



Hematology Profile of Broiler Chickens Affected by Mild Footpad Dermatitis and Hock Burn

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Abstract | Intensive broiler production often results in health issues like footpad dermatitis (FPD) and hock burn (HB), which negatively affect chicken welfare and health. While severe FPD and HB are well-studied, the impacts of mild cases are underexplored. This study hypothesized that birds with mild lesions would have hematology profiles closer to normal than those with severe lesions, but still display changes compared to unaffected birds. This study aims to describe the blood profiles of broilers with mild FPD and HB with a case study at an Indonesian farm. To reduce stress, 34 Lohmann and Ross birds were blood-sampled across two broiler houses, with lesion scores assigned using Welfare Quality standards (0: no lesions, 1-2: mild lesions, 3-4: severe lesions). The blood samples were collected from the brachial veins in vacutainer tubes with anticoagulants for hematological examination. The blood was drawn from 4-6 birds sampled from six sampling points and brought to a private lab in Bandung City to be analyzed using a hematology analyzer. The blood profiles of red blood cells (RBCs) and white blood cells (WBCs) were then described with descriptive analysis. Results showed that birds with mild FPD and HB had higher leukocytes compared to unaffected birds, alongside lower RBC counts with larger cell sizes. These findings suggest that even mild lesions are associated with stress or infection, which supports the importance of preventing them for chicken's health and welfare. However, as a case study, hematology profiles were also influenced by farm-specific factors such as management practices and environmental conditions, regardless of the lesion scores.

Keywords | Hematology profile, Broiler chicken, Animal welfare, Footpad dermatitis, Hock burn, Health

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INTRODUCTION

Broiler chicken production in Indonesia has reached nearly 4 billion tonnes, making the country the largest

producer in Southeast Asia (Indonesian Statistics, 2024). To meet growing demand, broilers are typically raised in intensive systems focused on rapid weight gain, but this has led to increased health issues, including gut infections,

respiratory and cardiovascular diseases, leg lesions, and skeletal abnormalities (EFSA AHAW Panel *et al.*, 2023). These problems result in reduced weight, poor feed conversion ratios, high slaughterhouse condemnation rates, and increased mortality, causing significant economic losses (Jones *et al.*, 2018).

Footpad dermatitis (FPD) and hock burn (HB) are prevalent foot lesions in broilers raised in intensive systems. Foot-pad dermatitis (FPD) and hock burn (HB) are characterized by discolouration of the skin, separation or enlargement of skin scales, and hyperkeratosis, which can progress to necrosis of the epidermis, ulcerations, and inflammation of the subcutaneous tissue at the footpad (FPD) or hock joint (HB) (Sherlock *et al.*, 2012). FPD affects up to 99.5% of broilers, with severe cases reaching 38.4% (de Jong *et al.*, 2014; Kyvsgaard *et al.*, 2013), while HB can reach up to 25.12% in intensive farms (Bergmann *et al.*, 2016). Both lesions are multifactorial, influenced by breed, diet, litter type, and management practices (Hunter *et al.*, 2017; Alabi *et al.*, 2024). One of the most common causes for these lesions is prolonged exposure to wet, dirty litter and poor air quality. Previous studies have shown that these inflammatory responses can lead to significant hematological changes, including increased white blood cell counts, increased heterophil-to-lymphocyte ratios, and reduced hematocrit levels, which are markers of physiological stress and immune system activation in broilers (Sherlock *et al.*, 2012; Nwaigwe *et al.*, 2020).

These conditions not only cause pain and discomfort but also lead to lameness and systemic infections, which adversely affect the welfare of chickens and the economic returns for farmers (Thofner *et al.*, 2019; Opengart *et al.*, 2018). Severe lesions frequently result in a high rate of condemnation for chicken feet, which are a valuable product in Asian markets (Shepherd and Fairchild, 2010; Jacob *et al.*, 2016). Consequently, Foot Pad Dermatitis (FPD) and Hock Burn (HB) are increasingly recognized as critical indicators when assessing the welfare of poultry production systems (Welfare Quality, 2009).

Hematology profile can provide insights into the health status of an animal through blood component analysis, which is crucial for assessing the clinical and nutritional health, disease diagnosis, and monitoring (Merck, 2012; Taer and Taer, 2023; Adegbenro *et al.*, 2024). Hematology profile provides a cheaper, faster, and more practical alternative to the corticosterone measurement method, but still maintains its reliability as a biomarker for measuring animal health and stress levels. For example, reductions in circulating eosinophils and hematocrit levels, and increases in white blood cell counts, heterophil-to-lymphocyte (H:L) ratios, and thrombocyte counts are reliable markers of stress in animals (Nowaczewski and Kontecka, 2012;

Nwaigwe *et al.*, 2020). Hemoglobin indices like erythrocytes are also valuable for disease prognosis and monitoring stress (Wicaksono *et al.*, 2020).

FPD and HB manifest in degrees of lesion severity, where increased scores increase the effect on broiler pain, stress, and alteration in blood parameters. While the impacts of severe lesions (scores 3-4) on the chicken's blood profile are well-studied (de Jong *et al.*, 2014; Alabi *et al.*, 2023), the effects of mild FPD and HB especially in Indonesian farms remain underexplored. We hypothesized that birds with mild lesions would have hematology profiles closer to normal than those with severe lesions, but farther than unaffected birds. This study aims to describe the hematology profile of broilers with mild FPD and HB with a descriptive analysis as a case study at an Indonesian farm related to its welfare.

MATERIALS AND METHODS

RESEARCH OBJECT

The subjects of this research consisted of 34 unsexed birds from two fast-growing strains: 17 Ross strain birds and 17 Lohmann strain birds. These birds were obtained from two different commercial hatcheries and were housed in two separate environments, with population sizes of 21,000 birds and 11,000 birds, respectively. The birds were selected from 6 specific sampling points (two near the inlet, two near the outlet, and two in the middle) in two broiler houses. The sampling points were designed to represent differing lengths of the house, as they often have differing litter quality, footpad dermatitis, and hock burn cases (Jaya *et al.*, 2022). A total of 34 birds were randomly selected from two farms, with a total of 4-6 birds from 6 sampling points in each house (Figure 1). The sample size was carefully selected to minimize stress on the flock, as multiple data collection activities were occurring on the farm simultaneously, which could have increased the risk of overhandling and stress.

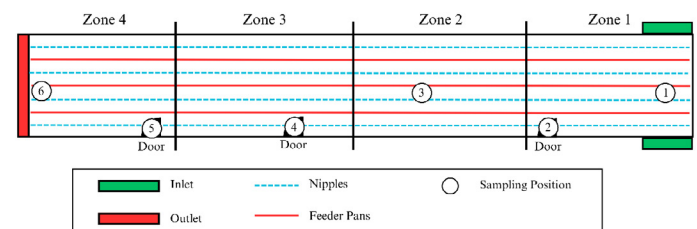


Figure 1: Sampling position points in both broiler houses.

The sampled birds were chosen based on their similarity to the overall condition of the population at the sampling points. The selection criteria included:

- Body weights: From a sample of 20 birds at each sampling point, 2 to 3 birds that were closest to the average

weight (± 50 grams) were selected.

- Gait score: All birds exhibited normal gait, without any clear signs of abnormality or lameness, based on the 0-2 gait scoring criteria outlined by Webster *et al.* (2008).
- General health: A brief physical health examination ensured that all sampled birds were healthy.

The birds were blood-sampled at 28 days of age, with an average weight of 1,526.65 grams, and had no history of contact with clinical diseases. None of the sampled birds showed signs of severe Footpad Dermatitis (FPD) or Hock Burn (HB), as indicated by a score of 4.

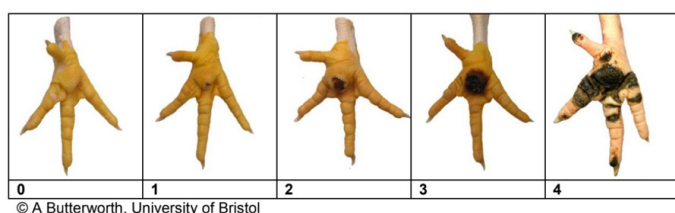


Figure 2: Footpad dermatitis scoring categories (Welfare Quality, 2009).

The chickens were fed ad libitum with commercial feed from PT New Hope Indonesia. The chicks received vaccinations for Newcastle Disease (ND), Infectious Bursal Disease (IBD), and Infectious Bronchitis (IB) at the hatchery and again when they were 12 days old. From 1 to 10 days old, they were given CB10 (fine-crumble) pre-starter feed, and from 11 days old until the end of production, they received C11 starter feed (crumble). The feed comprises approximately 13% moisture, 7% ash, 22.5-23% crude protein, 4.6-5% crude fiber, 1.1% crude fat, 0.8-1.1% calcium, and 0.6% phosphorus. The chickens were also provided with vitamins and medications through their drinking water, following a schedule in accordance with the standard managerial procedures set by the farmer.

ETHICAL STATEMENT

This research had been approved by the Research Ethics Committee of Padjadjaran University, Indonesia coded 70/UN6.KEP/EC/2024.

RESEARCH LOCATION

The research was conducted at Camara Broiler Farm, Camara Village, Pamulihan sub-district, Sumedang Regency, West Java, Indonesia. The farm is located on 814 meters above sea level, with hot and humid tropical weather (temperature of 20-30°C, relative humidity of 57-94%, and air velocity of 0.0 - 2.05 m/s). The farm consisted of two semi-closed houses of different lengths: 1,400 m² and 740 m², both under the same management. In contrast to closed houses, the semi-closed houses were covered with non-permanent roll-up tarpaulin and without cooling pads, al-

though still had automatic feeders, drinkers, and blower fans. Both house densities were kept at 20-21 kg/m². The substrate used was rice husks, without litter turning, and new litter only thinly spread conditionally to the wet and dirty area, with an initial thickness of 3 cm. The chickens were subjected to 24 hours of light (24-L, 0-D) throughout the whole production period.

DATA COLLECTION

Footpad dermatitis (FPD) and hock burn (HB) were scored based on the system outlined by Welfare Quality (2009). A score of 0 indicated that the birds were free from dermatitis (no evidence), scores of 1 and 2 indicated mild lesions (minimal evidence of FPD or HB), and scores of 3 and 4 indicated severe lesions (clear evidence of FPD and HB) (Figures 2 and 3). Each FPD scores were assigned based on the characteristics below (Sherlock *et al.*, 2012):

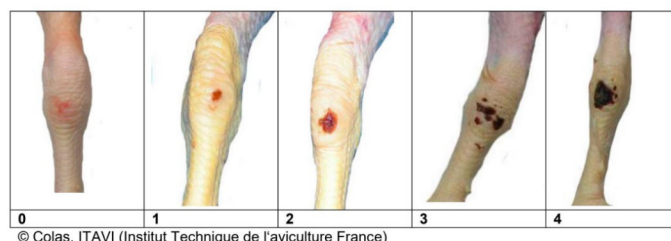


Figure 3: Hock burn scoring categories (Welfare Quality, 2009).

Score 0 = Skin of footpad and digital pads appears normal. Scale pattern clear and skin soft with no reddening/ scale enlargement/ discolouration.

Score 1 = Obvious scale enlargement and/or separation, with or without discoloration on or between scales on footpad or digital pads.

Score 2 = Small but distinct skin lesion(s) present, including scale separation and enlargement, redness and areas of discoloration/ black necrotic tissue on footpad or digital pads (total area up to 0.5 cm diameter).

Score 3 = As Score 2, but lesion area covers up to a third of the footpad area or less if there are significant lesions (score C or higher) on digital pads.

Score 4 = As Score 3, but lesion covers more than a third of the foot pad, with or without significant lesions on digital pads.

Meanwhile, each HB scores were assigned based on the characteristics below (Sherlock *et al.*, 2012):

Score 0 = Skin of hock appears normal. Scale pattern clear and skin soft with no discoloration.

Score 1 = Obvious reddening/bruised appearance of skin in hock area.

Score 2 = Small but distinct skin lesion(s); includes areas of discoloration/ scabs/black necrotic tissue on or between

scales (total area up to 0.5 cm diameter).
Score 3 = As Score 2, but lesion area covers up to a third of the hock area.
Score 4 = As Score 3, but lesion area covers more than a third of the hock area.

The observation was done by a master student with practical experience on broiler chickens on-farm for two years, and trained for this observation under a Ph.D animal-welfare researcher. To reduce bias, another undergraduate student with a short training helped the scoring. To minimize stress during sampling, a small pen (1 m²) made of PVC pipes and plastic nets was placed at each sampling position to prevent the birds from running away. The FPD and HB scores presented in the result tables (Table 1 and 2) were based on the highest score assessed from both feet.

Table 1: Haematology Profile of Chickens Exposed to FPD Based on Severity Score (Average ± SD).

Parameter	Severity Score		
	Score 0	Score 1	Score 2
n (bird)	14	9	11
Erythrocyte (10 ⁶ /mm ³)	3.29 ± 0.41	3.50 ± 0.48	3.44 ± 0.33
Hemoglobin (gr/dL)	12.70 ± 0.72	13.52 ±1.13	13.35 ±0.96
Hematocrit %	32.43 ± 2.44	33.33 ±3.32	32.64 ±3.72
Leucocyte (10 ³ /mm ³)	150.43±12.51	163.30±15.03	158.24±14.38
Heterophile %	29.14 ± 4.47	25.93 ±5.35	26.13 ± 5.19
Lymphocyte %	52.29 ± 9.64	57.33 ±8.34	59.09 ±9.32
Basophile %	4.21 ± 1.02	3.39 ±1.23	3.50 ±1.01
Eosinophil %	4.36 ± 1.32	3.55 ±1.33	3.68 ±1.40
Monocyte %	4.28 ± 1.14	3.88 ±1.32	3.43 ±0.47
H : L Ratio	0.59 ± 0.18	0.48 ±0.18	0.47 ±0.17
MCV (fL)	99.81±12.12	95.75±6.07	95.35±11.49
MCH (pg)	39.12±4.85	38.95±3.29	38.95±2.37

SD: Standard deviation.

BLOOD ANALYSIS

Blood sample was drawn on the farm at the 28th days of production. The selected birds were the same sampled birds labeled for FPD and HB assessment. After calming and laying the chickens on a soft surface, the chicken's pectoral wing vein blood was punctured and drawn using a sterile 21G x 3.12 cm syringe. The blood was collected into 3 mL EDTA-K2 tubes blood, and then homogenized (Onunkwo *et al.*, 2024). The blood samples were put into the cooling box, and brought to CV. Indosains Analysis Laboratory for analyzing with Genius KT6200 Vet MAXEL hematology analyser manufactured from Japan. The analysed blood parameters included: erythrocyte, hemaglobin, haematocrit,

leukocyte, heterophile, lymphocyte, basophil, eosinophil, monocyte, MCH, MCV, and then calculated for H:L ratio. The collected blood sample tubes were then loaded into the analyzer, where it automatically diluted the blood using the diluent for accurate cell counting. The collected blood sample tubes were then loaded into the analyzer, where it automatically diluted the blood using the diluent for accurate cell counting.

STATISTICAL ANALYSIS

The statistical analysis used was descriptive analysis. Each hematological parameter data obtained was measured for its median, modus, maximum, minimum, standard deviation, standard of error, and coefficient of variation using SPSS iBM 25, and then described.

RESULTS AND DISCUSSION

HEMATOLOGY PROFILE OF BIRDS CONTACTING FPD

The results of the ANOVA and descriptive analysis of the hematology profiles of chickens unaffected or affected by mild footpad dermatitis (FPD) scored 0, 1, and 2 are shown in Table 1 and 2.

Table 2: Haematology Profile of Chickens Exposed to HB Based on Severity Score (Average ± SD).

Parameter	Severity Score			
	Score 0	Score 1	Score 2	Score 3
n (bird)	10	20	2	2
Erythrocyte (10 ⁶ /mm ³)	3.18±0.40	3.49±0.41	3.55 ± 0.47	3.32 ± 0.83
Hemoglobin (gr/dL)	12.64±0.63	13.38±1.15	13.50±0.35	12.66±1.56
Hematocrit %	31.90±2.47	33.10±2.81	32.50±4.95	33.50±4.95
Leucocyte (10 ³ /mm ³)	150.19 ± 12.72	158.99 ± 13.84	159.00 ± 23.33	158.34 ± 26.72
Heterophile %	29.07±4.48	26.50±5.31	27.50±7.35	26.60±6.36
Lymphocyte %	51.40 ± 10.43	58.10±8.27	58.00±8.49	53.00 ± 15.56
Basophile %	4.17 ± 1.33	3.58±1.09	3.80 ± 0.57	3.50±0.99
Eosinophil %	4.29 ± 1.35	3.69±1.48	4.18 ± 1.53	4.18±1.81
Monocyte %	4.46 ± 1.30	3.69±1.10	3.20 ± 0.57	3.85±0.92
H : L Ratio	0.60 ± 0.19	0.48±0.17	0.49 ± 0.20	0.54±0.28
MCV (fL)	101.59 ± 13.25	95.21 ± 6.91	91.56 ± 1.73	102.38 ± 10.62
MCH (pg)	40.23 ± 4.70	38.50 ± 3.88	38.36 ± 4.13	38.79 ± 4.97

SD: Standard deviation.

RED BLOOD CELL (RBC) COUNT: Some inclining patterns were observed on the red blood cell (RBC) or erythrocyte count difference among chickens in each score and

dermatitis type. However, RBC for chickens scored 0 on FPD and HB averaged at $3.08 \pm 0.39 \times 10^6/\text{mm}^3$, lowest compared to birds scoring 0 on FPD and birds scoring 0 on HB. The RBC count increased in birds with FPD scores of 1 and a score of 2, which may indicate the effects of FPD lesions. These averages still align with the normal range for chickens ($2.5\text{--}3.5 \times 10^6/\text{mm}^3$) according to Bounous and Stedman (2000), however, birds with erythrocytosis ($3.87\text{--}3.90 \times 10^6/\text{mm}^3$) were also observed across the three score groups of FPD. Male chickens exhibited higher RBC counts ($3.48 \times 10^6/\text{mm}^3$) than females ($3.34 \times 10^6/\text{mm}^3$), a pattern consistent across different FPD and HB scores.

Accordingly, there is an incline for the hemoglobin and hematocrit count of birds contacting FPD on scores 1-2 to be higher than the birds without it. While the average hemoglobin and hematocrits were within the normal range of 7-13 g/dL and 22-35%, respectively (Bounous and Stedman, 2000), birds from poorly ventilated farms exhibited higher hemoglobins (13.45-15.4 g/dL) and hematocrits levels (32.64-33.44%). These results can be affected by the lack of oxygen, high ammonia concentration (20.96 ppm in compared to an average of 8.35 ppm in the other farm), erythrocyte count (Alfian *et al.*, 2017). Male birds also showed higher hematocrit % (33.06%) than female birds (32.5%).

In contrast, the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values of birds with feather plumage disorder (FPD) were lower than those of birds without FPD lesions. Among the three score groups, birds with a score of 0 for FPD had the highest MCV and MCH values. The average MCV and MCH of birds scoring 0 still fell within the normal ranges of 90-140 fL and 33-47 pg, respectively (Bounous and Stedman, 2000; Tanuwiria *et al.*, 2022). However, the lowest MCV (67.04 fL) and MCH (38.17 pg) were observed in the score 1 group, which was sampled in areas with the lowest airflow. In contrast, the highest MCV and MCH values were found in birds without any FPD lesions (score 0).

WHITE BLOOD CELL (WBC) COUNT: Similar to RBC, some inclinations were found on all WBC counts between each FPD and HB scores. The lowest leukocyte count was observed in birds without FPD, while the highest was found in birds with an FPD score of 1 especially those from particular sampling positions, and slightly decreased in birds with a score of 2. The normal range for white blood cells (WBC) in chickens is $12\text{--}30 \times 10^3/\text{mm}^3$ (Bounous and Stedman, 2000), indicating that the WBC levels in all samples were abnormally high (leucocytosis). Male birds also showed higher average leukocyte ($157.59 \times 10^3/\text{mm}^3$) than females ($155.12 \times 10^3/\text{mm}^3$), but lower average heterophile than females (26.43% compared to 28.04%).

Lymphocyte levels were lowest in birds without FPD ($52.29 \pm 9.64\%$), falling below the normal range of 55-69% according to Dukes (1995). The highest lymphocyte levels were found in birds with an FPD score of 2, followed by those with a score of 1, suggesting that inflammation or infections related to FPD lesions triggered an immune response, linearly with the increase in leukocyte. Birds with the highest lymphocytes were still those located in areas with the lowest airflow and high ammonia (20,96 ppm), as those with the highest WBC counts. However, the birds with lymphocytes below normal were sampled near the outlet in the larger farm, where higher microorganism accumulation likely occurred due to the denser bird population. These birds were also those located closest to the culling pen.

The highest average heterophile and basophil levels were found in birds without FPD, and lower heterophile and basophile % in birds with FPD scores of 1 and 2. Some birds sampled near the farm's outlet in the bigger farm showed higher heterophiles (33.50-33.70%) and basophiles (5.20-5.40%) than Dukes' (1995) normal ranges for broiler chicken (22-30% and 1-4%, respectively). However, these higher heterophile averages were still lower than those obtained by Baudojun *et al.* (2021) (26-63%) and Onyishi *et al.* (2017) (63-64%).

The highest average eosinophil ($4.36 \pm 1.32\%$) and monocyte ($4.28 \pm 1.14\%$) counts were also observed in birds without FPD, followed by birds with an FPD score of 2 and 1. Eosinophil counts fell within the normal range according to Dukes (1995) (3-8%) and Baudouin *et al.* (2021) (1-13%), and the lowest eosinophil levels (2.10-2.40%) were found in sampling points with the lowest airflow and highest humidity. The lowest monocyte levels were also found in such positions however, all monocytes were consistently below the typical 10% (Dukes, 1995) but within the normal range obtained in chicken by Baudouin *et al.* (2021) (1-8%), regardless of FPD scores (ranging from 2.70% to 6.70%).

According to Wicaksono *et al.* (2020), H:L ratio was categorized as high at 0.5-0.79 and normal at 0.2-0.49, therefore, the average H:L ratio in this research was still considered normal. The H:L ratio was highest in birds without FPD (0.59 ± 0.18) and decreased with increasing FPD severity. Extremely high H:L ratios (>0.8) were found in birds closest to the outlet in the larger farm, consistent with birds observed with heterophilia and lymphopenia (Table 1). This could indicate that these birds were under significant stress due to continuous exposure to environmental hazards along the production cycle (Krams *et al.*, 2012).

HEMATOLOGY PROFILE OF BIRDS CONTACTING HB

The results of the statistical analysis of the hematology profiles of chickens unaffected or affected by mild hock burn (HB) scored 0-3 is shown in Table 2.

RED BLOOD CELL (RBC): The average erythrocyte count from all HB score groups (0-3) was higher than the erythrocyte count for birds free from both FPD and HB ($3.08 \pm 0.39 \times 10^6/\text{mm}^3$). The average erythrocyte was higher than those obtained by [Odunitan-Wayas et al. \(2018\)](#), especially those birds from the areas with the highest average ammonia concentration (20,96 ppm). Birds scoring 0 in HB had the lowest erythrocyte counts ($2.71 \times 10^6/\text{mm}^3$) among all score groups, and the highest ($3.90 \times 10^6/\text{mm}^3$) was found in birds with the most severe HB (score 3).

The average hemoglobin in birds scoring 0 on HB was 12.64 ± 0.63 g/dL, increased to 13.36, 13.50, and dropped to 12.66 g/dL in birds scoring 3. This finding is higher than the maximum count obtained by [Odunitan-Wayas et al. \(2018\)](#) (11 g/dL), but was still within normal ranges (7-13 g/dl) according to [Dukes \(1995\)](#) and lower than the averages obtained by [Onyishi et al. \(2017\)](#) (13.67-15.00 g/dl). Consistent with erythrocytes, the lowest hemoglobin count (11.45 g/dL) was observed on birds near the inlets, and the highest hemoglobin was found on birds scoring 1 (15.40 g/dL) positioned near the outlet on the farm with the lowest airflow and high ammonia concentration (23,75 ppm).

The lowest hematocrit was found on birds scoring 0, 2, 1, and 3, respectively. These were still similar to the averages obtained by [Baudouin et al. \(2021\)](#) (15.30-44%), but higher than the averages from [Onyishi et al. \(2017\)](#) findings (27.87-32.75%). Higher hematocrit followed the higher erythrocyte counts. The highest hematocrit was 37% in birds scoring 3, and the lowest was found on birds scoring 1 (24%), found on birds in the smaller farm.

The highest average MCV was observed on birds scoring 3, 0, 1, and 2 on HB, respectively. The highest MCV was found on birds scoring 0 and the lowest was found on birds scoring 1. The highest MCH was observed on birds scoring 0, 3, 2, and 1 on HB, respectively. The lowest MCH was observed on birds scoring 1, and the highest on birds scoring 0, similar to MCV. These numbers were within the normal ranges for broilers.

WHITE BLOOD CELLS (WBC): The highest average leukocytes based on HB scorings were found within the group of birds scoring 2, 1, 3, and 0, sequentially. The lowest leukocyte count was found in birds scoring 1 ($137.40 \times 10^3/\text{mm}^3$) and the highest was found in birds scoring 3 on HB ($177.23 \times 10^3/\text{mm}^3$). The lowest leukocyte count in this research far exceeded the normal leukocyte count in broiler chickens obtained by [Odunitan-Wayas et al. \(2018\)](#) ($15.15\text{--}20.50 \times 10^3/\text{mm}^3$). Despite these high counts, birds with the highest leukocyte were found by the walls and

outlet with low airflow and high ammonia concentration (the smaller farm, with an average ammonia concentration of 30.95 ppm), similar to birds with FPD.

Lymphocyte % in this study was highest in birds scoring 2 ($58 \pm 8.49\%$) and lowest in birds scoring 0 with $51.40 \pm 10.43\%$. These numbers were still within the normal range (27-67%) according to [Baudouin et al. \(2021\)](#), but higher lymphocytes (68-69%) were found in birds scoring 1 in HB, and the lowest (34%) in group score 0 HB. In contrast, the heterophiles % in this study was highest in birds scoring 0, and lowest in birds scoring 1, followed by birds scoring 3 and 2. Despite these averages falling within the normal ranges, the highest heterophile (33.79%) and the lowest (21.90%) were both found within the group scoring 1 in HB.

The highest basophile % in this study was found in birds scoring 0, and lowest in birds scoring 3, followed by birds scoring 1 and 2. These numbers were still within normal range of 0-4% ([Baudouin et al., 2021](#)), except for most birds without HB. The highest basophile % found in all score groups were higher than 4 (4.20-5.40%), with the highest (5.40%) was found on bird scoring 0 in HB, same with lymphocyte, and the lowest in the birds scoring 1 (1.60%). The lowest basophile % found was still higher than the 0% basophil from [Onyishi et al. \(2017\)](#).

Consistent with the WBC count, the highest eosinophil % in this study was found in birds scoring 0, and followed by birds scoring 1, 2, and 3. The highest eosinophil % was found in group score 1 HB (6.10%). Meanwhile, the highest monocyte % in this study was also found in birds scoring 0, but it was followed by birds scoring 3, 1, and 2. The highest monocyte % was found in groups scoring 0 and 1 on HB (6.70%), similar to lymphocyte, eosinophil, and basophile. However, birds scoring 0, 1, and 2 had eosinophil and monocyte ranges below the normal ranges according to [Dukes \(1995\)](#) (3-8% eosinophil and 10% monocyte), but still within the normal range according to [Baudouin et al. \(2021\)](#) (1-13% eosinophil and 1-8% monocyte).

The highest average H:L ratio in this study was found in birds scoring 0 on HB (0.60 ± 1.19) followed by birds scoring 3 (0.54 ± 0.28), both having H:L ratio above 5, which indicated an abnormally high H:L ratio and possibly stressed ([Wicaksono et al., 2020](#)). Some birds with the highest H:L ratio (0.88-0.90), regardless of HB scores, were those from the culling pen, which directly contacted sick and lame birds, the place with relatively higher ammonia (11.63 compared to an average of 8.35 ppm), and possibly inadequate food and water supply. These stress factors were independent of FPD or HB, as normal H:L ratios (0.32-0.35) were also found across all score groups.

RED BLOOD CELL (RBC) COUNT

Higher erythrocytes were recorded in birds with FPD and HB, especially those sampled from areas within farm with low airflow, and therefore low oxygen supply, and high ammonia concentration (23,16 ppm). According to [Gorelik et al. \(2020\)](#), stress from environmental conditions affects the cellular composition of blood, including RBC levels. RBCs are critical for determining the rheological properties of blood, performing respiratory functions, transporting essential nutrients, and participating in humoral regulation during normal and pathological conditions ([Chachaj et al., 2019](#)). These functions are enabled by receptors on the erythrocyte membrane, which acts as a stress responder and the animal's adaptive potential. Consequently, the mobilization of red blood components in response to stress reflects the body's adaptive reactions and overall adaptive potential. Under oxygen deficiency, the chicken would increase erythropoietin production, which increases RBC levels, allowing chickens to compensate for lack of O₂ quickly and restore homeostasis. Besides low oxygen supply, oxygen deficiency could also be the result of cells' hemolysis due to exposure to said stress factors, which determined the subsequent activation of erythropoiesis and the mobilization of red cells into the bloodstream ([Gorelik et al., 2020](#)).

The observed increase in RBCs can be linked to the body's response to hypoxia, which increases oxygenation when the O₂ levels are low or demand rises. This occurs through the production of erythropoietin (EPO), a hormone triggered by hypoxia that boosts RBC production to improve oxygen transport. Hypoxia-inducible factors (HIFs) regulate genes that increase RBC production and hemoglobin levels, which are essential for oxygen delivery in blood. Hemoglobin's affinity for oxygen shifts depending on tissue demand (influenced by factors like temperature, pH, and 2,3-BPG compound) which help unload oxygen more effectively in stressed tissues ([Rhodes et al., 2022](#)).

Chronic exposure to elevated ammonia levels in particular sampling positions (average ammonia concentration reaching 23.75 ppm) can cause respiratory stress, which impairs oxygen exchange and leads to a hypoxic state. This ammonia-induced hypoxia, along with oxidative stress from environmental factors, may damage red blood cells and trigger compensatory erythropoiesis to maintain adequate oxygenation ([Etim et al., 2014](#)). Stress-induced hypoxia and oxidative stress further shift hemoglobin's affinity for oxygen, which contributes to the observed elevated RBC levels. Additionally, hemoglobin levels are influenced by age, stress, elevation, season, nutritional status, parasitism, high gaseous ammonia, and population density ([Minias, 2015](#)), all of which may have contributed to the observed findings.

In contrast, decreased MCV and MCH were observed in birds affected by those HB, especially in birds sampled under stressful environments. As MCV reflects the average size of erythrocytes, higher MCV (macrocytes) in those conditions enabled RBC to contain more hemoglobin to meet the body's oxygen requirements ([Gorelik et al., 2020](#)). During hypoxia-induced erythropoiesis, the erythroid maturation process involves cell size reduction due to division, which causes the decline in MCV in birds with the highest RBC counts ([Sarma, 1990](#)). MCH quantifies the hemoglobin content per red blood cell and, therefore, follows a similar trend as MCV ([Abdulazeez et al., 2016](#)).

Environmental stressors, such as oxidative stress and ammonia exposure, disrupt erythropoiesis and can exacerbate these changes in MCV and MCH. High ammonia recorded in the poultry housing can contribute to respiratory irritation, oxidative stress, and tissue inflammation, which may impair oxygen exchange and lead to compensatory erythropoiesis. This oxidative stress damages cellular components, including lipids and proteins within erythrocytes, accelerating their destruction and further triggering the need for increased erythropoiesis ([Obeagu et al., 2024](#)). Ammonia exposure can also lead to the accumulation of reactive oxygen species (ROS) ([Oke et al., 2024](#)), which exacerbates oxidative damage and alters erythroid maturation, thus impacting erythrocyte size ([Orrico et al., 2023](#)). Oxidative stress caused by high ammonia levels disrupts the normal erythropoiesis process, influencing both the maturation and the hemoglobin content of red blood cells ([Obeagu et al., 2024](#)), which may explain the reduced MCV and MCH values seen in birds affected by FPD and HB. Therefore, environmental stressors play a significant role in modulating the hematological response and ultimately affect the broilers' ability to cope with hypoxia.

WHITE BLOOD CELL (WBC) COUNT

Leukocyte counts increased with the severity of footpad dermatitis (FPD), consistent with the expectation that necrotic conditions would lead to infections, immune response, and stress ([Scanes, 2015](#)). In cases of FPD and HB, the innate immune system has two primary leukocyte cell types responding to infection and tissue damage: macrophages and neutrophils. Neutrophils migrate rapidly to the site of inflammation. They can release neutrophil extracellular traps (NETs) to trap and neutralize pathogens. At the same time, macrophages phagocytose pathogens and debris as well as secrete cytokines and growth factors that aid in tissue repair and regulate the immune response ([Rosowski, 2020](#)). Leukocytosis typically indicates infections, inflammation, injury, stress, trauma, or immune system disorders ([Mitchell and Johns, 2008](#)).

Despite the initial hypothesis, we found that birds with less severe lesions showed higher leukocyte counts than

those with more severe lesions. This suggests that other factors such as higher ammonia levels (40.85 ppm) and lower airflow (0.95 m/s wind speed) in specific sampling positions increased the leukocyte counts. A high concentration of ammonia gas (>25 ppm) can irritate the bird's eye corneas and mucous membranes of the respiratory tract, which eventually increases the bird's susceptibility to infection, especially *E. coli* (Olanrewaju *et al.*, 2008; Sheikh *et al.*, 2018). Furthermore, birds with the highest leukocyte counts exhibited lower eosinophil and monocyte levels, which reflects a shift in immune cell types as the immune response progresses or environmental stress affects immune modulation.

Similarly, higher lymphocyte counts were observed in birds with FPD and HB, which may indicate an ongoing immune response. An increase in lymphocytes is characteristic of long-term bacterial infections, parasitic infections, and lymphatic leukemia. At the same time, a decrease is observed in immunosuppression, acute viral infections, lack of parasites, and stress (Skwarska, 2019). While ammonia exposure is often linked to immunosuppression, which reduces lymphocytes, it can also trigger chronic low-level immune activation, leading to lymphocytosis as the body attempts to counter ongoing irritation or subclinical infections. In these birds, the continuously high ammonia levels (>25 ppm) may have contributed to both immunosuppressive effects and increased immune activation (Guo *et al.*, 2022). Chronic environmental stressors—such as inadequate water or nutritional supply (Etim *et al.*, 2014), high ammonia levels, temperature stress, and social interactions within the flock—are known to influence immune function and could have played a role in modulating lymphocyte levels (Schat and Skinner, 2008). Birds with the highest lymphocyte levels also exhibited the lowest monocyte levels, which could reflect an interaction between immune cell populations as the immune system responds to prolonged environmental stress or infection (Asmara *et al.*, 2019).

Contrary to leukocytes and lymphocytes, heterophiles tend to decrease with increasing foot dermatitis severity. Birds sampled from the outlet of the larger farm showed higher heterophiles, regardless of FPD or HB scores. Heterophiles are involved in the acute inflammatory response and phagocytosis (Genovese *et al.*, 2013). They play a significant role in innate immune responses and their numbers increase in response to bacterial and fungal infections, as well as stress. The observed decrease in heterophiles with increasing FPD and HB severity appears to contradict the expected outcome, as heterophiles typically rise in response to infection or inflammation. Inflammation from FPD could trigger compensatory mechanisms potentially due to the depletion of immune resources from chronic stress or inflammation (Hannoodie and Nasuruddin, 2024). However, due to the relatively low FPD and HB scores, this result might be

caused by prolonged exposure to possible infections and other inflammatory issues (Hofmann *et al.*, 2020). This result could also be caused by environmental stressors such as high humidity, low temperature, or overcrowding that limit the production or recruitment of heterophils, rather than being directly related to feet lesions (Abo-Al-Ela *et al.*, 2021). This could also explain the reduced lymphocyte counts, as the immune response shifts due to the continuous environmental and physiological stress.

The basophils, eosinophils, monocyte %, and H:L ratio from birds with FPD and HB were lower than those unaffected by it. Still, higher basophils were observed near the outlet of the larger house. Avian basophils are involved in inflammatory reactions, particularly those associated with hypersensitivity. They are more sensitive than mammals to environmental stressors and can indicate early inflammatory responses, diseases, or stress (Rahmania *et al.*, 2022). The observed basophilia in birds near outlets suggests that these birds may have been exposed to stressors not evident through clinical signs, such as having clear eyes, no signs of labored breathing or snoring, and normal activity levels, with no apparent signs of illness or distress. Additionally, these areas showed higher ammonia levels (11.63 ppm compared to an average of 8.35 ppm) and higher temperatures (26.45°C compared to 25.21°C), which may have contributed to the stress response (Hofmann *et al.*, 2020).

Lower eosinophils and monocytes were observed in sampling positions with low airflow and high ammonia concentration (Sampling Position 5, with an average ammonia concentration of 19.33 ppm). Eosinophils are abundant in submucosal and mucocutaneous areas, and eosinopenia may indicate stress, infection, or allergy (York, 2017) in birds from those sampling positions. Meanwhile, monocytes are precursors to macrophages and other immune cells and play a crucial role in inflammatory and anti-inflammatory processes. Low monocyte levels suggest a compromised immune system (Espinoza and Emmady, 2023), potentially due to environmental conditions or acute infection. Despite these low percentages, monocytopenia might hold little to no significance, as zero is the lower limit of the reference range for most avian species.

Higher H:L ratios are commonly associated with ongoing disease or stress. Higher H:L ratios indicates a stronger stress response, due to higher heterophiles and lower lymphocytes from more glucocorticoids in blood (Skwarska, 2019; Krams *et al.*, 2012). The birds from the smaller farm showed lower H:L ratio which might indicate that these birds have higher intestinal immunity (Thiam *et al.*, 2021). However, in this study, those same birds had lymphocyte levels above their normal ranges, regardless of FPD or HB scores. The higher lymphocyte could also indicate early infection (Gaunson *et al.*, 2000), possibly due to the low-

er oxygen supply and higher NH_3 , in contrast to the assumption of better chicken welfare with a lower H:L ratio. Higher H:L in the larger farm could be caused by stress from sub-clinical or acute infection, especially as birds with the highest H:L ratio were those sampled closest to the possibly sick birds from the culling area, near the outlet.

CONCLUSIONS AND RECOMMENDATIONS

Broilers with mild FPD and HB (scores of 0-2) generally showed normal blood profiles, except for leukocytes. Birds without dermatitis showed lower RBC and higher MCH and MCV than birds affected by foot lesions. In contrast, higher leukocytes, lymphocytes, and H:L ratio were associated with higher lesion scores, and the opposite was observed for the granulocytes and monocytes. The changes in RBC and WBC levels in birds with mild FPD and HB suggest that even mild cases of foot dermatitis can lead to stress and infections. However, these variations may be attributed more to environmental factors such as airflow, ammonia levels, and population density due to the studied farm condition. Other factors, including genetics, nutrition, and individual bird health, may have also contributed to the observed hematological profiles.

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NOVELTY STATEMENT

The novelty of this research is a hematological profile description with a focus on footpad dermatitis and hock burn in an Indonesian commercial semi-closed broiler farm.

AUTHOR'S CONTRIBUTIONS

Eluzia Aloina Barus: Conceptualization, Data Curation, Formal Analysis, Resources, Funding Acquisition, Investigation, Writing - original draft, Project Administration.
Indrawati Yudha Asmara and Novi Mayasari: Conceptualization, Methodology, Resources, Supervision, Validation, Writing - review & editing.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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