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Impact of environmental factors on milk β-hydroxybutyric acid and acetone levels in Holstein cattle associated with production traits

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Abstract

The objective of this study was to estimate the environmental factors affecting milk β-hydroxybutyric acid (BHBA) and acetone (Ac) concentrations in Holstein cattle. A total of 264,221 test-day records collected from the Korea Animal Improvement Association (KAIA) during the period of 2012 to 2014 were used in this study. Analysis of variance (ANOVA) was performed to determine the factors significantly affecting ketone body concentrations. Parameters considered in the model were season of test, season of calving, parity, lactation stage, and milk collecting time (AM and PM). According to the ANOVA, the R^2 for milk BHBA and Ac were 0.5226 and 0.4961, respectively. 'Season of test' showed a considerable influence on ketone body concentration. Least square (LS) means for milk BHBA concentrations was the lowest (39.04 μM) in winter while it increased up to 62.91 μM in summer. But Ac concentration did not significantly change along with 'season of test'. The means of milk BHBA and Ac concentrations were high at first lactation stage, low around second lactation stage, and then gradually increased. Cows milked in the morning had lower mean BHBA and Ac concentrations (48.49 μM and 121.69 μM, respectively) in comparison to those milked in the evening (53.46 μM and 130.42 μM, respectively). The LS means of BHBA and Ac slightly increased over parities. These results suggest that proper maintenance of milk collection, herd management programs, and evaluation of ketone body levels in milk should be considered for the efficient management of resistance to ketosis.

Keywords: environmental factors, ketone body, lactation stage, parity, test-day records

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Introduction

Acetoacetate (AcAc), β-hydroxybutyrate (BHBA), and acetone (Ac) are the main circulating ketone bodies in ruminants. The excess level of ketone bodies in blood, urine and milk is a metabolic disorder termed as ketosis (Andersson, 1988). Early identification of diseases in dairy health management systems has been based on the measurement of milk constituents (Mottram et

al., 2002) because milk is easier to sample than blood or urine. Furthermore, techniques have been developed for automated sampling and measurement of components in milk (Geishauser et al., 2000; Godden et al., 2002; Van Knegsel et al., 2010), which makes it a suitable medium for routine analysis.

Clinical ketosis has visible clinical symptoms and normally occurs within the first 6 to 8 weeks post calving. Clinical ketosis is easy to diagnose by its clinical symptoms. Subclinical ketosis (SCK) is an excess level of circulating ketone bodies in the absence of the clinical signs of ketosis (Zhang et al., 2012). The ketone bodies Ac, AcAc, and BHBA can all be measured in milk and are useful as direct indicators of physiological imbalance and subclinical ketosis (Geishauser et al., 2000; Enjalbert et al., 2001; Nielsen et al., 2003).

SCK becomes more severe in weakened lactating cows with insufficient energy reserves for milk production (McArt et al., 2012). This metabolic state diagnosed by elevated levels of the ketone bodies listed above, combined with negative energy balance around calving, occurs mainly within the early lactation period. The cow's ability to meet energy intake and demand through different transition periods like dry-period, calving, and lactation is an important contributor to the maintenance of ketone body concentrations (Duffield et al., 2009). Minimizing the occurrence, severity, and consequences of negative energy balance in the early lactation period has become an important issue for the dairy industry. The proper maintenance of milk collection, well-organized herd management programs and assessment of BHBA and Ac measurements in milk should be considered for the efficient management of ketosis resistance. Non-genetic factors such as season of calving, season of test, parity, lactation stage and milk collecting time could be highly correlated with the maintenance of the ketone body concentrations, because metabolic disorders in early lactation indicate that the ability of the animals to adapt to living conditions that do not appropriately provide its specific nutrient and energy requirements has been overstressed (Sundrum, 2015). The objective of this study was therefore to identify and quantify environmental factors affecting ketone body concentrations in milk.

Materials and Methods

Fourier transform infrared (FTIR) measurements for milk Ac and BHBA levels along with routine monthly test-day records were collected from April 2012 to August 2014 by the Korea Animal Improvement Association (KAIA). Test-day milk sampling and analysis were performed according to the Korean milk-recording procedures (Cho et al., 2013).

Test-day milk records included milk yield, fat, protein, and lactose percentages of cows that were 1 to 305 days in milk at sampling. Milk samples were taken in the evening and morning. Test-day milk samples were analyzed by FTIR spectroscopy using a CombiFoss™ FT + system (Foss Analytical A/S, Denmark) with previously developed calibration equations for milk BHBA and Ac from the manufacturer. The original data set consisted of 264,221 test-day records.

For descriptive statistics of all parameters and statistical analysis of environmental factors affecting milk BHBA and Ac concentrations, SAS 9.2 software package (SAS Institute Inc. Cary, NC, USA) was used. The following model was used for the analysis:

$$y = Xb + e$$

Where y is the vector of observations for milk BHBA and Ac concentrations, b is the vector of fixed effects, X is an incidence matrix relating fixed effects to milk BHBA and Ac concentrations, fixed effects are season of test, season of calving, parity, lactation stage, and milk collecting time (AM, PM), and e is the vector of random residual errors.

Cows from 1 to 305 days in milk at sampling were considered for the analysis. Lactation stages were defined from 1 to 6, according to the days in milk (DIM) in 50 day intervals as 1 - 50 DIM, 51 - 100 DIM, 101 - 150 DIM, 151 - 200 DIM, 201 - 250 DIM and 251 - 305 DIM. 'Season of test' and 'season of calving' were defined as summer (May to October) and winter (November to April). Least square (LS) means were used to compare the effect of seasons, parity, lactation stage and milk collecting time (AM and PM).

Results and Discussion

A total of 264,221 milk samples from KAIA were evaluated for their BHBA levels, Ac, protein, fat, lactose contents, and milk yield. This study concerns the energy metabolism of dairy cows and how it relates to milk composition including ketone bodies. The mean BHBA level for all cows studied was 43.48 μM with a range of 0 to 4,280 μM while the mean Ac level was 127.49 μM with a range of 0 to 3,570 μM (Table 1).

Table 1. Descriptive statistics for the traits including milk β-hydroxybutyrate (BHBA) and acetone (Ac).

Traits	Mean	Standard deviation	Minimum	Maximum
Protein %	3.27	0.35	0.40	15.63
Fat %	3.84	0.92	0.19	18.89
Lactose %	4.73	0.23	0.28	5.53
Milk BHBA (μM)	43.48	59.48	0.00	4,280.00
Acetone (μM)	127.49	91.76	0.00	3,570.00
Milk yield (kg/day)	16.11	5.15	11.80	98.00

Various thresholds of BHBA and Ac in milk have been used to define subclinical ketosis. Geishauser et al. (1998), proposed a cut-off point of 50 or 100 μM for milk BHBA, and 200 or 500 μM for making changes in the diet and, after that, Enjalbert et al. (2001) proposed 70 or 100 μM , as a reasonable minimal concentration for detection of cows with subclinical ketosis.

Very different thresholds have previously been proposed for milk acetone. Miettinen (1994) described the upper limit of milk Ac in relation to milk production as 50 μM. But, according to some other studies, the upper limit of milk Ac in relation to milk production has been set as 700 μM (Gustafsson et al., 1993; Steen et al., 1996). Later, with the effects of ketonemia on milk production on a large set of field data, Gustafsson and Emanuelson (1996) suggested that 1,400 μM should be used as the critical value.

Fluctuations in ketone body concentrations in this study were associated with individual factors and herd factors. According to the ANOVA, milk BHBA records along with season of test, season of calving, parity, lactation stage and milk collection time accounted for 52.26 % of the total variation while Ac records accounted for 49.61% of the total variation. All sources of variation for milk BHBA were highly significant ($p < 0.01$). Milk Ac mean squares according to ANOVA, were also highly significant except for calving season (Table 2). A similar study reported about non genetic effects on Ac concentration. According to that study, the main factors were DIM class, lactation stage, and test month. The R^2 of that analysis was 0.102. All the main factors had highly significant effects (Wood et al., 2004).

Least square (LS) means were obtained for environmental factors (Table 3). Estimates based on LS means, milk BHBA concentration for the season of test was 39.04 μM in the winter (December to May) while increased up to 62.91

Table 2. The analysis of variance (ANOVA) for milk β-hydroxybutyrate (BHBA) and acetone (Ac).

Source	Df[x]	Milk β-hydroxybutyrate		Milk acetone	
		MS[y]	F[z]	MS	F
Season of calving	1	5,000.02	13.38	83.02	0.09
Season of test	1	64,440.28	172.40	7,372.86	7.88
Lactation stage	5	8,949.38	23.94	65,095.88	69.54
Parity	3	2,887.40	7.72	4,559.40	4.87
Milking time	1	5,461.06	14.61	9,228.29	9.86

All sources of variation were highly significant ($p < 0.01$) except for calving season for milk acetone.
[x]Degrees of freedom.
[y]Mean squares.
[z]F-value.

Table 3. Least square (LS) means of milk β-hydroxybutyrate (BHBA) and acetone (Ac) scores.

Source	Milk BHBA (μM)	Milk Ac (μM)
Season of calving		
Winter (November to April)	51.88	124.27
Summer (May to October)	50.06	127.84
Season of test		
Winter (November to April)	39.04	132.24
Summer (May to October)	62.91	119.87
Parity		
1	38.49	133.96
2	41.65	126.25
3	46.16	130.10
4	46.22	128.57
Time at sampling		
AM (0500 - 0700 hours.)	48.49	121.69
PM (1700 - 1900 hours.)	53.46	130.42

μM in the summer (June to October). But Ac concentration slightly decreased from 132.24 μM to 119.87μM in winter and summer seasons respectively. Milk BHBA concentration in summer was nearly doubled compared to winter. This result is supported by the findings of Vosman et al., 2015.The changes in feeding regimes during different seasons are the main effect on fluctuations of ketone body concentrations. A higher percentage of grass products in the diet and grazing in pasture were related to a higher incidence of ketosis (Van der Drift, 2013). Possibly, heat stress also plays a role in this.

Parity is clearly indicated as a risk factor for ketosis when looking at epidemiological literature (Nielsen et al., 2005; McArt et al., 2012). The level of ketone body concentration slightly increased with parity. Milk ketone body concentrations were also sensitive to diurnal variations. Milk samples were taken in the evening and morning. Milking time was considered as AM (0500 - 0700 hours) and PM (1700 - 1900 hours). According to that, the LS means for milk BHBA were 48.49 μM and 53.46 μM, respectively, for AM and PM and, in relation to milk Ac, 121.69μM and 130.42 μM, respectively. However, diurnal variations of ketone body concentrations were affected by feeding restrictions like times of feeding, feeding frequency, and feed stuffs (Nielsen et al., 2003). This consequence can be minimized by using

commonly accepted feeding recommendations.

Cows with test days from 1 to 305 DIM at sampling were considered in this analysis. The means of milk BHBA and Ac concentrations were high at the first lactation stage (between 1 and 50 DIM), low around second lactation stage (51 to 100 DIM), and then, gradually increased (Fig. 1).

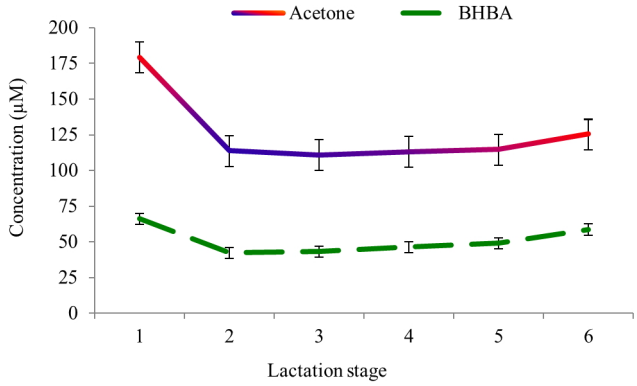

Fig. 1. Least square (LS) means of milk β-hydroxybutyrate (BHBA) and acetone (Ac) level (μM) across lactation stages. Lactation stages were defined from 1 - 6, according to the days in milk (DIM) with 50 day intervals as 1 - 50 DIM, 51 - 100 DIM, 101 - 150 DIM, 151 - 200 DIM, 201 - 250 DIM, and 251 - 305 DIM.

The result obtained was similar to data from Dohoo and Martin (1984) in that peak ketone levels were observed during the first 65 days of lactation. Geishauser et al. (2000), also reported that subclinical ketosis was highest in the first and second weeks after parturition. The variation in ketone body concentrations among lactation stages suggests that, if a program is set up to monitor milk ketone body levels, cows should better be tested during the first two months of lactation.

Conclusion

The risk of ketosis can potentially be predicted using measures of BHBA and Ac in milk and environmental risk factors. Elevated levels of ketone bodies can be due to lactation stage, parity, milk collecting time, and seasons of calving and testing. Individual detection of ketosis can be performed via quantitative determination of milk ketone bodies. The mean values of milk BHBA and Ac concentrations in this study were high during the period between 1 and 50 DIM and low around 51 to 100 DIM, after which they slightly increased with lactation stage. Diurnal variations and seasonal variations can be minimized by proper herd management programs. Further testing and thorough validation using a dataset from cows from differing management systems are needed.

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