

## **Genetic aspects of milk $\beta$ -hydroxybutyrate in Italian Holstein cows**

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### **Summary**

The aim of this study was to estimate heritability of milk  $\beta$ -hydroxybutyrate (BHB) and its genetic relationships with milk production and composition traits in early lactation (5 to 100 days in milk) of Italian Holstein cows. The dataset consisted of 67,131 test-day milk analyses from 21,223 cows of different parity. Estimate of heritability for milk BHB was  $0.08 \pm 0.01$ . The strongest genetic correlation was observed between BHB and fat-to-protein ratio ( $0.33 \pm 0.07$ ), whereas genetic relationships between milk BHB and other milk traits were weak, ranging from  $-0.07 \pm 0.07$  with urea to  $0.21 \pm 0.06$  with fat percentage. Although milk BHB routinely determined in milk samples during early lactation is a lowly heritable trait, genetic improvement can be made through appropriate breeding strategies to reduce the susceptibility of cows to ketosis in Italian Holstein population.

*Keywords:  $\beta$ -hydroxybutyrate, milk, genetic parameter, dairy cow*

### **Introduction**

Ketosis is one of the most frequent metabolic disorders in high-producing dairy cows, occurring when animals are unable to cope with the energy requirements of early lactation. This condition leads to a negative energy balance and an abnormal increase in circulating ketone bodies, known as hyperketonemia (Herdt, 2000; Duffield *et al.*, 2009).

The reference test for ketosis detection is the concentration of  $\beta$ -hydroxybutyrate (BHB) in blood (Oetzel, 2004) and BHB greater than 1.2 mmol/L is an indicator of ketosis (van Knegsel *et al.*, 2010). Despite blood BHB is the most accurate test to detect ketosis, BHB concentration in milk has been proposed as indicator of hyperketonemia due to its strong correlation with ketone bodies in blood (Denis-Robichaud *et al.*, 2014) and the advantage to be routinely available in milk recording system (Koeck *et al.*, 2014). Ketosis significantly impairs productive performance and health of dairy cows (Duffield *et al.*, 2009), and it has a high prevalence in Italian herds (Berge & Vertenten, 2014).

Milk BHB is a practical tool for both selecting cows with a low susceptibility to ketosis (Koeck *et al.*, 2014; Jamrozik *et al.*, 2016) and supporting farmers management practices.

Recent studies have reported that milk BHB in early lactation is a heritable trait, with estimates ranging from 0.14 to 0.29 (van der Drift *et al.*, 2012; Koeck *et al.*, 2014). Nevertheless, there is a paucity of studies that assessed genetic parameters of BHB in bovine milk worldwide and only one Italian study presented some results in Italian Holsteins (Penasa *et al.*, 2015). Therefore, this study aimed to estimate heritability of milk BHB and its genetic correlations with milk production and composition traits in Italian Holstein dairy cattle.

## **Material and Methods**

### **Data**

The dataset was provided by the Breeders Association of Veneto region (Padova, Italy) and consisted of test-day production records and predicted milk BHB values of Italian Holstein cows collected from May 2015 to June 2017. Milk fat, protein and lactose percentages, urea content and BHB concentration were predicted by MilkoScan FT6000 (Foss, Hillerød, Denmark) with calibration models developed by Foss. Somatic cell count was analyzed by Fossomatic (Foss, Hillerød, Denmark) and values were transformed to SCS through the formula  $SCS = 3 + \log_2(SCC/100,000)$ .

The original dataset was edited to retain cows with known sire and dam, between 5 and 100 days in milk (DIM), from parity 1 to 9, and with at least 2 test-day records in the first 100 DIM. The minimum number of cows per herd-test date (HTD) was set to 5. Moreover, records were discarded from the dataset if they represented inconsistent information or exceeded 3.5 standard deviations (SD) from the respective mean of milk yield, and fat, protein, and lactose percentages. Values of BHB were added with a constant of 1.00 and log<sub>e</sub>-transformed to achieve a normal distribution of the data. After editing procedure, a subset of 30% of herds (n = 261) was randomly selected in order to reduce computational demand for genetic analysis. The dataset contained 67,131 records from 21,223 cows. The pedigree file (79,539 individuals) included cows with phenotypic records and their ancestors up to 6 generations back.

### **Statistical analysis**

Milk yield, composition traits, fat-to-protein ratio (F:P), urea content, SCS and BHB were analysed through ASReml software (Gilmour *et al.*, 2015). Heritability for the studied traits was estimated using a single-trait repeatability animal model that included the fixed effects of parity (first, second, third, and fourth and later parities), classes of DIM (1 to 15, with the first 11 being classes of 5 d each, followed by 4 classes of 10 d each), season of calving (winter, December to February; spring, March to May; summer, June to August; autumn, September to November), and HTD (1 to 3,488 levels), and the random effects of additive genetic animal, permanent environment and residual. Genetic correlations between BHB and other traits were estimated using 7 sequential bivariate models in which BHB was analysed simultaneously with milk yield, fat percentage, protein percentage, lactose percentage, F:P, urea content or SCS.

## **Results and Discussion**

Milk BHB averaged 0.059 and it was the most variable trait, with coefficient of variation of

100% (Table 1). Large variability of milk BHB during early lactation has been already reported in previous studies (van der Drift *et al.*, 2012; Koeck *et al.*, 2014; Jamrozik *et al.*, 2016). Milk production and chemical composition were consistent with official data reported by the Italian Holstein Association (ANAFI, 2016).

Table 1. Descriptive statistics of  $\log_e$ -transformed milk  $\beta$ -hydroxybutyrate (BHB), milk yield, composition traits, and somatic cell score (SCS) in the first 100 days in milk ( $n = 67,131$ ).

Trait	Mean	SD	Minimum	Maximum
BHB	0.059	0.059	0	1.043
Milk yield, kg/d	37.38	9.42	4.10	64.70
Fat, %	3.71	0.79	0.90	6.84
Protein, %	3.10	0.32	2.00	4.89
F:P <sup>1</sup>	1.20	0.25	0.26	2.90
Lactose, %	4.92	0.19	4.04	5.61
Urea, mg/dL	22.65	6.06	10.00	66.30
SCS	2.50	2.04	-3.64	10.79

<sup>1</sup> F:P = fat-to-protein ratio.

Heritability of milk BHB in the first 100 DIM was 0.08 (Table 2). This estimate was lower than findings of Koeck *et al.* (2014), who reported heritability for milk BHB between 0.14 and 0.29 in the first 100 DIM of primiparous Canadian Holsteins, whereas Jamrozik *et al.* (2016) and Lee *et al.* (2016) reported similar estimates of heritability for Holstein cows between 5 and 40 DIM, and for the first 150 DIM, respectively. Heritabilities for other milk traits were comparable with those reported by Cassandro *et al.* (2008), Tiezzi *et al.* (2013) and Visentin *et al.* (2017). Conversely, in the present study heritability of fat percentage was lower than estimates observed in whole lactation (Tiezzi *et al.*, 2013; Visentin *et al.*, 2017). Indeed, heritability of fat percentage has been described to progressively increase from the beginning until the end of lactation (Jattawa *et al.*, 2016).

Table 2. Estimates<sup>1</sup> of additive genetic variance ( $\sigma_a^2$ ), heritability and repeatability for  $\log_e$ -transformed milk  $\beta$ -hydroxybutyrate (BHB), milk yield, composition traits, and somatic cell score (SCS), and genetic correlations ( $r_g$ ) between BHB and other milk traits in the first 100 days in milk.

Trait	$\sigma_a^2$	Heritability	Repeatability	$r_g$ of BHB with
BHB	0.00012	0.08	0.20	-
Milk yield, kg/d	4.29366	0.09	0.45	0.07
Fat, %	0.05511	0.12	0.24	0.21
Protein, %	0.01621	0.25	0.48	-0.12
F:P <sup>2</sup>	0.00355	0.07	0.19	0.33
Lactose, %	0.00894	0.34	0.51	-0.08
Urea, mg/dL	2.24204	0.12	0.28	-0.07
SCS	0.20814	0.06	0.39	0.16

<sup>1</sup> Standard errors ranged from 0.00001 to 0.48461 for additive genetic variance, 0.008 to 0.0015 for heritability, 0.0045 to 0.0050 for repeatability, and 0.05 to 0.08 for genetic correlations.

<sup>2</sup> F:P = fat-to-protein ratio.

Repeatabilities of BHB (0.20) and F:P (0.19) were the lowest among the investigated

traits (Table 2), suggesting that temporary effects play a strong role in determining the variation of BHB and F:P in early lactation and thus several observations per animal are necessary to assess their overall variability within lactation (Tyrisevä *et al.*, 2003). Low estimates of repeatability for milk BHB (0.08 to 0.16) were reported also by Cho *et al.* (2015) in Holstein dairy cattle. Repeatability estimates of other milk traits were similar to those reported by Tiezzi *et al.* (2013) and Visentin *et al.* (2017).

The strongest estimate of genetic correlation was obtained between BHB and F:P (0.33), confirming the complementary role of these traits in ketosis detection. Similarly, Penasa *et al.* (2015) observed that overall mean genetic relationship between BHB and F:P during the entire lactation was 0.31, whereas coefficients of 0.49 and 0.12 were reported by Jamrozik *et al.* (2016) for first- and later-parity cows, respectively. As expected genetic relationships of BHB with fat (0.21) and protein percentage (-0.12) were in opposite direction. A positive despite weak genetic correlation (0.16) was observed between BHB and SCS, suggesting that ketosis and mastitis are genetically related in early lactation. Finally, genetic correlations of BHB with other milk traits were less than 0.10.

## Conclusion

Results of the present study revealed that milk BHB routinely determined in milk samples during early lactation exhibits genetic variation and thus breeding strategies to reduce the susceptibility of cows to ketosis are possible in Italian Holstein population. Further research will focus on estimation of genetic parameters for milk BHB in different parities and exploiting random regression models on a larger dataset to model genetic parameters for milk BHB over early lactation.

## List of References

- ANAFI (Associazione Nazionale Allevatori Frisone Italiana), 2016. Official Statistics. Accessed Sep. 6, 2017. <http://www.anafi.it/english/>.
- Berge, A.C. & G. Vertenten, 2014. A field study to determine the prevalence, dairy herd management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds. *J. Dairy Sci.* 97: 2145–2154.
- Cassandro, M., A. Comin, M. Ojala, R. Dal Zotto, M. De Marchi, L. Gallo, P. Carnier & G. Bittante, 2008. Genetic parameters of milk coagulation properties and their relationship with milk yield and quality traits in Italian Holstein cows. *J. Dairy Sci.* 91: 371-376.
- Cho, K.H., C.I. Cho, J.H. Lee & K.D. Park, 2015. (Co)heritability of acetone and  $\beta$ -hydroxybutyrate concentrations in raw milk related to ketosis in Holsteins. *J. Korean Data Inf. Sci. Soc.* 26: 915-921.
- Denis-Robichaud, J., J. Dubuc, D. Lefebvre & L. DesCôteaux, 2014. Accuracy of milk ketone bodies from flow-injection analysis for the diagnosis of hyperketonemia in dairy cows. *J. Dairy Sci.* 97: 3364-3370.
- Duffield, T.F., K.D. Lissemore, B.W. McBride & K.E. Leslie, 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. *J. Dairy Sci.* 92: 571–580.
- Gilmour, A.R., B.J. Gogel, B.R. Cullis, S.J. Welham & R. Thompson, 2015. ASReml User 346 Guide Release 4.1, VSN International Ltd, Hemel Hempstead, UK [www.vsn.co.uk](http://www.vsn.co.uk)
- Herdt, T.H., 2000. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. *Vet. Clin. North Am. Food Anim. Pract.* 16: 215–230.

- Jamrozik, J., A. Koeck, G.J. Kistemaker & F. Miglior, 2016. Multiple-trait estimates of genetic parameters for metabolic disease traits, fertility disorders, and their predictors in Canadian Holsteins. *J. Dairy Sci.* 99: 1990-1998.
- Jattawa, D., M.A. Elzo, S. Koonawootrittriron & T. Suwanasopee, 2016. Genomic-polygenic and polygenic evaluations for milk yield and fat percentage using random regression models with Legendre polynomials in a Thai multibreed dairy population. *Livest. Sci.* 188: 133-141.
- Koeck, A., J. Jamrozik, F.S. Schenkel, R.K. Moore, D.M. Lefebvre, D.F. Kelton & F. Miglior, 2014. Genetic analysis of milk beta-hydroxybutyrate and its association with fat-to-protein ratio, body condition score, clinical ketosis, and displaced abomasum in early first lactation of Canadian Holsteins. *J. Dairy Sci.* 97: 7286-7292.
- Lee, S., K. Cho, M. Park, T. Choi, S. Kim & C. Do, 2016. Genetic parameters of milk  $\beta$ -hydroxybutyric acid and acetone and their genetic association with milk production traits of Holstein cattle. *Asian Australas. J. Anim. Sci.* 29: 1530-1540.
- Oetzel, G.R. 2004. Monitoring and testing dairy herds for metabolic disease. *Vet. Clin. North Am. Food Anim. Pract.* 20: 651-674.
- Penasa, M., D. Pretto, A. Varotto & M. De Marchi, 2015. Heritability of milk  $\beta$ -hydroxybutyrate and its genetic association with milk yield and fat-to-protein ratio in Italian Holstein cows. In Book of Abstracts of the 21st National Congress of the Animal Science and Production Association (ASPA), June 9-12, 2015, Milano, Italy. *Ital. J. Anim. Sci.* 14(Suppl. 1): 77. (Abstr. C-137).
- Tiezzi, F., D. Pretto, M. De Marchi, M. Penasa & M. Cassandro, 2013. Heritability and repeatability of milk coagulation properties predicted by mid-infrared spectroscopy during routine data recording, and their relationships with milk yield and quality traits. *Animal* 7: 1592-1599.
- Tyrisevä, A.M., T. Ikonen & M. Ojala, 2003. Repeatability estimates for milk coagulation traits and non-coagulation of milk in Finnish Ayrshire cows. *J. Dairy Res.* 70: 91-98.
- van der Drift, S.G.A., K.J.E. van Hulzen, T.G. Teweldemedhn, R. Jorritsma, M. Nielen & H.C.M. Heuven, 2012. Genetic and nongenetic variation in plasma and milk  $\beta$ -hydroxybutyrate and milk acetone concentrations of early-lactation dairy cows. *J. Dairy Sci.* 95: 6781-6787.
- van Knegsel, A.T.M., S.G.A. van der Drift, M. Horneman, A.P.W. de Roos, B. Kemp & E.A.M. Graat, 2010. Short communication: Ketone body concentration in milk determined by Fourier transform infrared spectroscopy: Value for the detection of hyperketonemia in dairy cows. *J. Dairy Sci.* 93: 3065-3069.
- Visentin, G., S. McParland, M. De Marchi, A. McDermott, M.A. Fenelon, M. Penasa & D.P. Berry, 2017. Processing characteristics of dairy cow milk are moderately heritable. *J. Dairy Sci.* 100: 6343-6355.