

Phenotypic and Genetic Aspects of Milk Freezing Point in Primiparous Holstein Friesian Cows

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Summary

Freezing point (FRP) of milk and its associations with milk traits have not been studied in Italian Holstein Friesian population yet. Moreover, in other countries, studies were based on small sample size. Therefore, the aim of the present paper was to investigate sources of variation of milk FRP, and to estimate its heritability and genetic correlations with milk yield and quality traits. An analysis of variance was carried out to identify sources of variation of FRP, and genetic parameters were assessed using test-day repeatability animal models on 37,331 records from 5,102 first-lactation cows in 435 herds. Only classes of days in milk and herd-test-date significantly affected FRP. Heritability and repeatability of FRP were low (0.12 and 0.23, respectively), and moderate genetic correlations of this trait with lactose percentage (-0.52), protein content (-0.32) and somatic cell score (0.29) were estimated. Despite low, heritability of FRP is comparable to that of other traits such as somatic cell score, suggesting that there is room for applying selection strategies to improve this trait, also exploiting its genetic relationships with other milk traits.

Key words

genetic parameter, freezing point, lactose, bovine milk

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Introduction

Milk FRP is the temperature at which milk freezes and due to milk solids concentration, it is lower than water FRP (0°C). The reference analysis for FRP is the thermistor cryoscopy (ISO 5764:2009), but in routine and field conditions it is determined by mid-infrared technology (ISO 9622:2013). According to Council Directive 92/46/EEC, the maximum limit in Europe is -0.520°C, whereas in other countries this threshold ranges from -0.525°C to -0.530°C.

The FRP is considered by dairy processors one of the most important milk quality traits and it is commonly used to detect the presence of extraneous water in milk, mainly due to voluntary addition (fraud) or trouble during cleaning of milking systems. Diluted milk is generally characterized by low density at 27°C, low concentration of fat and solids-non-fat and an increase of FRP. Given a certain FRP, it is possible to estimate the amount of added water in milk: for example, FRP equal to -0.491°C is associated to milk with 5% of extra water and FRP of -0.257°C indicates a dilution of 50% (FAO, 2009). Due to the importance of this trait, current payment systems penalize abnormal values of FRP in bulk milk.

Few studies have investigated sources of variation of milk FRP using individual milk and they were based on quite small sample size. Parity order, days in milk (DIM), somatic cell count (SCC), breed and season effects have been reported to significantly affect milk FRP (Slaghuis, 2001; Bjerg et al., 2005; Henno et al., 2008; Kedzierska-Matysek et al., 2011; Hanuš et al., 2012; Petrera et al., 2016). Nevertheless, there is a lack of information on genetic aspects of milk FRP in the scientific literature. Therefore, the aim of the present study was to investigate phenotypic variation and to estimate genetic parameters of milk FRP in first-lactation Holstein Friesian cows.

Material and methods

The initial dataset consisted of 91,493 records collected on 10,609 primiparous Italian Holstein Friesian cows from December 2010 to December 2014 in Bolzano province (Northern Italy) during official monthly milk testing. Daily milk yield (MY) was directly recorded in the dairy herds and FRP, composition traits and SCC were determined in the milk laboratory of the South Tyrol Dairy Association (Bolzano, Italy). Freezing point, and fat (FP), protein (PP) and lactose (LP) percentages were measured with MilkoScan™ FT6000 (Foss Electric A/S, Hillerød, Denmark) and SCC were recorded through Fossomatic™ (Foss Electric A/S, Hillerød, Denmark).

Records from cows with unknown sire and dam as well as records with MY and FRP that deviated more than 3 and 2 standard deviations from their mean, respectively, were removed from the dataset. Moreover, data were edited on composition traits following ICAR guidelines (2016), so that records were retained if FP was between 2.00 and 6.00%, PP between 2.50 and 4.50%, and LP between 4.0 and to 5.5%. Somatic cell count was restricted to values between 1,000 and 10,000,000 cells/ml and it was converted to somatic cell score (SCS) using the transformation: $SCS = 3 + \log_2(SCC/100,000)$. Cows with less than 3 herd-test-date (HTD) observations and HTD with less than 3 animals were discarded. Finally, only cows with age at calving between

20 and 40 months and between 6 and 480 DIM were considered. After editing of the data, 37,331 records from 5,102 cows in 435 herds were available for statistical analysis.

An analysis of variance was performed to investigate sources of variation of milk FRP, milk yield and quality traits. The model included the fixed effects of HTD (7,597 levels), DIM (13 classes of 30 days each, except for the first which was a class between 6 and 30 DIM, the second to last which was a class between 330 and 390 DIM, and the last which included records beyond 390 DIM), age at calving (3 classes, the first being a class between 20 and 27 months, the second being a class between 27 and 30 months, and the third being a class between 31 and 40 months), and season of calving (4 classes: spring, summer, autumn and winter). Random factors were cow and residual.

Variance components to estimate heritability of the studied traits were assessed through single-trait animal models, whereas covariance components to estimate genetic correlations of FRP with MY, composition traits and SCS were assessed using bivariate animal models. The general form of the model, in matrix notation, was as follows:

$$y = Xb + Z_1p + Z_2a + e,$$

where y is the vector of phenotypic records of the trait (FRP, MY, FP, PP, LP, SCS), b is the vector of fixed effects as previously described, p is the vector of random cow permanent environmental effects, a is the vector of random animal genetic effects, e is the vector of random residuals, and X , Z_1 and Z_2 are incidence matrices relating the corresponding effects to the dependent variable. The pedigree file (22,822 animals) was provided by the Italian Holstein Association (ANAFI, Cremona, Italy) and included individuals with phenotypic records and their ancestors up to 6 generations back. The software package VCE6 (Neumaier and Groeneveld, 1998; Groeneveld et al., 2010) was used to estimate variance and covariance components for the random effects through REML procedures. Heritability (h^2), repeatability (rep) and genetic correlations (r_a) were calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

$$rep = \frac{\sigma_a^2 + \sigma_p^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

$$r_a = \frac{\sigma_{a1,a2}}{\sigma_{a1} \cdot \sigma_{a2}}$$

where σ_a^2 is the additive genetic variance, σ_p^2 is the cow permanent environmental variance, σ_e^2 is the residual variance, $\sigma_{a1,a2}$ is the additive genetic covariance between trait 1 and 2, and σ_{a1} and σ_{a2} are the additive genetic standard deviations for traits 1 and 2, respectively.

Results and discussion

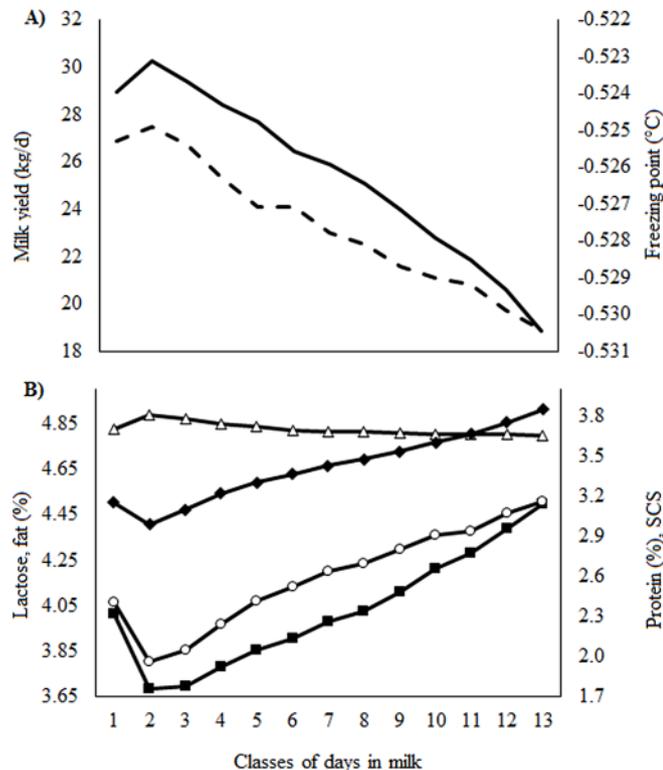
Descriptive statistics for studied traits are reported in Table 1. Means of MY, FP, PP, LP and SCS were 26.04 kg/d, 3.99%, 3.39%, 4.83% and 2.55, indicating good milk quality. Milk FRP averaged -0.528°C, which is an intermediate value compared to those reported in literature and it is far below the permitted

Table 1. Descriptive statistics of freezing point, milk yield and quality traits (n = 37,331)

Trait	Mean	SD	Minimum	Maximum
Freezing point (°C)	-0.528	0.007	-0.547	-0.507
Milk yield (kg/d)	26.04	5.96	5.20	44.60
Fat (%)	3.99	0.63	2.01	6.00
Protein (%)	3.39	0.36	2.50	4.50
Lactose (%)	4.83	0.15	4.04	5.38
Somatic cell score	2.55	1.68	-3.64	9.53

Table 2. Heritability (SE) and repeatability of investigated traits, and genetic correlations (SE) of freezing point with milk yield and quality traits

Trait	Heritability	Repeatability	Genetic correlation
Freezing point (°C)	0.12 (0.02)	0.23	-
Milk yield (kg/d)	0.19 (0.03)	0.43	0.15 (0.02)
Fat (%)	0.31 (0.03)	0.45	0.07 (0.02)
Protein (%)	0.39 (0.04)	0.54	-0.32 (0.08)
Lactose (%)	0.46 (0.04)	0.56	-0.52 (0.06)
Somatic cell score	0.12 (0.03)	0.54	0.29 (0.11)

**Figure 1.** Least squares means of A) freezing point (dashed line) and milk yield (continuous line), and B) lactose percentage (Δ), fat percentage (■), protein percentage (◆) and somatic cell score (○) across lactation

European threshold of -0.520°C (Council Directive 92/46/EEC). In fact, in the present study only first-lactation cows were available and it has been reported that primiparous generally showed lower milk FRP than multiparous cows (Emmett and Rogers, 1997; Henno et al., 2008; Forsbäck et al., 2010; Hanuš et al., 2010, 2012; Kedzierska-Matysek et al., 2011). Milk FRP exhibited the lowest coefficient of variation (1.33%), and SCS the highest (66%).

Classes of DIM and HTD effects were statistically significant ($P < 0.001$) in explaining the variation of all investigated traits. Age at calving was significant for MY, FP, PP and LP, with younger cows producing significantly higher LP and PP and lower MY and FP than older cows. Finally, season of calving significantly affected MY, FP, LP and SCS ($P < 0.05$).

Least squares means of FRP, MY and quality traits across classes of DIM are displayed in Figure 1. Similar to MY, FRP and LP increased immediately after calving, reached a peak in class 2 (30 to 60 DIM) and decreased thereafter, until the end of lactation. Therefore, milk FRP exhibited its worst value at the maximum milk production of the cow, probably because of a dilution effect: indeed, the peak of lactation corresponds not only to the greatest daily MY, but also to the lowest fat and protein percentages and thus to a less concentrated milk.

Heritability and repeatability of FRP, MY and quality traits are shown in Table 2. Milk FRP exhibited low heritability (0.12), but greater than that (0.06) assessed by Jonkus and Paura (2011) in Latvian Brown cows (2011). Repeatability of FRP (0.23) was the lowest among investigated traits, meaning that temporary environmental effects are important in explaining FRP variation and that several measurements within lactation are needed to estimate reliably breeding values for milk FRP in genetic evaluations. Estimates of heritability and repeatability of MY, FP, PP, LP and SCS were in accordance with previous studies (Tyrisevä et al., 2003; Tiezzi et al., 2013), with the greatest value estimated for LP (0.46) and the lowest for SCS (0.12).

Genetic correlations of FRP with LP (-0.52), PP (-0.32) and SCS (0.29) were moderate, and they were low between FRP and MY (0.15), and between FRP and FP (0.07) (Table 2). The negative genetic relationship between FRP and LP, and the positive one between FRP and SCS were somewhat expected. Indeed, FRP depends on the amount of particles in solution in milk and being lactose the major milk solid, when it decreases the FRP increases (Hanus et al., 2010; Zagorska and Ciprovica, 2013). In fact, Brouwer (1981) stated that LP was responsible for 53.8% of milk FRP, and Pinkerton and Peters (1958) reported that for LP equal to 5.00% and 4.05%, FRP was -0.558°C and -0.524°C , respectively. Moreover, cows producing milk with low LP are probably cows with high SCC (Lindmark-Månsson et al., 2006; Cinar et al., 2015), and this explains the positive correlation between FRP and SCS. To our knowledge, no other estimates of genetic relationships between FRP and milk traits are currently available.

Conclusions

Milk FRP is affected by DIM and HTD, and it is genetically correlated with LP, PP and SCS. Although heritability of milk FRP estimated in the present study is low, its value is large enough to set up selection strategies aimed to improve milk FRP. Further

studies should broaden the genetic analysis to multiparous cows. Moreover, it would be interesting to estimate genetic correlations between FRP and other economically important traits currently included in the selection index of Italian Holstein Friesian population such as fertility.

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