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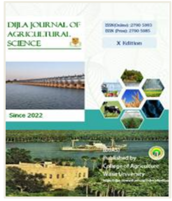

Research article

Physiological Adaptation and Stress Dynamics in Indigenous Chickens Following Transition from Natural to Intensive Rearing System: A Longitudinal Hematological, Biochemical, and Hepatic Biomarker Study

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ABSTRACT

In this study, the physiological adaptation and stress reaction of Iraqi indigenous chickens was assessed after the change from natural rearing system to intensive rearing system, related to the connection between housing system and health-status biomarkers. Twenty-five hundred birds were used, divided into five experimental replicates, and analyzed periodically for complete blood count (CBC) and blood glucose, lipids and liver enzymes, and specific stress indicators. The results showed that the conversion to the intensive system resulted in significant and clear changes: the RBC count decreased by 3.2×106/µL to 2.6×106/µL and the level of hemoglobin (Hb) went down from 12.5g/dL to 10.0g/dL. In contrast, there were consistent elevations in blood glucose (from 180 to 265 g/dL) and triglycerides (from 83 to 143 g/dL) while white blood cell (WBC) counts rose consistently from 20 to 32 ×103/µL. In addition, there was a noticeable metabolic stress load on the liver reflected by a significant increase in the H/L ratio (1.07) and a decrease in the A/G ratio (1.5 to 1.0), suggesting a shift in protein balance towards immune activity to deal with the rearing conditions. The overall results indicate that indigenous chickens are at risk of experiencing real physiological and metabolic stress due to intensive rearing systems, and require careful management of their hormonal status and productivity.

1. Introduction

Over the past few decades, poultry production systems have been greatly intensified to provide the high demand for animal protein in the world. All these changes have led to high housing density, feed management and environmental manipulation which are all physiological challenges imposed upon birds (Nassar, 2026; Narrod *et al.*, 2007; Kumar & Patyal, 2020). One of these problems is physiological stress, which can be a limiting factor that impacts health status, metabolic efficiency and productivity in poultry species. The hypothalamic–pituitary–adrenal (HPA) axis is the main regulatory system that triggers physiological stress in Chicken, which subsequently causes the

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release of corticosterone and changes to immune function, energy metabolism and organ integrity (Ncho *et al*., 2025; Ahmad *et al*., 2022). The prolonged activation of this axis is a risk to animal health and welfare, as well as to the efficiency of livestock production, due to its relationship with several adverse effects such as immunosuppression, oxidative imbalance, and metabolism deviation (Xiao *et al.*, 2026; Prates, 2025).

Hematological and biochemical parameters are used widely as good indicators of the physiological status and response to stress in poultry. The leukocytes profile with different ratios of heterophils and lymphocytes, such as the heterophil-to-lymphocyte ratio (H/L), is thought to be sensitive measures of exposure to chronic stress (Nwaigwe *et al*., 2020; Sokolenko *et al*., 2024). Likewise, biochemical parameters like glucose, cholesterol, triglycerides and total proteins are indicators of metabolic changes in response to environmental or nutritional stress (Khondowe *et al*., 2012; Café *et al*., 2012; Zálešáková *et al*., 2025). Furthermore, liver markers of avian integrity and metabolic burden, such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) are widely used markers in avian species (Silvestre-Ferreira, 2025; Borsa *et al*., 2006). The focus has shifted towards the serum protein fractions as integrative biomarkers of immune–nutritional status in recent years. The A/G ratio is important and has been correlated with various physiological processes, including albumin nutritional state and globulins immune activity, and may serve as a useful indicator to be analyzed in avian physiological studies (Wang *et al*., 2022; Bueno *et al*., 2017). Despite extensive research on commercial broiler chickens, relatively limited attention has been given to indigenous chicken populations, which are characterized by superior adaptability to harsh environmental conditions, fluctuating feed availability, and increased disease resistance (Kpomasse *et al*., 2023; Birhanu & Dessie, 2025). This adaptive advantage is however put to the test in intensive rearing conditions, where the natural or semi-natural environmental conditions of the indigenous birds are very different.

Therefore, the current study was conducted to explore the physiological response to stress and metabolic adaption of indigenous chickens under intensive rearing condition. This is realized by a detailed examination of hematological, biochemical, hepatic enzyme activities and serum protein fractions, especially the A/G ratio which is an integrated marker of physiological homeostasis. The study also aims to provide insights into the physiological mechanisms that mediate stress adaptation in indigenous chicken, thereby helping to enhance their understanding regarding their possible incorporation into the modern poultry production systems without having any impact on their health status and welfare.

2. Materials and Methods

2.1. Ethical Statement

All animal experiments were carried out following the internationally accepted standards for animal experiments. All procedures were conducted in accordance with the highest standards to reduce stress factors and ensure a suitable environment for the birds during the study, to maintain their welfare and to ensure the validity of the scientific results.

2.2. Study Site

This study was conducted at the Laboratory of the Department of Agricultural Biotechnology and the poultry breeding units of the Technical Institute/Kut, Middle Technical University under the roof of Iraq. The experimental trial period is from 1st March 2026 to 31st March 2026.

2.3. Experimental Animals and Design

In this study, 250 female local Iraqi chickens (pullets) at 20 weeks old were used. Birds were selected to ensure experimental homogeneity and were weighed an average of 1420 ± 25 g. The birds were randomly distributed into five equal replicates (50 Chicken/replicate). A study using a longitudinal experimental design was used to assess the physiological response of the chickens when they were reared in a free-range (natural) system and then transferred to an intensive cage system (battery cages) at the rate of 10 birds/m^2. The attention was directed towards the study of their physiological adaptation during one month.

2.4. Dietary and Environmental Management

2.4.1Diet.

The birds were fed a nutritionally balanced commercial basal diet containing a metabolizable energy (ME) of 2,900 kcal/kg and a crude protein (CP) content of 17 % (Fedco Company), consistent with the nutrient requirements for local poultry recommended by the National Research Council (NRC, 1994). There was no restriction on feed and water during the experiment.

2.4.2. Housing and Environmental Conditions.

Ambient Temperature: To avoid heat stress, the poultry house was kept within the comfortable temperature range of 20-24°C as recommended by Bell & Weaver, 2012. Relative Humidity: Humidity levels were kept between 50% and 70%. Ventilation and Oxygen: Ventilation machine was used to assure oxygen levels were maintained above 19.6% (Fairchild, 2017) and ammonia (NH3) was kept below 10 ppm. Lighting: A constant photoperiod of 16 hours of light and 8 hours of darkness was used each day.

2.5. Parameters Studied and Measurement Methods

Physiological parameters were assessed at the beginning of the study (Day 0) as reference data and then measured weekly (Draw blood from the wing 2ml / bird) during the study to track physiological changes that occurred because of the change in the housing system. Samples were consistently taken at the same time of the day, e.g., at 08:00 AM, to ensure consistency of data and to reduce the diurnal variation.

2.5.1. Hematological Parameters and Stress Index

Total Red Blood Cell (RBC) and White Blood Cell (WBC) counts were made by diluting with a diluting fluid and counting with a hemocytometer, as described by Natt & Herrick (1952). Hemoglobin (Hb) concentration was measured using the cyanmethemoglobin method, method based on Campbell (1995). The Giemsa-stained blood smears were used to calculate Stress Index, the Heterophil-to-Lymphocyte (H/L) ratio of the blood cells as described by Maxwell & Robertson (1998).

2.5.2. Biochemical Indicators

Glucose, cholesterol, triglycerides, total protein and albumin concentrations in serum were determined by standard colorimetric techniques (Tietz, 1995) with commercially available diagnostic kits. The value of globulin concentration was determined by subtracting the albumin value from the total protein value. The Albumin-to-Globulin (A/G ratio) was obtained by dividing the albumin concentration by the globulin concentration (Coles, 1986; Al-Salhi, 2025).

2.5.3. Liver Enzymes

The kinetic method of Reitman & Frankel (1957) was used to estimate the activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT). The alkaline Phosphatase (ALP) activity was determined by the method of KIND & KING (1954).

2.6. Statistical Analysis

The SPSS software (Version 26.0) was used to analyze the data. The Repeated Measures Design was used and One way Analysis of Variance (ANOVA) was carried out to compare the experimental periods. Means were compared for significant differences using Tukey's Honestly Significant Difference (HSD) test. A P value of ≤ 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Hematological parameters

The data presented in table 1 showed marked changes in haematological parameters as a result of the change-over from natural to intensive rearing conditions. The red blood cell count (RBC) was seen to be decreasing from W0 ($3.2\times10^6/\mu L$) to W4 ($2.6\times10^6/\mu L$) that was statistically significant (P = 0.030). Likewise, the concentration of hemoglobin (Hb) also significantly decreased from 12.5 to 10.0 g/dL (P = 0.020). White blood cell count (WBC) showed a gradual rise in the week 3 to 32 $\times10^3/\mu L$, with a peak value in week 4 (P = 0.041). From the results it can be seen that there is significant change in the hematological parameters with rearing time in response to rearing conditions imposed.

The physiological implication of the WBC elevation is the activation of the immune system in response to environmental stress that can be caused by intensive rearing. Stress-induced stimulation of the hypothalamic–pituitary–adrenal (HPA) axis results in increase in corticosterone levels that impact distribution of immune cells and promote the production of leukocytes, especially during chronic stress (Oluwagbenga *et al*., 2023; Hofmann *et al*., 2020). On the other hand, the decrease observed for RBC and Hb indicates a decrease in erythropoiesis and in the oxygen-carrying capacity. Intensive systems may create more stress hormones, oxidative stress and metabolic imbalance with the effect to suppress the compensatory mechanism of erythrocyte production, leading to a reduction in erythrocyte indices (Nawab *et al*., 2018).

The present findings agree with other reported studies on hematological changes that have occurred in poultry under environmental and management stress. Khan *et al*. (2023) found that there was a significant increase in the number of leukocytes as an indicator of stress response in poultry, and Lee *et al*. (2022) suggested that WBC is a good indicator of chronic stress in intensive production. Likewise, Nawab *et al.* (2018) proved that heat and density stress could negatively affect the erythropoiesis, even if the hypoxia was present; and Nawaz *et al.* (2021) indicated oxidative stress in the reduction of the erythrocyte lifespan. These findings taken together indicate that although indigenous chickens are able to cope with such conditions quite adaptively, they also suffer physiological stress under such conditions, especially if the conditions are stressful for extended periods.

Table 1. Temporal changes in hematological parameters of indigenous chickens following transition to intensive rearing system. (N=250)

Time / Week	Hematological parameters		
	RBC ($\times10^6/\mu L$)	Hb (g/dL)	WBC ($\times10^3/\mu L$)

W 0	^{a}3.2	^{a}12.5	^{b}20
W 1	^{a}3.0	^{a}11.8	^{b}25
W 2	^{ab}2.8	^{ab}11.0	^{ab}28
W 3	^{ab}2.7	^{b}10.5	^{a}30
W 4	^{b}2.6	^{b}10.0	^{a}32
MSE	0.05	0.17	2.11
P. Value	0.030	0.020	0.041

Different superscripts (a, b) within the same column indicate significant differences among weeks at ($P \leq 0.05$).

3.2. Biochemical parameters

The data presented in table 2 showed that the biochemical components were also affected significantly by the change from natural to intensive rearing system. Blood glucose levels increased steadily from baseline (W0) to Week 4 (265 g/dL), and significant differences were observed (P = 0.000). In the same way, there was a gradual increase in cholesterol levels from 132 to 192 g/dL (P < 0.000), and a significant rise in triglycerides from 83 to 143 g/dL (P = 0.011). Total protein levels on the other hand decreased gradually from 5.8 to 4.1 g/dL in the experimental periods, significant difference was seen between the weeks (P = 0.007). The results suggest that a change in metabolism occurs in response to the high levels of rearing stress.

Physiologically the progressive rise in glucose levels leads to activation of the stress response system, especially hypothalamo–pituitary–adrenal (HPA) axis, and a rise in corticosterone secretion along with increased gluconeogenesis (Khan *et al*., 2023; Nawab *et al*., 2018). Elevations in cholesterol and triglycerides indicate a change in lipid metabolism, which is probably related to increased energy mobilization and reduced lipid utilization in a stressed state. In chronic stress situations, when energy substrates are mobilized to ensure physiological homeostasis, these metabolic adaptations are often observed (Brugaletta *et al*., 2022; Nawaz *et al*., 2021). On the other hand, a decrease in total protein can suggest decreased protein synthesis and/or increased protein catabolism, which could be related to hepatic stress or nutritional imbalance under intensive conditions.

In the present results, the results obtained are in line with several recent studies that reported similar biochemical responses in poultry exposed to environmental and management stressors. Khan *et al*. (2023) reported that stress resulted in increased glucose and lipid profile in broilers, which were due to metabolic changes brought about by changes in the endocrine system. Nawab *et al*. (2018) showed that heat and density stress significantly increased levels of serum lipids, and Brugaletta *et al*. (2022) reported on the adverse effects of stress on protein metabolism and liver function. Moreover, Nawaz *et al*. (2021) highlighted metabolic dysregulation, such as hyperglycemia and hyperlipidemia, as a result of chronic stress. The results as a whole corroborate the idea that despite their adaptive ability, over time, intense rearing systems cause considerable metabolic changes in indigenous chickens.

Table 2. Temporal changes in biochemical parameters of indigenous chickens following transition to intensive rearing system. (N=250)

Time /week	Biochemical parameters			
	Glucose (g/dL)	**Cholesterol (g/dL)**	**Triglycerides (g/dL)**	**Total Protein (g/dL)**
W 0	^{a}180	^{c}132	^{c}83	^{a}5.8
W 1	^{a}207	^{c}143	^{c}95	^{a}5.3
W 2	^{ab}223	^{b}166	^{bc}110	^{ab}5.0

W 3	^{a}241	^{b}174	^{b}127	^{b}4.5
W 4	^{a}265	^{a}192	^{a}143	^{b}4.1
MSE	10.015	7.100	3.006	0.04
P. Value	0.000	<0.000	0.011	0.007

Different superscripts (a, b) within the same column indicate significant differences among weeks at (P ≤ 0.05).

3.3. Liver enzymes

In table 3, significant progressive increases in liver enzyme activities were observed in all the experimental groups, after the transition to intensive rearing condition. Increased aspartate aminotransferase (AST) was significant from baseline (W0, 141 U/L) to week 4 (246 U/L, P = 0.010). Likewise, alanine aminotransferase (ALT) markedly rose (from 22 to 55 U/L; P < 0.000) and alkaline phosphatase (ALP) rose (from 103 to 190 U/L; P = 0.001). Physiological responses to the imposed rearing stress showed a time-dependent response as the values at each week are distinctly indicated by the different superscripts.

In a physiological context, the increase of AST and ALT levels is indicative of hepatocyte stress, and may be due to damage to the hepatocyte membrane, leading to the release of these enzymes into the bloodstream. The gradual increase indicates the accumulation of metabolic stress and the chronic exposure of the fish to stress-inducing factors such as the high stocking density, the changed feeding regime and environmental restriction characteristic of intensive systems (Abdel-Moneim *et al*., 2021; McKinney, 2022). In addition, any increase in the activity of ALP can represent changes in hepatic function and may have implications for metabolic turnover, especially that of lipids, and possibly biliary involvement. These changes are in line with the stress induced liver dysfunction and rise in oxidative stress well observed in birds reared in intensive conditions (Khan *et al*., 2023; Nawaz *et al*., 2021).

The present results are in line with previous research, which also found that liver enzymes are good indicators of stress in poultry. Abdel-Moneim *et al*. (2021) showed that environmental stressors can greatly raise the AST and ALT levels because it causes damage to hepatocytes. Likewise, liver enzyme activities have been shown to be increased under intensive rearing conditions due to a metabolic overload and oxidative stress (McKinney, 2022). Khan *et al*. (2023) also noted that the changes in endocrine systems due to stress may affect the functioning of the liver and cause leakage of enzymes into the blood. Furthermore, Nawaz *et al*., (2021) pointed out the chronic stress situation produced long-term metabolic dysregulation, especially in hepatic pathways. Taken together, the results indicate that under the conditions of intensive breeding, the liver of indigenous chickens is subjected to significant stress, which increases with time and manifests itself as an acute reaction and then as a chronic physiological stress.

Table 3. Temporal changes in liver enzyme activities of indigenous chickens following transition to intensive rearing system. (N=250)

Time / Week	Liver enzymes		
	AST (U/L)	ALT (U/L)	ALP (U/L)
W 0	^{c}141	^{d}22	^{e}103
W 1	^{c}163	^{d}25	^{d}127
W 2	^{bc}182	^{c}30	^{c}145
W 3	^{b}210	^{b}41	^{b}168
W 4	^{a}246	^{a}55	^{a}190
MSE	0.05	0.22	2.54

P. Value	0.010	<0.000	0.001

Different superscripts (a, b) within the same column indicate significant differences among weeks at ($P \leq 0.05$).

3.4 physiological stress

The temporal course (Figure1; Figure2) of H/L ratio and A/G ratio showed different and contradicting physiological responses of indigenous chickens upon the change from their natural environment to intensive rearing conditions. The ratio of H/L rose steadily from 0.45 at week 0 to 1.07 at week 3 and then dropped a bit to 1.0 at week 4. The opposite is true for the A/G ratio, which dropped from 1.5 at week 0 to 1.0 at week 3 but only marginally rebounded to 1.1 at week 4. This separation suggests a gradual transition from a normal to a stress response physiological state with immune activation and changes in protein metabolism.

In terms of physiology, the increased H/L ratio is related to a stress related shift in the distribution of leukocytes; heterophilia and lymphopenia are responses to glucocorticoid activity. This is a common pattern that has been taken as a reliable marker of chronic stress in poultry, especially in the face of environmental and management challenges (Maxwell & Robertson, 1998; Gross & Siegel, 1983). At the same time, the drop in A/G ratio implies diminished production of albumin and/or higher of globulin fractions, which means a twofold effect of hepatic strain and immune system activation. The A/G (albumin/globulin) ratio is closely associated with protein metabolism and liver function, while globulins are immunologically active proteins, a decreased A/G ratio being an indication of shifting from metabolic equilibrium to immune priority (Lumeij, 2008; Thrall *et al*., 2012).

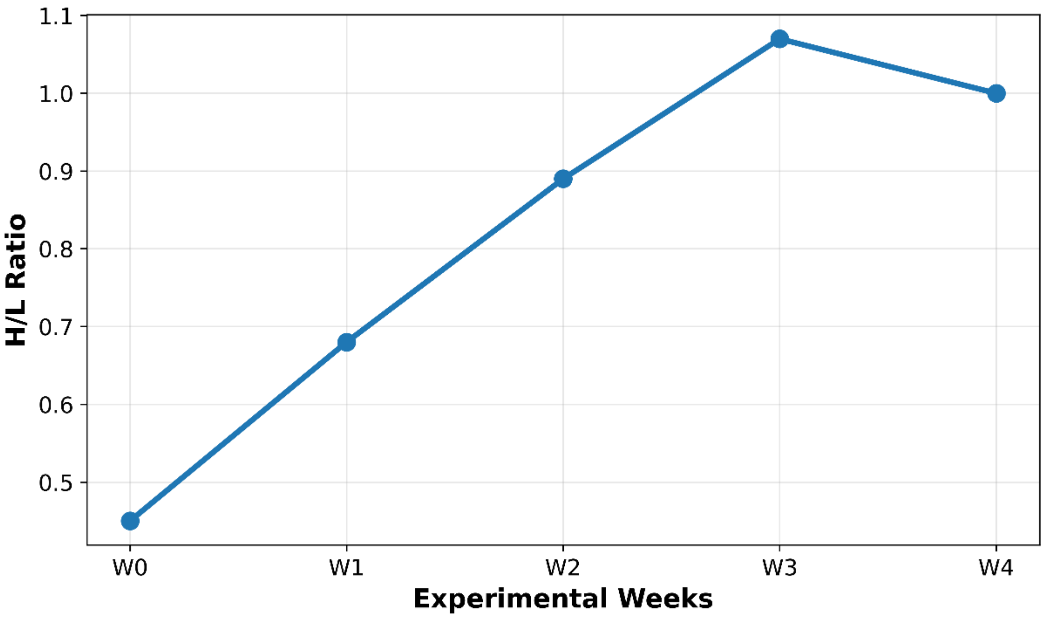

Figure 1. Temporal changes in Heterophil/Lymphocyte (H/L) ratio in indigenous chickens under intensiv e rearing. (N=250)

The inverse relationship between H/L ratio and A/G ratio in the current study is in line with the integrated stress response in poultry. Under intensive stocking and handling systems, where metabolism and immunity are disturbed by stressors like abnormal environmental conditions, high stocking density, and handling, similar responses have been reported (Scanes, 2016; Campbell *et al.*, 2019). The partial stabilization of certain values at week 4 could reflect initiation of physiological adaptation, but the continued elevation of other values would suggest that the values were not fully corrected during the course of the experiment. The results reveal that although indigenous chickens are adaptively resilient, their immuno-metabolic strain can be measured under intensive rearing conditions over time.

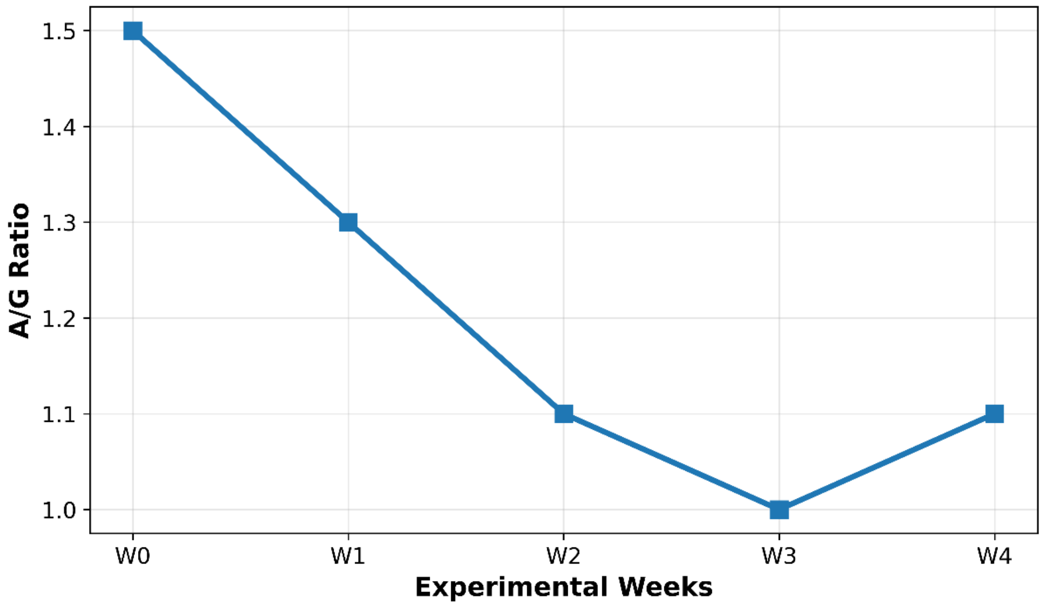

Figure 2. Temporal changes in Albumin/Globulin (A/G) ratio in indigenous chickens under intensive rearing. (N=250)

4. Conclusions

Physiological stress reactions were clearly observed with significant changes in the hematological, biochemical and hepatic parameters after the chickens were shifted from natural to intensive rearing systems. Increased H/L ratio and WBC with decreased RBC and Hb levels are markers of activation of the stress axis and immune modulation. At the same time, the increase in glucose and lipid profile as well as the decrease in total protein and A/G ratio represents metabolic imbalance and switch to immune prioritization. The elevated levels of liver enzymes also indicate hepatic stress and higher metabolic stress with intensive conditions. In conclusion, the results show that indigenous chickens can adapt to some degree but that this is only partial and measurable when they are exposed to intensive systems for a longer time.

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