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SHORT COMMUNICATION

Haematological Parameters of Lactating Dairy Cows Fed with Total Mixed Ration (TMR) Briquettes

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Abstract

The goal of this study was to explore the influence of haematological parameters of nursing dairy cows fed with Total Mixed Ration (TMR) briquettes. Nine, Jersey × Sahiwal crossbred dairy cows (Body weight = 275 ± 33 kg and parity = 3) were randomly assigned to three treatments in a Completely Randomized Design (CRD) with a 14-day adaptation period and a 21-day feeding trial. A mixture of Guinea grass (*Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs) and commercial cow feeds (CTL) which was the control diet and two TMR briquettes including a variety of air-dried forages with agro-industrial by-products (TMR1 and TMR2) were employed as treatments. Blood samples were taken from each cow at the end of the feeding trial and haematological parameters were measured. The blood was tested for total white blood cell count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), red blood cell count (haemoglobin and red blood cells), and platelet count by following standard laboratory guidelines. The haematological parameters were not significantly (p>0·05) different among the treatments. The total white blood cell count (× 10^9 L^{-1}) was measured for CTL, TMR1, and TMR2 as 9.7 ± 1.37, 10.20 ± 1.37, and 10.60 ± 1.37 while the red blood cells (× 10^{12} L^{-1}) were 5.88 ± 0.60, 6.35 ± 0.60, and 4.40 ± 0.60, respectively. The platelet count (× 10^9 L^{-1}) were observed as 213.33 ± 36.74, 215.33 ± 36.74, and 226.67 ± 36.74 in cows fed with CTL, TMR1, and TMR2, respectively. The study found that shifting the diet to TMR has no impact on haematological indicators in lactating dairy cows.

Keywords: *Haematological parameters, Lactating dairy cows, TMR briquettes*

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1. Introduction

Haematological parameters measure many components of the blood and indicate the physiological variables of an animal which determine the sub-clinical stress responses, health status, production, and welfare of animals. Furthermore, different factors such as age, sex, stress, diet, body condition, reproductive status, and exercise influence the haematological parameters (Astuti et al. 2021). Moreover, milk production and productivity of cows can be affected by the haematological parameters (Bezerra et al. 2017). Hence, the nutritional status, health, and stress condition of dairy cows could be evaluated by analysing the haematological parameters of animals (Astuti et al. 2021).

There are three main cell types as red blood cells (RBC), white blood cells (WBC), and platelets in blood. However, haematological parameters; erythrocytes, leukocytes, haemoglobin, erythrocyte index, and platelets are commonly examined in livestock to evaluate their health (Roland et al. 2014). The key function of RBC is the transport of oxygen to the tissues from the lungs, through binding erythrocytes to haemoglobin (Roland et al. 2014). In general, beef cattle breeds and bulls have higher RBC counts than dairy cows (Adili et al. 2014). White blood cells are essential for immune defence mechanism of the animal body. Therefore, WBC count is increased with disease conditions (Wagner et al. 2008). However, the total number of WBC decreases with age of cattle (Boule Diagnostics AB 2020). Platelets are cytoplasmic fragments and they are essential for haemostasis (Roland et al. 2014).

Current studies on haematology parameters of Jersey × Sahiwal crossbred dairy cows are limited (Regmi & Pande. 2018). Moreover, haematological parameters are not yet available for different feeding methods. Therefore, this study aimed to provide a complete profile of haematology in Jersey × Sahiwal crossbred dairy cows fed with Total Mixed Ration (TMR) briquettes.

2. Materials and Methods

Ethical Approval

The ethical approval was obtained from the Animal Ethics Committee, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka (VERC-19-09).

Experimental Animals and Treatments

Nine, Jersey x Sahiwal crossbred dairy cows (Body weight = 275 ± 33 kg) were randomly assigned to three treatments in a Completely Randomized Design (CRD). Cows were at third parity and early lactation when the experiments were commenced. During the experiment, each cow individually had access to *ad libitum* water and feed *via* an individual water bowl and feed bunk, respectively. The dairy cows were in cubicles in a tie-stall barn located at the Livestock Experimental Farm, Faculty of Agriculture, Rajarata University of Sri Lanka. These animals were introduced to the experimental site and 14 days of adaptation period for the environment was given. There was a 21-day feeding trial period. The three treatments were Guinea grass (*M. maximus*) with commercial cow feeds (CTL) which was

the control diet and two types of TMR briquettes containing a variety of air-dried forages with agro-industrial by-products (TMR1 and TMR2) with different nutrient composition (Tables 1 and 2). During the whole study period, all cows had individual access to *ad libitum* treatment diets and water. Each TMR briquette was prepared on as fed basis and pressed into 10.4 kg by applying hydraulic pressure using a briquette machine (Green Pack 09, Christo Creations Pvt Ltd, Negombo, Sri Lanka). The TMR briquette preparation are described in previous studies (Karunanayaka et al. 2021; Karunanayaka et al. 2022).

Blood Sample Collection and Analysis

Blood samples were collected from each cow at the end of the feeding trial period by a veterinary surgeon. The blood (10 mL) was drawn from the jugular vein into a vacuum tube containing Tripotassium (K3) Ethylene Diamine Tetraacetic Acid (EDTA). Blood samples were immediately stored in a cool box at a temperature around 4°C and transferred to the laboratory for examination within 1 hour after collection. The examination of the Jersey × Sahiwal crossbred dairy cattle haematological parameters was carried out in a Hematology Analyzer (CELL-DYN Emerald 22, Abbotte, CA, USA) at Asiri Medical Laboratory, Anuradhapura, Sri Lanka. The blood was analysed for total WBC count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), RBC count (haemoglobin and red blood cells), and platelet count by following standard laboratory guidelines.

Statistical Analysis

Data analysis was conducted using using one-way Analysis of variance (ANOVA) procedure in Statistical Analysis System (SAS), Ver 9.0. Treatment effects on total blood cell count were evaluated. Means were separated using Tukey's Studentized Range Test at $p \leq 0.05$ significance level.

3. Results and Discussion

The haematology parameters in Jersey × Sahiwal cross-bred dairy cows after the feeding trial period are given in Table 3. There were no significant ($p>0.05$) differences in the total WBC ($p=0.866$), RBC ($p=0.130$) and platelet count ($p=0.963$) of cows fed with CTL, TMR1, and TMR2. The total WBC counts of Jersey × Sahiwal cows were 9.57 ± 1.37, 10.20 ± 1.37, and 10.60 ± 1.37 ($\times 10^9$ L^{-1}), respectively for cows fed with CTL, TMR1, and TMR2.

Table 1: Composition of the control diet (CTL) and selected TMR briquettes on a dry matter basis

Ingredients	CTL (%)	TMR1 (%)	TMR2(%)
Gliricidia sepium	-	8.5	11
M. maximus	65	21.5	14
Zea mays	-	16	11
Napier grass[1]	-	13	19
Sorghum bicolour	-	13	21.5
Rice (*Oryza sativa)* bran	-	10	6.5
Maize (*Zea mays)* meal	-	2.5	7
Soybean (*Glycine max)* meal	-	2.5	3
Coconut (*Cocos nucifera)* poonac	-	11	5
Mineral mixture	-	2	0
Di Calcium Phosphate	-	0	2
Cattle feed[2]	35	-	-
Total dry matter	100	100	100

Source: Karunanayaka et al. (2021)

[1]*Pennisetum purpureum × Pennisetum americanum*

[2]*Cereals, cereal by-products, oil seed meal, vegetable oil, minerals, vitamins, and additives*

CTL = Animals fed with Guinea grass + commercial cow feed (control), TMR1 = Animals fed with pre-selected TMR briquette 1, TMR2 = Animals fed with pre-selected TMR briquette 2

Table 2: Nutrient composition of the control diet and selected TMR briquettes on a dry matter basis

Parameters (%)	Treatments		
	CTL	TMR1	TMR2
Dry matter	25.6±2.12	88.9±1.40	88.6±1.07
Ash	13.3±0.59	11.1±1.68	11.1±2.68
Crude protein	9.43±0.88	11.6±1.06	11.5±1.00
Acid detergent fiber	43.8±3.21	34.5±2.34	38.7±2.58
Neutral detergent fiber	52.7±3.70	48.8±3.30	48.6±2.66
Calcium	0.95±0.07	0.77±0.11	0.40±0.02
Phosphorous	0.65±0.06	0.79±0.09	0.34±0.05

Source: Karunanayaka et al. (2021)

CTL = Animals fed with Guinea grass + commercial cow feed (control), TMR1 = Animals fed with pre-selected TMR briquette 1, TMR2 = Animals fed with pre-selected TMR briquette 2

Table 3: White blood cell (WBC), red blood cell (RBC) and platelet counts in lactating dairy cows fed with TMR briquettes

Parameters	Treatments			SEM	*p* value
	CTL	TMR1	TMR2		
Total white cell count (10^9 L^{-1})	9.57	10.20	10.60	1.37	0.8658
Neutrophils (%)	33.20	32.30	40.37	6.94	0.6837
Lymphocytes (%)	62.13	65.37	51.33	6.30	0.3258
Monocytes (%)	0.70	0.37	1.03	0.22	0.1824
Eosinophils (%)	3.60	1.73	6.40	1.83	0.2699
Basophils (%)	0.37	0.23	0.90	0.27	0.2591
Neutrophils count (10^9 L^{-1})	3.23	3.17	4.20	0.81	0.6209
Lymphocyte count (10^9 L^{-1})	5.87	6.80	5.50	1.08	0.6977
Monocytes count (10^9 L^{-1})	0.07	0.03	0.10	0.04	0.5787
Eosinophils count (10^9 L^{-1})	0.33	0.17	0.73	0.22	0.2607
Basophils count(10^9 L^{-1})	0.07	0.03	0.07	0.03	0.7290
Haemoglobin (g dL^{-1})	9.63	10.27	7.60	0.91	0.1773
Red blood cells (10^{12} L^{-1})	5.88	6.35	4.40	0.60	0.1296
Mean cell volume (fl)	49.33	47.23	51.20	1.23	0.1541
Haemotocrit (%)	29.03	29.77	22.43	2.44	0.1423
Mean cell haemoglobin (pg)	16.37	16.20	17.37	0.51	0.2910
MCH Concentration (g dL^{-1})	33.20	34.37	33.93	0.54	0.3666
Red blood cells distribution width (%)	15.30	13.60	15.97	0.95	0.2654
Platelet count (10^9 L^{-1})	213.33	215.33	226.67	36.74	0.9627

CTL = Animals fed with Guinea grass + commercial cow feed (control), TMR1 = Animals fed with pre-selected TMR briquette 1, TMR2 = Animals fed with pre-selected TMR briquette 2
SEM = Standard error of means

White blood cells play an essential role in immune defence and protect the body against infections and invaders (Etim et al. 2014). Therefore, the number of WBC is one of the indicators to identify the disease conditions. Animals with high WBC counts generate antibodies to resist them against diseases and enhance their adaptability to stressful environmental conditions (Etim et al. 2014). According to Burke (1994), the WBC of 5 – 13 ($\times 10^9$ L^{-1}) is considered to be within the normal range, such that, values reported in the present study are within the acceptable range. This indicates good immunity levels and health conditions of the cows. Prior to the study, effective management practices were implemented to keep the cows in a less stressful environment. The accepted period of adaption was practised for the feeds and environment and recommended vaccination was practised prior to the experiment. During the experiment, all possible measures were taken to prevent diseases. Hence, these practices might have contributed to maintain good immunity levels.

Neutrophils are prominent in calves (Roland et al. 2014). However, lymphocytes become dominant in WBC as they grow old. Therefore, the neutrophil-to-lymphocyte ratio is approximately 1:2 in adult cows (Roland et al. 2014). Al-Shami (2003) recorded neutrophil and lymphocyte values as 58 ± 2.0% and 30 ± 1.2% for Hassawi cattle in Saudi Arabia. Research Animal Resource (2009), reference values for monocytes, eosinophils, and basophils were 1-6%, 0-4%, and 0-2%, respectively. According to Coroian et al. (2017), the lactation stage was affected by the lymphocyte concentration which vary within wide limits. Therefore, in first lactation, they were 2.8±1.56 ($\times 10^9$ L^{-1}) and in sixth lactation, the mean values of lymphocytes were 7.55 ± 1.80 ($\times 10^9$ L^{-1}) (Coroian et al. 2017). Significant differences in lymphocytes were not observed in the present study might be due to all cows were in the same lactation stage.

Red blood cells or erythrocytes are mainly responsible for carrying oxygen from the respiratory organs to the rest of the body and carrying carbon dioxide from those cells back to the lungs (Roland et al. 2014). Haemoglobin, which transports oxygen and carbon dioxide in RBCs, has a negative correlation with atmospheric temperature. Moreover, the RBC count is reported to decrease significantly in cattle under heat stress conditions (38°C) (Aggarwal et al. 2016). However, in the present study, haemoglobin and RBC have not shown any significant difference. During the whole study period, sprinklers were used in the daytime to reduce heat stress, which might have contributed to non-significant differences.

The hematopoietic cells differ between animal species and breeds. According to Boule Diagnostics AB (2020) reference values for monocytes ($\times 10^9$ L^{-1}), lymphocytes (10^9 L^{-1}) and neutrophils counts ($\times 10^9$ L^{-1}) in cow blood are 0.0-0.8, 2.5-7.5, and 0.6-4.0, respectively. The values in the current study were within recommended the range. Chen et al. (2022) record that haematological parameters of Holstein cows are 3.99 ± 1.23, 5.35 ± 2.70, 0.62 ± 0.22, 0.48 ± 0.28, and 0.12 ± 0.06, respectively for neutrophils ($\times 10^9$ L^{-1}), lymphocytes ($\times 10^9$ L^{-}

^{1}), monocytes (× 10^{9} L^{-1}), eosinophils (× 10^{9} L^{-1}) and basophils (× 10^{9} L^{-1}). Values reported in this study were closer to the recorded values by Chen et al. (2022). The mean cell volume for Holstein cows was 47.69 ± 4.59 fl (Chen et al. 2022). However, these values were lower than the recorded values in the present study. It might be due to the breed variation. In addition, different factors such as age, health status, diet and nutrition also affect the mean cell volume. However, observed mean cell volume for CTL, TMR1 and TMR2 were 49.33 ± 1.23 fl, 47.23 ± 1.23 fl, and 51.20 ± 1.23 fl, respectively and values were within the range recommended of 40.0-60.0 fl (Boule Diagnostics AB 2020).

Three weeks of a prepartum, prepartum day, and three weeks postpartum, haemotocrit contents in Bali cows were 30.50 ± 2.66%, 23.83 ± 3.18%, and 25.33 ± 2.50%, respectively (Merdana et al. 2020). At the end of the periparturient period, the hematocrit value increases due to the good condition for O_2 and CO_2 transportation and maintaining the stable blood pH. When cows become dehydrated, haemotocrit value increases. In addition, the blood fluid viscosity is affected by the haemotocrit values (Merdana et al. 2020). In the present study, haemotocrit percentage values were not significantly different among the treated cows might be due to all cows were in the same lactation stage and cows being free to access water throughout the study period. Wohlt et al. (1984) show a haemotocrit decreased with the lactation stage and younger cows had more haemotocrit than old cows. Therefore, the results observed in this study agreed with Wohlt et al. (1984).

The average mean cell haemoglobin (MCH) values of horned and polled cows were 14.57 ± 1.11 pg and 14.03 ± 1.23 pg, respectively (Fahrimal et al. 2020). The average MCH and MCH concentration values of Sahiwal cow in winter season are 17.14 ± 0.42 pg, and 38.45 ± 1.08 g dL^{-1}, respectively and in summer season they are reported as 17.31 ± 0.72 pg and 37.56 ± 1.32 g dL^{-1}, respectively (Parmar et al. 2013). The results of Parmar et al. (2013) and the present study values show a similar pattern, it might be due to the breed similarity. Therefore, this indicates that Sahiwal cows have a better tolerance to heat and cold stress. However, MCH and MCH concentrations increased during pregnancy, because they are provided with high-energy nutrient intake during pregnancy period (Merdana et al. 2020). Therefore, MCH and MCH concentrations are related to nutrient content. According to Ramin et al. (2011), average mean cell haemoglobin values are 16.0 ± 0.5 pg, 17.2 ± 0.34 pg, 7.1 ± 0.64 pg, 16.4 ± 0.54 pg, and 15.0 ± 0.61 pg for healthy cows, cows without anaemia, mild anaemia, moderate anaemia, and severe anaemia, respectively. In this study, cows were not in an anaemic condition. According to Boule Diagnostics AB (2020), reference values for RBC distribution width (%) were 0.0 – 99.9 and values of the current study were also within that range.

Platelets play an essential role in hemostasis and it is anuclear cytoplasmic fragments of megakaryocytes (Roland et al. 2014). Bovine platelets survive up to 10 days in blood and the amount of production, consumption, sequestration, and loss are affected by the total number of platelets (Roland et al. 2014).

Reference values for platelets in adult cattle range between 155–1022 × 10^9 L^{-1} (Strous et al. 2021), which tally with the current work.

4. Conclusions

The study reveals that the haematological parameters of the cows are not affected by changing the conventional diet to TMR briquettes made with multiple forages and concentrates.

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Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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