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ORIGINAL RESEARCH

Effect of total and differential somatic cell count on yield, composition and predicted coagulation properties from individual dairy cows

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This study aimed to investigate the effect of interaction between total (SCC) and differential somatic cell count (DSCC) on milk yield, composition and coagulation traits from individual dairy cows. A total of 159 224 test-day records (daily milk yield, composition and coagulation traits) have been collected during a period of 14 months from 12 849 Holstein-Friesian and 9275 Simmental cows farmed in 223 herds. The interaction between somatic cell traits was highly significant for almost all the evaluated traits. This study showed that the combined use of SCC and DSCC can be employed for assessing the performances of dairy cows and for monitoring the improvement of milk quality.

Keywords Differential somatic cell count, Milk coagulation properties, Milk quality.

INTRODUCTION

The costs of milk at herd level have been raised each year and breeders need to find strategies for improving the efficiency of their productions to overcome this issue. Disease management is one of the raising costs where mastitis is the main concern in the dairy sector. As a matter of fact, van Soest *et al*. (2016) estimated a variation of costs due to mastitis ranging from 17 to 261 EUR among herds. Furthermore, consumers' awareness on antibiotics resistance has been increased recently, rising more concerns on the use of antibiotics in dairy farms (Doehring and Sundrum 2019). Differential somatic cell count (DSCC) is a recently developed tool used by dairy herd improvement services worldwide (DHI) to monitor udder health (Damm *et al*. 2017), and it is defined as the combined proportion of polymorphonuclear neutrophil (PMN) and lymphocytes within the total somatic cell count (SCC) (Damm *et al*. 2017). In Europe, several authors have investigated the possibility to use DSCC combined with SCC as a supplementary indicator of intramammary infections (Schwarz *et al*. 2019). According to Zecconi *et al*. (2018, 2021), DSCC allows for a more accurate detection of subclinical mastitis implementing the information provided by SCC. Currently, the SCC is used for different purposes, such as for the milk payment systems, to check compliance with regulations in terms of milk quality and hygienic standard, and in milk recording for genetic evaluation and farm management (IDF 2013).

Recently, it has been evidenced that the combination of different levels of SCC and DSCC had significant effects on milk composition (Stocco *et al*. 2020) and milk coagulation properties (MCP) at individual cow level (Bobbo *et al*. 2020). Stocco *et al*. (2020) investigated the relationships between the interaction of SCC and DSCC with milk composition by applying thresholds indicated for cow's udder health status, commonly applied by the Italian Breeders Association for SCC and suggested by Zecconi

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et al. (2018) for DSCC. Bobbo *et al*. (2020) investigated the effect of four classes of udder health status (healthy, mastitic, chronic and susceptible), built upon the combination of SCC thresholds according to Dohoo and Leslie (1991) and DSCC according to the stage of lactation of the cows (Zecconi *et al*. 2018), on milk composition and MCP. Four different classes of DSCC based on the percentiles of the trait distribution (Pegolo *et al*. 2021) were tested on the variability of cheesemaking, coagulation and composition traits in Holstein-Friesian cows.

Despite the current scientific output, the knowledge on the relationship among milk production, quality, coagulation traits and the combination of SCC and DSCC is limited to: (i) a low number of observations and cows involved in the trial; (ii) the standardised subdivision of the total somatic and differential cells traits in a limited number of classes (i.e. two or four classes) and frequently based on thresholds provided by the literature; (iii) the lack of the interaction between SCC and DSCC in the statistical models used to test the variability of milk quality and production; and (iv) the unavailability of the information yielded by important factors influencing milk composition and production (i.e. breed, season) in the statistical models, which are key aspects to correct the results obtained for SCC and DSCC respectively. Since the variations of many milk-related traits (i.e. milk production, composition, and coagulation traits), considered together in a single large survey, combined with a proper evaluation of DSCC classes, together with SCC, have never been reported in the literature, it is very important to clarify these aspects to offer farmers' and cheesemakers' detailed information to monitor and improve the quality of the milk along the dairy chain. This is particularly relevant in those countries where milk production is mainly addressed to cheese manufacture and where milk payment systems and selection indices aim to improve cheesemaking-related traits. For those reasons, the aim of our study was to investigate the interaction between different classes of SCC and DSCC on yield, composition, and traditional coagulation traits of milk from individual cows.

MATERIALS AND METHODS

Animals and herd data

The initial data set comprised 195 784 data points from 15 617 Holstein-Friesian (HF) and 13 706 Simmental (Si) cows reared in 404 herds. We excluded herds with less than 30 cows under milk recording. Cows with less than three observations and less than five DIM were discarded from the data set. After the editing, a total of 12 849 HF and 9275 Si cows distributed across 223 herds in the Northeast of Italy (Friuli Venezia Giulia Region) were used. The herds were single ($N = 103$) and multibreeds ($N = 120$). The herd average size was 84 ± 54 cows. The data set consisted of 159 224 data points, including daily milk yield (DY_M) and composition, collected on dairy cows between July 2019 and September 2020 during the milk test-day. Each cow considered for this study was evaluated on average every 4 or 5 weeks, according to the test-day records schedule. On average, 7.9 milk samples were available from each cow, with a maximum of 20 samples. Information about milking system, average herd size, breed, days in milk (DIM) and parity were provided by the Breeders Association of Friuli Venezia Giulia (Codroipo, Italy). Herd milking system was classified into three classes: automatic milking system (AMS), free stall with milking parlour (i.e. tandem, herringbone and parallel) and tie stall with cow side milking (i.e. milking trolley or buckets, round-the-bar pipeline milking system).

Milk data

Breeders Association of Friuli Venezia Giulia (Codroipo, Italy) provided the milk data collected during the routine milk recording procedures and included DY_M, milk composition (fat, protein, casein, lactose and urea), MCP and somatic cell traits (SCC and DSCC). Milk samples were analysed in the laboratory of the Breeders Association of Friuli Venezia Giulia. All the milk samples were collected and analysed according to the International Committee for Animal Recording procedures (ICAR 2020). Milk composition (fat, protein, casein, lactose and urea) and milk coagulation traits [rennet coagulation time (RCT, min) is defined as the time from addition of the coagulant to the beginning of coagulation, k_{20} (min) is the interval from RCT to the time at which the curd firmness reaches 20 mm, and a_{30} (mm) is a measure of the extent of curd firmness 30 min after rennet addition] were predicted using a MilkoScan FT7 (FOSS Electric A/S, Hillerød, Denmark), according to ISO 9622/IDF 141:2013 for milk composition and according to the models developed by Visentin *et al*. (2016) for MCP. A Fossomatic 7DC (FOSS Electric A/S, Hillerød, Denmark; according to ISO 13366-2/IDF 148-2:2006) was used to measure SCC and DSCC (PMN + lymphocytes, %), and then, SCC was transformed into the logarithmic [$\log_{10}$(SCC)] to LSCC.

Fat-to-protein ratio (F:P) and casein index (CN-*in*) were then calculated as the ratio between fat and protein and casein and protein respectively.

For all milk traits, data within the range of mean ± 3 SD for each trait were considered for the statistical analysis.

Herd productivity

The HP was established on the average daily milk energy output (D_{MEO}) of the lactating cows, and herds were divided into two classes: high HP ($N = 112$, average D_{MEO} = 63.19 MJ/d) or low HP ($N = 111$, average D_{MEO} = 34.58 MJ/d), using the following method: the net energy level (NEL) of 1 kg of milk was estimated using the equation proposed by the National Research Council (2001):

$$\text{NEL (Mcal/kg)} = 0.0929 \times \text{fat, \%} + 0.0547 \times \text{protein, \%} + 0.0395 \times \text{lactose, \%}$$

Net energy level was converted to MJ per kg and multiplied by DY_M of each cow (MJ/d) to obtain the D_{MEO} at individual cow level. Then, D_{MEO} data were tested using an ANOVA (Mixed procedure; SAS Institute Inc., Cary, NC, USA) to obtain the least squares means (LSM) for all the herds, after correcting for the fixed effects of season, breed, DIM and parity, and the random effect of herd and animal. After ranking the D_{MEO} LSM of the 223 herds, they were categorised into high or low based on the D_{MEO} median value (40.71 MJ/d).

Statistical analysis

Data were analysed using a MIXED procedure (SAS version 9.4), according to the following model:

$$\begin{aligned} Y_{mnopqrstuvw} &= \mu + HP_m + Milking_n + Season_o + Breed_p \\ &+ Parity_q + DIM_r + LSCC_s + DSCC_t \\ &+ (LSCC \times DSCC)_{st} + Herd_u \\ &+ Animal\left(Breed_p\right)_v + e_{mnopqrstuvw} \end{aligned}$$

where $Y_{mnopqrstuvw}$ is the observed trait (DY_M and $D_{MEO;}$ milk composition traits: fat, protein, casein, lactose, urea, fat-to-protein ratio and casein index; milk coagulation traits: RCT, k_{20}, a_{30}); { is the overall intercept of the model; HP_m is the fixed effect of the mth class of herd productivity level [m = class 1: low (≤40.71 MJ/d); class 2: high (>40.71 MJ/d)]; $Milking_n$ is the fixed effect of the nth milking system (n = AMS, free and tie stall); $Season_o$ is the fixed effect of the oth season of sampling (o = winter, spring, summer and autumn); $Breed_p$ is the fixed effect of the pth breed (p = HF and Si); $Parity_q$ is the fixed effect of the qth parity (q = 1 to 5, with class 5 including cows of parity ≥5); DIM_r is the fixed effect of the r_{th} class of days in milk (r = 1 to 12; class 1: ≤30 d; class 2: 31–60 d; class 3: 61–90 d; class 4: 91–120 d; class 5: 121–150 d; class 6: 151–180 d; class 7: 181–210 d; class 8: 211–240 d; class 9: 241–270 d; class 10: 271–300 d; class 11: 301–330 d; class 12: >330 d); $LSCC_s$ is the fixed effect of the sth class of LSCC (s = 1 to 7: class 1 = <4.21; class 2 = 4.21–4.51; class 3 = 4.52–4.82; class 4 = 4.83–5.12; class 5 = 5.13–5.43; class 6 = 5.44–5.74; class 7 = ≥5.75); $DSCC_t$ is the fixed effect of the tth class of DSCC (t = 1 to 7: class 1 = <41.43; class 2 = 41.43–50.01; class 3 = 50.02–58.59; class 4 = 58.60–67.18; class 5 = 67.19–75.77; class 6 = 75.78–84.36; class 7 = ≥84.37); $(LSCC \times DSCC)_{st}$ is the fixed effect of the interaction between LSCC and DSCC effects; $Herd_u$ is the random effect of the uth class of herd (u = 1 to 223); $Animal(Breed_p)_v$ is the random effect of the vth animal (v = 1 to 22 124) within the pth breed; and $e_{mnopqrstuvw}$ is the random residual ~N (0, σ_e^2), where σ_e^2 is the residual variance.

The division of the classes of DSCC and LSCC was based on their respective distributions. Each class represented 0.5 SD of the variable and centred on the mean value, with the 1st and 7th class representing the tails of the distribution.

RESULTS AND DISCUSSION

As mentioned above, DSCC has been proposed as indicator of mastitis occurrence and more specifically, when combined with SCC, has been proven to be able to distinguish different status of inflammation (i.e. susceptible, acute or chronic) (Zecconi *et al*. 2018). Given the growing interest in this trait, some authors have also investigated the relationship between DSCC and milk composition, to eventually improve management practices at farm level (Stocco *et al*. 2020). In this context, our purpose was to study the interaction between the two somatic cell traits on milk yield, quality and coagulation traits to understand whether their combined use could be useful for better understanding the changes of performances in individual dairy cows, also considering the technological properties of milk.

As the investigation of the effects of herds, animals, HP, breed, milking type, season, parity and DIM was not within the aims of this study, these aspects were briefly summarised as results, but they were not discussed throughout the manuscript.

Descriptive statistics and analysis of variance

Descriptive statistics (mean, standard deviation, 5th and 95th percentiles) and analysis of variance for milk yield, composition and MCP from individual dairy cows are summarised in Tables 1 and 2, respectively. In the following sections, milk components are always reported as weight/volume percentages.

The average fat, protein, casein and lactose contents were 3.98%, 3.46%, 2.73% and 4.81%, respectively, with the first having the highest CV (19%) among these traits. The average CN-*in* was 78.9%. The average LSCC was 4.98, corresponding to about 95 500 total somatic cell/ml, whereas the average DSCC was 62.9% (Table 1), which corresponds to about 60 000 differential somatic cell/ml. As regard to milk coagulation traits, means of RCT, k_{20} and a_{30} were 27.6 min, 6.20 min and 15.8 mm respectively.

All the fixed effects included in the model were highly significant ($P < 0.001$) on milk yield, composition and coagulation traits, with few exceptions (Table 2). In particular, HP affected four out of 11 traits, and milking type affected eight out of the 12 traits considered in this study. Among the random effects, herd had the greatest explained variance for DY_M, urea and D_{MEO}, whereas animal had larger variance on the other traits, especially protein, casein, lactose and a_{30} (Table 2).

Table 1 Descriptive statistics[a] of milk yield, composition, somatic cell and coagulation traits of 159 224 data points collected from 22 124 individual dairy cows.

Item[b]	*N*	Mean	CV	P5th	P95th
DY_M, kg/d	159 224	20.2	54	7.60	42.5
D_{MEO} , MJ/d	157 931	57.9	52	23.3	117.8
Milk composition traits					
Fat, %	159 224	3.98	19	2.79	5.28
Protein, %	159 224	3.46	11	2.86	4.14
Casein, %	159 106	2.73	12	2.21	3.32
CN-*in*, %	156 979	78.9	2	76.5	81.3
Fat:Protein	158 774	1.15	17	0.85	1.49
Lactose, %	158 934	4.81	4	4.46	5.10
Urea, mg/dL	157 959	24.2	27	13.7	35.9
Somatic cell traits					
DSCC, %	159 224	62.9	27	32.0	86.7
LSCC	159 224	4.98	12	4.11	6.15
Milk coagulation traits					
RCT, min	115 430	27.6	23	18.2	38.6
k_{20}, min	115 792	6.20	39	2.76	10.5
a_{30}, mm	109 744	15.8	51	3.58	30.2

[a]Coefficient of variation = CV, %; Percentile = 5th and 95th percentiles, which indicate the upper and lower 5% limits in the 2-tailed distribution of data.

[b]DY_M, daily milk yield; D_{MEO}, daily milk energy output; CN-*in*, casein index; DSCC, differential somatic cell count; LSCC, logarithmic somatic cell count; RCT, rennet coagulation time; k_{20}, curd-firming time; a_{30}, curd firmness 30 min after rennet addition.

Effect of the interaction of LSCC with DSCC on daily milk yield and energy output

In Figure 1 are reported the LSM of DY_M (Figure 1a) and D_{MEO} (Figure 1b) for the effect of the interaction between LSCC and DSCC. It should be noted that LSM values presented here for a specific effect were corrected for all the other factors included in the statistical model. It is well known that high SCC is related to a reduction of performances of dairy cows both in terms of yield and energy output (Fox *et al*. 2017). Franzoi *et al*. (2020) reported that the increase of SCC does not affect linearly the DY_M, probably because SCC alone does not give any information about the immunosuppressed animals. In contrast, we observed an effect of the interaction between LSCC and DSCC on DY_M. Particularly, we found that when LSCC data were combined with DSCC, the increase showed different patterns. Up to 100 000 cells/mL (i.e. around the fourth class of LSCC; range: 4.83–5.12), the effect of the increase of LSCC across different levels of DSCC on DY_M and D_{MEO} was small and negligible, whereas moving to the highest LSCC content, we observed a decrease in the performances of dairy cows. In the case of the lowest DSCC content (<41.4%), moving from the lowest to the highest LSCC, we observed a reduction of DY_M of about 36% (−7.28 kg/d), whereas cows with the highest DSCC content (>84.37%) decreased their production by only 10% (−1.98 kg). Bobbo *et al*. (2020) evidenced that inflamed cows can maintain their production while reacting to the primary infection, whereas chronic cows decrease their production because the mammary gland tissue is damaged by previous mastitis.

Even though we observed high variations of cows' performances across classes of LSCC with low percentage of DSCC (i.e. larger proportion of macrophages), it is important to consider that the average of DY_M in our data set was not high. Therefore, it can be hypothesised that in high producing dairy cows, the relative decrease in DY_M due to the combined effect LSCC and DSCC may be lower. Indeed, although cows with high LSCC and high DSCC are usually identified as affected by subclinical mastitis (Zecconi *et al*. 2018, 2021), they are probably more able to respond promptly to the infection and, simultaneously, to sustain their production performances. In fact, the milk secretory system is also highly efficient in producing pro-inflammatory and antimicrobial molecules (Mazzilli and Zecconi 2010). In contrast, the group of cows with high LSCC and low DSCC, commonly defined as chronic (Bobbo *et al*. 2020), could also consist of cows not showing an active immune response.

As regard to D_{MEO}, the patterns among LSCC and DSCC classes were similar to those observed for DY_M (Figure 1b). This was expected because, as aforementioned, D_{MEO} was obtained multiplying NEL with DY_M. However, D_{MEO} provides more information over DY_M, as it can be seen as an indicator of the energy cost of the cow. Indeed, when the immune system is activated and the inflammatory response is triggered, there is a redistribution of the overall energy. If a temporary inflammatory response is normal and necessary at the onset of infection, an uncontrolled or a chronic inflammatory response may have adverse effects on cow's health, also compromising the productive performances. These conditions are clearly represented in Figure 1b. Indeed, large differences were observed among classes of DSCC starting from the fourth LSCC class, where cows belonging to the highest classes of DSCC and LSCC were characterised by a higher D_{MEO} compared to those belonging to the lowest DSCC (Figure 1b).

Effect of the interaction of LSCC with DSCC on milk composition

Moving on the combined effect of somatic cell traits on each milk component, we observed that the increase of LSCC was accompanied by a different increase of the fat, protein and casein percentages across DSCC classes (Figure 2a–c respectively), showing opposite patterns respect to the DY_M and D_{MEO}. This result was expected, since concentration of the main components (i.e. fat, protein and casein) in milk is affected by variation of DY_M (Fox *et al*. 2017).

Table 2 Analysis of variance of daily milk yield, milk composition[a] and coagulation[b] traits with significance for fixed effects[c] (Herd productivity, milking type, season, breed, parity, DIM, LSCC, DSCC and LSCC × DSCC) and the proportion of variance (in percentage) explained by herd and animal random effects.

		Milk composition traits								Milk coagulation traits		
Effects	DY_M	D_{MEO}	Fat	Protein	Casein	CN-*in*	F:P	Lactose	Urea	RCT	k_{20}	a_{30}
Fixed effects												
HP	***	–	NS	*	*	NS	NS	***	NS	NS	NS	NS
Milking type	***	***	*	NS	NS	**	***	NS	***	**	**	NS
Season	***	***	***	***	***	***	***	***	***	***	***	***
Breed	***	***	***	***	***	***	***	***	***	***	***	***
Parity	***	***	***	***	***	***	***	***	***	***	***	***
DIM	***	***	***	***	***	***	***	***	***	***	***	***
LSCC	***	***	***	***	***	***	***	***	***	***	***	***
DSCC	***	***	***	***	***	***	***	***	***	***	***	***
LSCC × DSCC	***	***	***	***	***	***	***	***	NS	***	***	***
Random effects												
Herd	49	62	7	9	8	6	8	8	30	5	6	7
Animal	19	13	30	44	45	29	20	46	11	32	20	40
RMSE[d]	3.91	11.2	0.56	0.21	0.18	1.05	0.16	0.11	5.13	4.80	2.03	5.75

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, nonsignificant.

[a]DY_M, daily milk yield; F:P, fat to protein ratio; CN-*in*, casein index; D_{MEO}, daily milk energy output.

[b]RCT, rennet coagulation time; k_{20}, curd-firming time; a_{30}, curd firmness 30 min after rennet addition.

[c]HP, herd productivity; DIM, days in milk; LSCC, logarithmic somatic cell count; DSCC, differential somatic cell count.

[d]RMSE, root mean square error.

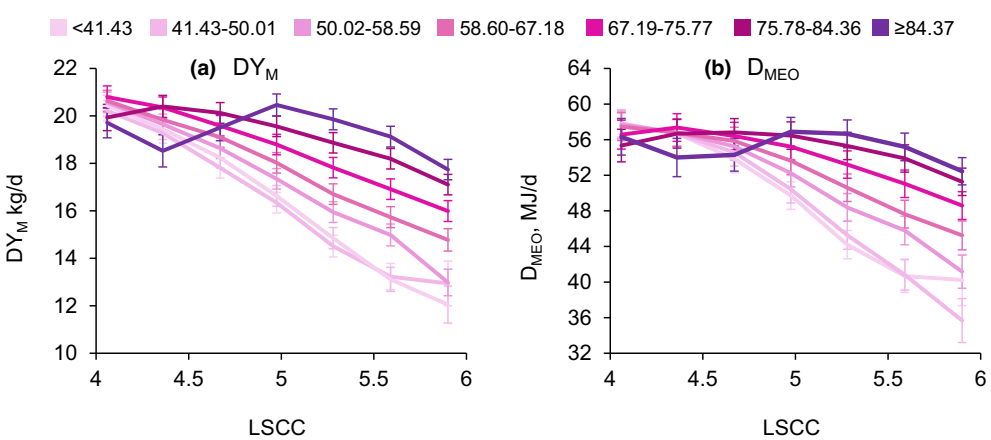

Figure 1 Least squares mean and standard error of the interaction between logarithmic somatic cell count (LSCC) and differential somatic cell count (DSCC) on daily milk yield (a) and daily milk energy output (b).

Respect to the lowest LSCC, the increase of those components became evident already at the third LSCC class, which corresponds to the range of about 33 000–66 000 total somatic cell/mL. Those findings demonstrated that fat, protein and casein are more susceptible to the changes in SCC at lower levels with respect to DY_M. Anyway, the rise of these milk components was lower compared to the reduction of DY_M. Indeed, when the actual yield (in kg) of those components was calculated multiplying them with DY_M, we found a decrease moving from low to high values of LSCC, as observed for D_{MEO}. Particularly, within the lowest percentage of DSCC (<41.43%), daily yield of fat, protein and casein decreased of −0.19, −0.21 and −0.17 kg/d, respectively, moving from the lowest to the highest content of LSCC (data not shown).

In the case of protein and casein concentration, usually, milk containing high LSCC also shows high proteolytic activity, due to protease released by PMN, that leads to a

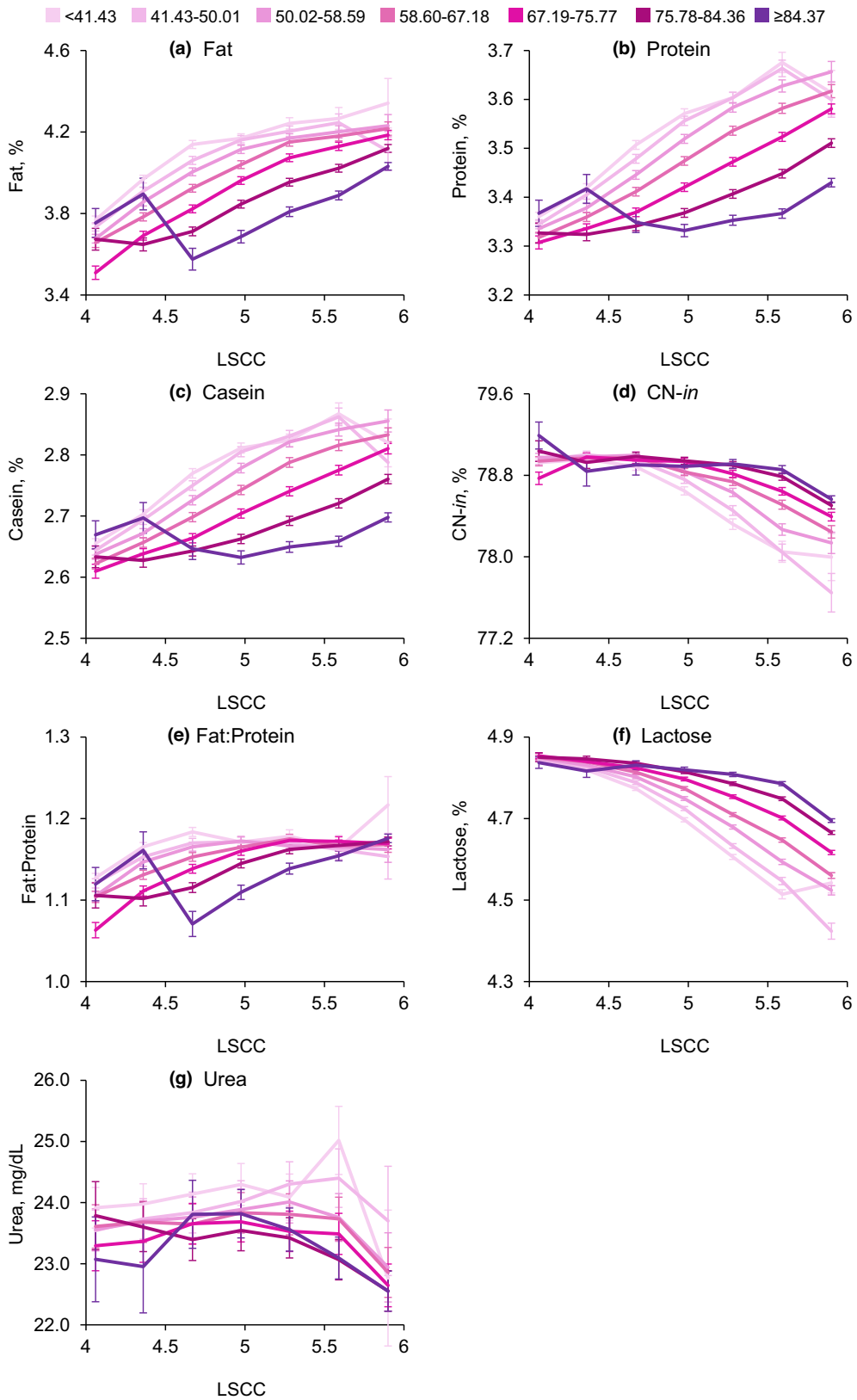

Figure 2 Least squares mean and standard error of the interaction between logarithmic somatic cell count (LSCC) and differential somatic cell count (DSCC) on fat (a), protein (b), casein (c), casein index (d), fat to protein ratio (e), lactose (f) and urea (g).

decrease in the relative proportion of some casein fractions (α- and β-caseins) together with an increase in γ-casein (Urech *et al*. 1999). In the present study, similarly to what we obtained for DY_M, the differences across classes of LSCC for milk protein and casein were negligible at the highest percentage of DSCC. Therefore, acquiring information on the proportion of the different cell traits in milk might be a key aspect to further define not only the health status of cows, but also the effects on their performances. Thus, DSCC might be used by the breeding associations in novel selection indexes to reduce udder health problems, as well as improve dairy cows' functionality. In this regard, Bobbo *et al*. (2019) showed that DSCC presents higher heritability compared to the traditional somatic cell score (0.08 ± 0.02), and it is positively correlated with LSCC.

Fat, protein and casein are pivotal in dairy industry, as they represent the main matrix for cheese production. In this way, not simply their content, but also their ratio is fundamental. Casein index had quite stable values across LSCC levels and in higher classes of DSCC (i.e. >58.6%) (Figure 2d), whereas it showed a remarkable decrease when DSCC were lower than 58.60% and when somatic cells were higher than 134 000 cells/mL (LSCC = 5.13–5.43). However, milk with the highest DSCC content was also characterised by higher CN-*in*, explained by the larger proportion of whey proteins within the total protein content, which are responsible for the immune system activation (i.e. β-lactoglobulin), and the augmentation of γ-casein over the other casein fractions (Urech *et al*. 1999). These findings are particularly attracting, as the variation of CN-*in* according to different level of LSCC combined with DSCC allows to identify a specific CN-*in* content to a hypothetical udder health condition. Indeed, very low DSCC and very high LSCC were characterised by the lowest CN-*in*, while the highest CN-*in* was in milk samples with opposite characteristics. Also, differences were found in the high DSCC samples with increasing LSCC content, although they were much larger in samples with low DSCC and increasing LSCC content. This result demonstrates that the modification extent of milk composition varies not only according to huge variations in LSCC and DSCC (i.e. low or high), but it also sensitive to small modifications (i.e. DSCC and LSCC classes, as shown in this study). These findings are important especially for those countries where milk is mostly used for cheese production, since in practice, with high LSCC and low DSCC, the performances of dairy cows seem to be even worse in terms of daily cheese yield compared with DY_M.

Moving to F:P, this ratio was characterised by a much more erratic trend, especially in the highest DSCC class and across LSCC classes (Figure 2e). In the literature, it is reported that F:P could be used as potential indicator for energy status in dairy cows, at least during the most metabolically stressful stage of lactation (i.e. early lactation; Buttchereit *et al*. 2010). If considered separately, the effect of different levels of LSCC on fat-to-protein ratio (positive) was opposite to that of DSCC (negative; Figure S2i and l) and mainly followed the patterns of fat and protein considered individually. The results obtained for milk fat, protein, casein and their ratios underline that the introduction of LSCC and DSCC in the milk payments systems, as a combined parameter, could be useful to improve the accuracy in the assessment of milk quality.

As expected, lactose content (Figure 2f) revealed a similar pattern to that of DY_M, although for the higher classes of DSCC (>67%) the decrease of this component became less linear compared to the trend displayed for the lower DSCC classes. This could be explained by the fact that during inflammation, glucose (the main substrate for lactose synthesis) is saved and redirected to boost the immune system, at the expense of milk production (Kvidera *et al*. 2017).

Milk urea tended to a curvilinear pattern, showing an overall lower content in milk with high DSCC and LSCC. However, milk produced by cows with low DSCC content and high LSCC showed more variability of this trait (Figure 2g), as highlighted by the standard error bars. Pegolo *et al*. (2021) found no significant associations between urea and SCC content in milk, or with different levels of DSCC. Nevertheless, focusing on the LSCC effect, we observed a decrease in milk urea in the highest class of LSCC (corresponding to ≥560 000 somatic cells/mL) (Figure S2o). This outcome is in accordance with a previous study where a negative association with milk urea concentration and SCC was found (Nyman *et al*. 2014).

Effect of the interaction of LSCC with DSCC on MCP

Nowadays, MCP are measured using computerised renneting meters, or alternative systems (i.e. optical, thermal, mechanical and vibrational methods), including their prediction via mid infrared spectroscopy (Visentin *et al*. 2016). This technique is particularly suitable for large-scale application (i.e. implementation in the milk test-day recording systems), being nondestructive and having reduced the time and costs of analysis. Although the accuracy of prediction of MCP is lower than that of milk components (i.e. fat and protein), this technique represents a valid tool for a fast-screening or cost-effective collection of phenotypes at population level (Visentin *et al*. 2016).

In Figure 3 are reported the LSM of MCP for the combined effect of LSCC and DSCC. The effect was less variable on RCT and k_{20} within the first four classes of LSCC, whereas the differences among classes became more evident when LSCC was higher than five (i.e. >135 000 cells/mL). Despite the last class of LSCC showed the greatest variability compared with the other classes, in general, RCT was longer in milk with low DSCC (about + 2 min) compared to those with high DSCC, regardless of the LSCC level, and longer in milk samples with low DSCC and high

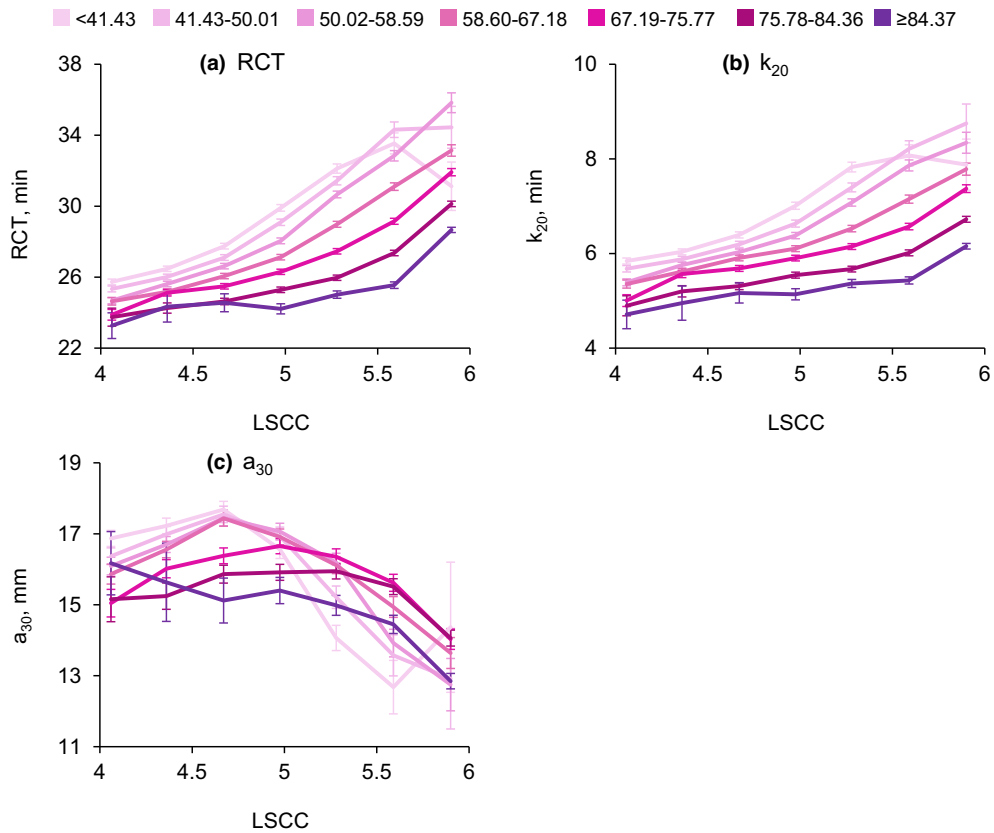

Figure 3 Least squares mean and standard error of the interaction between logarithmic somatic cell count (LSCC) and differential somatic cell count (DSCC) on rennet coagulation time (a), curd-firming time (b) and curd firmness 30 min after rennet addition (c).

LSCC, than those with high DSCC and high LSCC (about + 3 min; Figure 3a). The same pattern was evidenced for k_{20} (Figure 3b). It is widely recognised that milk gelation and curd firming are largely influenced by milk protein and casein contents (Fox *et al*. 2017), so the changes in milk composition previously observed are mirrored by MCP. Indeed, it is recognised that overall increases in milk protein levels (>3.1%) result in an increase in gelation time (Fox *et al*. 2017), and this effect can be ascribed to the decreasing rennet-to-casein ratio, which needs an increase in time required to generate enough hydrolysis of the k-casein to induce the aggregation of the micelles (Fox *et al*. 2017).

In the case of a_{30}, this trait had a more erratic decreasing trend, and this could be due to the much higher variability within each class of DSCC and LSCC. It is important to notice that the prediction accuracy of traits indirectly measured in milk, as a_{30}, is much lower than the directly measurable traits as fat and protein (Visentin *et al*. 2015). This could have contributed to the high variability within each class, and probably, it should be considered as a limitation of the study. However, the curd firmness was clearly lower in milk samples with high LSCC compared to those with low LSCC. In this case, the pattern of a_{30} is not reflected by the quantity of casein or protein but is probably a signal of the altered protein–casein profile. Indeed, high SCC are associated with higher levels of total protein, whey proteins and proteolytic activity of plasmin towards β-casein (Ismail and Nielsen 2010), one of the most important casein fractions affecting the technological quality of milk, in particular curd firmness (Urech *et al*. 1999). Since composition of milk affects the coagulation ability of milk, we further used the same statistical model described in the material and method section including also milk fat and protein as linear covariates, in order to quantify the true independent effect of LSCC and DSCC on the considered traits (data not shown). Nevertheless, the effect remained highly significant showing the same pattern of the MCP for the interaction between LSCC and DSCC. This outcome is particularly interesting, because it highlights that somatic cell traits are directly involved in milk coagulation process, and further confirms their importance not only for composition, but also for the technological quality of milk.

CONCLUSION

The results presented in this study evidenced that the inclusion in the statistical model of seven different classes for DSCC and LSCC and their interactions allowed us to better

understand the influence of their combined effect on milk yield, composition and technological properties. In particular, variations of fat, protein and CN-*in* due to different combinations of DSCC and LSCC allowed to observe the direct change in milk quality, to consider the aftermath related to the cheesemaking ability of milk and the potential ability of the individual cow to face an uncomfortable health situation. Also, results from the MCP clearly evidenced that slight modifications in the content of DSCC and LSCC are enough to worsen the technological ability of milk. Thus, these results give better understanding on the relationships between DSCC, LSCC and milk quality, and might be helpful to monitor the quality of milk intended for cheese production.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Elena Mariani: Writing – original draft; Writing – review & editing. **Claudio Cipolat-Gotet:** Conceptualization; Writing – review & editing. **Bruno Stefanon:** Resources; Supervision. **Alfonso Zecconi:** Supervision; Writing – review & editing. **Giorgia Stocco:** Writing – original draft; Writing – review & editing. **Misa Sandri:** Writing – review & editing. **Michela Ablondi:** Formal analysis; Writing – review & editing. **Maria Mountricha:** Writing – review & editing. **Andrea Summer:** Supervision; Writing – review & editing.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

The following supporting information is available for this article:

Fig S1. Least squares mean and standard error of LSCC and DSCC on daily milk yield (a, b), and daily milk energy output (c,d).

Fig S2. Least squares mean and standard error of LSCC and DSCC on fat (a, b), protein (c, d), casein (e, f), casein index (g, h), fat:protein (I, l), lactose (m, n) and urea (o, p) days in milk (a, b), fat:protein (c, d) and casein index (e, f).

Fig S3. Least squares mean and standard error of LSCC and DSCC on rennet coagulation time (a, b), curd-firming time (c, d), and curd firmness 30 min after rennet addition (e, f).

