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Original article

POSTPARTUM BIOCHEMICAL DIFFERENCES IN METABOLIC ADAPTATION BETWEEN HOLSTEIN-FRIESIAN AND SIMMENTAL COWS RELATED TO NEGATIVE ENERGY BALANCE AND SUBCLINICAL KETOSIS

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Summary

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Subclinical ketosis is a prevalent metabolic disorder in high-producing dairy cows, characterised by negative energy balance, excessive lipolysis, and altered hepatic metabolism. We hypothesised that Holstein-Friesian (HF) cows exhibit more pronounced hepatic lipid accumulation and metabolic stress than Simmental (SIM) cows during the transition period (TP). To test this, 240 postpartum cows were assigned to either ketosis (KET) or control (CON) groups in relation to the breed: HF-KET, HF-CON, SIM-KET, and SIM-CON groups (n = 60 each). Blood samples collected within the first 21 days postpartum were analysed for BHB, NEFA, TG, AST, ALT, glucose, and NEFA/BHB ratio. HF-KET cows displayed the highest BHB (2.35±0.41 mmol/L) and NEFA (0.92±0.18 mmol/L), elevated TG and hepatic enzyme activities, reduced glucose, and a low NEFA/BHB ratio, indicating intense ketogenesis and hepatic metabolic stress. SIM-KET cows showed moderate changes, reflecting partial metabolic compensation. Strong positive correlations between BHB and NEFA, TG, AST, and negative correlation with glucose in HF-KET cows confirmed the direct link between ketonaemia and hepatic lipid overload. These findings demonstrate clear breed-specific differences in hepatic metabolic adaptation to TP. Early detection and monitoring of high-risk HF cows are essential for preventing hepatic dysfunction and optimising postpartum energy requirements during the TP.

Key words: dairy breed comparison, hepatic lipid metabolism, metabolic disease, transition period physiology

INTRODUCTION

Postpartum subclinical ketosis (SCK) in dairy cows is a significant metabolic condition that occurs as a consequence of negative energy balance (NEB) during the first weeks after parturition, when the energy demands for milk production exceed dietary intake. SCK is characterised by asymptomatic elevations in blood β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA), reflecting intensified lipolysis and increased ketogenesis (Loiklung *et al.,* 2022; Ruda *et al.,* 2025). Although clinical signs of ketosis are absent, SCK is associated with a higher risk of metabolic disorders, reduced milk production, and increased incidence of postpartum diseases, leading to significant economic losses in dairy production systems (Suthar *et al.,* 2013; Loiklung *et al.,* 2022).

The global prevalence of subclinical ketosis in dairy herds has been reported to range between approximately 20–26%, depending on production system, geographic region, and management practices, highlighting its major impact on dairy cow health and productivity (Suthar *et al.,* 2013).

The integrity of hepatic metabolism is crucial for successful adaptation to NEB, as the liver serves as the central site for gluconeogenesis and ketogenesis (Rico, 2024; He, 2025). Excessive mobilisation of NEFA from adipose tissue leads to hepatic uptake and partial re-esterification into triglycerides, resulting in hepatic lipid accumulation and increased risk of hepatic overload. Elevated serum triglycerides (TG) and increased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities indicate hepatic metabolic stress, which may remain undetected without routine biochemical monitoring (Chapinal *et al.,* 2012). NEFA and BHB are commonly used indicators of lipolysis and ketogenesis, respectively, and reflect metabolic adaptation to NEB.

Leptin is a key metabolic hormone involved in the regulation of energy balance, appetite, and lipid mobilisation during the transition period. Altered leptin signalling during NEB contributes to enhanced lipolysis and increased susceptibility to ketosis in high-producing dairy cows (Strączek *et al.,* 2021).

Breed-related differences in metabolic adaptation are well documented. Holstein-Friesian (HF) cows, due to their high milk yield, are more prone to NEB, enhanced lipolysis, and metabolic stress, whereas Simmental (SIM) cows exhibit more moderate ketogenesis and a greater capacity to compensate for energy deficits (Bobe *et al.,* 2004; Arfuso *et al.,* 2016; Soares *et al.,* 2021; Magro *et al.,* 2025). Genetic factors also contribute to susceptibility to ketosis, with recent studies identifying candidate genes and genomic regions associated with circulating BHB levels and metabolic resilience in dairy cattle (Sejersen *et al.,* 2012; He *et al.,* 2025).

Comparative studies between HF, SIM, and other dairy breeds have demonstrated significant differences in metabolic profile, milk production, and susceptibility to postpartum disorders, emphasising the importance of breed-specific metabolic adaptation in dairy production systems (Knob *et al.,* 2021; Ha *et al.,* 2025).

Although numerous studies have investigated SCK in HF cows, comparative analyses of metabolic parameters and hepatic function between HF and SIM cows, including breed-specific controls, remain limited. Understanding breed-specific differences in BHB, NEFA, TG, AST, ALT, glucose, and the NEFA/BHB ratio enables an integrative assessment of metabolic adaptation and hepatic stress, which is of considerable practical and scientific importance (Contreras *et al.,* 2010; Knob *et al.,* 2021; Magro *et al.,* 2025; Song *et al.,* 2025).

The aim of the study was to evaluate breed-specific differences in metabolic adaptation to NEB in HF and SIM, focusing on biochemical parameters that indicate energy metabolism and liver function for SCK diagnosis in early postpartum.

MATERIALS AND METHODS

Ethical approval for this study was obtained from the Ministry of Agriculture, Forestry and Water Management – Veterinary Directorate, Republic of Serbia, which authorised sample collection and animal handling (approval number: 001328298 2024 14841 002 001 323 022; approval date: 11 April 2024, Belgrade, Serbia).

Study design, location, and animals

This study included a total of 240 postpartum dairy cows, divided into four experimental groups of 60 animals each: HF cows with SCK (HF-KET), SIM cows with SCK (SIM-KET), HF control cows without clinical or biochemical signs of ketosis (HF-CON), and SIM control cows (SIM-CON). Within each group, cows were further stratified according to parity into primiparous (PRP) and multiparous (MUL) animals. The distribution of PRP and MUL cows was as follows: HF-KET (PRP=24, MUL=36), HF-CON (PRP=25, MUL=35), SIM-KET (PRP=23, MUL=37), and SIM-CON (PRP=26, MUL=34). The mean parity (± SD) and range (min–max) were: HF-KET (2.9±1.3; 1–6), HF-CON (2.7±1.2; 1–5), SIM-KET (3.0±1.4; 1–6), and SIM-CON (2.8±1.3; 1–5), indicating a slightly higher proportion of MUL cows across all groups, consistent with SCK epidemiology.

The study was conducted between April 2024 and April 2025 on two commercial dairy farms located in the Vojvodina region, Republic of Serbia. Farm 1 was located in the vicinity of Novi Sad (45.2671° N, 19.8335° E), while Farm 2 was located in the wider Vojvodina region (45.0000° N, 20.0000° E). Both farms operated under intensive dairy production systems. The study period encompassed all seasonal conditions (spring, summer, autumn, and winter) characteristic of the temperate continental climate of the region, thereby minimising potential seasonal bias in metabolic status and environmental exposure.

From Farm 1, a total of 120 cows were enrolled, including 60 HF (PRP=25, MUL=35) and 60 SIM cows (PRP=24, MUL=36). From Farm 2, a total of 120 cows were included, comprising 60 HF (PRP=24, MUL=36) and 60 SIM cows (PRP=25, MUL=35). The distribution of PRP and MUL cows was balanced across farms and experimental groups to minimise potential confounding effects of parity and farm-level variation.

All cows were housed within the same farm environments per farm, ensuring identical management, housing, feeding, and environmental conditions for both breeds, thereby minimising confounding farm-related effects. Each farm contained approximately 300–350 lactating cows. Animals were maintained under standardised management and health-monitoring protocols throughout the study.

The postpartum period was defined as 5–21 days in milk (DIM), representing the critical window for the development of SCK. All blood samples were collected within this defined period, and cows were stratified according to exact DIM to reduce temporal variation in metabolic status.

Feeding management and ration composition

Dairy cows were managed under a total mixed ration (TMR) feeding system with diets formulated according to physiological stage (close-up and early lactation). Both Farm 1 and Farm 2 used the same basic ingredient sources; however, nutrient density and concentrate proportions were adjusted according to stage of lactation and energy requirements. Close-up cows received a controlled-energy diet formulated to prevent metabolic disorders during the transition period, while early lactation cows were fed a higher-energy diet to support milk production and prevent NEB.

All diets were offered as a TMR under *ad libitum* conditions, with feed delivered twice daily. Fresh water was freely available at all times. Tables 1 and 2 refer to both Farm 1 and Farm 2, as identical feeding regimes were applied.

Clinical examination and classification

All cows underwent clinical examination prior to sampling to exclude animals with acute infections, inflammatory conditions, or other metabolic disorders that could confound the results.

SCK was defined as a blood BHB concentration ≥ 1.2 mmol/L in the absence of clinical signs of ketosis. NEFA levels ≥ 0.7 mmol/L were used as an additional indicator of enhanced lipid mobilisation and NEB, but not applied as a diagnostic criterion for SCK.

Blood sampling and analysis

Blood samples were collected from the external jugular vein using sterile Vacutainer systems (18G needles) in the morning before milking to minimise diurnal variation and stress effects. From each cow, 20 mL of blood was collected: 10 mL in Li-heparin tubes (for NEFA and

Table 1. Ingredient composition of TMR for close-up (prepartum transition period) and early lactation dairy cows. The same diet formulation was applied in both Farm 1 and Farm 2 with identical ingredient sources

Ingredient (kg DM/day)	Close-up cows	Early lactation cows
Corn silage	10.0	12.0
Grass silage	5.5	6.0
Alfalfa hay	2.0	2.5
Concentrate mix	6.0	8.0
Mineral-vitamin premix	0.25	0.3

Table 2. Nutritional composition of the total mixed ration for close-up and early lactation cows at Farm 1 and Farm 2. Nutritional values were calculated based on standard feed formulation models

Parameter	Close-up cows	Early lactation cows
Dry matter intake (DMI, kg/day)	22.0	28.8
Net energy for lactation (NEL, MJ/kg DM)	6.1	6.7
Metabolisable energy (ME, MJ/kg DM)	10.2	10.8
Crude protein (CP, %)	15.5	17.2

glucose) and 10 mL in plain tubes (for serum BHB, TG, AST, ALT). Samples were transported at 4 °C to the laboratory within 2 hours, centrifuged at 3000 × *g* for 10 minutes, and stored at −20 °C until analysis.

Biochemical analyses were performed using standardised automated laboratory procedures. Plasma glucose, TG, BHB, AST, and ALT were quantified using a Cobas c311 fully automated clinical chemistry analyzer (Roche Diagnostics, Basel, Switzerland). Plasma NEFA were determined using Wako NEFA-HR enzymatic colorimetric assay kits (Wako Pure Chemical Industries, Osaka, Japan), strictly following the manufacturer's instructions.

Energy status indicator

In this study, the NEFA/BHB ratio was calculated as an original, integrative index developed to assess the relationship between lipid mobilisation and ketogenesis. This ratio was used to reflect metabolic adaptation to NEB during early lactation and was interpreted comparatively between HF and SIM cows in ketotic and control groups.

Statistical analysis

Statistical analyses were performed using SPSS version 28.0. Data distribution was tested using the Shapiro-Wilk test. Based on normality results, parametric tests were applied. Intergroup differences were analysed using one-way ANOVA, followed by Tukey's *post-hoc* test for multiple comparisons. Pearson correlation analysis was applied because all variables showed normal distribution (P>0.05 in Shapiro-Wilk test), confirming the appropriateness of parametric correlation methods. Correlation coefficients were interpreted according to Schober *et al.* (2018), classifying r values as weak (0.10–0.39), moderate (0.40–0.69), strong (0.70–0.89), and very strong (0.90–1.00) associations. Results are presented as mean ± standard deviation (SD), and significance was set at P<0.05.

Study design rationale

This study design enabled a controlled comparative evaluation of metabolic adaptation between HF and SIM cows under identical environmental and nutritional conditions. It allowed integrative assessment of lipid mobilisation, ketogenesis, and hepatic metabolic function during the critical early postpartum period, ensuring high reproducibility and minimal confounding variability.

RESULTS

Results presented in Table 3 show significant differences among groups for all measured metabolic parameters (P<0.001). Cows with SCK in both HF and SIM groups exhibited higher concentrations of NEFA, TG, AST, and ALT, while glucose concentrations were significantly lower compared with control cows. HF cows with SCK consistently showed the most pronounced metabolic alterations, followed by SIM cows with SCK.

The ratio between NEFA and BHB also differed significantly among groups (P<0.001), with the lowest values observed in HF cows with SCK and the highest values in control cows, indicating an altered balance between lipid mobilisation and ketogenesis during early lactation.

Importantly, BHB was used exclusively for group classification (greater than or equal to 1.20 mmol/L) and was not treated as a continuous comparative metabolic parameter. NEFA was included as an in-

Table 3. Serum and plasma metabolic parameters in Holstein-Friesian and Simmental dairy cows with and without subclinical ketosis. Values are presented as mean ± SD.

Parameter	HF-KET (N=60)	HF-CON (N=60)	SIM-KET (N=60)	SIM-CON (N=60)	P Value
BHB (mmol/L)	2.35 ± 0.41^{a}	0.80 ± 0.18^{c}	1.89 ± 0.36^{b}	0.77 ± 0.17^{c}	<0.001
NEFA (mmol/L)	0.92 ± 0.18^{a}	0.38 ± 0.09^{c}	0.71 ± 0.15^{b}	0.37 ± 0.08^{c}	<0.001
TG (mmol/L)	0.46 ± 0.09^{a}	0.23 ± 0.05^{c}	0.34 ± 0.07^{b}	0.22 ± 0.05^{c}	<0.001
AST (U/L)	124 ± 18^{a}	80 ± 12^{c}	102 ± 16^{b}	78 ± 11^{c}	<0.001
ALT (U/L)	41 ± 7^{a}	27 ± 5^{c}	34 ± 6^{b}	26 ± 5^{c}	<0.001
Glucose (mmol/L)	2.31 ± 0.28^{a}	3.05 ± 0.22^{c}	2.62 ± 0.25^{b}	3.08 ± 0.22^{c}	<0.001
NEFA/BHB ratio	0.39 ± 0.06^{a}	0.60 ± 0.08^{c}	0.48 ± 0.07^{b}	0.61 ± 0.08^{c}	<0.001

Different superscript letters within the same row indicate statistically significant differences ($P<0.05$). HF-KET = Holstein-Friesian cows with subclinical ketosis; HF-CON = Holstein-Friesian control cows; SIM-KET = Simmental cows with subclinical ketosis; SIM-CON = Simmental control cows; BHB – β-hydroxybutyrate; NEFA – non-esterified fatty acids; TG – triglycerides; AST – aspartate aminotransferase; ALT – alanine aminotransferase. Subclinical ketosis was defined as serum BHB ≥1.20 mmol/L. NEFA was measured as an indicator of lipid mobilisation but was not used for classification.

Table 4. Correlation analysis between blood β-hydroxybutyrate (BHB) and metabolic parameters in Holstein-Friesian and Simmental dairy cows with subclinical ketosis

Parameter	HF-KET (r)	SIM-KET (r)	P value (HF-KET)	P value (SIM-KET)
BHB – NEFA	0.74	0.61	<0.001	<0.001
BHB – triglycerides	0.69	0.54	<0.001	<0.001
BHB – AST	0.66	0.48	<0.01	<0.01
BHB – Glucose	−0.71	−0.58	<0.001	<0.001

Values represent Pearson correlation coefficients (r). Statistical significance (P values) is reported separately for HF-KET and SIM-KET groups. HF-KET = Holstein-Friesian cows with subclinical ketosis; HF-CON = Holstein-Friesian control cows; SIM-KET = Simmental cows with subclinical ketosis; SIM-CON = Simmental control cows; BHB – β-hydroxybutyrate; NEFA – non-esterified fatty acids; AST – aspartate aminotransferase.

dicator of lipid mobilisation but not used as a diagnostic criterion for SCK.

Results presented in Table 4 show significant correlations between BHB and metabolic parameters in both HF cows with SCK and SIM cows with SCK. In HF cows with SCK, strong correlations were observed between BHB and NEFA, TG, AST, and glucose. Similar but generally weaker correlations were observed in SIM cows with SCK. Overall, ketonaemia was positively associated with lipid mobilisation and hepatic enzyme activity, while showing a negative association with glucose concentration. Stronger correlations were consistently observed in HF cows compared with SIM cows.

DISCUSSION

SCK in dairy cows represents a complex metabolic disorder arising from an imbalance between energy demand and supply during early lactation, characterised by enhanced lipolysis, ketogenesis, and altered hepatic metabolic function. The results of this study demonstrate clear breed- and group-specific differences, with HF cows with ketosis exhibiting the most pronounced metabolic alterations.

HF cows with SCK showed elevated concentrations of NEFA and BHB, reflecting intensified mobilisation of body fat reserves and a pronounced NEB. This metabolic response is a well-established adaptation to early lactation energy deficit, in which increased lipolysis provides substrates for hepatic ketogenesis (Drackley, 1999; Grummer, 1993). The concurrent increase in TG and hepatic enzymes suggests reduced hepatic metabolic efficiency and impaired lipid processing capacity, consistent with previous findings in high-producing dairy cows (Overton & Waldron, 2004; Ha *et al.,* 2022). Reduced glucose concentrations further indicate increased utilisation of glucogenic precursors for ketone body synthesis (Bell, 1995; Mohsin *et al.,* 2022).

Nutritional management is a key factor influencing energy balance and metabolic adaptation during early lactation. In the present study, cows were fed a TMR with increased energy density in early lactation; however, this was still not sufficient to fully compensate for the high energy demands of milk production, leading to a state of NEB. Such conditions promote increased lipid mobilisation and ketogenesis, which is reflected in elevated NEFA and BHB concentrations in HF and SIM cows with SCK. Similar effects of dietary energy deficit on metabolic status in transition dairy cows have been previously described (Drackley, 1999).

In contrast, SIM cows with SCK showed milder metabolic disturbances, suggesting improved adaptation to NEB and reduced susceptibility to excessive ketone formation. These findings support the concept of breed-related metabolic resilience, where dual-purpose breeds exhibit more stable energy metabolism compared with specialised high-yielding dairy breeds (Strączek *et al.,* 2021; Magro *et al.,* 2025).

Environmental temperature and heat stress are critical external determinants of metabolic adaptation in dairy cows during early lactation. Exposure to elevated ambient temperature increases thermal load and disrupts thermoregulatory homeostasis, leading to reduced feed intake, altered nutrient partitioning, and impaired energy balance. These physiological adaptations may exacerbate NEB and increase susceptibility to metabolic disorders, including SCK, particularly in high-producing dairy cows (Becker *et al.,* 2020).

Epidemiological evidence indicates that both animal-related and environmental factors contribute to the risk of SCK. Increased parity has consistently been associated with higher susceptibility to SCK, as MUL cows experience greater metabolic demands during early lactation. In addition, seasonal and environmental temperature variations may influence feed intake and energy balance, thereby increasing the risk of NEB and ketone body formation during the transition period (Vanholder *et al.,* 2015). These factors may partially explain the inter-individual variability in metabolic responses observed in the present study.

Control cows of both breeds maintained stable metabolic profiles, with low concentrations of NEFA, TG, hepatic enzymes, and normal glucose levels, indicating adequate adaptation to the postpartum period without excessive lipid mobilisation or hepatic stress. These findings are consistent with previous descriptions of normal metabolic adaptation in dairy cows without NEB (Petit *et al.,* 2007; Pothmann *et al.,* 2026).

Correlation analysis confirmed the physiological interconnection between ketone body formation, lipid mobilisation, and glucose metabolism. Strong positive correlations between BHB and NEFA, TG, and liver enzymes, along with negative correlations with glucose in HF cows, indicate a tightly coordinated metabolic response under severe NEB. In SIM cows, weaker correlations suggest improved metabolic control and greater homeostatic stability (Van den Top *et al.,* 2005; Girma *et al.,* 2024).

The overall metabolic profile indicates a shift toward increased ketone body utilisation as an adaptive mechanism to sustain energy supply, although this occurs at the expense of increased hepatic metabolic load. This pattern has been associated with reduced hepatic efficiency and impaired physiological performance in high-producing dairy cows. The findings highlight the systemic nature of SCK as a metabolic condition affecting multiple physiological pathways.

Overall, the results emphasise that breed plays a key role in metabolic adaptation during early lactation. HF cows show greater metabolic instability and susceptibility to energy imbalance, while SIM cows demonstrate improved metabolic flexibility and resilience. In addition, parity and environmental conditions, including seasonal temperature variation, are important external factors influencing the risk and severity of metabolic adaptation during early lactation.

CONCLUSIONS

The present study demonstrates clear breed-specific metabolic differences between HF and SIM dairy cows during the early postpartum period. HF cows with SCK exhibited significantly higher concentrations of BHB, NEFA, triglycerides, AST, and ALT, along with lower glucose levels and reduced ratios between NEFA and BHB, indicating a more severe NEB and greater hepatic metabolic stress compared with SIM cows. Correlation analysis further revealed stronger associations between BHB and NEFA, as well as a stronger negative relationship between BHB and glucose in HF cows, while SIM cows showed weaker to moderate correlations, indicating improved metabolic adaptability. These results highlight that HF cows are more susceptible to metabolic disturbances associated with SCK, whereas SIM cows demonstrate greater metabolic resilience during early lactation.

REFERENCES

Arfuso, F., F. Fazio, M. Levanti, M. Rizzo, S. Di Pietro, E. Giudice & G. Piccione, 2016. Lipid and lipoprotein profile changes in dairy cows in response to late pregnancy and the early postpartum period. *Journal of Veterinary Science*, **17**, 555–563.

Bobe, G., J. W. Young & D. C. Beitz, 2004. Invited review: Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. *Journal of Dairy Science*, **87**, 3105–3124.

Bell, A. W., 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *Journal of Animal Science*, **73**, 2804–2819.

Becker, C. A., R. J. Collier & A. E. Stone, 2020. Physiological and behavioral effects of heat stress in dairy cows. *Journal of Dairy Science*, **103**, 6751–6770.

Chapinal, N., M. E. Carson, T. F. Duffield, M. Capel, S. M. Godden & T. R. Overton, 2012. The association of serum metabolites with clinical disease during the transition period. *Journal of Dairy Science*, **95**, 2567–2575.

Contreras Bravo, G. A. & L. M. Sordillo, 2010. Lipid mobilisation in transition dairy cows alters endothelial inflammatory response. *American Association of Bovine Practitioners Conference Proceedings*.

Drackley, J. K., 1999. Biology of dairy cows during the transition period: the final frontier? *Journal of Dairy Science*, **82**, 2259–2273.

Girma, M., S. Heirbaut, K. Hertogs, X. P. Jing, M. Q. Zhang, P. Lutakome, K. Geerinckx, S. Els, B. Aernouts, L. Vandaele & V. Fievez, 2024. Metabolic clusters of early-lactating dairy cows based on blood β-hydroxybutyrate trajectories and predicted from milk compounds. *Journal of Dairy Science*, **107**, 9719–9734.

Grummer, R. R., 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. *Journal of Dairy Science*, **76**, 3882–3896.

Ha, S., S. Kang, M. Han, J. Lee, H. Chung, S.-I. Oh, S. Kim & J. Park, 2022. Predicting ketosis during the transition period in Holstein Friesian cows using hematological and serum biochemical parameters on the calving date. *Scientific Reports*, **12**, Article 853.

Ha, S., S. Kang, M. Jung, S. B. Kim, S. Hwang, J. Lee, D. Kim, K. C. Choi & J. Park, 2025. Changes in haematological and serum biochemical parameter concentrations from the day of calving to ketosis onset in Holstein dairy cows during the postpartum period. *Irish Veterinary Journal*, **78**, Article 8.

He, Y. R., H. Y. Zhao, L. L. Li, Y. Wang, Y. C. Zhao & L. S. Jiang, 2025. Metabolic lipid alterations in subclinical ketotic dairy cows: A multisample lipidomic approach. *Journal of Dairy Science*, **108**, 8887–8903.

Janevski, D., M. Radinović, J. Krivokapić, J. Stanojević, P. Dodovski, V. Stojanoski & T. Gichova, 2025. The influence of ketosis as a risk factor on mastitis occurrence during early lactation period in dairy cows. *Veterinarija ir Zootechnika*, **82**, 1–7.

Knob, D. A., A. Thaler Neto, H. Schweizer, A. C. Weigand, R. Kappes & A. M. Scholz, 2021. Energy balance indicators during the transition period and early lactation of purebred Holstein and Simmental cows and their crosses. *Animals*, **11**, 309.

Loiklung, C., P. Sukon & C. Thamrongyoswittayakul, 2022. Global prevalence of subclinical ketosis in dairy cows: A systematic review and meta-analysis. *Research in Veterinary Science,* **144**, 66–76.

Magro, S., A. Costa, A. Cesarani, L. Degano & M. De Marchi, 2025. Genetic aspects of major blood metabolites in the Italian Simmental cattle population. *Journal of Dairy Science*, **108**, 1778–1789.

McArt, J. A. A., D. V. Nydam & G. R. Oetzel, 2012. Epidemiology of subclinical ketosis in early lactation dairy cattle and its association with production diseases and performance. *Journal of Dairy Science*, **95**, 5056–5066.

McArt, J. A. A., D. V. Nydam, G. R. Oetzel, T. R. Overton & P. A. Ospina, 2013. Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. *The Veterinary Journal*, **198**, 560–570.

Mohsin, M. A., H. Yu, R. He, P. Wang, L. Gan, Y. Du, Y. Huang, M. B. Abro, S. Sohaib, M. Pierzchala, P. Sobiech, K. Miętkiewska, C. S. Pareek & B. X. He, 2022. Differentiation of subclinical ketosis and liver function test indices in adipose tissues associated with hyperketonemia in postpartum dairy cattle. *Frontiers in Veterinary Science*, **8**, 796494.

Overton, T. R. & M. R. Waldron, 2004. Nutritional management of transition dairy cows: strategies to optimize metabolic health. *Journal of Dairy Science*, **87**, E105–E119.

Petit, H. V., M. F. Palin & L. Doepel, 2007. Hepatic lipid metabolism in transition dairy cows fed flaxseed. *Journal of Dairy Science*, **90**, 4780–4792.

Pothmann, H., M. Mitterer, F. Flicker, M. Sahebi, V. Havlicek, U. Besenfelder, A. Tichy & M. Drillich, 2026. Evaluation of hyperketonemia in the transition period of dairy Simmental cows and association with liver activity, uterine and oviductal health, and reproductive performance. *Dairy*, **7**, 2.

Rico, J. E. & M. A. Barrientos-Blanco, 2024. Invited review: Ketone biology—The shifting paradigm of ketones and ketosis in the dairy cow. *Journal of Dairy Science*, **107**, 3367–3388.

Ruda, L., C. Straub, A. M. Scholz & K. Huber, 2025. Metabolic adaptation to energetic demands of early lactation in Holstein Friesian and Simmental cows. *Animals*, **19**, 101608.

Suthar, V. S., J. Canelas-Raposo, A. Deniz & W. Heuwieser, 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. *Journal of Dairy Science*, **96**, 2925–2938.

Schober, P., C. Boer & L. A. Schwarte, 2018. Correlation coefficients: Appropriate use and interpretation. *Anesthesia & Analgesia*, **126**, 1763–1768.

Sejersen, H., M. T. Sørensen, T. Larsen, E. Bendixen & K. L. Ingvartsen, 2012. Liver protein expression in dairy cows with high liver triglycerides in early lactation. *Journal of Dairy Science*, **95**, 2409–2421.

Song, Y., X. Jiang, Y. Hao, R. Sun, Y. Bai, G. Shao, W. Ren & C. Xia, 2025. Effectiveness of a novel propylene glycol protocol in reducing ketosis in transition dairy cows. *Frontiers in Veterinary Science*, **12**, 1609300.

Soares, R. A. N., G. Vargas, M. M. M. Muniz, M. A. M. Soares, A. Cánovas, F. Schenkel & E. J. Squires, 2021. Differential gene expression in dairy cows under negative energy balance and ketosis: A systematic review and meta-analysis. *Journal of Dairy Science*, **104**, 602–615.

Strączek, I., K. Młynek & A. Danielewicz, 2021. The capacity of Holstein Friesian and Simmental cows to correct a negative energy balance in relation to their performance parameters, course of lactation, and selected milk components. *Animals*, **11**, 1674.

Van den Top, A. M., A. Van Tol, H. Jansen, M. J. H. Geelen & A. C. Beynen, 2005. Fatty liver in dairy cows postpartum is associated with decreased concentration of plasma triacylglycerols and decreased activity of lipoprotein lipase in adipocytes. *Journal of Dairy Research*, **72**, 129–137.

Vanholder, T., J. Papen, R. Bemers, G. Vertenten & A. C. B. Berge, 2015. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. *Journal of Dairy Science*, **98**, 880–888.

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