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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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 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...
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.
The herds are located in one of 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].

### 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 (x 10^3/mm³) | Lymphocytes (%) | Albumin Ab (g/dl) | Globulin Gb (g/dl) | Ab/Gb ratio | MBHB (mM) |
| Jan–Mar        | Media ±SD               | Reference value | 3.1 ± 0.5 | 2.9 ± 0.6 | 1.1 ± 0.4 | 0.0 ± 0.0 |
|                | 11.4 ± 5.2              | 5.1–26.7       | 19.9–97.4 | 2.1–4.2  | 1.6–4.2  | 0.6–2.2  |
|                | 4.6–34.7                | 2.1–37.5       | 13.4–99.0 | 1.9–4.5  | 1.6–4.5  | 0.6–2.7  |
| Apr–Jun        | Media ±SD               | Reference value | 35 ± 0.4 | 2.7 ± 0.6 | 1.3 ± 0.3 | 0.0 ± 0.0 |
|                | 9.5 ± 4.2               | 5.1 ± 2.4      | 55.1 ± 17.5 | 2.4–4.2b | 1.9–4.3b | 0.7–2.1b |
|                | 4.1–22.9b              | 1.5–10.5b      | 15.2–84.9 | 2.3–4.5  | 1.6–4.6  | 0.5–2.2  |
| Jul–Sep        | Media ±SD               | Reference value | 3.6 ± 0.3 | 2.6 ± 0.6 | 4.5 ± 0.6 | 0.0 ± 0.0 |
|                | 9.8 ± 3.7               | 4.6 ± 2.7      | 48.1 ± 17.6 | 2.9–4.3b | 1.6–4.1b | 0.8–2.4a |
|                | 4.0–18.9b              | 1.55–13.7b     | 18.0–86.0b | 2.4–4.4  | 1.5–4.1  | 0.7–2.5  |
| Oct–Nov        | Media ±SD               | Reference value | 3.9 ± 0.3 | 2.6 ± 0.8 | 1.6 ± 0.6 | 0.0 ± 0.0 |
|                | 7.8 ± 2.2               | 3.4 ± 1.4      | 46.9 ± 17.8 | 2.6–4.4b | 0.7–3.6a | 0.0–0.2b |
|                | 3.0–13.0b              | 1.2–6.7b       | 5.3–86.7  | 2.6–4.5  | 0.1–4.5  | 0.5–5.0  |
|                | 1.3–15.0               | 0.0–8.0        | 5.3–86.7  | 2.6–4.5  | 0.1–4.5  | 0.5–5.0  |

*Differences on reference value and 95% IC (P < 0.05), compared to primiparous cows in the Multi-Level Generalized Linear Model.*

### 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 differ (P < 0.05).

### 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 E60 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, Shenzhen, 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.
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). 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 – IC95%) the following analytes: Leucocytes [x10³/mm³] (2, 3, 4 trimester) Lymphocytes [x10³/mm³] (2, 3, 4 trimester),
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 second 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 could 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
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 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.

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.
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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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