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Blood Profile and Leukocyte Differentiation of *Coturnix coturnix japonica* with Addition of African Leaf Juice (*V. amygdalina*) in Drinking Water

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ABSTRACT

This study aimed to assess the impact of incorporating African leaf juice into drinking water on the blood profile. A completely randomized design was used with 120 laying quail divided into three treatments and five replicates. The treatments included a control group (without African leaf juice) and two groups with African leaf juice added to their drinking water at 3 ml/bird/day doses and 6 ml/bird/day. The results showed that adding African leaf juice 3 ml and 6 ml/bird/day indrinking water decreased haemoglobin. Adding 3 and 6 ml/bird/day of African leaf juice in drinking water did not affect leukocyte differentiation. It was concluded that adding 3 ml/bird/day of African leaf juice could be used in quail drinking water.

Keywords: African leaf, leukocyte Differentiation, quail blood profile, quail egg, *Vernonia amydalina*

INTRODUCTION

Quail eggs are a source of animal protein with a protein content of 14% (Hasbullah, 2020). Various herbs can be used as feed additives to maximize and efficiently produce quail eggs through feed or drinking water. One such herb is the African leaf or *Vernonia amygdalina*, which is easy to grow and widely cultivated by the community. African leaf plants are commonly used as yard fences, making them easily accessible. They are also utilized for medicinal purposes and as a feed additive due to their potential bioactive substances, such as flavonoids, saponins, tannins, steroids, and alkaloids (Mandey et al. 2020; Nnaoma et al. 2022).

African leaves contain phytochemicals such as 0.5% flavonoids, 4.86% total phenols, 0.05% tannins, and 0.46% beta carotene. Additionally, they have 274.09 ppm of antioxidant IC50-DPPH, 530.77 ppm of LC50 toxicity, and 4.78 ppm of vitamin C (Mandey et al., 2020). The concentration of bioactive substances in African leaves depends on the leaves' location. According to Ogundele et al. (2022), old leaves have higher concentrations of bioactive substances than young leaves. They reported that the levels of bioactive compounds in old leaves were 1.621 g/100g of alkaloids, 0.642 g/100g of phenol, 0.064 g/100g of saponins, and 0.371 of g/100g flavonoids, while in young leaves, the levels were 1.473 g/100g of alkaloids, 0.397 g/100g of phenol, 0.072 g/100g of saponins, and 0.281 g/100g of flavonoids. Similarly, Nnaoma et al. (2022) reported that African leaves contain 0.381% of phenol, 1.273% of flavonoids, and 48.40 mg/100g of Vitamin C.

African leaves contain bioactive substances and potential nutrients for feed ingredients. Mandey et al. (2020) reported that African leaves (DM 90.87%) contain 23.45% of crude protein, 4.15% of fat, 9.73% of crude fibre, 14.77% of ash, 1.438% of Ca, 0.426% of P, and 4034.47 kcal/kg of gross energy. Similarly, Usunomena and Ngozi et al. (2016) found that African leaves contain 22.81% of crude protein, 18.17% of crude fibre, 4.34% of crude fat, and 16.65% of ash. Old African leaves contain 19.75 mg/kg of phosphorus, 96.25 mg/kg of potassium, 117.6 mg/kg of calcium, and 141.45 mg/kg of magnesium. Meanwhile, young leaves contain 21.85 mg/kg of phosphorus, 105 mg/kg of potassium, 113.15 mg/kg of calcium, and 134.65 mg/kg of magnesium (Ogundele et al., 2022).

The supplementation of African leaf meal in broiler chickens has been found to have varying effects on ration consumption, body weight gain, carcass weight, and feed conversion ratio. Nur et al. (2022) reported a significant reduction in ration consumption, body weight gain, and increased ration conversion. However, Mandey et al. (2021) found that African leaf meal supplementation increased carcass weight and decreased feed conversion ratio. Including African leaf juice in drinking water did not affect the cholesterol, uric acid, or blood glucose levels

of broiler chickens or their feed conversion ratio (Syamsuddin et al.2022; Damayanti et al. 2019), but it did increase the heart and liver weight of chickens (Japhet and Godgift, 2021) and an increase in feed consumption and body weight gain (Damayanti et al., 2019). Additionally, the use of African leaf extract at a concentration of 20 mL/L resulted in an increase in carcass weight and a reduction in cholesterol levels in the meat (Kismiati et al., 2023).

African leaves have not been utilized as an alternative feed supplement in laying quail, particularly concerning the haematology profile and leukocyte differential. Therefore, this study evaluated the effect of adding African leaf juice on quail blood's haematology and leukocyte differential.

MATERIALS AND METHODS

Animals

A total of 120 laying quail (*Coturnix coturnix japonica*) of 57-day-old were allocated to 15 50 cm x 60 cm x 20 cm, and each cage consisted of 8 female quails.

Feed and Treatments

The feed was commercial quail layer feed of layer period (> 6 weeks of age) with 92% dry matter content, 12.47% of ash, 22.8% of crude protein, 5.69% of crude fat, and 2.97% of crude fibre. Cages were equipped with feed and drinking water containers. Anti-stress vitamins were provided upon arrival of the quail birds. The treatments used were control treatments, including adding 3 ml/bird/day and 6 ml/bird/day in drinking water.

African Leaf Juice Preparation

African leaf juice was prepared using a modified version of the method described by Halimah et al. (2019). The leaves were collected from Cibuntu Village, Ciampea District, Bogor Regency, West Java Province. Four to five leaves from the top of the plant were used. The African leaves were cleaned with water, soaked for 24 hours, sliced into small pieces, and ground until smooth using a blender for 3 minutes. The leaves were ground twice by adding 100 ml of water at each grinding stage. The resulting juice was filtered and collected in a container. The juice was added to the quails' drinking water according to the treatment.

Birds Rearing

Feed and drinking water were provided at 25 kg/bird/day and 65 ml/bird/day, respectively. The drinking water was treated and replaced in the morning and afternoon. Feed consumption was measured weekly, and drinking water consumption was measured daily in the morning. The amount of feed provided and the remaining feed were weighed weekly to measure feed consumption. Daily drinking water consumption was measured by weighing the water provided and the remaining water.

Blood Sampling and Hematology Profile Analysis

At week 6th, blood samples were collected from 9 quails at the neck. The neck of each quail was cleaned with 70% alcohol before 1-2 ml of blood was taken and placed into a Heparin tube. The number of red blood cells (BDM) and white blood cells (BDP) were counted using a counting chamber with a 40x10 magnification microscope. The Hayem diluent assisted in calculating the number of BDM, and the Rees Ecker diluent assisted in calculating the number of BDP. Furthermore, the erythrocyte count (million cells mm^{-3}) was calculated using the formula:

$$\text{Erythrocytes (million cells mm}^{-3}\text{)} = 200 \times 50 \times \text{number of BDM grains}$$

$$\text{Leukocytes (thousand cells mm}^{-3}\text{)} = 20 \times \frac{10}{4} \times \text{number of BDP grains}$$

Haemoglobin levels were analyzed using the Sahli method with 0.1N HCl as the reagent, and hematocrit values were measured using the microhematocrit method. Leukocyte differentiation was performed by creating blood review preparations stained with May Grunwald-Giemsa and observed under a microscope at 100 x 100 magnification. The observed differentiation included monocytes, lymphocytes, heterophils, basophils, and eosinophils.

Design and Data Analysis

This study used a completely randomized design (CRD) with three treatments, each comprising five replicates. Data were analyzed using analysis of variance (ANOVA) followed by Duncan's test if the data were significantly different ($P<0.05$).

RESULTS AND DISCUSSION

Feed and Drinking Water Consumption

Adding African leaf juice to drinking water did not affect feed consumption (Table 1). The average feed consumption in this study was

25 g/bird/day. Andari et al. (2018) reported that feeding laying quails herbs containing flavonoids, tannins, steroids, and alkaloids resulted in a feed consumption range of 21-22 g/bird/day. Similarly, Ariqoh et al. (2019) reported that feed consumption ranged from 14-16 g/bird/day when using Pegagan extract and carrot waste containing triterpenoids, steroids, and saponins. The African leaf juice contains bioactive substances such as flavonoids (+++), saponins (+++), tannins (++), alkaloids (+), phenol hydroquinone (+), and steroids (+).

The bioactive compounds of African leaf extract, including saponins, flavonoids, tannins, alkaloids, and phenol dihydro and steroids, did not affect drinking water consumption despite the treated water's bitter green to intense green colour. These results are consistent with the findings of Suci et al. (2023) that African leaf extract did not affect drinking water consumption up to a dose of 6 ml/bird/day, but contradict the report of Suci et al. (2019) that the addition of 5% and 7% Kemuning leaf extract to drinking water tended to decrease consumption. Furthermore, Andari et al. (2018) reported that drinking water with herbal medicine tended to increase at 10 ml and 30 ml doses, with drinking water consumption ranging from 543-592 ml/bird/week.

The feed-to-drinking water ratio in this study was 2:1, considered normal as it did not cause any adverse effects, such as stress on the quails. When quails are under heat stress, they consume more drinking water than feed. Additionally, quail health can be assessed by observing physiological conditions, such as leukocyte differentiation. According to Maheshwari et al. (2017), quail experiencing heat stress can also be known through increased leukocyte differentiation, especially heterophils and lymphocytes.

Table 1 Drinking water and feed consumption of quails

Treatment	Drinking waterconsumption (ml/bird/day)	Feed consumption(g/bird/day)
P0 (control)	56.83±3.30	24.48±0.45
P1 (3 ml/bird/day)	57.45±6.59	24.78±0.21
P2 (6 ml/bird/day)	56.22±2.86	24.56±0.41

Quail Blood Profile

The blood profile values provided with African leaf juice in drinking water were still within the normal range according to Sturkie and Griminger (1976), with an average erythrocyte count of 3.86 million/mm^3, haemoglobin of 12.3 g%, hematocrit of 37%, leukocyte of 20 - 40 thousand/mm^3. According to Campbell and Ellis (2012), normal quail haemoglobin levels range from 10.7 to 14.3 g/dl, hematocrit from 30.0 to 45.1%, and leukocyte from 12.5 to 24.6 million/mm^3. Sobingin et al. (2019) reported that quail blood erythrocyte levels were 1.53 million mm^3, haemoglobin was 10.29 g/dl, and hematocrit was 31.63%. Agina et al. (2019) found that quail blood has a hematocrit range of 25.00-66% with an average of 43.11 ± 1.08% and haemoglobin levels ranging from 8.62-20.57 g/dl with an average of 16.21 ± 0.27 g/dl.

Erythrocytes, also known as red blood cells, transport haemoglobin and deliver oxygen from the lungs to all body tissues. Hemoglobin's primary function is to distribute oxygen throughout the body, while hematocrit measures the percentage of red blood cell volume in the blood. The results of this study indicate that supplementation with 3 ml and 6 ml/day/bird of African leaf juice did not affect erythrocytes, hematocrit, or leukocytes, except for haemoglobin levels (Table 2). These results contradict the report by Mahbuba et al. (2022), who stated that using African leaves up to 75 ml/L of drinking water had no significant effect on haemoglobin levels. Due to the content of phenolics, flavonoids and terpenoids, herbal plants can facilitate the process of erythropoietin, which stimulates the kidneys to increase the production of red blood cells and haemoglobin.

Additionally, they can boost the immune system by increasing the production of leukocytes (Mahbuba et al., 2022). Garmana et al. (2014) stated that antioxidant substances could preserve the integrity of erythrocyte cells by inhibiting the use of oxygen by bacteria, which prevents membrane damage due to free radicals and preserves erythrocytes. Haemoglobin levels are positively correlated with red blood cell levels. The decrease in haemoglobin is caused by a slight decrease in normal red blood cells and by tannin compounds in African leaves

(Usunomena et al. 2016; Mandey et al. 2020). Tannin compounds can interfere with protein digestibility, creating protein-tannin complexes that interfere with haemoglobin formation.

Leukocyte Differentiation

The addition of African leaf juice did not significantly affect the mean number of lymphocytes, heterophils, monocytes, eosinophils, and basophils (Table 2). Leukocyte differential values are highly dependent on leukocyte values. According to Maheshwari et al. (2017), the temperature significantly impacts the quail blood profile, particularly on heterophils, lymphocytes, monocytes, eosinophils, basophils, and leukocytes. The study did not observe any heat stress, as evidenced by the similar performance of leukocyte differentials across all variables.

Table 2 Blood profile of laying quails supplemented with African leaf juice in drinking water

Variables	Normal levels*	African leaf juice (ml/bird/day)		
	Adult Females	control	3	6
Erythrocytes (10^6 /mm^3)	3.31± 0.38	1.64±0.42	1.51±0.28	1.57±0.16
Haemoglobin (g%)	15.5 ± 0.57	11.33±0.12[a]	10.33±0.31[b]	10.67±0.12[b]
Hematocrit (%)	38.3 ± 1.69	37.00±1.73	40.67±3.51	39±0.01
Leukocytes (103/mm^3)	26.6 ± 854.7	17.67±9.50	19.00±12.03	19.67±79.73
Leukocyte Differentiation (%)				
Lymphocytes	25	64.67±9.71	66.0±10.15	66.00±10.5
Heterophiles	71.5	24.67±3.51	24.0±1.0	24.0±1.0
Monocytes	0.8	1.33±0.57	1.0±0.01	1.0±0.01
Eosinophils	0.8	7.00±1.00	7.0±1.0	7.0±1.15
Basophils	2.2	2.33±0.57	2.0±0.01	2.0±0.01

*Costachescu et al. (2009)

Lymphocytes are a crucial immune system component, responding to antigens by producing antibodies. The lymphocyte counts in this study were within the normal range of 64-66%. According to Santoso et al. (2019), quail lymphocyte levels range from 50 to 69% at a temperature of 35°C. Campbell and Ellis (2012) also state that the standard normal lymphocyte level ranges from 50 to 70%. Lymphocytes respond to antigens and stress by increasing antibody circulation during immune system development. The number of lymphocytes is primarily affected by heat or environmental stress, which reduces the weight of the thymus and the bursa of Fabricius, ultimately leading to a decrease in lymphocyte count. The lymphocyte count is primarily affected by heat or environmental stress, which reduces the weight of the thymus and the bursa of Fabricius, ultimately leading to a decrease in lymphocyte count.

The heterophyll level in this study (24%) was lower than the heterophyll value reported by Campbell and Ellis (2021), which ranged from 29% to 52%. Santosa et al. (2019) also reported that normal quail heterophyll levels range from 25% to 46%. In this study, both the control and treatment groups that received African leaf juice had similar heterophyll levels. Therefore, it can be concluded that all quails were not stressed. Maheshwari et al. (2017) found that temperature affects the levels of quail blood profile, specifically heterophils and lymphocytes. Additionally, the adequacy of feed nutrients was identified as a factor affecting heterophil levels (Maheshwari et al. 2017). Providing African leaf juice up to a dose of 6 ml/bird/day did not inhibit feed digestion and absorption, thus preventing nutrient deficiencies.

Eosinophils respond to allergens and eliminate harmful microorganisms while neutralizing inflammatory factors. The study found that all treatments and controls had eosinophil levels of 7%. High eosinophil production may indicate the effective functioning of the body's defence system against pathogens. The present study found a significantly higher eosinophil count compared to previous studies by Santoso et al. (2019) and Sturkie and Griminer (1976), which reported counts of 0.75-1.25% and 3%, respectively. In contrast, Aengwanich and

Chinrasri (2003) and Costachescu et al. (2009) reported counts of 1.8% and 0.8%, respectively.

Monocytes fight non-acute infections caused by foreign objects, such as bacteria entering the body (Anggraeni, 2015). The study found that the average number of monocytes obtained ranged from 1%-1.33%, within the normal range. It suggests the absence of bacteria or infection in quail, as large monocytes are not required. Previous studies reported that monocyte levels ranging from 0-4% (Campbell and Ellis, 2012), 1.5- 3.75% (Santoso et al. 2019), 0.8 % (Costachescu et al. 2009), 3.60% (Kabir, 2013), 1.33% (Sturkie and Griminer (1976) and 0.8% (Costachescu et al. 2009).

Eosinophils are a type of white blood cell that is produced in response to parasitic infections and allergic reactions. They act as a defence mechanism against toxins, and an increase in the number of eosinophils indicates the presence of an infection in the body. The average eosinophil value ranges from 7%-8%, which is higher than the eosinophil level of 0.5-1.5% (Santoso et al. 2019), 0.8% (Costachescu et al. 2009) and 3-8% (Swenson,1984)

Basophils release heparin, histamine, some bradykinin, and serotonin when the tissue is inflamed (Aulia et al. 2017). The basophil number in all treatments ranged from 2% to 2.33%. Previous studies reported that basophil levels were 0.25-0.5% (Santoso et al. 2019), 2.2% (Costachescu et al. 2009), and 0.2% (Sturkie and Griminer, 1976), and 1.79% (Aengwanich and Chinrasri, 2003)

CONCLUSIONS

Adding 3 ml/bird/day of African leaf juice in drinking water can be a feed additive without adverse haematology and blood leukocyte differential of quail.

REFERENCES

Andari, A., E. N. Nis, R. F. Wulandari dan D. M. Suci. 2018. Efek suplementasi jamu rempah pada puyuh (*Coturnix coturnix* Japonica) terhadap performa dan kadar kolesterol telur. Jurnal Ilmu Nutrisi dan Teknologi Pakan 16 (2) : 34- 41.

Aengwanich, W.. and O. Chinrasri. 2003. Effect of dexamethasone on differential white blood cell counts and heterophyl/Lymposit ratioa in Japanese Qual (*Coturinx coturnix* Japonica). Songklanakarin Journal of Science Tecnnology 25 (2): 184 – 189.

Agina, O. A., W. Ezema, and E. M. Iwuoha. 2017. The haematology and serum biochemistry profile of adult Japanese quail (*Coturnix coturnis* Japonica). Not Science Biology 9(1):67-72.

Anggraeni, N., A. Farajallah, and D. A. Astuti. 2016. Blood profile of quails (*Coturnic coturnix japonica*) fed ration containing silkworm pupae (*Bombyx mori*) powder extract. Media Peternakan 39 (1) : 1-8.

Aulia, R., Sugito, M. Hasan, T. F. Karmil and R. Gholib. 2017. The number of leukocyte and leukocyte differential in broilers that infected with Eimeria tenella and given neem leaf and jaloh extract. Jurnal Medika Veterinaria 11(2):93-99.

Ariqoh, H., S. Prayoga, B. S. Hermanto, dan W. Hermana.2019. Suplementasi jus daun pegagan dan limbah wortel terhadap produktivitas puyuh jantan (*Coturnix coturnix* Japonica). Jurnal Ilmu Nutrisi dan Teknologi Pakan 17(2):54-58.

Campbell, T. W. and C. K. Ellis. 2013. Avian and exotic animal hematology and cytology. John Wiley & Sons.

Costachescu, D. F. I., P. C. Boisteanu, and R. Lazar. 2009. Characteristics of the hematological profile in the meat preparation. Scientific Paper-Animal Science Series: Lucrari-Seria Zootehnie 70 : 155-160.

Damayanti, P., Mihrani, dan M. Y. Surung. 2019. Pemanfaatan ekstrak daun Afrika *(Vernonia amygdalina*) terhadap performa broiler. Jurnal Agrisistem 15(1): 23-28.

Garmana, A. N., E. Y. Sukandara, and I. Fidriannya. 2014. Activity of several plant extracts against drug-sensitive and drug-resistant microbes. Procedia Chemistry 13:164- 169.

Halimah, H., D. M. Suci, dan I. Wijayanti. 2019. Studi penggunaan daun mengkudu (*Morinda citrifolia*) sebagai bahan antibakteri *Escherichia coli* dan *Salmonella typhimurium*. Jurnal Ilmu Peternakan Indonesia 24(1): 58-64.

Japhet, Y. B. and T. Godgift. 2021. Effect of *Vernonia amygdalina* leaf extract on

growth performance, carcass characteristics of pullet and broiler birds. International Journal of Science and Research Archive 3(2): 209-213.

Kabir, A. 2013. Blood chemistry analyses of Japanese quail (*Coturnix coturnix* Japonica). Scholarly Journal of Agricultural Science 3(4): 132-136

Kismiati, S., T. A. Sarjana, L. D. Mahfudz, and T. Prayitno. 2023. African leaf (*Vernonia amygdalina*) extracts Japanese Quail (*Coturnix coturnix* Japonica) carcass traits. Veterinary Word 16(4):773-778.

Mandey, J. S., M. Sompie, and C. J. Pontoh. 2020. Potensi nutrien dan bioaktif daun Afrika (*Vernonia amygdalina*) sebagai kandidat bahan pakan dan additive natural pada ayam broiler. Prosiding Seminar Nasional Biodiversity Indonesia 6(1): 507-511.

Mandey, J. S., M. Sompie, and F. R. Wolayan. 2021. Growth assessment of broiler chicken given bitter leaves (*Vernonia amydalina*) as phyto-additive, potential antimicrobial agents of lipid and amimo acid. Advances in Biological Sciences Research 18 : 21-25.

Mahbuba, A. M., A. A. B. Jabbar, and N. A. Mustafa. 2022. The effectiveness of some medicinal plants on body performance, hematological, ileum morphology and immune statue of Japanese quail. Iraqi Journal of Agricultural Sciences 53(4): 724-731.

Nnaoma, I. E., A. Obinna, O. Chizoba, J. Rich, A. Chisom, and N. Cecil. 2022. Phenolic contet, flavonoid content and antioksidant properties of *Vernonia amydalina* butanol leaf extract (Bitter Leaf). International Journal of Pharmaceutical Science and Research 7(2):1-5.

Nur, S. N., L. O. Nafiu, dan R. Badaruddin. 2022. Performa produksi ayam broiler yang diberi tambahan pakan tepung daun afrika (*Vernonia amydalina*). Jurnal Ilmiah Peternakan Halu Oleo 4(3): 225-230.

Suci, D. M., N. U. Nuha, dan Suryahadi. 2019. Pemberian ekstrak daun kemuning (*Murraya paniculata* (L.) Jack) dalam air minum terhadap performa dan kualitas fisik telur puyuh malon. Jurnal Ilmu Nutrisi dan Teknologi Pakan 17 (3): 73-77.

Suci, D. M., W. Hermana, D. Maik, dan I. Hasbullah. 2023. Profil asam lemak kuning telur puyuh Jepang yang disuplementasi jus daun afrika (*Vernonia amygdalina* Del) dalam air minum. Jurnal Ilmu Nutrisi dan Teknologi Pakan 21(1): 29-34.

Ogundele, O. D., O. D. Ajobiewe, and M. Z. Ojo. 2022. Phytochemicals and mineral elemant compositions of bitter-leaf (*Vernonia amygdalina*) young and matured leaves. International Journal of Interdisciplinary Invention & Inovation in Research 1(1):1-10.

Santoso, K., F. B. Harlimawan, A. Wijaya, Isdoni, H. Maheswari, D. R. Ekastuti, P. Achmadi, R. Tarigan, A. S. Satyaningtijas, A. S. Suparyogi dan W. Wanalu. 2022. Profil leukosit burung puyuh yang mengalami cekaman panas setelah pemberian asprin. Jurnal Peternakan Indonesia 24(2): 180-189.

Sobingin, A., Rinawidiastuti, dan F. Iskandar. 2019. Pengaruh pemberian sari jahe gajah (*Zingiber officinale* Rosc) pada air minum terhadap profil darah buruh puyuh (*Coturnix coturnix* Japonica). Jurnal Riset Agribisnis dan Peternakan 4(1) : 52-59.

Syamsuddin, D. Zulkarnain, Nasriati, R. Badaruddin, dan A. Indi. 2022. Pemberian jus Vernonia amygdlina terhadap kolesterol, asam urat, dan glukosa darah ayam broiler. Journal of Tropical Animal Science and Technology 4 (2): 103-109.

Sturkie, P. D. dan P. Griminger. 1976. Blood: physical characteristics, formed elements, hemoglobin and coagulation. 3rd ed. Springer, Verlag.

Tunsaringkarn, T., W. Tungjaroenchai, dan W. Siriwong. 2013. Nutrient benefits of quail (*Coturnix coturnix* Japonica) eggs. International Journal of Scientific and Research Publications 3(5): 1-8.

Usunomena, U. and O. P. Ngozi. 2016. Phytochemical analysis and proximate composition of *Vernonia amygdalina*. International Journal of Scientific World 4(1): 11-14.