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Nutrient status, hematological and blood metabolite profile of mid-lactating dairy cows during wet and dry seasons raised under Indonesian tropical environmental conditions

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Abstract This study was conducted to determine the nutrient status, changes in the hematological and selected blood metabolites profile of mid-lactating dairy cows during wet and dry seasons under Indonesia's tropical environment. Forty mid-lactating FH cows were selected from the Livestock Farm Complex (Dairy Community) in Yogyakarta, with an average body weight of 421.77±28.60 kg and 1st to 3rd lactation. They were housed in a semi-open housing system with concrete flooring covered with rubber mats and provided with feeders and drinkers. They were fed twice daily, and drinking water was given ad libitum. Measurements were made on the intakes of feed (and nutrients). The variables measured were nutrient intakes, blood hematology, and metabolites profile and measured over two seasons, namely wet and dry seasons. The results of the measurements made were compared using independent t-test analysis. The result indicated that intake of CP, RLYM, RNEUT, NEUT, RBC, HGB, MCV, MCHC, MPV, albumin, urea nitrogen, and total protein was differed significantly ($P < 0.05$) between wet and dry seasons, respectively. It was concluded that the nutrient status of mid-lactating dairy cows in the wet season was higher than during the dry season, especially the intake of CP. In the wet season, it has a positive effect on the basic hematological parameters.

Keywords: blood metabolite profile, dairy cows, hematology, mid-lactation, season

1. Introduction

The weather in Indonesia, which is situated in the tropics, is characterized by high temperature and humidity, ranging between 25.8 to 34.6 ^{o}C and 52.0 to 86.8%, respectively (BPS 2010; Hernawan 2014; Rochijan et al 2016a; Widyobroto et al 2019) and temperature humidity index (THI) value in Indonesia is between 76.2 to 84.7 (Widyobroto et al 2019). This condition is likely to affect dairy cows' production, health, and nutrition raised in Indonesia.

Hematological and profile of blood metabolites have been used widely to monitor health status, nutritional status, and metabolism, identify dietary causes of low productivity, and evaluate animal stress and welfare levels (Brucka-Jastrzębska et al 2007). Effect of physiological status significantly manifested on the blood metabolic profile and hormones (leptin, insulin, and thyroid hormones) (Antunovic et al 2011). Physiological changes in constituents of blood cells and blood metabolites occur dynamically during growth, pregnancy, and lactation.

The blood metabolites profiles are also considered important in evaluating the health (Khalili et al 2020) and nutritional status of cows. The present study examined the nutrient status, hematological, and some of the blood metabolites profiles of mid-lactating dairy cows under wet and dry seasons under Indonesia's tropical environment.

2. Materials and Methods

2.1. Ethical approval

The animal procedure herein used was approved by the Ethical Clearance Committee, and it was performed following the Guidelines of Animal Use of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia.

2.2. Animal, diets, and feed analysis

Forty Friesian Holstein cows in their mid-lactation were selected from the Livestock Farm Complex (Dairy Community) in Yogyakarta, Indonesia during the wet and dry seasons. The cows (average body weight 421.7±28.6 kg) were on 1st to 3rd lactation. The experimental animals were reared in a semi-open housing system with a concrete floor, covered with rubber mats, and supplied with feeders and automatic drinkers. The roofs were made of asbestos roofing material, and the house was constructed in an east-west direction. Daily temperature and humidity are generally high during the

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study, i.e., 31.3 °C and 64.1%; and temperature humidity index (THI) value is 82.1. The cows were fed twice daily, at 07.00 and 15.00 h, with forages and concentrate given separately, and drinking water was given ad libitum. The proportion and chemical composition of forage (*Pennisetum purpureum* and *Panicum maximum*) and commercial concentrate was presented in Table 1. Collecting of samples was conducted daily during the study. The proximate composition of feed sample (forage and commercial concentrate) and feed refusals were analyzed by the AOAC method (2005) to measure their moisture content, dry matter, organic matter, crude protein, crude fiber, extract ether, and total digestible nutrients.

Table 1 Proportion (%) and chemical composition forage and concentrate.

Parameters	Forages (%)		Concentrate (%)
	Pennisetum purpureum	*Panicum maximum*	
Season			
Wet season	57.00	-	43.00
Dry season	-	56.40	43.60
Nutrient composition (% DM)			
Dry matter	26.88	32.87	85.57
Organic matter	87.19	90.22	92.09
Crude protein	7.01	5.22	21.84
Crude fiber	34.87	33.64	27.66
Extract ether	1.96	0.92	0.11
Total digestible nutrients	50.98	55.14	60.87
Neutral detergent fiber (%)	66.70	70.11	44.39
Acid detergent fiber (%)	37.59	40.29	12.62

2.3. Blood sampling and analysis

During respective wet and dry seasons, blood samples from each animal (once in every two weeks) were collected aseptically from the caudal artery (3 to 4 h after the morning feeding) into 3 mL vacutainer K3 Ethylene Diamine Tetraacetic Acid (EDTA) tubes. The tubes were rolled several times gently to ensure enough anticoagulant mixing with Delany et al (2010) methods, modified by Rochijan et al (2016a) to sample preparation. Immediately after collection, the samples were transported to the laboratory over ice for further processing. The samples were analyzed for hematological parameters, namely white blood cell (WBC), the ratio of lymphocytes (RLYM), the ratio of eosinophil granulocytes, basophil granulocytes and monocytes (REBM), the ratio of neutrophil granulocytes (RNEUT), lymphocytes (LYM), eosinophil granulocytes, basophil granulocytes and monocytes (EBM), neutrophil granulocytes (NEUT), red blood cell (RBC), hemoglobin concentration (HGB), hematocrit value (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT) and mean platelet volume (MPV) using automated hematology analyzer (Make Mindrey; model BC 2800). Then the blood samples were centrifuged for 15 min at 3000 rpm, and the plasma was taken using a micropipette and then transferred into 1 mL Eppendorf tube. The blood plasma samples were then stored at a temperature of -20 °C freezer until blood urea nitrogen analysis. After being collected, the plasma concentration of glucose, albumin, urea nitrogen, total protein, and total cholesterol were determined using an auto-analyzer.

2.4. Statistical analysis

The data obtained were statistically analyzed by independent t-test analysis using Statistical Program for Social Science or SPSS version 24.0.

3. Results and Discussion

3.1. Microenvironment condition during wet and dry seasons

Many environmental factors such as the climate can interfere with the productivity of dairy cows, feed consumption, weight gain, body health, hematological and blood metabolite profile. Dairy cows are susceptible to a hot environment (heat stress) which can cause changes in the endocrine system in the blood. Table 2 shows the result of the study in the microenvironment condition during the wet and dry seasons (temperature, humidity, wind/velocity, and solar radiation).

Table 2 shows that the average temperature in the wet season was 27.1±3.5 °C and 26.7±3.8 °C in the dry season. According to Yousef (1985), the temperatures are above the Thermo Neutral Zone (TNZ) for dairy cattle ranging from 16 to 25 °C, so that the cows begin to experience stress. The average daily humidity in the wet season was 82.1±9.0% and 81.0±8.0% in the dry season. Humidity is relatively high because of high rainfall. The impact of low or high humidity causes the cow will make adjustments by increasing or decreasing the frequency of respiration, feed intake, and level productivity. There is a significant difference in solar radiation. According to De Rensis and Scaramuzzi (2003), environmental factors that affect reproductive efficiency, body health, nutrient consumption, and production include temperature, solar radiation, humidity, wind or velocity, and rainfall which contribute significantly to heat stress levels in dairy cows.

3.2. Nutrient status of cows during wet and dry seasons

The average intake of dairy cows during the wet and dry seasons is presented in Table 3. The intakes of DM, OM, CF, and TDN did not show significant differences between wet and dry seasons. However, the intakes of crude protein were significantly higher ($P < 0.05$) in cows during the dry season compared to the wet season. The consumption of OM is positively correlated with dry matter intake (DMI). The DMI has special importance to meet the nutrient requirement of fresh cows (early lactation) to maintain their health and production (Rochijan et al 2016b). Le Floc'h et al (2004) and Sejrsen et al (2006) reported that low DMI and deficiency in nutrient supply, especially protein and amino acids, have led to immunosuppression and incidence of metabolic disorders in cows. Thus, diets with higher CP and undegraded protein are effective in maintaining production and body condition score (BCS) (NRC 2001).

In this study, dietary concentrate:forage ratio of total feed consumed in the wet season (43.0:57.0) and dry season (43.6:56.4) (Table 3) were not significantly different. Results of the study are comparable with those obtained by Cantalapiedra-Hijar et al (2009) and Ramos et al (2009), who found no effect of dietary concentrate: forage ratios (30:70 and 70:30) on OM intake in both goats and sheep fed above maintenance. The differences in CF of the diets were mainly due to the variance in CF concentrations. However, Allen (2000) noted that no effect of CF ranging from 25 to 40% was found on DM intake in dairy cows, although feed intake generally decreases with increasing CF. Furthermore, the forages intake was low, negatively affecting the digestive process because the concentrates had a high CP and structural carbohydrate. Farmers should utilize this situation as a reference, especially during the dry season when forages are expensive and hard to find. Energy in feed could affect the efficiency of the ration used, especially in ruminants. Excess energy in the ration causes a reduction in feed efficiency, and it tends to build in the body fat. One of the disadvantages was that there were excesses in the supply of amino acids, which will be deaminated and excreted, with a consequent reduction in the energy value of the diet.

Table 2 Microenvironment condition during the wet and dry seasons.

Parameters	Wet Season			Dry Season		
	Min.	Max.	Average	Min.	Max.	Average
Temperature (°C)	21.9	33	27.1±3.5^{a}	20.3	32.5	26.7±3.8^{a}
Humidity (%)	64	95	82.1±9.0^{a}	62	93	81.0±8.0^{a}
Wind / Velocity (m/s)	2	14	5.2±3.8^{a}	2	12	4.5±3.3^{a}
Solar radiation (%)	26	77	51.8±12.9^{a}	50	78	64.4±9.2^{b}

^{ab} Different superscripts within rows indicate significant differences ($P < 0.05$).

Table 3 Nutrient intake of lactating dairy cows during the wet and dry seasons.

Nutrient Intake	Groups (kg DM/head/day)	
	Wet Season (Mean±SD)	Dry Season (Mean±SD)
Dry matter	18.72±3.73^{a}	19.06±2.69^{a}
Organic matter	16.72±3.28^{a}	17.33±2.44^{a}
Crude protein	2.52±0.37^{a}	2.22±0.43^{b}
Crude fiber	5.94±1.26^{a}	5.97±0.85^{a}
Total digestible nutrients	10.35±1.97^{a}	10.93±1.54^{a}

^{ab} Different superscripts within rows indicate significant differences ($P < 0.05$).

3.3. Blood hematological profile of cows during wet and dry seasons

Blood hematological profile in ruminants, especially for dairy cows, depends on many factors linked to the animal's physiological status and management system, including housing hygiene, health, and nutrition. For the dairy cows to perform normally and productively, appropriate management conditions are required. According to Brucka-Jastrzębska et al (2007), hematological blood tests primarily aim to monitor health status, detect possible diseases, and evaluate animal stress and welfare levels. Results of the hematological blood profiles in cows during the wet and dry seasons are shown in Table 4. It showed that in all groups, the values of the significant blood morphological indicators fell within the range of reference values for healthy dairy cows. WBC are the basic cells of the immune system in humans and animals, which determine normal body function. In both wet and dry seasons, the mean white blood cell count in all cows was fell within the reference range (Winnicka 2008; Quiroz-Rocha et al 2009; Patel et al 2017).

In this study, the cows in the wet season have a higher concentration of white and red cell counts, hemoglobin concentration, hematocrit value, and platelets compared to during the dry season. In this study, statistically significant differences ($P < 0.05$) were observed in the concentration on red cell counts, the ratio of lymphocytes, the ratio of neutrophil granulocytes, neutrophil granulocytes, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin concentration, and mean platelet volume were found in cows between the seasons. The present study showed that most hematological parameters were higher during the wet season than during the dry season in cows. White cell count was observed to increase significantly ($P < 0.05$) in cows during the wet season compared to the dry season. The white cells of animals observed in the wet season were significant higher than in the dry season, indicating that the cows' immune system was increased. However, there was no evidence of pathology in the observation. The result of leukocyte count in cattle may also increase due to stress, including blood collection (Brucka-Jastrzębska et al 2007).

Table 4 Concentrations of hematological blood profile in cows during the wet and dry seasons.

Parameters	Groups		Normal Values
	Wet Season	Dry Season	
WBC - White blood cell (x 10^3/µL)	15.35[a]	14.78[a]	5 – 13.3 (1)
RLYM - Ratio of lymphocytes (%)	24.08[a]	45.50[b]	-
REBM - Ratio of eosinophil granulocytes, basophil granulocytes and monocytes (%)	5.55[a]	2.16[a]	-
RNEUT - ratio of neutrophil granulocytes (%)	70.37[a]	52,35[b]	-
LYM - Lymphocytes (x 10^3/µL)	3.28[a]	9.45[a]	1.8 – 8.1 (1)
EBM - Eosinophil granulocytes, basophil granulocytes and monocytes (x 10^3/µL)	0.82[a]	0.19[a]	0.1 – 1.1 (1)
NEUT - Neutrophil granulocytes (x 10^3/µL)	10.31[a]	5.15[b]	1.7 – 6 (1)
RBC - Red blood cell (x 10^6/µL)	7.11[a]	5.74[b]	5 – 10 (2)
HGB - Hemoglobin concentration (g/dL)	12.20[a]	9.63[b]	8 – 15 (2)
HCT - Hematocrit value (%)	33.05[a]	27.89[a]	24 – 46 (2)
MCV - Mean corpuscular volume (fL)	46.50[a]	48.75[b]	40 – 60 (2)
MCH - Mean corpuscular hemoglobin (pg)	17.74[a]	16.84[a]	11 – 17 (2)
MCHC - Mean corpuscular hemoglobin concentration (g/dL)	38.21[a]	34.57[b]	30 – 36 (2)
PLT - Platelets (x 10^4/µL)	66.10[a]	38.71[a]	-
MPV - Mean platelet volume (fL)	6.89[a]	7.33[b]	-

[a,b] Different superscripts within rows indicate significant differences ($P < 0.05$).
(1) Hoff and Duffield (2003).
(2) Morris (2009).

There are studies on the effect of management systems blood parameters in the scientific literature, but their results are inconclusive. The previous study suggests that red blood cell parameters and hemoglobin concentration do not show significant seasonal differences (Deptuła and Dorynek, 1993). Furthermore, Kumar and Pachaura (2000) discovered that during the dry season, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean cell volume all increased in crossbred dairy cattle, but the hematocrit value decreased.

The mean corpuscular volume and the mean corpuscular hemoglobin concentration decreased in response to high temperature, but there were no changes in white and red blood cell counts, hematocrit value, and hemoglobin concentration (Casella et al 2013; Mazzullo et al 2014). Another study, Patel et al (2017) reported a reduction in hemoglobin, hematocrit value, mean corpuscular volume, and mean corpuscular hemoglobin concentration during the dry season, but no change was observed in red and white blood cell count and MCHC. The hemoglobin level, erythrocyte count, and hematocrit value are all factors that influence red blood cell parameters. According to Aengwanich et al (2009), MCV can be employed in the hematological analysis of a response to thermal and humidity stress in dairy cattle. The same study revealed that stress caused by high temperatures and humidity conditions had been shown to impact hemoglobin synthesis, most likely lowering the available metabolites affected the thyroid and body metabolic failure.

3.4. Blood metabolites profile of cows during wet and dry seasons

Based on Scamell's study (2006), biochemical blood tests are commonly used to assess a human's, TGE functions of the organ (e.g., kidneys and liver), and metabolic processes. It's critical to select the appropriate biochemical parameters that determine how various the functions of organs or systems. In cows, the concentration of glucose, free fatty acids, and beta-hydroxybutyric acid are considered an indicator of energy metabolism. In contrast, the indicators of protein metabolism are urea, total protein, and albumins. In this study, most of the biochemical parameters (Table 5) had values that fall within the range of reference values proposed by Winnicka (2008), Quiroz-Rocha et al (2009), and Patel et al (2017). In this study, no statistically significant differences in blood glucose and total cholesterol concentration were found in the cows, and this finding is supported by the findings of Brzóska (2005). However, the concentration of blood urea nitrogen, albumin, and total protein showed statistically significant ($P < 0.05$).

Urea nitrogen concentration in blood and milk is one of the indicators of the nutritional status of dairy cows. It was shown that the urea nitrogen and total protein concentration in the blood of experimental cows, which was relatively high but were within normal limits. The urea nitrogen of blood indicated that feed protein was efficiently utilized by rumen microflora. The high nitrogen level in forage may affect hepatic nitrogen metabolism, manifested by higher serum activity. The concentration of these enzymes can also be influenced by ambient temperature. According to Brzóska (2006) cows ingest relatively high amounts of dry matter with a high protein content during both the summer or dry season, which is rapidly metabolized to ammonia in the rumen. Cerutti et al (2018) found aminotransferase activity to increase during the dry season in response to high temperature, and lower cholesterol concentrations in the plasma of cows during the dry season compared with the wet season.

Table 5 Concentrations of blood metabolites profile in cows during the wet and dry seasons.

Parameters	Groups	
	Wet Season (Mean ± SD)	Dry Season (Mean ± SD)
Glucose (mg/dL)	36.20±5.85^{a}	37.24±8.04^{a}
Albumin (g/dL)	4.04±0.48^{a}	3.63±0.26^{b}
Urea nitrogen (mg/dL)	36.76±5.58^{a}	23.12±7.28^{b}
Total protein (g/dL)	6.06±1.56^{a}	8.05±0.60^{b}
Total cholesterol (mg/dL)	106.00±21.30^{a}	119.60±26.15^{a}

^{a,b} Different superscripts within rows indicate significant differences ($P < 0.05$).

4. Conclusions

It was concluded that the nutrient intake of mid-lactating dairy cows during the wet season was higher than during the dry season, especially on the intake of CP, probably due to the higher availability of concentrates and less forages supplied to the cows. The wet season positively affects the basic hematological parameters, and hence, affects dairy cows' welfare.

Conflict of Interest

The authors declare that there is no conflict of interest with this work.

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