Research Article

Flushing Diets Influence on Blood Mineral and Haematological Profile of Late-Pregnant Cows under Extensive Grazing

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Abstract | The nutrient requirement of the late-pregnant cows increases for three or two months before expected calving. One of the efforts to meet the nutrient requirement of the late-pregnant cows is by providing flushing diets. The study aims to reveal the effect of urea-impregnated zeolite inclusion in flushing diets on the blood mineral and hematological profiles of three-year-old pregnant Pasundan cows under extensive grazing. The study adopted a completely randomized design experiment with three treatments and five replicates. All the fifteen late-pregnant (two – three months before calving) cows were randomly assigned to three treatments (five cows per treatment) as follows: 1) extensive grazing, 2) extensive grazing and fed flushing diets without urea-impregnated zeolite supplementation (flushing-1), 3) extensive grazing and fed flushing diets supplemented urea-impregnated zeolite (flushing-2). The result showed that flushing diets increased blood Ca (from 2.95 ± 0.11 g/dL in extensive grazing to 3.95 ± 0.55 g/dL in extensive grazing + flushing-1 or to 4.28 ± 0.24 g/dL in in extensive grazing + flushing-2; P<0.05) and Fe (from 0.37 ± 0.08 g/dL in extensive grazing to 1.38 ± 0.42 mg/L in extensive grazing + flushing-1 or to 1.43 ± 0.36 mg/L in in extensive grazing + flushing-2; P<0.05), but not for Zn and Cr concentrations. There were negative correlations between Zn and Ca or Zn and Fe. Flushing-2 diets increased red blood cells, hemoglobin, platelet hematocrit, and platelet distribution width concentration. Both flushing diets decreased mean corpuscular hemoglobin. None of the flushing diets affected white blood cells, lymphocyte, monocyte, neutrophil, and eosinophil. However, flushing-2 diets decreased its basophil count. In conclusion, late-pregnant Pasundan cows raised in the extensive grazing show calcium and iron mineral deficiencies. Inclusion of urea-impregnated zeolite in flushing diets to the late-pregnant cows under extensive grazing improves the calcium and iron availability and most hematological parameters.

Keywords | Hematological parameters, Mineral correlation, Pasundan cows

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INTRODUCTION

One of the indigenous Indonesian beef cattle is Pasundan cattle that genetically have characteristic genes of Bali cattle, Javanese cattle, Ongole cattle and Madura cattle (Baharun et al., 2017). Most Pasundan cattle are reared in extensive grazing system where the cattle graze on a certain pasture area daily without shelter. In this condition, the cattle, especially late-pregnant cow which is the most susceptible to any environmental stress factors like heat stress, parasite infections, protein, and mineral deficiencies. Several researchers previously reported some cases of mineral deficiency in pregnant cows (Delima, 2008; Moeini et al., 2009; Pradhan et al., 2011; Eisenberg et al., 2019). Currently, a significant proportion of pasture plants contain less calcium (Ca) and zinc (Zn) than is required for growth and reproduction. Concentrations of Ca, Zn, and iron (Fe) were, in some species, below the requirements for high production in ruminants, with grasses the most likely to be deficient. In this case, grasses contained less (P <0.05) Ca, Zn, and Fe than legumes (Masters et al., 2019).

The mineral status of grazing cattle plays an important part in forage digestion, reproductive performance, and the development of bones, muscle, and teeth (Ndlovu et al., 2017). Therefore, determination of the mineral status of the grazing cattle is valuable in measuring the degree to which cattle are affected by nutrition, disease, or other environmental factors. One of the indicators to evaluate whether the cattle suffer from nutrient deficiencies or parasites infection is its hematological profiles. The hematological values are valuable indicators of various pathological and metabolic disorders, health status, stress, or welfare of cattle (Sripad et al., 2014), and indicators of physiological condition (Mariana et al., 2019).

During the last trimester of pregnancy, pregnant cows tend to buffer the adverse effects of undernutrition on their developing fetuses by utilizing body reserves, resulting in weight and condition loss from their own body. Increase nutrient intake 1 to 3 months before calving substantially improves pregnancy rate (Morrison et al., 1999). One of the preventive efforts to increase the late-pregnant cow's resistance to any adverse effects under the extensive grazing system is to fulfill the nutrient requirement of the late-pregnant cows for three or two months before expected calving, which is known as flushing. However, flush feeding is not common among the cattle farmers whose cattle are reared in extensive grazing. This is because flushing diets generally contain soybeans as a protein source, which is quite expensive. Urea-impregnated zeolite as a nonprotein nitrogen source can replace some of the protein source so that it can reduce the flushing ration price. The quality of most grasses in the tropics is generally low in crude protein and digestible energy (Bakrie et al., 1996). Therefore, supplementation of urea-impregnated zeolite in the flushing diet is needed to fulfill the crude protein and energy needs of late-pregnant cows under extensive grazing. In the previous study, the inclusion of urea-impregnated zeolite in rice straw-based diets improved live weight gain and feed efficiency in Bali bulls (Kardaya et al., 2018) and it may work for the flushing diets fed to late-pregnant cows under extensive grazing. In recent year, data of blood mineral status and hematological profile of Pasundan cattle are negligible. It is necessary to establish breed-specific reference ranges for blood parameters for indigenous cattle breeds (Sripad et al., 2014). Hence, a study on "the effect of urea-impregnated-zeolite inclusion in flushing diets on blood mineral status and hematological profiles of late-pregnant Pasundan cattle under extensive grazing" is very important to carry out.

This study focuses on the four blood minerals because these four blood minerals are very important for maintaining health, especially during the last trimester of pregnancy. Calcium minerals are mainly needed for fetal bone growth and to prevent hypocalcemia (Khachlouf et al., 2019). Fe minerals are needed for the synthesis of hemoglobin, preventing anemia, and abortion (Kumar et al., 2011; Onmaz et al., 2018). Zn minerals are an important component of more than 70 enzymes, play an important role in the metabolism of proteins, nucleic acids, carbohydrates, lipids, cell membrane stability, and functioning of the immune system (Djokovic et al., 2014). Chromium plays an important role in increasing growth rate, increasing the proportion of muscle to fat, improving reproductive function, and immune system function (Bernhard et al., 2012).

MATERIALS AND METHODS

The study used fifteen late-pregnant (two-three months before calving) Pasundan cows of three-year-old reared in extensive grazing system in the area around the Southern Coast of West Java, Indonesia. The forages that grow on these grazing lands are dominated by shorter-growing grasses such as *Paspalum* sp., *Eleusine indica*, *Chloris barbata*, and less leguminous plants such as *Indigofera* sp. and *Desmodimn* sp. The body condition was 2.95±0.1 on a 9-point scale (Nicholson and Sayers, 1987) and average of body weight was 190.3±22.6 kg. Every night all the cows are kept in a 75 square meter pen without a roof or shade surrounded by bamboo and wooden fences. Inside the paddock there are several trees that can serve as a shelter for the cattle. In the early morning, all cows were driven into the pasture for grazing until 07:00 in the evening. Afterward, all the cows were driven back to the paddock.

The study applied a completely randomized design by three treatments and five replicates. All the fifteen late-pregnant (two – three months before calving) cows were randomly assigned to three treatments (five cows per treatment) as follows: 1) extensive grazing, 2) extensive grazing and fed flushing diets without urea-impregnated zeolite supplementation (flushing-1), 3) extensive grazing and fed flushing diets supplemented urea-impregnated zeolite (flushing-2). The organic chromium (Cr-yeast; 3 mg Cr/kg Mineral mix; source: Animal Feed and Nutrition Laboratory, Department of Animal Science, Djuanda University, Bogor) and urea-impregnated zeolite (contain 36.49% nonprotein nitrogen; source: source: Animal Feed and Nutrition Laboratory, Department of Animal Science, Djuanda University, Bogor) were prepared based on the procedure of Sudrajat et al. (2011) and Kardaya et al. (2018), respectively. Each flushing diet sample was dried in a forced-air oven (Style V-23, Despatch oven Co. Minneapolis, MN) at 55 ^{0}C for 96 h, ground through a 1-mm screen in a Willey mill (Model 3; Arthur H. Thomas Co. Philadelphia, PA) to determine dry matter, crude protein, ether extract, crude fiber, and ash contents (AOAC, 1990). Table 1 presents the nutrient composition of both flushing diets.

Table 1: Nutrient composition of flushing diets.

Compositions	Diets (DM basis, %)	
	Flushing-1	Flushing-2
Ingredients, % DM basis	36	33
Rice bran	36	33
Cassava meal	-	12
Palm kernel meal	23	28
Coconut meal	30	23
Soybean meal	8	-
Urea-impregnated zeolite	-	1
Mineral mix†	3	3
Total	100	100
Nutrient composition, % DM basis	17.71	17.69
Crude protein	17.71	17.69
Ether extract	5	3.15
Crude fibre	13.33	12.48
Nitrogen free extract	53.2	56.16
Ash	10.76	10.52
Total digestible nutrient‡	60.19	60.28

† Composition (per kg): $ZnSO_4$ 1.24 g, di-calcium phosphate 20 g, organic chromium 3 mg. ‡TDN = 0.67 x DM (NRC, 1985).

The adaptation period took place within twelve days before feeding period. Because these cows had never been given concentrate diets before, the adaptation to flushing diets was done in stages. The first day to the second day, cows were given flushing diets and grass with a ratio of 25%: 75%, the third day to the fourth day 50%: 50%, the fifth day to the seventh day 75%: 25%, and the eighth day to the twelfth day was given 100% flushing ration. This adaptation period aims to make cattle become accustomed to flushing rations and to eliminate the cumulative effect of rations consumed before the adaptation period. Thus, the performance of cattle that appears reflects the effect of the experimental ration.

Cows on flushing diets; treatments 2 and 3 were fed the diets before allowed into the pasture area. Meanwhile, cows in treatment 1 were driven into the pasture areas as usually practiced by the cattle farmers daily. In a two-month feeding period, cows allocated to treatment 2 and 3 fed 2 kg of flushing diets at 07:00 daily before driving the cows to the pasture areas. All late-pregnant cows had access to drinking water that was available in the pond around the pasture area during the grazing time and afterward, all the cows were driven back to the paddock.

At the end of the 60 days of the feeding period, before morning feeding, five mL of venous blood from the coccygeal vein of each cow was collected in the EDTA-vacutainer and analyzed with atomic absorbance spectrophotometer Varian Spectra AA220 series for the blood mineral profile (Ca, Fe, Zn, and Cr) and *Vet Scan HM5* automatic analyser for the haematological profile including red blood cell, hemoglobin, hematocrit, mean platelet volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width concentration, platelets count, platelet hematocrit, mean platelet volume, platelet distribution width concentration, white blood cell, lymphocyte, monocyte, neutrophil, eosinophil, and basophil. The principle of blood mineral samples analysis by the AAS was based on absorption of light by free metallic ions. The study followed all protocols of the ethical guideline for animals arranged by the Directorate General of Livestock and Animal Health, Ministry of Agriculture of the Republic of Indonesia.

Statistical Analysis

The data were analyzed with the analysis of variance and means separated by Duncan's Multiple Range Test (Steel and Torrie, 1980). The differences were considered statistically significant if P <0.05. The Pearson correlation test was applied to evaluate the relationship between the blood mineral concentrations. The statistical analysis was conducted using IBM SPSS Statistics 24.0, 2018.

RESULTS AND DISCUSSION

Mean (± SD) values for blood mineral concentrations of Pasundan pregnant cows of three treatments are presented in Table 2. The higher (P <0.05) blood calcium (Ca) and iron (Fe) concentrations were obtained from the Pasundan pregnant cows fed either flushing-1 or flushing-2 diets. Meanwhile, the blood Zn and Cr showed similar concentrations among the treatments.

Blood calcium concentration in this study (Table 2) was lower than reported by Brscic et al. (2015) i.e. in the range of 8.2–10.8 mg/dL, and 11.45 ± 0.87 mg/dL (Onmaz et al., 2018). Study of Indonesia's exotic pregnant cows by Yuherman et al. (2017) showed higher blood Ca concentration (10.16 ± 0.44 mg/dL). Even though, feeding flushing diets increased (P <0.05) blood calcium concentration in the recent study, it was still below the critical limit of blood calcium level (8.00 mg/dL) as suggested by McDowell et al. (1993). Some authors suggested many factors, i.e. low dietary calcium, phosphorus, magnesium, vitamin D intake, and cows are unable to compensate for the dramatic increase in Ca needed for colostrum and milk production at calving affected the lower blood calcium concentration (Smith and Risco, 2005; Charbonneau et al., 2006; Gaignon et al., 2019; Hernández-Castellano et al., 2020). García et al. (2017) suggested that the lack of adequate levels of Ca increases the risk of developing postpartum diseases such as hypocalcemia. In addition, Masters et al. (2017) suggested that forages had complex mineral compositions meaning that grazing ewes may have an increased risk of direct or induced hypocalcemia. Apparently, the inclusion of 20 g di-calcium phosphate/kg mineral mix in flushing diets (Table 1) did not meet the calcium requirement of the late-pregnant cows two weeks before the predicted calving. Gelfert and Staufenbiel (2008) explained that the increase in calcium requirement of the late-pregnant cows because the concentration of ionized Ca [Ca^{2+}] increased in the blood of the cow in the last two to three weeks before calving. This increase leads to increase calcium urinary excretion, which is ensued by an increased calcium absorption in the intestine. Thus, the calcium diet level must be increased to meet the calcium requirement of the late pregnant cows in the last two to three weeks before calving.

Table 2: Blood mineral concentrations of Pasundan pregnant cows fed flushing diets.

Blood minerals†	Grazing	Grazing + flushing-1	Grazing + flushing-2
Ca (g/dL)	2.95^{b} ± 0.11	3.95^{a} ± 0.55	4.28^{a} ± 0.24
Fe (mg/L)	0.37^{b} ± 0.08	1.38^{a} ± 0.42	1.43^{a} ± 0.36
Zn (mg/L)	2.17 ± 0.98	1.36 ± 0.41	1.43 ± 0.36
Cr (µg/L)	1.54 ± 0.23	1.75 ± 0.12	1.68 ± 0.12

^{a,b}Different superscripts within similar row show significant differences (P <0.05); data presented as mean ± s.d.; Ca: Calcium, Fe: Ferrum (Iron), Zn: Zinc, Cr: Chromium.

Blood iron (Fe) concentration in grazing pregnant cows was lower than the critical level of 1.10 mg Fe/L recommended by McDowell (1985) for the tropical environment. Lower blood iron concentration results in anemia and affects reproduction adversely in the form of repeat breeding, requiring an increased number of inseminations per conception and occasionally leading to abortion (Kumar et al., 2011). The lower blood iron concentration in grazing pregnant cows in the recent study might indicate that the cows grazed low iron content forages. This result is supported by Onmaz et al. (2018) who reported that low serum iron concentration might be associated with long-term nutritional iron deficiency. Feeding flushing-1 or flushing-2 diets to the grazing pregnant cows increased (P <0.05) blood iron concentration (1.38 ± 0.42 mg/L, 1.43 ± 0.36 mg/L, respectively) over the critical level. This result is quite comparable to the blood iron concentration reported by Yuherman et al. (2017) and Hartman et al. (2018), i.e. 2.27 ± 0.19 mg/L and 1.86 – 2.09 mg/L, respectively. The results indicated that the iron content of both flushing diets fulfilled the iron requirement of the grazing pregnant cows.

Blood zinc concentration in grazing pregnant cows (2.17±0.98 mg/L) is slightly lower than the blood zinc concentration (2.5 ± 0.37 mg Zn/L) reported by Yuherman et al. (2017) but those are still higher than the blood zinc concentration in cows (0.14 – 0.22 mg/L) reported by Hüsamettin et al. (2015). Meanwhile, blood zinc concentration of pregnant cows fed either flushing-1 or flushing-2 diets (1.36 ± 0.41, 1.43 ± 0.36 mg/L, respectively) are in the normal range, but it is slightly higher than the blood zinc concentration (1.26 ± 0.019 mg/L) as reported by Hartman et al. (2018).

Blood chromium concentration in this study ranged from 1.54 ± 0.23 to 1.75 ± 0.12 µg Cr/L is lower than the blood chromium concentration of perinatal cows (3 – 5 µg Cr/L) reported by Pechova et al. (2002). Feeding chromium inclusion (3 mg Cr/kg) in the flushing diets did not change (P >0.05) the blood chromium concentration. This finding is alike previous study of Pechova and Paylata (2007) who reported that supplementation of 10 mg chromium per cow/day had no effect on the blood chromium concentration.

Table 3 shows the relationship between blood mineral concentrations. There were negative correlations between zinc and calcium (r= -562, P <0.05) or between zinc and iron (r= -0.679, P <0.05) when none of the control variables applied. The correlation did not occur between calcium and organic chromium (r= 0.429, P >0.05), iron and organic chromium (r= 0.480, P >0.05), or between zinc and organic chromium (r= -0.044, P >0.05). When organic chromium is applied as a control variable or it is excluded from the mineral mix, the negative correlations become worsen between zinc and calcium (r= -0.602, P <0.05) or between zinc and iron (r= -0.751, P <0.05) except for calcium and iron showed a positive correlation (r = 0.931, P <0.05). When zinc was applied as the control variable, a positive correlation occurred between calcium and iron (r= 0.926, P <0.05) or organic chromium and iron (r= 0.614, P <0.05) but not for organic chromium and calcium correlation (r= 0.489, P <0.05).

The negative correlations between zinc and calcium or between zinc and iron in this study is supported by the study of Saleh (2019) who reported that feeding high dietary calcium concentration reduced blood zinc concentration in sheep by decreasing the absorption of zinc. In addition, Bernhard et al. (2012) suggested that chromium suppressed iron availability. However, when organic chromium is excluded from the mineral mix as previously stated, the negative correlation occurred between zinc and calcium (r = - 0.602, P <0.05) or between zinc and iron (r = - 0.751, P <0.05). It was an implication that the inclusion of 3 mg organic chromium/kg mineral mix in both flushing diets reduced the adverse effect of negative correlation between calcium and zinc or between zinc and iron. Furthermore, when zinc was excluded from the analysis system or treated as the control variable, the negative correlation between minerals did not occur. The results indicated that ZnSO4, as an inorganic zinc source showed a low bioavailability when mixed with calcium or iron, but the bioavailability became higher when the chromium mineral was included. The adverse effect of calcium or iron on zinc might be reduced by using an organic zinc source as reported by some studies (Mallaki et al., 2015; Dresler et al., 2016; Marques et al., 2016; Harmanpreet et al., 2018).

Table 3: Correlation among blood mineral concentrations.

Correlations						
Control variables			Ca	Fe	Zn	Cr
none[†]	Ca	Correlation	1.000	0.944	-0.562	0.429
		Significance (2-tailed)	0.	0.000	0.029	0.111
		Df	0	13	13	13
	Fe	Correlation	0.944	1.000	-0.679	0.480
		Significance (2-tailed)	0.000	0.	0.005	0.070
		Df	13	0	13	13
	Zn	Correlation	-0.562	-0.679	1.000	-0.044
		Significance (2-tailed)	0.029	0.005	0.	0.877
		Df	13	13	0	13
	Cr	Correlation	0.429	0.480	-0.044	1.000
		Significance (2-tailed)	0.111	0.070	0.877	0.
		Df	13	13	13	0
Cr	Ca	Correlation	1.000	0.931	-0.602	
		Significance (2-tailed)	0.	0.000	0.023	
		Df	0	12	12	
	Fe	Correlation	0.931	1.000	-0.751	
		Significance (2-tailed)	0.000	0.	0.002	
		Df	12	0	12	
	Zn	Correlation	-0.602	-0.751	1.000	
		Significance (2-tailed)	0.023	0.002	0.	
		Df	12	12	0	
Zn	Ca	Correlation	1.000	0.926		0.489
		Significance (2-tailed)	0.	0.000		0.076
		Df	0	12		12
	Fe	Correlation	0.926	1.000		0.614
		Significance (2-tailed)	0.000	0.		0.020
		Df	12	0		12
	Cr	Correlation	0.489	0.614		1.000
		Significance (2-tailed)	0.076	0.020		0.

The correlations were statistically significant at $P < 0.05$. [†]Cells contain zero-order (Pearson) correlations. Ca: calcium, Fe: Ferrum (iron), Zn: Zinc, Cr: Chromium, Df: Degrees of freedom.

Mean (± SD) values for hematological profiles in Pasundan pregnant cows of three treatments and the normal reference values are presented in Table 4. The red blood cell (RBC), hemoglobin (Hb), and hematocrit (HCT) obtained from pregnant cows allowed extensive grazing plus flushing-2 showed higher values ($P < 0.05$) than the pregnant cows allowed extensive grazing or extensive grazing plus flushing-1. Both mean corpuscular hemoglobin (MCH) and platelet count (PLT) of pregnant cows treated with extensive grazing plus flushing-1 showed a lower value ($P < 0.05$) than the ones treated extensive grazing or extensive grazing plus flushing-2. The red blood cell distribution width concentration (RDWC) and PLT values were higher in pregnant cows treated with extensive grazing plus flushing-1 and extensive grazing plus flushing-2 than those of extensive grazing. The platelet distribution width concentration (PDWC) values were the highest ($P < 0.05$) in the pregnant cows treated with extensive grazing plus flushing-1 followed with extensive grazing plus flushing-2 and extensive grazing, respectively. Meanwhile, mean corpuscular volume (MCV), mean platelet volume (MPV), and mean corpuscular hemoglobin concentration (MCHC) showed similar values among the treatments. This similarity was also showed in white blood cell (WBC), Lymphocyte, Monocyte, Neutrophil, and Eosinophil. Basophil were lower ($P < 0.05$) in pregnant cows treated with extensive grazing plus flushing-2 than extensive grazing plus flushing-1 or extensive grazing, respectively.

Most haematological profiles of Pasundan pregnant cows were in hematological reference intervals of Merck Veterinary Manual (Merck, 2018), except for PLT ($61.22 \pm 13.99 \times 10^3/\mu L$) were below the normal reference intervals in the pregnant cows fed flushing-1 diets. Increased in RBC, Hb, HCT, RDWC, and PDWC of the pregnant cows fed flushing-2 diets ($P < 0.05$) revealed that urea-impregnated zeolite inclusion improved all blood parameters. Presumably, zeolite increases iron (Fe) absorption and impact positively on the RBC, Hb, HCT, RDWC, and PDWC. In the previous study, Katsoulos et al. (2005) concluded that feeding concentrate containing 1.25 and 2.5% clinoptilolite had no adverse effect on RBC, Hb, and WBC. The main function of RBC is to transport oxygen, which binds to Hb (Roland et al., 2014). Thus, higher RBC ($6.45 \pm 0.30 \times 10^6/\mu L$), Hb ($10.64 \pm 0.82$ g/dL), HCT (35.21 ± 0.92 %), RDWC (20.81 ± 0.39 %), and PDWC (36.05 ± 0.04 %) indicated that the flushing-2 diets containing urea-impregnated zeolite improved oxygen transport in blood. The RBC value of the current study was similar to the RBC value ($6.4 \pm 0.37 \times 10^6/\mu L$) of cows raised in lowland areas reported by Mariana et al. (2019) and within the range of pregnant dry period ($4.87 - 6.68 \times 10^6/\mu L$) reported by Halloz et al. (2015).

Lower platelet ($61.22 \pm 13.99 \times 103/\mu L$) below the normal reference intervals in the pregnant cows fed flushing-1 diets might generate an adverse effect on the platelet. Roland et al. (2014) reported that a decrease in the platelet fragments observed in iron-deficiency anemia.

Table 4: Haematological profiles of Pasundan late-pregnant cows fed flushing diets.

Haematological profiles†	Grazing	Grazing+flushing-1	Grazing+flushing-2	Normal Ref‡
RBC (10^6/μL)	$5.17^b \pm 0.29$	$5.29^b \pm 0.08$	$6.45^a \pm 0.30$	5 – 10
Hb (g/dL)	$8.29^b \pm 0.54$	$8.11^b \pm 0.54$	$10.64^a \pm 0.82$	8 – 15
HCT (%)	$26.57^b \pm 1.86$	$27.66^b \pm 3.03$	$35.21^a \pm 0.92$	24 – 45
MCH (pg)	$16.05^a \pm 0.12$	$15.26^b \pm 0.74$	$16.44^a \pm 0.58$	12.5 – 17.5
RDWC (%)	$19.91^b \pm 0.86$	$20.80^a \pm 0.23$	$20.81^a \pm 0.39$	19.4 – 24
PLT (10^3/μL)	$317.10^a \pm 103.81$	$61.22^b \pm 13.99$	$165.51^b \pm 96.43$	100 – 800
PDWC (%)	$35.25^c \pm 0.04$	$37.20^a \pm 0.01$	$36.05^b \pm 0.04$	-
MCV (fL)	50.99 ± 0.78	52.07 ± 4.67	54.52 ± 1.17	39 – 55
MPV (fL)	8.04 ± 0.42	7.55 ± 0.12	7.54 ± 0.66	4.5 – 7.5
MCHC (g/dL)	31.30 ± 0.23	29.58 ± 1.40	30.13 ± 1.59	30 – 36
WBC (10^3/μL)	9.80 ± 2.19	9.40 ± 0.90	9.61 ± 0.54	5.5 – 19.5
Lymphocyte (10^3/μL)	3.90 ± 0.47	3.91 ± 0.20	4.36 ± 0.55	1.5 – 7.0
Monocyte (10^3/μL)	0.09 ± 0.03	0.08 ± 0.01	0.09 ± 0.01	0 – 1.5
Neutrophil (10^3/μL)	4.92 ± 2.78	4.43 ± 0.90	4.28 ± 0.28	2.5 – 14
Eosinophil (10^3/μL)	0.66 ± 0.17	0.72 ± 0.10	0.79 ± 0.19	0 – 1
Basophil (10^3/μL)	$0.23^a \pm 0.11$	$0.25^a \pm 0.10$	$0.10^b \pm 0.05$	0 – 0.2

† Different superscripts within similar row show significant differences ($P < 0.05$); ‡ Merck (2018). RBC: red blood cell, Hb: haemoglobin, HCT: haematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDWC: red blood cell distribution width concentration, PLT: platelet count, MPV: mean platelet volume, PDWC: platelet distribution width concentration, WBC: white blood cell.

In this study, all flushing diets contain mineral mix of organic chromium and zinc to anticipate any stresses occurred during the late gestation period. Even though organic chromium has a high bioavailability as a source of chromium (Bernhard et al., 2012), it suppresses iron availability as both minerals compete for transferrin protein and zinc competes with iron for metal transporters (Bjørklund et al., 2017). However, when chromium is excluded from the mineral mix, worsens the negative correlation between zinc and iron and in turn, may deteriorate the iron deficiency. Thus, a negative interaction between zinc-iron elucidate lower platelet value in the late-pregnant cows fed the flushing-1 diets. Although the pregnant cows fed flushing-2 diets showed lower platelet (165.51 ± 96.43 × 103/μL) than the ones allowed in extensive grazing (317.10 ± 103.81 × 103/μL), the platelet value of pregnant cow fed flushing-2 diets was still within the normal reference interval (100 – 800 × 103/μL). Apparently, the urea-impregnated zeolite inclusion in the flushing-2 diets eliminates chromium-iron and zinc-iron antagonist because of the zeolite cation exchange capacities.

White blood cell, lymphocyte, monocyte, neutrophil, eosinophil, and basophil were in a range of normal references. These data indicate that feeding flushing-1 or flushing-2 diets have no negative effect on the parameters above. Higher basophil counts, as shown in the grazing late-pregnant cows or in the cows fed flushing-1 diets may indicate a parasitic infection as proposed by Karasuyama et al. (2018) that in several animal species, including cattle, guinea pigs, rabbits and mice, basophil accumulation is observed at the tick re-infestation site. Feeding flushing-2 diets decreased basophil counts even though the basophil counts were still in the normal reference interval. These data indicated that urea-impregnated zeolite inclusion in flushing diets might improve the defense mechanism of the cows although the mechanism was not clear yet. Probably, cation exchange capacity of zeolite may kill the infected parasites along gastrointestinal tracts of the cows (Papaioannou et al., 2005).

CONCLUSIONS AND RECOMMENDATIONS

Late-pregnant Pasundan cows raised in the grazing system showed calcium and iron mineral deficiency. Feeding urea-impregnated zeolite inclusion in flushing diets to the late-pregnant cows under extensive grazing improved the calcium and iron availabilities and most hematological parameters. To keep the late-pregnant cows healthy, it is recommended for cattle farmers to provide flushing diets for the late-pregnant cows before the cattle are grazed in the pasture even though the flushing diets did not improve the blood zinc and chromium concentration.

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AUTHOR'S CONTRIBUTIONS

Dede Kardaya designed the study, executed the project, and analyzed data. Elis Dihansih assisted in the design of the study and data analyses. Deden Sudrajat designed the research and edited the draft version of the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

REFERENCES

- AOAC (1990). Official methods of analysis, 16th edn. Association of official analytical chemists. Arlington, VA, USA.
- Baharun A, Arifiantini RI, Yusuf TL (2017). Freezing capability of pasundan bull sperm using tris-egg yolk, tris-soy, and andromed® diluents. J. Kedokteran Hewan, 11(1): 45-49. https://doi.org/10.21157/j.ked.hewan.v11i1.5810
- Bakrie B, Hogan J, Liang JB, Tareque AMM, Upadhyay RC (1996). Ruminant nutrition and production in the tropics and subtropics. Canberra, Australia. Australian Centre for International Agricultural Research.
- Bernhard BC, Burdick NC, Rathmann RJ, Carroll JA, Finck DN, Jennings MA, Young TR, Johnson BJ (2012). Chromium supplementation alters both glucose and lipid metabolism in feedlot cattle during the receiving period. J. Anim. Sci., 90: 4857-4865. https://doi.org/10.2527/jas.2011-4982
- Bjørklund G, Aaseth J, Skalny AV, Suliburska J, Skalnaya MG, Nikonorov AA, Tinkov AA (2017). Interactions of iron with manganese, zinc, chromium, and selenium as related to prophylaxis and treatment of iron deficiency. J. Trace Elem. Med. Biol., 41: 41-53. https://doi.org/10.1016/j.jtemb.2017.02.005
- Brscic M, Cozzi G, Lora I, Stefani AL, Contiero B, Ravarotto L, Gottardo F (2015). Reference limits for blood analytes in Holstein late-pregnant heifers and dry cows: Effects of parity, days relative to calving, and season. J. Dairy Sci., 98: 7886–7892. https://doi.org/10.3168/jds.2015-9345
- Charbonneau E, Pellerin D, Oetzel GR (2006). Impact of lowering dietary cation-anion difference in nonlactating dairy cows: a meta-analysis. J. Dairy Sci., 89(2): 537–548. https://doi.org/10.3168/jds.S0022-0302(06)72116-6
- Delima M (2008). The effect of urea molasses mineral block administration on mineral deficiency content of cattle's serum mineral. Agripet, 8 (1): 45-49. https://doi.org/10.17969/agripet.v8i1.608
- Djokovic, RD, Kurcubic, VS, Ilic ZZ (2014). Blood serum levels of macro - and micronutrients in transition and full lactation cows. Bulgarian J. Agric. Sci. 20 (3): 715-720.
- Dresler S, Illek J, Zeman L (2016). Effects of organic zinc supplementation in weaned calves. Acta Vet., 85: 49-54. https://doi.org/10.2754/avb201685010049
- Eisenberg SWF, Ravesloot L, Koets AP, Grünberg W (2019). Effect of dietary phosphorus deprivation on leukocyte function in transition cows. J. Dairy Sci., 102(2): 1559-1570. https://doi.org/10.3168/jds.2018-15417
- Gaignon P, Le Grand K, Laza-Knoerr AL, Hurtaud C, Boudon, A (2019). Effect of calcium intake and the dietary cation-anion difference during early lactation on the bone mobilization dynamics throughout lactation in dairy cows. PLoS One, 14(11): e0218979. https://doi.org/10.1371/journal.pone.0218979
- García CAC, Prado FMG, Galicia, LL, Borderas TF (2017). Reference values for biochemical analytes in Mexican dairy farms: interactions and adjustments between production groups. Arq. Bras. Med. Vet. Zootec., 69(2): 445-456.
- Gelfert CC, Staufenbiel R (2008). The role of dietary calcium concentration in the use of anionic salts to prevent parturient paresis in dairy cows. Berl. Munch Tierarztl. Wochenschr., 121(7-8): 256-262.
- Halloz HF, Meliani S, Benallou B, Ghazi K (2015). Haematological parameters during late gestation in dairy cows raised in Tiaret, Algeria. Global Vet., 15(1): 45-47.
- Harmanpreet S, Grewal RS, Kaur S, Kaur J, Singh C, Lamba JS, Malhotra P (2018). Effect of organic Cu and Zn on the performance of pre-ruminant buffalo calves. Int. J. Curr. Microbiol. App. Sci., 7(5): 763-769.
- Hartman S, Genther-Schroeder O, Hansen S (2018). Comparison of trace mineral repletion strategies in beef cattle to overcome a high antagonist diet. Anim. Ind. Rep., AS664: 1-5. https://doi.org/10.31274/ans_air-180814-566
- Hernández-Castellano L, Hernandez L, Bruckmaier R (2020). Review: Endocrine pathways to regulate calcium homeostasis around parturition and the prevention of hypocalcaemia in periparturient dairy cows. Animal, 14(2): 330-338.
- Hüsamettin E, Özkan Ş, Şevket A, Meryem E, Bayram G (2015). Comparing levels of certain heavy metals and minerals and antioxidative metabolism in cows raised near and away from highways. Turk J. Vet. Anim. Sci., 39: 322-327. https://doi.org/10.3906/vet-1412-33
- Karasuyama H, Tabakawa Y, Ohta T, Wada T, Yoshikawa S (2018). Crucial role for basophils in acquired protective immunity to tick infestation. Front. Physiol., 9: 1-8.
- Kardaya D, Wiryawan KG, Parakkasi A, Winugroho HM (2018). Effects of three slow-release urea inclusions in rice straw-based diets on yearling Bali bull performances. South Afr. J. Anim. Sci., 48(4): 751-757.
- Katsoulos PD, Roubies N, Panous N, Christaki E, Karatzanos P, Karatzias H (2005). Effects of long term feeding dairy cows on a diet supplemented with clinoptilolite on certain haematological parameters. Vet. Med. Czech. 50(10): 427-431. https://doi.org/10.17221/5644-VETMED
- Khachlouf K, Hamed H, Gdoura R, Gargouri A (2019). Effects of dietary Zeolite supplementation on milk yield and composition and blood minerals status in lactating dairy cows. J. Appl. Anim. Res., 47: 54–62.
- Kumar S, Pandey AK, Razzaque WAA, Dwivedi DK (2011).

Importance of micro minerals in reproductive performance of livestock. Vet. World, 4(5): 230-233. https://doi.org/10.5455/vetworld.2011.230-233
- Mallaki M, Norouzian MA, Khadem AA (2015). Effect of organic zinc supplementation on growth, nutrient utilization, and plasma zinc status in lambs. Turk. J. Vet. Anim. Sci., 39: 75-80. https://doi.org/10.3906/vet-1405-79
- Mariana E, Sumantri C, Astuti DA, Anggraeni A, Gunawan A (2019). Thermoregulation, haematological profile, and productivity of holstein friesian under heat stress at different land elevations. Bull. Anim. Sci., 43(1): 8-16. https://doi.org/10.21059/buletinpeternak.v43i1.37648
- Marques RS, Cooke RF, Rodrigues MC, Cappellozza BI, Mills RR, Larson CK, Moriel P, Bohnert DW (2016). Effects of organic or inorganic cobalt, copper, manganese, and zinc supplementation to late-gestating beef cows on productive and physiological responses of the offspring. J. Anim. Sci. 94: 1215–1226. https://doi.org/10.2527/jas.2015-0036
- Masters DG, Hancock S, Refshauge G, Robertson S, Bhanugopan M, Friend M, Thompson AN (2017). Mineral status of reproducing ewes grazing vegetative cereal crops. Anim. Prod. Sci., 58(11): 2049-2060. https://doi.org/10.1071/AN16530
- Masters DG, Norman HC, Thomas DT (2019). Minerals in pastures are we meeting the needs of livestock? Crop Pasture Sci., 70(12): 1184-1195. https://doi.org/10.1071/CP18546
- McDowell LR (1985). Nutrition of grazing ruminants in warm climates. San Diego: Academic Press. https://doi.org/10.1016/B978-0-12-483370-8.50025-2
- McDowell LR, Conrad JH, Glen-Hembry F (1993). Minerals for grazing ruminants in tropical regions. 2nd ed. Animal Science Department Center for Tropical Agriculture, University of Florida.
- Merck (2018). Merck Veterinary Manual: Hematologic reference range. Merck Sharp and Dohme Corp: A subsidiary of Merck and Co. Inc. Kenilworth NJ USA.
- Moeini MM, Karami H, Mikaeili E (2009). Effect of selenium and vitamin E supplementation during the late pregnancy on reproductive indices and milk production in heifers. Anim. Reprod. Sci., 114(1-3): 109-114.
- Morrison DG, Spitzer JC, Perkins JL (1999). Influence of prepartum body condition score change on reproduction in multiparous beef cows calving in moderate body condition. Anim. Sci., 77: 1048–1054. https://doi.org/10.2527/1999.7751048x
- Ndlovu T, Chimonyo, M Okoh AI, Muchenje V, Dzama K, Raats JG (2017). Assessing the nutritional status of beef cattle: current practices and future prospects. Afr. J. Biotechnol., 6 (24): 2727-2734. https://doi.org/10.5897/AJB2007.000-2436
- Nicholson MJ, Sayers AR (1987). Reliability, reproducibility, and sequential use of condition scoring of Bos indicus cattle. Trop. Anim. Health Prod., 19: 127-135.
- Onmaz AC, Güneş V, Çınar M, Çitil M, Keleş I (2018). Hematobiochemical profiles, mineral concentrations, and oxidative stress indicators in beef cattle with pica. Ital. J. Anim. Sci., pp. 1-6. https://doi.org/10.1080/1828051X.2018.1501283
- Papaioannou D, Katsoulos PD, Panousis N, Karatzias H (2005). The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: A review. Microporous and Mesoporous Mater., 84(1-3): 161-170. https://doi.org/10.1016/j.micromeso.2005.05.030
- Pechova A, Pavlata L (2007). Chromium as an essential nutrient: a review. Vet. Med., 52(1): 1-18. https://doi.org/10.17221/2010-VETMED
- Pechova A, Podhorsk A, Lokajova E, Pavlata L, Illek J (2002). Metabolic effects of chromium supplementation in dairy cows in the peripartal period. Acta Vet. Brno., 71: 9-18. https://doi.org/10.2754/avb200271010009
- Pradhan SM, Khanal B, Hamal N (2011). Effect of steaming up practice in late pregnant stage in dairy cows. Subarna Man Pradhan and Suresh Kumar Wagle (eds.). pp. 241.
- Roland L, Drillich M, Iwersen M (2014). Haematology as a diagnostic tool in bovine medicine. J. Vet. Diagn. Invest. 26(5): 592–598. https://doi.org/10.1177/1040638714546490
- Saleh WMM (2019). Clinical and haematological profiles due to cases of minerals deficiency in local ewes at Basra, Iraq. Adv. Anim. Vet. Sci., 7(4): 315-320.
- Smith BI, Risco CA (2005). Management of periparturient disorders in dairy cattle. Vet. Clin. North Am. Food Anim. Pract., 21: 503–521. https://doi.org/10.1016/j.cvfa.2005.02.007
- Sripad K, Kowalli S, Metri R (2014). Haematological profile of Khillar breed of cattle in Karnataka. Vet. World, 7(5): 311-314.
- Steel RGD, Torrie JH (1980). Principles and procedures of statistics. A biometrical approach, 2nd edn. McGraw-Hill Book Company, London.
- Sudrajat D, Toharmat T, Boediono A, Permana IG, Arifiantini I, Amir F (2011). Mineral utilization in rams fed ration supplemented with different chromium, calcium, and cation-anion balance. Med. Pet., 34(3): 212-218. https://doi.org/10.5398/medpet.2011.34.3.212
- Yuherman, Reswati, Yulianti FK, Indahwati K (2017). Haematological and mineral profiles of reproductive failure of exotic breed cattle in Payakumbuh, West Sumatra, Indonesia. Pak. J. Biol. Sci., 20: 390-396. https://doi.org/10.3923/pjbs.2017.390.396