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Impact of Subclinical Ketosis on Mastitis and Milk Parameters in Early Lactation Brown Swiss Cows

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ABSTRACT: The objective of this study was to determine beta-hydroxybutyrate (BHB) levels as an identifier of subclinical ketosis and its effect on milk parameters and mastitis in postpartum dairy cows raised under highland conditions. A total of 32 primiparous and 32 multiparous cows were evaluated to determine the proportion of cows with subclinical ketosis using a semiquantitative milk test, as well as the presence of mastitis, glucose levels, body condition, milk yield and milk parameters such as pH, density, milk fat and protein content. Data were analyzed using ANOVA and contingency tests for categorical variables. The prevalence of subclinical ketosis was 56% in multiparous cows and 14% in primiparous cows. Subclinical ketosis was significantly associated ($p < 0.05$) milk protein reduction, increased somatic cell counts, and higher milk fat content. Cows with subclinical ketosis showed subclinical mastitis rates of 9.3% in primiparous cows and 17% in multiparous cows. Glucose levels were significantly lower ($p < 0.05$) in cows with ketosis, with a greater reduction observed in multiparous cows. Body condition scores were lower in cows with subclinical ketosis, especially during early lactation ($p < 0.05$). Conversely, milk production increased in cows with subclinical ketosis, particularly after the second week in lactation. The findings suggest a potential effect of beta-hydroxybutyrate concentrations on the occurrence of mastitis and certain milk parameters in Brown Swiss cows raised at high altitudes.

Keyword: Beta-hydroxybutyrate; Ketone bodies; Body condition; Mastitis; Early lactation; Glucose.

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INTRODUCTION

Dairy farming in high-altitude regions of Peru relies on semi-intensive systems (Carhuas et al., 2025), with Brown Swiss cows being the predominant breed due to their hardiness. Nutritional management is based on forage composed of grasses and legumes, with only some herds incorporating concentrated feed (Estremadoyro et al., 2024). The success of dairy production depends on factors such as the physiological capacity of cows to utilize dietary nutrients, which is influenced by genetic, environmental, and management factors. Unchupaico et al. (2021) identified the presence of metabolic disorders in the central highlands of Peru, particularly subclinical ketosis in early lactation cows. This condition is not limited to intensive farming systems but is also influenced by poor nutritional management during the prepartum and postpartum periods, in addition to physiological changes inherent to these stages. Studies on metabolic disorders in dairy cows raised under semi-intensive and extensive systems are scarce, especially concerning subclinical ketosis, a condition often unnoticed by farmers. As a result, no corrective measures are implemented, further exacerbating health and reproductive problems (Benedet, 2019).

Subclinical ketosis is characterized by increased blood beta-hydroxybutyrate (BHB) concentrations, typically ranging between 1.4 and 2.9 mmol/L (Seely et al., 2021; Benedet, 2019). In intensive production systems, the clinical incidence of this condition ranges from 2% to 15% in postpartum cows (Seely et al., 2021), while the incidence of its subclinical form has been estimated between 40% and 60% (Renaud et al., 2019). Subclinical ketosis is a common metabolic disorder affecting more than 40% of dairy cows in early lactation (Zhang et al., 2020; Ford et al., 2024). Cows with elevated blood ketone body levels are at greater risk of developing displaced abomasum, metritis, mastitis, reduced milk production, and decreased fertility (Brunner et al., 2018; Zhang et al., 2020).

Metabolic rate is closely associated with lactation performance (Gorden and Timms, 2015; Themistokleous et al., 2023), particularly in cows experiencing subclinical ketosis, a condition characterized by elevated beta-hydroxybutyrate (BHB) concentrations. Increased BHB impairs glucose metabolism, leading to reduced milk production, altered milk composition, and increased susceptibility to health disorders such as mastitis and metritis (Grummer and Carrol, 1991; Sundrum, 2015; Tufarelli et al., 2024). There is substantial evidence that milk production is also negatively impacted (Holzhauer and Valarcher, 2024). Elevated BHB concentrations impair carbohydrate metabolism by reducing glucose concentrations through diminished gluconeogenesis (Chaput et al., 2020). Seely et al. (2021) reported reduced milk production in early lactation cows associated with elevated serum BHB levels, typically occurring within the first week postpartum. Additionally, Holzhauer and Valarcher et al. (2024) observed that BHB levels were higher in multiparous cows during the first two weeks postpartum compared to primiparous cows; however, both groups experienced reduced milk yields. Elevated BHB in early lactation cows has also been associated with increased milk fat content and decreased milk protein levels, although relationships with milk production and somatic cell counts remain controversial (Benedet, 2019). High somatic cell counts, potentially linked to the high incidence of mastitis in certain herds, may be associated with subclinical ketosis. The present study addresses this controversy to provide evidence of such associations. The first two weeks postpartum are considered the optimal period for testing subclinical ketosis (Tatone et al., 2017). Among the methods available for detecting BHB, most commercial tests are designed to measure acetoacetate and acetone levels in milk and urine through colorimetry. BHB detection in serum has also been achieved using spectrophotometry (Tatone et al., 2016). Permissible BHB levels in milk range from 100 μmol/L to 200 μmol/L (Geishauser et al., 2001).

In high-altitude dairy production systems, there is empirical evidence of disorders associated with subclinical ketosis, such as mastitis, metritis, and reduced milk production. However, these associations have not been scientifically corroborated. Within this context, the objective of this study was to determine beta-hydroxybutyrate levels as an identifier of subclinical ketosis and its effect on milk parameters and mastitis in postpartum dairy cows raised under highland conditions.

MATERIALS AND METHODS

This study adhered to the ethical standards for animal research, guaranteeing the welfare and humane treatment of all animals involved with CARTA LETTER N° 009-GRJ-DRA-AAC-PERÚ-2023. The

protocols were approved by the institutional ethics committee and complied with international guidelines for the use of animals in scientific research. Handling, sampling and management practices were carried out by trained personnel to minimize stress and discomfort, ensuring the welfare of the Brown Swiss cows throughout the study.

Area of study

The study was conducted during the dry season under apparent feed scarcity conditions in highland areas (May–August 2023) across four representative dairy farms in the Mantaro Valley, located at an average altitude of 3,587 meters above sea level in the central Andes of Peru. The average ambient temperature was 10 °C, with an average relative humidity of 65%. The animals were managed under a semi-intensive system with a feeding regimen based on grazing a grass-legume association containing approximately 17% crude protein, supplemented with balanced feed provided empirically, without a formulated protocol tailored to the animals' nutritional requirements. The balanced feed was supplied at a rate of 2 kg per cow per day, with feeding occurring in the early morning hours (5:00 to 7:00 a.m.).

Sampling and processing

The analyzed variables included the proportion of cows with mastitis (%) and subclinical ketosis (%), milk production, body condition score, and glucose levels. Among the milk parameters, somatic cell count, pH, density, total solids, protein, and fat content were evaluated.

Mastitis was evaluated using the California Mastitis Test (CMT, ImmuCell Corporation, Portland, ME, USA), considering cows positive if at least one quarter of the udder was affected. The determination of beta-hydroxybutyrate (BHB) in milk (µmol/L) was performed using the Porta BHB Milk Ketone Test kit (USA), a semi-quantitative method utilizing reactive strips in milk. The reactive pad of the test strip contains an enzyme that converts BHB from acetoacetate, producing a purple compound. A darker color indicates a higher concentration of BHB. The test interpretation is based on the following ranges: 0–99 µmol/L is normal (-); 100–199 µmol/L is questionable (+/-); 200–499 µmol/L is positive (+); >500 µmol/L is strongly positive (++). For the present study, subclinical ketosis was diagnosed in cows with BHB levels exceeding 200 µmol/L.

For glucose analysis (mg/dL), blood samples were collected from the jugular vein and subcutaneous abdominal vein using vacutainer tubes every 7 days from day 1 to day 60. All animals were fasted prior to sampling. Serum was obtained by centrifugation at 1000 × g for 10 minutes and stored in cryovials at -20 °C. Glucose analysis was performed through quantitative colorimetry using spectrophotometry with commercial kits (Valtek® - Chile). A UV-visible spectrum spectrophotometer (Toption UV-5100B UV/VIS – China) was used for the measurements. Milk parameters were analyzed with a milk analyzer (MILKOTESTER® - Bulgaria), using 5 mL of freshly collected milk in the analyzer tube. Milk production was measured in fasted animals during the first milking before feed administration. Finally, body condition was estimated through observation and palpation of the iliac fossa to assess fat reserves, scored on a scale of 1 (thin) to 5 (obese), following the methodology described by Edmonson et al. (1989).

Statistical analysis

The data were analyzed using descriptive statistics. An analysis of variance (ANOVA) was conducted under a completely randomized design with a 2x2 factorial arrangement for quantitative variables with a normal distribution. The Tukey test was used for mean comparisons, with a significance level of 95%. For the analysis of categorical variables, a contingency test was applied. The statistical software used was SAS v. 9.0.

RESULTS AND DISCUSSION

The results show a reduction in total milk protein in multiparous cows with subclinical ketosis or when beta-hydroxybutyrate concentrations exceeded 200 µmol/L ($p < 0.05$, Table 1). However, this effect was not observed in primiparous cows, even when subclinical ketosis was evident. Other milk parameters influenced by beta-hydroxybutyrate concentrations included milk fat and somatic cell count, both of which increased in cows with subclinical ketosis. For milk fat content, the effect was significant only in multiparous cows ($p < 0.05$, Table 1). In primiparous cows, although a numerical increase in milk fat was observed, it was not statistically significant. A marked increase in somatic cell count was noted in the milk of multiparous cows with subclinical ketosis compared to both multiparous and primiparous cows without subclinical ketosis. This suggests that subclinical ketosis in primiparous cows has a mild or negligible effect on these milk parameters.

Milk pH, density, and total solids were not in-

Table 1. Milk Parameters in Brown Swiss Cows with and without Subclinical Ketosis Under Highland Conditions

Variables	Primiparous cows (n=32)		Multiparous cows (n=32)	
	0-200 (µmol/l)	>200 (µmol/l)	0-200 (µmol/l)	>200 (µmol/l)
pH	6.71 ± 0.07[b]	6.75 ± 0.09[a]	6.74 ± 0.07[a]	6.77 ± 0.08[a]
Density	30.18 ± 0.68[a]	31.11 ± 0.44[a]	29.01 ± 0.98[a]	30.49 ± 0.88[a]
Total protein (%)	2.74 ± 0.07[a]	2.71 ± 0.09[a]	2.69 ± 0.10[a]	2.04 ± 0.12[b]
Total Solids (%)	8.43 ± 0.17[a]	8.32 ± 0.19[a]	8.51 ± 0.11[a]	8.17 ± 0.18[a]
Fat (%)	0.88 ± 0.16[b]	0.90 ± 0.11[b]	0.85 ± 0.14[b]	0.96 ± 0.19[a]

[a,b,c] Different letters within rows indicate significant differences between deliveries ($p<0.05$). µmol/l = micromoles per liter.

fluenced by beta-hydroxybutyrate concentrations or the productive status of the animals. There is limited information in the literature on the effect of subclinical ketosis on milk parameters, particularly considering the breed and conditions of semi-intensive dairy farming in the Peruvian highlands. In these systems, altitude and problematic feeding management based on empirical experience likely contribute to metabolic disorders, especially during the transition and peripartum periods. The findings of this study contrast with Benedet (2019), who reported that elevated beta-hydroxybutyrate concentrations are associated with higher milk fat content and lower protein and urea nitrogen levels. Additionally, Bénedet noted that the relationship between milk production and somatic cell count remains controversial. However, the present study provides evidence of a potential association between subclinical ketosis and increased somatic cell count, likely influenced by the presence of subclinical mastitis, as shown in Table 3.

Further supporting the findings, studies on subclinical ketosis have indicated that hyperketonemia negatively affects milk protein content. Animals with high BHB concentrations produce milk with 0.3% (Santschi et al., 2016) to 11.6% (Chandler et al., 2018) less protein compared to healthy animals. In this study, the effect was significant in multiparous cows. A discrepancy between this study and findings by Santschi et al. (2016), Chandler et al. (2018), and De Jong et al. (2023) is that greater reductions in milk protein have been observed in primiparous cows with high BHB levels, contrary to the findings of this study, where the effect was seen in multiparous cows. This may be due to breed differences, feeding practices, and varying metabolic rates influenced by altitude.

Regarding milk fat content, previous studies have reported increases ranging from 2.4% (Garro et al., 2014) to 23.9% (Santschi et al., 2016) in cows with subclinical ketosis compared to healthy cows. Moreover, it has been noted that the most significant differences in milk fat are observed in hyperketonemic cows during very early lactation (Koeck et al., 2014; De Jong et al., 2023). These reports contrast with the findings of this study in terms of milk fat.

Table 2 shows a significant decrease in glucose concentrations ($p < 0.05$) in both primiparous and multiparous cows with subclinical ketosis. This is likely due to the inability of early lactating cows to cope with the energy deficit characteristic of this period (Duque et al., 2011). Additionally, cows experience pronounced lipid mobilization at the onset of lactation, leading to low serum concentrations of glucose, total proteins, and urea, which could explain these results (De Jong et al., 2023). The high glu-

Table 2. Glucose concentrations, milk yield and body condition in lactating Brown Swiss cows with and without subclinical ketosis under range conditions.

Variables	Primiparous cows (n=32)		Multiparous cows (n=32)	
	0-200 (µmol/l)	>200 (µmol/l)	0-200 (µmol/l)	>200 (µmol/l)
Glucose (mg/dl)	57.65 ± 3.88[a]	50.03 ± 4.76[c]	53.60± 2.11[b]	49.67 ± 2.14[c]
Milk Px (liters)	10.02 ± 1.11[c]	12.98 ± 2.55[b]	13.01 ± 3.45[b]	15.66 ± 4.02[a]
Body condition (CC)	3.05 ± 0.11[a]	2.78 ± 0.59[b]	2.99 ± 0.17[ab]	2.56 ± 0.16[c]

[a,b,c] Different letters within rows indicate significant differences between deliveries ($p<0.05$).

Table 3. Frequency of primiparous and multiparous Brown Swiss lactating cows with subclinical ketosis and presence of mastitis according to β-hydroxybutyrate values in milk.

Lactation status	β-hidroxi-butitrato (µmol/l)	Cows with subclinical ketosis (%)	Cows with mastitis (%)
Primiparous (n=32)	>200	14[b]	9.3[b]
Multiparous (n=32)	>200	56[a]	17[a]

[a,b] Different letters within rows indicate significant differences between deliveries ($p<0.05$).

cose demand to sustain milk production, combined with low dry matter intake, also contributes to this glucose deficit. Moreover, there is an imbalance in glucose regulation by insulin, and elevated BHB concentrations impair carbohydrate metabolism by reducing glucose levels through decreased gluconeogenesis (Chaput et al., 2020). Regarding body condition, there is a clear relationship between subclinical ketosis or high BHB concentrations and body condition score (BCS), suggesting that BCS could be an effective predictor of subclinical ketosis without directly measuring BHB concentrations, provided the assessment is performed by experienced professionals. This is supported by the significant differences observed in Table 2 between cows with and without subclinical ketosis, in both primiparous and multiparous cows. These findings contrast with those reported by Roche et al. (2007), who noted that body condition decreases from the first week of lactation in both primiparous and multiparous cows, attributed to the increased energy demands at the onset of lactation. An additional study mentioned that mastitis presence could be related to body condition, although this association was not statistically significant ($p \leq 0.08$) (Zhang et al., 2020).

Figure 1a presents two notable scenarios. The first scenario illustrates a marked reduction in glucose concentrations during the first 14 days of lactation in cows with subclinical ketosis compared to healthy cows. This finding contrasts with reports by Chandler et al. (2018) and Garro et al. (2014), who highlight that the high demands of the first week of lactation significantly impact serum glucose concentrations, compounded by negative energy balance characterized by lipid mobilization. The second scenario reveals that glucose concentrations begin to stabilize from the third week of lactation, likely due to hepatic physiological adaptation, with restored gluconeogenesis and increased dry matter intake. This information is critical, as it underscores that strategies for controlling subclinical ketosis should be implemented during the first two weeks of lactation.

Table 3 confirms the presence of subclinical ketosis in both primiparous and multiparous cows, with a higher incidence observed in the latter. Brunner et al. (2018) reported a general mastitis prevalence of 3.4% in cows with ketosis, which is slightly lower than the findings of this study, where mastitis prevalence was 9% in primiparous and 17% in multiparous cows. This difference could be attributed to management conditions, the moderate level of milking technology, and the semi-intensive nature of the small herds studied. Additionally, the table provides slight evidence of a probable relationship between mastitis and subclinical ketosis in cows. This contrasts with reports from various authors who observed a higher frequency of subclinical ketosis in multiparous cows compared to primiparous cows (Santschi et al., 2016; De Jong et al., 2023; Chandler et al., 2018). Regarding mastitis, conflicting results have been reported. Berge and Vertenten (2014) found that cows diagnosed with subclinical ketosis were nearly twice as likely to experience a mastitis event within the first month of lactation compared to healthy cows. Conversely, Moyes et al. (2014) observed that udder inflammation led to increased BHB concentrations in milk. However, Seely et al. (2021) and Suthar et al. (2013) did not detect any association between mastitis and elevated BHB concentrations. Given that hyperketonemic cows have a higher incidence of clinical mastitis compared to healthy cows (Berge and Vertenten, 2014), higher somatic cell counts would be expected in hyperketonemic animals. This expectation, however, contrasts with the findings of the present study, as shown in Table 1.

Figure 1b illustrates milk production in cows with and without subclinical ketosis. An intriguing observation is that, during the first week postpartum, milk yield decreased in cows experiencing subclinical ketosis. Initially, this aligns with the biological expectation that elevated beta-hydroxybutyrate (BHB) levels impair gluconeogenesis, resulting in reduced glucose availability and thus limiting milk

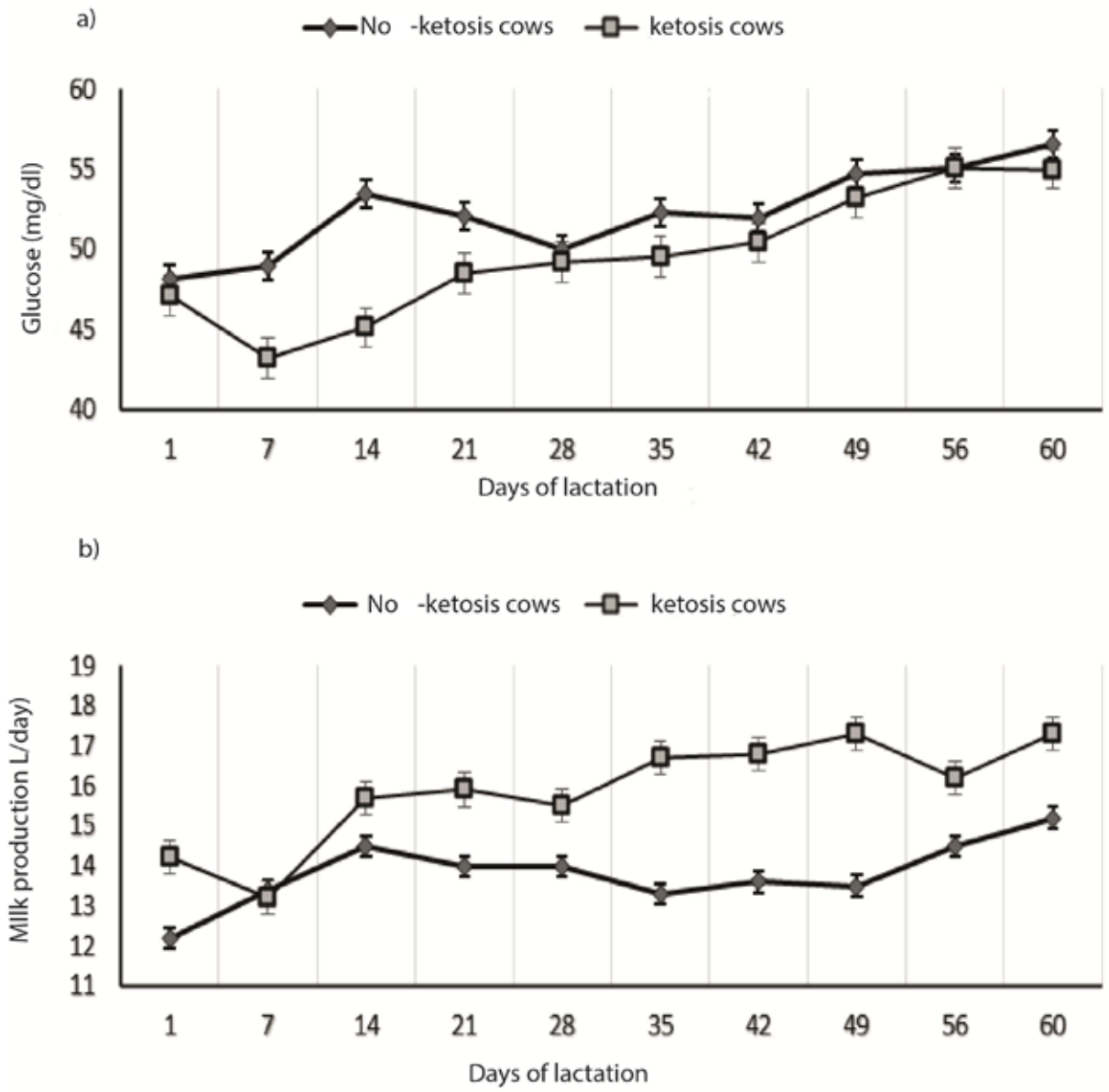

Figure 1. Glucose levels and milk production in Brown Swiss cows with and without subclinical ketosis during the first 60 days of lactation.

production capacity (Seely et al., 2021; Holzhauer and Valarcher, 2024). However, from the third week postpartum onward, cows with subclinical ketosis exhibited increased milk production compared to non-ketotic cows. This unexpected increase may be explained by a compensatory physiological adaptation, as cows mobilize substantial body fat reserves to sustain higher lactation demands, reflected by a maintained body condition score (BCS) not dropping below 2.5 points. Indeed, the transition to increased milk yield despite high BHB levels suggests metabolic plasticity and efficient utilization of body energy reserves, enabling cows to meet lactation demands even under metabolic stress conditions. This phenomenon is supported by Ha et al. (2023), who indicated that cows capable of effectively mobilizing body fat reserves could temporarily sustain elevated milk production despite compromised glucose metabolism and elevated serum BHB concentrations. The results presented show an initial trend, due to the sample size. Further research with larger sample sizes is recommended to reinforce these results and confirm the observed relationships.

CONCLUSIONS

There is evidence of subclinical ketosis in Brown Swiss cows raised under semi-intensive systems and high-altitude conditions. Elevated beta-hydroxybutyrate concentrations, particularly during the first two weeks of lactation, contribute to increased milk fat content and somatic cell counts, reduced milk protein levels, and are likely associated with the presence of mastitis. These effects are predominantly pronounced in multiparous cows, while the effects in primiparous cows remain controversial and require further investigation. Additionally, body condition score is a reliable parameter for identifying potential subclinical ketosis, particularly in multiparous cows, whereas glucose levels serve as a predictor of ketosis exclusively during the first two weeks postpartum.

Conflict of interest

None declared

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