



Association of Metabolic Profile and Body Condition Score with Productive and Reproductive Parameters in Dairy Cows in a High-altitude Environment

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ABSTRACT

High-altitude environments impact metabolic adaptation during the transition from late gestation to early lactation, thereby affecting milk production, metabolic health, and reproductive recovery. The present study aimed to evaluate the associations between metabolic status and body condition with milk yield and composition, metabolic disorders, and reproductive intervals in Brown Swiss cows grazing in high-altitude areas. A prospective longitudinal observational study was conducted at a single production facility in Peru, located at an altitude of 3,829 meters above sea level. Sixty-one multiparous cows, approximately 5.5 to 7 years old, with 4 to 5 recorded calvings, had an average prepartum body weight of 692.4 ± 123.6 kilograms. These cows were monitored from 4 weeks before calving until 9 weeks postpartum. Metabolic adaptation during the transition period was evaluated prepartum and at postpartum weeks 1, 3, 5, 7, and 9. Levels of β -hydroxybutyrate, glucose, cholesterol, triglycerides, albumin, total protein, ionized calcium, body condition score, estimated body weight, milk yield and composition, and reproductive intervals were measured. Energy-lipid imbalance was determined by measuring β -hydroxybutyrate, glucose, cholesterol, and triglycerides, whereas serum protein levels were assessed by measuring albumin and total protein. Both integrated indicators, energy-lipid imbalance and serum protein status, demonstrated non-linear patterns, peaking at their highest posterior levels around postpartum week 5. Postpartum week had a greater impact on milk production and composition than the animals' metabolic status. The body condition score demonstrated the strongest association with ketosis, where a 1-standard-deviation increase was associated with lower odds of ketosis. Ketosis prevalence was highest at week 5 (19.6%), whereas hypocalcemia persisted as a common condition throughout the follow-up period. Higher serum protein levels were associated with a shorter calving-to-conception interval, although this finding was imprecise. The postpartum period was more strongly associated with milk production, whereas body condition was the most indicative measure of ketosis risk.

Keywords: Body condition score, Dairy cow, Ketosis, Milk yield, Postpartum period, Transition period

INTRODUCTION

The interval from late pregnancy to the start of lactation involves significant physiological and metabolic changes in the dairy cow's production cycle (LeBlanc, 2010). Immediately following calving, lactation markedly increases nutrient demands, while feed intake and metabolic adaptation may be postponed during the initial weeks postpartum (Villar et al., 2024). In the early postpartum period, cows often mobilize fat reserves to balance the temporary mismatch between energy required for lactation and nutrient intake. This mobilization redirects nutrient utilization primarily toward maintenance and milk production, which may reduce resources available for reproduction (Nigussie, 2018). These coordinated metabolic changes can be detected in the bloodstream through levels of glucose, β -hydroxybutyrate, cholesterol, triglycerides, proteins, and minerals. Additionally, changes in body condition score (BCS) and body weight (BW) can serve as indicators of these integrated metabolic adaptations. Excessive or prolonged adjustments can increase the risk of metabolic disorders such as ketosis and may impair productivity and postpartum reproductive recovery (Çolakoğlu et al., 2019; Zhao et al., 2019).

Dairy cows raised at high altitude are exposed to additional environmental challenges, particularly reduced oxygen availability, low ambient temperatures, and fluctuations in forage availability, which may alter nutrient consumption and metabolic adaptation during the postpartum period (Estremadoyro et al., 2024). Reduced oxygen availability in high-altitude environments may affect tissue oxygenation and metabolic efficiency, whereas exposure to low ambient temperatures can increase the energy required for thermoregulation. In addition, seasonal changes in forage availability

and quality may limit nutrient intake, thereby increasing the metabolic challenge faced by dairy cows during early lactation (Bartl *et al.*, 2009). Consequently, the metabolic patterns observed in lowland or intensive production settings might not accurately reflect how cows in highland environments respond (Kong *et al.*, 2021). Evaluating Brown Swiss dairy cows raised under high-altitude grazing conditions within their local production environment is therefore necessary to differentiate physiological postpartum adaptation from metabolic changes that may adversely affect health, milk production, or reproductive performance.

Metabolic assessment is particularly relevant for evaluating the transition period because alterations in energy, protein, and mineral metabolism can be associated with changes in milk production, postpartum health, and the return of reproductive function during early lactation (LeBlanc, 2010; Caixeta and Omontese, 2021). β -Hydroxybutyrate and glucose are widely used to characterize energy metabolism, while cholesterol and triglycerides offer additional insights into lipid transport and liver metabolic adaptation (Puppel and Kuczyńska, 2016; Spaans *et al.*, 2022). Albumin and total serum protein contribute to assessing protein metabolism, while albumin may also reflect changes in hepatic synthetic activity and the inflammatory response during the transition period (Bertoni and Trevisi, 2013; Spaans *et al.*, 2022). Ionized calcium plays a crucial role around the calving period, as the start of colostrum and milk production sharply increases the mother's calcium requirements (Weaver *et al.*, 2016). In dairy cows, the BCS provides a practical estimate of body energy reserves and their mobilization during the transition from late gestation to early lactation (Roche *et al.*, 2009), supplementing data from circulating metabolic biomarkers (Ghaffari *et al.*, 2023). Nevertheless, the BCS does not fully reflect metabolic adaptation, and analyzing a single biomarker is complicated by factors such as lactation stage, feeding, herd dynamics, and individual variation. Therefore, considering indicators of energy and lipid metabolism, such as β -hydroxybutyrate, glucose, cholesterol, and triglycerides, along with serum proteins, ionized calcium, and BCS, may provide a broader assessment of dairy cows' metabolic status (Bertoni and Trevisi, 2013; Puppel and Kuczyńska, 2016).

Studies on transition dairy cows often assess metabolic biomarkers, body condition, production performance, and reproductive results, typically using separate or outcome-specific analyses (LeBlanc, 2010; Abdelli *et al.*, 2017; Kerwin *et al.*, 2022). Longitudinal studies have documented temporal changes in metabolic and inflammatory indicators, whereas other investigations have focused specifically on the relationships among energy balance, blood metabolites, and fertility (Wathes *et al.*, 2007; Spaans *et al.*, 2022). Previous studies conducted in high-altitude or high-tropical conditions have generally focused on specific sources of variation, including altitude and diet (Bartl *et al.*, 2009), geographic differences among high-altitude production areas (Kong *et al.*, 2021), and parity or seasonal effects during the early postpartum period (Vallejo-Timarán *et al.*, 2020). In contrast, there is a limited number of longitudinal studies that examine metabolic status alongside both productive and reproductive outcomes in the same cows from prepartum to early lactation. Consequently, the specific relationships among the integrated energy–lipid state, serum protein status, calcium concentration, and body condition in relation to milk yield, milk composition, metabolic disorders, and reproductive recovery under high-altitude conditions remain insufficiently defined. Multivariate approaches identify coordinated patterns among circulating metabolites in dairy cows, enabling biomarkers to be assessed within broader metabolic profiles rather than as independent measurements. These approaches help characterize metabolic adaptation and identify patterns linked to different health conditions during the transition period (Hailemariam *et al.*, 2014; Kayano and Kida, 2015). Accordingly, the present study aimed to investigate the associations between metabolic status and BCS and milk production, milk composition, and reproductive performance in dairy Brown Swiss cows grazing at high altitudes.

MATERIALS AND METHODS

Ethical approval

The present study and its field procedures received authorization from the Regional Directorate of Agriculture of Junín via the Agricultural Agency of Peru, under Letter No. 007-GRJ-DRA-AAC-PERÚ-2025. All procedures involving animal handling, clinical assessments, and the collection of biological samples were performed by trained personnel in accordance with animal welfare principles and to minimize stress and discomfort for the animals.

Study area

The study was conducted at a single dairy production unit in Yanacancha District, Chupaca Province, Junín Region, central Peruvian Andes, Peru, at approximately 3,829 m above sea level. The production unit was chosen because it maintained a Brown Swiss herd that met the study's criteria, allowing for repeated monitoring of the same cows from prepartum through early lactation. The study area was a dry, temperate high-Andean climate with temperatures ranging from approximately 3 to 18.5°C, a mean annual precipitation of approximately 726.6 mm, and marked seasonal variation, often experiencing low temperatures and frost (Figure 1; Marmolejo Gutarra and Ruiz Velásquez, 2018).

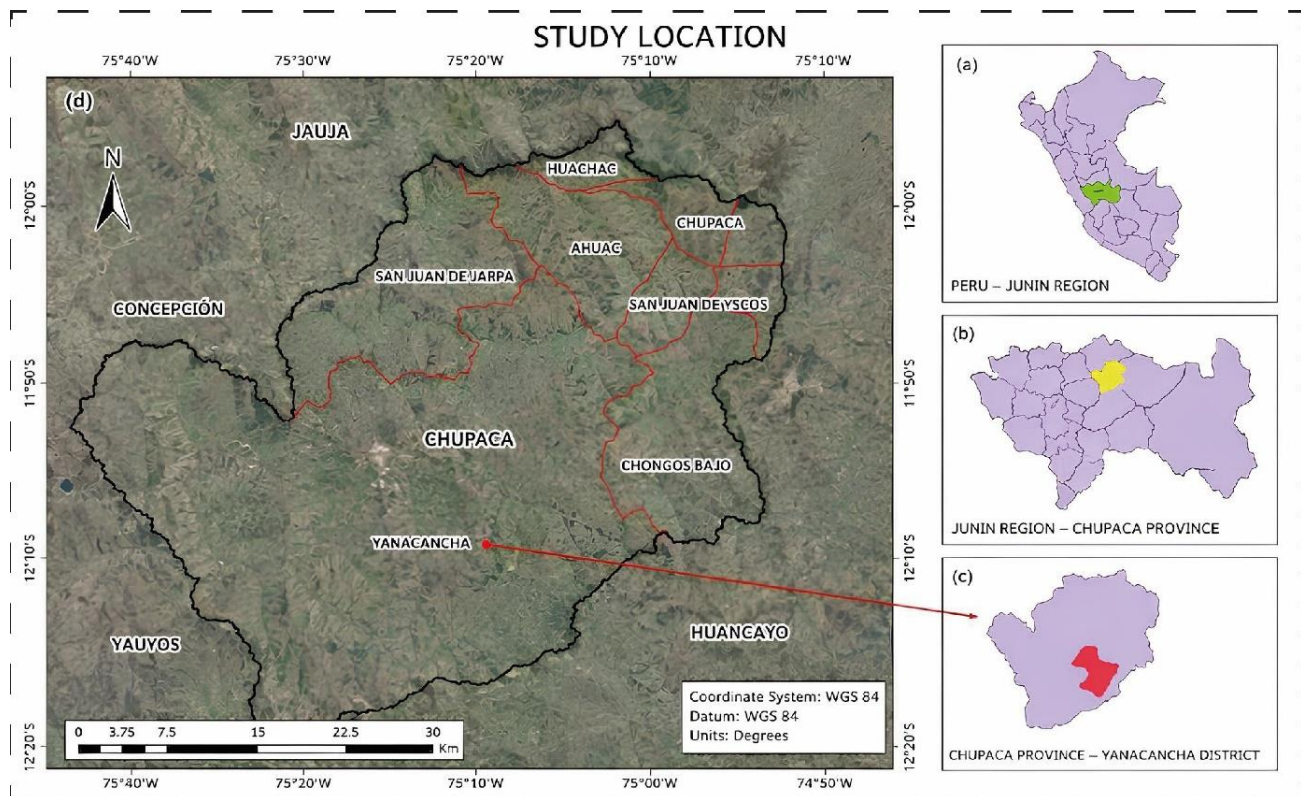


Figure 1. Geographic location of the study area in Yanacancha District, Chupaca Province, Junín Region, Peru

Animals and distribution

The study involved 61 multiparous Brown Swiss cows, aged around 5.5 to 7 years, each with four to five calvings and a full set of permanent teeth, including eight incisors. All 61 cows included in the study were available during the study period and met the preset inclusion criteria for breed, parity, reproductive status, and health; therefore, a formal a priori sample size calculation was not conducted. During the prepartum assessment, the average live weight was 692.44 ± 123.55 kg. Before the study, the cows received the routine antiparasitic treatment used in the herd, consisting of oral administration of Ranide Forte® (Tecnología Química y Comercio S.A., Lima, Peru) at 1 mL/10 kg of body weight, equivalent to 10.0 mg/kg of radoxanide and 9.0 mg/kg of albendazole, according to the manufacturer's recommendations (Tecnología Química Y Comercio S.A., 2024). The cows were maintained on a grazing-based feeding system, with alfalfa as the primary forage, and no experimental dietary treatment was imposed.

Cows were repeatedly evaluated from late gestation through week 9 postpartum. Six assessment intervals were scheduled at approximately four weeks prior to calving and at postpartum weeks 1, 3, 5, 7, and 9. Blood sampling, body condition scoring, and live-weight estimation were scheduled at all six assessment times. Milk yield was obtained from individual milking records, and milk composition was determined at postpartum weeks 1, 3, 5, 7, and 9 using an ultrasonic milk analyzer BOECO Milk Analyzer LAC-SP (Boeckel + Co. GmbH + Co. KG, Hamburg, Germany).

Blood sampling and biochemical parameters

Blood samples were collected at 06:00 a.m., before morning feeding. Approximately 4.5 mL of blood was obtained from the coccygeal vein using sterile needles and red-top evacuated Vacutainer tubes without anticoagulant. Samples were labeled with the individual cow identification code and sampling period and transported on the same morning to the Animal Health Laboratory of the Faculty of Animal Science, Universidad Nacional del Centro del Perú, where laboratory processing was performed between 10:00 and 11:00 a.m.

Blood samples were left to clot for approximately 30 minutes at room temperature and then centrifuged at 3,000 rpm for 10 minutes. The resulting serum samples were analyzed on the same day. Serum glucose, cholesterol, triglycerides, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), direct bilirubin, and total bilirubin were determined using a WP21A semi-automated chemistry analyzer (Genrui Biotech Inc., Shenzhen, China) with commercial reagents (QCA, Amposta, Spain), following the recommended procedures for each assay. The reagent reference codes were 998282 for glucose, 995282 for cholesterol, 992330 for triglycerides, 997180 for total protein, 997258 for albumin, 999500 for AST, 999200 for ALT, 990208 for direct bilirubin, and 992714 for total bilirubin.

Serum β -hydroxybutyrate (BHB) concentration was measured in mmol/L using a NovaVET Xpress ketone meter (Nova Biomedical Corporation, Waltham, MA, USA). Ionized calcium (iCa) was determined in mmol/L using a

LAQUAtwin portable calcium ion meter (HORIBA Advanced Techno Co., Ltd., Japan). The BHB and iCa were evaluated as continuous variables and as binary classifiers for metabolic disorders, with ketosis defined as a BHB of 1.4 mmol/L or greater ($\text{BHB} \geq 1.4 \text{ mmol/L}$; Denis-Robichaud *et al.*, 2014) and hypocalcemia operationally defined as iCa concentration below 0.86 mmol/L ($\text{iCa} < 0.86 \text{ mmol/L}$). At each assessment, prevalence was determined by dividing the number of cows meeting the specific criterion by the total number of cows with available measurements for that biomarker (Thrusfield *et al.*, 2018).

Milk sampling and productive measurements

Milk samples were collected during postpartum evaluations. Following udder and teat cleaning, the initial streams of milk were discarded, and approximately 50 mL of milk was collected per cow into an individually identified container. Samples were immediately placed in a cooler with ice packs and transported to the Microbiology Laboratory at the Universidad Nacional del Centro del Perú for physicochemical analysis. Milk fat, lactose, protein, and solids-not-fat (SNF) were measured using a BOECO Milk Analyzer LAC-SP ultrasonic milk analyzer (Boeco, Hamburg, Germany). The instrument was used and calibrated following the manufacturer's guidelines. The milk fat-to-protein ratio was calculated for each observation as the ratio of measured fat to protein percentages, following its conventional use in dairy cow metabolic assessment (Cabezas-Garcia *et al.*, 2021). Whenever the original ratio differed from the ratio calculated directly from the relevant fat and protein measurements, the calculated value was used. Prepartum milk data were not available in the dataset because the cows had not yet calved, so no zero values were imputed. Individual milk yield was recorded for each cow from their production records and linked to their postpartum assessment via animal identification code. The outcomes related to milk, including milk yield, fat, lactose, protein, SNF, and the fat-to-protein ratio, were assessed.

Body condition score and live-weight measurements

The BCS was evaluated on a five-point scale, with higher scores indicating greater body energy reserves. The BCS was treated as a continuous variable in the statistical analyses, and its scores were assigned in 0.25-point increments when appropriate, following the criteria described by Ferguson *et al.* (1994). The BCS assessment was performed by the same trained veterinarian throughout the study to minimize inter-observer variability (Edmonson *et al.*, 1989).

Body weight (kg) was estimated using a bovine heart-girth weight tape. The tape was placed around the thorax immediately behind the forelimbs, and the corresponding BW estimate was recorded for each cow, following the heart-girth approach described for cattle (Wood *et al.*, 2015).

Reproductive records

Reproductive information was collected from the individual records maintained for each cow, including calving date, first postpartum service, subsequent services, and the service associated with confirmed pregnancy. Pregnancy was confirmed by rectal palpation by a veterinarian and cross-checked with the reproductive record.

The calving-to-first-service interval was calculated as the number of days from calving to the first postpartum service, while the calving-to-conception interval represented the number of days from calving to the service that led to a confirmed pregnancy (Coleman *et al.*, 2009). Accordingly, the conception date was attributed to the recorded service that subsequently resulted in a positive pregnancy diagnosis. These reproductive intervals were utilized as outcome variables within the Bayesian reproductive models.

Statistical analysis

A conservative sensitivity analysis was conducted to assess the statistical power of the available sample. The analysis used a multiple-regression model with four predictors, assuming a two-sided alpha of 0.05, 80% power, and a sample size of 61 cows. Under these assumptions, the smallest detectable overall effect was Cohen's $f^2 = 0.213$, corresponding to approximately 17.6% of explained variance ($R^2 \approx 0.176$), which represents a roughly moderate effect. All analyses were conducted using R version 4.4.1. The data were structured in long format, with repeated measurements nested within each cow. Missing observations were not imputed, and prepartum milk measurements were regarded as structurally unavailable. Continuous predictors in the regression models were standardized by centering each observation on the overall mean and dividing by the corresponding standard deviation (SD; Schielzeth, 2010).

$$z_{ij} = \frac{x_{ij} - \bar{x}}{s_x} \quad (\text{Formula 1})$$

z_{ij} is the standardized value of predictor x for cow i at observation j , x_{ij} is the original observed value, $\bar{x}$ is the overall mean of the predictor, and s_x is its SD. Thus, continuous predictors were expressed on a common scale with a mean of

zero and a SD of one. This standardization enables interpretation of model coefficients in terms of a one-standard-deviation increase in each predictor.

Energy–lipid imbalance was represented as a latent factor derived from Bayesian confirmatory factor analysis of standardized levels of BHB, glucose, cholesterol, and triglycerides. The measurement model was defined as follows (Merkle and Rosseel, 2018).

$$z_{kij} = v_k + \lambda_k F_{EL,ij} + \varepsilon_{kij}, \quad \varepsilon_{kij} \sim N(0, \theta_k) \quad \#(\text{Formula 2})$$

z_{ijk} is the standardized concentration of metabolic indicator k for cow (i) at assessment (j), k represents BHB, glucose, cholesterol, or triglycerides; v_k is the intercept of indicator k , λ_k is its factor loading; $F_{EL,ij}$ is the latent energy–lipid imbalance score for cow (i) at assessment (j), ε_{ijk} is the indicator-specific residual error, and θ_k is the corresponding residual variance. For model identification and orientation, the BHB loading was fixed at $\lambda_{\text{BHB}} = 1$.

A broader hepatic–protein latent construct was initially assessed but was not retained due to its inability to provide a sufficiently stable factor solution. Therefore, serum protein status was indicated by a composite score derived from standardized measurements of albumin and total serum protein concentrations. For each cow and assessment, this composite was calculated according to the following formula (Merkle and Rosseel, 2018).

$$P_{ij}^* = \left(\frac{1}{m_{ij}} \right) \sum_{k \in \{\text{Alb, TP}\}} z_{kij} \quad \#(\text{Formula 3})$$

P_{ij}^* is the raw serum protein composite for cow (i) at assessment (j), z_{kij} is the standardized value of albumin (Alb) or total serum protein (TP), and m_{ij} is the number of available protein markers for that observation. When both markers were available, $m_{ij} = 2$, when only one marker was available, $m_{ij} = 1$, and when neither marker was available, the composite was treated as missing. The resulting composite was subsequently standardized according to Formula 1 and used as a predictor of serum protein status.

Milk yield and milk-composition outcomes were analyzed using Bayesian mixed-effects models implemented in the brms package (Bürkner, 2017). A Student-t likelihood was used for milk yield, fat, lactose, solids-not-fat, and the fat-to-protein ratio, whereas milk protein was modeled using a Gaussian likelihood.

$$Y_{ij} \sim t_v(\mu_{ij}, \sigma) \quad \#(\text{Formula 4})$$

$$Y_{ij} \sim N(\mu_{ij}, \sigma) \quad \#(\text{Formula 5})$$

$$\mu_{ij} = \beta_0 + \beta_{\text{week}(j)} + \beta_1 F_{EL,ij} + \beta_2 P_{\text{ser},ij} + \beta_3 zCa_{ij} + \beta_4 zBCS_{ij} + b_i \quad \#(\text{Formula 6})$$

Y_{ij} is the observed milk outcome for cow (i) at assessment (j), μ_{ij} is its expected value, v is the degrees-of-freedom parameter of the Student-t distribution, σ is the residual SD, β_0 is the model intercept, $\beta_{\text{week}(j)}$ represents the fixed effect of postpartum week at assessment (j), β_1 to β_4 are the regression coefficients associated with the established predictors, $F_{EL,ij}$ is the established latent energy-lipid imbalance score, $P_{\text{ser},ij}$ is the established serum protein status, zCa_{ij} is established ionized calcium, $zBCS_{ij}$ is established BCS, and b_i is the cow-specific random intercept, assumed to follow $b_i \sim N(0, \sigma_b^2)$, in which σ_b^2 represented the between-cow variance. Posterior means and pairwise contrasts were then estimated for each postpartum week. The overall metabolic-productive analysis framework is summarized in Figure 2.

Ketosis and hypocalcemia were analyzed separately using Bayesian logistic mixed-effects models in brms (Bürkner, 2017). The binary response for each metabolic disorder was calculated using the following formulas.

$$Y_{ij} \sim \text{Bernoulli}(p_{ij}) \quad \#(\text{Formula 7})$$

$$\text{logit}(p_{ij}) = \beta_0 + \beta_{\text{week}(j)} + \beta_1 zBCS_{ij} + \beta_2 zBW_{ij} + b_i \quad \#(\text{Formula 8})$$

Y_{ij} represents the binary indicator of metabolic-disorder status in cow (i) at assessment (j), where 1 indicated the presence of a disorder, and 0 indicates its absence. p_{ij} is the probability of the disorder, β_0 is the intercept, $\beta_{\text{week}(j)}$ represents the fixed effect of sampling period, β_1 and β_2 are the regression coefficients for standardized body condition score ($zBCS_{ij}$) and standardized body weight (zBW_{ij}), respectively, and b_i is the cow-specific random intercept, with $b_i \sim N(0, \sigma_b^2)$. Regression coefficients were exponentiated and expressed as odds ratios ($OR = \exp[\beta]$). Values greater than 1 indicated higher odds, while those less than 1 indicated lower odds of the respective disorder.

The calving-to-first-service and calving-to-conception intervals were analyzed using Bayesian lognormal regression models implemented in brms (Bürkner, 2017). For each cow, early-postpartum metabolic predictors were summarized as the mean of available standardized measurements from postpartum weeks 1, 3, and 5.

$$T_i \sim \text{Lognormal}(\mu_i, \sigma) \quad \#(\text{Formula 9})$$

$$\mu_i = \beta_0 + \beta_1 \bar{F}_{EL,i} + \beta_2 \bar{P}_{\text{ser},i} + \beta_3 \bar{zCa}_i + \beta_4 \bar{zBCS}_i \quad \#(\text{Formula 10})$$

T_i is the reproductive interval in days for cow (i), μ_i is the expected value of the natural logarithm of the reproductive interval, σ is the residual SD on the log scale, β_0 is the intercept, β_1 to β_4 are the regression coefficients, $\bar{F}_{EL,i}$ is the mean standardized energy-lipid imbalance score, $\bar{P}_{\text{ser},i}$ is the mean standardized serum protein status, $\bar{zCa}_i$ is the mean standardized ionized calcium concentration; and $\bar{zBCS}_i$ is the mean standardized body condition score across postpartum weeks 1, 3, and 5. Exponentiated coefficients were reported as time ratios ($TR = \exp[\beta]$); values greater than 1 indicated a longer reproductive interval, whereas values below 1 indicated a shorter interval.

Models were fitted using four Markov chains and model-specific weakly informative priors to provide regularization while allowing the observed data to primarily determine the posterior estimates (Gelman et al., 2008; Bürkner, 2017). Posterior distributions were summarized by their means, 95% credible intervals (CrI), and probability of direction (Pdir), following the guidelines of Makowski et al. (2019).

$$P_{\text{dir}} = \max[P(\beta > 0 | \text{data})P(\beta < 0 | \text{data})] \quad (\text{Formula 11})$$

β is the model coefficient of interest, $P(\beta > 0 | \text{data})$ is its posterior probability of being positive, and $P(\beta < 0 | \text{data})$ is its posterior probability of being negative. Therefore, Pdir calculated the posterior probability that the estimated effect aligned with the most probable direction. Convergence was evaluated with the rank-normalized $\hat{R}$ diagnostic, effective sample sizes (ESS) for bulk and tail, and the presence of divergent transitions (Vehtari et al., 2021). Convergence was considered satisfactory when $\hat{R} < 1.01$, both bulk and tail ESS exceeded 400 posterior samples, and no divergent transitions occurred. The ESS referred to the effective number of approximately independent posterior samples generated by the Markov chains and did not represent the number of cows or observations included in the study. Model adequacy was examined using posterior predictive checks (Gabry et al., 2019), while out-of-sample predictive performance was evaluated using Pareto-smoothed importance-sampling leave-one-out cross-validation (PSIS-LOO; Vehtari et al., 2017).

Bayesian architecture of the metabolic-productive system

The energy-lipid state is latent; serum protein status is an observed composite

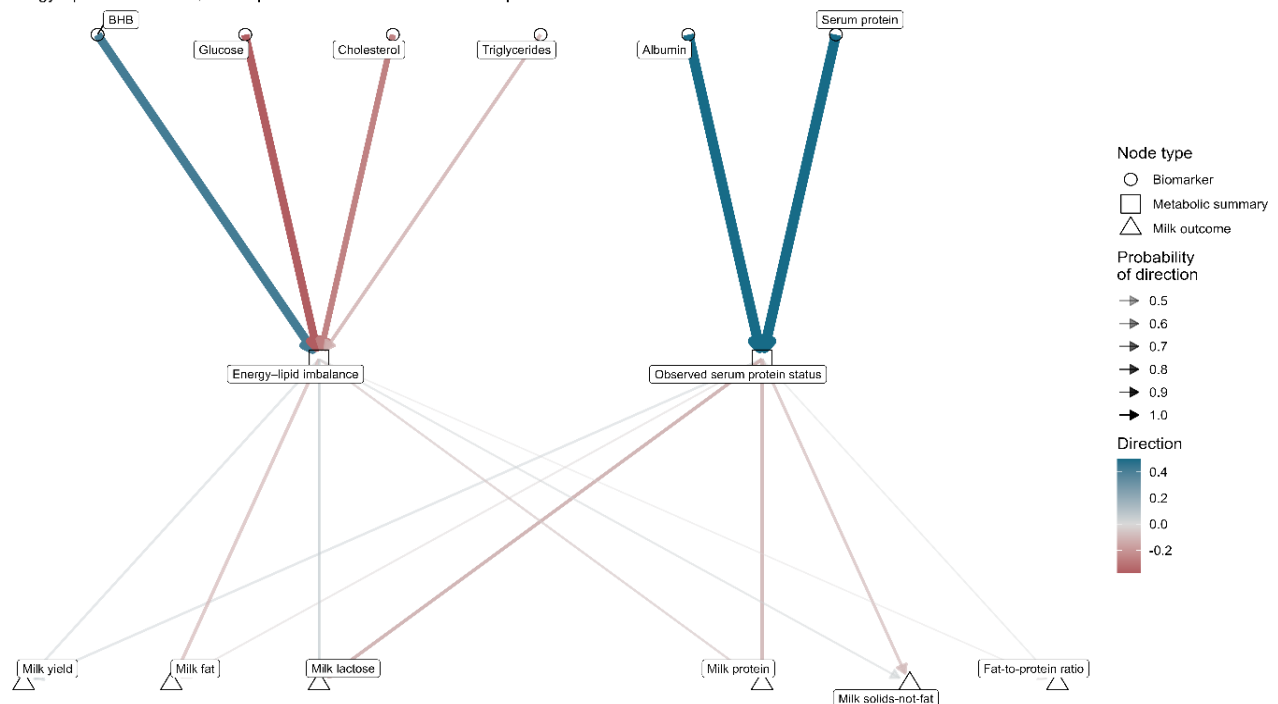


Figure 2. Bayesian architecture of the metabolic-productive system in Brown Swiss dairy cows from approximately 4 weeks prepartum to 9 weeks postpartum under high-altitude grazing conditions in Peru. β -Hydroxybutyrate, glucose, cholesterol, and triglycerides were integrated into a latent energy-lipid imbalance score, whereas albumin and total serum protein were combined into an observed serum protein status index.

RESULTS

Descriptive characteristics and metabolic profile

The main temporal changes in serum biochemical indicators, body measurements, and milk traits are summarized in Table 1. Body condition score had its lowest mean at postpartum week 1 (2.66 ± 0.08) and its highest at the prepartum assessment (3.40 ± 0.20), then increased progressively to 3.19 ± 0.30 at week 9. Body weight was highest prepartum (692.44 ± 123.55 kg), declined to its lowest value at week 5 (618.57 ± 101.76 kg), and subsequently increased to 632.05 ± 100.34 kg by week 9, although it remained below the prepartum mean. Among the metabolic indicators, β -hydroxybutyrate reached its highest concentration at week 5 (1.04 ± 0.57 mmol/L) and its lowest concentration at week 9 (0.83 ± 0.25 mmol/L). Triglycerides decreased from a maximum of 51.85 ± 25.53 mg/dL prepartum to a minimum of 30.39 ± 9.54 mg/dL at week 5, whereas cholesterol increased from its lowest mean prepartum (82.80 ± 22.59 mg/dL) to its highest value at week 9 (104.86 ± 38.39 mg/dL). Total bilirubin declined from 0.59 ± 0.29 mg/dL prepartum to 0.33 ± 0.21 mg/dL at week 9, while AST decreased from 126.46 ± 48.11 U/L prepartum to its lowest mean of 97.38 ± 37.64 U/L at week 7. Serum glucose differed across assessments, with the lowest mean at week 5 (50.55 ± 26.51 mg/dL) and the highest at week 9 (67.95 ± 24.69 mg/dL).

Table 1. Serum biochemical, metabolic, body condition, and milk parameters of Brown Swiss dairy cows across the prepartum and early postpartum periods under high-altitude grazing conditions in Peru

Parameters	PRE	S1	S3	S5	S7	S9
Serum biochemical and metabolic indicators						
Albumin (g/dL)	3.89 ± 0.86	4.27 ± 1.70	4.08 ± 1.86	4.68 ± 2.24	4.31 ± 2.06	3.22 ± 1.69
Direct bilirubin (mg/dL)	0.29 ± 0.24	0.31 ± 0.35	0.18 ± 0.20	0.23 ± 0.26	0.19 ± 0.28	0.34 ± 0.68
β-Hydroxybutyrate (mmol/L)	0.85 ± 0.46	0.88 ± 0.32	0.90 ± 0.32	1.04 ± 0.57	0.93 ± 0.27	0.83 ± 0.25
Total bilirubin (mg/dL)	0.59 ± 0.29	0.45 ± 0.19	0.42 ± 0.22	0.38 ± 0.23	0.34 ± 0.17	0.33 ± 0.21
Ionized calcium (mmol/L)	0.71 ± 0.10	0.78 ± 0.28	0.73 ± 0.24	0.79 ± 0.23	0.77 ± 0.24	0.75 ± 0.21
Cholesterol (mg/dL)	82.80 ± 22.59	89.51 ± 41.25	98.11 ± 46.92	104.28 ± 46.67	93.88 ± 38.88	104.86 ± 38.39
Glucose (mg/dL)	53.71 ± 20.25	51.45 ± 29.10	61.96 ± 28.35	50.55 ± 26.51	55.98 ± 28.55	67.95 ± 24.69
Total serum protein (g/dL)	6.21 ± 3.34	9.61 ± 5.19	8.73 ± 5.31	9.68 ± 5.62	10.11 ± 6.62	7.71 ± 6.01
Aspartate aminotransferase (U/L)	126.46 ± 48.11	118.51 ± 40.04	110.49 ± 44.62	112.85 ± 49.13	97.38 ± 37.64	100.56 ± 35.41
Alanine aminotransferase (U/L)	32.30 ± 6.65	30.03 ± 9.46	33.27 ± 14.26	30.79 ± 11.45	30.15 ± 12.90	29.67 ± 8.85
Triglycerides (mg/dL)	51.85 ± 25.53	39.39 ± 29.91	33.94 ± 19.69	30.39 ± 9.54	32.26 ± 15.72	31.21 ± 10.14
Body measurements						
BCS (1–5)	3.40 ± 0.20	2.66 ± 0.08	2.93 ± 0.17	3.05 ± 0.33	3.09 ± 0.27	3.19 ± 0.30
BW (kg)	692.44 ± 123.55	653.70 ± 103.85	620.04 ± 99.39	618.57 ± 101.76	625.13 ± 104.42	632.05 ± 100.34
Milk production and composition						
Milk yield (L)	NA	15.34 ± 7.52	14.63 ± 6.86	14.63 ± 6.86	11.43 ± 6.59	9.08 ± 6.12
Milk fat (%)	NA	4.33 ± 2.53	4.89 ± 2.09	4.46 ± 2.58	3.90 ± 2.45	4.00 ± 2.03
Milk lactose (%)	NA	4.67 ± 1.19	4.03 ± 0.77	4.00 ± 1.11	3.98 ± 1.41	3.77 ± 1.60
Milk protein (%)	NA	3.15 ± 1.16	2.91 ± 1.30	3.22 ± 1.65	3.83 ± 2.04	3.79 ± 2.21
Milk solids-not-fat (%)	NA	8.30 ± 2.26	7.06 ± 2.00	6.80 ± 2.69	6.64 ± 3.22	6.30 ± 3.18
Fat-to-protein ratio	NA	1.59 ± 1.21	1.95 ± 1.04	1.73 ± 1.49	1.27 ± 1.17	1.35 ± 1.07

BCS: Body condition score, BW: Body weight, PRE: Assessment approximately 4 weeks before calving, S1, S3, S5, S7, and S9 represent assessments at postpartum weeks 1, 3, 5, 7, and 9. Values are presented as mean ± standard deviation. NA indicates that the variable was not assessed during that period. The effective sample size may vary among variables because of missing observations.

Milk yield was highest at 15.34 ± 7.52 L in week 1 and progressively declined to its lowest mean at 9.08 ± 6.12 L in week 9. Milk fat reached its highest value at week 3 ($4.89 \pm 2.09\%$) and its lowest level at week 7 ($3.90 \pm 2.45\%$), whereas lactose decreased from $4.67 \pm 1.19\%$ at week 1 to $3.77 \pm 1.60\%$ at week 9. Milk protein level was $2.91 \pm 1.30\%$ at week 3, the lowest value, while its highest level was $3.83 \pm 2.04\%$ at week 7. Solids-not-fat decreased from $8.30 \pm 2.26\%$ at week 1 to $6.30 \pm 3.18\%$ at week 9. The fat-to-protein ratio reached its maximum level at week 3 (1.95 ± 1.04) and its minimum value at week 7 (1.27 ± 1.17).

Reproductive outcomes are summarized separately in Table 2. The calving-to-first-service interval averaged 75.48 ± 37.62 days for 25 cows, whereas the calving-to-conception interval averaged 104.44 ± 40.78 days for 23 cows.

Both integrated metabolic indicators changed non-linearly across the transition period (Figure 3). The energy-lipid imbalance remained close to the prepartum level at postpartum week 1 (S1) and postpartum week 3 (S3), increased at S5, and then decreased to its lowest estimate after S9. Serum protein level demonstrated a similar temporal pattern, increasing after calving, peaking at S5, remaining elevated at S7, and declining by S9. Compared with the prepartum period (PRE), the most evident directional increase was observed at S5 for both energy-lipid imbalance (Pdir = 95.2%) and serum protein status (Pdir = 97.4%). At S9, the posterior distributions were generally lower than the prepartum observations. When both metabolic indicators were considered simultaneously, cows at S5 and S7 tended to exhibit higher values of energy-lipid imbalance and serum protein levels, whereas at S9 energy-lipid imbalance and serum protein levels were lower. However, the distributions across the different assessment periods overlapped significantly, indicating that metabolic responses differed among cows and that the temporal changes did not clearly distinguish all sampling periods.

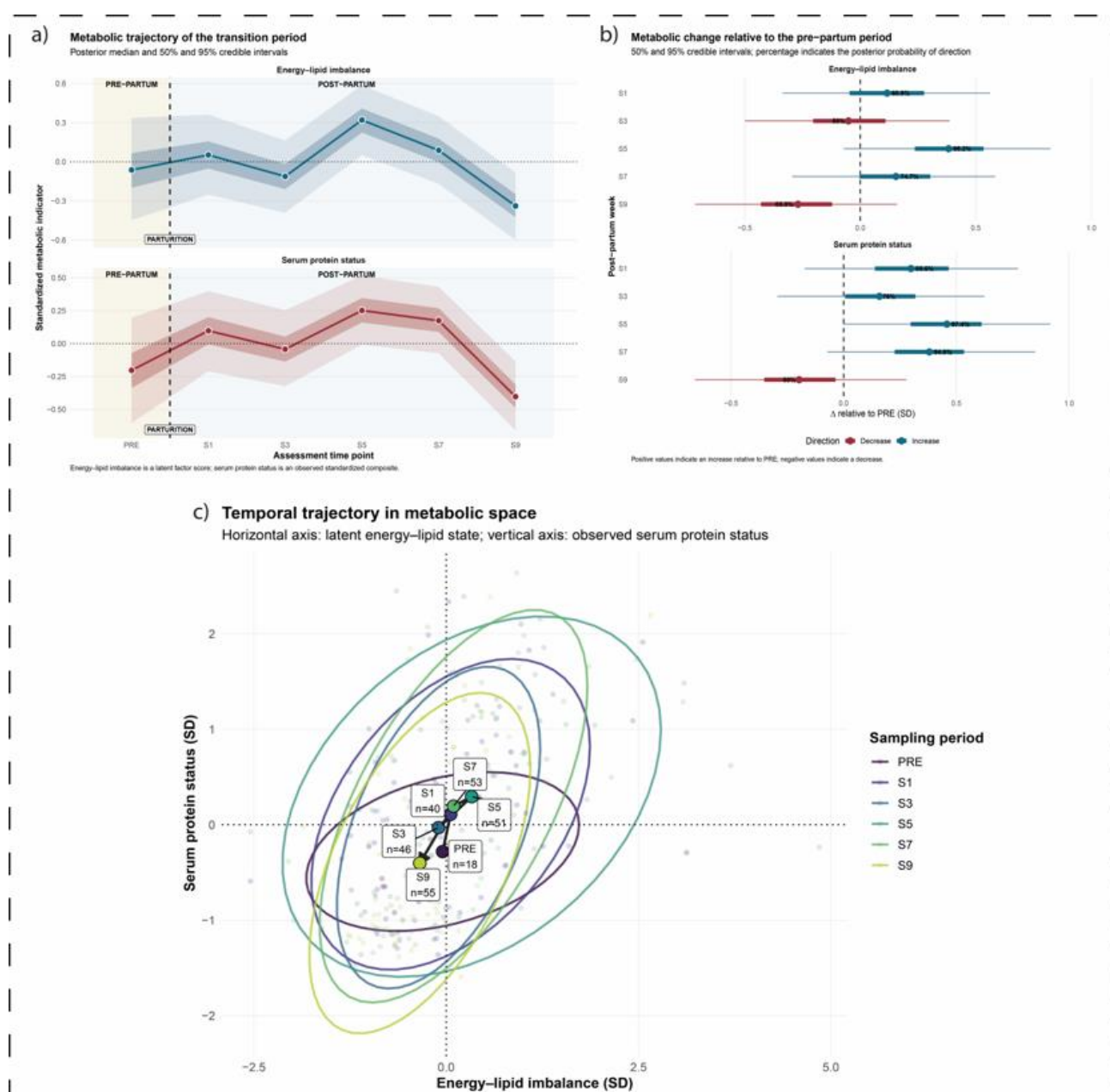


Figure 3. Posterior trajectories and joint distribution of energy-lipid imbalance and serum protein status in Brown Swiss cows in Peru, from prepartum to postpartum week 9. **a:** Temporal trajectories with 50% and 95% credible intervals (CrIs). **b:** Changes relative to the prepartum period (PRE); percentages indicate the posterior probability of direction (Pdir). **c:** Joint distribution of standardized indicators; points show period centroids and sample sizes, and ellipses show within-period dispersion.

Table 2. Reproductive intervals of Brown Swiss dairy cows during the early postpartum period under high-altitude grazing conditions in Peru

Reproductive outcome	n	Mean $\pm$ SD (days)
Calving-to-first-service interval	25	75.48 $\pm$ 37.62
Calving-to-conception interval	23	104.44 $\pm$ 40.78

The calving-to-first-service interval was calculated from calving to the first recorded postpartum service. The calving-to-conception interval was calculated from calving to the service that resulted in a subsequently confirmed pregnancy. n: Number of cows included in the analysis for each reproductive outcome. Values are presented as mean $\pm$ standard deviation (SD)

Associations between metabolic indicators and milk outcomes

Table 3 and Figure 4 summarize the posterior associations between metabolic indicators and milk outcomes. Overall, the estimated effects were modest and uncertain, with all 95% credible intervals including zero. Milk yield demonstrated no clear association with any of the evaluated predictors. The present results indicated that serum protein level was consistently associated with lactose and milk protein, ionized calcium with solids-not-fat, and BCS with milk fat. However, these patterns remained uncertain and were not interpreted as conclusive effects. Associations with the fat-to-protein ratio were generally weak. A visual overview of the direction and relative strength of these posterior associations, emphasizing that the evidence was primarily directional rather than conclusive, is presented in Figure 4.

Postpartum changes in milk production and composition

Milk production and composition differed across postpartum weeks, whereas model-predicted differences associated with changes in the integrated metabolic indicators were comparatively small (Table 4 and Figure 5). Adjusted milk yield increased from 9.97 L at S1 to 11.08 L at S3, remained similar at S5 (11.02 L), and subsequently decreased to 9.96 L at S7 and 8.30 L at S9. Compared with postpartum week 9 (S9), milk yield was higher at S3 by 2.76 L (95% HPD interval of 1.04 to 4.61 L), at S5 by 2.69 L (95% HPD interval of 0.98 to 4.49 L), and at S7 by 1.64 L (95% HPD interval of 0.10 to 3.36 L). These posterior differences indicate the estimated variation in the adjusted mean milk yield between the two postpartum weeks. Milk fat reached its highest adjusted mean at S3 (5.05%) and was higher at S3 than at S7 and S9. Lactose concentration was highest at S1, decreased at S3, then increased again at S5 and S7. Milk protein levels were lowest at S3 and highest at S7, with an estimated increase of 0.33 percentage points between these stages. Solids-not-fat exhibited a similar pattern, whereas the fat-to-protein ratio peaked at S3 and decreased at S7 and S9.

Figure 5 illustrated model-based predictions across three predefined metabolic conditions, including the average level of each metabolic indicator, one SD above the mean (+1 SD), and one SD below the mean (−1 SD). For energy-lipid imbalance, predictions under these three conditions were very similar for milk yield, solids-not-fat, and the fat-to-protein ratio, with only small differences in milk fat, lactose, and protein. Predictions based on serum protein levels demonstrated limited differentiation among the three conditions. The significant overlap of the 95% credible intervals indicated that differences across postpartum weeks are more distinctly identifiable than the predicted changes resulting from a one-standard-deviation increase or decrease in either metabolic indicator.

Ketosis and hypocalcemia

Ketosis remained relatively infrequent throughout the study, with a temporary increase at postpartum week 5 to 19.6%, then declining to 3.6% at week 9 (Table 5). In contrast, hypocalcemia was frequent throughout the study, with prevalence decreasing from 88.9% prepartum to its lowest value of 60.8% at week 5. The percentages indicated the observed frequency of each disorder, while the Bayesian logistic mixed-effects models assessed the adjusted associations with postpartum period and animal-level predictors (Table 6).

Body condition score demonstrated the clearest association with ketosis. A one-standard-deviation increase in BCS was associated with substantially lower odds of ketosis (OR = 0.207; 95% credible interval: 0.103-0.386), corresponding to approximately a 79% reduction in odds. No other ketosis predictor had a 95% credible interval that excluded 1. Regarding hypocalcemia, none of the predictors had a 95% credible interval that excluded 1; however, the posterior estimates indicated lower odds in postpartum weeks 5 and 9 compared to the prepartum period, with Pdir values of 96.6% and 95.1%, respectively. Overall, the strongest model-supported result was the inverse relationship between BCS and ketosis, while the evidence for temporal changes in hypocalcemia was consistent in direction but not conclusive.

Early metabolic status and reproductive recovery

Table 7 and Figure 6 illustrate the relationships between early-postpartum metabolic health and reproductive recovery. None of the evaluated predictors had a 95% credible interval for the time ratio that excluded 1, indicating that the estimated associations remained uncertain. For the calving-to-first-service interval. The posterior estimates generally suggested longer intervals characterized by greater energy-lipid imbalance and increased BCS, while higher serum protein levels were generally linked to shorter intervals. For the calving-to-conception interval, serum protein level demonstrated the strongest directional association, with a time ratio of 0.771, a 95% credible interval of 0.579-1.035, and a Pdir value of 96.3%, indicating a shorter interval as serum protein level increased, although the credible interval included 1. Figure 6 illustrates the combined predictions for energy-lipid imbalance and serum protein status. Regarding reproductive outcomes, longer predicted intervals were predominantly observed with early energy-lipid imbalance and reduced serum protein levels, with the effect most pronounced for the calving-to-conception interval. Conversely, a higher serum protein level combined with a lower energy-lipid imbalance was associated with shorter predicted intervals.

However, the gradual changes across the predictions, along with the credible intervals reported in Table 7, indicated that these associations were uncertain rather than conclusive.

Table 3. Posterior associations of metabolic indicators and body condition score with milk yield and composition in Brown Swiss dairy cows from postpartum weeks 1 to 9 under high-altitude grazing conditions in Yanacancha, Junín, Peru

Milk outcome	Predictor	β	95% CrI	P_{dir}
Milk yield	Energy–lipid imbalance	0.06	[−0.65, 0.74]	56.9%
	Serum protein status	0.04	[−0.71, 0.70]	57.3%
	Ionized calcium	−0.04	[−0.73, 0.62]	53.5%
	BCS	0.13	[−0.79, 1.07]	61.0%
Milk fat	Energy–lipid imbalance	−0.12	[−0.48, 0.24]	74.4%
	Serum protein status	−0.03	[−0.35, 0.29]	56.9%
	Ionized calcium	−0.16	[−0.48, 0.16]	83.7%
	BCS	0.23	[−0.13, 0.62]	88.8%
Milk lactose	Energy–lipid imbalance	0.07	[−0.11, 0.24]	77.3%
	Serum protein status	−0.12	[−0.27, 0.03]	94.6%
	Ionized calcium	−0.04	[−0.18, 0.11]	69.9%
	BCS	−0.08	[−0.26, 0.09]	82.1%
Milk protein	Energy–lipid imbalance	−0.05	[−0.20, 0.10]	75.1%
	Serum protein status	−0.10	[−0.24, 0.03]	92.6%
	Ionized calcium	−0.07	[−0.21, 0.06]	86.2%
	BCS	−0.11	[−0.26, 0.05]	91.4%
Milk solids-not-fat	Energy–lipid imbalance	0.04	[−0.25, 0.32]	59.8%
	Serum protein status	−0.09	[−0.33, 0.14]	78.3%
	Ionized calcium	−0.18	[−0.42, 0.06]	93.4%
	BCS	−0.15	[−0.43, 0.13]	85.4%
Fat-to-protein ratio	Energy–lipid imbalance	−0.00	[−0.19, 0.18]	51.8%
	Serum protein status	0.01	[−0.16, 0.18]	54.3%
	Ionized calcium	0.02	[−0.14, 0.18]	60.8%
	BCS	0.06	[−0.15, 0.27]	72.3%

BCS: Body condition score, β : Posterior mean, CrI = Credible interval, P_{dir} : Posterior probability of the estimated direction. All continuous predictors were standardized; therefore, β represents the expected change in the outcome associated with a one-standard-deviation increase in the predictor.

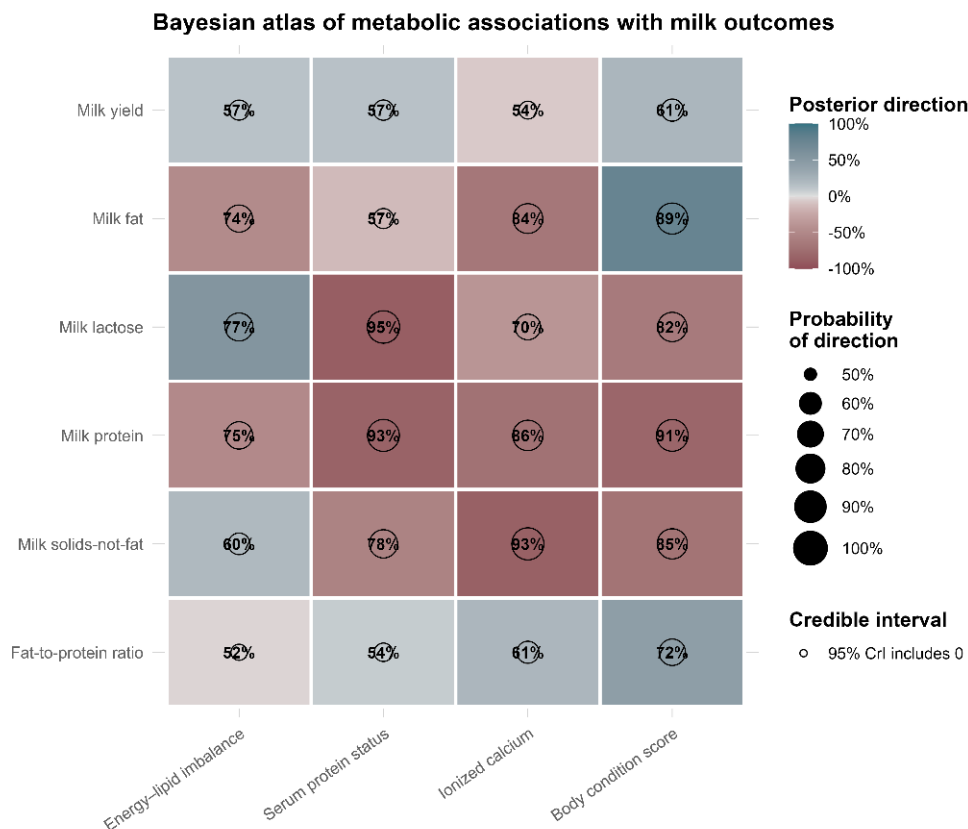


Figure 4. Bayesian atlas of posterior associations between metabolic indicators and milk production traits in Brown Swiss dairy cows from postpartum weeks 1 to 9 under high-altitude grazing conditions in Peru. Cell color indicates the direction and relative magnitude of the posterior association, with blue representing positive effects and red representing negative effects. Circle size and the percentage shown within each cell represent the posterior probability of direction (P_{dir}). Open circles indicate that the 95% credible interval included zero.

Table 4. Adjusted posterior estimates of milk production and composition from postpartum weeks 1 to 9 in Brown Swiss dairy cows under high-altitude grazing conditions in Yanacancha, Junín, Peru

Panel A. Adjusted posterior marginal means					
Outcome	S1	S3	S5	S7	S9
Milk yield (L/day)	9.97 [6.27, 13.66]	11.08 [7.72, 14.37]	11.02 [7.86, 14.47]	9.96 [6.54, 13.06]	8.30 [4.95, 11.60]
Milk fat (%)	4.73 [3.97, 5.55]	5.05 [4.37, 5.73]	4.61 [3.95, 5.25]	3.99 [3.39, 4.65]	3.95 [3.25, 4.55]
Milk lactose (%)	4.17 [3.71, 4.60]	3.60 [3.20, 4.00]	3.86 [3.46, 4.25]	4.03 [3.65, 4.40]	3.76 [3.36, 4.15]
Milk protein (%)	3.85 [3.29, 4.41]	3.56 [2.98, 4.11]	3.70 [3.15, 4.27]	3.89 [3.36, 4.45]	3.77 [3.22, 4.34]
Milk solids-not-fat (%)	6.70 [5.72, 7.76]	6.02 [4.99, 6.91]	6.26 [5.32, 7.19]	6.58 [5.66, 7.49]	6.40 [5.46, 7.30]
Fat-to-protein ratio	1.59 [1.17, 2.03]	1.98 [1.59, 2.32]	1.71 [1.36, 2.08]	1.31 [1.00, 1.63]	1.38 [1.05, 1.71]

Panel B. Pairwise posterior contrasts with 95% HPD intervals excluding zero			
Outcome	Comparison	Posterior difference [95% HPD]	Interpretation
Milk yield (L/day)	S3 vs. S9	2.76 [1.04, 4.61]	Higher at S3
Milk yield (L/day)	S5 vs. S9	2.69 [0.98, 4.49]	Higher at S5
Milk yield (L/day)	S7 vs. S9	1.64 [0.10, 3.36]	Higher at S7
Milk fat (%)	S3 vs. S7	1.05 [0.17, 1.85]	Higher at S3
Milk fat (%)	S3 vs. S9	1.11 [0.26, 1.94]	Higher at S3
Milk lactose (%)	S1 vs. S3	0.57 [0.19, 0.97]	Higher at S1
Milk lactose (%)	S3 vs. S7	−0.43 [−0.78, −0.07]	Higher at S7
Milk protein (%)	S3 vs. S7	−0.33 [−0.66, −0.01]	Higher at S7
Milk solids-not-fat (%)	S3 vs. S7	−0.56 [−1.13, −0.01]	Higher at S7
Fat-to-protein ratio	S3 vs. S7	0.66 [0.22, 1.09]	Higher at S3
Fat-to-protein ratio	S3 vs. S9	0.59 [0.14, 1.05]	Higher at S3

S1, S3, S5, S7, and S9: Postpartum weeks 1, 3, 5, 7, and 9. HPD: Highest posterior density. Values in Panel A are adjusted posterior marginal means (95% HPD interval). In Panel B, values are posterior differences (95% HPD interval). Positive values indicate a higher mean in the first week, and negative values indicate a higher mean in the second week. Only contrasts whose 95% HPD intervals exclude zero are shown.

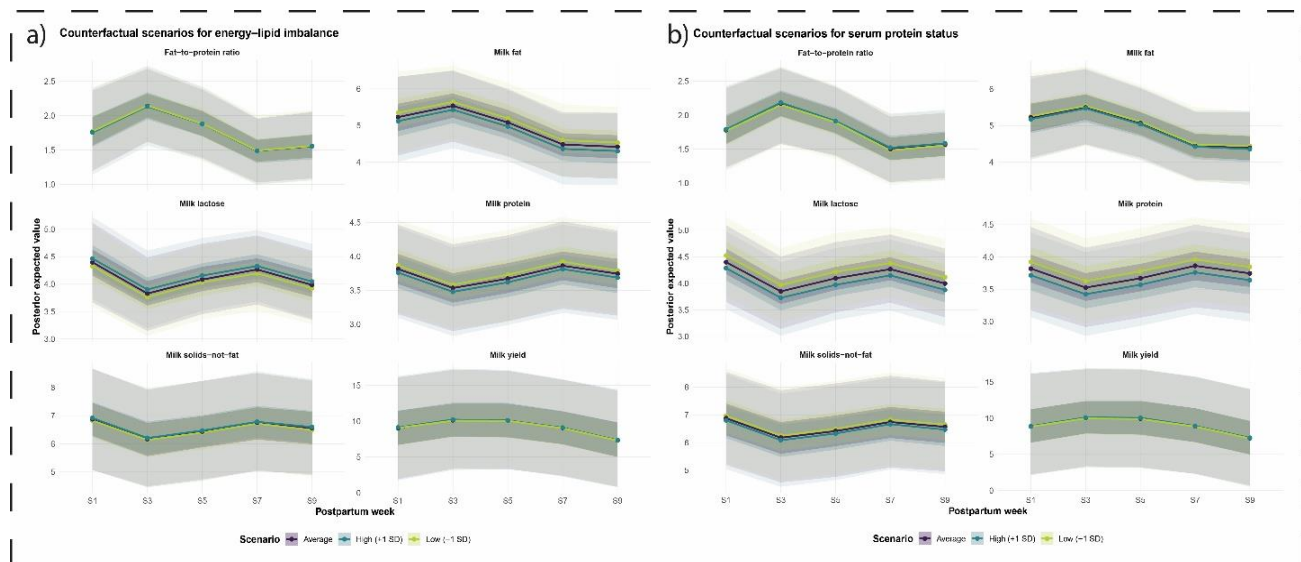


Figure 5. Posterior counterfactual trajectories of milk yield and composition according to energy–lipid imbalance and serum protein status in Brown Swiss dairy cows from postpartum weeks 1 to 9 under high-altitude grazing conditions in Yanacancha, Junín, Peru. **a:** Posterior expected trajectories of milk yield, milk fat, lactose, protein, solids-not-fat, and the fat-to-protein ratio under average, high (+1 SD), and low (−1 SD) energy–lipid imbalance scenarios. **b:** Corresponding posterior trajectories under average, high (+1 SD), and low (−1 SD) serum protein status scenarios. Points represent posterior expected values and shaded bands represent 95% credible intervals. Predictions were obtained for postpartum weeks 1, 3, 5, 7, and 9, with the remaining covariates held constant.

Table 5. Prevalence of ketosis and hypocalcemia across sampling periods in Brown Swiss dairy cows under high-altitude grazing conditions in Yanacancha, Junín, Peru

Sampling period	Ketosis, n/N (%)	Hypocalcemia, n/N (%)
Prepartum	1/18 (5.6)	16/18 (88.9)
Postpartum week 1	4/40 (10.0)	31/40 (77.5)
Postpartum week 3	4/46 (8.7)	31/46 (67.4)
Postpartum week 5	10/51 (19.6)	31/51 (60.8)
Postpartum week 7	3/53 (5.7)	37/53 (69.8)
Postpartum week 9	2/55 (3.6)	35/55 (63.6)

Values are presented as n/N (%), n: The number of cows classified as cases, N: The total number of cows evaluated at each sampling period. Ketosis was defined as a serum β -hydroxybutyrate concentration ≥ 1.4 mmol/L, and hypocalcemia as an ionized calcium concentration < 0.86 mmol/L. Prevalence was calculated using only cows with a valid measurement for the corresponding biomarker.

Table 6. Bayesian logistic mixed-model associations with ketosis and hypocalcemia from prepartum to postpartum week 9 in Brown Swiss dairy cows under high-altitude grazing conditions in Yanacancha, Junín, Peru

Outcome	Predictor	β (95% CrI)	OR	Pdir (%)
Ketosis	Week 1 vs. prepartum	-0.992 (-2.249, 0.213)	0.371	94.5
	Week 3 vs. prepartum	-0.219 (-1.480, 1.007)	0.803	63.6
	Week 5 vs. prepartum	0.987 (-0.152, 2.137)	2.683	95.5
	Week 7 vs. prepartum	-0.260 (-1.558, 0.991)	0.771	65.6
	Week 9 vs. prepartum	-0.341 (-1.745, 1.019)	0.711	68.9
	BCS, per 1 SD	-1.575 (-2.272, -0.951)	0.207	100.0
	Body weight, per 1 SD	0.263 (-0.389, 0.862)	1.301	79.7
Hypocalcemia	Week 1 vs. prepartum	0.086 (-1.047, 1.229)	1.090	56.2
	Week 3 vs. prepartum	-0.497 (-1.474, 0.477)	0.608	83.8
	Week 5 vs. prepartum	-0.901 (-1.868, 0.053)	0.406	96.6
	Week 7 vs. prepartum	-0.428 (-1.349, 0.518)	0.652	81.6
	Week 9 vs. prepartum	-0.796 (-1.733, 0.142)	0.451	95.1
	BCS, per 1 SD	-0.018 (-0.407, 0.373)	0.982	54.4
	Body weight, per 1 SD	0.212 (-0.266, 0.711)	1.236	80.9

β values are posterior mean regression coefficients on the log-odds scale, with the numbers in parentheses representing the lower and upper limits of the 95% credible interval (CrI). OR: Posterior mean odds ratio, calculated as $\exp(\beta)$, Pdir: Posterior probability of the estimated direction, SD: Standard deviation. Week effects were estimated relative to the prepartum period. Body condition score and body weight were standardized, and models included a cow-specific random intercept. Bold values indicate that the 95% CrI for β excluded zero.

Table 7. Bayesian lognormal regression of early-postpartum metabolic status (weeks 1, 3, and 5) and reproductive intervals in Brown Swiss dairy cows under high-altitude grazing conditions in Yanacancha, Junín, Peru

Outcome	Predictor	β (95% CrI)	Time ratio	Pdir (%)
IP-PC	Energy-lipid imbalance	0.121 (-0.227, 0.471)	1.129	76.3
	Serum protein status	-0.175 (-0.553, 0.209)	0.839	82.5
	Ionized calcium	0.034 (-0.414, 0.481)	1.035	56.2
	BCS	0.175 (-0.146, 0.491)	1.191	86.7
IP-C	Energy-lipid imbalance	0.068 (-0.195, 0.331)	1.070	69.7
	Serum protein status	-0.260 (-0.547, 0.034)	0.771	96.3
	Ionized calcium	-0.030 (-0.373, 0.308)	0.970	57.2
	BCS	0.126 (-0.120, 0.368)	1.134	84.7

BCS: Body condition score, β values are posterior mean regression coefficients, and the numbers in parentheses represent the lower and upper limits of the 95% credible interval (CrI); for example, 0.121 (-0.227, 0.471) indicates a posterior mean β of 0.121 with a 95% CrI from -0.227 to 0.471. Time ratios were obtained as $\exp(\beta)$ and are presented as posterior means; values > 1 indicate longer reproductive intervals and values < 1 indicate shorter intervals. Pdir: Posterior probability of the estimated direction; IP-PC: Calving-to-first-service interval; IP-C: Calving-to-conception interval. All continuous predictors were standardized.

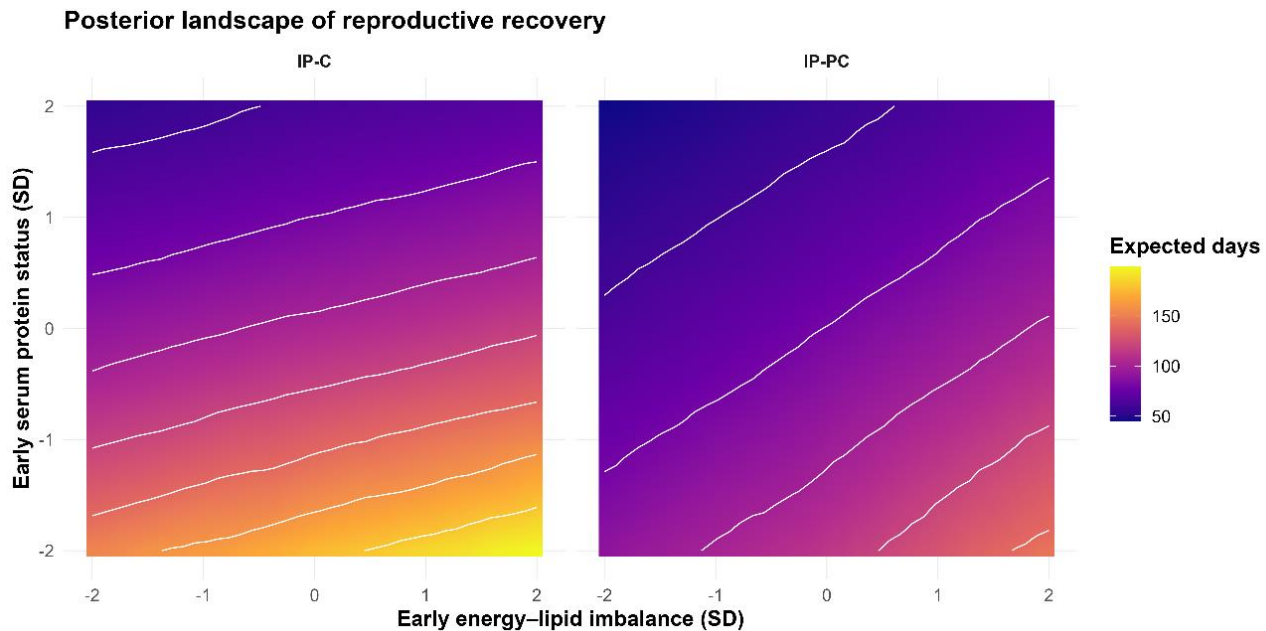


Figure 6. Posterior landscape of reproductive recovery according to early-postpartum energy–lipid imbalance and serum protein status in Brown Swiss dairy cows from postpartum weeks 1 to 5 under high-altitude grazing conditions in Yanacancha, Junín, Peru. IP–C = calving-to-conception interval; IP–PC = calving-to-first-service interval.

DISCUSSION

Energy-lipid imbalance and serum protein levels reached their highest posterior values around postpartum week 5 and subsequently declined, whereas BCS and BW decreased after calving. These patterns were consistent with the substantial metabolic adjustments that occur during early lactation (LeBlanc, 2010), when nutrient requirements for milk synthesis increase more rapidly than feed intake and metabolic capacity (Bertoni and Trevisi, 2013; Caixeta and Omontese, 2021; Spaans et al., 2022). The increase in energy-lipid imbalance around week 5 may reflect a greater reliance on endogenous energy reserves at the initiation of lactation. When nutrient intake does not fully meet energy requirements, adipose tissue mobilization increases the supply of fatty acids to the liver, alters circulating glucose and lipid metabolites, and may promote ketone body production (Roche et al., 2009; LeBlanc, 2010; Zhao et al., 2019). The observed concurrent decline in BCS and BW in the present study supported this finding. However, the energy-lipid indicator, comprising β -hydroxybutyrate, glucose, cholesterol, and triglycerides, reflected a broader metabolic pattern rather than a direct measure of negative energy balance. Similar multimetabolite methods have been employed to analyze metabolic adaptation and disease-related profiles in transition dairy cows (Hailemariam et al., 2014; Kayano and Kida, 2015). The considerable overlap in sampling periods indicated that the extent of metabolic adaptation differed among cows. Under high-altitude grazing conditions, this variability may be affected by factors such as environmental temperature, forage availability, diet, and hypoxic exposure (Bartl et al., 2009; Kong et al., 2021).

The postpartum week had a more pronounced influence on milk production and composition than the metabolic conditions. Milk yield remained comparatively higher during the middle of the observation period before declining at week 9, whereas fat, lactose, protein, solids-not-fat, and the fat-to-protein ratio demonstrated different temporal patterns. These changes were expected, as nutrient distribution, mammary demand, dry matter intake, and body reserve mobilization vary considerably during early lactation (Roche et al., 2009; LeBlanc, 2010; Caixeta and Omontese, 2021). Predictions at the mean and ± 1 SD for energy-lipid imbalance and serum protein level overlapped extensively. This finding suggested that differences in these integrated indicators, within the observed range, had a smaller effect on milk-trait variability than the postpartum period. This finding did not suggest that metabolism was unimportant for lactation; instead, metabolic effects on milk production occur through different interconnected pathways and can be influenced by diet, physiological stage, individual capacity, and environmental factors (Bertoni and Trevisi, 2013; Puppel and Kuczyńska, 2016; Spaans et al., 2022). Studies conducted in high-altitude and high-Andean environments similarly demonstrated that factors such as diet, breed, season, and production environment can influence milk yield and metabolic characteristics (Bartl et al., 2009; Estremadoyro et al., 2024).

The correlation between higher serum protein levels and decreased lactose and milk protein should be interpreted with caution. The serum protein indicator used in the present study combined albumin and total serum protein, thereby

representing the systemic biochemical status of proteins rather than directly indicating the supply of metabolizable protein or amino acids to the mammary gland. Albumin and total protein levels can change due to hepatic synthesis, inflammatory responses, hydration status, and physiological stages (Bertoni and Trevisi, 2013; Puppel and Kuczyńska, 2016; Spaans *et al.*, 2022). Therefore, the observed changes might reflect relationships among systemic metabolic adaptation, lactation stage, and milk production, rather than a direct inhibitory impact of circulating proteins. The negative association between ionized calcium and solids-not-fat had a credible interval of zero. The present findings indicated that individual biomarkers need to be interpreted within a wider metabolic framework, rather than evaluated only by the sign of an estimated coefficient (Hailemariam *et al.*, 2014; Caixeta and Omontese, 2021).

The strongest animal-level link observed in the present study was the inverse relationship between BCS and ketosis. Within the range observed in these cows, higher BCS was associated with substantially lower odds of ketosis, possibly because lower scores during early lactation reflect greater prior mobilization of adipose reserves, thereby increasing fatty acid delivery to the liver and favoring ketogenesis (Roche *et al.*, 2009; Çolakoğlu *et al.*, 2019; Zhao *et al.*, 2019; Ghaffari *et al.*, 2023). Cows with a high BCS may have reduced feed intake and increased postpartum lipid mobilization. However, early lactation and the extent of body condition loss are distinct indicators of changes in energy reserves (Roche *et al.*, 2009; Çolakoğlu *et al.*, 2019; Ghaffari *et al.*, 2023). The peak in energy-lipid imbalance at week 5, coinciding with the highest observed prevalence of ketosis, further supported the link between increased lipid mobilization and ketogenesis during this period (LeBlanc, 2010; Caixeta and Omontese, 2021).

In contrast, a high number of cows were classified as hypocalcemic throughout the study, which could not be explained solely by the conventional periparturient decline in calcium. Calcium demand increases abruptly around calving due to colostrum and milk synthesis; therefore, disturbances in calcium homeostasis are most commonly associated with the immediate periparturient period (Weaver *et al.*, 2016; Caixeta and Omontese, 2021). The persistence of low ionized calcium levels in later postpartum assessments indicated that additional factors, such as mineral intake, individual calcium regulation, analytical methods, or the suitability of the threshold, could be contributing. Ionized and total calcium are related but not diagnostically suitable, and their relationship may change in early lactation (Weaver *et al.*, 2016; Cohrs *et al.*, 2023). Therefore, the high prevalence observed in the present study should be interpreted as a deficiency of ionized calcium, not as clinical milk fever.

The associations between early postpartum metabolic state and reproductive recovery were less strong and less precise. A greater energy-lipid imbalance was associated with longer reproductive intervals, while higher serum protein levels generally reduced the duration, particularly in the calving-to-conception interval. Prolonged negative energy balance impairs insulin/IGF signaling, follicular development, steroidogenesis, and resumption of ovarian activity (Roche *et al.*, 2009; Nigussie, 2018; Çolakoğlu *et al.*, 2019). Conversely, a more favorable overall metabolic condition might facilitate faster recovery of reproductive function (Wathes *et al.*, 2007). Nevertheless, serum protein levels should not be viewed as a direct indicator of dietary protein sufficiency. However, since all 95% credible intervals for the reproductive time ratios include 1, these associations should be considered suggestive rather than conclusive. Additionally, their uncertainty might be due to the fewer cows with full reproductive results.

CONCLUSION

Brown Swiss cows raised in high-altitude grazing areas exhibited a heterogeneous, non-linear metabolic transition from the prepartum period through early lactation, with the greatest energy-lipid imbalance occurring approximately during the postpartum week 5. Changes in milk yield and composition were more closely linked to postpartum stage than to variations in energy-lipid imbalance and serum protein status. Body condition score demonstrated the strongest association with ketosis, with higher BCS associated with lower odds of ketosis. Associations between hypocalcemia and reproductive intervals were less precise. The current findings were limited by the observational design, evaluation within a single high-altitude production setting, a limited number of cows with complete reproductive data, and the lack of a low-altitude comparison group. Future studies should include larger populations from multiple high-altitude herds, verify ionized calcium reference thresholds specifically for cows living at high altitudes and explore whether integrating repeated metabolic assessments with BCS improves early detection of cows at higher metabolic risk.

DECLARATIONS

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Authors' contributions

Lucy Remigio-Lorenzo was responsible for funding acquisition, project administration, field sample collection, laboratory analyses, and the preparation of the original draft. Fernando Arauco Villar contributed to the study design, methodology, funding acquisition, project administration, review, and editing. Noemí Mayorga Sánchez performed the laboratory analyses and provided resources. Yakelin Mauricio-Ramos performed the laboratory analyses. Ide Unchupaico-Payano reviewed and critically revised the manuscript. Jordan Ninahuanca performed the statistical analyses and drafted the manuscript. All authors reviewed and approved the final edition of the manuscript.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

Ethical considerations

Generative artificial intelligence was used only to support English-language editing, improve grammatical accuracy, and enhance sentence clarity. Artificial intelligence tools were not used to generate, modify, or analyze the study data, nor to make scientific decisions or independently interpret the results. All AI-assisted content was critically reviewed, verified, and revised by the authors. The authors accept full responsibility for the accuracy, originality, integrity, and scientific content of the manuscript, both before submission and after acceptance.

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