Influence of Different Rearing Systems and Sex on the Hematological Profile of IPB D3 Chickens

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ABSTRAK

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Sistem pemeliharaan berpengaruh signifikan terhadap kesejahteraan, produktivitas, dan status fisiologis ayam, termasuk respons hematologi yang mencerminkan kondisi kesehatan dan sistem imun. Penelitian ini bertujuan untuk menganalisis interaksi antara sistem pemeliharaan dan jenis kelamin terhadap profil hematologi IPB-D3 chicken. Sebanyak 60 ekor ayam umur 12 minggu dipelihara selama 12 minggu dalam dua sistem, yaitu intensif dan free-range, masing-masing terdiri atas 30 ekor (15 jantan dan 15 betina). Pada umur 24 minggu, sampel darah diambil melalui vena brakialis menggunakan tabung EDTA. Analisis hematologi dilakukan dengan metode standar meliputi penghitungan eritrosit dan leukosit menggunakan hemocytometer, pengukuran hemoglobin, penentuan hematokrit dengan metode mikrohematokrit, serta diferensial leukosit melalui preparat apus darah yang diwarnai menggunakan Giemsa stain. Hasil penelitian menunjukkan bahwa jumlah eritrosit, kadar hemoglobin, dan indeks eritrosit tidak berbeda nyata antara sistem pemeliharaan (P>0,05). Nilai hematokrit tidak dipengaruhi secara signifikan oleh sistem pemeliharaan, namun terdapat interaksi nyata antara sistem pemeliharaan dan jenis kelamin (P=0,038), di mana ayam jantan pada sistem free-range memiliki nilai hematokrit lebih tinggi dibandingkan sistem intensif. Jumlah leukosit total dipengaruhi secara signifikan oleh sistem pemeliharaan (P= 0,010), dengan nilai lebih tinggi pada sistem intensif (19,52×10³/µL) dibandingkan free-range (14,12×10³/µL), tanpa interaksi dengan jenis kelamin. Persentase leukosit diferensial dan rasio heterofil-limfosit tidak menunjukkan perbedaan nyata (P>0,05). Kesimpulannya, meskipun beberapa parameter hematologi dipengaruhi oleh sistem pemeliharaan dan interaksinya dengan jenis kelamin, ayam IPB-D3 menunjukkan kemampuan adaptasi fisiologis yang baik pada sistem intensif maupun free-range.

Kata Kunci: Sistem free-range, profil hematologi, Ayam IPB-D3, sistem pemeliharaan, interaksi jenis kelamin

ABSTRACT

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Rearing systems significantly influence the welfare, productivity, and physiological status of poultry, including hematological responses that reflect immune and health conditions. This study analysed the interaction between rearing system and sex on the hematological profile of IPB-D3 chicken. A total of 60 twelve-week-old chickens were reared for 12 weeks under two systems: intensive and free-range, with 30 birds per system (15 males and 15 females). At 24 weeks of age, blood samples were collected from the brachial vein into EDTA tubes. Hematological analysis was performed using standard methods, including erythrocyte and leukocyte counts using a hemocytometer, hemoglobin determination, hematocrit measurement by the microhematocrit method, and differential leukocyte counts using blood smears stained with Giemsa stain. Results showed that erythrocyte count, hemoglobin concentration, and erythrocyte indices did not differ significantly between rearing systems (P>0.05). Hematocrit was not significantly affected by rearing system alone, but a significant interaction between system and sex was observed (P = 0.038), with free-range males exhibiting higher hematocrit than intensively reared males. Total leukocyte count was significantly influenced by rearing system (P = 0.010), with intensively reared chickens showing higher leukocyte levels (19.52×10³/µL) than free-range chickens (14.12×10³/µL), without interaction with sex. Differential leukocyte percentages and the heterophil-to-lymphocyte ratio were not significantly different (P>0.05). In conclusion, although certain hematological parameters were influenced by rearing system and its interaction with sex, IPB-D3 chicken demonstrated good physiological adaptability under both intensive and free-range conditions.

Key Words: Free-Range System, Hematological Profile, IPB-D3 Chickens, Rearing System, Sex Interaction

INTRODUCTION

The IPB-D3 chicken is a selectively bred strain developed from the seventh generation of IPB D1 chickens and was officially released as an Indonesian local chicken in 2019 (Sumantri et al. 2020). The IPB D1 population originated from a composite cross involving Pelung chicken × Sentul chicken and Kampung chicken × Broiler chicken, each contributing approximately 25% to the genetic composition, and was selected primarily for superior body weight. The parental lines exhibit distinct productive and adaptive characteristics. Pelung chickens are known for their large body size, with adult males reaching 2.5–3.5 kg and, in some cases, up to 5–6 kg (Asmara et al. 2019). Sentul chickens show moderate growth performance (1.5–2.0 kg) and good adaptability and disease resistance (Depison et al. 2019). Kampung chickens are characterized by high environmental adaptability but relatively slower growth, typically ranging from 0.8–1.2 kg at 12–24 weeks of age (Rahardja et al. 2015). In contrast, broiler chickens exhibit rapid growth, reaching 1.6–2.0 kg within 5 weeks under intensive management (Kuswandi et al. 2024). The combination of these parental traits through crossbreeding results in heterosis, which enhances growth rate and overall performance. Consequently, IPB D3 chickens demonstrate rapid growth, exceeding 1 kg before three months of age (Salsabila et al. 2022), with males reaching 1,256 g and females 1,042 g at 10 weeks. In addition, like other local chickens, IPB D3 exhibits strong environmental adaptability and improved feed efficiency, making it a promising genetic resource for sustainable poultry production (Gheyas et al. 2021). However, the expression of these superior genetic and growth characteristics may vary across rearing environments. Therefore, evaluating how different rearing systems influence physiological responses, particularly hematological parameters and productivity, is essential to optimize performance under diverse management conditions.

In addressing global challenges in the livestock sector, optimizing poultry-rearing systems is key to improving animal welfare and productivity. Various approaches, including intensive and free-range systems, offer distinct advantages and challenges, particularly their impact on chicken health. Environmental factors, feed quality, and stress levels due to stocking density and management practices significantly affect the physiological condition of chickens, including hematological parameters (Kim & Lee 2023). For instance, while the intensive system enhances production efficiency, it raises concerns about increased stress and disease risk. In contrast, the free-range system, with its more natural environment, has the potential to reduce stress but faces challenges such as feed variability and pathogen exposure (Sanchez-Casanova et al. 2020). Proper environmental and nutritional management is crucial to balancing productivity and poultry health. Therefore, an in-depth study of various rearing systems and their effects on hematological parameters is essential to developing sustainable, optimal poultry farming practices.

Blood hematology represents a critical indicator of poultry health status, physiological responses, and stress conditions. Key parameters, including leukocyte and erythrocyte counts, hemoglobin (Hb), hematocrit (Hct), and erythrocyte indices, provide insight into the internal physiological state of chickens and may vary in response to infections, stress, and nutritional imbalances (Griss et al. 2019). Elevated leukocyte counts are commonly associated with immune responses triggered by pathogenic challenges or environmental stressors. Normal Hb and Hct values indicate adequate oxygen-carrying capacity and the absence of anemia (Horhoruw & Rajab, 2024). Hematological profiles are also strongly influenced by management-related factors such as rearing systems, hygiene practices, and housing conditions (Gorelik et al. 2020; Tantikositruj et al. 2022). Moreover, suboptimal environmental conditions, including high stocking density and inadequate ventilation, can induce heat stress, thereby altering hematological parameters, particularly Hb and Hct levels (Dadgar et al. 2021). Therefore, hematological assessment serves as a reliable and integrative tool for evaluating poultry health and assessing the effectiveness of rearing systems and nutritional management in supporting optimal welfare and productivity.

Factors such as rearing systems, nutrition, growth, sex, breed, and age significantly influence chicken performance, with sex being a key factor in improving genetic traits (Cygan-Szczegielniak & Bogucka 2021). Blood parameter analysis is crucial for assessing chickens' physiological condition and overall health, and for providing insights into their responses to environmental and nutritional factors (Angoua et al. 2021). Sex differences can affect various hematological parameters through physiological and hormonal variations, thereby altering metabolism and immune responses across different rearing environments (Taha & Aljumaily 2021). However, the interaction between sex and rearing systems, such as intensive and free-range systems, in shaping hematological parameters remains underexplored. Previous studies have shown that strain and sex significantly influence several hematological parameters (Adewole et al. 2021), underscoring the importance of accounting for genetic factors and sex differences in poultry management to optimize health and productivity.

Studies on IPB-D3 chickens have primarily focused on growth performance and carcass quality, with limited attention given to their hematological responses under different rearing conditions (Ratnawati et al. 2024). In IPB-D3 chickens, total leukocyte counts have been reported to range from 39.9 to 51.07×10³/µL, while

hematocrit values were recorded at 27.25% (Romantis et al. 2024). However, these findings were obtained exclusively under intensive rearing systems. To date, no studies have specifically investigated the interaction between sex and rearing systems on hematological parameters in IPB-D3 chickens. Therefore, evaluating hematological responses under different rearing systems and sex variations is essential to better understand the adaptability and physiological resilience of this locally improved chicken line. This study provides new insight into the interaction between rearing system and sex on hematological responses in IPB-D3 chickens. The findings are expected to support the future commercialization of IPB-D3 chickens by improving their adaptability across diverse rearing environments and by contributing to the development of optimized, sex-specific poultry management strategies to enhance health and productivity.

MATERIALS AND METHODS

Ethical statement

The present study was conducted in accordance with animal welfare principles and received approval from the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia, under permit number 209/KEH/SKE/IV/2024.

Animal management and experimental design

The present study involved 60 twelve-week-old IPB-D3 chickens, a dual-purpose line developed by IPB University for both egg and meat production. The chickens were raised for 12 weeks under two rearing systems: intensive and free-range. Each system consisted of 30 birds, with equal numbers of males and females. The nearly equal number of males and females was chosen because the birds were in the growth phase rather than the breeding period, allowing a comparative evaluation of hematological responses between the sexes under different rearing conditions. At the start of the trial, chickens in both rearing systems were in the same growth phase to ensure initial uniformity. A commercial grower feed (B-12 L, PT. New Hope Indonesia, Tangerang, Indonesia) was offered twice daily. The diet contained approximately 14.0% crude protein, 3.0% crude fat, 8.0% crude fiber, 8.0% ash, 0.90–1.20% calcium, and 0.55–1.00% total phosphorus (with phytase enzyme). The amino acid composition included 0.70% lysine, 0.27% methionine, 0.45% methionine + cystine, and 0.17% tryptophan. This commercial feed was provided to chickens reared under both intensive and free-range systems. Ambient temperature and relative humidity in both the intensive (IN) and free-range (FR) housing systems were monitored three times daily (06:00, 12:00, and 17:00) using a digital thermo-hygrometer (HTC-1, Equipslab, China). In the FR system, the device was placed on a wooden platform positioned in a shaded area within the chicken environment to accurately capture the surrounding microclimate. Environmental conditions were maintained within the optimal comfort range for chickens, with temperatures between 20 °C and 30 °C and relative humidity levels of 60–70%. Housing facilities were equipped with feeders, drinkers, perches, and rice husk bedding. Chickens in the intensive system were kept fully confined, whereas those in the free-range system were released daily from 06:00 to 17:00 with access to shaded areas and natural vegetation, including *Indigofera* and wild grasses. Throughout the experimental period, no vaccinations were administered; health management focused on maintaining environmental hygiene, regularly replacing litter, and minimizing stress through gentle handling practices.

Preparation of the free-range and intensive rearing systems

The rearing experiment was carried out at the Laboratory of Animal Breeding and Genetics, Department of Animal Production and Technology, IPB University, Bogor, Indonesia. The free-range rearing area measured 20 m × 10 m (200 m²) and included a 2.5 m × 2.5 m enclosure for nighttime housing. A total of 30 chickens were allocated to the free-range system, resulting in a daytime stocking density of approximately 0.15 birds/m², well below the maximum limit of 1 bird per square meter recommended by the Australian free-range standards (McCormack, 2017). The area was enriched with natural vegetation, including banana trees (*Musa* spp.), *Indigofera*, and various native tropical grasses. These vegetation types, along with the presence of naturally occurring insects and earthworms, may contribute to additional nutrient intake and influence foraging behavior and activity patterns of the chickens. Approximately 4 meters of wooden perches were provided to facilitate roosting and support natural behaviors. The entire area was enclosed with 2-meter-high nylon netting to prevent escape and protect against predators. However, the contribution of natural feed sources, including vegetation, insects, and earthworms, was not quantitatively measured and was considered part of the free-range system conditions. The intensive housing unit measured 6x 1.5 m (9 m²) and accommodated 30 chickens, resulting in a stocking density of approximately 3.33 birds/m². The housing was designed with adequate ventilation and an easy-to-clean floor, and each enclosure was equipped with feeders and

drinkers to ensure animal welfare during the rearing period.

Sample collection

At 24 weeks of age, blood samples were collected from 60 IPB-D3 chickens, comprising 30 birds from each rearing system (intensive and free-range), with equal numbers of males and females per group. This sampling design ensured balanced sex representation within each system, thereby minimizing sex-related variation in hematological parameters. The 24-week sampling point was selected to represent a physiologically mature stage following 12 weeks of exposure to the respective rearing conditions, reducing age-related variability, and enabling standardized comparisons between systems and sexes. Additionally, selecting 30 individuals per group provided sufficient statistical power to detect biologically meaningful differences while adhering to ethical principles of animal use (3Rs: Replacement, Reduction, and Refinement). Approximately 2 mL of blood was collected from the brachial vein using a 3 mL syringe fitted with a 23-gauge needle (0.6×30 mm). The puncture site was disinfected with 70% alcohol prior to venipuncture, and no anesthesia was administered. Blood samples were immediately transferred into ethylenediaminetetraacetic acid (EDTA)-coated tubes to prevent coagulation, then gently inverted to ensure proper mixing with the anticoagulant.

Hematological analyses were performed to evaluate blood composition and cellular profiles according to the protocol described by Onunkwo et al. (2019). Parameters measured included packed cell volume (PCV), erythrocyte count (RBC), hemoglobin concentration (Hb), and total leukocyte count (WBC). Differential leukocyte counts, including lymphocytes (LYM), heterophils (HET), eosinophils (EOS), monocytes (MON), and basophils (BAS), were determined using a Neubauer improved hemocytometer (Marienfeld Superior, Germany) and standard blood smear staining techniques with Giemsa stain (Merck, Darmstadt, Germany). Collectively, these parameters provided comprehensive insights into the physiological status, stress response, and immune function of chickens under different rearing systems.

Variables observed

The variables observed in the present study encompass the following.

Erythrocytes

The number of Erythrocytes was counted using a hemocytometer (Improved Double Neubauer counting chamber, Germany). EDTA blood was diluted with Hayem's solution (France), homogenized using a figure-eight motion, and placed on a hemocytometer for observation under a 10× microscope (Pujiastuti et al. 2017).

Hemoglobin and hematocrit

Hemoglobin and hematocrit levels were measured using the Mindray BC-2800 Vet Auto Hematology Analyzer (Mindray, China), where the sample tube was placed under the nozzle, and results were automatically displayed on the screen.

Erythrocyte index calculation

The erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), are used to evaluate red blood cell (RBC) characteristics and to detect anemia. These indices are calculated using standard formulas based on RBC count, hemoglobin concentration, and hematocrit values (Pujiastuti et al. 2017):

$$\text{MCV} = \frac{\text{Hematocrit (\%}\times 10)}{\text{Erythrocyte (million/}\mu\text{L)}} \quad \text{Formula (1)}$$

$$\text{MCH} = = \frac{\text{Hemoglobin (g/dL)}}{\text{Erythrocyte count (million/}\mu\text{L)}} \quad \text{Formula (2)}$$

$$\text{MCHC} = = \frac{\text{Hemoglobin (g/dL)}}{\text{Hematocrit (\%}\times 10)} \quad \text{Formula (3)}$$

Total leukocytes (leukocyte count)

The leukocyte count was performed using a leukocyte diluting pipette. Blood samples were mixed with Rees and Ecker's solution to stabilize leukocytes. After thorough homogenization of the mixture, the sample was loaded into a Neubauer counting chamber, manufactured by companies such as Marienfeld (Germany) or Thermo Fisher Scientific (USA). Leukocytes were manually counted under a microscope at 400× magnification in four large squares of the chamber. The observed count was multiplied by 50 to calculate the total leukocyte concentration per milliliter of blood. This multiplication factor represents a standard conversion factor for the chamber, accounting for its volume and dilution. (Maheshwari et al. 2017).

Leukocyte differentiation

Leukocyte differentiation was performed by preparing a blood smear, fixing it with methanol (Merck, Germany), staining it with Giemsa solution (Sigma-Aldrich, USA), and rinsing it with distilled water. The prepared slide was examined under a microscope

(Olympus, Japan) at 100× magnification. Leukocyte types, including lymphocytes, monocytes, heterophils, eosinophils, and basophils, were counted using the battlement method. At least 100 cells were counted per slide, and the percentage of each leukocyte type was calculated based on the total cell count (Nurhayati et al. 2019).

Heterophil/Lymphocyte (H/L) ratio

The H/L ratio is calculated by dividing the number of heterophils by the number of lymphocytes observed in the leukocyte differential count. The formula used is:

$$H/L = \frac{\text{Heterfophils (\%/dL)}}{\text{Limphocyte count (million/µL)}} \qquad \text{Formula (4)}$$

Data analysis

Hematological data from IPB-D3 chickens, along with environmental temperature and humidity, were analyzed using Student's t-test to compare mean differences between rearing systems (intensive versus free-range). To assess the effects of rearing system (intensive vs. free-range) and sex on hematological parameters, multiple regression analysis with dummy variables was performed, allowing the evaluation of potential interactions between rearing system and sex. Additionally, Pearson's correlation analysis was used to examine relationships among hematological parameters, providing insight into possible physiological interconnections. All statistical analyses were conducted at the 5% significance level ($P<0.05$), and the assumptions for each test were checked in RStudio 4.4.0. This approach ensured that each analysis was directly linked to the appropriate statistical method, clearly specifying the purpose and model used for each dataset.

RESULTS AND DISCUSSION

Environmental conditions during rearing

The temperature and humidity conditions in the rearing environments of IPB-D3 chickens under the intensive and free-range systems during the study showed significant variation throughout the day. The following table presents the temperature and humidity conditions observed during the study.

As presented in Table 1, midday temperature (12:00 p.m.) in the free-range system (34.56±3.06°C) was significantly higher ($P<0.05$; $P= 0.006$) than in the intensive system (33.46±2.63°C). Similarly, midday humidity differed significantly ($P<0.05$; $P= 0.006$), with higher values in the intensive system (67.69±11.89%) than in the free-range system (62.93±13.12%). No significant differences ($P>0.05$) were detected in temperature or humidity during morning (6:00 a.m.) and afternoon (5:00 p.m.) measurements across both rearing systems.

These findings indicate distinct microclimatic conditions between rearing systems, particularly during midday when the risk of heat stress is greatest. The observed temperature range (25–34°C) exceeded the thermoneutral zone for poultry (20–24°C), potentially impairing physiological functions and productivity (Ulupi & Afnan, 2016). Furthermore, the higher humidity observed in the intensive system is likely associated with limited ventilation in enclosed housing, which can increase thermal load and reduce evaporative heat loss. Humidity levels recorded (90.11%–90.43%) exceeded the optimal range for poultry (40%–60%), potentially increasing the risk of respiratory disorders and reducing productivity (Wolkoff et al. 2021; Li et al. 2023). The combined effect of elevated temperature and

Table 1. Environmental temperature and humidity conditions observed during the study

Parameter	Intensive ($\bar{x}$ ± SEM)	Free-range ($\bar{x}$ ± SEM)	P-value
Temperature (°C)			
Morning (6.00 a.m)	25.23±1.10	25.10±1.68	0.521^{ns}
Midday (12.00 p.m)	33.46±2.63	34.56±3.06	0.006^{*}
Afternoon (5.00 p.m)	28.39±2.20	27.98±2.08	0.169^{ns}
Humidity (%)			
Morning (6.00 a.m)	90.23±5.04	90.43±6.02	0.891^{ns}
Midday (12.00 p.m)	67.69±11.89	62.93±13.12	0.006^{*}
Afternoon (5.00 p.m)	81.70±6.65	81.17±6.86	0.575^{ns}

$\bar{x}$= mean value; SEM= Standard error of the mean; P-value= significance of the t-test between chickens raised under intensive and free-range systems; *= significantly different ($P<0.05$); ns= not significantly different. Temperature and humidity measurements were recorded three times daily throughout the rearing period

humidity may exacerbate heat stress and negatively affect poultry performance (Li et al. 2023). Therefore, these environmental variations highlight the critical importance of microclimate control in maintaining poultry welfare and minimizing heat stress under different rearing systems.

Hematological parameters of IPB-D3 chickens in different rearing systems

Hematological parameters are used to assess the physiological condition and health status of chickens, which are influenced by both genetic and environmental factors. This analysis provides an overview of the hematological profile of IPB-D3 chickens reared under different rearing systems. The indices are presented in Table 2.

The results indicated that none of the measured hematological parameters differed significantly (P>0.05) between IPB-D3 chickens raised under intensive and free-range rearing systems (Table 2). The erythrocyte count averaged 2.84±0.65 × 10^6/mm³ in the intensive system and 2.99±0.61 × 10^6/mm³ in the free-range system. Hematocrit values ranged from 28.60±3.89% in the intensive system to 29.77±4.16% in the free-range system, while hemoglobin concentrations were 9.06±1.22 g/dL and 9.69±1.46 g/dL, respectively. Similarly, no significant differences were observed in erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), between the two rearing systems. MCV values were 104.35±22.15 fl in the intensive system and 103.12±23.02 fl in the free-range system, whereas MCH values were 33.15±6.49 pg and 33.55±7.63 pg, respectively. MCHC values were recorded at 32.08±3.80% for intensive rearing and 32.63±2.72% for free-range rearing.

All measured values fell within or close to the normal physiological ranges for healthy chickens, indicating that differences in rearing systems did not adversely affect the hematological status of IPB-D3 chickens. Erythrocyte count, hemoglobin concentration, and hematocrit are recognized as key indicators of oxygen-carrying capacity and physiological responses to environmental stress (Wang et al. 2018). The stability of these parameters in the present study suggests that IPB-D3 chickens maintained physiological homeostasis despite environmental variation. This finding is consistent with previous reports indicating that IPB chickens possess good adaptability to different rearing conditions (Sumantri et al. 2020). In addition, proper management practices, including adequate ventilation and balanced nutrition, play an important role in maintaining hematological stability in poultry (Fayzrakhmanov et al. 2020; Hollemans et al. 2020).

Leukocyte profile and immune response in different rearing systems

Leukocyte and differential leukocyte counts are essential parameters for assessing the immune response of chickens to environmental conditions and rearing systems. The results of leukocyte and differential leukocyte analysis, including the heterophil to lymphocyte (H/L) ratio, are presented in Table 3.

Table 2. Overview of total erythrocytes, hematocrit, hemoglobin, and erythrocyte indices of IPB-D3 chickens in different rearing systems

Parameter	IPB-D3 Chicken		P-value
	Intensive n=30 ($\bar{x}$ ± SEM)	Free-range n=30 ($\bar{x}$ ± SEM)	
Erythrocyte (10^6/mm ^{3})	2.84±0.65	2.99±0.61	0.374^{ns}
Hematocrit (%)	28.60±3.89	29.77±4.16	0.266^{ns}
Hemoglobin (g/dL)	9.06±1.22	9.69±1.46	0.103^{ns}
Erythrocyte Indices			
MCV (fl)	104.35±22.15	103.12±23.02	0.834^{ns}
MCH (pg)	33.15±6.49	33.55±7.63	0.829^{ns}
MCHC (%)	32.08±3.80	32.63±2.72	0.521^{ns}

N= number of samples; $\bar{x}$= mean value; SEM= standard error of the mean; P-value= significance of the t-test between chickens raised in intensive and free-range systems; *= significantly different (P<0.05); ns: not significantly different

Table 3. Total leukocytes, differential leukocytes, and H/L ratio in the blood of IPB-D3 chickens under different rearing systems

Parameter	IPB-D3 Chicken		
	Intensive n=30 ($\bar{x}$ ± SEM)	Free-range n=30 ($\bar{x}$ ± SEM)	p-value
Leukocyte (10^3 mm^{-3})	19.52±8.83	14.12±6.77	0.010*
Leukocyte Differential			
Lymphocyte (%)	49.55±7.60	46.97±8.07	0.207ns
Heterophil (%)	36.46±7.48	39.66±8.28	0.122ns
Eosinophils (%)	8.58±2.12	7.82±2.70	0.233ns
Monocytes (%)	4.57±2.14	4.71±1.64	0.775ns
Basophils (%)	0.83±0.07	0.83±0.06	0.688ns
Heterophil/Lymphocyte Ratio (H/L)	0.78±0.30	0.90±0.33	0.149ns

N= number of samples; $\bar{x}$= mean value; SEM= standard error of the mean; P-value= significance of the t-test between chickens raised in intensive and free-range systems; *= significantly different (P<0.05); ns: not significantly different

Table 4. Hematological parameters of IPB-D1 and IPB-D2 chickens compared with published reference ranges.

Parameter	IPB D1 Chicken	IPB D2 Chicken	Publish Range	Reference
Erythrocyte (10^6/mm^{-3})	1.95±0.38(1)	1.93-2.59(2)	2.5-3.9	Samour (2015)
Hematocrit (%)	22.40±5.04(1)	27.67-28.44(2)	24-43	Samour (2015)
Hemoglobin (g/dL)	7.25-8.03(1)	9.96-10.89(2)	10.2-15.1	Samour (2015)
Erythrocyte Indices				
MCV (fl)	-	-	90-140	Jain (1993)
MCH (pg)	-	-	33-47	Jain (1993)
MCHC (%)	-	-	26-35	Jain (1993)
Leukocyte (10^3 mm^{-3})	11.68±0.88(3)	15.29±4.28(4)	13-32	Hendro *et al*. (2013)
Leukocyte Differential				
Lymphocyte (%)	65.20±18.20(3)	50.96±11.91(4)	63-73	Hendro (2013)
Heterophil (%)	30.71±18.13(3)	42.76±12.23(4)	20-40	Lokapirnasari *et al*. (2016)
Eosinophils (%)	1-2(3)	1.61±0.9(4)	0-7	Lokapirnasari *et al*. (2016)
Monocytes (%)	4.00±4.48(3)	4.85±0.95(4)	2-8	Lokapirnasari *et al*. (2016)
Basophils (%)	-	1.40±0.61(5)	0-1	Lokapirnasari *et al*. (2016)
Heterophil/Lymphocyte Ratio (H/L)	0.58-0.64(3)	0.48±.39(5)	0.2-0.8	Hendro *et al*. (2013)

(1)Susanti et al. (2020),(2)Khairunnisa et al. (2021); (3)Al-Habib *et al.* (2020); (4)Lestari *et al.* (2025); (5)Falah et al. (2022). *Pu*blished range refers to reference values for normal hematological parameters in chickens from the cited literature

As shown in Table 3, the total leukocyte count differed significantly between rearing systems (P= 0.010). Chickens raised under the intensive system exhibited a significantly higher leukocyte count (19.52±8.83 × 10^3 mm^{-3}) compared with those raised in a free-range system (14.12±6.77 × 10^3 mm^{-3}), indicating a notable hematological response to the production environment. Elevated leukocyte counts in intensively reared chickens may reflect a physiological response to environmental pressure, such as restricted movement and limited air circulation, which can stimulate immune activity (Elsherbeni et al. 2024).

In contrast, the relative proportions of lymphocytes, heterophils, eosinophils, monocytes, and basophils did not differ significantly (P>0.05), suggesting that the distribution of immune cells remained stable across rearing systems. The average lymphocyte proportion was 49.55±7.60% in the intensive system and 46.97±8.07% in the free-range system, while heterophil proportions were 36.46±7.48% and 39.66±8.28%, respectively. Eosinophil, monocyte, and basophil values also remained within normal physiological ranges. The stability of differential leukocyte profiles indicates that, despite differences in total leukocyte counts, the overall immune balance was maintained under both management conditions, which may reflect adaptive physiological responses rather than pathological changes (Kamel et al. 2017).

The heterophil-to-lymphocyte (H/L) ratio did not differ significantly between systems, with values of 0.78±0.30 in the intensive system and 0.90 ± 0.33 in the free-range system (P>0.05). However, both values exceeded the typical physiological threshold of 0.5, indicating a mild stress response in chickens from both rearing environments. This elevation in H/L ratio may be associated with environmental challenges, including fluctuating microclimatic conditions and exposure to external stressors in free-range systems, as well as confinement-related stress in intensive systems (Wlazlak et al. 2023).

Hematology of male and female IPB-D3 chickens under different rearing systems

The hematological status of chickens is influenced by the rearing system and sex, reflecting their physiological condition and response to the environment. The hematological parameters of male and female IPB-D3 chickens under different rearing systems are presented in Table 5.

Table 5 shows that no significant differences (P>0.05) were observed between male and female chickens across all measured hematological parameters in both intensive and free-range systems. In the intensive system, females exhibited numerically higher leukocyte counts (22.64±2.63 × 10^3/mm^3) than males (18.11±2.25 × 10^3/mm^3), although the difference was not statistically significant (p > 0.05). A similar trend was observed in the free-range system, where females also showed slightly higher leukocyte counts (14.87±2.25 × 10^3/mm^3) compared to males (13.27±1.26 × 10^3/mm^3).

This consistent numerical pattern suggests potential sex-related variation in immune responses, despite the absence of statistical significance. Such differences may be influenced by physiological and hormonal factors, as estrogen is known to enhance immune function, whereas androgens tend to exert immunosuppressive effects (Sciarra et al. 2023) . This mechanism may explain the tendency for higher leukocyte counts in females across both rearing systems.

Furthermore, the slightly higher lymphocyte proportions and lower heterophil-to-lymphocyte (H/L) ratios observed in females, particularly under free-range conditions, may indicate a more balanced immune status. Previous studies have also reported that female poultry generally exhibit stronger humoral immune responses than males, although such differences are not always statistically significant (Sellau et al. 2022). In contrast, erythrocyte-related parameters, including erythrocyte count, hematocrit, hemoglobin concentration, and erythrocyte indices (MCV, MCH, MCHC), did not differ between sexes, indicating similar oxygen-carrying capacity in both males and females. Likewise, other differential leukocyte parameters, such as eosinophils, monocytes, and basophils, remained comparable, suggesting that overall hematological stability was maintained regardless of sex. Although the observed differences were not statistically significant, the consistent trend of higher leukocyte counts in females highlights the possibility of subtle sex-related differences in immune function. With a larger sample size, these differences may become statistically detectable, indicating the presence of sex-specific immune responses in IPB-D3 chickens.

Interaction of rearing systems and sex on the hematological profile of IPB-D3 chickens

To understand the interaction between rearing systems and sex on the hematological profile of IPB-D3 chickens, we analyzed hematological parameters. The results presented in Table 6 indicate an interaction between rearing systems, sex, and hematological values in IPB-D3 chickens, with two parameters showing significant effects. The following section provides a detailed analysis of the affected parameters.

The present study demonstrated that the rearing system significantly affected leukocyte count (P= 0.039), while a significant interaction between rearing system and sex was observed for hematocrit (P= 0.038). In contrast, erythrocyte count, hemoglobin concentration, erythrocyte indices, differential leukocyte percentages,

Table 5. Total erythrocyte, hematocrit, hemoglobin, and erythrocyte index parameters of IPB-D3 chickens under different rearing systems based on sex.

Parameter	IPB-D3 Chicken			
	Intensive		Free-range	
	Male n=15 ($\bar{x}$ ± SEM)	Female n=15 ($\bar{x}$ ± SEM)	Male n=15 ($\bar{x}$ ± SEM)	Female n=15 ($\bar{x}$ ± SEM)
Erythrocyte (10^6/mm^{-3})	2.77±0.17	2.80±0.19	3.08±0.18	2.89±0.16
Hematocrit (%)	27.80±1.10	29.33±0.93	32.25±1.14	28.67±0.91
Hemoglobin (g/dL)	8.92±0.40	9.09±0.24	10.55±0.45	9.29±0.28
Erythrocyte Indices				
MCV (fl)	104.19±5.96	108.37±6.24	107.70±5.83	103.38±6.53
MCH (pg)	33.29±1.86	34.04±1.69	35.20±2.09	33.45±2.04
MCHC (%)	32.33±1.25	31.59±0.68	32.76±0.93	32.52±0.67
Leukocyte (10^3 mm ^{3})	18.11±2.25	22.64±2.63	13.27±1.26	14.87±2.25
Leukocyte Differential				
Lymphocyte (%)	48.94±2.07	49.13±2.26	49.44±2.15	45.47±2.21
Heterophil (%)	36.78±1.96	37.84±2.19	37.31±2.21	41.32±2.31
Eosinophils (%)	8.63±0.50	8.25±0.72	8.04±0.76	7.54±0.72
Monocytes (%)	4.80±0.50	3.94±0.68	4.39±0.43	4.83±0.46
Basophils (%)	0.84±0.02	0.84±0.02	0.82±0.02	0.83±0.02
Heterophil/Lymphocyte Ratio (H/L)	0.80±0.08	0.81±0.09	0.79±0.09	0.97±0.09

N= number of samples; $\bar{x}$= mean value; SEM= standard error of the mean

and H/L ratio were not significantly influenced (P>0.05). These findings suggest that IPB-D3 chickens maintain hematological stability across different rearing systems, reflecting strong physiological resilience under varying environmental conditions. Erythrocyte-related parameters are closely associated with oxygen-carrying capacity and are highly sensitive to environmental stress such as temperature and humidity (Wang et al. 2018; Nwaigwe et al. 2020). However, the absence of significant differences indicates that IPB-D3 chickens can maintain stable erythropoiesis despite suboptimal environmental conditions, consistent with previous findings in IPB-derived chickens (Sumantri et al. 2020).

The significant effect of rearing system on leukocyte count likely reflects an adaptive physiological response to environmental conditions, particularly in intensive systems characterized by higher environmental pressure (Elsherbeni et al. 2024). Elevated leukocyte levels may be associated with increased microbial exposure, as reported by Kuswandi et al. (2025), although values remained within normal physiological ranges, indicating adaptation rather than pathological stress. The absence of significant differences in H/L ratio further supports that the chickens were not under severe chronic stress (Kamel et al. 2017). Additionally, the interaction between rearing system and sex on hematocrit suggests sex-specific physiological responses, likely influenced by hormonal differences affecting oxygen transport and immune regulation (Frerichs et al. 2022). Overall, these findings indicate that IPB-D3 chickens are well adapted to different rearing systems, with important implications for sustainable poultry production, although further studies on molecular and stress-related indicators are needed to confirm these adaptive mechanisms.

Relationship between hematological parameters, rearing systems, and sex in IPB-D3 chickens

A correlation heatmap (Figure 1) is presented for hematological parameters in IPB-D3 chickens, rearing systems, and sex. A positive correlation was observed between hemoglobin (Hb) and hematocrit (HCT),

Table 6. Interaction of blood hematology in IPB-D3 chickens under different rearing systems and sexes

Parameter Hematology IPB-D3 Chickens	Intensive and Free-range Housing	Sex	Interaction
Erythrocyte (106/mm 3)	0.834	0.283	0.221
Hematocrit (%)	0.527	0.065	0.038*
Hemoglobin (g/dL)	0.236	0.391	0.097
Erythrocyte Indices			
MCV (fl)	0.878	0.976	0.996
MCH (pg)	0.839	1.000	0.943
MCHC (%)	0.493	0.965	0.745
Leukocyte (103 mm 3)	0.039*	0.560	0.786
Leukocyte Differential			
Lymphocyte (%)	0.147	0.464	0.415
Heterophil (%)	0.091	0.311	0.383
Eosinophils (%)	0.192	0.275	0.492
Monocytes (%)	0.529	0.846	0.547
Basophils (%)	0.980	0.778	0.657
Heterophil/Lymphocyte Ratio (H/L)	0.099	0.320	0.354

The data presented above are derived from Table 5. Values with an asterisk (*) indicate a significant parameter difference (P<0.05)

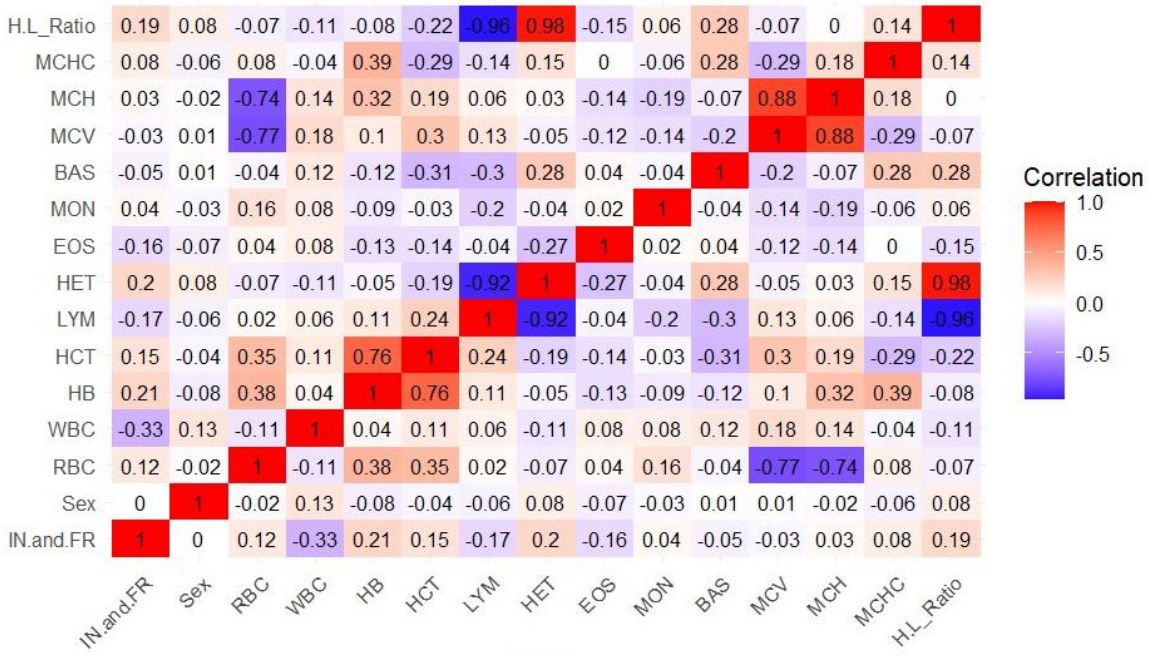

Figure 1: Pearson's correlation between hematological parameters in IPB-D3 chickens. IN and FR= Rearing system (Intensive and Free-range), RBC= Erythrocytes, WBC= Leukocytes, HB: Hemoglobin, HCT= Hematocrit, LYM= Lymphocytes, HET= Heterophils, EOS= Eosinophils, MON= Monocytes, BAS= Basophils, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Hemoglobin, MCHC= Mean Corpuscular Hemoglobin Concentration, H.L_Ratio Heterophil/Lymphocyte Ratio.

indicating that higher red blood cell volume is associated with higher hemoglobin levels, reflecting their close physiological relationship in oxygen transport. Similarly, heterophil (HET) counts showed a positive correlation with the H/L ratio, indicating that an increase in heterophils directly increases the H/L ratio, which supports its use as an indicator of stress in chickens.

The correlation analysis revealed several significant relationships among hematological parameters in IPB-D3 chickens, reflecting coordinated physiological regulation between oxygen transport and immune function. A strong positive correlation was observed between hemoglobin (Hb) and hematocrit (HCT) (r= 0.76), indicating a close physiological association in oxygen-carrying capacity. This relationship is expected, as hematocrit represents the proportion of red blood cells in circulation and directly affects hemoglobin concentration and oxygen transport efficiency (Ivanov 2022).

Similarly, heterophils (HET) exhibited a very strong positive correlation with the heterophil-to-lymphocyte (H/L) ratio (r= 0.98), suggesting that variation in the H/L ratio is primarily driven by heterophil dynamics rather than lymphocyte fluctuation alone. Conversely, HET showed a strong negative correlation with lymphocytes (LYM) (r= −0.92), reflecting a classical leukocyte response pattern under physiological stress conditions. Heterophils function as the primary innate immune cells in poultry, responding rapidly to environmental stressors, whereas lymphocytes represent adaptive immune function. Therefore, a shift toward heterophil dominance indicates activation of stress-related immune pathways (Kamel et al. 2017; Thiam et al. 2021).

Erythrocyte count (RBC) was negatively correlated with mean corpuscular volume (MCV) (r= −0.77) and mean corpuscular hemoglobin (MCH) (r= −0.74), suggesting a compensatory regulatory mechanism between erythrocyte number and cell size or hemoglobin content. This inverse relationship may represent an adaptive strategy to maintain efficient oxygen transport under varying physiological demands, where increased erythrocyte numbers are balanced by reduced cell size to optimize blood viscosity and circulation efficiency (Udroiu 2023). Moderate positive correlations were also observed between Hb and MCH (r= 0.38) and between HCT and mean corpuscular hemoglobin concentration (MCHC) (r= 0.35), further supporting the coordinated regulation of erythrocyte characteristics.

In contrast, the correlation between rearing system and sex was weak (r= 0.19), and most other relationships ranged from weak to moderate. This indicates that hematological profiles in IPB-D3 chickens are predominantly regulated by intrinsic physiological mechanisms rather than external management factors. Such stability suggests the presence of effective homeostatic buffering systems that maintain hematological balance despite environmental variation. Although environmental temperatures in this study exceeded the thermoneutral zone for poultry, the stability of erythrocyte-related parameters indicates that IPB-D3 chickens possess efficient physiological adaptation mechanisms to cope with moderate heat stress. These mechanisms may involve regulation of erythropoiesis, blood viscosity, and oxygen transport efficiency to maintain metabolic homeostasis under fluctuating environmental conditions.

It is important to note that the absence of strong correlations involving rearing system and sex may also be influenced by biological variability among individuals, as reflected in the standard error values. Additionally, hematological responses are multifactorial and may require complementary indicators, such as hormonal stress markers (e.g., corticosterone), to fully elucidate physiological stress responses. Overall, these findings demonstrate that hematological parameters in IPB-D3 chickens are regulated by an integrated physiological network that involves erythrocyte dynamics and immune cell balance, enabling adaptive responses to environmental and internal challenges.

CONCLUCION

The rearing system and sex influenced hematological responses in IPB-D3 chickens, with most parameters remaining within normal physiological ranges and not significantly different, indicating strong adaptability across different management conditions. Free-range chickens showed higher erythrocyte-related parameters, while intensively reared chickens exhibited higher leukocyte counts. Females showed slightly higher leukocyte levels, suggesting potential sex-related differences in immune regulation. Overall, IPB-D3 chickens demonstrate good hematological stability across rearing systems.

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