Jurnal Nukleus Peternakan
pISSN : 2355-9942, eISSN:2656-792X
Terakreditasi Nasional, Dirjen Pendidikan Tinggi, Riset, dan Teknologi
Kementerian Pendidikan Tinggi, Sains dan Teknologi RI
S.K. No. 10/C/C3/DT.05.00/2025

Juni 2025, Vol. 12 No. 1: 89 – 96
Received: 18 November 2024, Accepted: 14 Juni 2025
Published online: 7 Agustus 2025
https://doi.org/10.35508/nukleus.v12i1.19530
https://ejurnal.undana.ac.id/index.php/nukleus/article/view/19530

EFFECTS OF WATER RESTRICTION AND EXERCISE ON HEMATOLOGICAL PARAMETERS IN BALI CALVES (*Bos javanicus*) FED A COMPLETE *Clitoria ternatea*-BASED DIET

(Pengaruh Pembatasan Air Minum dan Exercise Terhadap Parameter Hematologis Pada Pedet Sapi Bali (Bos javanicus) yang Diberi Pakan Komplit Berbasis Clitoria ternatea)

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ABSTRACT

This study aimed to evaluate the effects of water restriction and exercise on hematological parameters in Bali calves (*Bos javanicus*) fed a complete diet based on *Clitoria ternatea*. Four post-weaning Bali calves were subjected to four treatments: T0: control (*ad libitum* water and feed), T1: water restriction (50% of daily water intake), T2: exercise (30 minutes 2 km daily), and T3: a combination of water restriction and exercise. The experimental design followed a Latin Square arrangement with four treatments and four periods as replication. Blood samples were collected at the end of each period to assess erythrocyte, leukocyte, hemoglobin, and haematocrite concentrations. The results showed no significant effects (P>0.05) of water restriction or exercise on any of the measured hematological parameters. Erythrocyte, leukocyte, hemoglobin, and haematocrite levels remained stable across all treatments, with haematocrite values ranging from 34.23% to 35.58%. These findings suggest that feeding Bali calves a nutritionally balanced diet containing *Clitoria ternatea* can help mitigate the physiological impact of environmental stressors such as water restriction and exercise. The results highlight the resilience of Bali cattle in maintaining hematological health under challenging conditions, indicating their suitability for eco-farming systems in arid and water-limited regions. Further research is recommended to explore additional physiological responses and long-term effects on animal productivity.

Keywords: Bali cattle, Clitoria ternatea, water restriction, exercise, hematological parameters, environmental stress

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi pengaruh pembatasan air minum dan exercise terhadap parameter hematologis pada pedet Sapi Bali (*Bos javanicus*) yang diberi pakan komplit berbasis *Clitoria ternatea*. Empat ekor pedet Sapi Bali pasca-sapih diberi empat perlakuan: kontrol (air minum dan pakan ad libitum), pembatasan air minum (50% dari kebutuhan air harian), exercise (2 km per hari), serta kombinasi pembatasan air minum dan exercise. Desain percobaan menggunakan Rancangan Bujur Sangkar Latin dengan empat periode pengumpulan data. Sampel darah diambil pada akhir setiap periode untuk mengukur konsentrasi eritrosit, leukosit, hemoglobin, dan hematokrit. Hasil penelitian menunjukkan tidak ada pengaruh signifikan (P>0,05) dari pembatasan air minum dan exercise terhadap parameter hematologis yang diukur. Konsentrasi eritrosit, leukosit, hemoglobin, dan hematokrit tetap stabil pada semua perlakuan, dengan nilai hematokrit berkisar antara 34,23% hingga 35,58%. Pakan berkualitas tinggi yang kaya protein dari *Clitoria ternatea* diduga berperan penting dalam membantu pedet Sapi Bali mengatasi stres yang disebabkan oleh pembatasan air dan exercise. Temuan ini menunjukkan bahwa pemberian pakan yang seimbang secara nutrisi dapat membantu menekan dampak fisiologis dari stres lingkungan seperti pembatasan air minum dan exercise pada pedet Sapi Bali. Hasil ini menyoroti kemampuan adaptasi Sapi Bali dalam menjaga kesehatan hematologis di bawah kondisi yang menantang, yang mengindikasikan kesesuaian mereka untuk sistem peternakan ramah lingkungan di wilayah kering dan terbatas air. Penelitian lebih lanjut direkomendasikan untuk mengeksplorasi respons fisiologis lainnya serta dampak jangka panjang terhadap produktivitas ternak.

Kata-kata kunci: Sapi Bali, Clitoria ternatea, pembatasan air, exercise, parameter hematologis, cekaman lingkungan

INTRODUCTION

Bali cattle, when grazed on natural pastures during the dry season, are often exposed to various environmental stressors that significantly reduce their productivity. The extended dry season results in severe limitations in both the quantity and quality of available feed. Additionally, access to drinking water near grazing areas is often inadequate, forcing calves and their mothers to travel long distances in search of water sources, such as lakes or rivers. This condition leads to high calf mortality rates, historically reported to range between 30% and 40% (Baco *et al*., 2019; Krova *et al*., 2020). These findings underscore the critical impact of environmental stressors on livestock survival and productivity.

Although there is limited data on the direct impact of water scarcity on Bali cattle productivity, several studies have demonstrated how water restriction affects physiological responses in other ruminants (Ahlberg *et al*., 2019). For instance, Al-Ramamneh *et al*. (2011) reported that sheep and goats subjected to water restriction for 24 hours per day or 48 hours over two days did not show significant differences in feed and water intake. However, sheep exhibited higher rectal temperatures and increased respiratory rates compared to goats, suggesting that sheep may be more sensitive to water scarcity. Similar findings were reported by Gupta *et al*. (2016), who noted that sheep subjected to a 40% reduction in water intake showed significant physiological changes, including elevated respiratory rates and rectal temperatures, indicating substantial stress. Kasa *et al*. (1995) further demonstrated that exercise in heat-stressed goats led to marked stress responses, with male goats showing significantly higher respiratory rates, rectal temperatures, and skin temperatures than females.

Efforts to reduce high calf mortality have focused on improving nutritional supplementation. Concentrate-based feed supplements have been reported to successfully lower calf mortality rates from 30% to as low as 1% (Copland *et al*., 2011). However, the high cost of these feeds limits their widespread use among farmers. As a sustainable alternative, the use of legumes like *Clitoria ternatea* (butterfly pea) as a base for complete feed has been proposed. *C. ternatea* can be produced using intercropping systems with maize, which not only improves soil fertility and maize yields but also provides high-quality cattle feed. The crude protein content of *C. ternatea* is approximately 21.5%, while its crude fiber content is around 29%, making it a highly nutritious feed option (Hartati *et al*., 2019). Recent studies have reinforced the role of legume-based feeds in enhancing livestock health and productivity in resource-limited environments (Mayberry *et al*., 2019).

Despite the susceptibility of Bali calves to environmental stress, providing high-quality feed may alleviate some of the adverse effects caused by water scarcity and physical exertion. However, no comprehensive studies have yet examined the combined effects of these environmental stressors on the physiological responses of Bali calves. Therefore, this study aims to evaluate the impact of water restriction and exercise on key physiological parameters such as hemoglobin levels, erythrocyte counts, leukocyte counts, and haematocrit levels in Bali calves fed a *Clitoria ternatea*-based complete diet.

EXPERIMENTAL METHOD

Animal Selection and Management

Four weaned Bali calves, aged 6 to 8 months, were selected for this study. The animals were clinically healthy and had an average initial body weight typical for their age range. Upon arrival, the calves were placed in individual pens for a 2-week acclimatization period, during which they were fed a standard diet based on *Clitoria ternatea* and natural grass hay to adapt them to the experimental conditions. Each calf was housed in an individual pen measuring 1.20 m x 2.10 m, which allowed for isolation and specific feeding regimes. The pens were constructed with cement floors and zinc roofs to protect the animals from environmental conditions. Feed and water

troughs were provided for each animal to allow the accurate measurement of feed and water intake.

Feed Preparation and Distribution

The preparation and distribution of feed were carried out meticulously to ensure that the cattle received a balanced and nutritious diet. The concentrate consisted of rice bran (8%), corn meal (10%), fish meal (6%), pollard (5%), and minerals (1%). The ingredients were measured and mixed based on the protein level required in the diet to meet the nutritional needs of the animals. Once the ingredients were measured, they were thoroughly homogenized to ensure an even distribution of nutrients throughout the feed. This mixing process ensured that each animal received a consistent diet when the feed was distributed.

In addition to the concentrate, natural grass hay was also prepared as part of the basal diets. The grass was harvested and chopped into small pieces, approximately 1 to 3 cm in length, using a chopper machine. After chopping, the grass was spread out under the sun to dry, a process that reduced its moisture content to around 20-30%. The hay was then packed into sacks and stored in a cool, dry area until it was ready to be fed to the cattle. A similar preparation process was undertaken for *Clitoria ternatea*.

Experimental Design

The study employed a Latin Square Design (LSD) with 4 treatments and 4 periods, each serving as a replicate. This design was chosen to account for variation between animals and to ensure that each calf experienced each treatment over the course of the experiment.

The four treatments were:

T0: Control group.

T1: Water restriction (60% of the *ad libitum* intake) to simulate water scarcity.

T2: Exercise (30 minutes daily walking) to simulate physical exertion similar to that experienced when walking to water sources or grazing areas.

T3: Water restriction (60% of *ad libitum* intake) and exercise (30 minutes daily walking).

Each treatment period lasted for 12 days, followed by a 3-day washout period, during which all calves were returned to the control diet (*ad libitum* feed and water) to minimize carryover effects between treatments. The total study period, including acclimatization, data collection, and washout phases, lasted 14 weeks.

Exercise Protocol

Before the start of the experimental period, the cattle underwent a 2-week acclimatization phase to adjust to the exercise treatment. During this time, the animals were gradually introduced to walking routines to ensure they could handle the daily exercise sessions without experiencing excessive stress or fatigue. The acclimatization process helped the animals become familiar with the walking distance, pace, and environmental conditions of the designated walking area. The exercise intensity was gradually increased, starting with shorter distances and slower walking speeds, which were progressively adjusted to reach the targeted exercise level by the end of the acclimatization period. Once the cattle were fully acclimatized, the exercise protocol was implemented during the experimental period. The exercise involved walking the cattle within a prepared and controlled area at the research site. The walking sessions took place twice a day for 30 minutes each.

The total daily walking distance for each animal was approximately 2 km, split between the morning and afternoon sessions. The cattle were led at a moderate pace on flat terrain to simulate the typical physical exertion they would experience when walking to graze or reach water sources in natural grazing conditions. The walking area was designed to allow uninterrupted movement, with minimal distractions and obstacles to maintain a steady pace.

Parameters and Sampling Procedure

The following were variables measured in this experiment. Those included:

- **Hemoglobin (Hb)**: Measured in g/dL using a spectrophotometer.
- **Erythrocyte Count**: Expressed as the number of cells per microliter of blood using a hemocytometer.
- **Leukocyte Count**: Measured as the number of white blood cells per microliter of blood.
- **Haematocrit (Ht)**: Expressed as a percentage of red blood cells in total blood volume, measured using a microhaematocrit centrifuge.

Blood samples were systematically collected from each animal at the conclusion of every experimental period. This timing was crucial to ensure that the blood parameters reflected the physiological state of the animals under the specific treatment conditions. Blood sampling was conducted 4 hours after the morning feeding and watering, allowing sufficient time for the animals to metabolize the feed and water, thus providing an accurate representation of their metabolic state during the study.

The blood samples were drawn from the jugular vein, which provides a reliable and accessible site for venipuncture in cattle. A sterile venoject needle was used for this purpose, and each needle was fitted with a collection tube containing heparin, an anticoagulant that prevents blood from clotting. The use of heparin ensured that the blood samples remained viable for the necessary tests without coagulation interfering with the analysis.

After the blood was collected, it was immediately placed in an ice-filled thermos to preserve the integrity of the sample by maintaining a low temperature. This step was critical in preventing the degradation of blood components, ensuring that the measurements of various parameters such as urea, haemoglobin, leukocytes, and haematocrit would be accurate. The cooled blood samples were then promptly transported to the laboratory, where they underwent further analysis using appropriate biochemical and haematological methods. This entire process was designed to maintain the highest standards of sample quality and accuracy, ensuring that the results of the blood analysis reflected the true physiological responses of the animals to the experimental treatments.

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) using the Latin Square Design model to determine the treatment effects. Significant differences between treatments were further analysed using post-hoc tests Duncan Multiple Range Test, with significance set at $p < 0.05$. All statistical analyses were performed using statistical software (SPSS 23).

RESULTS AND DISCUSSION

Effect of Treatment on Red Blood Cell (Erythrocyte) Concentration in Bali Calves

The mean erythrocyte concentrations in Bali calves subjected to water restriction and exercise are presented in Table 1. The results of this study indicate that there was no significant effect ($P>0.05$) of water restriction and exercise on the erythrocyte concentrations of Bali calves fed a complete diet based on *Clitoria ternatea*. Our results differs with previous several reports showing that water deprivation leads to extensive variations in physiological parameters, including weight loss, blood metabolites, hematic parameters, and feed intake (Qinisaa *et al*. 2011; Mpendulo *et al*. 2017; Gohler *et al*., 2020).

This finding suggests that providing high-quality feed can mitigate the stress experienced by Bali calves under conditions of limited water supply and physical exercise. This could be attributed to the fact that despite restricting water intake to 50% of the required amount and subjecting the calves to 30 minutes of exercise per day, their dry matter intake did not significantly decrease (Gohler *et al*., 2020). Erythrocytes, or red blood cells, are essential components of the blood responsible for transporting haemoglobin, which facilitates the distribution of nutrients derived from digestion and the supply of oxygen to various tissues in the body (Escalera-Valente *et al*., 2021). Since nutrient intake did not decrease in the calves that experienced water restriction and exercise, it is understandable that their erythrocyte concentration remained relatively unchanged. This finding implies that a balanced and nutrient-rich diet may help maintain physiological stability, even under potentially stressful conditions such as limited water intake and moderate exercise.

Table 1. The Effect of Treatment on the Blood Components of Balinese Calves

Parameter	Treatment				SEM	P-Value
	T0	T1	T2	T3		
Erythrocytes (µ/L)	8.263	8.488	8.018	8.190	0.87	0.93
Leukocytes (µ/L)	15.68	13.20	11.35	13.93	3.42	0.51
Hemoglobin (g/dl)	13.83	14.13	19.33	13.63	5.19	0.50
Hematocrit (%)	34.225	35.125	34.590	35.575	3.06	0.95

Source: Processed from Primary Data

Blood plays a crucial role in maintaining the physiological balance of the body, particularly in the transport of nutrients and oxygen to cells, removal of metabolic waste and carbon dioxide, and defense against bacteria, viruses, and other harmful organisms. The composition of blood can vary depending on factors that either inhibit or stimulate the circulatory system (Merdana *et al*., 2020). In this study, variations in blood components may have been caused by stress induced by water restriction and exercise. However, the results show no significant effect of the treatments on the red blood cell (RBC) or erythrocyte concentrations in Bali calves. This lack of significant change suggests that the physiological mechanisms of the calves were able to compensate for the environmental stressors, likely due to the high-quality nutrition provided by the complete diet, which helped stabilize their metabolic functions. Thus, although the calves were subjected to potentially stressful conditions, their erythrocyte levels remained stable, underscoring the importance of good nutrition in mitigating the effects of environmental stress.

Effect of Treatment on Leukocyte Concentration in Bali Calves

Leukocytes, or white blood cells (WBCs), are essential components of the immune system, acting as the body's primary defence against harmful microorganisms such as bacteria, viruses, and fungi. These cells are crucial in recognizing and neutralizing infections, thus maintaining the overall health of the animal. In livestock, changes in leukocyte counts are often used as indicators of the animal's immune response to stressors such as environmental conditions, nutritional deficiencies, water restriction, and physical stress like exercise. This makes leukocyte concentration an important physiological parameter to monitor, particularly when animals are subjected to conditions that could provoke an immune or stress response. In this study, the statistical analysis revealed no significant effect ($P>0.05$) of feed treatment, water restriction, or exercise on the leukocyte concentration in Bali calves (Table 1). This result suggests that the imposed water restriction and exercise did not trigger an immunological stress response in the calves, as leukocyte levels remained stable across all treatment groups. This finding contrasts with what is typically expected, as leukocyte counts often increase in response to physical and environmental stressors, which signal the body to prepare for potential infections or injuries.

The lack of a significant rise in leukocyte levels in this study can be attributed to several factors, most notably the provision of high-quality feed based on *Clitoria ternatea*. Adequate and balanced nutrition plays a critical role in supporting the immune system of animals, ensuring that their physiological responses remain stable even under stress. Previous studies have demonstrated that diets rich in essential nutrients, particularly protein, can help modulate the immune response and reduce the impact of environmental stress (Wang *et al*., 2022; Zhang *et al*., 2021). In this study, the complete feed based on *Clitoria ternatea* likely provided sufficient nutritional support to maintain immune homeostasis, allowing the calves to cope with the imposed exercise and water restriction without a significant change in leukocyte concentrations.

Leukocytes, particularly neutrophils and lymphocytes, are key players in the animal's ability to fight infections. Under stress conditions such as water deprivation or physical exertion, one would typically expect an increase in leukocyte production as part of the body's response to perceived threats (Gupta *et al*., 2020). However, the stable leukocyte levels observed in this study suggest that the Bali calves were not under substantial physiological stress, likely due to the mitigating effects of their nutritional intake. Additionally, the restricted water intake (50% of normal consumption) and the exercise regimen (30 minutes of walking twice daily) did not appear

to induce significant stress, as measured by leukocyte concentrations. This is consistent with research suggesting that moderate exercise and controlled dehydration may not always provoke an acute immune response in well-nourished animals (Singh *et al*., 2019). Rather, it seems that the combination of high-quality feed and the relatively mild nature of the exercise and water restriction treatments enabled the calves to maintain normal immune function. The findings from this study highlight the importance of nutrition in mitigating the effects of environmental stressors such as water deprivation and exercise on leukocyte concentration in Bali calves. The stable leukocyte levels observed across all treatment groups suggest that the high-quality complete feed, rich in *Clitoria ternatea*, played a protective role in maintaining immune system balance, preventing any significant stress response. Future research could further explore the role of specific nutrients in enhancing livestock resilience to environmental stress, as well as the long-term effects of such dietary interventions on immune function and overall health.

Effect of Treatment on Hemoglobin Concentration in Bali Calves

Hemoglobin is the primary component of red blood cells responsible for transporting oxygen molecules from the lungs to various tissues throughout the body. It consists of four subunits, each containing a heme group conjugated to a peptide. Hemoglobin plays a crucial role in ensuring that tissues receive adequate oxygen for cellular respiration and metabolism, which is particularly important for growing animals like calves (Gohler *et al*., 2020).

In this study, statistical analysis revealed that the different treatments, including water restriction and exercise, did not have a significant effect on the hemoglobin concentration in the blood of Bali calves ($P>0.05$) (Table 3). This suggests that despite the physical and environmental stressors imposed, the oxygen-carrying capacity of the calves' blood remained stable. A possible explanation for this stability is the provision of a high-quality diet, which could have mitigated the impact of water restriction and exercise on hemoglobin levels.

Previous research has shown that dietary quality can significantly influence hemoglobin synthesis and the overall health of livestock. Adequate nutrition, particularly with sufficient protein and iron intake, supports the production and maintenance of hemoglobin in the bloodstream (Garg *et al*., 2021). The complete feed based on *Clitoria ternatea* used in this study may have provided the necessary nutrients to sustain hemoglobin levels, even when the calves were subjected to moderate dehydration and exercise.

Haemoglobin concentration is often affected by factors such as dehydration, where a reduction in water intake can lead to haemoconcentration, artificially elevating haemoglobin levels (Smith *et al*., 2019). However, in this study, despite a 50% reduction in water intake in some treatment groups, hemoglobin concentrations did not increase significantly. This suggests that the calves were able to maintain normal hydration levels, likely due to the high water content of the complete feed and the physiological adaptations of Bali cattle, which are known for their resilience in harsh environmental conditions.

Furthermore, physical exercise can increase the demand for oxygen in tissues, leading to a potential increase in haemoglobin concentration to meet this demand (Patel *et al*., 2020). However, the exercise regimen in this study appears to have been mild enough that it did not induce a significant physiological change in haemoglobin levels. This suggests that Bali calves are well-adapted to moderate physical activity without experiencing substantial shifts in blood oxygen transport capacity. Therefore, In conclusion, the absence of a significant change in haemoglobin concentration across the treatment groups indicates that the calves' ability to transport oxygen to tissues was not compromised by water restriction or exercise. This highlights the potential of high-quality feed to buffer livestock against environmental and physical stress, ensuring stable physiological function. Future research could explore the long-term effects of such treatments on oxygen transport and metabolic efficiency in Bali cattle.

Effect of Treatment on Haematocrit Concentration in Bali Calves

Haematocrit, also known as packed cell volume (PCV), is the proportion of red blood cells in the blood, typically measured through centrifugation. Haematocrit levels are an important indicator of the animal's health, particularly in assessing oxygen-carrying

capacity and the potential presence of conditions such as anaemia. A lower-than-normal haematocrit level is commonly associated with anaemia, which can result from nutritional deficiencies, dehydration, or other stressors. In this study, the average haematocrit levels in Bali calves ranged between 34.23% and 35.58%, showing no significant difference among the treatment groups (P>0.05). These values are consistent with the findings of Benu *et al*. (2016), who reported haematocrit concentrations between 34.75% and 37.00% in *Bos indicus* cattle supplemented with nitrates and exercised for 24 minutes at a speed of 3.3 km/h over a distance of 2.8 km. The similarity in results indicates that moderate exercise and water restriction did not significantly alter the haematocrit levels in Bali calves, likely due to the quality of the diet provided.

Haematocrit levels in cattle are strongly influenced by dietary factors. Adequate nutrition ensures that red blood cell production remains within a healthy range, preventing conditions such as anemia. The results of this study suggest that the complete feed based on *Clitoria ternatea* provided sufficient nutrients to support normal haematocrit levels, even in calves subjected to water restriction and exercise. This indicates that the calves were neither anemic nor dehydrated, which could have caused a reduction in haematocrit levels.

Previous studies have shown that cattle under dehydration or nutritional stress often exhibit lower haematocrit values, as a result of reduced red blood cell production or haemolysis (Smith *et al*., 2019). However, the stable haematocrit levels observed in this study suggest that the calves adapted well to the imposed stressors, possibly due to the protein-rich and highly digestible nature of the *Clitoria ternatea*-based feed (Patel *et al*., 2020). Moreover, Bali cattle are known for their resilience in challenging environments, and their ability to maintain stable haematocrit levels under water restriction and physical activity further underscores their adaptability.

It is therefore the results of this study indicate that the treatments, including water restriction and exercise, did not negatively impact the haematocrit levels of Bali calves. The provision of a nutritionally balanced diet played a crucial role in maintaining the calves' physiological health, allowing them to cope effectively with environmental stressors without experiencing significant changes in their red blood cell concentration.

CONCLUSION

A well-balanced diet containing *Clitoria ternatea* can buffer the negative effects of environmental stress such as water restriction and exercise on haematological health, ensuring the well-being and productivity of Bali calves, particularly in arid or water-scarce areas. Further research is recommended to explore the long-term effects of combined environmental stressors and to assess additional physiological and production parameters in Bali cattle under varying management conditions.

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