Research article

Parity and season affect hematological, biochemical, and milk parameters during the early postpartum period in grazing dairy cows from high-tropics herds

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ABSTRACT

Improving the early detection of animals or herds at increased risk for diseases, reduced milk yield or impaired reproductive performance should be an essential component of herd health programs. The present study aimed to describe the findings of hematological, biochemical, and milk analytes of grazing cows from tropical dairy herds during the early postpartum period, and the effects of parity and calving season. In the North of Antioquia, Colombia which is the major area of specialized dairy production in the country, a longitudinal study comprising 260 dairy cows selected at calving, was conducted. Blood and milk sampling were made to establish red blood cell count, white blood cell count, minerals, protein traits and, milk composition. Variables were checked for normal distribution. The Box-Cox transformation was used when necessary. Variables were statistically analyzed using a GLM model considering parity and calving season as fixed effects. Using a parametric or non-parametric method based on the distribution of the variables, single average values, and 90% confidence intervals were determined considering fixed effects founds in the GLM model. Blood values affected by parity in lactating dairy cows grazing in highland tropical herds included serum calcium levels, red blood cell counts, hematocrit, hemoglobin, mean corpuscular hemoglobin, and total eosinophil counts, whereas blood values affected by season included: total leukocyte counts, total and differential lymphocyte counts, serum albumin and globulin concentration, and serum albumin:globulin ratio (P < 0.05). Data from our study could be used for comparison studies between lactating dairy cows within tropical herds or between tropical and seasonal dairy herds.

1. Introduction

In dairy cows, metabolic and hormonal changes that occur during the transition period are required for milk yield during lactation, rendering high metabolic output as a priority that is maintained at the cost of other reproductive and metabolic processes. Therefore, it occurs a corporal redistribution of nutrients, reduction in dry matter intake and, negative energy balance with immunosuppression, and predisposition to several diseases [1, 2]. Although the transition period is associated with a peak incidence of diseases, their effects on dairy cow health and milk yield extend far into the following lactation [3]. At the cow-level, the

occurrence of the disease may result in lower milk yield, impaired fertility, and, subsequently, increased culling rates. Improving the early detection of animals or herds at increased risk for disease, reduced milk yield, or poor reproductive performance should be an essential component of herd health programs. Laboratory blood analysis must be promoted as a necessary tool for dairy veterinarians in their assessment of cow health status [4, 5]. The first step through the evaluation of corporal analytes is the comparison of laboratory results with a set of reference values representing those values of healthy animals. Population-based reference intervals are commonly used in the clinical decision-making process. In veterinary medicine, the diversity of species and complexity

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Table 1. Hematological and biochemical parameters, and some milk analytes in lactating dairy cows grazing in highland tropics.

Analytes	n	Mean	SD	Min.	Max.	W
Minerals						
Calcium (mg/dl)	202	9.2	1	6.27	11.67	0.97
Phosphorus (mg/dl)	202	7.8	1.7	3.16	10.76	0.94
Magnesium (mg/dl)	202	2.7	0.7	1.2	4.9	0.97
Red Blood cell count						
Erythrocytes (x 10^6/mm^3)	202	7.1	0.9	4.28	7.9	0.99
Hematocrit (%)	202	34.2	4.3	21.6	45.5	0.98
Hemoglobin (g/dl)	201	11.1	1.2	7.5	14.4	0.96
Mean Corpuscular Volume (fl)	194	49.3	3.4	42	57	0.94
Mean Corpuscular Hemoglobin (pg)	197	15.9	1.2	11.6	18.9	0.88
Mean Corp. Hb. Concentration (g/dl)	195	32.5	2	30	44.8	0.39
White Blood cell count						
Leucocytes (x 10^3/mm^3)	197	9.5	3.6	3.8	20.7	0.89
Neutrophils (x 10^3/mm^3)	202	4.1	2.9	0.47	16.3	0.85
Neutrophils (%)	202	40.4	18.1	1	89	0.98
Lymphocytes (x 10^3/mm^3)	194	4.8	2.3	1.2	13.73	0.89
Lymphocytes (%)	202	52.2	18.1	7	88	0.98
Eosinophils (x 10^3/mm^3)	202	0.5	0.4	0	2	0.88
Eosinophils (%)	202	5.2	5.3	0	26	0.86
Monocytes (x 10^3/mm^3)	199	0.1	0.2	0	1	0.82
Monocytes (%)	199	1.5	2.1	0	10	0.78
Basophils (x 10^3/mm^3)	202	0	0	0	0.19	0.76
Basophils (%)	202	0.1	0.2	0	1	0.87
Bands (x 10^3/mm^3)	202	0	0.1	0	0.73	0.73
Bands (%)	202	0.3	1.1	0	8	0.75
Platelets (x 10^3/mm^3)	202	550.1	205	162	1092	0.96
Protein traits						
Total Protein (g/dl)	202	6.8	0.6	5.36	8.61	0.99
Albumin (g/dl)	202	3.5	0.4	2.32	4.45	0.97
Globulin (g/dl)	202	2.7	0.6	1.5	4.55	0.98
Albumin/Globulin ratio	202	1.4	0.4	0.54	2.8	0.97
Fibrinogen (mg/dl)	202	642	253.3	200	1200	0.98
Milk analytes						
Milk Fat (%)	202	3.4	2.7	0.41	13.62	0.86
Milk Protein (%)	202	3.6	1	2.37	10	0.69
Fat: Protein Ratio	202	0.94	2.7	0.17	1.36	0.66
Total Solids (%g/100g)	202	10.9	3.7	2.49	21.3	0.94
Lactose (%g/100g)	202	6.4	1.41	1.41	26.9	0.55
Milk Urea Nitrogen (mg/dl)	201	16.4	5	4.5	29.5	0.98
Acetone (mM)	193	0.1	0.1	0	0.45	0.8
Beta-Hydroxy-Butyrate (mM)	202	0	0	0	0.24	0.76

W = Saphiro Wilk Test (<0,9 was Box-Cox transformed).

in how they can be sub-grouped must be considered, making necessary to understand the characteristics of the population that is being sampled to achieve a more accurate interpretation of the results [6, 7].

Some studies describe the values for different biochemical and hematological analytes in dairy cows during the late pregnancy, the transition period [6, 7, 8], and the postpartum period [7, 9, 10]. Variations in these values in dairy cows and late-pregnant heifers considering the effects of parity, stage of lactation, days relative to calving, and season were established [4, 11]. However, we found no information for biochemical analytes during the early postpartum period in dairy cows from grazing in tropical herds. The present study aimed to describe the findings for hematological, biochemical and milk analytes (Using the methods reported to define the reference limits and 90% confidence intervals in veterinary medicine) [12, 13], of lactating grazing cows from high tropics dairy herds during the first week postpartum, by testing the effects of parity and calving season.

2. Materials and methods

2.1. Ethical statement

All animal studies were conducted following the experimental practices and standards approved by the Institutional Board an Animal Subjects of Experimentation, University of Antioquia, Colombia (Act # 111, June 8, 2017). All cows were managed according to the standard procedures for clinical exam and blood and milking sampling, avoiding or minimizing the conditions affecting animal welfare.

2.2. Study population and experimental design

In the dairy region of Northern Antioquia, Colombia, a longitudinal study comprising 260 dairy cows selected at calving was conducted between January 1st to November 30th, 2018. Seven dairy herds were

Table 2. Reference values for hematological and biochemical parameters, and some milk analytes in lactating dairy cows grazing in highland tropics.

Analytes	Media ±SD	Reference value	90% CI
Minerals			
Phosphorus (mg/dl)	7.8 ± 0.7	4.0–10.4	3.4–10.7
Magnesium (mg/dl)	2.7 ± 0.7	1.6–4.3	1.5–4.5
Blood cell counts (Red)			
Mean Corpuscular Volume (fl)	49.3 ± 3.4	43.0–56.0	42.3–57.0
Mean Corp. Hb. Concentration (g/dl)	32.5 ± 2.0	30.1–36.7	30.0–43.0
Blood cell counts (White)			
Neutrophils (x 10^3/mm^3)	4.1 ± 2.9	0.7–12.5	0.7–16.2
Neutrophils (%)	40.4 ± 18.1	9.2–77.9	6.0–88.0
Monocytes (x 10^3/mm^3)	0.2 ± 0.2	0.0–0.9	0.0–1.1
Monocytes (%)	1.7 ± 2.6	0.0–9.9	0.0–12.0
Platelets (x 10^3/mm^3)	564.4 ± 226.8	208.4–1090.3	185–1292
Protein traits			
Fibrinogen (mg/dl)	642 ± 253.3	151–1143.2	97.9–1185.3
Total Protein (g/dl)	6.8 ± 0.6	5.6–8.1	5.4–8.2
Milk analytes			
Milk Fat (%)	3.4 ± 2.7	0.5–11.1	0.5–13.6
Milk Protein (%)	3.6 ± 1.0	2.5–6.5	2.5–9.0
Fat/Protein Ratio	1.0 ± 1.1	0.1–3.8	0.1–5.5
Total Solids (%g/100g)	10.9 ± 3.7	3.9–18.8	2.6–21.0
Lactose (%g/100g)	6.4–4.7	3.4–20.9	2.3–23.9
MUN (mg/dl)	16.4 ± 5.0	8.0–26.7	6.2–28.4
Acetone (mM)	0.1 ± 0.1	0.0–0.4	0.0–0.4

SD = Standard Deviation.
CI = Confidence Interval.
Media, CI 90% and suggested reference values according to the data from our study for hematological and biochemical parameters, and some milk analytes in lactating dairy cows grazing in highland tropics.

Table 3. Reference values for analytes affected by parity in lactating dairy cows grazing in highland tropics.

Parity		Minerals	Blood cell counts (Red)				Blood cell counts (Withe)			
		Calcium	Erythrocytes	Hematocrit	Hemoglobin	MCH	Eosinophils	Eosinophils	Basophils	Basophils
		(mg/dl)	(x 10^6/mm^3)	(%)	(g/dl)	(pg)	(x 10^3/mm^3)	(%)	(x 10^3/mm^3)	(%)
1	Media ±SD	9.6 ± 0.8	7.5 ± 0.8	36.1 ± 3.4	11.6 ± 0.9	15.5 ± 1.4	3.8 ± 4.7	0.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
	Ref. value	7.2–11.6	5.7–9.6	27.3–43.1	8.9–13.7	11.7–18.3	0.0–21.6	0.0–1.9	0	0
	90% CI	7.1–11.7	5.7–9.7	27.1–44.3	8.8–13.7	11.7–18.3	0.0–22.0	0.0–1.9	0	0
2	Media ±SD	9.1 ± 0.8	7.1 ± 0.7	34.7 ± 4.4	11.3 ± 1.4	15.7 ± 1.1	0.5 ± 0.4	5.6 ± 5.2	0.0 ± 0.0	0.1 ± 0.3
	Ref. value	7.5–10.8[a]	5.2–8.5[a]	22.0–43.1	8.6–16.5[a]	13.6–17.7	0.0–19.0[a]	0.0–17.8[a]	0.0–0.2[a]	0.0–1.0[a]
	90% CI	7.4–10.8	5.2–8.5	21.6–43.1	8.5–17.5	13.5–17.8	0.0–2.0	0.0–18.0	0.0–0.2	0.0–1.0
3	Media ±SD	9.3 ± 0.9	6.9 ± 0.8	33.8 ± 4.3	11.1 ± 1.2	16.0 ± 1.0	0.4 ± 0.4	6.4 ± 6.3	0.0 ± 0.0	0.1 ± 0.3
	Ref. value	6.4–11.1[a]	5.2–8.6[a]	23.4–45.1[a]	8.5–14.3	13.6–18.4[a]	0.0–1.5	0.0–17.9	0.0–0.1[a]	0.0–1.0[a]
	90% CI	6.3–11.2	5.2–8.7	22.9–45.5	8.4–14.4	13.5–18.4	0.0–1.5	0.00–22.8	0.0–0.1	0.0–1.0
4	Media ±SD	8.9 ± 1.1	7.2 ± 1.0	34.3 ± 4.3	11.3 ± 1.2	15.9 ± 1.2	0.6 ± 0.5	4.4 ± 4.2	0.0 ± 0.0	0.1 ± 0.2
	Ref. value	6.1–10.7[a]	4.7–8.9[a]	25.5–43.2[a]	8.8–13.8[a]	13.3–18.5[a]	0.0–3.0[a]	0.0–16.8[a]	0	0.0–0.6
	90% CI	4.6–11.0	4.7–9.3	23.2–45.2	8.2–14.5	12.7–19.1	0.0–4.7	0.0–17.0	0.0–1.0	0.0–0.9
5 to 8	Media ±SD	9.1 ± 1.1	6.5 ± 1	31.8 ± 4.3	10.4 ± 1.3	16.5 ± 1.2	0.5 ± 0.4	6.4 ± 5.9	0.0 ± 0.0	0.0 ± 0.0
	Ref. value	6.3–11.1[a]	4.4–8.6[a]	22.9–40.6[a]	7.7–13.2[a]	13.8–18.9[a]	0.0–1.5[a]	0.0–18.4[a]	0	0
	90% CI	5.1–11.5	3.9–9.1	20.8–42.9	7.1–13.8	13.2–19.4	0.0–1.8	0.0–22.0	0	0

[a]Differences on reference value and 95% IC (P < 0,05), compared with primiparous cows in the Multi-Level Generalized Linear Model.
SD = Standard Deviation; CI = Confidence Interval. MCH = Mean Corpuscular Hemoglobin.

purposively selected considering the following selection criteria: (i) having similar nutritional management, (ii) similar herd size (number of cows), (iii) milk yield ranging from 23 to 32 kg/day, (iv) the existence of standard records of milk yield and cows reproduction, and (v) the commitment of producers to provide all information requested. All lactating cows in each herd were selected at calving, according to the following criteria: (i) healthy status at clinical exam performed one week before the expected calving day, (ii) normal calving without the occurrence of dystocia, fetal membranes retention, mastitis, or postpartum uterine disease, (iii) clinically healthy at the semiological exam after calving, (iv) postpartum period without the occurrence of metabolic diseases such as ketosis, hypocalcemia or downer cow syndrome, and (v) Not having received medical treatment or administration of drugs before or during calving.

Table 4. Reference values for analytes affected by calving season (CS) in lactating dairy cows grazing in highland tropics.

Calving Season		White cell counts (Red)			Protein traits				Milk
		Leucocytes	Lymphocytes	Lymphocytes	Albumin Ab	Globulin Gb	Ab/Gb		MBHB
		(x 10³/mm³)	(x 10³/mm³)	(%)	(g/dl)	(g/dl)	ratio		(mM)
Jan–Mar	Media ±SD	11.4 ± 5.2	6.8 ± 4.6	57.4 ± 19.0	3.1 ± 0.5	2.9 ± 0.6	1.1 ± 0.4		0.0 ± 0.0
	Reference value	5.1–26.7	2.4–37.5	19.9–97.4	2.1–4.2	1.6–4.2	0.6–2.2		0.0–0.1
	90% CI	4.6–34.7	2.1–37.5	13.4–99.0	1.9–4.5	1.6–4.5	0.6–2.7		0.0–0.1
Apr–Jun	Media ±SD	9.5 ± 4.2	5.1 ± 2.4	55.1 ± 17.5	35 ± 0.4	2.7 ± 0.6	1.3 ± 0.3		0.0 ± 0.0
	Reference value	4.1–22.9[b]	1.5–10.5[b]	15.2–84.9	2.4–4.2[b]	1.9–4.3[b]	0.7–2.1[b]		0.0–0.2[b]
	90% CI	4.0–23.3	0.7–15.8	11.0–86.0	2.3–4.5	1.6–4.6	0.5–2.2		0.0–0.2
Jul–Sep	Media ±SD	9.8 ± 3.7	4.6 ± 2.7	48.1 ± 17.6	3.6 ± 0.3	2.6 ± 0.6	1.5 ± 0.4		0.0 ± 0.0
	Reference value	4.0–18.9[b]	1.55–13.7[b]	18.0–88.0[b]	2.9–4.3[b]	1.6–4.1[b]	0.8–2.4[b]		0.0–0.1
	90% CI	3.8–23.2	1.2–16.1	7.0–88.0	2.4–4.4	1.5–4.1	0.7–2.5		0.0–0.1
Oct–Nov	Media ±SD	7.8 ± 2.2	3.4 ± 1.4	46.0 ± 17.8	3.9 ± 0.3	2.6 ± 0.8	1.6 ± 0.6		0.0 ± 0.0
	Reference value	3.0–13.0[b]	1.2–6.7[b]	5.3–86.7	2.6–4.4[b]	2.6–4.4	0.7–3.6[b]		0.0–0.2[b]
	90% CI	1.3–15.0	0.0–8.0	5.3–86.7	2.6–4.5	0.1–4.5	0.5–5.0		0.0–0.2

[b]Differences on reference value and 95% IC (P < 0,05), compared to primiparous cows in the Multi-Level Generalized Linear Model.

n = Number of samples after outlier elimination. CI = Confidence Interval. MBHB = Milk Beta-Hydroxy-Butyrate.

The herds are located in one ot the most important dairy regions of Colombia [15], sharing several characteristics to the other dairy regions in Colombia regarding nutritional and productive management: predominantly Holstein cows grazing in *kikuyu grass* (*Pennisetum clandestinum*) *ad libitum*, plus twice daily concentrate food supplementation during milking (commercial concentrate food containing 16-to-8% crude protein, 1.75 kcal Net Energy of Lactation, for every 4–5 L milk yield)

plus mineral-supplemented salt (100–150 gr/cow/day of mineral salt with 26% sodium chloride, 15% calcium, 5% phosphorus, 5% sulfur and 1% magnesium is supplied *ad libitum*). The northern Antioquia, Colombia, represents 80% of the total dairy cows in Antioquia and produces 85% of the total milk production of the region [14]. The farms are located in an area ranges at an altitude from 2.600 to 3.129 m, temperature between 3 to 16 °C [15]. The average milk production is higher than 20 L in a grazing system with an energy supplementation twice per day. The management of the feeding is made with open grazing systems, with a harvest period that can vary from 25 to 45 days according to the exploitation and type of fertilization [14, 16].

2.3. Sampling and data collection

Animals were enrolled beginning one month before expected calving using lists generated from each herd records, with subsequent visits one week before the expected calving date for the semiological exam and 48–72 h after calving for semiological exam and blood/milk sampling. Information about peripartum diseases, mainly fetal membrane retention, dystocia, clinical ketosis, mastitis, metritis, milk fever, and lameness, were recorded. Cows with clinical signs of disease were excluded from the study. A 10-ml blood sample was collected once at 48 h after calving from the coccygeal vein into two sterile tubes with and without anticoagulant respectively (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ). A milk sample (20 ml) was collected once at 72 h after calving. The samples were kept stored at -5 °C, 3–4 h during the transport to the veterinary diagnostic laboratory.

Analyses performed on blood samples included: *red blood cell profile* (erythrocytes; hematocrit; mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); and mean corpuscular hemoglobin concentration (MCHC); *white blood cell count* (leucocytes; neutrophils; band cells; lymphocytes; eosinophils, monocytes, basophils) and platelets. Data were examined on an automated analyzer ABX Micros ESV 60 equipment (HORIBA ABX SAS, Kyoto, Kyoto Prefecture, Japan). For differential counting, slides were evaluated under optical microscopy. In serum samples it was quantified minerals (calcium, phosphorus, and magnesium concentrations) and *proteins* (total protein; albumin; globulin; fibrinogen), using a kinetic/colorimetric method on a semi-automated chemistry analyzer (Mindray BA 88A, Mindray Bio-Medical Electronics, Shenzen, Japan) (All reagents supplied by Randox). Milk samples were analyzed for the percentage of fat, protein, lactose, and total solids; milk urea nitrogen (MUN); ketone bodies and, beta-hydroxybutyrate (BHB) in milk using spectrometry.
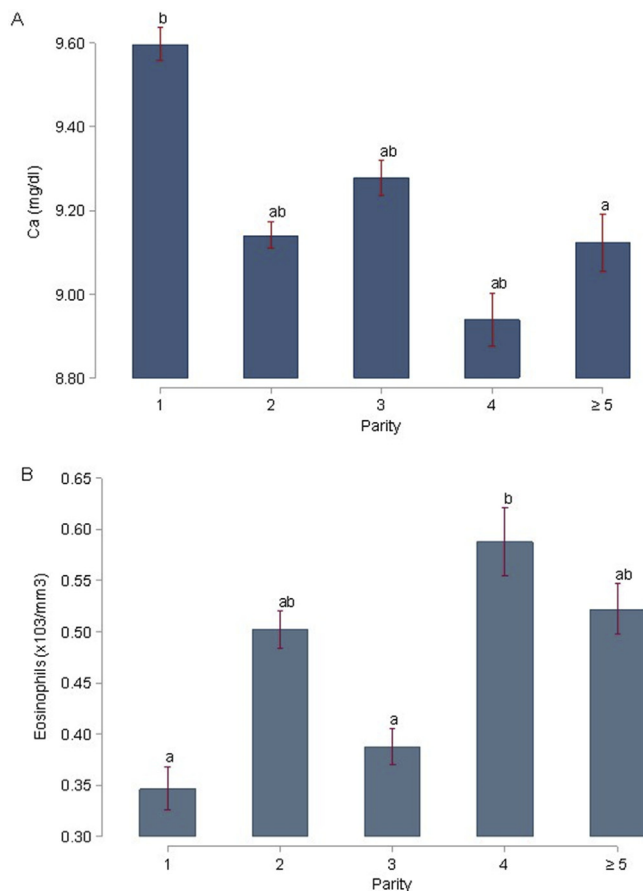
Figure 1. Serum calcium levels (A) and total eosinophil counts (B) (Mean ± SEM) affected by parity in lactating dairy cows grazing in highland tropical herds. Means without a common superscript differed (P < 0.05).
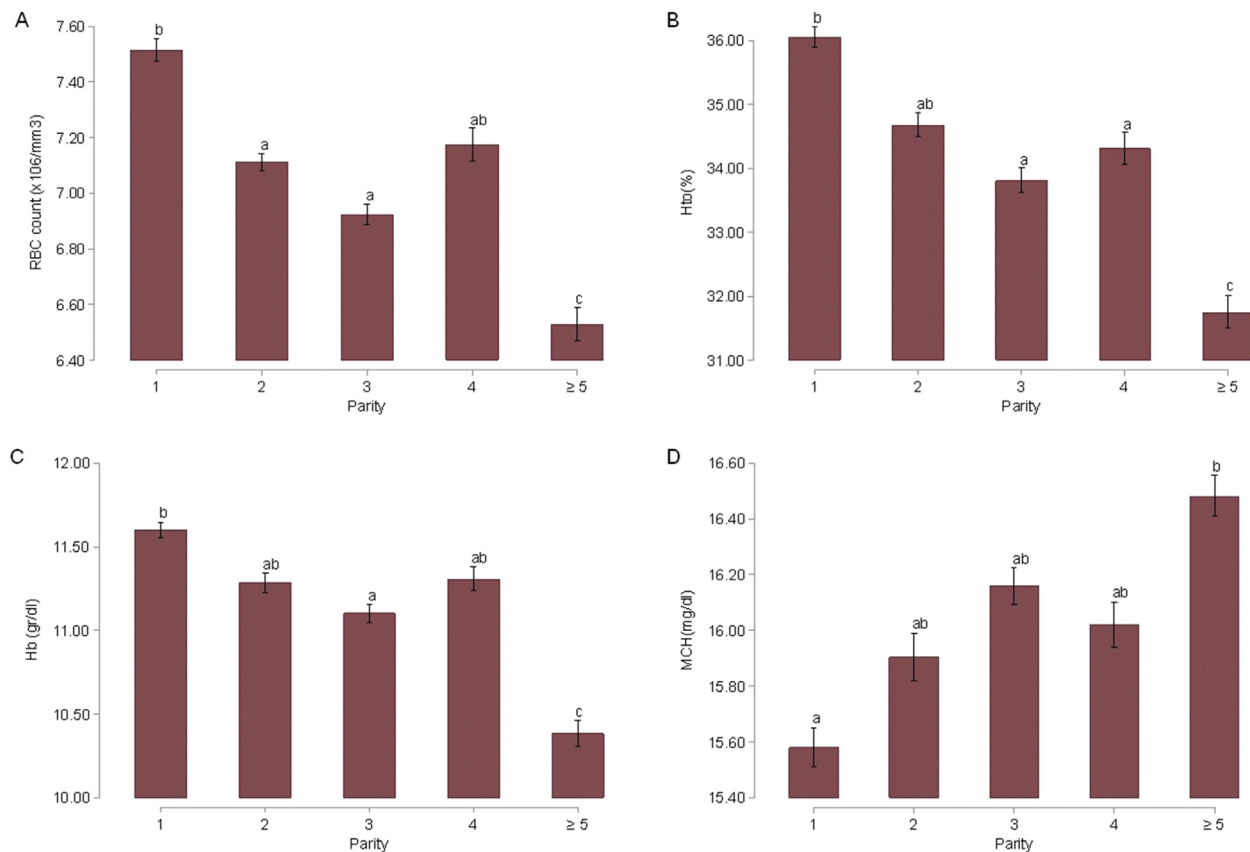
Figure 2. Red blood cell count (A); Hematocrit (B); Hemoglobin (C) and Mean Corpuscular Hemoglobin (D) (Mean ± SEM) affected by parity in lactating dairy cows grazing in highland tropical herds. Means without a common superscript differed (P < 0.05).

2.4. Data analysis

Using a parametric or non-parametric method based on the distribution of the variables, single average values, and 90% confidence intervals were determined considering fixed effects found in the GLM model. For grazing dairy cows in high tropic herds, this study found changes in average values and confidence intervals on different metabolites, including variations dependent on parity or calving season. Statistical analysis of variables was performed using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria) and, Reference Value Advisor Software [17]. Normal distribution of all variables was checked using histogram with a Gaussian distribution graph, a graph of the cumulative distribution of data, and Shapiro-Wilk (W) test. Through Tukey test, outliers (values more than 1.5 times the interquartile range from the quartiles, either below Q1 or above Q3) were removed from the data set, and variables with a W value <0.9 were Box-Cox transformed and checked for normality using Andersson Darling test (P > 0.05). Independent variables were parity (primiparous cow and a multiparous cow with 2, 3, 4 or 5 to 8 calving); calving season per trimester (January–March; April–June; July–September; October–November). Breed (Holstein, Ayrshire, Crossbreed) and farm. Breed and farm were considered as the main factors to decrease variability through a level approach. Each one of the variables was statistically analyzed using a Mixed Multi-Level Linear Generalized Model – MMGLM that considered parity, calving season and interaction between parity and calving season as fixed effects and; Farm and Breed as the random effect of the model with the cows at the lowest level of the hierarchy. The statistical criterion to consider a significant fixed effect was P < 0.05. In the variables that after MMGL model a significant effect was found, a post-estimation pairwise comparisons of marginal linear predictions was performed to find specific differences related with season or parity. Using a parametric

or non-parametric method based on the distribution of the variables, single average values, and 90% confidence intervals were determined for the analytes according to ASVCP [12]. Whenever a fixed effect of the model significantly affected (P < 0.05) a given analyte, this was partitioned into subclasses and specific average values and confidence intervals were generated for each of its levels.

3. Results

The number of samples after outlier's elimination and descriptive statistics after the normalization procedure for each analyte is reported in Table 1. For the MMGLM model, the random effect of the breed and farm present effect (Prob chi2 <0.05) on variability of: Phosphorus (P = 0.001); Leucocytes – x10³/mm³ (P = 0.002); Neutrophils – x10³/mm³ (P = 0.001); Lymphocytes – x10³/mm³ (P = 0.01); Milk fat % (P = 0.001); Milk fat/protein ratio (P = 0.001); Milk Total Solids (P = 0.001); Lactose – % (P = 0.001); and MUN g/dl (P = 0.001). In the model, parity and calving season did not show any significant (P > 0.05) effect on the following analytes: Phosphorus (mg/dl), Magnesium (mg/dl), MCV (fl), MCHC (g/dl), Neutrophils (x10³/mm³), Monocytes (x10³/mm³), Platelets (x10³/mm³), Fibrinogen (mg/dl), Total Protein (g/dl) and any milk analytes (with exception of Milk Beta-Hydroxy-Butyrate – MBHB). Table 2 describes the media and 90% confidence interval CI for these variables. Regarding parity, the model showed a statistically significant effect (P < 0,05 – IC95%) on the following analytes: Calcium [mg/dl] (2, 3, 4, 5 to 8 calving), Erythrocytes [x10⁶/mm³] (2, 3, 4, 5 to 8 calving), Hematocrit [%] (3, 4, 5 to 8 calving), Hemoglobin [g/dl] (3, 5 to 8 calving), MCH (3, 4, 5 to 8 calving), Basophils [x10³/mm³] (2, 3 calving), and Eosinophils [x10³/mm³] (2, 4 calving). Calving season significant affected (P < 0.05 – CI95%) the following analytes: Leucocytes [x10³/mm³] (2, 3, 4 trimester) Lymphocytes [x10³/mm³] (2, 3, 4 trimester),
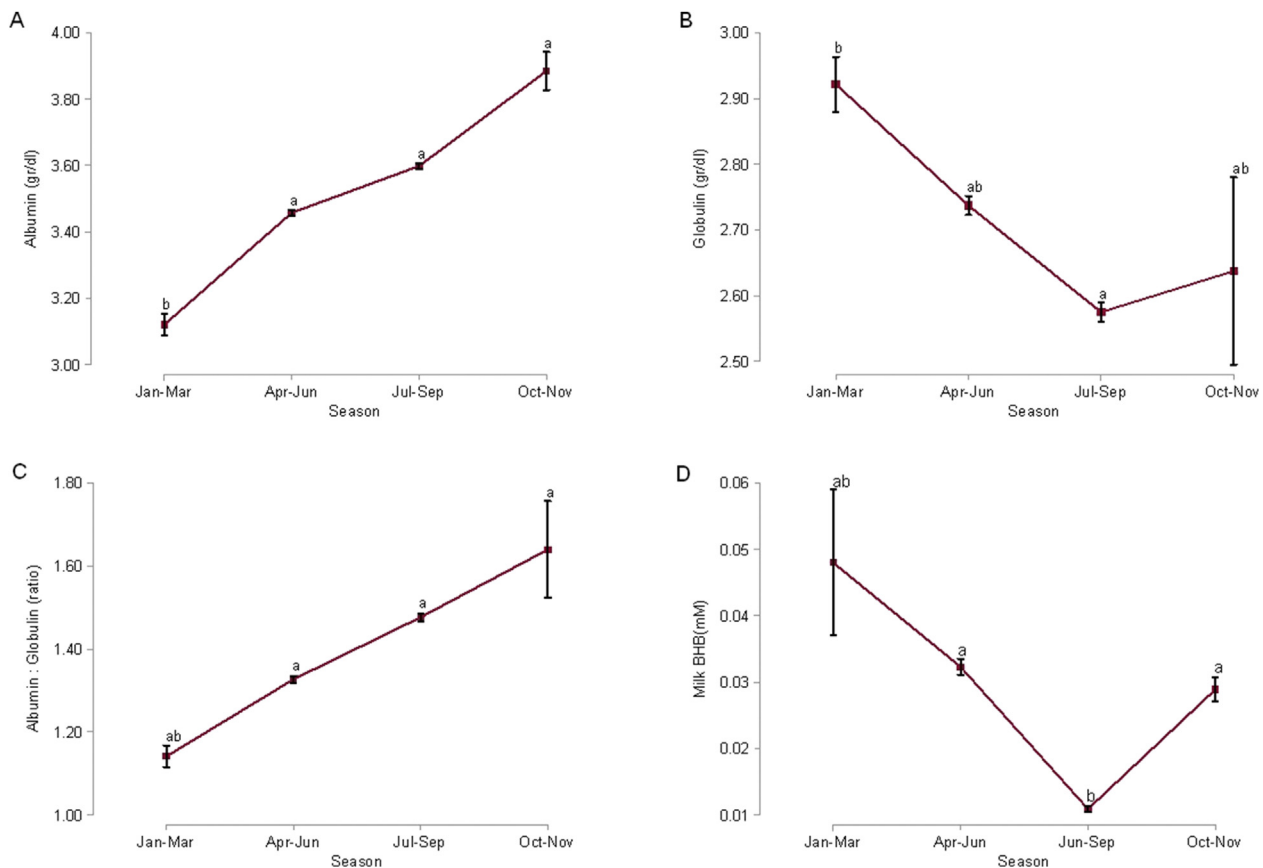
Figure 3. Variations by calving seasons of Albumin (A); Globulin (B); Albumin: Globulin ratio (C) and Milk Beta-Hydroxy-Butyrate (D) (Mean ± SEM). Means without a common superscript differed (P < 0.05).

Albumin [g/dl] (2, 3, 4 trimester), Globulin [g/dl] (3 trimester), Albumin/Globulin Ratio (2, 3, 4 trimester), and MBHB [mM] (2, 4 trimester). Tables 3 and 4 describe the media and CI 90% of the variables affected by parity and calving season, respectively.

Post-estimation pairwise comparisons of marginal linear predictions for metabolites affected by parity found differences (P < 0.05) for calcium levels on first and ≥5 calvings (Figure 1, A). In the case of red blood cell count, hematocrit and Hemoglobin (Figure 2A, B, C respectively) the marginal linear predictions found (P < 0.05) the higher levels at first calving and the lower levels after fourth calving. In addition, eosinophils concentrations (P < 0.05) differ regarding to parity (Figure 1, B). In the case of season, blood leukocyte and lymphocyte counts was higher (P < 0.05) in the first trimester (Jan–Mar) and progressively decreasing its values during the rest of the year. Milk values for Albumin; Globulin; and Albumin: Globulin ratio (Figure 3 – A, B, C) differ between first and fourth trimester (P < 0.05). Milk Beta-Hydroxy-Butyrate (Figure 3 - D) significantly differ regarding to season with variations throughout the year.

4. Discussion

The appropriate management of cows during the transition period for maintaining cows-health, fertility, and milk yield during every new lactation period, are the most difficult tasks to accomplish in dairy herds. Approximately 75% of disease in dairy cows typically happens in the first month after calving [18]. In dairy herds in highland tropics of Colombia, 47% of cases occur within the first two weeks after calving [19]. Consequently, attention to feeding and recognized nutritional guidelines during the transition and postpartum periods is the first step in managing the postpartum diseases. However, additional strategic tools may be useful in managing the postpartum period at herd and cow-level, one of

which is an appropriate application of blood and milk analysis. In this order, the use of adequate reference values, considering animal subgroup and the region is a critical point. The present study focuses on regional variations related to dairy cows from high tropics grazing herds. Previous data have been reported on reference values for analytes of dairy cows during the transition period [6, 7, 9, 10, 20], and lactating dairy cows [4, 11, 21]. Regarding minerals, our results showed that the upper value for P and Mg is two to three times higher than the reference values reported by other authors (Table 5). Calcium values are similar compared with other data but, our study found that Ca levels were significantly higher in primiparous cows compared with multiparous cows, mainly ≥ fourth parity (Table 3, Figure 1-A), compared with multiparous cows (higher milk production, major ca mobilization and osteoclast proportion with high levels of PTH), primiparous cows tend to produce a less amount of colostrum and to have a significant portion of osteoblast considering that the bone structure still growing. For Russel (2007), Ca, P, and common homeostatic mechanisms influence mg homeostasis [22]; therefore, changes in their serum concentrations can be interrelated and affected by blood pH, protein concentration and mineral loss after calving. Clinical exam and other conditions should be further considered before making clinical/herd decisions.

For red blood cell count (RBC), Hematocrit (HCT), Hemoglobin (Hb) and mean corpuscular hemoglobin (MCH), it was found variations related to parity with higher levels of RBC, HCT and Hb at first calving and the lower levels after fourth calving (Figure 2). This behavior coul be explained considering that an animal may have a significant deficit in the total circulating hemoglobin in the presence of normal RBC and HCT in states such as pregnancy where the total mass of circulating hemoglobin may be average, but the concentration values decreased because the plasma volume has expanded. However specific metabolic adaptations related to high milk yield in cows grazing in herds located in the high

Table 5. Comparison of data from grazing dairy cows bred in highland tropical herds to relevant clinical data reported by other authors.

Analytes	Present Study	Gerardo et al., 2009 [6]	Cozzi et al., 2011 [11]	Bertoni and Travesi, 2013 [20]	Smith, 2016 [21]	Moretti et al., 2017 [7]
Minerals						
Calcium (mg/dl)	6.1–11.7*	6.57–10.46	8.82–10.4	9.14–10.78	9.7–12.4	6.41–10.18
Phosphorus (mg/dl)	4.0–10.4	3.22–8.15	3.72–7.43*	3.96–7.62	5.6–6.5	2.54–6.26
Magnesium (mg/dl)	1.6–4.3	NE	1.92–2.6	2.07–2.62	1.8–2.3	1.51–3.04
Red Blood cell count						
Packed cell volume (x 10^6/mm^3)	4.4–9.6	NE	NE	NE	5–10	5.3–8.3
Hematocrit (%)	22–45.1	NE	NE	27–34*	24–46	25–38
Hemoglobin (g/dl)	7.7–16.5	NE	NE	NE	8–15.0	9.33–13.78
Mean Corpuscular Volume (fl)	43.0–56.0	NE	NE	NE	40–60	40.1–53.2
Mean Corpuscular Hemoglobin (pg)	11.7–18.9	NE	NE	NE	11–17	13.7–19.5
Mean Corp. Hb. Concentration (g/dl)	30.1–36.7	NE	NE	NE	30–36	32.8–37.4
White Blood cell count						
Leucocytes (x 10^3/mm^3)	3.0–26.7	3.9–17	NE	NE	4.0–12.0	4.7–13.9
Neutrophils (x 10^3/mm^3)	0.7–12.5	0.7–9.0	NE	NE	0.6–4.0	2.6–12.7
Lymphocytes (x 10^3/mm^3)	1.2–37.5	2.1–10	NE	NE	2.5–7.5	3.9–13.8
Eosinophils (x 10^3/mm^3)	0.0–21.6	0.0–0.7	NE	NE	0.0–2.4	0.0–1.6
Monocytes (x 10^3/mm^3)	0.0–0.9	0.0–0.3	NE	NE	0.025–0.84	0.0–2.8
Basophils (x 10^3/mm^3)		NE	NE	NE	0.0–0.2	NE
Protein traits						
Platelets (x 10^3/mm^3)	208.4–1090.3	NE	NE	NE	100–800	189–510
Total Protein (g/dl)	5.6–8.1	NE	7.0–9.2*	NE	6.74–7.46	4.83–8.34
Albumin (g/dl)	2.1–4.4**	NE	3.3–4.1	NE	3.0–4.3	2.92–3.95
Globulin (g/dl)	1.6–4.4**	NE	3.3–7.2*	NE	3.0–4.9	1.6–4.48
Albumin/Globulin ratio	0.6–3.6**	NE	NE	NE	0.84–0.94	0.71–1.90
Fibrinogen (mg/dl)	150–1140	NE	NE	NE	200–700	NE
Milk analytes						
Acetone (mM)	0.0–0.4	NE	NE	NE	0	NE
Beta-Hydroxy-Butyrate (mM)	0.0–0.2	NE	NE	NE	0.49	NE

NE = No evaluated in the reported study.
* Values affected by parity.
** Values affected by calving season.

tropics could explain this behavior. Compared to other authors [7, 21], the values for these analytes were similar in the present study (Table 5). The HCM does not provide additional diagnostic value, because it depends on the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) which had values not affected by parity. For White Blood cell count and protein traits, the present study found that these analytes were affected by the fixed (parity, calving season), and random effects (breed, area), which explain, at least in part, the variation. In comparison with other reports, the analytes had a wide range, either in the upper or lower reference values (Table 5). Events related to calving, uterine involution, or the presence of subclinical disorders could explain this finding. No relevant data using milk samples information for making clinical decisions was found. Finally, MBHB was significantly affected by the calving season with a tendency to decrease BHB concentrations during the first six months of the year to posteriorly increasing in the second semester, this fact could be explained by the weather conditions where there is a greater dry matter production in the pasture related with a major solar luminous intensity (regardless of whether the season is dry or rainy) in the period June to November. In this period the plant produce not only a larger volumen of grass but also a major amount of dry matter per square meter of pasture. Values of MBHB reported by Smith [21], were higher than those of the present study. The opposite occurs with ketones, which exhibited a wide range. Interestingly, we did not found changes in MUN values.

Milk fat and protein percentage average reported in 2017 in the same area of study was 3.77% and 3.06% respectively [23], these results differ of our findings for milk fat (3.4%) and protein (3.6%). It could be explained because the sampling was performed between 3 to 72 h after calving when milk present high protein levels (immunoglobulins, globulins and colostrum compounds) and a low percentage of milk fat related with a reduction of dry matter intake during the peripartum. However, mean value for milk fat/protein ratio was 0.94 and was not associated with an increase in MBHB levels, which suggests that the cows in this study did not suffer during peripartum a high mobilization body fat to milk. This finding may explain the low values of MHBH that did not exceed the 0.2 mmol/Lt. Montoya et al. reported that a body condition score <3.5 at calving could explain this phenomenon [23] and Garcia et al. suggest that values of milk fat/protein >1.19 can be related to subclinical ketosis [24]. Further research is required to evaluate the combined effect of body condition score, milk fat/protein ratio, and MBHB levels on metabolic profiles in lactating dairy cows grazing in highlands tropics.

Declarations

Author contribution statement

Dario Vallejo-Timarán: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Juan Maldonado-Estrada: Conceived and designed the experiments; Wrote the paper.

John Montoya-Zuluaga: Analyzed and interpreted the data.

Viviana Castillo-Vanegas: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] F.J. Mulligan, M.L. Doherty, Production diseases of the transition cow, Vet. J. 176 (2008) 3–9.

[2] G.R. Oetzel, Monitoring and testing dairy herds for metabolic disease, Vet. Clin. North Am. Food Anim. Pract. 20 (2004) 651–674.

[3] D. Raboisson, M. Mounié, E. Maigné, Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: a meta-analysis and review, J. Dairy Sci. 97 (2014) 7547–7563.

[4] M. Brscic, G. Cozzi, I. Lora, et al., Short communication: 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 (2015) 7886–7892.

[5] J.M. Huzzey, S. Mann, D.V. Nydam, R.J. Grant, T.R. Overton, Associations of peripartum markers of stress and inflammation with milk yield and reproductive performance in Holstein dairy cows, Prev. Vet. Med. 120 (2015) 291–297.

[6] F.Q.R. Gerardo, S.J. LeBlanc, T.F. Duffield, D. Wood, K.E. Leslie, R.M. Jacobs, Reference limits for biochemical and hematological analytes of dairy cows one week before and one week after parturition, Can. Vet. J. 50 (2009) 383–388.

[7] P. Moretti, S. Paltrinieri, E. Trevisi, et al., Reference intervals for hematological and biochemical parameters, acute phase proteins and markers of oxidation in Holstein dairy cows around 3 and 30 days after calving, Res. Vet. Sci. 114 (2017) 322–331.

[8] N. Chapinal, M. Carson, T.F. Duffield, et al., The association of serum metabolites with clinical disease during the transition period, J. Dairy Sci. 94 (2011) 4897–4903.

[9] S. Nazifi, M.R. Ahmadi, H.R. Gheisari, Hematological changes of dairy cows in postpartum period and early pregnancy, Comp. Clin. Pathol. 17 (2008) 157–163.

[10] G. Piccione, V. Messina, S. Marafioti, S. Casella, C. Giannetto, F. Fazio, Changes of some haematochemical parameters in dairy cows during late gestation, post partum, lactation and dry periods, Vet ir Zootech 58 (2012) 59–64.

[11] G. Cozzi, L. Ravarotto, F. Gottardo, et al., Short communication : reference values for blood parameters in Holstein dairy cows : effects of parity, stage of lactation, and season of production, J. Dairy Sci. 94 (2011) 3895–3901.

[12] K.R. Friedrichs, K.E. Harr, K.P. Freeman, et al., ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics, Vet. Clin. Pathol. 41 (2012) 441–453.

[13] P.S. Horn, A.J. Pesce, Reference intervals: an update, Clin. Chim. Acta 334 (2003) 5–23.

[14] O. David, M. Bedoya, L.D. Cassoli, M. Olivera, M. Fernando, C. Muñoz, Characterization of dairy farms with mechanical milking in Antioquia, Colombia, LRRD Newsletter 30 (2018) 1–15.

[15] J.J. Chaparro, N.F. Ramírez, D. Villar, et al., Survey of gastrointestinal parasites, liver flukes and lungworm in feces from dairy cattle in the high tropics of Antioquia, Colombia, Parasite Epidemiol Control 1 (2016) 124–130.

[16] J.M. Astaíza, M.R. Muñoz, C.J. Benavides, D.A. Vallejo, C.A. Chaves, Technical and productive characterization of milk production systems in the Sibundoy Valley (Putumayo, Colombia), Rev. Med. Vet. 34 (2017) 31–34.

[17] D. Concordet, J. Braun, C. Trumel, A. Geffr, Reference Value Advisor : a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel, Vet. Clin. Pathol. 40 (2011) 107–112.

[18] R.R. Daros, M.J. Hötzel, J.A. Bran, S.J. Leblanc, M.A.G. Von Keyserlingk, Prevalence and risk factors for transition period diseases in grazing dairy cows in Brazil, Prev. Vet. Med. 145 (2017) 16–22.

[19] D.A. Vallejo, C.J. Benavides, D.P. Morillo, J.M. Astaiza, C.A. Chaves, Effect of early postpartum diseases on calving – conception interval : a cohort study in dairy cows from Pasto, Colombia, Ces. Med. Vet. Zootec. 12 (2017) 33–43.

[20] G. Bertoni, E. Trevisi, Use of the liver activity index and other metabolic variables in the assessment of metabolic health in dairy herds, Vet. Clin. North Am. Food Anim. Pract. 29 (2013) 413–431.

[21] B.P. Smith, D. Van Metre, N. Pusterla, Large Animal Internal Medicine, fifth ed., Elsevier Science & Technology, Oxford, 2017.

[22] K.E. Russell, A.J. Roussel, Evaluation of the ruminant serum chemistry profile, Vet. Clin. North Am. Food Anim. Pract. 23 (2007) 403–426.

[23] J.J. Montoya, O.D. Munera, M.F. Ceron, Factors associated with milk urea nitrogen in dairy cows, LRRD Newsletter 29 (2017) 1–7.

[24] C.A.C. García, R.L.A. Montiela, T.F. Borderasa, V. Girard, Relationship between β-hydroxybutyrate and the fat : protein ratio of milk during early lactation in dairy cows, Arch. Med. Vet. 47 (2015) 21–25.