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Heritability and genetic correlations of total and differential somatic cell count with milk yield and composition traits in Italian Simmental cows

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ABSTRACT

Costs of production have deeply increased each year in the last decades, breeders are continuously looking for more cost effective and more efficient ways to produce milk. Despite the major signs of progress in productivity, it is fundamental to optimize rather than maximize the performances of the dairy cows. Mastitis is still a highly prevalent disease in the dairy sector which causes several economic losses and environmental effect. Its accurate and early diagnosis is crucial to improve profitability of dairy cows and contribute to a more sustainable dairy industry. Among mastitis reduction strategies, there is the urgent need to implement breeding objectives to select cows displaying mastitis resistance by investigating the genetic mechanisms at the base of the inflammatory response. Therefore, in this study we aimed to further understand the genetic background of the differential somatic cell count (DSCC), which provides thorough insights on the actual inflammatory status of the mammary glands. The objectives of this study were to estimate on a cohort of 20,215 Italian Simmental cows over a 3-yr period: (1) the heritability and repeatability values of somatic cell score (SCS) and DSCC, (2) the genetic and phenotypic correlations between these 2 traits and milk production and milk composition traits, (3) the heritability and repeatability values of SCS and DSCC within class of udder health status. Heritability was low both for SCS (0.06) and DSCC (0.08), whereas the repeatability values for these traits were 0.43 and 0.36, suggesting that the magnitude of cow permanent environmental effect for these traits is remarkable. The genetic and phenotypic correlation of SCS with DSCC was 0.612 and 0.605, respectively. Because both significantly differed from the unit, we must consider those traits as different ones. This latter aspect corroborates the need to consider the DSCC as a further indicator of inflammatory status which might be implemented in the Simmental breed genetic evaluation. It is worthy to mention that heritability estimates for SCS and DSCC were the highest in healthy cows compared with the other udder health classes. This implies that when the udder health status changes, it is most likely due to environmental factors rather than aspects related to the animal's genetics. In contrast, the highest additive genetic variance and heritability found for SCS and DSCC in the healthy group might reveal the potential to further implement breeding strategies to select for healthier animals.
Key words: differential somatic cell count, heritability, mastitis, genetic correlations, udder health

INTRODUCTION

Novel breeding goals and management practices are crucial to improve health, resilience, and welfare of animals for a sustainable dairy chain. For this purpose, the selection strategies in dairy cows have started to simultaneously include both productive and functional traits. In several cattle breeds, breeding programs have increased the role of functional traits but there are still room for improvement in the light of the animal welfare (Brito et al., 2021). Among these aspects, infectious and noncommunicable diseases are main issues affecting the profitability and the sustainability of dairy farms (Seegers et al., 2003; Hogeveen et al., 2011), as they affect productive and reproductive performances, welfare and longevity, produce direct costs associated with the treatments, and indirect costs because of the higher amount of waste (i.e., contaminated milk), increased labor, veterinary fees, and drugs (Cha et al., 2011; Doehring and Sundrum, 2019). Moreover, diseases alter the culling policies based on changes in the potential value of dairy cows. Due to its high occurrence, mastitis is still the most challenging infectious disease that significantly affects global and local dairy sectors (Cobirka et al., 2020). The accurate and early diagnosis of mastitis

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is key to reduce its incidence, guarantee profitability of dairy cows and contribute toward a more sustainable dairy industry (Puerto et al., 2021). In the past, to ameliorate the monitoring of mastitis in dairy cattle, SCC (or its log-transformation, SCS) has been introduced as an informative indicator of udder health and nowadays is extensively used in the milk testing programs (ICAR, 2022) and in several quality-based milk payment schemes. Despite this, SCS only quantifies the variability in number of somatic cells in the milk without distinguishing among different cell populations. A few years ago, a rapid method of analysis has been introduced in the dairy market, allowing the measure of differential SCC (**DSCC**) to be routinely recorded at dairy cattle population level as already performed for SCC. The collection of DSCC in milk can improve the monitoring of udder health conditions as it provides more insights on the actual inflammatory status of the mammary glands, which is represented by the immune cells system as PMN and lymphocytes with respect to the total SCC (Wall et al., 2018; Halasa and Kirkeby, 2020). When used in combination with SCC, the differentiation of the percentage of each cell population allows for earlier and more accurate detection of healthy cows, with low DSCC and low SCC, and those affected by subclinical mastitis, with high DSCC and low SCC, as well as more accurately identifying chronic udder infections, with low DSCC and high SCC (Zecconi et al., 2019).

The collection of health-related traits in large populations is commonly unfeasible because of the cost and time-consuming procedures for measurement, sampling, and analysis. As aforementioned, this is not the case with SCC, which is already implemented in the milk test-day recording system, and nowadays DSCC can also be introduced. Despite the low to moderate heritability of SCS (Heringstad et al., 2006), the use of this trait in genetic schemes is recommended to decrease susceptibility to mastitis in dairy cattle. So far, for the DSCC trait, genetic parameters have been investigated in a limited number of studies in the Holstein Friesian breed only. Although these studies opened new opportunities for the genetic improvement of udder health traits in dairy cattle, they were characterized by a low period of sampling (Bobbo et al., 2019) and a low number of animals (Pegolo et al., 2021). Thus, heritability and genetic correlations of DSCC, SCS, and various production traits (e.g., milk yield, percentage of fat and protein in milk) need to be further investigated (e.g., different breeds, higher number of animals and records; longer period of sampling). In addition, because the combination of DSCC and SCC has been proposed to define an udder health condition (e.g., cow susceptible to mastitis or at risk for chronic disease; Zecconi et al., 2019), a further exploration from a genetic perspective could unravel novel applications of these traits.

Therefore, the objectives of this study were to estimate on a cohort of 20,215 Italian Simmental cows over a 3-yr period: (1) the heritability and repeatability values of SCS and DSCC, (2) the genetic and phenotypic correlations between these 2 traits and with milk production and milk composition traits, (3) the heritability and repeatability values of SCS and DSCC within class of udder health status.

MATERIALS AND METHODS

Animal Data

A total of 249,806 test-day records were collected during the routine recording scheme in a time span of 3 years (2019–2021) on 35,642 dual-purpose Italian Simmental cows reared in 6 regions in the North of Italy. The data were provided by the Italian Simmental breeders association (**ANAPRI**) and included daily milk yield (kg/d), milk components (fat, casein, lactose, and urea), and somatic cell traits (SCC and DSCC). All milk samples were collected and analyzed according to the guidelines for dairy cattle milk recording and analysis (ICAR, 2022). Percentage of milk fat, casein and lactose, and urea (mg/dL) were measured using MilkoScan 7 calibrated according to the International Standard ISO 9622 and IDF 141:2013 (ISO, 2013), while SCC and DSCC were measured using Fossomatic 7DC according to the International Standard ISO 148–2:2006 (ISO, 2006).

To achieve normality and homogeneity of variances, SCC was log-transformed $[Log_2(SCC \times 10^{-5}) + 3]$ to SCS according to Ali and Shook (1980) and also DSCC was multiplied with SCC and converted via log-transformation $\left[Log_2\left(\frac{DSCC}{100} \times SCC \times 10^{-5}\right) + 3\right]$ into differential SCS (**DSCS**). As regards the editing of the data, because the instrument has a potential limitation related to the accuracy and repeatability of measurements when the SCC is below 50,000 cells/mL and does not report the DSCC values for those samples, we first excluded those observations. Then, all the evaluated traits underwent quality control and those observations falling outside the interval of the mean ±3 standard deviations were excluded from further analyses. Only cows with known sire and dam were selected and cows sired by bulls with less than 5 daughters were removed. Cows with less than 5 DIM and over 450 DIM were discarded from the analysis of the data. Herds and cows with less than 9 and 3 observations, respectively, were

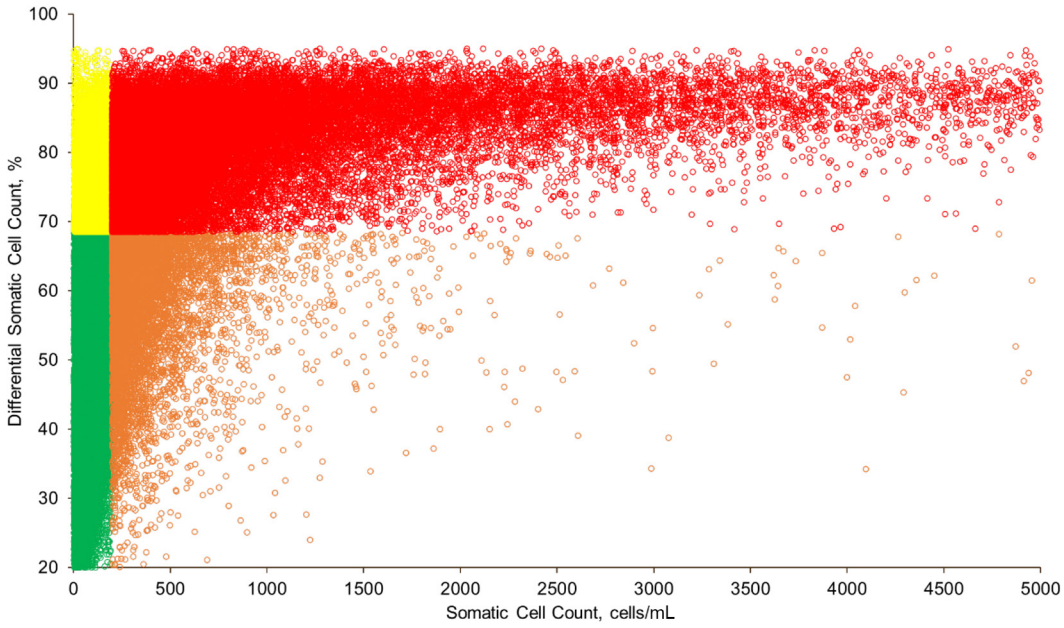

Figure 1. Distribution of cows in the 4 udder health classes based on the combination thresholds of SCC (200,000 cells/mL) and DSCC (68.5%; Zecconi et al., 2019). Cows are classified as healthy (lower left, green color; SCC <200,000 cells/mL and DSCC <68.5%), at risk of mastitis (upper left, yellow color, SCC <200,000 cells/mL and DSCC ≥68.5%), inflamed (upper right, red color, SCC ≥200,000 cells/mL and DSCC ≥68.5%), and chronic (lower right, orange color, SCC ≥200,000 cells/mL and DSCC <68.5%).

discarded from the data set and were not included in the study. The final data set accounted for 169,847 observations from 20,215 cows. On average, herds' size was about 20 cows. Each cow provided 8.4 milk samples, with a minimum of 3 and a maximum of 22 observations. Pedigree information was also provided by ANAPRI and included 1,151,660 records of which 78,453 sires and 675,404 dams.

Genetic Parameter Estimations

Single-trait animal models with repeated measurements were run to estimate variance components and heritability of all the above-mentioned traits in ASReml 4.2 (Gilmour et al., 2015). The following model was used:

$$y_{ijklmno} = \mu + region_i + DIM_j + parity_k + DIM_j \times parity_k + HTD_l + animal_m + pe_n + e_{ijklmno},$$

where y is the trait under evaluation for the mth animal; μ is the population mean; $region_i$ is the fixed effect of the ith class of the region where the analyses took place ($i = 6$ classes), which accounts for the variability existing among instruments; DIM_j is the fixed effect of the jth class of DIM (12 classes of 30 d); $parity_k$ is the fixed effect of kth class of parity order (5 classes; 1 to 5); $DIM_j \times parity_k$ is the fixed effect of the interaction between the jth class of DIM and the kth class of parity order; HTD_l is the fixed effect of the lth class of herd-test-day; $animal_m$ is the random additive genetic effect of the mth animal; pe_n is the random permanent environmental effect and $e_{ijklmno}$ is the random residual effect.

Univariate analyses were also performed on animals grouped according to the 4 classes of udder health status (i.e., healthy, inflamed, at risk of infection, and chronic) built upon the combination of SCC and DSCC thresholds as previously described by Zecconi et al. (2019) and as depicted in Figure 1. Briefly, these thresholds classified dairy cows as follows: healthy (SCC ≤200,000 cells/mL; DSCC ≤68.5%); at risk of mastitis (SCC ≤200,000 cells/mL; DSCC >68.5%); with udder inflammation (SCC >200,000 cells/mL; DSCC >68.5%); chronic (SCC >200,000 cells/mL; DSCC ≤68.5%). For this specific analysis, only one observation (the first one recorded) per cow per udder health status was considered to obtain a more balanced number of observations across udder health classes.

Moreover, bivariate animal models were run to estimate phenotypic and genetic correlations of DSCC and SCS with the other milk traits, between DSCC and SCS, and between DSCC and DSCS. Fixed and random effects were the same as reported above and included in the univariate analysis.

Table 1. Descriptive statistics (mean, SD, minimum, and maximum) and number of observations for somatic cell, production, and composition traits of individual milk samples

Trait[1]	n	Mean	SD	Minimum	Maximum
SCS	168,870	2.76	2.04	−1.52	8.08
DSCC, %	162,161	63.4	16.5	20.0	95.0
DSCS	162,020	2.16	2.25	−4.05	8.51
Milk yield, kg/d	168,979	25.4	8.77	5.20	49.6
Fat, %	166,915	3.97	0.76	1.79	6.18
Lactose, %	162,921	4.77	0.18	4.28	5.30
Casein, %	168,253	2.76	0.33	1.91	3.71
Urea, mg/dL	168,267	22.5	7.05	1.45	42.65

[1]SCS: [$\log_2$(SCC × 10^{-5}) +3]; DSCC = differential SCC; DSCS = differential SCS.

RESULTS AND DISCUSSION

Milk Yield, Milk Composition Traits, SCS, and DSCC

Descriptive statistics for udder health traits, milk yield, and milk composition are reported in Table 1. The SCS averaged 2.76 (±2.04 SD) corresponding to about 85,000 cells/mL of milk, although, due to the non-normal distribution of SCC, the average SCC was equal to 261,000 cells/mL. The DSCC percentage averaged 63.4% (±16.5 SD), whereas when expressed as log count it averaged 2.16 (±2.25). Based on the combination of SCC and DSCC thresholds, 50.6% of the cows were classified as healthy, followed by similar proportion between those with an inflammation (22.2%) and those at risk of a mastitis (22.1%), while only 5.1% of cows were classified as chronic (Figure 1). Because the ability to categorize cows according to their individual udder health state became possible only in recent years (Zecconi et al., 2019; Halasa and Kirkeby, 2020), it is hard to compare these results with those of previous studies. However, these percentages are consistent with a recent study where the overall prevalence of subclinical mastitis across the entire cohort was estimated equal to 22.5% (Zecconi et al., 2020).

Heritability and Repeatability Estimates

All the evaluated factors included in the model were significant, and the trend of SCS and DSCC during lactation (Supplemental Figure S1; https://doi.org/10.6084/m9.figshare.24187317; Stocco, 2023a) and across parities (Supplemental Figure S2; https://doi.org/10.6084/m9.figshare.24187323; Stocco, 2023b) agreed with previous findings (Zecconi et al., 2020; Stocco et al., 2023). Heritability was low for SCS (0.06), DSCC (0.08), and DSCS (0.06) and comparable with the estimates previously reported in Holstein dairy cattle (Bobbo et al., 2019; Pegolo et al., 2021), but with a lower standard error due to the high number of cows and observations included in the present study. Repeatability values for these traits were 0.43, 0.36, and 0.41, suggesting that the magnitude of cow permanent environmental effect for these traits was remarkable (Table 2). This indicates that the variation usually observed in SCS and DSCC is influenced by environmental conditions and management practices. Therefore, it appears that interventions aimed at specific environmental factors, such as increasing hygiene, optimizing nutrition, or reducing stressors, could have a major influence on SCC and DSCC and supporting better udder health in cows (Dufour et al., 2011; Neculai-Valeanu and Ariton, 2022).

Heritability was low for MY (0.09), and moderate for fat and urea (0.18), lactose (0.24) and casein (0.31), respectively. The above-mentioned heritability estimates were in the range of those reported in a previous study where records from 19,000 Italian Simmental cows were used (Cecchinato et al., 2015). Using genomic information, a previous study found greater values of heritability for daily milk yield (0.26) but with a higher standard error (0.02) (Cesarani et al., 2020). In the present study, the repeatability values were moderate, ranging from 0.28 to 0.47, and they were slightly lower than those reported for the same traits recorded for Holstein cows (Costa et al., 2019).

Table 2. Heritability and repeatability estimates for somatic cell, production, and composition traits of individual milk samples

Trait[1]	σ^2_a	σ^2_{pe}	σ^2_e	h^2	SE	rep	SE
SCS	0.214	1.293	1.990	0.06	0.007	0.43	0.003
DSCC, %	19.83	65.12	154.0	0.08	0.008	0.36	0.004
DSCS	0.290	1.610	2.680	0.06	0.007	0.41	0.003
Milk yield, kg/d	2.850	10.38	19.43	0.09	0.009	0.41	0.004
Fat, %	0.079	0.040	0.311	0.18	0.009	0.28	0.004
Lactose, %	0.006	0.006	0.013	0.24	0.013	0.47	0.004
Casein, %	0.018	0.011	0.029	0.31	0.013	0.49	0.004
Urea, mg/dl	3.350	2.499	12.97	0.18	0.010	0.31	0.004

[1]SCS: [$\log_2$(SCC × 10^{-5}) +3]; DSCC = differential SCC; DSCS = differential SCC: [$\log_2$(DSCC/100 × SCC × 10^{-5})+3].

Genetic and Phenotypic Correlations

Investigation of the genetic background of DSCC and its correlation with milk quality traits could open new scenarios in genetic selection programs aimed at improving mastitis resistance combined with milk quality in dairy cattle. The phenotypic and genetic correlations between DSCS and SCS were both over 90% (0.98 and 0.94 respectively), indicating that they can be practically considered as the same trait when they are both log-transformed. In Table 3 are reported the genetic and phenotypic correlations among SCS, DSCC and all the other investigated milk traits. As expected, most of the genetic correlations were of larger magnitude than the phenotypic ones. The low phenotypic correlations among SCS and DSCC with milk quality traits agree with previous studies confirming the nonlinear relationships of milk quality traits and the different content of SCS (Bobbo et al., 2017) and DSCC (Stocco et al., 2020). The biological explanation for these relationships lies in the alteration of animal physiological mechanisms induced by mastitis infection. During intramammary inflammation, the secretion of milk components is altered due to both a reduced synthetic ability of mammary epithelial cells and an increased influx of blood components into the milk (Ogola et al., 2007; Pegolo et al., 2021). These blood components include a variety of enzymes, which alter milk composition through the breakdown of milk casein and fat (Santos et al., 2003; Kelly et al., 2006). The genetic correlation of SCS with DSCC was of 0.61. This value, calculated in Holstein Friesian cows, was equal to that reported by Pegolo et al. (2021) (0.61) and slightly lower compared with Bobbo et al. (2019) (0.66). Because their phenotypic and genetic correlations significantly differed from unit, we must consider these traits as different ones. This latter aspect corroborates the need to consider the DSCC as an additional indicator of inflammatory status, which might be implemented in the Simmental breed genetic evaluation. The DSCC was phenotypically uncorrelated with milk yield and milk composition traits, with correlation values ranging from −0.06 for urea to −0.005 for casein. From a genetic point of view, the DSCC was almost uncorrelated with casein percentages (0.03) but weakly associated with milk yield (0.13), fat and lactose percentage (0.12, 0.13). Thus, from this study, DSCC showed genetically positive correlations, although of limited magnitude, with milk yield and milk composition, which contrasted with findings previously reported in Holstein breed (Bobbo et al., 2019; Pegolo et al., 2021). Nevertheless, the differences in terms of data structure as well as of the sample size between the present study and the previous ones performed in the Holstein breed justify the obtained results. On top of that, we can suppose that the observed differences might also be due to a potential breed-specific response to inflammation. Indeed, it has been shown that, in infection-induced mastitis, the presence of different patterns of neutrophil migration to the mammary gland is different among breeds (Bannerman et al., 2008). Therefore, we cannot exclude that the differences found in this study compared with the previous one observed in the Holstein, might be due to the Simmental's different response toward the infection. In addition, a study conducted on 3 dairy cow breeds hypothesized that different levels of somatic cells in milk could have different meanings according to the considered breeds, reporting that Holstein cows experienced a greater increase of SCS for less-severe mastitis events compared with Brown Swiss and Simmental breeds (Franzoi et al., 2020). Those authors also reported that Simmental cows revealed maximum productivity at higher SCS (in terms of milk and components yield) than the other breeds, suggesting that Simmental breed copes better with potential mammary infections. The positive genetic correlations between DSCC and milk yield as well as some milk composition traits found in the current study confirm this hypothesis.

As expected, SCS was unfavorably correlated with lactose percentage in agreement with previous findings in Holstein breed (Miglior et al., 2007; Stoop et al., 2007). In contrast, DSCC seems to be slightly genetically correlated with lactose percentage (0.13). It has been proposed that an increase in lactose might be caused by the first responses of the cow toward inflammation due to augmented glucose demand (Naidu and Newbould, 1973; Verdi and Barbano, 1991; Stocco et al., 2020). Thus, the positive genetic correlation between DSCC and lactose percentage, although moderate, might reveal that both are genetically responsible

Table 3. Genetic (r_a) and phenotypic correlations (r_p) of DSCC and SCS among them and with milk production and composition characteristics[1]

	DSCC[2]		SCS[3]	
Trait	r_a	r_p	r_a	r_p
SCS	0.612	0.605	—	—
Milk yield	0.130	−0.007	0.120	−0.190
Fat	0.121	−0.011	0.110	−0.055
Lactose	0.126	−0.014	−0.253	−0.332
Casein	0.030	−0.005	0.110	−0.035
Urea	−0.067	−0.063	−0.099	−0.082

[1]SE ranging between 0.05 and 0.09 for the r_a and between 0.004 and 0.01 for r_p.
[2]DSCC = differential SCC.
[3]SCS: $[\log_2(SCC \times 10^{-5}) + 3]$.

Table 4. Heritability estimates of SCS and DSCC calculated in each of the 4 udder health classes based on the combination thresholds of SCC (200,000 cells/mL) and DSCC (68.5%; Zecconi et al., 2019)

		SCS[1]		DSCC[2]	
Group	n	h^2	SE	h^2	SE
Healthy	16,348	0.060	0.010	0.040	0.010
At risk	13,079	0.020	0.010	—	—
Chronic	4,777	0.020	0.035	—	—
Mastitic	11,520	0.010	0.010	0.001	0.010

[1]SCS: $[\log_2(\text{SCC} \times 10^{-5}) +3]$.
[2]DSCC = differential SCC.

to primary response to inflammation in cows. These results provide further evidence of the importance of combining different indicators of udder health into a multitrait selection index with the goal of improving mastitis resistance.

Heritability of SCS and DSCC Within Cow's Health Status

Because it is reasonable to expect that SCC and DSCC values taken on healthy, mastitic, at risk, and chronic cows display different distributions, means, and variances, we also performed univariate analyses on animals grouped according to the udder health status. In Table 4 are reported the heritability estimates of SCS and DSCC calculated in each of the 4 udder health classes based on the combination thresholds of SCC (200,000 cells/mL) and DSCC (68.5%). Interestingly, the heritability estimates for SCS and DSCC were higher in healthy cows compared with the other udder health classes that showed very low (e.g., SCS = 0.01) or null (e.g., DSCC = 0.00) heritability values. Similarly, Heringstad et al. (2006) showed that heritability of SCS was 0.03 for mastitic cows and 0.08 for healthy cows. This suggests that when a modification of the udder's health status occurs, it is highly affected by environmental factors rather than aspects related to the genetics of the animal. In contrast, the highest additive genetic variance and heritability found for SCC and DSCC in the healthy group might reveal the potential to further implement breeding strategies to select for healthier animals. Thus, DSCC might be used as an indirect selection criterion for improving mastitis resistance in combination with SCC. Nevertheless, the nature and strength of the genetic relationship between DSCC and mastitis have to be further investigated. Therefore, it would be interesting to assess the degree of agreement between genetic evaluations based on novel indirect criteria, such as DSCC, and evaluations based on clinical mastitis measured directly.

CONCLUSIONS

This study evaluated the genetic parameters of the DSCC for the first time in the Simmental breed. Although the understanding of the genetic mechanisms underlying mastitis is difficult because of the complex nature of the 2 investigated milk udder health traits, as well as the overwhelming environmental effects, the DSCC trait is heritable in the Simmental breed. This would offer new opportunities in the genetic improvement of udder health for dairy cattle. Moreover, because the genetic correlation between DSCC and SCS was different from unit, it is important to consider these 2 traits separately. The weak positive genetic correlations of DSCC with milk yield and milk composition traits contrasted with previous results found in the Holstein breed, suggesting the existence of breed-dependent responses to inflammation. Our findings might pave the way for further analyses aimed at identifying genomic regions associated with these traits and deepening the biological pathways involved in their regulation.

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