

Genetic parameter estimation for milk β -hydroxybutyrate and acetone in early lactation and its association with fat to protein ratio and energy balance in Korean Holstein cattle

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Objective: The objective of this study was to estimate the genetic parameters for milk β -hydroxybutyrate (BHBA), acetone (Ac), fat protein ratio (FPR), and energy balance (EB) using milk test day records and investigate the effect of early lactation FPR and EB on milk ketone body concentrations.

Methods: Total 262,940 test-day records collected from Korea Animal Improvement Association during the period of 2012 to 2016 were used in this study. BHBA and Ac concentrations in milk were measured by Fourier transform infrared spectroscopy (FTIR). FPR values were obtained using test day records of fat and protein percentage. EB was calculated using previously developed equation based on parity, lactation week, and milk composition data. Genetic parameters were estimated by restricted maximum likelihood procedure based on repeatability model using Wombat program.

Results: Elevated milk BHBA and Ac concentrations were observed during the early lactation under the negative energy balance. Milk FPR tends to decrease with the decreasing ketone body concentrations. Heritability estimates for milk BHBA, Ac, EB, and FPR ranged from 0.09 to 0.14, 0.23 to 0.31, 0.19 to 0.52, and 0.16 to 0.42 respectively at parity 1, 2, 3, and 4. The overall heritability for BHBA, Ac, EB and FPR were 0.29, 0.32, 0.58, and 0.38 respectively. A common pattern was observed in heritability of EB and FPR along with parities.

Conclusion: FPR and EB can be suggested as potential predictors for risk of hyperketonemia. The heritability estimates of milk BHBA, Ac, EB, and FPR indicate that the selective breeding may contribute to maintaining the milk ketone bodies at optimum level during early lactation.

Keywords: Energy Balance; Fat Protein Ratio; Heritability; Repeatability Model; Genetic Parameters

INTRODUCTION

Subclinical ketosis in dairy cattle can be defined as the presence of elevated levels of circulating ketone bodies in blood, milk, and urine, without expression of typical clinical signs. It has been suggested that early lactation energy status and test-day fat and protein percentages should be considered as the potential indicators for the monitoring of sub clinical ketosis risk (SCK) [1,2].

Milk fat protein ratio (FPR) is a potent, easily quantifiable trait which can be utilized to differentiate cows with higher adoptability to early lactation challenges. The early lactation energy deficit leads to lipolysis and body fat mobilization resulting in an increased fat synthesis in the udder. Simultaneously, inadequate intake of fermentable carbohydrates can cause an insufficient protein synthesis by ruminal bacteria and the flow of amino acids to the udder is compromised and milk protein content decreases. Both of these processes result

in an increased FPR.

An FPR >1.5 indicates abnormally high lipolysis and has proven to be a good predictor of SCK [3].

Most dairy cows experience a negative energy balance (NEB) in early lactation, because their dietary intake cannot support the required energy, due to metabolic priority for milk production. The continuous mobilization of body fat reserves to balance the deficit between energy intake and energy production leads to ketogenesis, which ultimately exhausts the cow [4]. So excess level of ketone bodies are excreted to blood, which can be detected even in milk and urine.

Serum ketone body analysis is the major reference test to diagnose hyperketonemia. However due to practical limitations and close relationship between blood and milk ketone body concentrations, milk composition records become a convenient approach for routine analysis. The concentration of milk acetone (MAc) is similar to that of blood acetone (BAc), with mean MAc/BAc ratio of 1.04 [5]. Furthermore, a low correlation coefficient ($r = 0.45$) between blood AcAc and milk AcAc have been recorded [6]. The correlation between blood β -hydroxybutyrate (BHBA) and milk BHBA ranged from 0.00 to 0.87 [7]. The mean milk AcAc to BAcAc ratio and the mean milk BHBA to blood BHBA ratio were reported as 0.40 and 0.12, respectively [5]. Accordingly the use of milk ketone body concentrations to discriminate ketotic cows proved to be almost as reliable as blood ketone body concentrations.

For the use and application of milk ketone bodies in effective genetic evaluation and improvement, the knowledge of genetic parameters for the economically important traits is essential. Dairy cows display individual variation in metabolic adaptation during NEB [8]. This variation can be used for the selection of high producing cows with a lower hyperketonemia risk. Considerable genetic variation in heritability estimates of milk BHBA concentration ranging from 0.14 to 0.29 has been previously reported [9].

Therefore, this study was conducted to estimate the genetic parameters for milk BHBA, acetone (Ac), FPR, and energy balance (EB) and to further investigate the effect of early lactation FPR and EB on raising the milk ketone body levels in Korean Holsteins by utilizing milk test day records.

MATERIALS AND METHODS

Data

Fourier transform infrared (FTIR) measurements for milk Ac and milk BHBA levels along with routine test day records were collected from April 2012 to August 2016 by the Korea Animal Improvement Association (KAIA). Test-day milk sampling and analysis were performed according to the Korean milk-recording procedures [10]. The dairy population was managed under recommendations of KAIA. Herds are usually monitored for hyperketonemia by regular assessment of the percentage

of fresh cows having ketone body concentrations above a defined threshold value. The test day milk records included milk yield, fat and protein percentages of cows that were 1 to 100 days in milk at sampling. The cows were milked twice daily at 0500 and 1600 h. Milk composition was analyzed weekly based on samples collected from 2 consecutive milkings. Season of calving was defined as summer (May to October) and winter (November to April). The age at first calving ranged from ≤ 23 months, 24 to 25 months, 26 to 28 months, and ≥ 29 , respectively. The morning and evening records of registered cows with pedigree information of test days from cows that were 5 to 305 days in milk (DIM) at sampling were included in the analysis. Samples with missing sire or dam information were excluded from the analysis. Outliers over ± 3 standard deviations (SD) for milk production measurements were considered to be incorrect recordings and excluded from the analysis.

Test-day milk samples were analyzed by FTIR spectroscopy using a CombiFoss FT+ system (Foss Analytical A/S, Hilleroed, Denmark) with previously developed calibration equations for milk BHBA and milk Ac. The original data set consisted of 262,940 test-day records. Fat to protein ratio data were obtained from test day records.

This EB equation was based on parity, lactation week, and milk composition volume as follows [11,12]:

Equation

$$\begin{aligned} eEB = & 217.8 - wk2 \times 31.9 - wk3 \times 20.6 - wk4 \times 15.6 - wk5 \times 11.5 \\ & - wk6 \times 8.0 - wk7 \times 10.6 - wk8 \times 7.2 - wk9 \times 5.3 \\ & - wk10 \times 4.0 - wk11 \times 2.7 - wk12 \times 0 - par1 \times 34.9 \\ & - par2 \times 7.2 - par3 \times 6.7 - par4 \times 0 - milk \times 2.11 \\ & - prot \times 15.36 - FP \times 49.24 \text{ (MJ nel/d)} \end{aligned}$$

Where; wk2, wk3 . . . to wk12 = lactation wk 2 to 12; par1 - par4 = parity categories 1 to 4; milk = milk yield (kg/d); prot = % milk protein; FP = ratio of % fat to % protein in milk.

Statistical analysis

For descriptive statistics of all parameters SAS 9.2 package (SAS Institute Inc. Cary, NC, USA) was used. The test day records of milk BHBA, Ac, EB, and FPR were analyzed for each parity. Genetic parameters, including genetic (co)variance components, were estimated by the restricted maximum likelihood procedure based on repeatability model using the Wombat program [13]. Heritability was analyzed within each parity and across all parities using two models.

The linear model for across all parities and for individual parities were illustrated as follows (Model 1 and Model 2);

$$\begin{aligned} \text{Model 1: } Y_{ijklmn} = & \mu + DIM + Age_i + Season_j + ampm_k \\ & + parity_l + a_m + p_m + e_{ijklm} \end{aligned}$$

$$\text{Model 2: } Y_{ijkl} = \mu + \text{DIM} + \text{Age}_j + \text{Season}_k + \text{ampm}_l + a_m + p_m + e_{ijkl}$$

Where, Y_{ijklmn} is BHBA, Ac, FPR, or EB observation; μ is the overall mean; DIM is the covariate describing the effect of days in milk, Age_i is the fixed effect of calving age i , Season_j is the fixed effect of calving season j , ampm_k is the fixed effect of milk collecting time k . The parity $_l$ is the fixed effect of parity l . The a_m is the additive genetic effect of cow m , p_m is the permanent environmental effect of cow m , e is the random residual effect associated with each record.

Heritability was calculated using this equation:

$$h^2 = \frac{\sigma_A^2}{\sigma_p^2}$$

Where h^2 is heritability, σ_A^2 is additive genetic variance and σ_p^2 is phenotypic variance.

RESULTS

A total of 262,940 milk samples were evaluated for BHBA, Ac, protein, fat and milk yield at KAIA. The mean BHBA level for above data set was 43.48 $\mu\text{mol/L}$ with the range of 0 to 4,280 $\mu\text{mol/L}$ while the mean Ac level was 127.49 $\mu\text{mol/L}$ with the range of 0 to 3,570 $\mu\text{mol/L}$. Average milk yield was 16.11 kg/d while mean fat and protein percentages were 3.27 and 3.84 respectively (Table 1).

This study evaluated the relationship between milk ketone bodies (BHBA and Ac) with the indicator traits (FPR and EB). Mean milk BHBA and Ac level declined with lactation week. In the early lactation (up to 6th week) both traits declined at a higher rate. During that period, Ac shows higher slope than BHBA. But after that the mean BHBA and Ac concentration become static. The FPR ranged from maximum value of 1.30 and minimum 1.15. DIM were split into 7 day intervals. The mean FPR was >1.2 up to 5th week of lactation. Cows with a $\text{FPR} \geq 1.2$ had greater milk BHBA and Ac up to 6th week of lactation, then the milk BHBA and Ac level became static. Highest mean BHBA and AC concentrations were 73.78 $\mu\text{mol/L}$ and 158.05 $\mu\text{mol/L}$ while lowest level was 33.48 $\mu\text{mol/L}$

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

Traits	No of records	Mean	SD	Minimum	Maximum
Protein (%)	262,940	3.27	0.35	0.4	15.63
Fat (%)	262,940	3.84	0.92	0.19	18.89
BHBA ($\mu\text{mol/L}$)	262,940	43.48	59.48	0	4,280
Acetone ($\mu\text{mol/L}$)	262,940	127.49	91.76	0	3,570
Milk yield (kg/d)	262,940	16.11	5.15	11.8	98

SD, standard deviation.

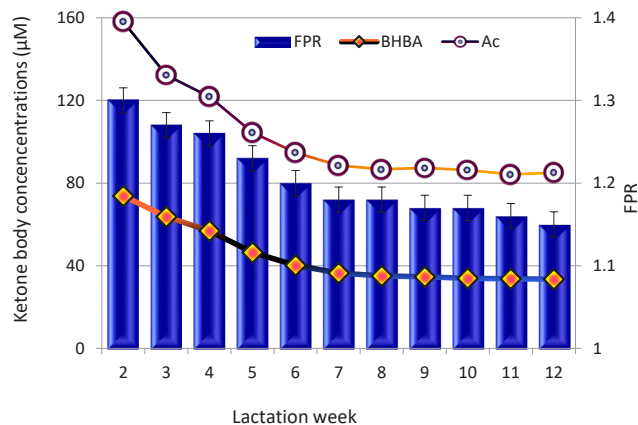


Figure 1. The relationship between milk BHBA and Ac concentration with FPR along with lactation week (FPR, fat to protein ratio; Ac, acetone; BHBA, β -hydroxybutyrate). The bar graph represents the FPR values and line graphs represent the ketone body concentrations.

and 85.01 $\mu\text{mol/L}$ respectively (Figure 1).

Figure 2 shows the relationship between mean EB and ketone body concentrations. In the 2nd week of lactation EB was -13.18 MJ nel/d. The negativity was reduced with lactation week and became positive at 5th week of the lactation (35 DIM). In the 5th week of lactation EB was 1.81 MJ nel/d and it increased up to 15.1 MJ nel/d by the 12th week of lactation. During the phase of NEB, elevated levels of milk BHBA and milk Ac concentrations can be observed. Afterward mean EB becomes positive and ketone body concentrations stabilize and even out. In this study, the estimated heritability for milk BHBA and milk Ac for all parities was 0.19 and 0.28 respectively. Heritability estimates for EB and FPR were 0.50 and 0.46 respectively. Genetic and phenotypic relationships of milk BHBA, Ac, EB, and FPR were studied. Phenotypic correlations among the traits considered were lower than genetic correlations with the exception of BHBA and Ac. Genetic and

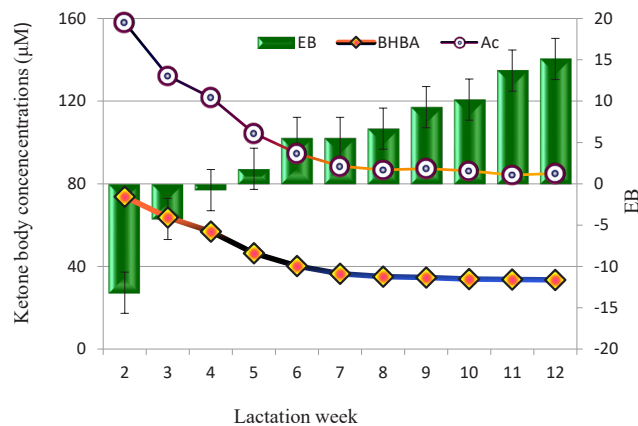


Figure 2. The relationship between milk BHBA and acetone concentration with energy balance along with lactation week (EB, energy balance; Ac, acetone; BHBA, β -hydroxybutyrate). The bar graph represents the EB values and line graphs represent the ketone body concentrations.

Table 2. Heritabilities (in bold in the diagonal) and genetic correlations (above the diagonal) and phenotypic correlations (below diagonal)

Item	BHBA	Ac	EB	FPR
BHBA	0.19	0.52	-0.28	0.40
Ac	0.57	0.28	0.22	-0.15
EB	-0.24	0.09	0.50	-0.86
FPR	0.35	-0.01	-0.81	0.46

BHBA, β-hydroxybutyrate; Ac, acetone; EB, energy balance; FPR, fat protein ratio.

phenotypic correlations between Milk BHBA and EB, Ac and FPR, EB and FPR were negative while other correlations were positive. The highest negative correlation was observed between EB and FPR (Genetic correlation of -0.86 and phenotypic correlation of -0.81) (Table 2).

The heritability estimates for milk BHBA, EB, and FPR declined with the parity while heritability for Ac deviated from that pattern. The highest heritability for milk BHBA (0.14) was observed at parity1 and it declined to 0.09 at the 4th parity. The observed 0.52 heritability for EB at parity 1 declined to 0.19 at parity 4 and 0.43 heritability for FPR at parity 1 declined to 0.16 at parity 4. Milk Ac showed the lowest heritability at the 1st parity (0.31) then it ranged from 0.29 to 0.31 during the subsequent parities (Table 3, Figure 3).

Variance components (additive genetic variance and phenotypic variance) and heritability estimates for different parities are shown in Table 3. The proportion of phenotypic variance was larger than additive genetic variance.

There was an apparent trend of increasing heritability with increasing parity.

DISCUSSION

The energy metabolism of dairy cows along with the milk composition including ketone bodies was the concern of this study. Dairy herds are monitored for the prevalence of hyper-

Table 3. Additive genetic variance (σ^2_A), phenotypic variances (σ^2_p), and heritability (h^2) estimates of different parities

Item		Parity 1	Parity 2	Parity 3	Parity 4	All
BHBA	(σ^2_A)	178.68	145.38	140.58	155.78	109.66
	(σ^2_p)	1,203	1,319.8	1,559.1	1,626.8	832.14
	(h^2)	0.14	0.11	0.09	0.09	0.19
Ac	(σ^2_A)	1,085.4	1,320.7	1,650.9	1,537.1	1,726.86
	(σ^2_p)	4,710.2	4,446.7	5,164.7	5,226.3	5,780
	(h^2)	0.23	0.29	0.31	0.29	0.29
EB	(σ^2_A)	117.88	116.44	86.343	54.839	115.88
	(σ^2_p)	224.82	277.15	291	277.38	222.28
	(h^2)	0.52	0.42	0.29	0.19	0.52
FPR	(σ^2_A)	0.0338	0.0352	0.0203	0.0154	0.0438
	(σ^2_p)	0.0788	0.0938	0.0958	0.0956	0.0983
	(h^2)	0.42	0.37	0.21	0.16	0.44

BHBA, β-hydroxybutyrate; Ac, acetone; EB, energy balance; FPR, fat protein ratio.

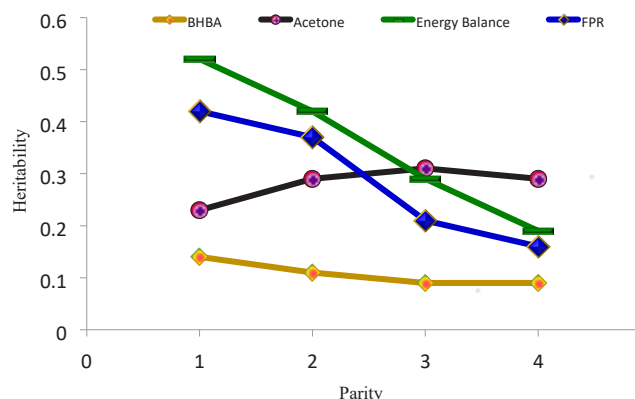


Figure 3. Heritability estimates of milk BHBA, milk Ac, EB, and FPR according to parities from parity 1-4 (FPR, fat to protein ratio; EB, energy balance; Ac, Acetone, BHBA-β-hydroxybutyrate).

ketonemia by regular assessment milk composition volumes above a defined threshold value.

Various thresholds of BHBA and Ac in milk have been used to define SCK. Consequently, the concentrations rather than presence or absence of hyperketonemia in cows were used to investigate genetic variations in ketosis susceptibility.

Data from the first week of lactation were excluded in estimating FPR and EB, because of possible effect of the colostrum component of milk. FPR was considered as an indicator of lipo-mobilization and NEB status of postpartum cows. Several studies have indicated different values for the optimal FPR, whereas 1.33 was set as a cut off value [1] while FPR values higher than 1.5 suggest a great energy deficiency and SCK if ketone bodies are present [3]. Animals with FPR greater than 2.0 in early lactation showed an increase in postpartum diseases [2]. A positive correlation of BHBA levels with the fat: protein ratio ($R^2 = 0.42$; $p < 0.001$) was evident [14]. Values recorded in this study suggest that FPR of 1.3 indicates the likelihood of elevated levels of ketone bodies with the probable risk of SCK.

Dairy cows exhibit variation in the adaptation to NEB with respect to individual and herd factors. This study shows, elevated levels of ketone body concentrations during the period of NEB (up to 4 week). It suggests that heavy fat mobilization and ketogenesis in this high metabolism period causes a reduction of energy reserves. Buttchereit et al [15] suggested that animals change to a state of positive EB around DIM 40 to 80, which coincides with findings of de Vries and Veerkamp [16].

The genetic parameter estimation focused on the challenge of using the existing genetic variation for genetic selection of high-performing dairy cattle with a lower hyperketonemia susceptibility. This study estimated heritabilities for milk BHBA, milk Ac, FPR, and EB in early lactation to assess the susceptibility to hyperketonemia and investigate the possibility of using test day records in milk for genetic improvements. The

previous studies suggested that the prevalence of hyperketonemia varies across parity, stage of lactation and season in cows [1,8], Therefore these factors were included in the models and heritability was analyzed within each parity ($j = 1, 2, 3, 4$) individually and across all parities.

The heritability estimates were somewhat higher when compared with previous studies on genetic variation. So, we can suggest that a considerable part of the observed variation could be allocated to fixed and random effects included in the model. Residual variance could be low. But our heritability estimates are also within the range of heritabilities in several studies on clinical ketosis. The heritability estimates of a previous study for milk BHBA and milk Ac were 0.04 to 0.17 and 0.22 to 0.29 respectively [17]. Koeck et al [9] found that heritabilities for milk BHBA at different stages of early lactation from 5 to 100 DIM were between 0.14 and 0.29. High genetic merit cows experience more severe NEB in early lactation [18]. Heritability estimates of EB ranged from, 0.12 to 0.49 over monthly intervals [19]. In this study high heritability was observed in EB (0.50). Thus, it suggests that a favorable metabolic adaptation to the NEB in early lactation could be used for genetic selection.

Generally, tests for fat and protein content of milk as a basis for regular dairy herd improvement have been considered as alternate measures for determining the early lactation nutritional status of dairy cows [1]. According to the breed and herd factors, milk fat and protein levels can vary tremendously. Holsteins have the lowest fat and protein content when compared with other breeds (USDA- AIPL summary of herds DHI test during 2004). Heritability estimate for FPR in this study was 0.46. Such a high heritability estimate indicates inheritance accounts for considerable difference between cows in protein and fat content in milk. German Holstein cattle showed heritability estimates of 0.06 and 0.30 for EB and FPR respectively [15].

The highest negative phenotypic correlation -0.81 was observed between EB and FPR while highest negative genetic correlation -0.86 also observed between EB and FPR. So, variation between cows exists for fat protein mobilization during the metabolic adaptation to NEB in early lactation, which increases the hyperketonemia risk.

Animals with higher FPR have less body reserves and NEB. Variation may exist in cows for dietary intake, fat mobilization, metabolic gene expression which all influence the metabolic adaptation to NEB in early lactation.

CONCLUSION

Milk BHBA, Ac, EB, and FPR can be used as indicator traits for hyperketonemia. Milk test day records, provide practical routine data collection for breeding programs. FTIR predictions for milk BHBA and Ac can be used for screening cows

for hyperketonemia. Milk BHBA, Ac, EB, and FPR showed a considerable genetic variation with heritability along with parity. Milk test day records provide useful data for selective breeding programs. Selective breeding may contribute to maintaining the milk ketone bodies at optimum level during early lactation.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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