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Prevalence of increased milk BHB and its association with SCC and milk components

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Abstract

The aim of this study was to investigate the prevalence of subclinical ketosis in dairy cows based on elevated levels of milk ß-hydroxybutyrate (BHB), somatic cell count (SCC), and other milk components. The analyzed data were obtained through routine analyses Dairy Herd Improvement (DHI) milk samples, by Fourier-transform infrared (FTIR) spectroscopy. The initial dataset included a total of 23,492 Holstein cow milk samples collected between January 2022 and January 2025 on six farms located in the Province of Vojvodina, Serbia. After outlier removal, final data set included 22,915 milk samples. Based on the concentration of ß-hydroxybutyrate (BHB), 75 % of the samples were in the first negative group (BHB<0.10 mmol/L), with an average concentration of 0.03 mmol/L. The second, suspicious-negative group (BHB 0.11-0.15 mmol/L) comprised 15 % of the samples, with an average of 0.13 mmol/L. In addition, 6 % of the samples fell into the third, suspicious-positive group (BHB 0.16-0.20 mmol/L), with an average of 0.18 mmol/L, while the fourth, positive group (BHB>0.20 mmol/L) comprised 4 % of the samples, with an average concentration of 0.34 mmol/L. Through the analysis of the milk components, it was determined that in the first period, at 5-42 days in milk (DIM), significantly affected all observed parameters. The lactose content is decreasing, while fat, protein, total solids, milk fat/protein (F/P) ratio, SCC and urea are increasing. The results highlight the potential of using milk samples from Dairy Herd Improvement (DHI) programs to monitor elevated levels of milk BHB and SCC at the herd level. This approach may serve as a useful tool for estimating the prevalence of subclinical ketosis in dairy cows. Additionally, different BHB thresholds might be needed for different periods of lactation.

Keywords: ß-hydroxybutyrate; somatic cell count; fat/protein ratio; ketosis; Holstein

Introduction

Ketosis is recognized as a significant metabolic disorder, primarily affecting highly productive dairy cows during the first two months of lactation. During this period, there is a high metabolic demand for milk production, which typically coincides with reduced feed intake, leading to a negative energy balance (Drackley, 1999). At the start of lactation, animals attempt to adjust to the energy deficit by utilizing fat reserves for energy. In the liver, fatty acids are converted into acetyl-CoA. However, when the amount of acetyl-CoA surpasses the capacity of the Krebs cycle, some is transformed into ketone bodies, including acetoacetate (AcAc), ß-hydroxybutyrate (BHB), and acetone (Ac) (White, 2015; Rico and Barrientos-Blanco, 2024). The blood BHB test is considered the gold standard for diagnosing hyperketonemia (Oetzel, 2004), as BHB is the primary ketone body circulating in ruminants. Multiple studies have assessed the BHB concentration threshold at which health, production, or reproduction may be negatively affected. Ketosis is typically diagnosed by measuring the concentration of BHB in the blood, with a blood BHB level greater than 1.2 mmol/L being classified as ketosis (Duffield et al., 1997; Geishauser et al., 1997; Geishauser et al., 1998; Jorritsma et al., 1998; McArt et al., 2012). Tools for early detection of ketosis in dairy cows should be user-friendly, non-invasive, and cost-effective. Blood sampling can be challenging for farmers, making milk the most practical option for sampling each cow. In this regard, the routine DHI program serves as an effective tool for detecting ketosis in dairy cows during early lactation (Duffield et al., 1997; Čejna and Chladek, 2005; Jenkins et al., 2015). Calibrations for BHB analyses from milk samples were developed in accordance with Denis-Robichaud et al. (2014) and de Roos et al. (2007). It is significantly easier to diagnose subclinical ketosis in milk than in blood, as evidenced by the strong correlation (r=0.66 to 0.96) between ketone body concentrations in milk and blood, as shown in the research by Enjalbert et al. (2001). Few studies directly compare blood BHB (BBHB) and milk BHB (MBHB) using FTIR analysis. Although FTIR spectrometry is quick and cost-effective, it can be easily integrated into large-scale operations. The accuracy of the Ac and BHB measurements is sufficient to offer a herd-level parameter for detecting subclinical ketosis and classifying cows as potentially ketotic or healthy (de Roos et al., 2007). Since the mammary gland can utilize BBHB for fatty acid synthesis, and AcAc can be converted to butyrate, lower concentrations of BHB are typically found in milk compared to blood (Dodds et al., 1981).

The threshold for detecting ketosis in milk samples, as part of the DHI control, is a BHB level above 0.15 mmol/L (de Roos et al., 2007; Tatone et al., 2017). Besides the presence of ketone bodies, both subclinical and clinical ketosis are also linked to reduced milk production, higher milk fat content, and lower milk protein content during the early days of the DHI test (Vanholder et al., 2014; Rico and Barrientos-Blanco, 2024). Additionally, peripartum health complications associated with ketosis increase the risk of premature culling, leading to economic losses for milk producers. Even in the absence of clinical signs, ketosis can impact both milk production (Gustafsson et al., 1993) and reproduction (Andersson and Emanuelson, 1985; Andersson et al., 1991; Geishauser et al., 1997). Due to the economic impact, detecting cows with subclinical ketosis is crucial for either treating individual cows or adjusting their diet. According to Duffield et al. (1997), the milk fat/protein (F/P) ratio can be an indicator of ketosis. Nir Markusfeld (2003) found that if the F/P ratio exceeded 1.38, the likelihood of cows being clinically ketotic increased by 2.1 times, while Richardt (2004) reported that an F/P ratio above 1.5 likely increased the risk of ketosis by 3.5 times. King et al. (2019) identified strong linear associations between blood BHB and the F/P ratio in milk, but the coefficients of determination were low, and no specific F/P ratio threshold offered sufficient sensitivity for detecting ketosis.

It is hypothesized that hyperketonemic cows will have a higher somatic cell count, since they are more likely to develop clinical mastitis than healthy cows (Berge and Vertenten, 2014). The relationship between somatic cells and BHB in milk is very complex, as Santschi et al. (2016) reported a 61.3 % increment of mastitis incidence in hyperketonemic cows compared with healthy animals, supporting the previously mentioned. According to research from Cascone et al. (2022), an increase in SCC was observed in cows with higher BHB levels, which suggests a correlation between elevated BHB and increased SCC may indicate a higher risk of mastitis.

The present research aimed to investigate the amount of raw milk's ß-hydroxybutyrate, somatic cell count and other components of milk to analyse mutual dependency and their correlation with the prevalence of subclinical ketosis in dairy cows.

Materials and methods

The Laboratory for Milk Quality Control, at the Faculty of Agriculture in Novi Sad, Department of Animal Science (Serbia), collected cow production data during the regular Dairy Herd Improvement (DHI) control. The initial data set included a total of 23,492 milk samples of Holstein cows during the period from January 2022 to January 2025 from 6 farms located in the Province of Vojvodina (Report, Main breeding organization 2024). Samples of milk were taken from 5 to 399 days in milk (DIM), for fat, protein, lactose, and MU content, BHB concentrations and SCC. The variability of milk parameters was observed in relation to BHB status at 5-42, 43-84 and 85-399 DIM. The F/P ratio is calculated. Analyses were performed using Fourier-transform infrared spectrophotometry (FTIR) (Foss MilkoScan) and flow cytometry (Fossomatic FC).

The cow groups were classified according to milk BHB concentration during lactation using the following thresholds: <0.1 mmol/L (negative-NEG), 0.11-0.15 mmol/L (suspect-SUSP negative), 0.16-0.20 mmol/L (suspect-SUSP

positive), >0.20 mmol/L (positive-POS). The outcomes of the present study are in accordance with the Foss Ketosis application note (Foss, 2009), relative to elevated milk BHB concentrations alone as a herd-level screening tool for assessment of energy status in early-lactation cows. After classification, all samples with milk fat, protein, lactose and urea content deviating more than 3 standard deviation from the group mean were removed from data set as outliers, total of 577 samples. Final data set included 22,915 milk samples.

Statistical analysis

Statistical data processing was performed using the TIBCO Statistica™ software package (ver. 14 StatSoft., 2020). Analysis of covariance (ANCOVA) models for all milk parameters included BHB group as the independent categorical variable, and DIM as the independent continuous variable (covariate), and was followed with Duncan multiple range Post-hoc test to separate the means. Differences were considered significant at $p<0.05$. Correlation analysis was performed using partial linear correlation with two independent predictors: BHB concentration and DIM. Correlation coefficients were considered significant at $p<0.05$.

Results and discussion

Table 1 shows results of variability for milk fat, protein, lactose, total solids (TS), fat-to-protein ratio (F/P), somatic cell count (SCC), milk urea (MU) and ß-hydroxybutyrate concentration (BHB) based on the analysis of 22,915 raw milk samples.

Analyzed milk samples contained on average: milk fat 3.69 %, protein 3.36 %, lactose 4.79 %, total solids 12.56 %, fat to protein ratio 1.10, somatic cell count 485,000/mL, MU 25.03 mg/dL and BHB 0.07 mmol/L. The BHB, MU and SCC content ranged from 0.001 to 2.99 mmol/L, 1.10 to 53.60 mg/dL, 5,000 to 15,102,000/mL, respectively. The level of variability (SD and CV) of BHB, MU and SCC is notably high, indicating the presence of large differences primarily in applied farm management and non-genetic factors. The obtained average results are in accordance with the current Regulation on the quality of raw milk (2017).

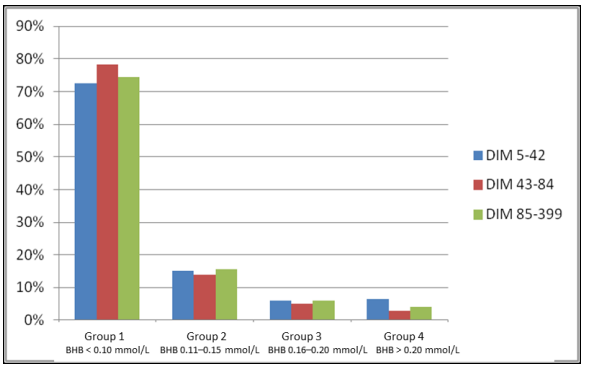

Figure 1. *Prevalence of milk BHB in different lactation stages according to the established thresholds of BHB groups*

Other authors have reported similar but usually higher values for average BHB content. Kowalski et al. (2021) in their research determined higher values of the mean content of BHB in milk (0.081 mmol/L), as well as a slightly higher content of fat (4.12 %) but lower protein content (3.11 %), also a higher ratio of fat to protein 1.33. Van Soest et al. (2024) also found a higher average content of milk fat (4.18 %), and BHB (0.11 mmol/L) and lower protein content (3.26 %). Santschi et al. (2016) looked at BHB, fat and protein content according to lactation and found higher milk fat content (4.94-5.08 %) and fat-protein ratio (1.53-1.68) in positive cows (BHB>0.20 mmol/L) compared to negative cows (BHB<0.15 mmol/L).

The average prevalence of BHB concentration according to the established thresholds of BHB groups related to DIM is shown in Figure 1. During early lactation period, average prevalence of elevated milk BHB concentration for the first group (BHB<0.1 mmol/L) was 75.0 % (average BHB 0.03 mmol/L), regarding the second group (BHB between 0.11-0.15 mmol/L) was 15 % (average BHB 0.13 mmol/L), the third group, (BHB between 0.16-0.20 mmol/L)

Table 1. *Descriptive statistics of the analysed milk parameters*

Parameters	N	Mean	Minimum	Maximum	SD	CV (%)
Milk fat (%)	22915	3.69	1.01	8.23	0.94	25.43
Protein (%)	22915	3.36	2.14	4.96	0.40	11.89
Lactose (%)	22915	4.79	3.39	5.50	0.24	5.01
TS (%)	22915	12.56	8.68	17.90	1.15	9.14
F/P	22915	1.10	0.27	3.31	0.26	23.99
SCC (*1000/mL)	22915	485	5	15102	1036	213.54
MU (mg/dL)	22915	25.03	1.10	53.60	9.11	36.38
BHB (mmol/L)	22915	0.07	0.00	2.99	0.09	138.20

TS - total solids, F/P - fat to protein ratio, SCC - somatic cell count, MU - milk urea, BHB - β-hydroxybutyrate, SD - Standard deviation; CV - Coefficient of variation.

was 6 % (average BHB 0.18 mmol/L) and the fourth (BHB >0.20 mmol/L) was 4 % (average BHB 0.34 mmol/L).

The milk parameters were considered in relation to the BHB content-groups (Table 2). The BHB positive group had BHB levels above 0.20 mmol/L, while the BHB negative group had BHB values below 0.10 mmol/L, as was previously reported. Two groups of suspect animals were divided: those with BHB levels between 0.11 and 0.15 mmol/L (suspect negative) and those with BHB levels between 0.16 and 0.20 mmol/L (suspect positive). Some authors use different cut-points of milk BHB. Tatone et al. (2017) stated that the optimal threshold based on association with clinically relevant outcomes has not been established, but values above 0.15 mmol/L can be considered positive. However, Santschi et al. (2016) classified cows as positive on ketosis if they had BHB above 0.20 mmol/L. Based on the comparison of the content of BHB in blood and milk, Renaud et al. (2019), conclude that the optimal cut point for determining ketosis on the DHI milk BHB test is above 0.14 mmol/L.

In the first period of lactation (Table 2), BHB groups significantly differ in all observed parameters except milk urea. Differences between first two groups are not statistically significant, fat and F/P ratio being the exceptions.

During the second period of lactation (Table 3), protein level and MU are not affected by BHB group, while all other parameters are. Contrary to the first period, differences between first two groups are mostly significant, while differences between last two are not (except lactose).

During the rest of lactation (Table 4), all parameters significantly differ between BHB groups. Differences between first two groups are almost always significant (except MU), and differences between last two groups are most often not significant.

Based on the statistical significance of observed differences, it can be assumed that, from the standpoint of milk parameters, different BHB thresholds might be needed for different periods of lactation. During the first 6 weeks of lactation, cows with BHB of 0.15, or under, can be considered negative, 0.16-0.20 suspect and cows over 0.20 positive. In the rest of lactation lower threshold could be used, with 0.10, or under, negative, 0.11-0.15 suspect and over 0.15 positive. The obtained results are in accordance with the statements of other authors (Duffield

Table 2. *Milk parameters according to BHB groups for milk samples at DIM 5-42*

BHB groups (mmol/L)	N	Milk fat (%)	Protein (%)	F/P	Lactose (%)	TS (%)	MU (mg/dL)	SCC (*1000/mL)
<0.1	1730	3.77^{a}	3.16^{a}	1.20^{a}	4.83^{a}	12.51^{a}	23.32	133^{a}
0.11-0.15	369	3.98^{b}	3.12^{a}	1.29^{b}	4.82^{a}	12.68^{a}	24.14	157^{a}
0.16-0.20	145	4.53^{c}	3.14^{a}	1.46^{c}	4.67^{b}	13.16^{b}	23.68	235^{b}
>0.20	154	5.07^{d}	3.22^{b}	1.59^{d}	4.48^{c}	13.69^{c}	22.91	217^{b}
Total/Mean	2398	3.93	3.16	1.25	4.80	12.65	23.45	146

*F/P - fat to protein ratio, TS - total solids, MU - milk urea, BHB - β-hydroxybutyrate, Log SCC - somatic cell log transformed. Values in the same column marked with different letters (a, b, c, d) are statistically different; *p<0.05, **p<0.01.*

Table 3. *Milk parameters according to BHB groups for milk samples at DIM 43-84*

BHB groups (mmol/L)	N	Milk fat (%)	Protein (%)	F/P	Lactose (%)	TS (%)	MU (mg/dL)	SCC (*1000/mL)
<0.1	2249	3.38^{a}	3.08	1.10^{a}	4.88^{a}	12.06^{a}	23.55	122^{a}
0.11-0.15	400	3.64^{b}	3.05	1.20^{b}	4.85^{a}	12.27^{b}	24.12	178^{b}
0.16-0.20	142	3.98^{c}	3.08	1.30^{c}	4.77^{b}	12.59^{c}	25.00	225^{bc}
>0.20	84	4.09^{c}	3.15	1.32^{c}	4.65^{c}	12.66^{c}	23.31	253^{c}
Total/Mean	2875	3.47	3.08	1.13	4.86	12.13	23.69	137

*F/P - fat to protein ratio, TS - total solids, MU - milk urea, BHB - β-hydroxybutyrate, SCC Log - somatic cell log transformed. Values in the same column with the same lower case letters do not differ statistically significantly (p>0.01); Between arithmetic means in the same column, with different labels (a, b, c, d), there is a statistically highly significant difference; *p<0.05, **p<0.01.*

Table 4. *Milk parameters according to BHB status for milk samples at DIM 85-399*

BHB groups (mmol/L)	N	Milk fat (%)	Protein (%)	F/P	Lactose (%)	TS (%)	MU (mg/dL)	SCC (*1000/mL)
<0.1	13135	3.58^{a}	3.41^{a}	1.05^{a}	4.80^{a}	12.50^{a}	25.68^{a}	177 ^{a}
0.11-0.15	2741	3.87^{b}	3.44^{b}	1.12^{b}	4.77^{b}	12.81^{b}	25.18^{ab}	250 ^{b}
0.16-0.20	1067	4.21^{c}	3.54^{c}	1.19^{c}	4.71^{c}	13.22^{d}	24.58^{b}	305 ^{c}
>0.20	699	4.23^{c}	3.55^{c}	1.19^{c}	4.57^{d}	13.14^{c}	23.76^{c}	329 ^{c}
Total/Mean	17642	3.69	3.43	1.08	4.78	12.61	25.46	197

*F/P - fat to protein ratio, TS - total solids, MU - milk urea, BHB - β-hydroxybutyrate, SCC Log - somatic cell log transformed. Values in the same column with the same lower case letters do not differ statistically significantly (p>0.01); Between arithmetic means in the same column, with different labels (a, b, c, d), there is a statistically highly significant difference; *p<0.05, **p<0.01.*

Table 5. Coefficients of correlation of milk parameters with DIM and BHB content from partial correlation analysis with two predictors, BHB and DIM

Parameters	Period	Milk fat (%)	Protein (%)	F/P	Lactose (%)	TS (%)	MU (mg/dL)	SCC (*1000/mL)
r BHB	5-42	0.21*	n.s.	0.20*	-0.29*	0.15*	n.s.	0.08*
	43-84	0.10*	n.s.	0.09*	-0.16*	0.07*	0.06*	0.10*
	85-399	0.13*	0,02*	0.13*	-0.18*	0.09*	0.02*	0.10*
r DIM	5-42	-0.25*	-0.40*	-0.07*	0.26*	-0.33*	-0.05*	-0.07*
	43-84	-0.06*	0.09*	-0.10*	n.s.	n.s.	n.s.	ns
	85-399	0.22*	0.48	n.s.	-0.18*	0.30*	0.03*	0.12*

*F/P - fat to protein ratio, TS - total solids, MU - milk urea, BHB - β-hydroxybutyrate; SCC - somatic cell count; significant difference *p<0.05*

et al., 1997; Nir Markusfeld, 2003; Vanholder et al., 2014, Santschi et al., 2016; King, et al., 2019; Rico et al., 2024) who state that with an increase in the concentration of ketone bodies, there is an increase in the content of milk fat and the ratio of the fat to protein, SCC count as well as a decrease in the content of lactose and MU. Hammon et al., (2006) and Santschi et al. (2016) also determined the highest somatic cell counts in suspect (BHB 0.15-0.19 mmol/L) and positive cows (BHB>0.20 mmol/L). Koeck et al. (2016) concluded that somatic cell score would improve if early lactation was selected for lower milk BHB. The negative effect of hyperketonemia and increased SCC has been confirmed in studies of Suthar et al., (2013), but not with increased risks for mastitis. Also, Santschi et al. (2016) found that suspect cows (BHB between 0.15 and 0.20 mmol/L) generally have intermediate values for milk fat, lactose and MUN. They also reported that the protein percentage was the lowest for suspect cows and the highest for negative cows.

As presented in Table 5, a positive statistically significant correlation was found between the BHB content and the milk fat, fat/protein ratio, TS, MU and SCC ($p<0.01$). Furthermore, a negative statistically significant correlation was found between the BHB and content of lactose, and correlation with protein is being insignificant except a weak link in the last period. The strongest correlations are observed with lactose, F/P ratio being only second to it in all phases. This finding indicates a possibility that low lactose content might be better correlated with energy deficit than usually used increased F/P ratio. An overall agreement stated by (Koeck et al., 2016; Jamrozik et al., 2016; Lee et al., 2016; Mehtiö et al., 2020) is that there was a strongly positive correlation among milk BHB and F/P. Contrary to the BHB effect, that is generally similar in all three periods, DIM effect is showing an obvious nonlinearity, as expected. Most parameters (fat, protein, total solids, MU, SCC) are decreasing in the first period, being stable or almost stable in the second, and then increasing during the third. Lactose is showing quite the opposite behavior, increasing during the first 6 weeks, being stable during the next 6 weeks, and decreasing after that. F/P ratio is decreasing during the whole early lactation, and being stable after that.

Conclusions

The results of this study show that elevated milk ß-hydroxybutyrate (BHB) concentrations, somatic cell count (SCC), altered fat-to-protein ratio, and reduced lactose levels are indicative of subclinical ketosis in dairy cows. Based on the observed BHB distribution, the study suggests that threshold values for subclinical ketosis detection may need to be adjusted according to the stage of lactation. In early lactation (first 6 weeks), cows with BHB concentrations ≤0.15 mmol/L can be considered negative, 0.16-0.20 mmol/L as suspect, and >0.20 mmol/L as positive. In later stages of lactation, lower thresholds may be more appropriate, with ≤0.10 mmol/L considered negative, 0.11-0.15 mmol/L suspect, and >0.15 mmol/L positive. Timely identification of at-risk animals enables producers to adjust nutritional management and feeding strategies to reduce the likelihood of disease progression. Furthermore, targeted monitoring of cows in the "suspect-positive" category may help refine herd-level interventions and improve individual health outcomes.

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Koncentracija ß-hidroksibutirata i broj somatskih stanica kao prevalencija subkliničke ketoze mliječnih krava

Sažetak

Cilj istraživanja bio je istražiti prevalenciju i učinak povišene razine ß-hidroksibutirata u mlijeku (BHB), SCC (broj somatskih stanica) i drugih komponenti sirovog mlijeka koje je detektirano rutinskom Fourier-transform infracrvenom (FTIR) analizom u uzorcima mlijeka. Skup podataka je prikupljen u razdoblju od siječnja 2022. do siječnja 2025. i prvobitno je brojao 23.492 uzoraka sirovog mlijeka od holstein krava sa 6 različitih farmi smještenih u Vojvodini, Srbija. Poslije uklanjanja ekstremnih vrijednosti, konačni skup podataka brojao je 22.915 uzoraka mlijeka. Prosječna prevalencija povišene koncentracije BHB u mlijeku za prvu negativnu skupinu (<0,1 mmol/L) iznosila je 75 % (između BHB 0,03 mmol/L), za drugu suspektno negativnu (između 0,11-0,15 mmol/L) bila je 15 % (prosječna BHB 0,13 mmol/L), suspektno pozitivnu (između 0,16-0,20 mmol/L) 6 % (prosječna BHB 0,18 mmol/L), a za četvrtu pozitivnu grupu (>0,20 mmol/L) bilo je 4 % (prosječna BHB 0.34). Analizom sastava mlijeka utvrđeno je da je prvo razdoblje od 5-42 dana značajno utjecalo na sve promatrane parametre kvalitete mlijeka. Sadržaj laktoze se smanjuje, međutim, sadržaj proteina, ukupne suhe tvari, F/P, SCC i uree raste. Rezultati ističu potencijal korištenja uzoraka mlijeka iz programa za poboljšanje mliječnog stada (DHI) za praćenje povišenih razina BHB-a i SCC-a u mlijeku na razini stada. Ovaj pristup može poslužiti kao koristan alat za procjenu prevalencije subkliničke ketoze kod mliječnih krava. Osim toga, za različita razdoblja laktacije mogu biti potrebni različiti pragovi detekcije BHB-a.

Ključne riječi: ß-hidroksibutirat; broj somatskih stanica; omjer masti/proteina; ketoza; holstein

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