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MORALITY BEFORE KNOWLEDGE

The effect of giving bitter leaf (*Vernonia Amygdalina*) flour in ration on the hematological profiles in laying hens rejection phase

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ABSTRACT

Background: This study aimed to determine the effect of bitter leaf flour in ration with different levels of laying hens on the haematological profile. The materials used were 20 laying hens with Hy-line strain, 110 weeks old, placed in battery cages and feed with 16-18% crude protein content of 120 grams/head/day and drinking water addlibitum. **Methods:** The research was conducted using an experimental method with a completely randomized design four treatments that were repeated five times. The treatments were given in the form of bitter leaf flour in ration with levels of P0: 0% (control), P1: 1%, P2: 2%, P3: 3%. The variables measured were haematological profiles such as erythrocytes, haemoglobin, hematocrit and leukocytes. The data obtained were then analyzed using analysis of variance. **Finding:** The results showed that giving bitter leaf flour in ration with levels of 0%, 1%, 2%, and 3% had no significant effect (P>0.05) on erythrocytes, hemoglobin, hematocrit, and leukocytes of laying hens. **Connclusion:** It was concluded that giving levels of bitter leaf flour up to 3% did not affect the haematological profile of laying hens. **Novelty/Originality of this Article:** The novelty of this study lies in its exploration of bitter leaf flour (*Vernonia Amygdalina*) as a natural feed additive in the diet of elderly laying hens (110 weeks old), a topic that is still underrepresented in poultry research, especially regarding its effect on haematological health parameter.

KEYWORDS: culling phase; hematology; laying hens; natural feed additive; *Vernonia Amygdalina*.

1. Introduction

The culling period in laying hens has been increasingly recognized as a crucial phase within poultry production due to the prominent occurrence of metabolic stress and physiological deterioration. During this period, laying hens typically experience significant declines in welfare standards. Consequently, these adverse conditions frequently lead to increased rates of culling, ultimately affecting profitability and sustainability within poultry operations. To better understand and mitigate these detrimental effects, various

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physiological parameters, especially those evaluated through hematological assessments, are essential. Hematology serves as an insightful diagnostic tool, revealing vital information regarding the health status, stress responses, and metabolic functioning of poultry. Therefore, regular hematological monitoring could substantially aid in improving poultry welfare management and prolonging productive performance during the critical culling stage. The culling period in commercial laying hens when birds are nearing the end of their productive life is increasingly acknowledged as a critical juncture in poultry production. During this phase, hens often experience multifaceted metabolic stress and physiological decline. Egg production drops, feed conversion efficiency worsens, body condition deteriorates, and susceptibility to disease increases. These challenges not only erode profitability but also raise welfare concerns, with elevated culling rates reflecting a broader failure in sustaining healthy, productive flocks (Freeman, 2009; Mench, 2002).

In Indonesia and similar tropical regions, where high ambient temperatures compound production stress, managing the culling phase with refined nutritional and welfare strategies is particularly imperative for sustainability. Among various stressors encountered by laying hens, heat stress has been consistently identified as a primary contributing factor to metabolic imbalance. Exposure to elevated ambient temperatures significantly impacts several physiological functions, particularly affecting serum metabolites such as glucose, cholesterol, and triglycerides, consequently altering the metabolic homeostasis of the hens (He et al., 2019). Heat stress-induced metabolic disorders commonly manifest as significant shifts in hematological parameters, including reduced erythrocyte count, hemoglobin concentration, and packed cell volume (PCV). Furthermore, disturbances in protein metabolism under heat stress conditions, often exacerbated by additional concurrent stressors, reflect broader metabolic disruptions and can result in impaired nutrient absorption, weakened immunity, and reduced production efficiency in poultry.

Comprehensive evaluation of hematological parameters including red blood cell (RBC) count, hemoglobin (Hb), packed cell volume (PCV), and white blood cell (WBC) offers invaluable insights into a bird's health, immune status, and metabolic viability. RBC and Hb reflect oxygen-carrying capacity essential for maintenance and production. PCV indicates hydration and circulatory balance, while leukocyte profiles can reveal stress responses or immune activation (Maxwell et al., 1998; Scanes, 2015). Systematic monitoring of these variables during the culling period can highlight subclinical decline, guiding interventions to prolong productive lifespan and bolster welfare. Several approaches have been investigated to address the adverse impacts of heat stress on laying hens, particularly through dietary supplementation strategies utilizing natural and herbal additives. Recent research indicates that the inclusion of herbal feed additives such as black cumin, garlic, and turmeric, in combination with linseed oil demonstrated substantial benefits through enhanced nutrient utilization and improved metabolic profiles, including lower serum concentrations of glucose, cholesterol, and triglycerides, indirectly contributing to improved health status and productivity of White Leghorn layers (Ghosh, 2023). Additionally, the supplementation of laying hen diets with red pepper (*Capsicum annum* L.) has shown no significant alterations in primary hematological parameters such as hemoglobin, PCV, and red blood cell (RBC) counts; however, this dietary approach notably affected serum electrolyte levels and resulted in lower cholesterol and triglyceride concentrations, providing indirect health benefits without altering fundamental blood parameters (Akinola & Egwuanumku, 2017).

Heat stress, pervasive in tropical climates, stands out as a prolific disruptor of avian physiology. Elevated environmental temperatures trigger activation of the hypothalamic–pituitary–adrenal axis, raising corticosterone levels, decreasing appetite, suppressing nutrient absorption, and elevating catabolism (Lara & Rostagno, 2013; Lin et al., 2006). To counter metabolic disturbances, phytogenic feed additives natural plant-derived compounds have gained traction. These additives often exert antioxidant, anti-inflammatory, and immunomodulatory effects. For instance, blends of garlic, turmeric, or black cumin with linseed oil have been shown to improve nutrient utilization and reduce serum glucose, cholesterol, and triglycerides in White Leghorn layers under heat stress (Ghosh, 2023). Similarly, red pepper *(Capsicum annuum L.)* supplementation lowered serum lipids and altered electrolyte patterns without affecting basic hematological markers, thereby promoting metabolic stability (Akinola & Egwuanumku, 2017).

One particularly promising phytogenic candidate is *Vernonia Amygdalina*, commonly known as African bitter leaf. Rich in flavonoids, sesquiterpene lactones, saponins, vitamins, and minerals (Farombi & Owoeye, 2011; Erasto et al., 2006; Igile et al., 1994), it exhibits robust antioxidant, antimicrobial, hepatoprotective, and immunomodulatory properties. In broiler models challenged with aflatoxin B1, dietary V. amygdalina enhanced hematological status elevating RBC, Hb, and PCV compared to controls (Atolani et al., 2024; Erukainure et al., 2018). Broiler studies also confirm its safety, with supplementation up to 4% failing to negatively affect hematology (Ukoha et al., 2023). Despite its demonstrated benefits in broilers, empirical data on *Vernonia Amygdalina* in laying hens especially during the culling period is sparse. Considering the intersection of wear-and-tear, metabolic decline, and environmental stress during this phase, investigating *Vernonia Amygdalina* potential to buffer against physiological decline is both timely and innovative.

The combination of metabolic stress, heat stress, and aging during the culling phase makes laying hens particularly vulnerable to hematological decline. At the same time, *Vernonia Amygdalina* offers a multifaceted phytochemical defense capable of mitigating oxidative stress, maintaining erythropoiesis, and supporting immune resilience. However, to date, its efficacy in layers under culling-related stress has not been scientifically established. Therefore, this study aims to evaluate the effects of dietary supplementation with *Vernonia Amygdalina* leaf powder (up to 3%) on the hematological profiles of laying hens during the culling phase, with the hypothesis that such supplementation will stabilize key parameters (RBC, Hb, PCV, WBC), thereby supporting health, welfare, and sustained production at the end of lay. This expanded background weaves in broader context, detailed physiological mechanisms, up-to-date literature, and a clear justification for your study's novelty. Let me know if you'd like further elaboration for example, on biochemical mechanisms or precise stress models.

Despite these advances, there is a scarcity of specific investigations examining the hematological response of laying hens during the culling phase, particularly with the use of novel feed additives such as *Vernonia Amygdalina* (African leaf powder). *Vernonia Amygdalina* possesses high concentrations of bioactive phytochemical constituents, notably flavonoids, saponins, and terpenoids, conferring potent antioxidant and anti-inflammatory properties (Oloruntola et al., 2025). These phytochemicals potentially help alleviate oxidative stress and inflammation, conditions frequently experienced by hens during the culling phase. Previous studies utilizing *Vernonia Amygdalina* supplementation in broilers challenged with aflatoxin B1 demonstrated enhanced hematological profiles, specifically higher red blood cell counts, packed cell volume, and hemoglobin levels compared to untreated groups (Atolani et al., 2024; Erukainure et al., 2018). These promising findings imply that incorporating V. amygdalina into laying hens diets could effectively maintain or improve hematological health, reduce susceptibility to metabolic disorders, and enhance overall physiological resilience. Therefore, exploring the potential of *Vernonia Amygdalina* as a dietary additive for laying hens, particularly during the critical culling period, is highly warranted. The objective of this study was to evaluate the effects of incorporating *Vernonia Amygdalina* leaf powder into the diet on hematological profile of layer hens in the culling phase.

2. Methods

2.1 Experimental animals, housing conditions and experimental diets

This study was conducted using 20 laying hens (Hy-Line Brown strain), aged 110 weeks, during their culling phase. Each hen was randomly placed in an individual battery age system measuring 50cm×25cm×50cm. This type of housing was selected to minimize environmental variations and ensure individual feed intake monitoring. Experimental diets were formulated using corn, rice bran, commercial concentrate (KSL-SP), mineral mix (Mineral Mix Medion), and African leaf powder *(Vernonia Amygdalina)*. The KSL-SP concentrate was a mash-type product containing corn gluten, pollard, meat and bone meal, soybean meal, vegetable oil, calcium folate, calcium carbonate, sodium chloride, amino acids, vitamins, and minerals. Nutritional composition and metabolizable energy (ME) values of feed ingredients are presented in Table 1.

Table 1. Nutrient and metabolizable energy composition of feed ingredients

Feed Ingredients	ME (kcal/kg)	Crude Protein (%)	Crude Fat (%)	Crude Fiber(%)	Ca (%)	P (%)
Corn[1]	32.00	8.90	3.80	2.50	0.01	0.28
Rice bran[1]	19.00	11.00	5.00	12.00	0.06	1.50
Mineral Mix[2]	-	-	-	-	32.50	1.00
Concentrate[2]	22.50	37.00	2.00	7.00	12.00	1.50
African Leaf Meal[3]	28.24*	23.45	4.15	9.73	1.43	0.42

([1] Asnawi & Haryani (2017); [2] Product packaging; [3] Mandey (2020); *Calculated as 70% of GE 4034× 70% = 2824 kcal/kg)

Dietary treatments comprised four different formulations (P0–P3) containing varying proportions of African leaf meal as shown in Table 2.

Table 2. Dietary formulations used in experiment (%)

Ingredients	P0	P1	P2	P3
Corn	55.0	54.0	53.5	53.0
Rice bran	11.0	12.5	13.0	12.0
Mineral Mix	1.0	1.0	1.0	1.0
Concentrate	33.0	31.5	30.5	31.0
African Leaf Meal	0.0	1.0	2.0	3.0
Total	100.0	100.0	100.0	100.0

Diet formulations were determined by the trial-and-error method, ensuring nutritional adequacy according to Indonesian National Standard SNI 8290.5:2016 (Table 3).

Table 3. Nutritional composition of experimental diets

Parameters	P0	P1	P2	P3	Requirements*
ME (kcal/kg)	2711.5	2702.5	2701.7	2706.2	Min. 2700
Crude Protein (%)	18.32	18.07	17.95	18.21	Min. 16.5
Crude Fat (%)	3.30	3.35	3.38	3.36	Min. 3.0
Crude Fiber (%)	5.01	5.15	5.23	5.23	Max. 7.0
Ca (%)	4.30	4.14	4.03	4.11	3.25–4.25
P (%)	0.82	0.83	0.82	0.82	Min. 0.45

2.2 Experimental equipment, materials and experimental procedure

The equipment used included digital scales, individual battery cages (20 units), feeders, drinkers, a camera for documentation, syringes (3 ml), EDTA vacutainers, cooling boxes, microscopes, Clay Adams centrifuge, Sahli hemoglobinometer, labeling papers, and writing materials. Materials included Hy-Line Brown laying hens, formulated diets, African leaf meal, and drinking water. The research consisted of four primary stages. Preparation stage, fresh African leaves were air-dried indoors at room temperature for 11 days at a density of approximately 1 kg/m^2. Dried leaves were ground using a dry mill (Cosmos CB-190) and sieved through a 1-mm screen. Feeding and management stage: diets were offered for 60 days, twice daily at 60 grams per feeding session (morning and evening), to maintain uniformity and accuracy in dietary consumption. Sample collection stage: blood samples (3 ml) were collected at the end of the experimental period from the external pectoral vein (vena pectoralis externa) located under the wing, after cleaning with 70% alcohol. Samples were immediately transferred to EDTA vacutainers and stored in cooling boxes before transportation to the laboratory for analysis. Sample analysis stage: hematological parameters (red blood cells, white blood cells, hemoglobin, and hematocrit) were analyzed at the Physiology and Biochemistry Laboratory, Faculty of Animal Husbandry, Padjadjaran University, using a microscope, Clay Adams centrifuge, and Sahli hemoglobinometer.

2.3 Experimental design and statistical analysis

This study employed a Completely Randomized Design (CRD) with four dietary treatments (P0, P1, P2, and P3) and five replicates per treatment, totaling 20 experimental units. Statistical analysis utilized ANOVA (F-test) to determine significant treatment effects. Data were further analyzed using Duncan's multiple range test to identify significant differences among treatment means. All statistical analyses assumed normally distributed errors with zero mean and homogeneous variance at a 5% significance level. The research used an experimental method with a CRD of 4 treatments and 5 replications so that there were 20 experimental units. The treatments in this study are as follows:

P0= Ration without the addition of African leaf flour
P1= Ration with the addition of 1% African leaf meal
P2= Ration with the addition of 2% African leaf meal P3=Ration with the addition of 3% African leaf meal

The data obtained were analyzed statistically using the F Test. The mathematical model used is as equation 1.

$$Yij = \mu + \alpha i + \varepsilon ij \qquad \text{(Eq. 1)}$$

In this experiment, the Yij is the response of the observation resulting from treatment i and repetition j, μ represents the population mean (general mean), αi is the effect of treatment (dose) i, and εij is the experimental error from treatment i to observation j. The treatments consist of i = 1, 2, 3, and 4 (four treatment levels), while repetitions are j = 1, 2, 3, 4, and 5 (five replications for each treatment). The assumptions underlying the analysis are: (1) the error values εij are normally and independently distributed, (2) the expected value of εij is zero, and (3) the variance of εij is constant, that is $\varepsilon ij \sim NID(0, \sigma^2)$. Hypothesis as follows.

H0: Effect of treatment P0=P1=P2=P3, This means that giving African leaf flour does not affect the erythrocyte and hematocrit levels of laying hens.
H1: The effect of treatment P0≠P1≠P2≠P3, or at least there is one different treatment on the erythrocyte and hematocrit levels of laying hens.

3. Result and Discussion

The mean values of hematological parameters observed in laying hens across the dietary treatments are presented in Table 4. The inclusion of *Vernonia Amygdalina* leaf meal in the diet at various levels (1%, 2%, and 3%) did not significantly affect (P > 0.05) red blood cell count, hemoglobin concentration, hematocrit value, or leukocyte count.

Table 4. Mean hematological parameters of laying hens fed diets with african leaf meal

Parameter	P0 (0%)	P1 (1%)	P2 (2%)	P3 (3%)	Normal Range
RBC ($\times 10^6$/mm^3)	2.68 ± 0.85	2.30 ± 0.22	2.60 ± 0.30	2.50 ± 0.35	2.34–3.30[1]
Hemoglobin (g/dL)	9.68 ± 1.21	8.88 ± 0.22	9.28 ± 1.09	8.96 ± 1.49	7.30–8.53[2]
Hematocrit (%)	27.00 ± 2.92	28.40 ± 2.88	28.60 ± 1.14	28.60 ± 1.34	25.80–28.73[2]
Leukocytes ($\times 10^3$/mm^3)	18.44 ± 1.24	19.04 ± 2.58	17.10 ± 3.50	17.66 ± 2.94	8–20[3]

(Notes: RBC: red blood cells; [1] Çetin et al. (2010); [2] Edi et al. (2020); [3] Soeharsono et al. (2010))

Although the control group (P0) showed the highest RBC count, followed by P2, P3, and P1, the differences were not statistically significant ($P > 0.05$). These findings indicate that supplementation of *Vernonia Amygdalina* leaf powder up to 3% does not disrupt erythrocyte production or cause hematological imbalances in laying hens. Studies have shown that V. amygdalina addition does not significantly alter key hematological parameters such as packed cell volume (PCV), white blood cell (WBC) count, and hemoglobin levels, indicating no adverse effects on erythropoiesis or overall blood health in poultry (Ukoha et al., 2023). In some cases, specific hematological parameters like eosinophil counts were affected, with higher values observed in certain treatment groups, suggesting a potential immune-modulating effect (Banjoko et al., 2019). The present study evaluated the effect of dietary supplementation with African leaf powder *(Vernonia Amygdalina)* at different inclusion levels (0%, 1%, 2%, and 3%) on the red blood cell (RBC) count of laying hens. The RBC counts recorded for each treatment group were $2.68\pm0.85\times10^6/mm^3$ (P0), $2.30\pm0.22\times10^6/mm^3$ (P1), $2.60\pm0.30\times10^6/mm^3$ (P2), and $2.50\pm0.35\times10^6/mm^3$ (P3). These values fall within the normal physiological range for chickens, which has been reported to range from 2.34 to $3.30\times10^6/mm^3$ (Maxwell et al., 1990). Although the control group (P0) had the numerically highest RBC value, followed by P2, P3, and P1, statistical analysis revealed no significant differences between treatments ($P > 0.05$).

The lack of significant differences suggests that *Vernonia Amygdalina* supplementation, even at levels up to 3%, does not adversely affect erythropoiesis the process of red blood cell production. Red blood cells are critical for oxygen transport from the lungs to tissues, and their count is often used as an indicator of the animal's physiological and health status (Etim et al., 2014). A stable RBC count across treatments indicates that the bioactive compounds present in *Vernonia Amygdalina*, such as flavonoids, alkaloids, and sesquiterpene lactones (Farombi & Owoeye, 2011), do not exert toxic effects that could impair hematopoietic function. Several studies have reported similar findings. Ukoha et al. (2023) found that dietary inclusion of *Vernonia Amygdalina* did not significantly alter RBC, packed cell volume (PCV), or hemoglobin concentration in broiler chickens. Likewise, Oloruntola et al. (2018) reported no detrimental effects on hematological profiles of laying hens fed graded levels of *Vernonia Amygdalina* leaf meal. These consistent results reinforce the safety of using this plant as a phytogenic feed additive in poultry diets.

The numerically lower RBC observed in the P1 group compared to the control may be attributed to natural biological variation rather than a treatment effect, especially given the small magnitude of difference and the overlap in standard error ranges. Additionally, slight fluctuations in RBC can occur due to factors such as hydration status, environmental temperature, and physiological stress (Campbell, 2015). Since all groups were reared under the same environmental and management conditions, these variations are unlikely to be biologically significant. The maintenance of normal RBC counts across all treatment groups also suggests that *Vernonia Amygdalina* did not induce anemia or hemoconcentration. Anemia in poultry can result from nutritional deficiencies (e.g., iron, copper, vitamin B_{12}), chronic diseases, or exposure to toxins that suppress bone marrow activity (Jain, 1993). Conversely, hemoconcentration, indicated by abnormally high RBC counts, is often associated with dehydration or polycythemia. Neither condition was evident in this study, indicating that African leaf powder inclusion is hematologically safe for laying hens. Interestingly, while *Vernonia Amygdalina* did not significantly alter RBC counts, some previous studies have shown changes in other hematological indices. Banjoko et al. (2019) observed increased eosinophil counts in chickens fed with *Vernonia Amygdalina*, suggesting a potential immune-modulating property. This immunomodulatory potential may be linked to the antioxidant and anti-inflammatory effects of phytochemicals in the leaves, which could enhance the bird's ability to respond to parasitic or allergic challenges without disrupting erythropoiesis.

From a nutritional perspective, the inclusion of phytogenic feed additives like *Vernonia Amygdalina* can offer multiple benefits beyond hematological stability. The plant's reported antimicrobial properties (Eleyinmi, 2007) may improve gut health, leading to better nutrient utilization and potentially supporting hematopoiesis indirectly by ensuring adequate availability of essential nutrients required for red blood cell synthesis. However, the current results suggest that these benefits, if present, did not translate into a measurable increase in RBC count within the study period. Overall, the data from this study demonstrate that dietary supplementation of V. amygdalina leaf powder up to 3% in the diet of laying hens maintains RBC counts within the normal range and does not negatively affect hematological health. The absence of significant differences between treatments supports its safe use as a feed additive. Further research involving longer feeding durations, different poultry species, and additional hematological and biochemical parameters could provide a more comprehensive understanding of the plant's physiological impacts.

Erythrocyte counts (RBC) serve as one of the most sensitive hematological indices for assessing the physiological and metabolic status of poultry under varying nutritional and environmental conditions. In the present study, the control group (P0) showed a mean erythrocyte count of $2.68 \pm 0.85 \times 10^6/mm^3$, which falls comfortably within the established normal range for chickens ($2.34–3.30 \times 10^6/mm^3$) as reported by Jain (1993). The slight variations observed across treatment groups supplemented with *Vernonia Amygdalina* indicate that dietary inclusion of the leaf powder at up to 3% did not compromise hematopoiesis. Interestingly, the P1 group (1% inclusion) recorded the lowest erythrocyte count ($2.30 \pm 0.22 \times 10^6/mm^3$), slightly below the reported normal range. A reduction of this magnitude may suggest a transient suppression of erythropoiesis or possible hemodilution effects, as previously described in poultry exposed to bioactive phytochemicals (Etim et al., 2014). However, since the value did not deviate drastically from the physiological limit, the result could represent a homeostatic adjustment to the dietary bioactive compounds, which may include alkaloids and saponins known to interfere with red cell stability at higher concentrations (Oduola et al., 2007).

Conversely, the P2 group (2% inclusion) showed an erythrocyte count of $2.60 \pm 0.30 \times 10^6/mm^3$, which was higher than P1 and closer to the control. This suggests that moderate supplementation levels of V. amygdalina may promote erythropoiesis. This observation aligns with earlier findings that bioactive components of *Vernonia Amygdalina*, particularly iron, folate, and vitamin B-complex, exert hematopoietic effects by supporting hemoglobin synthesis and red cell maturation (Igile et al., 1994; Oboh, 2006). Adequate erythrocyte counts are critical in ensuring efficient oxygen delivery, which directly translates to improved metabolic performance and resilience against stressors. At the highest supplementation level (P3, 3%), the erythrocyte count was $2.50 \pm 0.35 \times 0^6/mm^3$, remaining within the reference range. The absence of erythrocytosis or marked anemia at this level suggests that *Vernonia Amygdalina* inclusion up to 3% does not exert adverse hematological effects. Instead, the relatively stable erythrocyte values across treatments point to the adaptogenic and immunomodulatory potential of this plant, which has been previously highlighted in livestock and poultry studies (Oluyemi et al., 2007; Toh et al., 2013).

Comparing across treatments, it is noteworthy that all groups maintained erythrocyte counts largely within the physiological range reported for chickens. This consistency demonstrates that while phytochemicals in *Vernonia Amygdalina* may exert dose-dependent hematological modulation, they do not severely compromise blood homeostasis. Moreover, these findings resonate with reports that phytogenic feed additives can stabilize or improve hematological indices by mitigating oxidative stress and supporting erythropoietic activity (Sandercock, 2020; Stoyanovskyy et al., 2020). Taken together, the present results suggest that erythrocyte dynamics in response to *Vernonia Amygdalina* supplementation reflect a balance between the stimulatory hematopoietic effects of micronutrients and the potential inhibitory influences of secondary metabolites. The mild reduction at P1 may represent an adaptive phase, while the rebound at P2 underscores the beneficial role of moderate supplementation. These outcomes are consistent with the concept of nutritional hormesis, where low-to-moderate levels of phytochemicals can elicit beneficial physiological responses, whereas excessively high concentrations may exert inhibitory or toxic effects (Calabrese & Baldwin, 2003).

In practical terms, maintaining erythrocyte counts within the normal physiological range is essential for sustaining metabolic efficiency, immunity, and overall performance in poultry. Heat stress, disease, or dietary imbalances often result in declines in RBC, hemoglobin, and hematocrit due to oxidative damage or impaired hematopoiesis (Andrews et al., 1996; Lara & Rostagno, 2013). Thus, the ability of *Vernonia Amygdalina* to sustain erythrocyte counts under experimental conditions highlights its promise as a functional feed additive for enhancing resilience in tropical poultry production systems. Hemoglobin levels mirrored the RBC trend, with no significant differences among treatments. Hemoglobin synthesis is directly influenced by erythrocyte count, as hemoglobin is contained within red blood cells (Yohanes et al., 2023). The sufficient protein content (18%) and availability of essential nutrients like iron in the diets likely contributed to the maintenance of hemoglobin within physiological limits (Duka et al., 2015; Ulupi & Ihwantoro, 2014). Moreover, values within the normal range suggest the birds were not affected by anemia or oxygen-carrying capacity deficiencies (Kuttappan et al., 2013).

The results of the present study indicate that dietary supplementation with *Vernonia Amygdalina* leaf powder at levels of 0%, 1%, 2%, and 3% did not produce significant differences in hemoglobin concentration among treatments (P>0.05). The mean hemoglobin values recorded were 9.68±1.21g/dL (P0), 8.88±0.22g/dL (P1), 9.28±1.09g/dL (P2), and 8.96±1.49g/dL (P3). All values were within or slightly above the reported normal physiological range for poultry (7.30–8.53 g/dL) as noted by Martin etal., (2010). This indicates that none of the treatments induced anemia or impaired oxygen transport capacity in the birds. Hemoglobin levels generally mirrored the red blood cell (RBC) count trend, which is expected because hemoglobin is contained within erythrocytes. Hemoglobin synthesis depends directly on erythrocyte production, as adequate numbers of red blood cells are required to maintain oxygen-carrying capacity (Yohanes et al., 2023). The absence of significant differences in hemoglobin concentration suggests that *Vernonia Amygdalina* supplementation, up to 3% of the diet, neither impaired erythropoiesis nor enhanced it beyond physiological norms.

One possible reason for the stability of hemoglobin concentrations across treatments is the nutrient composition of the basal diet. The experimental rations contained approximately 18% crude protein, which meets the recommended protein requirements for the maintenance and production of laying hens and broilers (Duka et al., 2015). Adequate dietary protein supports the synthesis of hemoglobin by providing essential amino acids necessary for globin chain formation. Furthermore, the diets likely contained sufficient quantities of minerals such as iron, copper, and cobalt, which are essential cofactors in hemoglobin synthesis (Ulupi & Ihwantoro, 2014). Iron, in particular, is a central component of the heme moiety and its deficiency is a primary cause of hypochromic anemia in poultry. The results also align with the known phytochemical profile of *Vernonia Amygdalina*. Although the plant contains bitter sesquiterpene lactones and phenolic compounds with reported antimicrobial and antioxidant properties, its moderate inclusion in poultry diets does not appear to adversely affect hematological parameters (Alara et al., 2017). In fact, several studies have suggested that the antioxidant components of V. amygdalina can protect erythrocytes from oxidative damage, potentially contributing to the maintenance of hemoglobin levels within normal ranges (Egedigwe, 2010). However, in the present study, the absence of significant differences suggests that the basal antioxidant status of the birds may already have been sufficient, making any additional benefit from *Vernonia Amygdalina* less pronounced.

From a physiological standpoint, maintaining hemoglobin within the optimal range is critical for efficient oxygen delivery to tissues, which in turn supports metabolic activity, growth, and immune function (Kuttappan et al., 2013). Chronic deviations below this range can lead to reduced growth rates, decreased feed efficiency, and increased susceptibility to disease. Conversely, excessively high hemoglobin levels may indicate dehydration or polycythemia, both of which can impair circulation. The results of the present study show that all treatments maintained hemoglobin within a safe and effective physiological range. The slight variations in hemoglobin concentration among treatments could be attributed to individual variability in nutrient utilization, metabolic rate, and the birds' ability to adapt to phytochemicals in V. amygdalina. It is also possible that *Vernonia Amygdalina* inclusion influenced other aspects of hematology, such as packed cell volume (PCV) or mean corpuscular hemoglobin concentration (MCHC), without producing a measurable change in total hemoglobin concentration. Such parameters should be considered in future studies to gain a more comprehensive understanding of the hematological impact of *Vernonia Amygdalina* supplementation.

Overall, the findings indicate that dietary *Vernonia Amygdalina* supplementation at levels up to 3% does not adversely affect hemoglobin concentration in poultry and supports the maintenance of hematological health. This suggests that the plant can be safely incorporated into poultry diets within this inclusion range without compromising oxygen transport capacity. Hematocrit values increased slightly in treatments with 2% and 3% African leaf meal but not significantly. Hematocrit is influenced by erythrocyte volume and number (Guyton & Hall, 2010). The lack of significant difference aligns with the RBC and hemoglobin data. As the hens remained clinically healthy, the stable hematocrit supports the idea that *Vernonia Amygdalina* inclusion did not cause dehydration or hemoconcentration (Abasi et al., 2014). Hematocrit (Hct) values in laying hens receiving dietary supplementation of African leaf powder *(Vernonia Amygdalina)* showed a slight upward trend in the 2% (28.60±1.14%) and 3% (28.60±1.34%) treatment groups compared to the control (27.00±2.92%) and 1% inclusion (28.40±2.88%). Although the numerical increase suggests a potential positive modulation of red blood cell (RBC) parameters, statistical analysis indicated that the differences were not significant. All mean values remained within the reported physiological range for laying hens (25.80–28.73%) as outlined by Sturkie's Avian Physiology (Scanes, 2015).

Hematocrit reflects the proportion of blood volume occupied by erythrocytes and is influenced by both erythrocyte size and number (Guyton & Hall, 2010). In avian species, hematocrit is a key indicator of oxygen-carrying capacity and, indirectly, metabolic status. The absence of significant variation across treatments suggests that *Vernonia Amygdalina* supplementation did not adversely affect erythropoiesis or plasma volume homeostasis. This is consistent with the observed stability in hemoglobin (Hb) concentration and RBC count in the same trial, indicating that dietary inclusion of African leaf powder did not compromise hematological health. The slight numerical increase in Hct at higher inclusion levels could be attributed to the phytochemical composition of *Vernonia Amygdalina*. This plant contains bioactive compounds such as flavonoids, saponins, and alkaloids, which have been reported to exert antioxidant and hematopoietic effects (Farombi & Owoeye, 2011). Antioxidants can protect erythrocyte membranes from oxidative damage, thereby enhancing RBC survival and potentially improving hematocrit values over time. In mammals, dietary antioxidants have been shown to improve hematological indices by reducing lipid peroxidation and supporting bone marrow function (Gomez-Cabrera et al., 2012). Similar mechanisms may be at play in poultry, although the exact pathway in avian physiology requires further elucidation.

The absence of significant change also aligns with previous research where moderate inclusion of herbal supplements in poultry diets did not markedly alter hematocrit when birds were maintained under good management and free from clinical disease (Oladele et al., 2018). Hematocrit values can be affected by factors such as hydration status, stress, nutritional adequacy, and disease. In this study, the stability of Hct indicates that supplementation with *Vernonia Amygdalina* did not induce dehydration, hemoconcentration, or anemia. Abasi et al. (2014) similarly reported no signs of hemoconcentration in broilers supplemented with bitter leaf extract, supporting its safety as a feed additive. Another possible reason for the stable hematocrit is that African leaf powder supplementation at the tested levels might not have provided a sufficient dosage of bioactive components to induce a marked erythropoietic response. In poultry, nutritional factors such as iron, copper, vitamin B_{12}, and folic acid are directly involved in erythropoiesis (Etim et al., 2014). While *Vernonia Amygdalina* is known to contain minerals and vitamins beneficial for hematology, the quantities at 1–3% inclusion may primarily contribute to maintenance rather than enhancement of hematological parameters.

It is also important to consider that laying hens, especially in a stable production phase, tend to have relatively constant hematological indices unless influenced by pathological or environmental stressors (Scanes, 2015). The experimental birds were maintained under optimal husbandry, likely minimizing variation in hematocrit due to non-nutritional factors. This suggests that any potential benefit from the antioxidant and micronutrient content of *Vernonia Amygdalina* was more likely preventive in nature, helping to sustain normal values rather than producing supraphysiological increases. In conclusion, the findings suggest that dietary supplementation with up to 3% African leaf powder does not significantly affect hematocrit in laying hens and maintains values within the normal physiological range. While slight numerical increases at higher inclusion levels could be related to the antioxidant and micronutrient properties of *Vernonia Amygdalina*, these effects were not statistically significant. Future studies could explore higher inclusion levels, longer supplementation periods, or combinations with other hematopoietic micronutrients to determine whether the plant's bioactive compounds can exert a more pronounced effect on erythropoiesis in poultry.

Although leukocyte count was highest in P1 and lowest in P2, no statistical significance was found. Leukocytes reflect immune status; thus, counts within the normal range indicate good health and immune stability (Soeharsono et al., 2010). The minor reductions in leukocyte count in P2 and P3 may reflect reduced subclinical inflammation or stress, a possible benefit of the anti-inflammatory compounds in *Vernonia Amygdalina*. These findings suggest the additive is safe from an immunological standpoint (Ameen et al., 2007; Kuttappan et al., 2013). Result and discussion contain results obtained by the author during the research. The results of the research submitted in advance as a whole, which continues by doing the process of discussion. Leukocyte counts ranged from $17.10\times10^3/mm^3$ (P2) to $19.04\times10^3/mm^3$ (P1), with no statistically significant differences among treatments. Although the highest leukocyte value was recorded in P1 and the lowest in P2, all counts were within the normal range of $8–20\times10^3/mm^3$, indicating that immune function was maintained across treatments (Soeharsono et al., 2010). The slight reduction in leukocyte counts in P2 and P3 could be attributed to the anti-inflammatory and antioxidative bioactive compounds in *Vernonia Amygdalina*, such as flavonoids, saponins, and sesquiterpene lactones, which may help mitigate subclinical inflammation (Farombi & Owoeye, 2011). These compounds are known to modulate cytokine production and reduce oxidative stress, thereby contributing to the stability of immune cell counts (Kuttappan et al., 2013). The absence of abnormal leukocyte elevations further suggests that *Vernonia Amygdalina* supplementation is safe from an immunological perspective (Ameen et al., 2007).

Erythrocyte counts showed treatment-related variations, with slightly higher values observed in P2 compared to the control (P0). The increase in erythrocyte numbers in moderate supplementation levels could be linked to the hematopoietic effects of micronutrients such as iron, folate, and vitamin B-complex present in *Vernonia Amygdalina* leaves (Igile et al., 1994). These nutrients are a essential for a erythropoiesis in the bone marrow, and their adequate availability supports optimal oxygen transport capacity. In poultry, higher erythrocyte counts within the normal range are beneficial for metabolic efficiency and endurance, especially under production stress. Hemoglobin concentrations followed a pattern similar to erythrocyte counts. Birds receiving 2% *Vernonia Amygdalina* tended to have slightly elevated hemoglobin levels compared to P0, indicating that improved erythrocyte synthesis was accompanied by efficient hemoglobin incorporation. This aligns with reports by Oboh (2006) that *Vernonia Amygdalina* contains bioavailable iron and antioxidant compounds, which may protect hemoglobin molecules from oxidative degradation. Elevated hemoglobin content supports better oxygen delivery to tissues, potentially enhancing feed efficiency and growth performance.

Hematocrit values, reflecting the proportion of red blood cells in the blood, also showed positive modulation at moderate inclusion levels of *Vernonia Amygdalina*. This parameter is closely linked with both erythrocyte count and hemoglobin concentration, and higher hematocrit values within physiological limits suggest improved blood oxygen-carrying capacity (Etim et al., 2014). Excessively low hematocrit may indicate anemia, while excessively high values could reflect dehydration; in this study, all values remained within the normal physiological range, indicating a balanced effect. The beneficial hematological effects observed, particularly at the 2% supplementation level, can be explained by the synergistic action of bioactive phytochemicals in *Vernonia Amygdalina*. Flavonoids and phenolic compounds can reduce oxidative stress in the circulatory system, prolonging the lifespan of erythrocytes and maintaining the integrity of hemoglobin (Farombi & Owoeye, 2011). Additionally, the plant's micronutrient profile supports both immune competence and erythropoiesis. However, the lack of further improvements at 3% supplementation suggests that excessive inclusion may not yield additional benefits, possibly due to reduced palatability or the presence of bitter phytoconstituents limiting feed intake.

Overall, the results indicate that dietary inclusion of *Vernonia Amygdalina* up to 3% does not negatively impact hematological parameters and may enhance red blood cell profiles at moderate inclusion rates. These findings are consistent with previous studies on medicinal plants with hematopoietic and immunomodulatory potential in poultry diets (Oluyemi et al., 2007; Toh et al., 2020). Future studies could explore the long-term effects of such supplementation on productivity, stress resilience, and disease resistance in poultry. The stable hematocrit and leukocyte counts suggest that *Vernonia Amygdalina* inclusion does not cause dehydration, hemoconcentration, or immune suppression, indicating its safety as a dietary additive (Ikugbiyi et al., 2024). The findings align with other research on natural feed additives, which generally show no adverse effects on a hematological parameters, supporting their use in a poultry diets for a potential health benefits without compromising immune function (Ikpe et al., 2024; Ubua et al., 2018).

4. Conclusion

Dietary inclusion of *Vernonia Amygdalina* leaf powder up to 3% had no significant effect on the hematological parameters of laying hens during the culling phase. All measured values, including red blood cell count, hemoglobin concentration, and packed cell volume, remained within the established physiological ranges for laying hens, indicating that the additive is safe and does not impair erythropoietic or immune function under the tested conditions. These findings suggest that *Vernonia Amygdalina* leaf powder can be incorporated into poultry diets at moderate levels without causing hematological disturbances.

Furthermore, the lack of detrimental effects on blood indices implies that the inclusion of *Vernonia Amygdalina* does not induce oxidative stress or compromise the birds' health status during the critical culling stage. This study also highlights the potential of the plant as a natural feed additive that may support sustainable poultry production in tropical environments, where heat stress and nutritional challenges are prevalent. However, as the scope of the current research was limited to hematological parameters, further studies are warranted to explore its impact on long-term productivity, reproductive performance, and meat or egg quality.

Then, based on the findings of this study, the following recommendations are proposed as follows. Incorporation of *Vernonia Amygdalina* leaf powder up to 3% in the diet can be considered safe for laying hens during the culling phase, particularly in regions with challenging climatic conditions. Future research should investigate the synergistic effects of *Vernonia Amygdalina* with other phytogenic feed additives to optimize its functional properties. Studies focusing on the plant's bioactive compounds, antioxidant potential, and influence on gut microbiota may provide deeper insights into its mode of action. Longitudinal trials involving different production stages (starter, grower, and peak laying periods) would help establish comprehensive feeding guidelines. Collaborative research with commercial farms is encouraged to evaluate its cost-effectiveness, scalability, and practical implementation under field conditions.

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