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Short Communication

Turmeric Flour Supplementation Modulates Blood Profile in Murrah Buffalo

Salam N. Aritonang[1*], Yulia Yellita[1], Dwita Saningtias[2], Eka Putr[12], Rizqan[1]

[1]Department Animal Production and Technology, Faculty of Animal Science, Andalas University Padang, West Sumatera Indonesia; [2]Faculty of Animal Science Student, Andalas University Padang, West Sumatera Indonesia.

Abstract | Turmeric flour is rich in antioxidants and natural antibiotic compounds that support buffalo health. It is predicted that turmeric flour supplements will enhance erythrocytes, hematocrit, hemoglobin, and leukocytes, as observed in the health of female Murrah buffaloes. Parameters measured in this study included hematology (erythrocytes, hemoglobin, hematocrit, and leukocytes) in Murrah buffaloes. The study involved four lactating Murrah buffaloes aged 3 to 5 years, following a Latin Square Design consisting of four treatments: A (0% turmeric flour), B (0.015% turmeric flour), C (0.03% turmeric flour), and D (0.045% turmeric flour), added to the basal feed comprising field grass, king grass, and 2 kg of withered cassava leaf. The results showed that turmeric flour supplementation significantly increased (P<0.05) erythrocytes (4.8 - 6.51 x 10^6/mm^3), hemoglobin (9.85 - 13.22 g/dl), hematocrit (25.82 - 33.32%), and leukocytes (8.55 - 11.17 x 10^3/mm^3), while maintaining values within normal ranges. Based on the results, supplementation of turmeric flour up to 0.03% (C) was found to be the most optimal for blood profile improvement and can enhance the health of Murrah buffaloes.

Keywords | Murrah buffalo, Turmeric flour, Blood profile, Supplement, Indonesia

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***Correspondence** | Salam N Aritonang, Department Animal Production and Technology, Faculty of Animal Science, Andalas University Padang, West Sumatera Indonesia; **Email:** snaritonang@ansci.unand.ac.id
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INTRODUCTION

Buffalo is a potential animal to develop since it has high economic value. *Bubalus bubalis* is one of the buffalo species to improve buffalo genetics in Indonesia. Buffalo produces meat, milk, and leather. The Indonesian Directorate General of Veterinary and Statistical Central Bureau documented buffalo population in Indonesia in 2020 was around 1.154.226 heads, and in 2021 around 1.189.260 heads.

There are two types of buffalo in Indonesia which are swamp buffalo and river buffalo. River buffalo are only located in North Sumatra, while swamp buffalo spread almost in entire Indonesia. Swamp buffalo generally care for meat and work force; while river buffalo is plotted as a dairy producer. Buffalo milk has higher fat and protein content around 6-8% and 4-8% respectively than cow's milk (Mihaiu et al., 2011; Roza et al., 2015). River buffalo produce 6-8 L/head/day while swamp buffalo 1.5-2.0 L/head/day. Murrah buffalo's average milk production rate is 1.764 kg/294 lactation days (Halgberg and Lind, 2003) that has the potential to be expanded.

The blood profile condition describes the health status of the body, represented by erythrocytes, hemoglobin, hematocrit, and leukocytes. Mammals' blood volume constitutes 7-8% of body weight. Blood plasma consists of erythrocytes, leukocytes, and platelets (Ganong, 2003). Erythrocytes play a crucial role in oxygen transportation by binding to hemoglobin (Roland, 2014). Normally, buffalo have an erythrocyte level of 7.8±0.38 x10^6/mm^3 (Willis, 2010).

Formation and disruption of erythrocytes led to variations in hemoglobin levels, however, it also significantly affected by the size and number of erythrocytes (Wardhana, 2001). Hemoglobin consists of protein complex and iron. The complexity has a red color inside of the erythrocyte. Without hemoglobin, the blood only carries oxygen for 1 ml/100 ml blood (Isnaeni, 2006). Typically, the hemoglobin level in buffalo is around 12.10±1.36 g/dl. Hematocrit, also known as packed cell volume (PCV), represents the percentage of blood plasma to total blood volume, including erythrocytes and hemoglobin. Erythrocytes, hematocrit, and hemoglobin change in tandem due to physiological interactions (Meyer, 2004). The normal hematocrit level in buffalo is 39.80±3.79%. Leukocytes are formed in the vertebrae, with some originating in lymphatic tissues before being transported throughout the body to actively participate in the body's defense system. Leukocytes serve a dual function: they destroy attackers through phagocytosis and participate in antibody formation. The normal level of leukocytes in buffalo is around $10.7x10^3/mm^3$ (Willis, 2010). To maintain normal blood conditions indicative of good health, feed additives such as turmeric supplements are necessary.

Turmeric (*Curcumin longa*) is a rhizome plant used as antibiotic, antivirus, and antioxidants (Hartati, 2013). Furthermore, turmeric healed digestives tract (Shan and Iskandar, 2018) contained demetoxycurcumin, bisdemetxykurkumin, and essential oil (2.5 - 6 %) (Li et al., 2011). Curcumin in turmeric has an antioxidant activity to protect hemoglobin from oxidation (Fachrurozi et al., 2013). Curcumin is a major antioxidant that has a role in cutting off radicalization and has an immunomodulatory effect on optimizing animal health. Hence, it triggers the gall bladder to produce bile fluid and then improves appetizing (Mulani, 2015).

Supplementation of turmeric flour up to 1 % to Ongole crossbreeds improves body weight and feed efficiency (Wati and Miki, 2020). An extra turmeric flour for 0.03% of total body weight in-vitro improves propionate to 4.24 mMol/l (Ramandhani et al., 2017). Bali cow with an extra 1.5 % turmeric flour improves body weight by 38.24% and FCR by 47.20% (Ni Luh Gede et al., 2020). Furthermore, Satyaningtijas et al. (2010) explored a feed supplement single herbal (Turmeric or garlic) combined with zinc to improve erythrocyte, hematocrit, and hemoglobin levels in the avian. Napirah (2013) showed erythrocyte, hematocrit (PCV), and hemoglobin levels to quail in normal conditions, indicating that turmeric is not a toxic ingredient. This study aimed to show that giving turmeric flour can improve hematology and health levels in Murrah buffaloes.

MATERIALS AND METHODS

Location

This research was carried out at the Murrah buffalo farm in Nagari Kapau, Agam Regency, West Sumatra, Indonesia, and implemented from June 2023 to September 2023.

Experimental Design

This study used four lactating Murrah buffaloes aged 3-5 years, weighing 350-400kg, originating from North Sumatra and reared in Tilatang Kamang. The primary feed to support this study was field grass and king grass with cassava leaves as much as 2 kg/head/day. The method used in this study was a Latin Square Design consisting of four treatments and four replicates, namely the addition of turmeric flour obtained from local farmers where the research location area is one of the agricultural centers in West Sumatra, Indonesia. The treatments given in the study, namely:

A = Basal feed (field grass, king grass, cassava leaf) + 0% turmeric flour (control)
B = Basal feed (field grass, king grass, and 2 kg withered cassava leaf) + 0.015% turmeric flour
C = Basal feed (field grass, king grass, and 2 kg withered cassava leaf) + 0.03% turmeric flour
D = Basal feed (field grass, king grass, and 2 kg withered cassava leaf) + 0.045% turmeric flour
Turmeric flour was supplemented concurrently with 100 grams of diluted palm sugar in 250 ml of water. The duration of turmeric flour supplementation in this study lasted for 68 days.

Parameter Studied

The parameters observed included blood profiles such as erythrocytes, hemoglobin, hematocrit, and leukocytes, which were analyzed using a hematology analyzer (Auto Hematology Mindray BC-3200). Blood was collected from the jugular vein using a 5 ml syringe, with 3 ml drawn and then stored in a vacutainer EDTA K3 tube for homogenization before being transferred to the hematology analyzer for analysis, which took approximately 2 minutes to obtain results.

Data Analysis

Data were analyzed by Minitab 14 through Analysis of Variance (ANOVA). The blood profile data were subjected to Multivariate Analysis of Variance (MANOVA) and further analyzed using the Duncan's New Multiple Range Test (DNMRT) if significance was indicated.

RESULT AND DISCUSSIONS

Murrah buffalo's blood profile after being supplemented by

turmeric flour in this study was described in Table 1.

Table 1: The blood profile of Murrah buffalo

Parameter	A	B	C	D
Erythrocytes (10^6/mm^3)	4.8^a	5.63^b	6.33^{bc}	6.51^c
Hemoglobin (g/dl)	9.85^a	10.95^a	12.57^b	13.22^b
Hematocrit (%)	25.82^a	27.47^a	31.56^{ab}	33.32^b
Leukocytes (10^3/mm^3)	8.55^a	10.42^b	10.61^b	11,17^b

Noted: The different letter in the same row indicates significant differences (P<0.05)
A = Basal feed (field grass, king grass, cassava leaf) + 0% turmeric flour (control)
B = Basal feed (field grass, king grass, and 2 kg withered cassava leaf) + 0.015% turmeric flour
C = Basal feed (field grass, king grass, and 2 kg withered cassava leaf) + 0.03% turmeric flour
D = Basal feed (field grass, king grass, and 2 kg withered cassava leaf) + 0.045% turmeric flour

Erythrocytes

Table 1 illustrates the erythrocyte count following turmeric flour supplementation, with 0.045% (Treatment D) demonstrating the highest level. However, Treatment D does not show a significant difference compared to Treatments B and C, while the lowest erythrocyte level is observed in Treatment A. This indicates that an increase in turmeric flour supplementation correlates with an elevation in the erythrocyte count of Murrah buffalo. The presence of curcumin, a component of turmeric, is responsible for improving erythrocyte levels. Curcumin serves as an antioxidant, mitigating the effects of free radicals. If left unchecked, free radicals can induce reactive oxidative stress, potentially damaging the erythrocyte cell membrane and leading to lipid peroxidation. Curcumin's antioxidant properties play a crucial role in preventing hemoglobin oxidation and erythrocyte cell lysis due to its phenolic OH structure (Venkatesan, 2003). Through turmeric supplementation, erythrocyte formation remains undisturbed, resulting in an increase in erythrocyte count while maintaining standard levels.

The highest level of erythrocytes in Murrah buffalo was observed in Treatment D (0.045%), attributed to curcumin, a bioactive compound in turmeric. Curcumin acts by reducing free radicals, thus preventing damage to erythrocyte cells while not disrupting erythrocyte formation. Consequently, the erythrocyte count in Treatment D was the highest, although it did not significantly differ from Treatment C. The non-significant difference between Treatments D and C can be attributed to the fact that at the 0.03% level, curcumin in the turmeric supplement reaches its maximum efficacy in reducing free radicals and preventing erythrocyte cell damage and formation disturbance. Therefore, when turmeric flour supplementation was increased to 0.045% in Treatment D, the erythrocyte count in Murrah buffalo was not significantly different from that in Treatment C.

The erythrocyte count in Treatment A was the lowest due to the absence of antioxidants entering the body of Murrah buffalo. Consequently, erythrocyte cells underwent lysis due to free radicals, leading to a reduction in erythrocyte count in Treatment A. The erythrocyte level observed in this study ranged from 4.8 to 6.51 x 10^6/mm^3, which is below the standard level of 7.8±0.38 x 10^6 /mm^3 (Wills et al., 2010). In a related study, Murrah buffalo supplemented with another source of antioxidants, such as cassava leaves, exhibited an erythrocyte count of around 5.52 x 10^6/mm^3 (Saningtyas, 2022). Furthermore, the study highlights the use of turmeric feed supplementation to maintain buffalo health.

Hemoglobin

Table 1 displays the hemoglobin levels of Murrah buffalo supplemented with turmeric at 0.045% (Treatment D), which is significantly (P<0.05) higher, although not significantly different from Treatment C. Treatment A does not significantly differ from Treatment B. The trend suggests that turmeric flour improves hemoglobin levels in Murrah buffalo. The highest hemoglobin level observed in Murrah buffalo in Treatment D is attributed to the effect of curcumin, a component of turmeric, which exhibits antioxidant activity, protecting erythrocyte cell membranes from free radicals. As a consequence, hemoglobin levels increase, as demonstrated in this study. Fahrurrozi et al. (2014) suggest that curcumin defends hemoglobin from oxidation. Turmeric also contains flavonoids that inhibit enzyme activities such as cytochrome and cyclooxygenase, responsible for the passage of lipophilic polyphenols through the erythrocyte plasma membrane and the oxygenation of several hemes in the cytoplasm, thereby preventing erythrocyte hemolysis. The increase in erythrocyte levels is followed by an increase in hemoglobin levels. Kitagawa (2004) supports the notion that flavonoids and morin have the ability to increase hemoglobin levels.

In addition to curcumin and flavonoids, turmeric contains Vitamin C, which supports iron absorption. Iron substances are absorbed through electrophoresis processes to become hemoglobin in the spinal cord, transforming into hemoglobin. As demonstrated in this study, supplementation with turmeric flour at 0.045% improves hemoglobin levels, which remain within the normal range. This aligns with Patricia et al. (2013), who argue that vitamin C accelerates iron mineral absorption from intestinal mucus, facilitating its transfer from blood vessels to the spinal cord for hemoglobin production.

Hemoglobin levels are closely related to erythrocytes. Ganong (2003) states that hemoglobin is influenced by oxygen, and as erythrocyte levels tend to increase, hemoglobin levels rise, triggering the production of erythrocytes and hemoglobin improvement. In this study, supplementation with 0.045% turmeric flour in Treatment D increased Murrah buffalo erythrocyte levels, followed by hemoglobin levels, albeit not significantly different from Treatment C. The lowest hemoglobin level observed in this study belongs to Treatment A due to the absence of turmeric flour supplementation. Consequently, Treatment A, lacking additional curcumin, flavonoids, and vitamin C, does not protect erythrocyte cell membranes from free radicals. Moreover, the absence of heme guardian ions and degradation from heme contribute to membrane breakage, hindering iron ion acceleration.

The improvement in hemoglobin levels in Murrah buffalo observed in this research remains within the normal range, which is 9.8 - 13.22 (g/dl). Similar conditions were reported by Riyan (2016) with values of 9.67–13.82 (g/dl) when Swamp buffalo were fed cassava leaf, *Gliricidia sepium* leaf, and *Sauropus androgynous* leaf. Cassava leaf contains antioxidants such as flavonoids and phenolics, which protect erythrocyte cells (Faezah et al., 2013). *Gliricidia sepium* leaf and *Sauropus androgynous* also exhibit antioxidant effects (Roza et al., 2015; Hayati et al., 2016).

Hematocrit

Table 1 illustrates the hematocrit levels of Murrah buffalo supplemented with turmeric flour at 0.045% in Treatment D, which is significantly higher ($P<0.05$), although not significantly different from Treatment C. The percentage of hematocrit in Murrah buffalo in Treatment A is the lowest and statistically similar to Treatment B, indicating that turmeric flour supplementation increases hematocrit levels in Murrah buffalo.

The highest hematocrit level in Murrah buffalo in Treatment D (0.045% turmeric flour supplementation) is attributed to the role of turmeric flour as an antioxidant, supporting the digestive process by reducing the protozoa population in the rumen (Li et al., 2011). This reduction in protozoa and improvement in bacteria population enhances feed conversion efficiency (Bhandary et al., 2021), leading to improved digestive system function and increased nutrient absorption. Additionally, Vitamin C in turmeric flour supports iron absorption to produce erythrocytes and hematocrit. Sloane (2003) emphasizes that nutrition plays a crucial role in erythrocyte production to support normal growth.

Flavonoids in turmeric flour also contribute to improving erythrocyte levels. As antioxidants, flavonoids accelerate erythropoiesis (the erythrocyte formation process), which consists of tetrameric protein to release oxygen in hemoglobin to tissue cells. As a result, erythrocyte and hemoglobin levels rise (Zacaria and Ampode 2021). This study demonstrates that turmeric flour supplementation up to 0.045% in Treatment D increases Murrah buffalo hematocrit levels to the highest level, although not significantly different from Treatment C.

The hematocrit level in Murrah buffalo in Treatment A is the lowest in the absence of turmeric flour supplementation. Consequently, the cell membrane undergoes lysis due to increased free radicals and protozoa population, resulting in decreased nutrient absorption. As mentioned, erythrocytes are related to hematocrit, with hematocrit representing the percentage of erythrocytes in 100 ml of blood (Rohman et al., 2020). Hematocrit levels in this study remain within the normal range, approximately 25.82% - 33.32%. As Wills (2010) noted, the normal hematocrit level in buffalo is 39.80 ± 3.79%. In this study, the hematocrit level in Murrah buffalo is comparable to that reported by Riyan (2016) at 31.3%.

Leucocytes

Table 1 indicates that the leukocyte level of Murrah buffalo in Treatment D is the highest, although not significantly different from Treatments C and B. The lowest leukocyte level is observed in Treatment A. This phenomenon, demonstrated by turmeric flour, increases the leukocyte level of Murrah buffalo while still within standard values. The leukocyte levels in Murrah buffalo between Treatments D, C, and B did not show significant differences, but all three are higher than in Treatment A. This is attributed to curcumin, a component of turmeric flour, which is rich in antioxidants that stimulate the immune system by enhancing chemotaxis from lymphocytes, promoting lymphocyte adhesion, and activating T and B cells (Nuryanti et al., 2022). B lymphocytes produce antibodies (humoral immune response), while T lymphocytes play a role in cellular responses. Turmeric can not only modulate the function of lymphocytes but also have capability to act as antibiotic growth promoter (Lagua and Ampode, 2021). Consequently, leukocyte levels increase. This study demonstrates that turmeric flour supplementation at a level of 0.045% in Treatment D increased leukocyte levels to the highest point, although not significantly different from Treatments C and B, remaining within the standard levels around 10.7 x 10^3/mm^3 (Wills, 2010). The leukocyte level of Murrah buffalo in this study remains within the normal range, 8.55–11.1 x 10^3/mm^3. Saningtias (2022) reported a lower level, around 7.86 x 10^3/mm^3, compared to this research. The study confirms that turmeric flour has maintained animal health by improving leukocyte levels within normal ranges.

CONCLUSION

Turmeric flour supplementation in Murrah buffalo improves erythrocytes, hemoglobin, hematocrit, and leucocytes at a normal level. Turmeric flour supplementation up to 0.03% was the optimum level for better blood profile of Murrah buffalo.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest

NOVELTY STATEMENT

The novelty of this study is that the supplementation of turmeric flour as much as 0.03% in Murrah buffalo feed can increase erythrocytes, hemoglobin, hematocrit, and leukocytes at average levels so that it can be made as a feed supplement for Murrah buffalo.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the manuscript.

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