $\pm$ 0.08 ^b	1.86 $\pm$ 0.09 ^b	1.79 $\pm$ 0.07 ^b
Medium weight	2.31 $\pm$ 0.10 ^a	2.38 $\pm$ 0.11 ^a	2.29 $\pm$ 0.09 ^a
Heavy weight	2.07 $\pm$ 0.09 ^{ab}	2.13 $\pm$ 0.10 ^{ab}	2.05 $\pm$ 0.08 ^{ab}
Significance	*	*	*

*The heterogenous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$)

The observed increase in serum total protein and albumin concentrations in medium-weight hens may indicate improved protein metabolism, enhanced hepatic synthetic activity, and better nutritional status associated with optimal physiological balance during the laying period. Serum proteins, particularly albumin, play a fundamental role in maintaining osmotic pressure and in transporting nutrients, metabolites, and reproductive hormones, thereby supporting metabolic homeostasis and egg formation. In laying hens, adequate protein availability is essential for albumen synthesis and the production of reproductive tissues, which directly influences productive performance and egg quality.

In contrast, the lower total protein and albumin concentrations observed in light-weight hens may reflect insufficient nutrient intake, reduced amino acid availability, and limited hepatic synthetic capacity, which may negatively affect productive efficiency and reproductive activity. Conversely, although heavy-weight hens exhibited moderate

protein levels, excessive body weight may shift nutrient partitioning toward lipid deposition rather than protein synthesis, thereby reducing the efficiency of metabolic utilization for productive functions. These findings agree with previous studies reporting that the serum protein profile is closely associated with body weight, dietary protein intake, and productive performance in laying hens (Muir et al., 2022). Collectively, the results suggest that medium body weight provides an optimal physiological condition for efficient protein utilization, hepatic function, and sustained reproductive performance in commercial ISA Brown laying hens.

3.13 Effect of Body Weight Variation on Serum Cholesterol Concentration in ISA Brown Laying Hens

The results presented in Table 12 revealed significant differences ($p \leq 0.05$) in serum cholesterol concentration

among the different body weight groups throughout the experimental period. Heavy-weight hens consistently

recorded the highest cholesterol concentrations, whereas light-weight hens showed the lowest values.

Table 12. Serum total cholesterol concentration (mg/dL) of laying hens during the experimental period (Mean±SE)

Weight Groups	42 weeks	46 weeks	50 weeks
Light weight	128.6±4.8 ^b	132.1±5.1 ^b	135.4±5.0 ^b
Medium weight	146.9±5.3 ^{ab}	150.7±5.5 ^{ab}	154.1±5.4 ^{ab}
Heavy weight	168.4±5.8 ^a	173.6±6.1 ^a	178.9±6.0 ^a
Significance	*	*	*

*The heterogenous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$)

3.14 Effect of Body Weight Variation on Serum Triglyceride Concentration in ISA Brown Laying Hens

The results in Table 13 demonstrated significant differences ($p \leq 0.05$) in serum triglyceride concentration

among the experimental groups. Heavy-weight hens recorded significantly higher triglyceride concentrations compared with light-weight hens, while medium-weight hens remained intermediate.

Table 13. Serum triglyceride concentration (mg/dL) of laying hens during the experimental period (Mean±SE)

Weight Groups	42 weeks	46 weeks	50 weeks
Light weight	112.5±4.2 ^b	115.7±4.5 ^b	118.4±4.4 ^b
Medium weight	129.8±4.8 ^{ab}	133.6±5.0 ^{ab}	137.2±4.9 ^{ab}
Heavy weight	149.6±5.4 ^a	154.1±5.6 ^a	159.5±5.5 ^a
Significance	*	*	*

*The heterogenous letters within the same column indicate significant differences among treatments at ($p \leq 0.05$)

The observed alterations in the serum lipid profile, including cholesterol and triglyceride concentrations, across body weight categories indicate significant variation in lipid metabolism and energy partitioning in ISA Brown laying hens. The increased cholesterol levels observed in heavy-weight hens may be associated with enhanced hepatic lipogenesis and increased lipid deposition, both of which are associated with higher body weight and energy intake. Cholesterol plays a crucial physiological role in steroid hormone synthesis and yolk precursor formation; however, excessive circulating levels may reflect metabolic imbalance and increased fat accumulation in peripheral tissues and the reproductive system, potentially affecting metabolic efficiency during the laying period.

Similarly, the elevated triglyceride concentrations in heavy-weight hens may reflect intensified hepatic synthesis of very-low-density lipoproteins (VLDL) and increased lipid mobilization to support yolk formation. Although triglycerides are essential for egg production and yolk development, excessively high levels may indicate metabolic overload and impaired lipid homeostasis. In contrast, medium-weight hens maintained moderate cholesterol and triglyceride levels, suggesting a more stable lipid metabolic state that supports efficient nutrient

utilization, balanced energy distribution, and sustained reproductive performance without excessive fat deposition.

Conversely, light-weight hens exhibited lower lipid concentrations, which may reflect reduced energy reserves, limited feed intake, and decreased hepatic lipid synthesis capacity, ultimately restricting substrate availability for egg formation and reproductive processes. These findings support previous observations that body weight variation significantly influences lipid metabolism, where excessive body weight is frequently associated with increased lipid deposition and altered metabolic efficiency in laying hens, whereas moderate body weight is linked to more stable biochemical profiles and improved productive persistency (Muir et al., 2022).

3.15 Relationships between Physiological Blood Parameters and Productive Performance Traits in Light-Weight ISA Brown Laying Hens

It is evident from Table 14 that varying relationships existed between physiological blood parameters and productive performance traits in light-weight ISA Brown laying hens during the experimental period (42–50 weeks of age). Reproductive hormones, particularly FSH, LH, and estradiol, showed significant positive correlations ($p \leq 0.05$)

with hen-day egg production and egg mass, indicating the important role of hormonal activity in supporting reproductive efficiency and productive performance in light-weight hens. Estradiol also exhibited a significant positive correlation with egg weight. In contrast, cholesterol and triglyceride concentrations showed positive correlations with egg weight and negative associations with some

productive traits, suggesting that lipid metabolism influences production efficiency. Furthermore, total protein and albumin demonstrated positive correlations with productive performance traits, suggesting the importance of protein balance and metabolic status in maintaining productive activity in laying hens.

Table 14. Pearson correlation coefficients between physiological blood parameters and productive performance traits in light-weight ISA Brown laying hens during the experimental period (42–50 weeks of age)

Parameter	HD %	Egg weight	Egg mass
FSH	+0.74*	+0.32	+0.71*
LH	+0.69*	+0.28	+0.66*
Estradiol	+0.77*	+0.41*	+0.73*
Glucose	-0.26	+0.38	-0.14
Total protein	+0.71*	+0.35	+0.69*
Albumin	+0.66*	+0.31	+0.64*
Cholesterol	-0.39	+0.52*	-0.21
Triglycerides	-0.34	+0.48*	-0.18

*Correlation is significant at $p \leq 0.05$

3.16 Relationships between Physiological Blood Parameters and Productive Performance Traits in Medium-Weight ISA Brown Laying Hens

It is evident from Table 15 that strong relationships existed between physiological blood parameters and productive performance traits in medium-weight ISA Brown laying hens during the experimental period (42–50 weeks of age). Reproductive hormones, particularly FSH, LH, and estradiol, showed significant positive correlations ($p \leq 0.05$) with hen-day egg production and egg mass, indicating enhanced reproductive and metabolic efficiency in medium-

weight hens. Estradiol and total protein also demonstrated positive associations with egg weight, suggesting improved physiological status and nutrient utilization associated with optimal productive performance. In contrast, cholesterol and triglyceride concentrations exhibited positive correlations with egg weight and weak negative associations with hen-day egg production and egg mass. Overall, the results suggest that medium body weight provides the most balanced physiological condition for sustaining productive efficiency and metabolic stability in ISA Brown laying hens.

Table 15. Pearson correlation coefficients between physiological blood parameters and productive performance traits in medium-weight ISA Brown laying hens during the experimental period (42–50 weeks of age)

Parameter	HD %	Egg weight	Egg mass
FSH	+0.86*	+0.46*	+0.84*
LH	+0.82*	+0.41	+0.80*
Estradiol	+0.89*	+0.55*	+0.87*
Glucose	-0.18	+0.44	-0.09
Total protein	+0.83*	+0.49*	+0.81*
Albumin	+0.79*	+0.43	+0.77*
Cholesterol	-0.31	+0.58*	-0.17
Triglycerides	-0.27	+0.54*	-0.12

*Correlation is significant at $p \leq 0.05$.

3.17 Relationships between Physiological Blood Parameters and Productive Performance Traits in Heavy-Weight ISA Brown Laying Hens

It is evident from Table 16 that physiological blood parameters were variably associated with productive performance traits in heavy-weight ISA Brown laying hens during the experimental period (42–50 weeks of age). Reproductive hormones, including FSH, LH, and estradiol, showed significant positive correlations ($p \leq 0.05$) with hen-day egg production and egg mass, indicating the contribution of hormonal activity to reproductive performance. Estradiol also exhibited a significant positive correlation with egg

weight. In contrast, glucose, cholesterol, and triglyceride concentrations demonstrated significant positive correlations with egg weight and negative associations with hen-day egg production and egg mass, suggesting that excessive lipid metabolism and increased metabolic load associated with higher body weight may adversely affect productive efficiency. Additionally, total protein and albumin showed positive correlations with productive traits, reflecting the importance of metabolic and physiological balance in maintaining productive performance in heavy-weight laying hens.

Table 16. Pearson correlation coefficients between physiological blood parameters and productive performance traits in heavy-weight ISA Brown laying hens during the experimental period (42–50 weeks of age)

Parameter	HD %	Egg weight	Egg mass
FSH	+0.63*	+0.39	+0.58*
LH	+0.59*	+0.34	+0.54*
Estradiol	+0.68*	+0.47*	+0.62*
Glucose	-0.42*	+0.61*	-0.29
Total protein	+0.57*	+0.36	+0.52*
Albumin	+0.53*	+0.31	+0.49*
Cholesterol	-0.61*	+0.74*	-0.41*
Triglycerides	-0.56*	+0.69*	-0.37

*Correlation is significant at $p \leq 0.05$.

The correlation analyses presented in Tables 14, 15, and 16 revealed clear and consistent physiological relationships among reproductive hormones, biochemical blood parameters, and productive performance traits in ISA Brown laying hens during the mid-laying period. Overall, reproductive hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol, showed positive correlations with hen-day egg production and egg mass, highlighting the central role of the hypothalamic–pituitary–gonadal (HPG) axis in regulating ovarian activity, follicular development, ovulation, and sustained laying performance. The positive correlation between estradiol and egg weight further reflects its key role in hepatic synthesis of yolk precursors and oviductal function, which directly influences egg formation and size. These findings are in agreement with Anene et al. (2020), who reported that reproductive endocrine activity is closely linked to productive efficiency and physiological stability in laying hens (Anene et al., 2020).

Medium-weight hens exhibited the strongest positive correlations between reproductive hormones and productive traits, suggesting that moderate body weight provides an

optimal physiological and metabolic environment that enhances endocrine responsiveness and supports efficient nutrient partitioning toward reproductive functions. This observation is consistent with Muiret al. (2022), who demonstrated that optimal body weight and balanced nutrition improve egg production performance and metabolic efficiency in ISA Brown hens (Muir et al., 2022). Similarly, Narinc et al. (2014) emphasized that metabolic stability and physiological homeostasis are critical determinants of sustained egg production in commercial laying hens (Narinc et al., 2014).

Elevated lipid metabolites may be associated with metabolic alterations and increased adiposity, which could contribute to less favorable ovarian function and reduced egg production persistence. However, body composition and ovarian morphology were not directly measured in the present study, and further investigations are required to confirm these mechanisms. Nevertheless, the observed relationships are consistent with previous reports indicating that increased body weight and lipid concentrations are associated with metabolic imbalance and reduced productive efficiency in laying hens (Brelaz et al., 2021; Yenilmez &

Atay, 2023). Furthermore, positive correlations between total protein, albumin, and productive traits underline the importance of efficient protein metabolism and hepatic synthetic activity in supporting reproductive performance. Serum proteins play essential roles in nutrient transport, osmotic regulation, and the synthesis of egg components required for sustained production. These results are consistent with Al-Jaryan et al. (2021) and Al-Salhi and Al-Shatty (2023), who highlighted the close relationship between biochemical blood parameters and productive efficiency in laying hens (Al-Jaryan et al., 2021; Al-Salhi & Al-Shatty, 2023).

From a practical management perspective, the present findings suggest that maintaining ISA Brown hens within a moderate body-weight range may improve productive performance and favor a more favorable physiological status during the mid-laying period. These results may help poultry producers optimize flock management strategies to maximize egg production efficiency while maintaining metabolic balance.

This study has several limitations that should be considered when interpreting the results. The experiment was conducted over a relatively short period and included only one commercial laying strain (ISA Brown). In addition, direct measurements of body composition, ovarian morphology, liver lipid metabolism, and insulin-related metabolic indicators were not performed. Furthermore, body-weight categories were based on naturally occurring variation within the flock rather than experimentally imposed treatments, and feed intake was not individually measured. These factors should be considered when interpreting the observed associations. Therefore, caution should be exercised when extrapolating the present findings to other laying strains, production stages, or management systems. Further studies incorporating these physiological measurements are warranted to better elucidate the mechanisms underlying the observed relationships.

4 Conclusion

The study demonstrated significant associations between body weight categories, productive performance, reproductive hormones, and biochemical parameters in ISA Brown laying hens during the mid-laying period. Medium-weight hens exhibited the best overall physiological and productive status, indicating a more favorable metabolic and endocrine balance. In contrast, heavy-weight hens showed higher egg weight but also elevated lipid metabolites,

suggesting differences in metabolic status. Correlation analysis confirmed strong associations between reproductive hormones and productive traits, especially in medium-weight hens, highlighting the importance of the HPG axis in egg production. Biochemical parameters further reflected clear links between metabolic status and productivity. Overall, the findings suggest that moderate body weight is associated with improved metabolic stability, endocrine balance, and productive efficiency in ISA Brown laying hens.

Acknowledgements

The author gratefully acknowledges the University of Thi-Qar, Iraq, for its valuable support in conducting this study. The University's provision of laboratory facilities, scientific resources, and technical assistance is sincerely appreciated and has contributed significantly to the successful completion of this manuscript.

Use of Artificial Intelligence

Artificial intelligence (AI) tools were solely used for language editing and improvement of the visual presentation of Figure 1. The AI tools were not used for study design, data collection, data analysis, interpretation of results, or generation of scientific conclusions. All scientific content, analyses, interpretations, and final manuscript revisions were reviewed and approved by the author.

Conflict of Interest

The author declares no conflict of interest.

Data Availability Statement

Data are available from the corresponding author upon request.

Ethical Considerations

All experimental procedures involving animals were conducted in accordance with established national and international regulations for the care and use of experimental animals. The study protocol was reviewed and approved by the Scientific Committee of the Department of Animal Production, College of Agriculture and Marshes, University of Thi-Qar, under ethical approval No. 58 issued on February 25, 2026.

Funding

The author declares that they received no external financial support for this study.

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