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Peripartum Ketone and its Relationship with Milk Quality in Dairy Cattle

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ABSTRACT

Ketosis is a common metabolic disorder in dairy cows and has been associated with alterations in milk composition and a decrease in milk quality, impacting both the economic and nutritional value of dairy products. The purpose of this study was to evaluate ketone levels before and after calving and their effect on milk quality in cattle in the district of El Mantaro, Jauja in the Peruvian highlands. Multivariate analyses, including Principal Component Analysis (PCA) and clustering, were employed to explain the variability in the data better. The study was conducted at the Instituto de Investigaciones Tropicales y de Altura (IVITA) and El Mantaro experimental stations cattle in the district of El Mantaro, Jauja, using a total of 72 Brown Swiss cattle, all of which received similar management. Blood and milk samples were collected from all cows studied and analyzed in the laboratory for ketone levels and milk quality such as density, non-fat solids, protein, freezing point, solids, and lactose. The descriptive analysis revealed significant variations in the evaluated variables, highlighting a reduction in ketone levels after calving and consistency in milk composition, such as non-fat solids and density. The PCA showed that the first two principal components explained 49.8% of the total variability, dominated by compositional variables, while subsequent components contributed smaller proportions, reaching 100% with 11 components. The reduction in ketone levels after calving suggested metabolic stabilization associated with energy recovery during this stage, while differences in compositional variables such as protein and non-fat solids reflected the influence of factors such as diet, genetics, and physiological status. Although ketones showed weak to moderate correlations with the evaluated variables, the negative relationships with body condition and non-fat solids indicated that better nutritional and metabolic status might be associated with lower ketone levels.

Keywords: Brown Swiss cattle, Correlation, Milk composition, Milk quality, Subclinical ketosis

INTRODUCTION

Dairy cattle farming in the “Valle del Mantaro” Valley is characterized by being managed in small production units, which typically exhibit lower milk yield per cow compared to the major dairy regions of the country (Diego et al., 2024; Estremadoyro et al., 2024). The fresh milk produced is primarily directed towards a nascent artisanal or semi-industrial dairy industry, mainly focused on the production of cheese, yogurt, and other dairy derivatives, as well as for self-consumption (Carhuas et al., 2024; Payano et al., 2024). However, the pressure to increase milk production imposes greater metabolic demands on the animals, predisposing them to a higher incidence of metabolic diseases, commonly referred to as production diseases (Garcia-Olarte et al., 2024). These conditions arise from an imbalance between the intake, circulation, and excretion of one or more metabolites within the organism, pushing their concentrations beyond physiological limits.

Ketosis is a common metabolic disorder in dairy cows during the peripartum period, characterized by elevated levels of ketone bodies, particularly *β-hydroxybutyrate* (BHB), in blood, urine, or milk (Lei and Simões, 2021; Cascone et al., 2022). This condition arises from the high energy demands of late gestation and early lactation, combined with limited feed intake, leading to excessive mobilization of body fat reserves (Tufarelli et al., 2024). Beyond its direct health implications, ketosis has been associated with alterations in milk composition and a decline in its quality, impacting both the economic and nutritional value of dairy products (Cainzos et al., 2022). Milk quality, encompassing parameters such as fat, protein, lactose, and somatic cell count, is a critical indicator of herd productivity and consumer satisfaction (Pegolo et al., 2022). Emerging evidence suggests that ketone levels during the peripartum period can influence these parameters, underscoring the importance of early identification and management of ketosis to maintain optimal dairy production performance (Guliński, 2021). However, obtaining specific information for each production

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area is challenging due to environmental variations affecting quality. This highlights the necessity of understanding these values, particularly in the Junín region of Peru, where such data is lacking.

The analytical focus of this study was to evaluate the relationship between ketone levels during the peripartum period and milk quality parameters in dairy cattle, using descriptive and multivariate statistical analyses, models, and correlation analysis. This design enables the identification of patterns and associations between blood ketone concentrations and key quality variables, such as fat, protein, lactose content, and somatic cell count (Chapman et al., 2001). Although ketosis in cattle has been extensively studied, most research has focused on its impact on health and overall production, leaving a gap in the literature regarding its specific influence on milk quality. This study contributed to the understanding of metabolic health in dairy herds, offering potential strategies for the early detection and mitigation of ketosis and its impacts on milk quality.

MATERIALS AND METHODS

The procedures and ethical standards for animal use in this study were conducted in strict accordance with the "International and National Guidelines for the Care and Use of Research Animals," as outlined in LETTER No. 002-GRJ-DRA-AAC-PERÚ-2024. This ensured full compliance with established animal welfare protocols throughout the research process.

Study area and distribution

The study was conducted at the Instituto de Investigaciones Tropicales y de Altura (IVITA) and El Mantaro experimental stations, located in the district of El Mantaro, province of Jauja, Junín region, at an altitude of 3,200 meters above sea level (Senamhi, 2023). The study included 57 cows from the IVITA station and 15 cows from the El Mantaro Experimental Station (EEA), with five replicates for each cow. All of them were Brown Swiss. All cattle were fed a diet composed exclusively of alfalfa and received the same management. The cattle were selected 30 days before calving to measure ketone levels before calving, and the same animals were evaluated 30 days later to determine ketone levels after calving.

Data collection

Ketone

Blood samples (ml), one per cow, were collected using vacutainer tubes from the coccygeal artery of the animals and transported to the laboratory for processing. To ensure proper preservation, serum was separated and stored in cryovials at a freezing temperature of -40°C. Samples requiring additional processing were centrifuged prior to analysis. For ketone analysis, a NovaVET Xpress ketone/glucose meter (ket-mmol/L) was used to measure β-hydroxybutyrate (BHBA) levels in the blood (Zhuang et al., 2023). This parameter is widely regarded as the gold standard for detecting subclinical ketosis in dairy cattle. The ketone test strips contain a chemical reagent that reacts with the sample once inserted into the meter, and the reaction in the test strip's cell generates an electric current, which the device measures to calculate BHBA concentration.

Body condition and age

Body condition score (BCS) was assessed using the method described by Paul et al. (2020), which employs a scale from 1 to 5. Evaluations were performed by the same observer at 30 days before calving, at calving, and 15, 30, 45, and 60 days postpartum. The age of the cows was recorded by the experimental station records.

Milk quality

Milk quality was assessed using one sample per animal, collected at 6:00 a.m. before milking. The samples were analyzed with a Boeko Lactoscan (Germany) SP ultrasonic milk analyzer, which measured fat content (FAT), density, solids-not-fat (SNF), protein, pH, freezing point, total solids, and lactose levels (Kasmi et al., 2021).

Statistical analysis

A descriptive analysis was performed to examine the behavior of the data, followed by a Principal Component Analysis (PCA) to enable clustering and capture the maximum variability in the dataset. All analyses were conducted using R-Studio (Team et al., 2018) using version 4.3.0.

RESULTS

Table 1 summarizes the descriptive statistics for Age, Body Condition, Ketone Levels Before Delivery (KBD), Ketone Levels After Childbirth (KAC), Fat Content, Milk Density, Non-Fat Solids (NFS), Protein (PROT), Freezing Point (FP), Total Solids, and Lactose (LACT). The mean age of the subjects was 4.61 years, with a standard deviation of 2.81 years. The youngest individual was 1.9 years old, while the oldest was 15.4 years. The median age was 4.24 years. Body condition scores averaged 2.55, with variability of ±2.69, spanning from 2.0 to 3.0, with a median value of 2.60. Regarding ketone levels, the prepartum average was 0.91 ± 4.88 mM/L (range: 0.30–2.10; median: 0.80), while postpartum levels decreased to 0.60 ± 3.85 mM/L, with a range of 0.10–2.10 and a median of 0.45. The average fat content was 4.26 ± 1.97 (%) with a minimum of 0.08, a maximum of 9.45, and a median of 4.39. Milk density had an average of 26.83 ± 4.24 kg/m^3 (range: 14.52–39.54; median: 26.77), while solids-not-fat (SNF) reached an average of 7.58 ± 1.56 (%), with values between 3.26 and 11.72 and a median of 7.58. Protein concentration had a mean of 3.59 ± 9.72 (%) (range: 1.38–6.98; median: 3.46). Table 2 presents the findings from the Principal Component Analysis (PCA) conducted on milk quality indicators, including fat, protein, lactose content, and somatic cell count, alongside ketone body concentrations during the peripartum period, as well as variables such as age, calving condition, and seasonal grouping of the cows. The initial principal component (PC1) captured 35.12% of the overall variability, followed by the second component (PC2) with 14.70%, together explaining 49.80% of the variability. The third and fourth components (PC3 and PC4) contributed an additional 11.95% and 9.31%, respectively, reaching a cumulative 71.09%. From PC5 onward, the contribution of each component decreased, with variance proportions below 8%, until PC11 accounted for 100% of the variability in the dataset.

Table 1. The metabolic and milk quality parameters in dairy cattle during the peripartum period

Variable	Mean	sd	Min	Max	Median
Age	4.61	2.81	1.9	15.4	4.24
Body condition	2.55	2.69	2.0	3.00	2.60
Ketone before delivery (KBD) mM/L	0.91	4.88	0.30	2.10	0.80
Ketone after childbirth (KAC) mM/L	0.60	3.85	0.10	2.10	0.45
Fat (%)	4.26	1.97	0.08	9.45	4.39
Density kg/m^3	26.83	4.24	14.52	39.54	26.77
Non-fat solids (NFS) (%)	7.58	1.56	3.26	11.72	7.58
Protein (PROT) (%)	3.59	9.72	1.38	6.98	3.46
Freezing point (FP)	1.69	8.75	0.36	0.77	3.46
Solids (%)	0.67	1.18	0.41	0.98	0.66
Lactose (LACT) (%)	4.97	1.03	2.98	7.95	4.82

sd: Standard deviation; min: Minimum data value, max: Maximum data value.

Table 2. The principal component in the PCA analysis of Brown Swiss cows in the Mantaro district, to take the best PCs

Importance component	Proportion of Variance	Cumulative Proportion	SD
PC1	0.3512	0.3512	1.9654
PC2	0.1470	0.4980	1.2717
PC3	0.1195	0.6177	1.1467
PC4	0.0931	0.7109	1.0125
PC5	0.0760	0.7869	0.9144
PC6	0.0588	0.0845	0.8048
PC7	0.0493	0.0895	0.7366
PC8	0.0324	0.9276	0.5966
PC9	0.0278	0.9554	0.5533
PC10	0.0252	0.9806	0.5267
PC11	0.0193	1.0000	0.4610

PC1–PC11 represents the principal components generated from the principal component analysis (PCA), where each component captures a decreasing proportion of the total variability in the data, with PC1 explaining the largest share of the variance and subsequent components contributing progressively less. sd: Standard deviation.

The correlation matrix revealed significant relationships among the variables studied (Figure 1a). Notable correlations included Lactose (LACT) and Protein (PROT), with a positive correlation of 0.67, indicating that an increase in lactose was directly associated with an increase in protein levels. Non-fat Solids (NFS) and density (DENSITY) exhibited a positive correlation of 0.62, highlighting that higher non-fat solids content was associated with increased sample density. Fat (FAT) and Protein (PROT) showed a positive correlation of 0.56, suggesting that both variables were interconnected in their behavior. Ketones Before Calving (KBC) and Ketones After Calving (KAC) displayed a positive correlation of 0.39, indicating a direct relationship between ketone levels during both periods. LACT and density demonstrated a positive correlation of 0.58, showing that increased lactose levels had a direct impact on sample density. The strongest correlations ($p < 0.05$) were found among variables related to non-fat solids, density, lactose, and protein, underscoring their importance in the dataset. Conversely, variables such as ketones (KBD and KAC) exhibited weaker correlations with other variables, suggesting a lesser influence on the system studied. Before calving, ketone levels demonstrated moderate and weak correlations with the analyzed variables. A moderate negative correlation was observed with body condition (-0.15) and non-fat solids (-0.21), suggesting that better physical condition and higher non-fat solids content could be associated with lower ketone levels during this period. On the other hand, weak positive correlations were observed with variables such as fat (0.06), density (0.09), and total solids (0.06), indicating that these characteristics had no significant relationship with ketone levels. Correlations with lactose (0.04) and protein (0.08) were also too weak, suggesting a marginal influence of these variables before calving. After calving, ketone levels followed a similar trend, with a moderate negative correlation with body condition (-0.14), reaffirming that better body condition could be associated with lower ketone levels, moderate correlation is mentioned because it does not follow a strong correlation but there is some correlation. Correlations with non-fat solids (-0.10) and lactose (-0.02) were also negative but at a weak level, indicating a limited relationship. Conversely, correlations with protein (0.09), density (0.04), and total solids (0.06) were positive but at a weak level, suggesting an insignificant impact of these variables on ketone levels after calving. In general, ketone levels, both before and after calving, appeared to be inversely related to indicators of optimal metabolic health, although the relationships were mostly at a weak level. These correlations suggest that their variation may depend on other factors not evaluated in the current study.

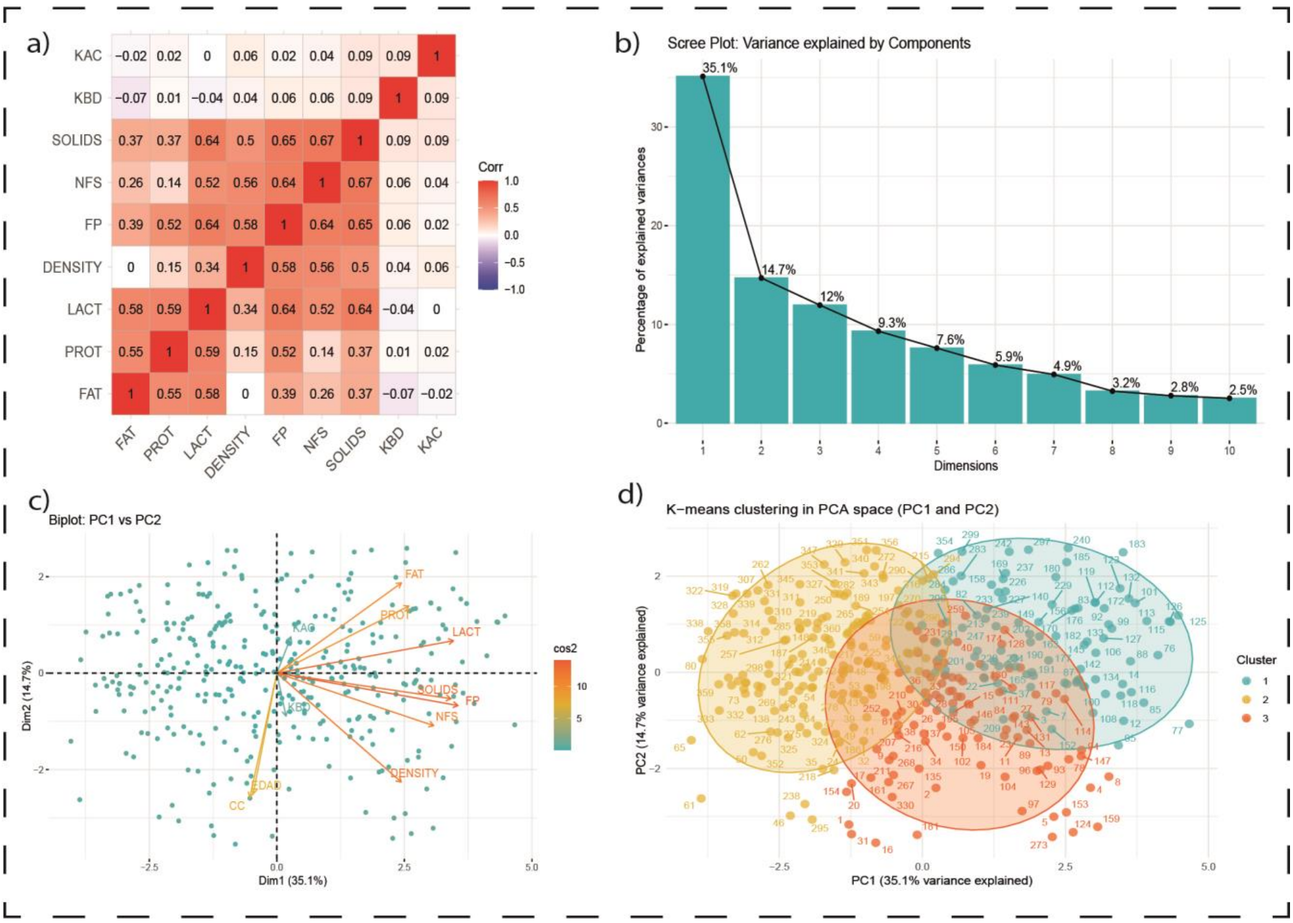

Figure 1. Comprehensive visualization of Principal Component Analysis (PCA) and clustering results for metabolic and milk quality parameters. **a:** Correlation Matrix, **b:** Scree Plot, **c:** Biplot (PC1 versus PC2), and **d**: K-means Clustering in PCA Space.

From Figure 1b, the Scree Plot, the variance analysis showed that the initial (PC1 and PC2) two principal components accounted for 35.1% and 14.7% of the total variance, respectively, accounting for a combined total of 49.8%. This suggested that a significant portion of the variability in the data could be accounted for through these two components. The Biplot (PC1 vs. PC2) observed how the variables contributed to the first two principal components (Figure 1c). The arrows indicated the direction and magnitude of each variable's contribution, highlighting that LACT, PROT, and NFS were highly associated with PC1, while density had an intermediate association. In contrast, ketones (KBD and KAC) showed minimal influence on the first components (PC1). Figure 1d, the K-means Clustering, displayed three main clusters in the space defined by PC1 and PC2. The colors distinguished the clusters, and the ellipses indicated the density areas of the groups. This clustering highlighted patterns in the data that would not be evident through univariate analyses, suggesting potential structural differences among the observations based on the evaluated variables.

DISCUSSION

The descriptive analysis and PCA provided insights into the variability and relationships within the studied dataset. The results revealed significant differences among the variables, emphasizing their relevance in evaluating the metabolic and productive status of the animals. The average age and BCS suggest a relatively uniform group. However, the large standard deviation observed in ketone levels, both KBC and post-KAC, underscores the significant metabolic heterogeneity among individuals. This variability aligns with findings from previous studies, which also report notable inter-individual differences in metabolic responses (Martens, 2020; Michalopoulou et al., 2024). It is important to note that factors like genetic predisposition, differences in energy metabolism, or variations in physiological states could influence such heterogeneity. The reduction in postpartum ketone levels suggested a shift in metabolic priorities, likely related to the stabilization of energy balance, as documented in previous studies (Parrettini et al., 2020; Wathes et al., 2021). Compositional variables such as FAT, PROT, and NFS exhibited patterns consistent with optimal nutritional management. However, the significant dispersion in protein levels suggested the influence of factors, such as lactation stage or genetics (Cheng et al., 2022; 2023). The PCA indicated that the first two principal components (PC1 and PC2) captured 49.8% of the total variability, with PC1 dominated by compositional variables (LACT, PROT, and NFS) and PC2 likely associated with metabolic indicators such as ketones. This finding underscored the value of PCA as a tool for identifying patterns in complex systems (Ellis et al., 2020). The negative correlations, although weak, suggest that higher levels of ketone bodies (KBC or KAC) could be inversely related to parameters such as lactose, fat, or milk density, reflecting potential adverse metabolic effects.

On the other hand, the graphical analysis of the figures provided additional insights into the relationships among variables and the structure of the dataset. The correlation matrix highlighted strong relationships, such as LACT with PROT (0.67) and NFS with density (0.62), underscoring the importance of these factors in milk composition. However, ketones showed weaker correlations with other variables, particularly before calving, where moderate negative correlations were observed with body condition (-0.15) and non-fat solids (-0.21). These relationships suggested that better metabolic status was associated with lower ketone levels, although their influence was limited (Torres et al., 2020; Hubner et al., 2022; Mohsin et al., 2022). The biplot provided a clear representation of how the variables contributed to the first principal components. The long arrows of LACT, PROT, and NFS toward PC1 confirmed their high influence, while the shorter arrows of ketones indicated a marginal contribution. These results aligned with studies that emphasized the importance of compositional components in milk quality and production efficiency (Magan et al., 2021; Timlin et al., 2021). Additionally, the K-means clustering analysis (Figure 1d) identified three main clusters in the space defined by PC1 and PC2, suggesting significant structural differences among the groups. These clusters could have been linked to genetic or management factors, as previous studies demonstrated that these variables were critical determinants in differentiating metabolic and productive patterns (Zhang et al., 2021; Adamik et al., 2022). The integration of these multivariate analyses with correlation data provided a comprehensive perspective, enabling a more precise characterization of the factors influencing animal productivity and metabolic status. This highlighted the potential of PCA and clustering analysis as essential tools for advanced studies in animal sciences.

CONCLUSION

The reduction in ketone levels after calving suggested metabolic stabilization associated with energy recovery during this stage, while differences in compositional variables such as protein and non-fat solids reflected the influence of factors such as diet, genetics, and physiological status. Although ketones showed weak to moderate correlations with the evaluated variables, the negative relationships with body condition and non-fat solids indicated that better nutritional and metabolic status might be associated with lower ketone levels. The utility of multivariate analysis to simplify complex data with models that would otherwise be difficult to interpret proved crucial in explaining variability, allowing the identification of fundamental patterns that optimize the assessment of milk quality in cattle. Strategies to monitor and control ketone body levels during the peripartum period are recommended, such as improving nutritional management with diets balanced in energy and effective fiber, supplementing with glucogenic compounds to reduce the risk of ketosis, and regular monitoring of metabolic parameters. These measures could improve both cow metabolic health and milk quality.

DECLARATIONS

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Availability of data and materials

The original data presented in the study are included in the article. For inquiries, please contact the corresponding author.

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Authors' contributions

Fernando Arauco Villar was in charge of supervising the overall research; Noemí Mayorga Sánchez was in charge of programming and follow-up; Milk samples and laboratory analysis were performed by Leonor Guzman Estremadoyro and Yakelin Mauricio-Ramos, Blood sampling and laboratory analysis for ketone was performed by Ronald Damas Huaman and Carlos Arana de la Cruz; Ide Unchupaico Payano was in charge of data collection and cleaning; Jordan Ninahuanca Carhuas was in charge of the statistical analysis and writing of the article. All authors have read and approved the final version of the manuscript before publication in the present journal.

Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

The authors confirm that all authors have reviewed and submitted the manuscript to this journal for the first time. Additionally, all authors checked the originality of data and sentences via plagiarism checkers.

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