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Udder health-related traits in cow milk: phenotypic variability and effect on milk yield and composition

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ABSTRACT

The milk differential somatic cell count (**DSCC**) has been proposed in recent years as a mean by which to better monitor the udder health status (**UHS**) in dairy cows. Milk DSCC is the amount of polymorphonuclear neutrophils and lymphocytes contributing to the total somatic cell count (**SCC**) and can be determined on a routine basis in individual milk samples subjected to official analysis. In the present study, 522 865 milk test-day records of 77 143 cows were scrutinised to identify factors affecting the variability of both DSCC and SCC in Holstein Friesian, Jersey, Simmental and Rendena cows through linear mixed models. The fixed effects were breed, parity, lactation stage, sampling season, and all the first-order interactions of breed. Cow and herd-test-date were considered as random. Subsequently, four UHS groups were created (1: SCC $\leq$ 200 000 cells/mL and DSCC $\leq$ 65%; 2: SCC $\leq$ 200 000 cells/mL and DSCC > 65%; 3: SCC > 200 000 cells/mL and DSCC > 65%; 4: SCC > 200 000 cells/mL and DSCC $\leq$ 65%) to compare milk yield and quality. Milk SCS and DSCC differed across lactation, parity, sampling season and breed. In particular, Simmental cows had the lowest SCC and Jersey the lowest DSCC. Depending on the breed, UHS affected daily milk yield and composition to a different extent. The UHS group 4, i.e. the one grouping test-day records with high SCC and low DSCC, presented the lowest estimate of milk yield and lactose content no matter the breeds. Our findings support that udder health-related traits (SCS and DSCC) are useful information to improve udder health at individual cow and herd levels. Moreover, the combination of SCS and DSCC is useful to monitor milk yield and composition.

Implications

In addition to milk somatic cell count, which is a historical indicator of udder health in cattle, the differential somatic cell count has been introduced as an attempt to improve the accuracy of mastitis detection. In this study, we investigated the variability of total and differential somatic cell count, and found out that stage of lactation, parity, season of sampling and breed affect their variability. The monitoring of total and differential somatic cell count is useful for the management of udder health at cow and herd levels, especially near the dry-off.

Introduction

Mastitis, an inflammation of the udder commonly caused by intramammary infection is one of the most prevailing and prominent diseases occurring in dairy cows (Farre et al., 2022). Inflammation of mammary gland tissue impairs the health status at both herd and individual cow levels and is often responsible for massive economic losses for the farmer, especially in the presence of the latent subclinical form (Ruegg, 2017; Sharma et al., 2011). Accurate and timely identification of mastitis was, is, and will be always crucial for the farmers (Farre et al., 2022).

In addition to milk somatic cell count (**SCC**, cells/mL), which historically has been used as an indicator of mammary gland health (de Haas et al., 2007), the differential somatic cell count (**DSCC**, %) has been introduced in more recent years as a promising novel trait to improve mastitis detection accuracy when used in conjunction with SCC (Damm et al., 2017). New technologies commercially available allow the simultaneous determination of SCC and DSCC. In particular, DSCC is the proportion of polymorphonuclear neutrophils and lymphocytes out of the total SCC (Damm et al., 2017). Flow cytometry can satisfactorily distinguish polymorphonuclear neutrophils and lymphocytes from macrophages in cow milk. While lymphocytes regulate the induction and suppression of immune responses, macrophages ingest foreign microorganisms and cellular debris. The latter also recognise invading

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pathogens and trigger an immune response by the rapid recruitment of polymorphonuclear neutrophils which act as the first defence against invading bacteria at the onset of an infection (Damm et al., 2017; Halasa and Kirkeby, 2020). Therefore, in milk of healthy quarters, lymphocytes and macrophages prevail over polymorphonuclear neutrophils (Schwarz et al., 2011), meaning that the SCC of a healthy quarter is low, while DSCC becomes inflated upon infection of the mammary gland (Schwarz et al., 2020a; 2020b). The DSCC also varies along a temporal axis in relation to the inflammation event, that is, before, during, and after the inflammation. In fact, DSCC is very high in the early stages of infection, while in chronic cases, the proportion of macrophages increases and DSCC tends to decrease (Leitner et al., 2000; 2003). According to Ariel et al. (2020), cows with one or more quarters suffering from chronic inflammation are expected to have a dampened immune response, particularly in the presence of certain pathogens. Activation of the immune response in newly infected animals in fact occurs rapidly, while a certain degree of tolerance is observed in cows subjected to chronic infection.

Although proposed as a promising phenotype, the variability of milk DSCC and its impact on milk-related performance have been scarcely explored (Bobbo et al., 2020; Mariani et al., 2022) in cattle breeds less cosmopolitan than Holstein Friesian (**HF**). In the present study, we aimed to investigate the factors determining the variability of udder health-related traits in HF, Jersey (**JE**), Simmental (**SI**) and Rendena (**RE**) breeds. In addition, by combining both SCC and DSCC, we attempted to estimate the effect of the overall udder health status (**UHS**) on milk yield and composition traits.

Material and methods

Data editing

A total of 924 432 milk test-day records collected on 100 619 HF, JE, SI and RE cows reared in Veneto region (Italy) were retrieved from the official routine milk recording testing data set of the Breeders Association of Veneto Region (ARAV, Vicenza, Italy). The data set covered the period from January 2019 to December 2021 and included exclusively all-year-round calving herds.

Test-day records included information on days in milk (**DIM**), parity, milk yield (kg/d), as well as fat, protein, casein, and lactose content (%), SCC (cells/mL), DSCC (%) and β-hydroxybutyrate concentration (**BHB**, mmol/L), a biomarker of negative energy balance and ketosis in dairy cows. All milk quality traits were determined using the CombiFoss™ 7 DC machine (Foss, Hillerød, Denmark). The casein index was calculated as the ratio between casein and protein and, having both milk SCC and DSCC available, the number of polymorphonuclear neutrophils and lymphocytes excreted ($DSCC_N$, cells/mL) were calculated as: $DSCC_N = (SCC \times DSCC)/100$ (Costa et al., 2021). To achieve a more normal distribution of data points and homogeneity of variance, the score of SCC was obtained through the formula of Ali and Shook (1980): $SCS = 3 + \log_2(SCC/100\ 000)$. Similarly, $DSCC_N$ was $\log_2$-transformed through the same formula to obtain a new variable, i.e., the differential somatic cell score (**DSCS**). Finally, following Benedet et al. (2020), the BHB was $\log_{10}$-transformed. The major loss of records (n = 185 346) was due to incomplete milk data; in fact, DSCC started to be routinely recorded on all test-day samples at the ARAV laboratory from January 2020.

The parameter known as 'good separation', conventionally known as "**GOSE**", is a binary trait (1/0) provided by the FOSS instrument and defines the reliability of the SCC-related traits of each milk sample (Schwarz et al., 2020a). Based on this quality control, 22 416 test-day records with no good separation were discarded from the analysis. Only samples with SCC ≥ 10 000 and ≤5 000 000 cells/mL were considered, leading to a loss of 17 241 test-day records. All samples were analysed within 5 days from the sampling date, in line with CombiFoss™ 7 DC guidelines and Damm et al. (2017).

Values of milk yield, content of fat, protein, casein, lactose, and BHB deviating more than 3 SD from the respective mean were removed as outliers. Only records of cows from 5 to 360 DIM and parities 1 to 8 were included, leading to a loss of 90 977 test-day records. In addition, only the lactations with at least three test-days records were kept, resulting in a final dataset of 522 865 test-day records available for statistical investigation.

According to SCC and DSCC, each test-day record was assigned to a specific UHS group according to Schwarz et al. (2020a):

- healthy (UHS1): SCC ≤ 200 000 cells/mL and DSCC ≤ 65%;
- suspicious (UHS2): SCC ≤ 200 000 cells/mL and DSCC > 65%;
- mastitis (UHS3): SCC > 200 000 cells/mL and DSCC > 65%;
- chronic (UHS4): SCC > 200 000 cells/mL and DSCC ≤ 65%.

In particular, UHS2 is indicative of suspected mastitis, since polymorphonuclear neutrophils are predominant in the presence of a normal level of SCC; this is typical of the onset of inflammation in dairy cows (Schwarz et al., 2020b). On the other hand, milk of UHS3 is considered mastitic as both SCC and DSCC are above the conventional cut-off. Finally, the UHS4 group comprises test-day records with high SCC but low DSCC: the most accredited interpretation is that this situation characterises animals with one or more quarters suffering from chronic inflammation (Leitner et al., 2000; 2003).

Statistical analysis

The software R v. 4.1.2 (R Core Team, 2022) was used to manipulate data, calculate Pearson's correlations and perform statistical analyses. Data were also visually inspected to evaluate potential non-linear associations between the traits. For each trait, the raw mean and SD were calculated for each breed.

To identify the statistically significant factors determining the variability of udder health-related traits, the analysis of variance was performed through the following linear mixed model:

$$y_{ijklmn} = \mu + B_i + M_j + P_k + D_l + \beta(T_{ijklmn}) + (B \times M)_{ij} + (B \times P)_{ik} + (B \times D)_{il} + C_m + H_n + e_{ijklmn}, \quad (1)$$

where y_{ijklmn} is the dependent variable; μ is the overall intercept of the model; B_i is the fixed effect of the ith breed (i = HF, JE, SI, RE); M_j is the fixed effect of the jth sampling period (j = January–February, March–April, May–June, July–August, September–October, November–December); P_k is the fixed effect of the kth parity (k = 1, 2, 3, ≥4, with the last class including cows of parity 4 to parity 8); D_l is the fixed effect of the lth stage of lactation (l = 11 classes, with the first being a class from 5 to 30 DIM, followed by nine classes of 30 d each, and the last being a class from 301 to 360 DIM); T is a covariate accounting for the effect of sample age, calculated as the difference (in days, from 0 to 5) between the sampling date and the date of analysis with regression coefficient β; $(B \times M)_{ij}$ is the fixed interaction effect between breed and bimonthly sampling period; $(B \times P)_{ik}$ is the fixed interaction effect between breed and parity; $(B \times D)_{il}$ is the fixed interaction effect between breed and stage of lactation; C_m is the random effect of the mth cow (77 143 levels) assumed to be distributed as $\sim N(0, \sigma^2_C)$, where σ^2_C is the cow variance; H_n is the random effect of the nth herd-test-date (13 699 levels) assumed to be distributed as $\sim N(0, \sigma^2_H)$, where σ^2_H is the herd-test-date variance; and e_{ijklmn} is the random residual assumed to be distributed as $\sim N(0, \sigma^2_e)$, where σ^2_e is the residual variance. Model diagnostics were checked through analysis of distri-

Table 1
Number of test-day records, cows and herds, and means (SD) of milk yield, composition traits, and udder health indicators for the breeds included in the study.

Trait[1]	Holstein Friesian	Jersey	Simmental	Rendena
Records, n	463 917	3 867	38 408	16 673
Cows, n	68 374	526	5 786	2 457
Herds, n	897	48	364	73
Milk yield, kg/d	33.5 (9.7)	23.8 (7.3)	25.2 (8.3)	20.0 (6.8)
Fat, %	3.87 (0.77)	4.96 (0.92)	3.88 (0.74)	3.57 (0.58)
Protein, %	3.39 (0.37)	3.95 (0.44)	3.51 (0.38)	3.34 (0.35)
Casein index	0.79 (0.02)	0.79 (0.02)	0.79 (0.02)	0.79 (0.01)
Lactose, %	4.83 (0.19)	4.73 (0.21)	4.77 (0.20)	4.85 (0.21)
SCS	2.85 (1.90)	3.24 (1.73)	2.83 (1.88)	3.16 (1.77)
DSCC, %	63.4 (16.4)	55.9 (16.4)	63.4 (16.1)	67.4 (14.3)
DSCS	2.14 (2.18)	2.33 (1.99)	2.11 (2.15)	2.55 (2.01)
BHB	−1.33 (0.35)	−1.46 (0.60)	−1.30 (0.36)	−1.30 (0.35)

[1] SCS = somatic cell score, calculated as SCS = 3 + $\log_2$(SCC/100 000), where SCC is somatic cell count (cells/mL); DSCC = polymorphonuclear neutrophils and lymphocytes (%) out of the total SCC; DSCS = differential somatic cell score, calculated as DSCS = 3 + $\log_2$($DSCC_N$/100 000), where $DSCC_N$ is the concentration of polymorphonuclear neutrophils and lymphocytes in milk (cells/mL); BHB = $\log_{10}$ transformation of β-hydroxybutyrate concentration in milk (mmol/L).

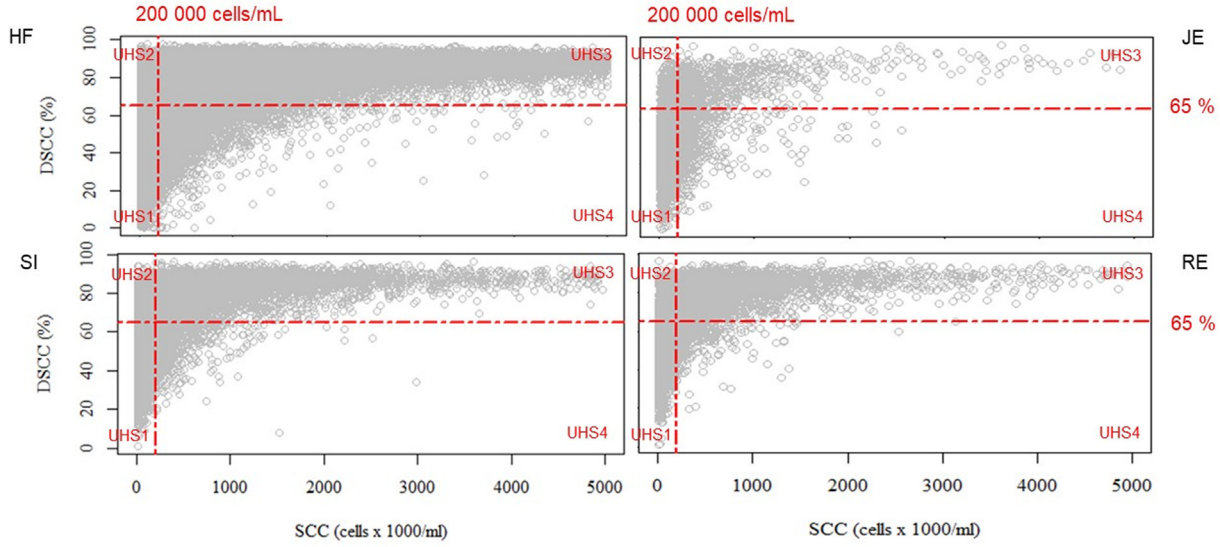
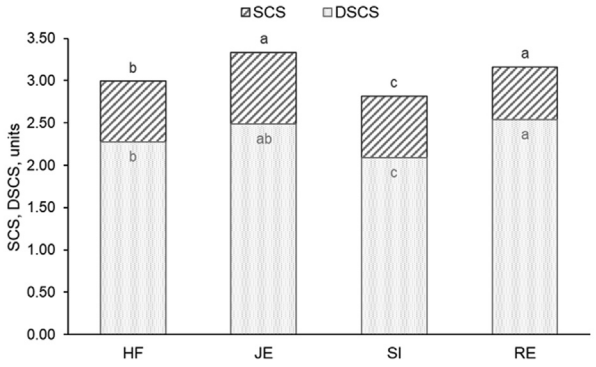


Fig. 1. Udder health status groups (UHS) as defined by plotting somatic cell count (SCC, cells/mL) and differential SCC (DSCC)[1] in Holstein Friesian (HF), Jersey (JE), Simmental (SI) and Rendena (RE) cows. [1]DSCC = polymorphonuclear neutrophils and lymphocytes (%) out of the total SCC.



DSCC, %	63.7 b	59.0 d	63.1 c	67.0 a

Fig. 2. Least squares means of SCS[1] (cross-hatched bar), DSCC[2] (table), and DSCS[3] (grey bar) for Holstein Friesian (HF), Jersey (JE), Simmental (SI) and Rendena (RE) cows. Means with different superscript letters within trait are statistically significantly different ($P \leq 0.05$). [1]SCS = somatic cell score, calculated as SCS = 3 + $\log_2$(SCC/100 000), where SCC is somatic cell count (cells/mL). [2]DSCC = polymorphonuclear neutrophils and lymphocytes (%) out of the total SCC. [3]DSCS = differential somatic cell score, calculated as DSCS = 3 + $\log_2$($DSCC_N$/100 000), where $DSCC_N$ is the concentration of polymorphonuclear neutrophils and lymphocytes in milk (cells/mL).

bution, variance homogeneity, and independence of residuals (Supplementary Figs. S1 and S2).

Least squares means (**LSMs**) of udder health-related traits for each breed and breed's first-order interaction with stage of lactation, parity and bimonthly sampling period have been estimated to build up lactation curves and to evaluate parity and seasonal trends. As regards the three interactions, a representative amount of test-day records (n ≥ 300) was guaranteed for each combination.

To assess the effect of UHS on milk yield, milk composition (fat, protein, casein index) and health biomarkers (lactose, BHB), we adopted the same linear mixed model as in (1) with inclusion of the fixed effect of UHS group and its interaction with breed:

$$y_{ijklmno} = \mu + B_i + M_j + P_k + D_l + \beta(T_{ijklmn}) + UHS_m + (B \times M)_{ij} + (B \times P)_{ik} + (B \times D)_{il} + (B \times UHS)_{im} + C_n + H_o + e_{ijklmno}. \quad (2)$$

For both (1) and (2), multiple comparisons of LSM were performed using the Bonferroni adjustment with significance set at $P \leq 0.05$. The LSMs are presented together with their SE. To explore the distribution of test-day records across the UHS groups, the frequencies were calculated within breed and parity.

Results

Sources of variation of udder health-related traits

A total of 522 865 test-day records from 77 143 cows in 713 single-breed and 320 multi-breed herds were available to explore UHS of four cattle breeds. Table 1 provides an overview of the number of records, cows, and herds for each breed and the raw means of milk yield and quality traits. The breed-specific distribution of records across the four UHS groups is depicted in Fig. 1. The average of SCS and DSCS ranged from 2.83 and 2.11 (SI cows) to 3.24 and 2.55 (RE cows). On the other hand, the mean DSCC was 56.0% (lowest) for JE and 67.4% (highest) for RE cows. In each breed, milk SCS demonstrated a strong correlation (Supplementary Table S1) with both DSCC and DSCS, and, as observed for DSCS, it was inversely correlated with milk yield, lactose, and casein index.

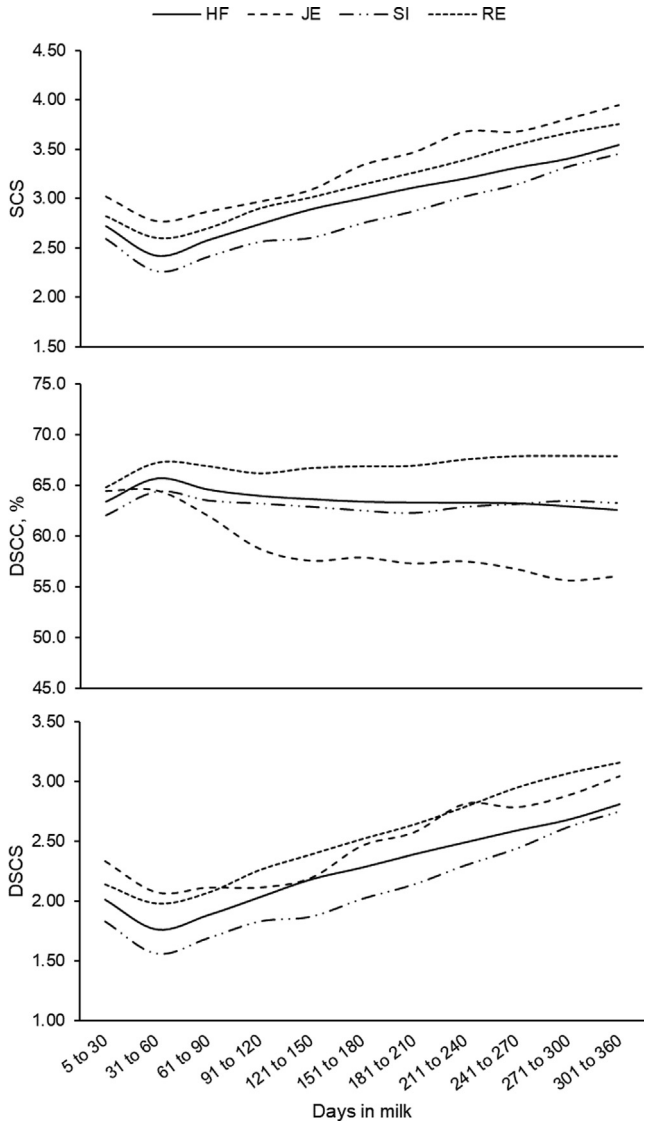


Fig. 3. Least squares means of udder health-related traits[1] for the fixed interaction effect between breed and stage of lactation ($P \leq 0.001$) in Holstein Friesian (HF), Jersey (JE), Simmental (SI) and Rendena (RE) cows. [1]SCS = somatic cell score, calculated as SCS = 3 + $\log_2$(SCC/100 000), where SCC is somatic cell count (cells/mL); DSCC = polymorphonuclear neutrophils and lymphocytes (%) out of the total SCC; DSCS = differential somatic cell score, calculated as DSCS = 3 + $\log_2$($DSCC_N$/100 000), where $DSCC_N$ is n of polymorphonuclear neutrophils and lymphocytes in milk (cells/mL).

All the fixed effects in (1) were statistically significant ($P \leq 0.05$; Supplementary Tables S2, S3 and Supplementary Figs. S1, S2) in explaining the variability of SCC, DSCC, and DSCS. The random effect of individual cow explained 42.3, 36.3, and 42.8% of the total variance of SCS, DSCC, and DSCS, respectively, whereas the amount given by the herd-test-date random effect was rather smaller, being just 3.9% for SCS and DSCS and 5.8% for DSCS.

In line with raw means (Table 1), the greatest SCS was estimated for RE (3.16 ± 0.03) and JE (3.33 ± 0.08), and the lowest for SI (2.82 ± 0.02; Fig. 2); LSM of the HF breed was intermediate (2.99 ± 0.01). The RE cows had the greatest DSCC and JE the lowest (Fig. 2). Results for DSCS were similar, in terms of pattern, to those of SCS, except for JE whose LSM was intermediate between HF (2.28 ± 0.01) and RE (2.54 ± 0.04) at 2.49 ± 0.09.

The breed-specific lactation curves of SCS, DSCC, and DSCS are depicted in Fig. 3. Regardless of the breed, SCS and DSCS showed the lowest LSM at the peak of milk yield (Fig. 3; Supplementary Fig. S3), where after both traits steadily increased until the end of lactation. Overall, the JE breed was characterised by the greatest level of SCS along the whole lactation and SI the lowest. The four breeds presented variability of DSCC within lactation, as indicated by the lactation curves in Fig. 3. The trait was fairly constant throughout the lactation in HF, with the maximum (65.8 ± 0.1%) and the minimum (62.6 ± 0.1%) occurring in early and late lactation, respectively. Among all the breeds, the most pronounced change in DSCC was observed in JE, with LSM moving from a minimum of 55.7 ± 1.0% (late lactation) to a maximum of 62.0 ± 1.0% (early lactation). As noted for HF, the dual-purpose breeds showed a low variability of DSCC across DIM. However, the LSMs of RE were generally the greatest (consistently > 65%) along the whole lactation compared to the other breeds.

In all breeds, udder health was worse in older than younger cows (Table 2), as both SCS and DSCS slightly increased from parity 1 onwards. Such a trend, however, is only numerically confirmed in JE. The presence of large SE, likely due to fewer cows for JE compared to the sample size of the other breeds, may have led to no statistically significant LSM comparisons in JE. In the case of DSCC, the lowest LSMs were estimated for parity 2 in all breeds (Table 2).

The SCS, DSCC and DSCS exhibited seasonal variation (Fig. 4). In HF cows, the greatest SCS was observed between July and October while the lowest in the first semester of the year. Similarly, SCS stayed high from July to December in SI and from July to October in RE. As a result of the large SE, the SCS of the JE breed did not differ statistically significantly across sampling seasons. The DSCC was greater in late spring and summer compared to autumn and winter. The greatest LSMs of DSCS were found in the class July-August for HF, SI, and RE, and in May-June for JE.

Milk yield and composition according to the udder health status

All the fixed effects included in model (2) were statistically significant ($P \leq 0.05$; Supplementary Tables S4 and S5) in explaining the variability of milk yield and the composition traits, with the exception of the interaction between breed and UHS which was not statistically significant for fat content. The LSMs estimated for the first-order interaction between breed and UHS are depicted in Table 3. The LSM demonstrated that HF was associated with higher milk yield, especially when compared to RE and JE (Fig. 5). The UHS4 group was characterised by lower milk yield in all breeds (Fig. 5). The greatest milk yield in the case of HF and SI cows was observed in UHS2, i.e. in test-day records with SCC ≤ 200 000 cells/mL and DSCC > 65%. In RE and JE breeds, the LSMs of UHS1 and UHS2 (both with a SCC ≤ 200 000 cells/mL) were similar and were the greatest in terms of milk yield. The loss of daily milk yield when moving from UHS2 to UHS4 was −5.34, −3.87, −2.74, and −3.44 kg/d for HF, SI, RE, and JE, respectively.

Table 2
Least squares means[1] (95% confidence intervals) of udder health-related traits for the fixed interaction effect between breed and parity in Holstein Friesian, Jersey, Simmental and Rendena cows. Means with different superscript letters within a row are statistically significantly different ($P \leq 0.05$).

Trait[2]	Breed	Parity							
		1		2		3		≥4	
SCS	Holstein Friesian	2.36^{d}	(2.34; 2.38)	2.77^{c}	(2.75; 2.79)	3.22^{b}	(3.20; 3.24)	3.61^{a}	(3.59; 3.64)
	Jersey	3.01^{b}	(2.77; 3.24)	3.16^{b}	(2.92; 3.39)	3.26^{b}	(2.98; 3.54)	3.90^{a}	(3.59; 4.21)
	Simmental	2.41^{d}	(2.34; 2.48)	2.62^{c}	(2.55; 2.69)	2.95^{b}	(2.88; 3.02)	3.28^{a}	(3.21; 3.36)
	Rendena	2.61^{d}	(2.50; 2.72)	2.97^{c}	(2.87; 3.08)	3.30^{b}	(3.18; 3.41)	3.76^{a}	(3.65; 3.87)
DSCC	Holstein Friesian	63.2^{c}	(63.1; 63.4)	61.1^{d}	(60.9; 61.3)	63.7^{b}	(63.5; 63.9)	66.8^{a}	(66.5; 67.0)
	Jersey	59.7^{b}	(57.6; 61.9)	54.4^{c}	(52.3; 56.6)	58.6^{b}	(56.1; 61.1)	63.1^{a}	(60.4; 65.9)
	Simmental	64.1^{b}	(63.5; 64.7)	60.4^{d}	(59.8; 61.0)	62.6^{c}	(61.9; 63.2)	65.3^{a}	(64.6; 65.9)
	Rendena	67.1^{b}	(66.1; 68.1)	65.3^{c}	(64.3; 66.2)	66.4^{b}	(65.4; 67.5)	69.4^{a}	(68.4; 70.4)
DSCS	Holstein Friesian	1.65^{d}	(1.63; 1.67)	1.99^{c}	(1.97; 2.02)	2.51^{b}	(2.48; 2.53)	2.98^{a}	(2.95; 3.01)
	Jersey	2.19^{b}	(1.92; 2.46)	2.18^{b}	(1.91; 2.46)	2.41^{b}	(2.09; 2.73)	3.16^{a}	(2.80; 3.52)
	Simmental	1.72^{d}	(1.64; 1.80)	1.83^{c}	(1.75; 1.91)	2.22^{b}	(2.13; 2.30)	2.61^{a}	(2.53; 2.70)
	Rendena	2.00^{d}	(1.87; 2.13)	2.32^{c}	(2.19; 2.44)	2.66^{b}	(2.53; 2.80)	3.19^{a}	(3.06; 3.32)

[1] SE ranged from 0.01 to 0.12 for SCS, 0.1 to 1.1 for DSCC, and 0.01 to 0.14 for DSCS.
[2] SCS = somatic cell score, calculated as SCS = 3 + $\log_2$(SCC/100 000), where SCC is somatic cell count (cells/mL); DSCC = polymorphonuclear neutrophils and lymphocytes (%) out of the total SCC; DSCS = differential somatic cell score, calculated as DSCS = 3 + $\log_2$($DSCC_N$/100 000), where $DSCC_N$ is the concentration of polymorphonuclear neutrophils and lymphocytes in milk (cells/mL).

Regardless of the UHS group, JE milk had the greatest protein content, followed by milk from SI, HF, and RE (Table 3). Protein content of HF and SI followed the same pattern across the UHS groups, with the minimum and the maximum in UHS2 and UHS4, respectively. Although the baseline protein content was greater in SI than HF, the relative reduction when comparing these two UHS groups was similar (Table 3). Also, in the case of RE, the greatest LSM of protein content was estimated for UHS4. The smallest LSM of casein index was observed in UHS4 and; in general, the greatest LSMs were estimated in groups where SCC was below 200 000 cells/mL (Table 3). As regards the fat content across UHS groups, the greatest LSMs were found for UHS3 and UHS4 (4.13 ± 0.01 and 4.16 ± 0.01%, respectively), while the lowest for UHS2 (4.01 ± 0.01%).

The lowest lactose content was observed in UHS4, i.e., in milk obtained from cows affected by 'chronic' inflammation. Despite the apparent low variability of the trait, lactose content was the greatest in UHS2 for HF and SI, and in UHS1 and UHS2 for RE and JE cows. On a relative basis, the reduction in this trait moving from UHS2 to UHS4 was the largest in the SI breed. Similarly, milk BHB was unfavourably high in UHS4 and low in UHS2. The relative increase in BHB when comparing the two UHS groups was the greatest in JE cows.

Discussion

In the present study, we exploited data from commercial farms retrieved by the local breeders' association. The frequency of the breeds in our data set (Table 1) mirrored the dairy cattle population currently present in the Veneto region. In fact, in 2021, the JE population in this area consisted of 561 officially registered heads compared to the 74 764 HF, 6 454 SI and 2 560 RE (Italian Breeders Association, AIA, 2021). In Italy, HF accounted for 86% of total lactating cows under routine milk recording programmes in 2021, while SI and JE for just 5 and 1%, respectively.

Variability of udder health-related traits

The main aim of this study was to investigate the variability of udder health-related traits (SCC, DSCC and DSCS) in cattle breeds. These traits are characterised by high variability. Indeed, in agreement with Stocco et al. (2023; another region, without JE and RE, and different transformations of data), a large part of the variability of udder health-related traits is controlled by individual cow. In SCS and DSCS, this high variability led to a positive kurtosis of residuals, probably due to the presence of cows with intramammary inflammation in the dataset (Supplementary Fig. S1).

Besides individual cow, other factors such as sampling period, stage of lactation, parity and breed affected SCS, DSCS and DSCC. Based on the literature, we expected milk SCC to differ across breeds (Sharma et al., 2011). In agreement with the findings of De Marchi et al. (2007), the dual-purpose RE breed had greater SCC compared to HF but lower compared to JE. In line with studies of Rupp and Boichard (2003) and Vicario et al. (2005), we confirm SI to be the breed with the lowest SCS. The dual-purpose SI breed, which comprises various populations (e.g., Italian Pezzata Rossa or German and Austrian Fleckvieh), is widely known for its resilience, robustness, and its strong resistance to disease, including mastitis (Rupp and Boichard, 2003). ZuchtData EDV-Dienstleistungen (2021) reported the median SCC between 64 000 and 74 000 cells/mL in the Austrian Fleckvieh and HF population. Their means were 186 716 and 231 237 cells/mL, respectively.

We observed that breed was a factor affecting DSCC (Fig. 2). This is a relevant result as, to the best of our knowledge, only a limited number of studies compared DSCC of different breeds. The vast majority of papers investigating this trait, in fact, focused on the cosmopolitan HF (Schwarz et al., 2020a). Using 10 709 HF test-day records, Bobbo et al. (2019) found DSCC to average 62.07% with a SD of 16.91%. Here, the RE breed presented greater DSCC than HF and SI (Table 1, Fig. 2), in agreement with the findings of Bobbo et al. (2020). In that study, the average and SD of DSCC were 66.6 and 15.6% in RE, 61.8 and 16.7% in HF, and 61.6 and 16.6% in SI. Despite showing the highest level of SCC, JE was the breed with the lowest LSM of DSCC in the present study. Most likely due to physiological reasons, JE cows secrete a greater number of somatic cells in milk. Therefore, the number of polymorphonuclear neutrophils and lymphocytes has a reduced impact on the total number of somatic cells. Moreover, the JE breed had the greatest number of records with DSCC ≤ 65% even with high SCC (Supplementary Table S6).

In addition to breed, udder health indicators are influenced by temporary effects such as management, stage of lactation, parity, and season of sampling, whose effect is similar in all breeds (Sharma et al., 2011).

Both SCS and DSCC presented different patterns across lactations (Fig. 3). Regardless of the breed, SCS tended to be elevated the first days after calving due to the innate immune response and enhanced mammary gland defence mechanisms around calving (Sharma et al., 2011; Fig. 3). The SCS subsequently decreased,

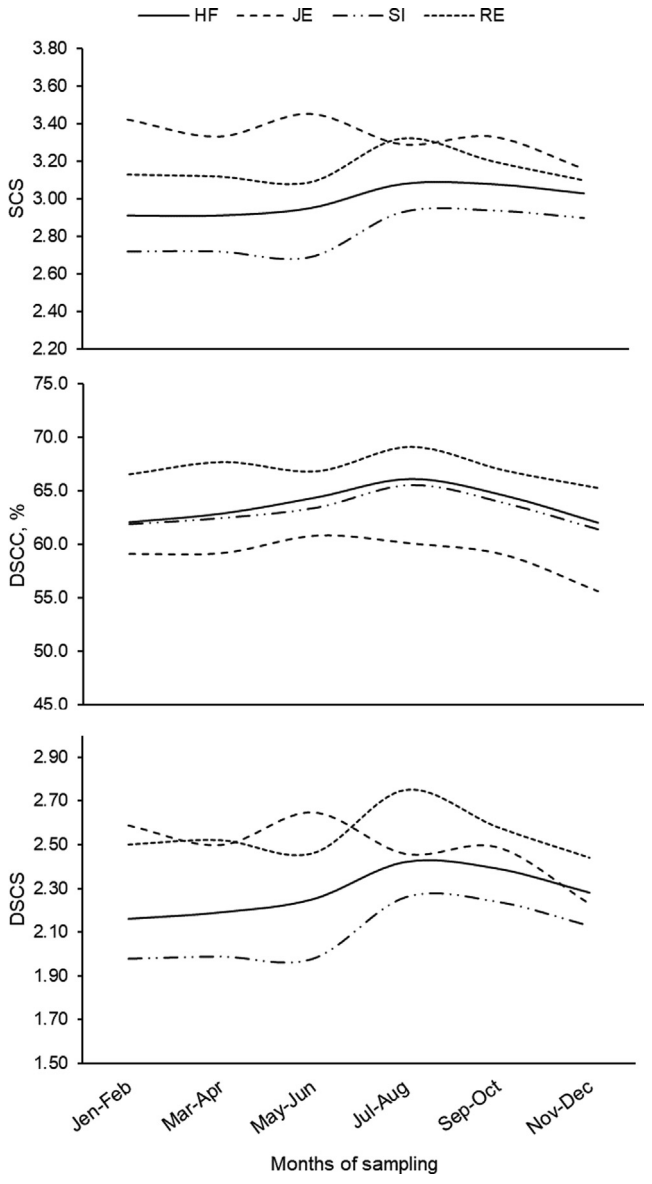


Fig. 4. Least squares means of udder health-related traits[1] for the fixed interaction effect between breed and sampling period ($P \leq 0.001$) in Holstein Friesian (HF), Jersey (JE), Simmental (SI) and Rendena (RE) cows. [1]SCS = somatic cell score, calculated as SCS = 3 + $\log_2$(SCC/100 000), where SCC is somatic cell count (cells/mL); DSCC = polymorphonuclear neutrophils and lymphocytes (%) out of the total SCC; DSCS = differential somatic cell score, calculated as DSCS = 3 + $\log_2$($DSCC_N$/100 000), where $DSCC_N$ is the concentration of polymorphonuclear neutrophils and lymphocytes in milk (cells/mL).

reaching a minimum at the peak of milk yield to thereafter finally, return again to elevated levels in late lactation due to a concentration effect (Fig. 3; Supplementary Fig. 3). Moreover, Suntinger et al. (2022) reported that the prevalence of cows with mastitis increased along lactation and reached the peak at the end of lactation. On the contrary, DSCC follows the lactation curve and seems to be less affected by the dilution effect (Kirkeby et al., 2020).

As parity increases, the number of secretory cells tends to augment, resulting in greater milk yield but also an increased exposure to major stress and thus a greater risk of intramammary infection (Schwarz et al., 2020a). This occurs in all breeds. According to Bobbo et al. (2019) and Zecconi et al. (2020), the lowest DSCC can be found in parity 2. To validate this, we calculated the frequency of records within the UHS groups for each parity: regardless of breed, the last parity class had the greatest number of records in UHS3 and UHS4. This was clearly evident in JE, where 20% of test-day records were UHS4 in cows of parity ≥ 4.

As also reported by Schwarz et al. (2020a), milk DSCC and SCS of all breeds included in the current study were higher in the warmer months likely due to exposure to heat. In the Po Valley, which is partly situated in the Veneto region, the area of this study, the average temperature recorded by a representative weather station was fairly high both years: 26.3 °C in August 2019 and 26.2 °C in August 2020 (night and day included; source: https://www.scia.isprambiente.it). Heat stress in dairy cattle is responsible for changes in both behaviour (reduced feed intake) and health condition (greater susceptibility to disease). Moreover, high temperatures coupled with high humidity are fertile grounds in summer for the proliferation of bacteria present in the environment, e.g., bedding material of housed stock (Sharma et al., 2011).

The conventional cut-off at 65% for DSCC was identified by Schwarz et al. (2020a) in HF breed from the combination of SCC and DSCC data to classify the UHS. Our findings suggest that the baseline and the phenotypic behaviour of DSCC differed among breeds (Fig. 2, Supplementary Table S7), and thus, the cut-off of DSCC may not be the same for HF, JE, SI and RE. However, we analysed milk data with no possibility to validate the results with health/clinical events such as veterinary observations of clinical/subclinical mastitis and other supporting data such as cultures and antibiograms (Halasa and Kirkeby, 2020). Only specific experimental trials can confirm that a single DSCC cut-off can be used across breeds. So far, dedicated studies have relied on HF data (Zecconi et al., 2019; Schwarz et al., 2020a) rather than data on dual-purpose and/or local breeds.

Effect of udder health on milk yield and composition

The second aim of this study was to evaluate how UHS affects cows' milk yield and composition. Specialised high-producing cows, like HF, are at higher risk of metabolic stress and mastitis compared to less selected populations (Oltenacu and Broom, 2010). The genetic correlations estimated between milk yield and mastitis in dairy cows support this concept (Costa et al., 2019b). It has been demonstrated that mastitis pathogens and immune response bacteriostatic and bactericidal factors are responsible for damages of the epithelium in the udder, particularly if mastitis is subclinical or recurrent (Zhao and Lacasse, 2008; Costa et al., 2020). This latent damage might further explain the reduced milk yield of cows in UHS4 which are expected to suffer from a chronic form of mastitis.

Intramammary infection causes an influx of leukocytes from the blood, which is accompanied by an alteration of the secretory function of cells. Moreover, due to a loss of epithelial permeability, the osmotic equilibrium of alveolar structures changes, consequently modifying milk composition (Ruegg, 2017). An increase in total fat content has been reported for cows with high milk SCC (Erwin and Randolph, 1975).

As reported by Bobbo et al. (2020), cows suspicious of intramammary infection - here UHS2 – are characterised by the lowest fat content. This probably happens as a result of a greater lipolytic activity stimulated by neutrophil recruitment. On the other hand, milk from UHS3 and UHS4 groups presented high content of fat due to a concentration effect resulting from low milk yield. The proteolytic activity that modifies the protein composition increases with high SCC, i.e. in the presence of mastitis. This reduces those proteins directly synthesised within the mammary gland (α-casein, β-casein, α-lactalbumin, and β-lactoglobulin), leaving space for the protein fractions transferred from blood (e.g., serum albumin and immunoglobulins; Haenlein et al., 1973). Therefore, the concentration of solids due to reduced milk

Table 3
Least squares means[1] (95% confidence intervals) of milk composition for the fixed interaction effect between breed and udder health status[2] (UHS) in Holstein Friesian, Jersey, Simmental and Rendena cows. Means with different superscript letters within a row are statistically significantly different ($P \leq 0.05$).

Trait[3]	Breed	UHS1		UHS2		UHS3		UHS4	
Protein, %	Holstein Friesian	3.39^{c}	(3.38; 3.39)	3.36^{d}	(3.36; 3.36)	3.41^{b}	(3.41; 3.41)	3.47^{a}	(3.47; 3.48)
	Jersey	3.93^{a}	(3.89; 3.97)	3.87^{b}	(3.82; 3.91)	3.94^{a}	(3.90; 3.99)	3.96^{a}	(3.91; 4.01)
	Simmental	3.52^{c}	(3.51; 3.53)	3.49^{d}	(3.48; 3.50)	3.54^{b}	(3.53; 3.55)	3.60^{a}	(3.59; 3.62)
	Rendena	3.35^{c}	(3.33; 3.37)	3.34^{c}	(3.32; 3.36)	3.40^{b}	(3.38; 3.42)	3.44^{a}	(3.41; 3.47)
Casein index	Holstein Friesian	0.7876^{b}	(0.7874; 0.7878)	0.7880^{a}	(0.7878; 0.7883)	0.7848^{c}	(0.7846; 0.7850)	0.7806^{d}	(0.7803; 0.7810)
	Jersey	0.7970^{a}	(0.7956; 0.7990)	0.7980^{a}	(0.7960; 0.7998)	0.7940^{b}	(0.7917; 0.7954)	0.7920^{b}	(0.7901; 0.7942)
	Simmental	0.7871^{a}	(0.7867; 0.7876)	0.7873^{a}	(0.7869; 0.7878)	0.7839^{b}	(0.7834; 0.7844)	0.7789^{c}	(0.7780; 0.7800)
	Rendena	0.7864^{a}	(0.7855; 0.7872)	0.7869^{a}	(0.7860; 0.7877)	0.7829^{b}	(0.7820; 0.7838)	0.7780^{c}	(0.7766; 0.7795)
Lactose, %	Holstein Friesian	4.82^{b}	(4.82; 4.82)	4.83^{a}	(4.82; 4.83)	4.75^{c}	(4.75; 4.75)	4.69^{d}	(4.68; 4.69)
	Jersey	4.74^{a}	(4.71; 4.76)	4.76^{a}	(4.73; 4.77)	4.64^{b}	(4.61; 4.66)	4.60^{c}	(4.57; 4.62)
	Simmental	4.79^{b}	(4.78; 4.79)	4.79^{a}	(4.78; 4.80)	4.70^{c}	(4.69; 4.70)	4.62^{d}	(4.61; 4.63)
	Rendena	4.86^{a}	(4.85; 4.87)	4.86^{a}	(4.86; 4.87)	4.78^{b}	(4.77; 4.79)	4.72^{c}	(4.70; 4.73)
BHB	Holstein Friesian	−1.327^{c}	(−1.332; −1.321)	−1.331^{d}	(−1.337; −1.325)	−1.291^{b}	(−1.297; −1.285)	−1.223^{a}	(−1.231; −1.215)
	Jersey	−1.488^{c}	(−1.524; −1.452)	−1.492^{c}	(−1.535; −1.449)	−1.375^{b}	(−1.415; −1.334)	−1.319^{a}	(−1.365; −1.272)
	Simmental	−1.308^{c}	(−1.318; −1.299)	−1.318^{c}	(−1.329; −1.307)	−1.265^{b}	(−1.276; −1.253)	−1.184^{a}	(−1.206; −1.163)
	Rendena	−1.294^{c}	(−1.313; −1.275)	−1.308^{c}	(−1.327; −1.288)	−1.272^{b}	(−1.292; −1.251)	−1.207^{a}	(−1.243; −1.170)

[1] SE ranged from 0.001 to 0.02 for protein, 0.0001 to 0.001 for casein index, 0.001 to 0.01 for lactose and 0.002 to 0.01 for BHB.
[2] Milk test-day records were classified according to somatic cell count (SCC) and differential SCC (DSCC, % = polymorphonuclear neutrophils and lymphocytes out of the total SCC) as follows: UHS1, if SCC ≤ 200 000 cells/mL and DSCC ≤ 65%; UHS2, if SCC ≤ 200 000 cells/mL and DSCC > 65%; UHS3, if SCC > 200 000 cells/mL and DSCC > 65%; UHS4, if SCC > 200 000 cells/mL and DSCC ≤ 65%.
[3] BHB = $\log_{10}$ transformation of β-hydroxybutyrate concentration in milk (mmol/L).

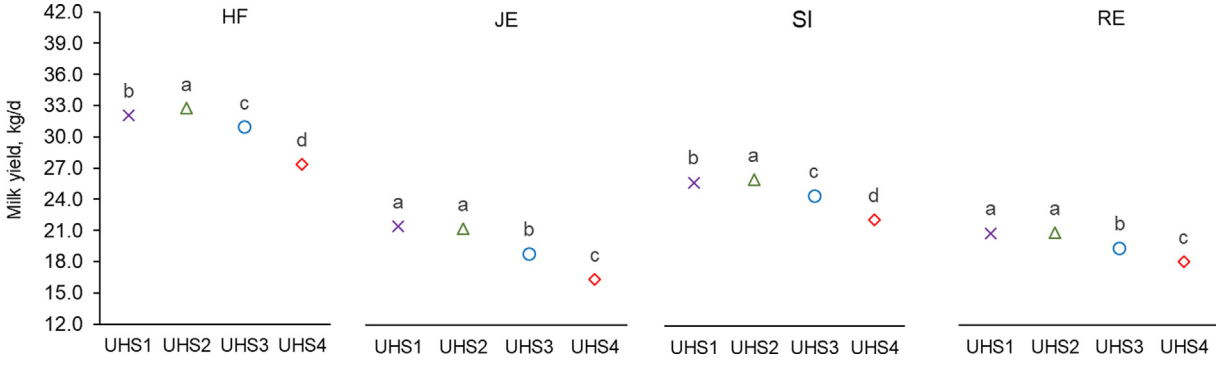


Fig. 5. Least squares means of milk yield for the fixed interaction effect between udder health status group[1] and breed in Holstein Friesian (HF), Jersey (JE), Simmental (SI) and Rendena (RE) cows. Means with different superscript letters within the breed are statistically significantly different ($P \leq 0.05$). [1]Milk test-day records were classified according to somatic cell count (SCC) and differential SCC (DSCC, % = polymorphonuclear neutrophils and lymphocytes out of the total SCC) as follows: UHS1, if SCC ≤ 200 000 cells/mL and DSCC ≤ 65%; UHS2, if SCC ≤ 200 000 cells/mL and DSCC > 65%; UHS3, if SCC > 200 000 cells/mL and DSCC > 65%; UHS4, if SCC > 200 000 cells/mL and DSCC ≤ 65%.

yield is also observed for the total protein content, but not in terms of fractions of interest. Supporting this, Mariani et al. (2022) observed the lowest casein index in cows with high SCC and low DSCC (UHS4), whereas the greatest index was reported for the UHS2 group. Lactose is the only solid of milk not subject to dilution or concentration effect; in agreement with Costa et al. (2020), cows with high SCC presented lower lactose content due to a leakage caused by compromised alveolar epithelial integrity (Herve et al., 2018; Costa et al., 2019a; 2020). Although further investigation into lactose is needed, reduction of this compound is expected to be greater in cows suffering from chronic mastitis due to the permanently compromised epithelial permeability of the mammary gland.

The association between cow's udder health and infrared-predicted milk BHB has been marginally investigated in a limited number of studies. We demonstrated that in all breeds, the concentration of this ketone body was the greatest in the UHS4 group, indicating the major risk of metabolic disorders in 'chronic' cows. This finding agrees with the results of Moyes et al. (2014) who observed that udder inflammation was associated with an increase in milk BHB. In general, cows suffering from mastitis and/or those with constantly high milk SCC throughout their productive life are more susceptible to metabolic disorders. Costa et al. (2019b) estimated positive genetic correlations between mastitis and common early lactation diseases (first 150 DIM), namely ketosis (0.36 ± 0.14) and milk fever (0.46 ± 0.10) in the Austrian Fleckvieh population.

Despite the largest part of variability of udder health indicators in milk was controlled by individual cow, they varied also according to the stage of lactation, parity, season of sampling and breed. In particular, cows belonging to SI breed had lower SCC compared to JE, HF and RE, and JE had the lowest DSCC. We also observed that in all breeds, milk yield and composition traits were affected by UHS. The monitoring of SCC and DSCC is useful for the management of udder health at both individual and herd levels, especially in proximity of the dry-off.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100823.

Ethics approval

Not applicable.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study are available from the corresponding author upon reasonable request.

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Declaration of interests

None.

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