J. Dairy Sci. TBC
https://doi.org/10.3168/jds.2024-25357
© TBC, The Authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association®.
This is an open access article under the CC BY license (https://creativecommons.org/licenses/by/4.0/).

Factors influencing somatic cell count and leukocyte composition in cow milk: a field study

S. Mondini,[1] G. Gislon,[1] M. Zucali,[1]* A. Sandrucci,[1] A. Tamburini,[1] and L. Bava[1]
[1]Department of Agricultural and Environmental Sciences, University of Milan, Milan, Italy 20133

ABSTRACT

In recent years, there has been a proliferation of studies investigating the composition of somatic cell count (SCC) of milk, focusing on neutrophils (NEU), lymphocytes (LYM), and macrophages (MAC). These 3 components are indeed crucial for the animal's immune response to mastitis-causing pathogens. The study examined various factors influencing somatic cell count and leucocyte components in cow milk, including lactation stage, parity, and milk electrical conductivity, using data from 179 dairy cows across 6 farms throughout the entire lactation. Statistical analyses, including mixed models and logistic regression, were employed to investigate the relationships between these variables and identify risk factors for high SCC levels. Results showed that factors such as parity and lactation stage were significantly associated with somatic cell composition. In particular, the highest milk NEU values (>60% of the total leucocytic fraction) and lowest MAC values (<20%) were found at the beginning and the end of lactation, that are the critical periods for udder health. High milk electrical conductivity, low milk production, number of parity, and poor hygiene scores were identified as contributing to increased SCC. Additionally, elevated percentages of NEU and LYM in milk were associated with increased risk of high SCC values, indicating potential udder health issues.
Keywords: Stage of lactation, parity, neutrophils, macrophages, lymphocytes, differential count

INTRODUCTION

Mastitis is one of the major problems in dairy herds worldwide, being one of the main causes of culling in dairy farms (De Vries and Marcondes, 2020). In response to mastitis, the udder brings back white blood cells against bacteria, leading to an increase of the milk somatic cell count (SCC) (Wall et al., 2018). To monitor and manage udder health, SCC milk concentrations at quarter, cow, and herd levels are widely used. In the last years, the evaluation of different types of cells that compose SCC has been rapidly expanding, enabling the differentiation between neutrophils (NEU), lymphocytes (LYM), and macrophages (MAC) in milk.

The number and distribution of leukocyte components, indeed, are crucial for the efficiency of udder defenses against invading pathogens. The main function of NEU is to defend the organism against bacteria in the early stage of an acute inflammatory process: their numbers can constitute more than 90% of the total mammary leukocyte population during mastitis (Sordillo and Streicher, 2002). On the other hand, LYM regulate the induction and suppression of immune responses, thanks to the identification of antigens through membrane receptors specific for pathogens (Sordillo and Streicher, 2002).

Additionally, MAC are active phagocytic cells and capable of ingesting bacteria, cellular debris, and accumulated milk components in the mammary gland. Milk or tissue macrophages recognize the invading pathogens and begin an immune response by the release of chemoattractants, inducing the rapid recruitment of NEU into the mammary gland (Oviedo-Boyso et al., 2007).

In many studies, the combination of NEU and LYM as a percentage of the total SCC was used as a complex indicator, known as differential somatic cell count (DSCC) (Damm et al., 2017). Swartz et al. (2020) and Kirkeby et al. (2021) described significant effects of endogenous factor as days in milk (DIM) and number of parity on DSCC trend.

The introduction of Precision Livestock Farming technologies could assist farmer to improve efficiency in different aspects of farm management. In particular, it could help in the identification of herd and individual health and welfare issues. The adoption of technology on dairy farms enables the collection of different kind of data, from different sources, that, with a proper integration, could aid to prevent mastitis, even in its subclinical form. Indeed, some studies showed that models using complex indicators and multiple data sources are most effective

Received June 28, 2024.
Accepted November 5, 2024.
*corresponding author: Maddalena Zucali, Via Celoria 2, 20133 Milan, Italy, +39 02503 16452 maddalena.zucali@unimi.it

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

for mastitis detection (van der Voort et al., 2021; Zucali et al., 2021; Lardy et al., 2023).

The aim of the present study was to analyze the relations between number of parity, stage of lactation and various milk production parameters (milk yield, electrical conductivity and milking duration) with somatic cell count (SCC) and its 3 leucocyte components (neutrophils, lymphocytes and macrophages) to better understand the sources of variation. Additionally, the risk factors associated with high milk SCC at the cow level (≥100,000 cells/ml) were investigated. The novelty of the study lies in the analysis of milk samples collected from the same cows throughout lactation, using a rapid and portable instrument to measure leucocyte types (neutrophils, lymphocytes and macrophages).

MATERIALS AND METHODS

Farm description

In the present study, 6 farms (farm A, B, C, D, E, F) located in Northern Italy were involved. The lactating cows were housed indoor throughout the year on cubicles with straw or compost materials, or with mattresses and straw, without access to pasture. Farms were equipped, in the lactating dairy cow housing area, with fans or fans and showers as cooling systems. Cows were milked twice a day (with the exception of farm C with 3 milkings) in milking parlors (parallel or herringbone) and the milking routine was different for each farm. However, all the farms had in common the use of individual towels for udder cleaning and they all performed fore-stripping. Five out of the 6 farms used post-dipping products. In all 6 farms, lactating cows were fed a total mixed ration, once a day, after the morning milking. The average daily dry matter intake was 24.0 ± 1.4 kg, with the diet consisting of 47 ± 3% forages. The total mixed ration was characterized by 15 ± 1% of crude protein and 31 ± 4% of neutral detergent fiber.

Experimental unit

The study was performed between March 2022 and January 2023. Initially, the experimental unit was composed by 179 Holstein cows from the 6 farms (from 23 to 37 cows per farm) (Table 1), the choice of the farm were done on the base of the willingness to be involved the study and on the farm characteristics for example the presence of milking parlor. A total of 67 primiparous, 59 secondiparous and 53 multiparous cows were monitored throughout the entire lactation, from calving from calving to 1 d before dry-off. On the average, the last milk sample was collected at 272 ± 22 Days In Milk (DIM). During each farm visit, individual udder milk samples, visual assessment of animals cleaning and milking parameters data were collected. All the evaluations and milk collection were performed on each farm 5 times per cow, throughout the entire lactation, at regular time intervals of 60 d (Figure 1), for a total of 848 samples. The 2.5% (n = 21) of the samples were collected within the 10 d before or after a mastitis diagnosis. The fifth sample was collected only from 152 cows, because the remaining 27 cows had already been dried-off.

Milk analyses

Milk collection was carried out during the afternoon milking using a milk sampler applied after the cluster, ensuring that the sample was representative of the entire udder (including both cisternal and alveolar milk, excluding foremilk) and the entire milking session. All samples were stored at 4°C and analyzed within one hour on the following day (within a 14-h window from collection) using the Vetscan DC-Q Milk Analyzer (AAD Advanced Animal Diagnostics, NC, USA) and the DeLaval Cell Counter (DeLaval, Tumba, Sweden). The Vetscan DC-Q Milk Analyzer uses a differential count method and provides the concentration as count (cells/ml) and the percentage (%) of the 3 components (NEU, MAC and LYM) on the total leucocyte count (TLC). The TLC is defined as the sum of the 3 somatic cell types (Mondini et al., 2023) or as SCC without epithelial cells (Lozada-Soto et al., 2020) and is expressed as cells/ml. The sensibility of Vetscan permits to establish exact percentage between 2 to 95%. Values higher than 95% was rounded to as 98%, and values lower than 2% was expressed as 1%, to have always the sum of the 3 percentage that is 100%. Moreover, the DeLaval Cell Counter provides the SCC (cells/ml). Milk collection was carried out during the afternoon milking using a milk sampler applied after the cluster, ensuring that the sample was representative of the entire udder (including both cisternal and alveolar milk, excluding foremilk) and the entire milking session. All samples were stored at 4°C and analyzed within one hour on the following day (within a 14-h window from collection) using the Vetscan DC-Q Milk Analyzer (AAD Advanced Animal Diagnostics, NC, USA) and the DeLaval Cell Counter (DeLaval, Tumba, Sweden).

Table 1. Number of cows involved in the study for each farm

Farm	Numbers of cows
A	26
B	23
C	36
D	37
E	32
F	25

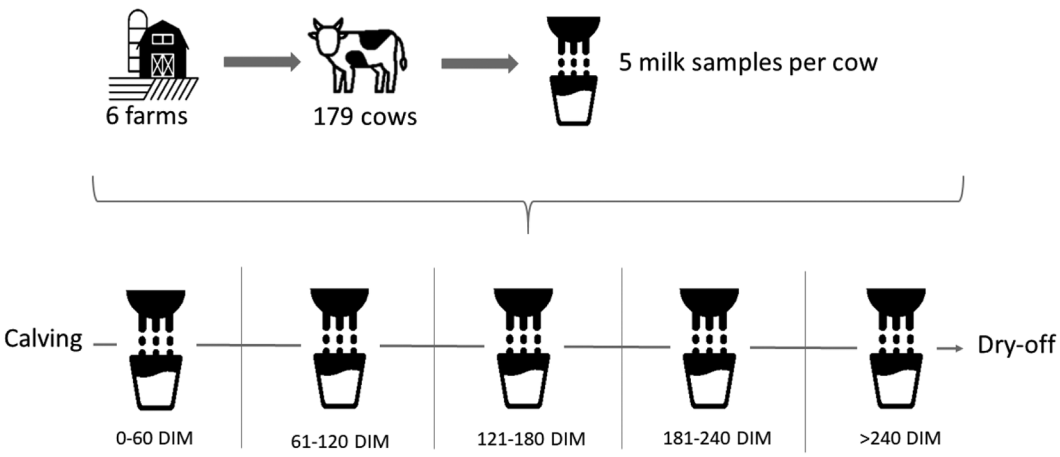

Figure 1. Experimental unit and timing of samples

Milking parameters and Hygiene Scores

Data on milk production per milking (kg) and daily milk production (kg), were obtained from the milking parlor software (Afimilk Agricultural Cooperative Ltd., Kibbutz Afikim, Israel) in all farms. Only 4 farms (farm A, B, C, F) were equipped with sensors in the parlor for the detection of milking duration (s) and milk electrical conductivity (mS/cm^2).

The cleanliness conditions of the cow (udder, legs and flank) were evaluated using a 4-point Hygiene Score (HS) chart (1 very clean - 4 very dirty) (Cook and Reinemann, 2007). In the final analysis, each body area was categorized as 'clean' if HS was ≤2; otherwise, it was considered 'dirty'. The assessment of HS was conducted by 2 trained evaluators before attaching the cluster on the days of milk sample collection. A training period was performed by researchers, before the beginning of the experimental trial.

Environmental parameters

Temperature (T, °C) and Relative Humidity (RH, %) were collected every 15 min on all the 6 farms using HOBO data loggers (Onset Computer Corporation, Pocasset, MA). The sensors collected data from March 2022 to December 2022. Environmental sensors were located 2 m from the ground in the resting area of lactating dairy cows. The Temperature and Humidity Index (THI) was calculated with the formula reported by Segnalini et al. (2013):

$$THI = (1.8 * T + 32) - (0.55 - 0.55 * (RH / 100)) * ((1.8 * T + 32) - 58)$$

T = Temperature; RH = Relative Umidity.

Moreover, hours with an average THI > 68, calculated from hourly recordings (at 00, 15, 30 and 45 min), were classified as hot, according to the study of Zimbelman et al. (2009) which suggested that cows could experience heat stress under these conditions. The total number of hours with THI > 68 was calculated for each day and, subsequently, the mean number of daily hot hours was computed for each month.

Statistical analysis

Normalization of data, distribution and relationships between variables. To perform a statistical analysis of the data set, data about SCC and TLC count were normalized using a $\log_{10}$ transformation. Data on NEU, MAC and LYM were used both as percentage and as count; the count underwent a $\log_{10}$ transformation before analysis. Skewness and kurtosis were used for the description of the data distributions. All statistical analyses were performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). A Pearson correlation matrix between variables was performed with PROC CORR.

Mixed model. The effect of lactation stage and parity on SCC and leucocyte components was assessed using a mixed model with repeated measurements. The model, referred to as Model 1, evaluated SCC (log10 cells/ml), TLC (log10 cells/ml), NEU (log10 cells/ml, % of TLC), LYM (log10 cells/ml, % of TLC), and MAC (log10 cells/ml, % of TLC), as well as daily milk production (kg), milking time (s), and milk conductivity (mS/cm) of the cows. The analysis considered different stages of lactation and parity numbers.

$$Y_{ijklmn} = m + Stage_i + Parity_j + Farm_k + LAC\text{-}TLC_l + SP_{ij} + Cow(Farm*Parity)_m + e_{ijklmn} \quad (1)$$

Where:

- Y_{ijkl} = dependent variables (SCC, TLC, NEU, LYM, MAC, daily milk production, milking time and milk electrical conductivity)
  m = average of dependent variables
- $Stage_i$ = stage of lactation divided for days in milk (1–60 DIM, 61–120 DIM, 121–180 DIM, 181–240 DIM and > 240 DIM, i = 1–5)
- $Parity_j$ = number of parity (primiparous, secondiparous and multiparous, j = 1–3)
- $Farm_k$ = farm involved in the study (Farm A, B, C, D, E, F, k = 1–6)
- $LAC\text{-}TLC_l$ = Lactation average TLC (< = 5 $\log_{10}$ cells/ml, > 5 $\log_{10}$ cells/ml, l = 1–2)
- SP_{ij} = interaction between Stage of lactation and number of parity
- $Cow(Farm*Parity)_m$ = cow as subject repeated effect
- e_{ijklmn} = error

In the mixed model, the Lactation Average TLC (LAC-TLC) for each cow was included as a fixed effect. The LAC-TLC was calculated as the average of the $\log_{10}$ TLC of the 5 milk samples collected during lactation. Cows were divided into 2 LAC-TLC groups: cows with udder health issues throughout entire lactation (average > 5 $\log_{10}$ cells/ml) and cows without apparent persistent udder health issues (average < = 5 $\log_{10}$ cells/ml).

The season effect was not added in the last model as fixed effect, due to the lack of statistical significance for all dependent variables. This decision was made because of the experimental design of the study, which resulted in an overlap between the season effect and the stage of lactation effect. This overlap occurred because all the farms were monitored simultaneously.

Comparison of sample group and subsample group. In this study only 4 farms were equipped with milking sensors (referred to as 4FG group) and 500 milk sample were collected. So, to analyze and make assumption on this data set that could be generalized to the total cow sample (179 cows), a comparison between these 2 groups was performed to investigate if there are significant differences for all the variables or if the subgroup is similar to the total sample group. The comparison was performed using Mixed Model 1, with Sample $Group_n$ (total cow sample, 4FG group, n = 1–2) as an additional fixed effect. The results highlighted no significant differences ($P > 0.5$) for all variables between the 2 groups and this highlighted that the 4FG subgroup was similar to the total cow sample.

Factor analysis. Multivariate factor analysis was performed on the 4FG group using PROC FACTOR. The number of factors was reduced to 4 (Cells as count, Neutrophils and macrophages, Endogenous factors, Lymphocytes), accounting for 76% of the variability. The Measure of Sampling Adequacy was calculated and the Harris-Kaiser rotation was applied using the VARIMAX method with a power equal to 1. Only Rotated Factor Patterns (Standardized Regression Coefficients) > 0.50 (absolute value) were considered. More details of the method were reported in Conte et al., 2021.

Risk factors for high SCC. A risk factor analysis of having high SCC (>5 $\log_{10}$ cells/ml) was performed on the 4FG group using a logistic regression (PROC LOGISTIC), with stepwise selection and an entry criterion of $P < 0.20$. Dependent classes entered into the model were:

- Stage of lactation (1–60 DIM, 61–120 DIM, 121–180 DIM, 181–240 DIM and > 240 DIM)
- Number of parity (primiparous, secondiparous and multiparous)
- NEU (<63 and ≥ 63%)
- LYM (<14 and ≥ 14%)
- MAC (<18 and ≥ 18%)
- Average HS (average values of flank, legs and udder, ≤ 2 and > 2)
- Milk electrical conductivity (≤8.8 and > 8.8 mS/cm^2)
- Individual daily milk production (<35 and ≥ 35 kg/day)

The threshold definitions for ranges were established using the median values for NEU, LYM, MAC, milk electrical conductivity, and individual daily milk production. For the other variables thresholds were based on sampling time (stage of lactation) or the most commonly used classification in literature (number of parity and average HS) were used.

Dependent variables not entered into the model ($P > 0.20$) were milking duration, milk production per milking, average milk flow, HS udder, HS legs and HS flank.

RESULTS

Sample description

Table 2 presents health milk indicators as well as milking performance measurements. Data appeared normally distributed.

Hygiene Score for flank, legs and udder, were calculated and legs were found to be the dirtiest area monitored (59% clean *vs* 41% dirty). Udder and flank were clean in the 62% and 82% respectively. Hygiene Score for flank, legs and udder during lactation were reported in Figure 2. The mean HS, calculated across all the 3 areas, was calculated per each cow observation: 50% of the animals

Table 2. Main indicators of data distributions for each variable

	N	Mean	Median	Minimum	Maximum	Std Dev	Skewness	Kurtosis
Somatic Cell Count (log_{10} cells/ml)	848	4.87	4.81	3.00	6.64	0.67	0.30	−0.43
Total Milk Leucocyte Count (TLC) (log_{10} cells/ml)	848	4.97	4.89	3.95	7.00	0.58	0.63	0.00
Neutrophils (log_{10} cells/ml)	848	4.67	4.61	1.95	6.88	0.76	−0.38	1.58
Lymphocites (log_{10} cells/ml)	848	3.99	4.08	1.95	6.15	0.85	−0.60	−0.05
Macrophages (log_{10} cells/ml)	848	4.14	4.26	1.95	6.18	0.74	−1.02	1.32
Neutrophils (% TLC)	848	58	64	1	98	21	−0.81	0.49
Lymphocites (% TLC)	848	17	14	1	98	14	1.93	6.75
Macrophages (% TLC)	848	25	19	1	98	22	1.26	1.36
Individual milk production (kg/d)	832	35.7	34.5	11.5	66.7	10.3	0.39	−0.13
Milk electrical conductivity (mS/cm^2)	613	9.27	9.20	6.20	13.70	1.16	0.58	1.07
Milking duration (s)	510	361	340	100	1140	121	1.81	6.44

appeared clean (HS mean ≤2) while the others 50% were classified as dirty (HS mean >2). In particular, 2 farms had less than 25% of dirty animals (HS mean >2) and other 2 farms had more than 60% of dirty animals.

Average, maximum and minimum THI trends for each month and the monthly mean of number of daily hours with THI ≥ 72 for each farm were calculated. During the summer months (June, July and August), the mean THI was always above 72, with maximum THI above 76. In July the minimum THI exceeded 71 and the maximum THI approached 79. Conversely, during the winter months (November and December) the mean THI was 52 and 46 respectively. In all the farms, the number of daily hours with THI ≥ 72 peaked from June to July (ranging from 22 to 23 h) and then decreased in October at 3–7 h. In farm B, the daily number of hours with THI ≥ 72 appeared lower than in the other farms, with maximum peak in June and July with 18 h.

Relationships among variables

Pearson correlation matrix among the monitored variables is reported in Table 3. The analysis highlighted a strong correlation between SCC and TLC (r = 0.88). The counts of NEU, MAC and LYM were positive associated with SCC (r > 0.70 except for MAC, r = 0.58) and TLC (r > 0.70), but the correlation between MAC and LYM counts was relatively weak (r = 0.41). The percentages of MAC and NEU showed a strong negative association; conversely, the correlations between NEU percentage and LYM percentage, and MAC percentage and LYM percentage were poor (r ≤ 0.4).

The correlations between milking parameters of the 500 samples of the 4FG farms subgroup indicated that milk conductivity was positively associated with NEU (%) and negatively associated with MAC (%) and LYM (%), although r values were low (<0.15). On the other hand, the correlation coefficients between milk conduc-

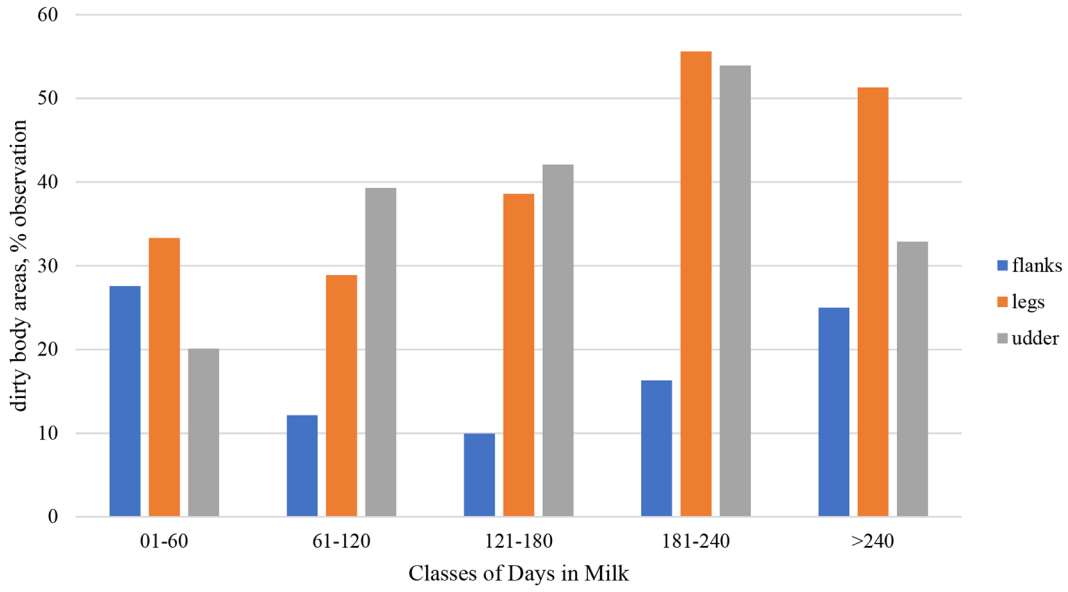

Figure 2. Trends of dirty body areas (flank, legs and udder) expressed as percentage of the total observations during lactation

ARTICLE IN PRESS—UNCORRECTED PROOF

tivity and SCC, as well as conductivity and TLC were around 0.2. Milking duration showed a positive correlation with milk production (r = 0.36) and weak negative correlations with all somatic cell types with r ranging from −0.16 to 0.10, as reported in Table 3 also for individual milk production.

Effect of stage of lactation and parity on SCC and leucocyte components

The tendencies of milk immune cell types were analyzed for DIM classes (Figure 3). The results for cell counts showed an increase after 120 DIM, for TLC (from 4.9 to 5.1 $\log_{10}$ cells/ml) ($P < 0.0001$) and NEU (from 4.6 to 4.8 $\log_{10}$ cells/ml) ($P = 0.003$), while LYM increased after 180 DIM ($P < 0.0001$). Conversely, MAC count varied across DIM classes with values of 3.8, 4.0, 4.5, 4.4 and 4.1 $\log_{10}$ cells/ml for the classes 1–60, 61–120, 121–180, 181–240 and > 240, respectively ($P < 0.0001$).

The percentages of NEU and MAC exhibited opposite trends. The percentage of NEU reached the lowest values in the 121–180 and 181–240 DIM classes (55.6 and 55.4%, respectively), while the highest values were observed in the extreme classes, 1–60 and > 240 DIM (64.3 and 62.2%, respectively) ($P < 0.0001$). Conversely, MAC percentage increased from the first class of DIM (17.3%) to the 121–180 and 181–240 DIM classes (31.7 and 30.0%, respectively), followed by a decreasing trend thereafter (17.0%) ($P < 0.0001$). The trend of LYM percentage was similar to that of NEU, with the highest values around 18–21% and the lowest values around 13–15% ($P < 0.0001$).

The effect of parity is reported in Table 4. With the increase of number of parity, there was a rise in SCC, TLC, NEU and MAC expressed as count, although statistically significant difference was only observed between primiparous cows and the other 2 groups (secondiparous and multiparous). Lymphocytes as count, NEU and MAC percentages did not change across the different groups of parity, but LYM, expressed as percentage, decreased with increasing parity number. As expected, there was an increase of milk conductivity, individual milk production, and consequently milking duration with the increase of parity number.

Moreover, cows with udder health issues (Lactation Average TLC, LAC-TLC > 5 $\log_{10}$ cells/ml) had higher values of TLC, SCC, NEU count, LYM count and MAC count than healthy cows (LAC-TLC ≤ 5 $\log_{10}$ cells/ml): 4.57 *vs* 5.28, 4.70 *vs* 5.33, 4.32 *vs* 5.15, 3.64 *vs* 4.46 and 3.94 *vs* 4.41 $\log_{10}$ cells/ml, respectively ($P < 0.0001$). At the same time NEU percentage was higher in problematic cows than in healthy cows (66.2 *vs* 52.7%; $P < 0.0001$) while MAC percentage was lower (30.4 *vs* 17.4%; $P < 0.0001$). Percentage of LYM was not statistically dif-

Table 3. Pearson correlation matrix between the three leucocyte components (milk neutrophils - NEU, milk lymphocytes - LYM - and milk macrophages - MAC), total milk leucocyte count (TLC), milk somatic cell counts (SCC) and milk production

	NEU (% TLC)	LYM (% TLC)	MAC (% TLC)	Individual milk Production (kg/day)	SCC ($\log_{10}$ cells/ml)	TLC ($\log_{10}$ cells/ml)	NEU ($\log_{10}$ cells/ml)	LYM ($\log_{10}$ cells/ml)	MAC ($\log_{10}$ cells/ml)
NEU (% TLC)	1								
LYM (% TLC)	−0.26	1							
MAC (% TLC)	−0.78	−0.40	1						
Individual milk production (kg/day)	−0.02	−0.05	0.05	1					
SCC ($\log_{10}$ cells/ml)	0.44	−0.12	−0.35	−0.10	1				
TLC ($\log_{10}$ cells/ml)	0.39	−0.11	−0.30	−0.13	0.88	1			
NEU ($\log_{10}$ cells/ml)	0.67	−0.17	−0.52	−0.11	0.82	0.91	1		
LYM ($\log_{10}$ cells/ml)	0.26	0.41	−0.51	−0.16	0.71	0.81	0.74	1	
MAC ($\log_{10}$ cells/ml)	−0.11	−0.40	0.36	−0.09	0.58	0.71	0.53	0.41	1

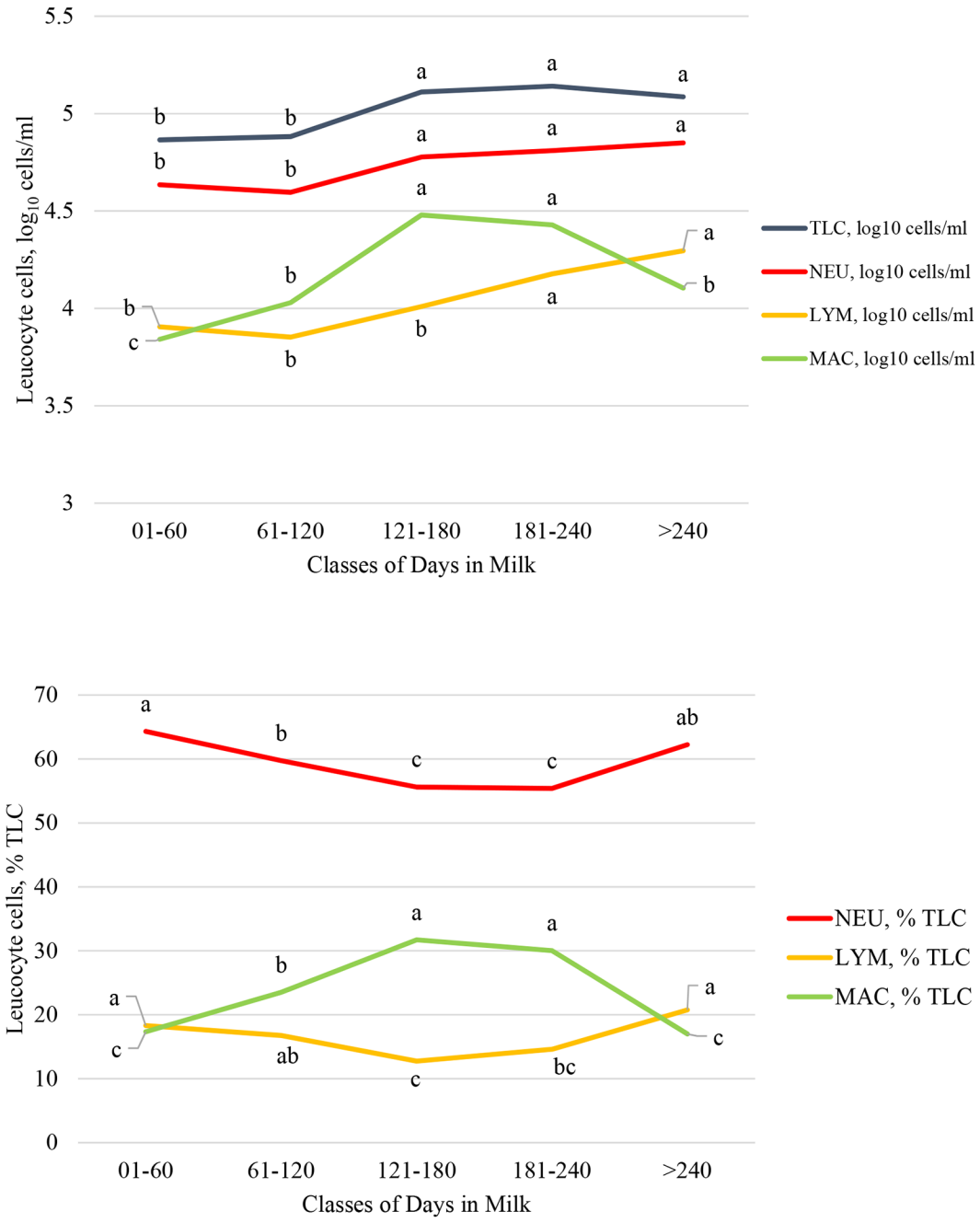

Figure 3. Trends of total milk leucocyte count (TLC), neutrophils (NEU), lymphocytes (LYM) and macrophages (MAC), the latter 3 expressed both as counts/ml and percentage of TLC for different classes of Days in Milk. Different letters expressed statistically difference between the stages of lactation. The letters are referred singularly to each variable.

ferent between the 2 groups of cows (around 16–17%) nor did milk production, milk electrical conductivity and milking time. The Farm effect resulted statistically significant only for MAC, expressed both as count ($P = 0.03$) and as percentage ($P = 0.001$), and for milking parameters (milk production, milk conductivity and milking duration) ($P < 0.0001$).

Factor analysis

In Table 5, the rotated factor patterns (Standardized Regression Coefficients) for each variable are presented. The variables are associated with each factor based on the higher absolute rotate factor pattern of the 4 presented in the table. In particular, SCC, TLC and leucocyte components (expressed as count) were grouped in Factor 1.

Table 4. Effect of parity on somatic cell count (SCC), Total milk leucocyte count (TLC), milk neutrophils (NEU), milk lymphocytes (LYM), milk macrophages (MAC), milk production, milk conductivity, milking time (LS means)

	Primiparous cows	Secondiparous cows	Multiparous cows	SE	*P model*	*P* 1–2	*P* 1–3	*P* 2–3
SCC (log_{10} cells/ml)	4.82	4.94	5.01	0.03	0.0004	0.01	0.0001	0.16
TLC (log_{10} cells/ml)	4.94	5.03	5.08	0.03	0.001	0.01	0.0003	0.20
NEU (log_{10} cells/ml)	4.64	4.73	4.83	0.04	0.003	0.09	0.001	0.07
LYM (log_{10} cells/ml)	4.01	4.07	4.07	0.05	0.53	0.33	0.34	0.99
MAC (log_{10} cells/ml)	4.01	4.23	4.29	0.04	<0.0001	<0.0001	<0.0001	0.26
NEU (% TLC)	59	58	60	1.2	0.51	0.56	0.54	0.25
LYM (% TLC)	18	17	14	0.9	0.004	0.19	0.001	0.04
MAC (% TLC)	22	25	25	1.2	0.15	0.13	0.07	0.72
Individual milk production (kg/d)	31.0	37.2	38.9	0.4	<0.0001	<0.0001	<0.0001	0.01
Milk electrical conductivity (mS/cm^2)	8.96	9.37	9.65	0.7	<0.0001	<0.0001	<0.0001	0.002
Milking duration (s)	348	366	386	9.9	0.02	0.14	0.004	0.11

In the columns of orthogonal contrasts: 1 = primiparous cows; 2 = secondiparous cows; 3 = multiparous cows.

Factor 2 was associated with NEU and MAC expressed as percentage, while LYM (%) was the only exponent of Factor 4. Factor 3 grouped milk production, milking time, parity and days in milk, while milk conductivity was not associated with any of the Factors.

Risk factors of high milk SCC

In Table 6, the results of logistic analysis, used for the detection of risk factors associated with high SCC level (>100.000 cells/ml), are presented. The concordant observations accounted for 80.4% of total ones. The increase in number of parity (multiparous) showed a greater risk of increasing SCC, as evidenced by the odds ratio lower than 1 of the others 2 classes of parity. The higher risk of lactation stage was between 121 and 180 DIM. High NEU (>63%), low MAC (≤18%) and high LYM (>14%) in milk, increased the possibility of observing an increase in SCC in milk. Clean cows (HS ≤ 2) had lower risk of having high SCC, as cows with low milk electrical conductivity (≤8.8 mS/cm^2) and high milk production (≥35 kg/day).

DISCUSSION

Sample description

The average milk production level of the cows involved in the study was higher than the average values in Northern Italy (>35 kg/d) (Bellingeri et al., 2019) and the average low level of SCC suggested a general good mammary condition of the cows involved in the study (SCC and TLC < 100,000 cells/ml) (Cobirka et al., 2020). However, significant variations in the leucocyte composition were observed in this study. The average NEU (%) was comparable to the concentration reported by Damm et al. (2017) and the LYM (%) with those presented by Alhussien et al. (2015). On the other hand, the range of MAC and NEU percentages (67% and 19%, respectively), reported in the research of Alhussien et al. (2015), were very different from the findings of this study. Our results are in contrast with those reported by Pilla et al. (2013), who found the percentages of 43% and 30% for NEU and LYM, respectively. Conversely, the MAC percentage is consistent with our findings. The results reported in all these studies are widely different with regard to the 3 immune cell fractions in milk. One possible explanation could be due the different milk analyzed (cisternal or alveolar milk) (Sarikaya et al., 2005; Sarikaya and Bruckmaier, 2006) or the method used for detection. As suggested by Leiner et al. (2000), different methods for the cell differentiation in milk (e.g., light microscopy and flow cytometry) could yield different results.

Moreover, after 2017 in most of the studies leucocyte components were expressed as differential somatic cell count (DSCC), that is the sum of NEU and LYM expressed as percentage of SCC. However, the use of DSCC in other studies allowed us to compare MAC concentration with our data, by calculating MAC percentage even if it is not directly reported in the previous studies, by subtracting DSCC to 100%. The percentage of MAC, found in the present study, was lower than that presented by Bisutti et. (2022) (around 31%) and higher than that reported by Farschtschi et al. (2022) (around 10%). This difference could be explained by the different instrument used or perhaps due to the different breeds involved in the study (Magro et al., 2023; Stocco et al., 2023). Average milk electrical conductivity was higher than that presented by Norberg et al. (2004) but similar to the findings reported by other authors (Gaspardy et al., 2012; Jensen et al., 2015; Paudyal et al., 2020). On the other hand, milking duration appeared similar to those presented by Tamburini et al. (2010), indicating the correct use of milking machine by farmers, without overmilking. The HS was used to describe the cleanliness of the cows in

Table 5. Rotated factor patterns (Standardized Regression Coefficients) of variables. SCC = somatic cell count; TLC = Total milk leucocyte count; NEU = milk neutrophils; LYM = milk lymphocytes; MAC = milk macrophages

	Factor1	Factor2	Factor3	Factor4
	Cells as count	Neutrophils and macrophages	Endogenous factors	Lymphocytes
Parity	0.256	−0.174	**0.545**	−0.145
Days in milk	0.277	−0.261	**-0.594**	−0.094
NEU (% TLC)	0.252	**0.916**	0.022	−0.279
LYM (% TLC)	−0.048	−0.011	−0.017	**0.989**
MAC (% TLC)	−0.211	**-0.878**	−0.009	−0.388
milk production	−0.156	−0.032	**0.805**	−0.014
milk electrical conductivity	0.296	0.0005	0.466	−0.137
milking time	−0.179	0.103	**0.575**	−0.029
SCC	**0.890**	0.228	−0.0161	−0.027
TLC	**0.960**	0.147	−0.058	−0.012
NEU count	**0.850**	0.459	−0.039	−0.104
LYM count	**0.809**	0.167	−0.084	0.517
MAC count	**0.764**	−0.455	−0.069	−0.312

the different farms. Fifty percent of cows appeared clean (based on the average score of flanks, legs and udder), but almost 40% of the udder were dirty indicating a need for improvement. The dirt on teats increases the probability of pathogen penetration through the teat canal and the consequent risk of mastitis (Schreiner and Ruegg, 2003). The hygiene levels of the herds were similar to ones reported by Sandrucci et al. (2014).

Regarding the mean values of THI during summer season, they were higher than 68, the threshold value to identify heat stress in dairy cows, according to Zimbelman et al., 2009. Moreover, in July, even the minimum THI was higher than the cut-off value of 68. The use of fans and water sprinklers, that were present in all 6 farms, was not enough to contrast the extremes temperatures during that period. The situation was further

Table 6. Risk factors of having high Somatic Cell Count (SCC) (≥100,000 cells/ml) in milk

Effect	Coefficient	Standard Error	*P*	Odds ratio Point Estimate	Odds ratio 95% Confidence Limits	
Intercept	−0.78	0.11	<0.0001			
Parity						
Primiparous cows	−0.27	0.15	0.08	0.50	0.30	0.85
Secondiparous cows	−0.14	0.14	0.30	0.57	0.35	0.92
Multiparous cows	Referent					
Stage of lactation						
1–60	−0.62	0.20	0.00	0.47	0.24	0.91
61–120	−0.40	0.21	0.05	0.58	0.29	1.17
121–180	0.73	0.23	0.00	1.81	0.89	3.70
180–240	0.16	0.20	0.43	1.02	0.55	1.88
>240	Referent					
Milk electrical conductivity						
≤8.8 mS/cm^2	−0.34	0.11	0.00	0.50	0.32	0.78
>8.8 mS/cm^2	Referent					
Daily milk production						
<35 kg	0.21	0.12	0.10	1.51	0.93	2.45
≥35 kg	Referent					
Milk neutrophils						
≤63%	−0.93	0.13	<0.0001	0.16	0.09	0.26
>63%	Referent					
Milk lymphocites						
≤14%	−0.22	0.11	0.05	0.65	0.42	0.99
>14%	Referent					
Milk macrophages						
≤18%	0.22	0.13	0.10	1.54	0.91	2.60
>18%	Referent					
Average Hygiene score						
≤2	−0.29	0.10	0.00	0.56	0.37	0.83
>2	Referent					

exacerbated by the absence of cooling hours during the summer days. An exception was Farm B, that is located near the mountains.

Relationships between variables

The results of the study highlighted that there was a positive relation among the increase in TLC or SCC and the increase in the count of the 3 leucocyte components, although the proportions vary for each of them. In particular, a strong correlation emerged between SCC/TLC and leucocyte components, as reported in a previous study (Mondini et al., 2023), that considered the level of somatic cell count at the beginning and at the end of lactation. The percentage of NEU was moderately correlated with TLC or SCC, as reported by Damm et al. (2019) and Mondini et al. (2023). On the other hand, the NEU percentage was strongly correlated with MAC percentage while the LYM percentage was not strongly correlated with other variables. The negative correlation between somatic cells and milk production was reported also by Magro et al. (2023) and could be due to the dilution factor in healthy cows or/and it could be due to a loss of mammary function in milk production caused by subclinical mastitis.

Effect of stage of lactation and parity on SCC and leucocyte components

In the international literature (since Wiggans and Shook, 1987), the highest values of milk somatic cells was detected during the first (Hagnestam-Nielsen et al., 2009) and last days of lactation (Cardozo et al., 2015), due to the heightened susceptibility of udder to mastitis. In fact, during the first stage of lactation, cows experience stress post-calving with increased risk of mastitis (Steeneveld et al., 2008). Additionally, improper management during the dry period is a risk factor for contracting mastitis before or during the onset of lactation (Winder et al., 2019; Cattaneo et al., 2023). Conversely, in the last days of lactation, cows are often subjected to over-milking (Sandrucci et al., 2014) or to prolonged exposure to incorrect vacuum system pressure (Mein et al., 2003), with the damage of the teats and the formation of hyperkeratotic rings at the teat end, that increase the risk of mastitis events (Cardozo et al., 2015).

In this study, different trends were observed, with the increase of TLC after 120 DIM. This may be partly attributed to the generally favorable mammary sanitary conditions of the cows involved in the study, as indicated by the low percentage (only 2.5%) of milk samples collected during mastitis events. Additionally, the previous studies focused on SCC rather than TLC, so the absence of epithelial cells in TLC could explain the different trend, because epithelial cells increased at the end of lactation. The lower values of TLC and NEU count, before and during the peak of lactation could be attributed also to the dilution effect of milk production (Green et al., 2006) or due to the better functionality of healthy udder. Moreover, the increase of LYM count occurred later than that of NEU count, after 180 d, possibly indicating an immune response. MAC count increased similarly to NEU, but in the last days of lactation there was a decrease, in contrast with the proliferation of LYM.

The percentages of the 3 fractions exhibited different trends throughout lactation. Although TLC did not follow a trend similar to that reported in the literature for SCC (Wiggans and Shook, 1987), NEU percentage showed a trend closely resembling it, with higher values at the beginning and at the end of lactation. In addition, LYM percentage had the same curve of NEU, although more flattened. The trend of DSCC (sum of NEU and LYM), reported in some studies, could be reported to NEU and LYM, individually. Although DSCC trends during lactation were different across the various studies (Moore et al., 2023; Stocco et al., 2023) and in some studies no discernible trend was detected (e.g., Kirkeby et al., 2020), Schwarz et al. (2020) reported higher values of DSCC at the beginning of lactation and at the end of lactation, similarly to NEU and LYM in this study. The percentage of MAC had the opposite trend of NEU, possibly due to the different activities of the immune cell components (Sordillo and Streicher, 2002). In fact, NEU increased at the beginning of an inflammation and MAC raised at the end to clear the infected area of debris.Inizio modulo

The rise of SCC, with the number of parity, may be attributed to the accumulation of epithelial cell debris with the aging of the mammary gland. Another possible explanation, suggested by Green et al. (2008), could be linked to the consequences of a previous mastitis episodes in previous lactations, that may have led to an increase of SCC without the return to its original content in milk.

Moreover, as expected, in the present study, the parity number had an effect on milk production and milking duration. At the same time, the shorter milking duration for primiparous cows were correlated to their lower milk production. In addition, the increase of milk electrical conductivity with parity number could be linked to the little increase of SCC, as reported by Bansal et al. (2005).

However, the average counts of TLC and SCC were around 100,000 cells/ml, indicating non-problematic udder conditions. In fact, NEU (%), that when present in high percentage is an indicator of critical mammary conditions, and MAC (%), which exhibits the opposite trend of NEU (%), were observed in similar percentage in milk in different number of parities. On the other side, there was a little decrease of LYM (%) with increasing parity number.

Cows, that presented higher average concentrations of SCC/TLC in milk during lactation, also had higher values of NEU (%) and lower MAC (%), highlighting a potentially critical udder condition. This finding confirms that the trends of NEU (%) or MAC (%) could be used to detect udder heath issues, as this study highlighted for the beginning and ending of lactation. This is true even for cows with lower TLC and SCC values, despite no apparent changes in milk conductivity, milk production, and milking time. The farm effect highlighted that various management techniques, such as feeding administration, diet or breeding, could affect milking parameters (milk production and milking duration). However, TLC, SCC, NEU and LYM showed no significant differences across the different farms, suggesting that animal management practices, that were not specifically investigated in this study, may not significantly influence these parameters. Further specific investigations are needed to understand the farm effect on the somatic cell types.

Factor analysis

The analysis of rotated factor patterns is useful to evaluate relationships with variables closely correlated (Conte et al., 2021), such as the different percentages and counts of leucocyte components. Factor 1 highlighted that with the increase of TLC and SCC, there was a corresponding increase in the count of each somatic cell types, confirming the correlation observed in the study. As highlighted in the correlation matrix, NEU and MAC percentages were strongly correlated and for this were grouped together in the second factor, with contrasting values indicating opposite trends as reported by Damm et al. (2019). On the contrary, LYM (%), as highlighted with MIXED model for parity class, had a trend that is not linked to other patterns. The third factor groups parity, milk production and milking time. Also DIM were included in the third factor with a negative sign, as simplifying the Wood milk curve reveals a descending trend in milk production over time.

Risk factors of having high SCC

The results from logistic regression confirmed some findings obtained using the mixed model approach. Multiparous cows had a higher risk of having high SCC due to the higher risk of mastitis contraction (Steeneveld et al., 2008). As previously highlighted, the highest risk of increased SCC is in the first 120 DIM and during the last stages of lactation (>240 DIM). High milk electrical conductivity was another risk factor; in fact, when cows had high SCC, milk conductivity was high, as also reported by Bansal et al. (2005), due to the different concentrations of electrolytes. For milk concentration factor (Green et al., 2006) and maybe for over-milking of udder, the cow with lower milk production was suggested a higher risk of high SCC than cows with higher milk production and also there is a 2-way relationship between the functioning of udder and the possible presence of subclinical mastitis. High NEU (%), low MAC (%) and high LYM (%) were the risk factor for having high SCC: these ratios between somatic cell components are often associated with udder critical conditions as suggested by Mondini et al. (2023). An average Hygiene Score > 2, indicating that cows were characterized as dirty in all body areas, represented an additional risk factor. This highlights the importance of management techniques applied in the dairy farms and highlights how cows that live in dirty and wet environments are at higher risk of high SCC and likely mastitis due to the favorable conditions of bacterial growth (Cardozo et al., 2015).

CONCLUSION

The study highlighted that during lactation cows with low somatic cell count experience changes in udder physiology with the modification of the trends of the leucocyte components: high percentages of neutrophils and low of macrophages were observed at both the beginning and the end of lactation. Deviations from these typical trends could serve as early indicators for potential udder issues, also in relation with parity number. Moreover, poor hygiene conditions, high percentages of neutrophils and lymphocytes and reduced macrophages are identified as risk factors associated to high somatic cell count. Multiparous cows with poor hygiene, especially in early and late lactation stages were found to be more susceptible to increased somatic cell count. These findings suggest that monitoring leucocyte component trends throughout lactation could enhance the ability to detect early signs of udder health problems. The study was conducted using a rapid and low-cost tool, which could also be employed in field conditions in large-scale dairy farms. This approach allows for practical monitoring of udder health, making it feasible to implement early intervention strategies directly in the herd. Further studies are needed to explore additional factors that can affect leucocyte counts, such as different health status of the udder or different farm management practices.

Notes

Funding information This study is part of the MAGA project supported by Regione Lombardia (PSR 2014-2020, operation 16.1.01 Gruppi Operativi PEI, ID 201802523261) “Farm models for efficient and sustainable management of the dry period’ and received funding from the Agritech National Research Center the Euro-

ARTICLE IN PRESS—UNCORRECTED PROOF

pean Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

ACKNOWLEDGMENTS We thank farmers and students for the availability to participate at this project.

Ethics statement This study is part of the MAGA project supported by Regione Lombardia (PSR 2014-2020, operation 16.1.01 Gruppi Operativi PEI, ID 201802523261) "Farm models for efficient and sustainable management of the dry period." The protocols for the MAGA project were designed with a strong emphasis on animal welfare and ethical considerations. In particular, the protocol for this study did not involve any direct contact with the animals. Specifically, the project activities included only the collection of milk samples automatically gathered by the milking machine, the collection of animal data (such as parity and lactation stage), and the assessment of the animals' cleanliness through hygiene scoring. The hygiene scoring was conducted visually without any physical interaction or handling of the animals. This approach ensured that the animals were not subjected to any additional stress or discomfort beyond their regular routine, adhering to the highest standards of ethical research practices in dairy science.

Conflict of interest statement The authors have not stated any conflicts of interest.

Nonstandard abbreviations: TLC = total leucocyte count, NEU = neutrophils, LYM = lymphocytes, MAC= macrophages, DSCC = Differential Somatic Cell Count, 4FG = group composed by the four farms equipped with milking sensors, LAC-TLC = lactation average TLC

REFERENCES

Alhussien, M., M. Kaur, P. Manjari, S. P. Kimothi, A. K. Mohanty, and A. K. Dang. 2015. A comparative study on the blood and milk cell counts of healthy, subclinical, and clinical mastitis Karan Fries cows. Vet. World 8:685–689. https://doi.org/10.14202/vetworld.2015.685-689.

Bansal, B. K., J. Hamann, N. T. Grabowski, and K. B. Singh. 2005. Variation in the composition of selected milk fraction samples from healthy and mastitic quarters, and its significance for mastitis diagnosis. J. Dairy Res. 72:144–152. https://doi.org/10.1017/S0022029905000798.

Bellingeri, A., V. Cabrera, A. Gallo, D. Liang, and F. Masoero. 2019. A survey of dairy cattle management, crop planning, and forages cost of production in Northern Italy. Ital. J. Anim. Sci. 18:786–798. https://doi.org/10.1080/1828051X.2019.1580153.

Bisutti, V., A. Vanzin, A. Toscano, S. Pegolo, D. Giannuzzi, F. Tagliapietra, S. Schiavon, L. Gallo, E. Trevisi, R. Negrini, and A. Cecchinato. 2022. Impact of somatic cell count combined with differential somatic cell count on milk protein fractions in Holstein cattle. J. Dairy Sci. 105:6447–6459. https://doi.org/10.3168/jds.2022-22071.

Cardozo, L. L., A. Thaler Neto, G. N. Souza, L. C. A. Picinin, N. C. Felipus, N. L. M. Reche, F. A. Schmidt, D. Werncke, and E. E. Simon. 2015. Risk factors for the occurrence of new and chronic cases of subclinical mastitis in dairy herds in southern Brazil. J. Dairy Sci. 98:7675–7685. https://doi.org/10.3168/jds.2014-8913.

Cattaneo, L., A. Minuti, G. E. Dahl, and E. Trevisi. 2023. Graduate Student Literature Review: The challenge of drying-off high-yielding dairy cows. J. Dairy Sci. 106:6416–6426. https://doi.org/10.3168/jds.2022-23113.

Cobirka, M., V. Tancin, and P. Slama. 2020. Epidemiology and classification of mastitis. Animals (Basel) 10:2212. https://doi.org/10.3390/ani10122212.

Cook, N. B., and D. Reinemann. 2007. A toolbox for assessing cow, udder and teat hygiene. Pages 31–43 in Proc. NMC. Annu. Mtg. National Mastitis Council (NMC) Inc., Verona, WI. National Mastitis Council (NMC) Inc.

Damm, M., C. Holm, M. Blaabjerg, M. N. Bro, and D. Schwarz. 2017. Differential somatic cell count—A novel method for routine mastitis screening in the frame of Dairy Herd Improvement testing programs. J. Dairy Sci. 100:4926–4940. https://doi.org/10.3168/jds.2016-12409.

De Vries, A., and M. I. Marcondes. 2020. Overview of factors affecting productive lifespan of dairy cows. Animal 14:155–164. https://doi.org/10.1017/S1751731119003264.

Farschtschi, S., A. Hildebrandt, M. Mattes, B. Kirchner, and M. W. Pfaffl. 2022. Using High-Resolution Differential Cell Counts (HRDCCs) in Bovine Milk and Blood to Monitor the Immune Status over the Entire Lactation Period. Animals (Basel) 12:1339. https://doi.org/10.3390/ani12111339.

Gaspardy, A., G. Ismach, A. C. Bajcsy, G. Veress, S. Markus, and I. Komlosi. 2012. Evaluation of the online electrical conductivity of milk in mastitic dairy cows. Acta Vet. Hung. 60:145–155. https://doi.org/10.1556/avet.2012.012.

Green, L. E., Y. Schukken, and M. Green. 2006. On distinguishing cause and consequence: do high somatic cell counts lead to lower milk yield or does high milk yield lead to lower somatic cell count? Prev. Vet. Med. 76:74–89. https://doi.org/10.1016/j.prevetmed.2006.04.012.

Green, M. J., A. J. Bradley, G. F. Medley, and W. J. Browne. 2008. Cow, farm, and herd management factors in the dry period associated with raised somatic cell counts in early lactation. J. Dairy Sci. 91:1403–1415. https://doi.org/10.3168/jds.2007-0621.

Hagnestam-Nielsen, C., U. Emanuelson, B. Berglund, and E. Strandberg. 2009. Relationship between somatic cell count and milk yield in different stages of lactation. J. Dairy Sci. 92:3124–3133. https://doi.org/10.3168/jds.2008-1719.

Jensen, D. B., H. Hogeveen, and A. De Vries. 2015. Bayesian integration of sensor information and a multivariate dynamic linear model for prediction of dairy cow mastitis. J. Dairy Sci. 99:7344–7361. https://doi.org/10.3168/jds.2015-10060.

Kirkeby, C., D. Schwarz, M. Denwood, M. Farre, S. S. Nielsen, M. Gussmann, N. Toft, and T. Halasa. 2021. Dynamics of somatic cell count (SCC) and differential SCC during and following intramammary infections. J. Dairy Sci. 104:3427–3438. https://doi.org/10.3168/jds.2020-19378.

Lardy, R., Q. Ruin, and I. Veissier. 2023. Discriminating pathological, reproductive or stress conditions in cows using machine learning on sensor-based activity data. Comput. Electron. Agric. 204:107556. https://doi.org/10.1016/j.compag.2022.107556.

Leitner, G., E. Shoshani, O. Krifucks, M. Chaffer, and A. Saran. 2000. Milk leucocyte populations patterns in bovine udder infection of different aetiology. J. Vet. Med. B Infect. Dis. Vet. Public Health 47:581–589. https://doi.org/10.1046/j.1439-0450.2000.00388.x.

Lozada-Soto, E., C. Maltecca, K. Anderson, and F. Tiezzi. 2020. Analysis of milk leukocyte differential measures for use in management practices for decreased mastitis incidence. J. Dairy Sci. 103:572–582. https://doi.org/10.3168/jds.2019-16355.

Magro, S., A. Costa, and M. De Marchi. 2023. Total and Differential Somatic Cell Count in Italian Local Cattle Breeds: Phenotypic Variability and Effect on Milk Yield and Composition. Animals (Basel) 13:1249. https://doi.org/10.3390/ani13071249.

Moore, S., A. Costa, M. Penasa, S. Callegaro, and M. De Marchi. 2023. How heat stress conditions affect milk yield, composition, and price

ARTICLE IN PRESS—UNCORRECTED PROOF

in Italian Holstein herds. J. Dairy Sci. 106:4042–4058. https://doi.org/10.3168/jds.2022-22640.
Norberg, E., H. Hogeveen, I. R. Korsgaard, N. C. Friggens, K. H. M. N. Sloth, and P. Løvendahl. 2004. Electrical Conductivity of Milk: Ability to Predict Mastitis Status. J. Dairy Sci. 87:1099–1107. https://doi.org/10.3168/jds.S0022-0302(04)73256-7.
Oviedo-Boyso, J., J. J. Valdez-Alarcón, M. Cajero-Juárez, A. Ochoa-Zarzosa, J. E. López-Meza, A. Bravo-Patino, and V. M. Baizabal-Aguirre. 2007. Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. J. Infect. 54:399–409. https://doi.org/10.1016/j.jinf.2006.06.010.
Paudyal, S., P. Melendez, D. Manriquez, A. Velasquez-Munoz, G. Pena, I. N. Roman-Muniz, and P. J. Pinedo. 2020. Use of milk electrical conductivity for the differentiation of mastitis causing pathogens in Holstein cows. Animal 14:588–596. https://doi.org/10.1017/S1751731119002210.
Pilla, R., M. Malvisi, G. G. M. Snel, D. Schwarz, S. König, C.-P. Czerny, and R. Piccinini. 2013. Differential cell count as an alternative method to diagnose dairy cow mastitis. J. Dairy Sci. 96:1653–1660. https://doi.org/10.3168/jds.2012-6298.
Sandrucci, A., L. Bava, M. Zucali, and A. Tamburini. 2014. Management factors and cow traits influencing milk somatic cell counts and teat hyperkeratosis during different seasons. Rev. Bras. Zootec. 43:505–511. https://doi.org/10.1590/S1516-35982014000900008.
Sarikaya, H., and R. M. Bruckmaier. 2006. Importance of the Sampled Milk Fraction for the Prediction of Total Quarter Somatic Cell Count. J. Dairy Sci. 11:4246–4250. https://doi.org/10.3168/jds.S0022-0302(06)72470-5.
Sarikaya, H., C. Werner-Misof, M. Atzkern, and R. M. Bruckmaier. 2005. Distribution of leucocyte populations, and milk composition, in milk fractions of healthy quarters in dairy cows. J. Dairy Res. 72:486–492. https://doi.org/10.1017/S0022029905001317.
Schreiner, D. A., and P. L. Ruegg. 2003. Relationship Between Udder and Leg Hygiene Scores and Subclinical Mastitis. J. Dairy Sci. 86:3460–3465. https://doi.org/10.3168/jds.S0022-0302(03)73950-2.
Schwarz, D., D. E. Santschi, J. Durocher, and D. M. Lefebvre. 2020. Evaluation of the new differential somatic cell count parameter as a rapid and inexpensive supplementary tool for udder health management through regular milk recording. Prev. Vet. Med. 181:105079. https://doi.org/10.1016/j.prevetmed.2020.105079.
Segnalini, M., U. Bernabucci, A. Vitali, A. Nardone, and N. Lacetera. 2013. Temperature humidity index scenarios in the Mediterranean basin. Int. J. Biometeorol. 57:451–458. https://doi.org/10.1007/s00484-012-0571-5.
Sordillo, L. M., and K. L. Streicher. 2002. Mammary gland immunity and mastitis susceptibility. J. Mammary Gland Biol. Neoplasia 7:135–146. https://doi.org/10.1023/A:1020347818725.
Steeneveld, W., H. Hogeveen, H. W. Barkema, J. van den Broek, and R. B. M. Huirne. 2008. The Influence of Cow Factors on the Incidence of Clinical Mastitis in Dairy Cows. J. Dairy Sci. 91:1391–1402. https://doi.org/10.3168/jds.2007-0705.
Stocco, G., C. Cipolat-Gotet, B. Stefanon, A. Zecconi, M. Francescutti, M. Mountricha, and A. Summer. 2023. Herd and animal factors affect the variability of total and differential somatic cell count in bovine milk. J. Anim. Sci. 101:1–10. https://doi.org/10.1093/jas/skac406.
Tamburini, A., L. Bava, R. Piccinini, A. Zecconi, M. Zucali, and A. Sandrucci. 2010. Milk emission and udder health status in primiparous dairy cows during lactation. J. Dairy Res. 77:13–19. https://doi.org/10.1017/S0022029909990240.
Van der Voort, M., D. Jensen, C. Kamphuis, I. N. Athanasiadis, A. De Vries, and H. Hogeveen. 2021. Invited review: Toward a common language in data-driven mastitis detection research. J. Dairy Sci. 104:10449–10461. https://doi.org/10.3168/jds.2021-20311.
Wall, S. K., O. Wellnitz, R. M. Bruckmaier, and D. Schwarz. 2018. Differential somatic cell count in milk before, during, and after lipopolysaccharide and lipoteichoic acid induced mastitis in dairy cows. J. Dairy Sci. 101:5362–5373. https://doi.org/10.3168/jds.2017-14152.
Winder, C. B., J. M. Sargeant, D. F. Kelton, S. J. Leblanc, T. F. Duffield, J. Glanville, H. Wood, K. J. Churchill, J. Dunn, M. D. Bergevin, K. Dawkins, S. Meadows, and A. M. O'Connor. 2019. Comparative efficacy of blanket versus selective dry-cow therapy: A systematic review and pairwise meta-analysis. Anim. Health Res. Rev. 20:217–228. https://doi.org/10.1017/S1466252319000306.
Zimbelman, R. B., R. P. Rhoads, M. L. Rhoads, G. C. Duff, L. H. Baumgard, and R. J. Collier. 2009. A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. Pages 158–169 in Proceedings 24th Annual Southwest Nutrition and Management Conference, Reno, NV.
Zucali, M., L. Bava, A. Tamburini, G. Gislon, and A. Sandrucci. 2021. Association between udder and quarter level indicators and milk somatic cell count in automatic milking systems. Animals (Basel) 11:3485. https://doi.org/10.3390/ani11123485.

ORCIDS

S. Mondini, https://orcid.org/0000-0002-2446-7399
G. Gislon, https://orcid.org/0000-0003-0389-7960
M. Zucali, https://orcid.org/0000-0002-2380-3274
A. Sandrucci, https://orcid.org/0000-0002-1014-0088
A. Tamburini, https://orcid.org/0000-0002-3371-6254
L. Bava https://orcid.org/0000-0001-6521-3195