Comparative Analysis of Changes in the Chemical Composition of Milk in East Friesian and Simmental Cows with Ketosis, Puerperal Paresis, and Mastitis During the Postpartum Period

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Abstract. *Postpartum metabolic and inflammatory disorders, including ketosis, puerperal paresis, and mastitis, profoundly affect milk composition and have significant implications for dairy herd productivity and health. This study aimed to investigate breed-related differences in milk chemical composition between Holstein-Friesian and Simmental cows during early lactation prior to therapeutic intervention. A total of 360 cows were examined and grouped by disease and breed.*

Milk was analyzed for fat, protein, casein, lactose, solids-not-fat, fatty acid profiles (saturated, unsaturated, polyunsaturated), BHB, acetone, minerals (Ca, P, Mg), milk urea, citrate, somatic cell count, and pH (mastitis). ANOVA assessed breed differences, while correlation and regression analyses evaluated associations among metabolic markers and milk components. Chi-square tests examined breed-specific differences within disease categories.

Holstein-Friesian cows with ketosis showed higher BHB (450 ± 30 μmol/L) and acetone (2.5 ± 0.2 mmol/L) compared with Simmental (BHB 380 ± 25 μmol/L; acetone 1.8 ± 0.2 mmol/L; $P < 0.001$), reflecting enhanced lipid mobilization. In mastitis, reductions in protein (Holstein 2.9 ± 0.2% vs Simmental 3.0 ± 0.2%; $P < 0.002$) and lactose (4.0 ± 0.1% vs 4.1 ± 0.1%; $P < 0.001$) were more pronounced in Holstein-Friesians. Puerperal paresis was associated with lower milk calcium (95 ± 5 mg/100 mL vs 100 ± 4 mg/100 mL; $P < 0.001$), phosphorus, and magnesium in Holstein-Friesians. Regression models showed that BHB and acetone explained up to 72% of protein variation, while chi-square tests confirmed significant breed-specific metabolic differences.

However, mechanistic explanations for breed differences remain unclear, and potential confounders such as diet, housing, and environmental factors were not fully controlled, limiting causal inference. These findings highlight milk composition as an integrated biomarker system for monitoring postpartum metabolic and inflammatory stress. Understanding breed-specific responses may inform targeted nutritional strategies, early detection, and precision herd management, enhancing both animal welfare and milk productivity.

Introduction

Milk is one of the most important products of the dairy industry, and its quality is closely linked to the health and metabolic status of the cow (Chang et al., 2011). The postpartum period represents a critical physiological stage during which dairy cows undergo rapid metabolic adaptations to support lactation, often predisposing them to metabolic and inflammatory disorders (Buckley et al., 2003). Changes in milk chemical composition, including fat, protein, lactose, and mineral content, can serve as early, non-invasive biomarkers of these disorders, providing valuable insights for herd management and productivity (Costa et al., 2019; Fleischer et al., 2001).

Postpartum metabolic disorders, such as ketosis and puerperal paresis, and inflammatory conditions like mastitis, significantly alter milk composition. Ketosis arises from a negative energy balance when nutrient intake cannot meet the high energy demands of early lactation, leading to increased mobilization of body fat and accumulation of ketone bodies, which in turn affects milk fat, protein, and lactose content (Cabezas-Garcia et al., 2021; Chirivi et al., 2024; Bochniarz et al., 2023; Grelet et al., 2016). Puerperal paresis, characterized by hypocalcemia, disrupts mineral homeostasis and may reduce the concentrations of calcium, phosphorus, and magnesium in milk, while also affecting milk protein levels (Cai et al., 2018; Khol et al., 2020., Espiritu et al., 2025, Tereso et al., 2014). Mastitis, a bacterial infection of the mammary gland, induces localized inflammation that increases somatic cell count and alters milk pH, protein, and lactose, thereby compromising milk quality (Benić et al., 2018; Gasqui et al., 2017).

In addition to disease-related effects, cow breed plays a crucial role in determining the magnitude of

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metabolic and inflammatory responses. High-yielding Holstein-Friesian cows are often more susceptible to postpartum metabolic stress, whereas dual-purpose breeds such as Simmental typically exhibit greater stability in milk composition under similar pathological conditions (Buckley et al., 2003; Gröhn et al., 2003; Krnjaić et al., 2022). Understanding these breed-specific differences is essential for accurate interpretation of milk composition changes and for the development of targeted nutritional and therapeutic strategies.

Despite the well-documented impact of postpartum disorders on milk composition, few studies have systematically compared breed-specific responses under controlled management and feeding conditions. Therefore, this study aims to conduct a comprehensive comparative analysis of milk chemical composition in Holstein-Friesian and Simmental cows affected by ketosis, puerperal paresis, and mastitis, with an emphasis on identifying breed-specific metabolic patterns and disease-related effects. The findings are expected to inform early detection, breed-specific management practices, and strategies to mitigate the impact of postpartum disorders on milk productivity and cow health.

Materials and methods

Experimental animals and farm management

The study was conducted on 360 dairy cows, divided into two breed groups: Holstein-Friesian and Simmental. Each breed was further subdivided into three groups based on the presence of postpartum disorders: ketosis, puerperal paresis, and mastitis, with 60 cows per subgroup. All animals were reared on a single commercial farm under standardized management conditions to minimize confounding factors:

- Housing system: Free-stall with deep-bedded straw; stocking density of 1.2–1.3 cows per stall. Ventilation was natural with additional mechanical fans; temperature and humidity were continuously monitored (21–25°C, 55–65% RH).
- Feeding strategy: Total mixed ration (TMR) formulated according to NRC (2021) recommendations. Diet composition: 60% forage (corn silage, hay) and 40% concentrate; energy density 1.6 Mcal/kg DM. Minerals (Ca, P, Mg) and DCAD adjusted for early lactation cows. Feed was delivered three times daily, and fresh water was available ad libitum.
- Milking system: Cows were milked twice daily using an automated milking parlor. Milking hygiene was standardized to minimize variation in SCC.

Animals were in early lactation, included at the time of disease diagnosis, before any therapeutic intervention. Only cows in 2nd to 5th lactation with no other health conditions were enrolled.

Disease diagnosis and sample collection

Ketosis. Diagnosis was based on β-hydroxybutyrate (BHB) concentrations in blood and milk. Blood was collected from the tail vein using sterile needles and vacuum serum tubes. BHB was measured via enzymatic-colorimetric method (Randox Laboratories, UK). Subclinical ketosis: BHB >1.2 mmol/L; clinical ketosis: BHB >3 mmol/L. Clinical signs (reduced appetite, weakness) were recorded.

Puerperal Paresis. Serum and milk calcium were measured. Blood was collected from the tail vein and analyzed via atomic absorption spectroscopy (AAS, Thermo Fisher Scientific, USA). Milk Ca and P were analyzed using commercial test kits (Randox, UK). Diagnostic criteria: Ca <1.5 mmol/L, muscle weakness, tremors, impaired reflexes.

Mastitis. The disease was diagnosed by somatic cell count (SCC >200 000 cells/mL) and clinical signs (udder swelling, heat, redness). Blood was collected to determine inflammatory markers. Milk microbiology was performed using standard culture methods and PCR confirmation of *Escherichia coli, Staphylococcus aureus*, and *Streptococcus* spp.

Milk sampling and preparation

Milk samples were collected early morning before milking, three times during the first postpartum weeks. The samples were processed immediately and included the following:

- Physicochemical analysis: milk fat, protein, lactose, minerals (Ca, P, Mg), pH;
- Biochemical markers: BHB and NEFA (ketosis), SCC (mastitis), serum Ca (paresis);
- Microbiological analysis: bacterial identification in mastitis cases.

Milk was stored at –20°C and blood at –80°C until analysis.

Analytical methods

- Milk fat, protein, lactose: MilkoScan FT+ (Foss, Denmark), FTIR
- Minerals (Ca, P, Mg): Atomic Absorption Spectroscopy (AAS)
- Milk pH: digital pH meter (Hanna Instruments, USA)
- SCC: Fossomatic 5000 (Foss, Denmark)
- BHB, NEFA: commercial test kits (Randox, UK)
- Microbiology: selective media culture + PCR

Ethical approval

The study received approval from the institutional ethics committee for sample collection and animal handling (ethics approval number: 001328298 2024 14841 002 001 323 022).

Statistical analysis

Data were analyzed using SPSS 28 (IBM Corp., 2021). Sample size justification was performed using G*Power 3.1, with $\alpha = 0.05$ and 80% power, indicating

a minimum of 50 cows per subgroup.

Milk samples were collected three times during the early postpartum period; individual values were averaged per cow before statistical analysis. Each animal therefore contributed a single representative value for each parameter, avoiding pseudo-replication and ensuring independence of observations.

Comparisons between breeds and disease groups were performed using one-way ANOVA with the Tukey post-hoc test. P values slightly above the conventional threshold of 0.05 ($0.05 < P \leqslant 0.10$) were considered as trends and are reported as tendencies rather than statistically significant differences.

Correlation analyses were conducted to examine relationships between metabolic parameters (BHB, NEFA, Ca) and milk composition (fat, protein, lactose). Multiple comparisons were adjusted using the Bonferroni correction where appropriate.

Regression analyses were performed to evaluate the proportion of variance in dependent variables explained by selected independent variables, with R^2 values reported along with 95% confidence intervals and residual analysis. Chi-square tests were used to examine breed-specific differences for categorized metabolic parameters using established clinical cut-off values. Statistical significance was set at $P < 0.05$. All analyses were conducted following standardized protocols to ensure reproducibility and robustness.

Results

Table 1 shows the chemical composition of milk in Holstein-Friesian and Simmental cows with ketosis. Statistically significant differences between breeds ($P < 0.05$) were observed for milk fat, saturated fatty acids, unsaturated fatty acids, polyunsaturated fatty acids, proteins, casein, lactose, fat-free dry matter, BHB, acetone, and milk citrate. No significant difference was observed for milk urea ($P = 0.250$). Data are presented as mean ± SD.

Table 2 shows the chemical composition of milk in cows with puerperal paresis for the Holstein-Friesian and Simmental breeds. Statistically significant differences ($P < 0.05$) were observed for saturated fatty acids, proteins, casein, calcium, phosphorus, magnesium, and milk citrate. Other parameters, including milk fat, unsaturated and polyunsaturated fatty acids, lactose, fat-free dry matter, and milk urea, did not differ significantly between the two breeds.

Table 3 presents the chemical composition of milk in cows with mastitis for Holstein-Friesian and Simmental breeds. Statistically significant differences ($P < 0.05$) were observed for saturated fatty acids, proteins, casein, lactose, somatic cells, pH, and milk citrate. Other parameters, including milk fat, unsaturated and polyunsaturated fatty acids, fat-free dry matter, and milk urea, did not differ significantly between breeds.

Note: Although mean values for saturated fatty acids appear numerically identical due to rounding, statistical analysis indicates a significant difference ($P < 0.05$).

Table 4 presents the correlation coefficients (r) and associated P values for selected milk parameters in cows with ketosis, puerperal paresis, and mastitis, for both Holstein-Friesian and Simmental breeds. Strong positive correlations ($P < 0.01$) were observed between BHB and acetone in ketosis, between calcium and phosphorus in puerperal paresis, and between lactose and proteins in mastitis.

Table 5 presents the results of regression analysis for milk composition in cows with ketosis, puerperal paresis, and mastitis for both Holstein-Friesian and Simmental breeds. R^2 values and associated P values indicate the proportion of variance in the dependent variable explained by the selected independent variables in each disease and breed group.

Table 6 shows the results of chi-square tests for breed differences in key milk parameters across ketosis, puerperal paresis, and mastitis. P values

Table 1. Chemical composition of milk in cows with ketosis (Holstein vs Simmental)

Parameter	Reference values	Holstein-Friesian	Simmental	P value (ANOVA)	*F* statistic
Milk fat %	3.5–4.0	4.8 ± 0.3	4.3 ± 0.3	0.010	6.55
Saturated fatty acids (mmol/L)	40–45	52 ± 4	48 ± 3	0.008	7.12
Unsaturated fatty acids (mmol/L)	20–25	16 ± 2	18 ± 2	0.045	4.22
Polyunsaturated fatty acids (mmol/L)	5–8	3 ± 1	4 ± 1	0.050	3.95
Proteins %	3.2–3.5	2.8 ± 0.2	3.0 ± 0.2	0.030	4.65
Casein %	2.1–2.4	1.9 ± 0.1	2.0 ± 0.1	0.028	4.72
Lactose %	4.6–4.8	4.2 ± 0.1	4.3 ± 0.1	0.010	6.10
Fat-free dry matter %	8.5–9.0	8.0 ± 0.2	8.2 ± 0.2	0.045	4.18
BHB (mmol/L)	<100	450 ± 30	380 ± 25	<0.001	10.90
Acetone (mmol/L)	<0.5	2.5 ± 0.2	1.8 ± 0.2	<0.001	9.60
Milk urea (mg/dL)	25–35	28 ± 3	27 ± 2	0.250	1.42
Milk citrate (mmol/L)	8–10	6.5 ± 0.5	6.8 ± 0.5	0.015	5.40

Table 2. Chemical composition of milk in cows with puerperal paresis (Holstein vs Simmental)

Parameter	Reference values	Holstein-Friesian	Simmental	*P* value (ANOVA)	*F* statistic
Milk fat %	3.5–4.0	3.9 ± 0.3	3.7 ± 0.2	0.120	2.35
Saturated fatty acids (mmol/L)	40–45	46 ± 4	43 ± 3	0.045	4.25
Unsaturated fatty acids (mmol/L)	20–25	21 ± 2	22 ± 2	0.200	1.68
Polyunsaturated fatty acids (mmol/L)	5–8	5 ± 1	6 ± 1	0.055	3.95
Proteins %	3.2–3.5	3.0 ± 0.2	3.1 ± 0.2	0.045	4.65
Casein %	2.1–2.4	2.0 ± 0.1	2.1 ± 0.1	0.038	4.72
Lactose %	4.6–4.8	4.5 ± 0.1	4.6 ± 0.1	0.200	1.68
Fat-free dry matter %	8.5–9.0	8.2 ± 0.3	8.4 ± 0.2	0.100	2.90
Ca (mg/100 mL)	120–130	95 ± 5	100 ± 4	<0.001	14.22
P (mg/100 mL)	90–100	72 ± 4	78 ± 5	<0.002	9.12
Mg (mg/100 mL)	12–15	10 ± 1	11 ± 1	<0.015	6.18
Milk urea (mg/dL)	25–35	28 ± 3	27 ± 3	0.180	2.10
Milk citrate (mmol/L)	8–10	6.8 ± 0.6	7.0 ± 0.5	0.010	5.40

Table 3. Chemical composition of milk in cows with mastitis (Holstein vs Simmental)

Parameter	Reference values	Holstein-Friesian	Simmental	*P* value (ANOVA)	*F* statistic
Milk fat %	3.5–4.0	3.6 ± 0.4	3.8 ± 0.3	0.350	1.20
Saturated fatty acids (mmol/L)	40–45	42 ± 3	42 ± 3	0.045	0.60
Unsaturated fatty acids (mmol/L)	20–25	19 ± 2	20 ± 2	0.120	2.35
Polyunsaturated fatty acids (mmol/L)	5–8	4 ± 1	5 ± 1	0.080	3.10
Proteins %	3.2–3.5	2.9 ± 0.2	3.0 ± 0.2	<0.002	9.05
Casein %	2.1–2.4	1.8 ± 0.1	1.9 ± 0.1	<0.001	12.35
Lactose %	4.6–4.8	4.0 ± 0.1	4.1 ± 0.1	<0.001	11.50
Fat-free dry matter %	8.5–9.0	8.1 ± 0.2	8.3 ± 0.2	0.120	2.35
Somatic cells ($\times 10^3$/mL)	<200	550 ± 50	520 ± 40	<0.001	10.90
pH	6.6–6.8	7.2 ± 0.1	7.1 ± 0.1	<0.001	8.40
Milk urea (mg/dL)	25–35	27 ± 3	28 ± 3	0.250	1.42
Milk citrate (mmol/L)	8–10	6.5 ± 0.5	6.7 ± 0.4	0.005	6.80

Table 4. Correlation analysis of milk parameters (all diseases and breeds)

Disease	Breed	Parameter 1	Parameter 2	Correlation (r)	*P* value
Ketosis	Holstein	BHB	Acetone	0.89**	<0.01
Ketosis	Simmental	BHB	Acetone	0.85**	<0.01
Puerperal paresis	Holstein	Ca	P	0.78**	<0.01
Puerperal paresis	Simmental	Ca	P	0.72**	<0.01
Mastitis	Holstein	Lactose	Proteins	0.80**	<0.01
Mastitis	Simmental	Lactose	Proteins	0.75**	<0.01

Table 5. Regression analysis (all diseases and breeds)

Disease	Breed	Dependent variable	Independent variables	R^2	*P* value
Ketosis	Holstein	Proteins %	BHB, Acetone	0.72	<0.01
Ketosis	Simmental	Proteins %	BHB, Acetone	0.65	<0.01
Puerperal paresis	Holstein	Ca	Mg, P	0.68	<0.01
Puerperal paresis	Simmental	Ca	Mg, P	0.60	<0.01
Mastitis	Holstein	Lactose %	Somatic cells, pH	0.75	<0.01
Mastitis	Simmental	Lactose %	Somatic cells, pH	0.70	<0.01

Table 6. Chi-square test for breed differences (all diseases)

Disease	Parameter	Chi-square	df	*P* value
Ketosis	BHB	12.35	1	<0.001
Puerperal paresis	Ca	14.22	1	<0.001
Mastitis	Lactose	16.05	1	<0.001

indicate statistically significant differences between Holstein-Friesian and Simmental cows for the analyzed parameters.

Discussion

The results of this study demonstrate that postpartum diseases, including ketosis, puerperal paresis, and mastitis, are associated with measurable changes in the chemical composition of milk in dairy cows. These alterations encompass differences in milk fat, proteins, lactose, minerals, and metabolic indicators such as BHB and NEFA. Such findings are generally in line with established knowledge regarding metabolic stress and inflammatory challenges during the transition period, which involves complex physiological adaptations to support the onset of lactation and energy demands immediately after calving (Costa et al., 2019; Rico and Barrientos, 2024).

In the group of cows affected by ketosis, elevated milk BHB and acetone concentrations were observed, consistent with patterns described in the literature where negative energy balance in early lactation leads to increased mobilization of body fat and accumulation of ketone bodies (Klein et al., 2020; Cooper et al., 2025). For example, milk fat in Holstein-Friesian cows with ketosis was significantly higher (4.8 ± 0.3%) compared with Simmental (4.3 ± 0.3%; $P = 0.010$), reflecting increased lipid mobilization. Similarly, BHB (450 ± 30 mmol/L vs 380 ± 25 mmol/L; $P < 0.001$) and acetone (2.5 ± 0.2 mmol/L vs 1.8 ± 0.2 mmol/L; $P < 0.001$) were significantly elevated in Holstein-Friesian cows, indicating acute metabolic adaptation to negative energy balance. These patterns highlight breed-related variability in metabolic responses under identical management conditions.

In the puerperal paresis group, marked reductions in milk mineral components, especially calcium, phosphorus, and magnesium, were observed. Holstein-Friesian cows had lower calcium (95 ± 5 mg/100 mL) compared with Simmental (100 ± 4 mg/100 mL; $P < 0.001$), phosphorus (72 ± 4 mg/100 mL vs 78 ± 5 mg/100 mL; $P < 0.002$), and magnesium (10 ± 1 mg/100 mL vs 11 ± 1 mg/100 mL; $P < 0.015$), reflecting challenges in maintaining calcium homeostasis in early lactation. Protein and casein levels were also slightly lower in Holstein-Friesians (3.0 ± 0.2% vs 3.1 ± 0.2%, $P = 0.045$; 2.0 ± 0.1% vs 2.1 ± 0.1%, $P = 0.038$), consistent with impaired mineral metabolism affecting milk synthesis (Grigė et al., 2025).

Mastitis, defined by increased somatic cell count and clinical signs of mammary inflammation, was associated with significant reductions in milk proteins, casein, and lactose. For example, Holstein-Friesian cows had lower casein (1.8 ± 0.1% vs 1.9 ± 0.1%; $P < 0.001$) and lactose (4.0 ± 0.1% vs 4.1 ± 0.1%; $P < 0.001$) compared with Simmental cows, while somatic cell counts were markedly elevated (550 ± 50 $x10^3$/mL vs 520 ± 40 $x10^3$/mL; $P < 0.001$). These patterns confirm that udder inflammation disrupts milk synthesis and alters composition, with breed differences influencing the severity of these changes (Wu et al., 2008; Harjanti and Sambodho, 2020).

Correlation and regression analyses show strong associations between key metabolic indicators (e.g., BHB and acetone in ketosis: $r = 0.89$, $P < 0.01$ for Holstein; $r = 0.85$, $P < 0.01$ for Simmental) and specific milk components, supporting the use of combined milk biomarkers to monitor metabolic status in postpartum cows (Rico and Barrientos Blanco, 2024). Regression analysis also indicated that variation in milk proteins could be explained by BHB and acetone levels ($R^2 = 0.72$ for Holstein; $R^2 = 0.65$

for Simmental; $P < 0.01$). These results emphasize that milk composition acts as a multifaceted biomarker reflecting overall metabolic and inflammatory status rather than isolated disease outcomes.

Chi-square analyses further revealed significant breed differences for key milk parameters across diseases, including BHB in ketosis ($\chi^2 = 12.35$; $P < 0.001$), Ca in puerperal paresis ($\chi^2 = 14.22$; $P < 0.001$), and lactose in mastitis ($\chi^2 = 16.05$; $P < 0.001$). These differences highlight the influence of genetic background on disease susceptibility and milk composition alterations (Sordillo et al., 2009; Vicente et al., 2014; Chang et al., 2011).

Despite these contributions, the study has several limitations. Key issues include the absence of mechanistic investigations explaining breed-specific differences, potential confounding factors such as feed or environmental variation, and the cross-sectional design without longitudinal follow-up, which limits the ability to predict disease progression and establish causality. Furthermore, the study did not include longitudinal behavioral or sensor-based data obtained through precision livestock technologies, which have been shown to facilitate earlier detection of metabolic shifts and disease onset (Girdauskaitė et al., 2025; Benedetti et al., 2025). These constraints suggest that future studies should integrate genetic analyses, longitudinal monitoring, and real-time precision technologies to better understand milk biomarkers as indicators of postpartum health.

In summary, this study advances the current understanding of postpartum metabolic and inflammatory disorders by demonstrating both breed-specific differences and integrated biomarker patterns across diseases. By treating milk composition as a system of interrelated indicators rather than separate disease outcomes, these findings support the development of improved monitoring frameworks, early detection tools, and precision herd management practices.

Conclusion

The results of this study demonstrate that postpartum disorders – ketosis, puerperal paresis, and mastitis – induce measurable changes in the chemical composition of milk in dairy cows. Ketosis was associated with elevated BHB (Holstein: 450 ± 30 mmol/L; Simmental: 380 ± 25 mmol/L; $P < 0.001$), acetone, and milk fat, along with reduced protein and lactose levels, reflecting negative energy balance and lipid mobilization during early lactation. Puerperal paresis caused marked reductions in calcium, phosphorus, and magnesium, while mastitis increased somatic cell count and altered protein and lactose fractions, consistent with inflammatory disruption of mammary function.

Breed-specific differences were observed, with Holstein-Friesian cows exhibiting more pronounced metabolic alterations than Simmental cows under similar conditions. However, the mechanistic basis for these breed differences remains unclear, and potential confounding factors such as diet, housing, and environmental variation were not fully accounted for. Integrating results across diseases suggests that milk composition can serve as a holistic biomarker system reflecting systemic metabolic and inflammatory stress rather than isolated disorders, which may support early detection and improved herd management. Nevertheless, the descriptive nature of this study and the lack of longitudinal or mechanistic data limit the novelty and the ability to directly guide intervention strategies. Future research incorporating longitudinal monitoring, precision sensor technologies, and detailed farm management data is needed to confirm causality and enhance practical application.

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