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Exploring the sources of variation of electrical conductivity and total and differential somatic cell count in Italian Mediterranean buffaloes

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ABSTRACT

In the buffalo dairy sector, a huge effort is still needed to improve mastitis prevention, detection, and management. Electrical conductivity (EC) and total somatic cell count (SCC) are well-known indirect indicators of mastitis. Differential somatic cell count (DSCC), which represents the proportion of neutrophils and lymphocytes on the total SCC, is instead a novel phenotype collected in the dairy cattle sector in the last lustrum. As little is known about this novel trait in dairy buffalo, in the present study we explored the nongenetic factors affecting DSCC, as well as EC and total somatic cell score (SCS), in the Italian Mediterranean buffalo. The data set used for the analysis included 14,571 test-day (TD) records of 1,501 animals from 6 herds, and climatic information of the sampling locations. The original data were filtered to exclude animals with less than 3 TD per lactation and, for the investigated traits, outliers beyond 4 standard deviations. In the statistical model we included the fixed effects of herd (6 classes), days in milk (DIM; 10 classes of 30 d, with the last being an open class until 360 d), parity (6 classes, from 1 to 6+), year-season of calving (11 classes, from summer 2019 to winter 2021/2022), year-season of sampling (9 classes, from spring 2020 to spring 2022), production level (4 classes based on quartiles of average milk yield by herd), and temperature-humidity index (THI; 4 classes based on quartiles, calculated using the average temperature and relative humidity of the 5 d before sampling). Average EC, SCS, and DSCC vary across herds. Considering DIM, greater EC values were observed at the beginning and the end of lactation; SCS was slightly lower, but DSCC was greater around the lactation peak. Increased EC, SCS, and DSCC levels with increasing parity were reported. Year-season calving and year-season sampling only slightly affected the variation of the investigated traits. Milk of high-producing buffaloes was characterized by lower EC and SCS mean values, nevertheless it had slightly greater DSCC percentages. Buffaloes grouped in the highest THI classes (classes 3 and 4) showed, on average, greater EC, SCS, and DSCC in comparison to the lower classes, especially to class 2. Results of the present study represent a preliminary as well as necessary step for the possible future inclusion of EC, SCS, or DSCC in breeding programs aimed to improve mastitis resistance in dairy buffaloes.
Key words: mastitis, electrical conductivity, differential somatic cell count, Italian Mediterranean buffaloes

INTRODUCTION

Mastitis is one of the most important diseases affecting the dairy sector worldwide as it impairs the animals' welfare, as well as their milk production and quality, with a negative effect on the dairy product industry (Halasa et al., 2007). In dairy cattle, important advances have occurred for improving hygienic conditions in the herd, milking procedures, screening tests, and antimicrobial usage (Ruegg, 2017). Although the morphology of the teat canal and sphincter seems to make Italian Mediterranean buffaloes (**IMB**; *Bubalus bubalis*) less susceptible to udder infections than dairy cows (Fagiolo and Lai, 2007), a huge effort is still needed to improve mastitis prevention, identification, and management in this species. In fact, most of the knowledge about mastitis management is transferred from the bovine sector (Puggioni et al., 2020). However, the routine measurement of indirect indicators of mastitis in the frame of the monthly test-day (**TD**) recording procedure should be better exploited to prevent and monitor the onset and development of the disease. Total SCC is the international standard indicator of mastitis and is commonly used to control udder health and milk quality in many countries (Harmon, 2001). Nevertheless, literature about dairy buffalo SCC is

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limited in comparison to dairy cattle SCC (Fagiolo and Lai, 2007). Also, electrical conductivity (**EC**) has been recognized as a useful trait to monitor inflammation of the mammary gland (Norberg et al., 2004; Matera et al., 2022b) and in Italy is currently provided in the TD reports of both dairy cows and dairy buffaloes. Differential SCC (**DSCC**) is a novel phenotype, representing the proportion of neutrophils and lymphocytes on the total SCC (Damm et al., 2017), measured in the dairy cattle sector in the last lustrum. Several studies have been performed in dairy cows, exploring the sources of variation of DSCC (Zecconi et al., 2020b; Stocco et al., 2023), its effect on milk composition (Bobbo et al., 2020; Stocco et al., 2020; Zecconi et al., 2020a; Pegolo et al., 2021), its possible application to improve mastitis detection (Gussmann et al., 2020; Halasa and Kirkeby, 2020; Schwarz et al., 2020), and its possible inclusion in breeding scheme (Bobbo et al., 2019). Little is known instead about this novel phenotype in dairy buffaloes, although it has been recently introduced in their routine milk recording scheme. Nevertheless, in a recent study, Bobbo et al. (2023b) identified DSCC as one of the most important features to predict the presence of subclinical mastitis at the subsequent TD using machine learning analyses.

So far, udder health indicators have not been considered as breeding objectives in the IMB selection index. For a possible future inclusion of such traits to improve resistance to mastitis, it is first necessary to explore their sources of variation. For this reason, in the present study, we explored the nongenetic factors affecting DSCC, as well as EC and SCC, in dairy buffalo.

MATERIALS AND METHODS

Ethics Statement

Data were obtained from pre-existing databases based on routine milk recording procedures. No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Data Collection and Editing

The Italian Breeders Association (Rome, Italy) provided monthly TD data of buffaloes reared in 6 commercial herds located in Basilicata Region (South Italy). Records included information about herd, animals (ID, calving date, parity order, and lactation stage), sampling date, daily milk production (kg/d), milk composition [e.g., fat (%), protein (%), casein (%), and lactose (%)], pH, EC (mS/cm), SCC (cells/mL), and DSCC (%). Milk traits were determined using the CombiFoss 7 DC (Foss, Hillerød, Denmark). The original data set, which included records collected from August 2019 to April 2022, was edited to select only animals that calved in the years from 2019 to 2022, with at least 3 TD within lactation, and with less than 360 DIM. Among milk traits, outliers beyond 4 standard deviations were considered as missing values, as well as records with SCC and DSCC equal to zero. In addition, SCC was log-transformed to SCC [$\log_2$ (SCC/100,000) + 3] according to Ali and Shook (1980) to achieve normality, whereas DSCC did not require any transformation. After editing, the data set included 14,571 records of 1,501 animals.

Climatic information (i.e., temperature at 2 m [**T**, °C] and relative humidity at 2 m [**RH**, %]) were retrieved from the NASA Prediction of Worldwide Energy Resource Data Access Viewer (Sparks, 2018). This tool allowed us to download daily averaged data by providing latitude and longitude of the 6 herds and the desired date range. A temperature-humidity index (**THI**) was then calculated according to (Vitali et al., 2009), using the average T and RH of the 5 d before sampling (**T5d** and **RH5d**, respectively):

$$\mathrm{THI} = (1.8 \times \mathrm{T5d} + 32) - (0.55 - 0.55 \times \mathrm{RH5d}) \times [(1.8 \times \mathrm{T5d} + 32) - 58].$$

With respect to the original formula, this equation was modified to account for the conversion of temperature degrees from Celsius to Fahrenheit, given that most of the literature is based on temperature values measured on a Fahrenheit scale.

Statistical Analysis

Data of EC, SCS, and DSCC were analyzed using the lmerTest package (Kuznetsova et al., 2017) of R Software v. 4.1.2 (R Core Team, 2022). In the statistical models, we included the fixed effects of herd (6 classes, A to F), DIM (10 classes of 30 d, with the last being an open class until 360 d), parity (6 classes, from 1 to 6+), year-season of calving (11 classes, from summer 2019 to winter 2021/2022), year-season of sampling (9 classes, from spring 2020 to spring 2022), production level (4 classes based on quartiles of average milk production by herd), and THI (4 classes based on quartiles). Animal ID was included as a random effect.

Least squares means (**LSM**) for the investigated fixed factors and all pairwise comparisons were estimated using the R emmeans package v. 1.7.0 (Lenth, 2021). The complete list of *P*-values is available as Supplemental Data Set S1 (https://data.mendeley.com/datasets/jk3ybmhs5v/1; Bobbo et al., 2023a). Proportion of

Table 1. Descriptive statistics of individual milk production, composition, mastitis indicators, and climatic parameters

Trait[1]	Number of records	Mean	SD	Minimum	Maximum
Milk production (kg)	14,528	10.36	4.05	0.40	26.50
Milk composition (%)					
Fat	14,510	8.04	1.66	1.60	15.05
Protein	14,541	4.68	0.48	2.67	6.68
Casein	14,537	3.83	0.46	1.94	5.73
Lactose	14,464	4.67	0.32	3.09	5.83
pH	14,509	6.64	0.19	5.85	7.23
Mastitis indicator					
EC, mS/cm	14,413	592.75	68.69	327.70	942.10
SCS, units	14,569	3.92	1.81	−2.06	10.73
DSCC, %	14,571	54.45	17.39	5.00	97.00
Climatic parameter					
T5d, °C	14,571	14.52	7.74	1.66	31.47
RH5d, %	14,571	69.86	15.25	29.58	92.97
THI, units	14,571	57.03	11.09	38.00	77.00

[1]EC = electrical conductivity; DSCC = differential SCC (i.e., the proportion of neutrophils and lymphocytes on the total SCC); T5d = average temperature of the 5 d before sampling; RH5d = average relative humidity of the 5 d before sampling; THI = temperature-humidity index.

variance explained by animal ID was calculated by dividing the corresponding variance component by the total variance. Pearson correlations among the mastitis indicators, based on the residuals extracted from the previous models, were computed using the R cor.test function.

RESULTS

Descriptive Statistics

Buffaloes had an average milk production of 10.36 kg and their milk was characterized by an average content of fat and protein of 8.04% and 4.68%, respectively (Table 1). Among the 3 mastitis indicators, EC averaged 592.75 mS/cm and SCS 3.92 cells/mL. In the studied population, the novel DSCC traits ranged from 5% to 97%, with a mean value of 54.45%. The average values of T5d and RH5d were 14.52°C and 69.86%, respectively. Finally, THI ranged from 38 to 77, with an average value of 57.03. Intervals of the 4 classes of the THI fixed effect were established, based on quartiles, as follow: class 1 = 38 to 46, class 2 = 47 to 56, class 3 = 57 to 66, class 4 = 67 to 77. For the production level's effect, classes were constructed based on quartiles of milk production in each herd: for herd A, $1 \leq$ class $1 < 8.8$, $8.8 \leq$ class $2 > 11.2$, $11.2 \leq$ class $3 < 14.5$, and $14.5 \leq$ class $4 < 26.5$; for herd B, $0.4 \leq$ class $1 < 6$, $6 \leq$ class $2 > 8.2$, $8.2 \leq$ class $3 < 10.8$, and $10.8 \leq$ class $4 < 23.9$; for herd C, $1.8 \leq$ class $1 < 7.05$, $7.05 \leq$ class $2 > 9$, $9 \leq$ class $3 < 11.5$, and $11.5 \leq$ class $4 < 25.3$; for herd D, $0.5 \leq$ class $1 < 8.2$, $8.2 \leq$ class $2 > 10.8$, $10.8 \leq$ class $3 < 13.6$, and $13.6 \leq$ class $4 < 25.2$; for herd E, $1 \leq$ class $1 < 7.5$, $7.5 \leq$ class $2 > 9.9$, $9.9 \leq$ class $3 < 12.5$, and $12.5 \leq$ class $4 < 20.5$; for herd F, $1 \leq$ class $1 < 6.7$, $6.7 \leq$ class $2 > 8.9$, $8.9 \leq$ class $3 < 11.4$, and $11.4 \leq$ class $4 < 18.5$.

Sources of Variation of EC, SCS, and DSCC

With the exception of year-season of calving, association between mastitis indicators and all the explanatory variables was observed (Table 2). In addition, the proportion of variance explained by the random effect of animal ID ranged from 26% (DSCC) to 32.8% (SCS).

After adjusting for the other effects included in the model, LSM of investigated traits showed variation across herds (Figure 1). In particular, herd B was characterized by the greatest value of all 3 mastitis indicators (628.98 mS/cm for EC, 5.69 cells/mL for SCS, and 64.9% for DSCC). The lowest LSM of EC, SCS, and DSCC were observed all in herd F (575.61 mS/cm, 2.57 cells/mL, and 43.8%, respectively), although average EC and DSCC values of herd F did not statistically differ from those of herd C (585.19 mS/cm and 47.5%, respectively). Considering the effect of DIM (Figure 1), greater EC values were observed at the beginning and the end of lactation; SCS was slightly lower, but DSCC was greater around the lactation peak (class 2). Increased EC, SCS, and DSCC levels with increasing parity were reported, with a clear separation between primiparae and pluriparae for EC and SCS (Figure 1). Year-season of calving only slightly affected EC; indeed, a significant ($P < 0.001$) difference was observed between spring and summer 2021 with both autumn 2021 and winter 2021/22 (Figure 1).

Considering year-season of sampling, LSM of EC showed variation across levels, with estimates of spring and summer months differing from estimates of autumn and winter months of the same year (Figure 1). The

Table 2. Results from ANOVA for mastitis indicators[1]

	EC		SCS		DSCC	
Effect	F-value	P-value	F-value	P-value	F-value	P-value
Fixed						
Herd	46.961	<0.001	294.424	<0.001	178.225	<0.001
DIM	375.241	<0.001	4.854	<0.001	22.938	<0.001
Parity	90.529	<0.001	103.333	<0.001	14.575	<0.001
Year-season calving	4.815	<0.001	1.282	0.234	2.091	0.022
Year-season sampling	13.955	<0.001	6.595	<0.001	11.144	<0.001
Production level	214.942	<0.001	139.738	<0.001	3.474	0.015
THI[2]	27.942	<0.001	6.765	<0.001	7.843	<0.001
Random						
Animal ID,[3] %	30.1		32.8		26.0	

[1]EC = electrical conductivity; DSCC = differential SCC (i.e., the proportion of neutrophils and lymphocytes on the total SCC).

[2]THI = temperature-humidity index.

[3]Proportion of variance explained by animal ID was calculated by dividing the corresponding variance component by the total variance.

LSM of SCS and DSCC revealed statistically significant greater values in winter 2021/22 (Figure 1). Milk of low-producing animals (class 1) had greater EC and SCS values (Figure 1); nevertheless, milk of highly producing buffaloes (class 4) was characterized by lower EC and SCS average values, but slightly greater DSCC percentages. Buffaloes grouped in the 2 THI classes above the mean (classes 3 and 4) showed, on average, greater EC, SCS, and DSCC values in their milk in comparison to the lower classes, especially to class 2 (Figure 1).

Correlations Among Residuals

The Pearson correlation coefficients, based on model residuals, among mastitis indicators are presented in Table 3. After correction for the most important sources of variations, the highest correlation was observed between SCS and DSCC (0.54, $P < 0.001$), whereas the coefficient of correlation between EC and DSCC was almost close to zero (0.009, $P > 0.05$).

DISCUSSION

Mastitis is an inflammatory response of the mammary gland to infection, resulting from pathogens' colonization of the udder by entering through the teat canal. Milk produced by infected animals is characterized by alterations in yield and chemical composition, and by increased SCC, which is considered the standard indirect indicator of udder health and milk quality (Harmon, 1994). Milk cellular components of SCC, whose proportion vary during mastitis, are mostly leukocytes (i.e., neutrophils, macrophages, and lymphocytes). These cell types play different roles during the various stages of inflammation and the knowledge of their variation in milk provides additional information to better define the mammary gland inflammatory status. Therefore, DSCC represents a valuable indirect indicator of mastitis, to be used in combination with SCC (Bobbo et al., 2020). In addition, as a result of the damage of the blood-milk barrier during mastitis, changes in the ionic udder environment can lead to increased milk EC (Harmon, 1994), making this trait another useful phenotype for mastitis screening.

Milk produced by mastitic animals presents also altered acidity and poor technological properties (Costa et al., 2020b), negatively affecting the cheese-making process. Given the high economic value and market demand of buffalo milk used for the production of the Mozzarella di Bufala, a Protected Designation of Origin (**PDO**) cheese, special attention should be paid to udder health of IMB. For this reason, mastitis indicators, such as EC, SCC, and DSCC, should be considered as breeding objective. To this purpose, factors affecting the variation of these traits have been explored.

Descriptive statistics of milk composition traits reported in the present study are comparable with results of Costa et al. (2020b) and Matera et al. (2022b) on dairy buffaloes. The average DSCC of IMB was lower (54.45%) than the mean values reported in the literature for dairy cows, generally above 60% (Bobbo et al., 2020; Stocco et al., 2023). According to Moroni et al. (2006), despite the elevated prevalence of intramammary infections in dairy buffaloes, their overall mean SCS is lower than the typical value reported for dairy cattle. The difference between the 2 species can be due to different selective pressure for milk production, as well as to different neutrophils' phagocytic activity (Moroni et al., 2006).

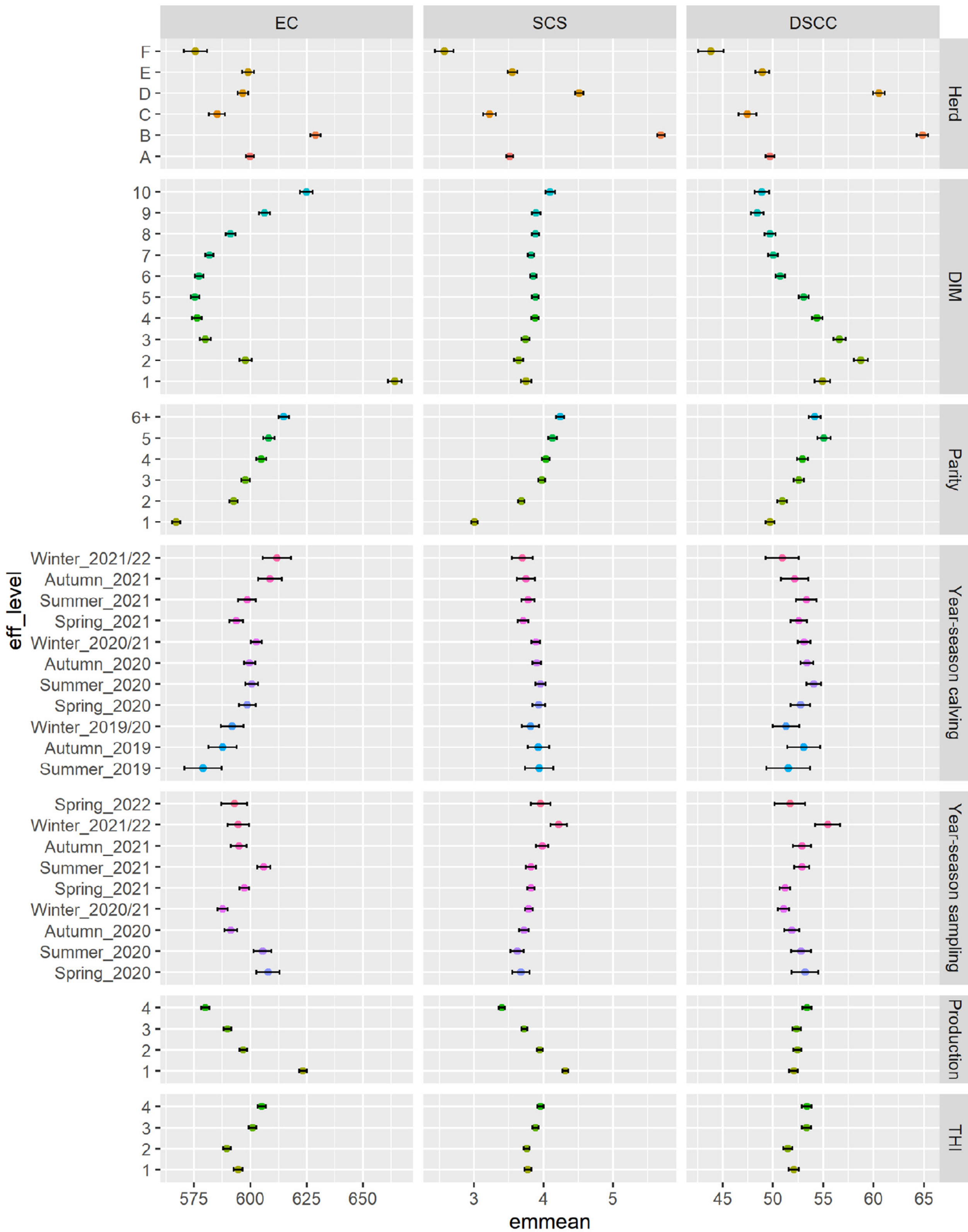

Figure 1. Least squares means (emmeans) of mastitis indicators (EC = electrical conductivity [mS/cm]; SCS [units]; DSCC = differential SCC [%; i.e. the proportion of neutrophils and lymphocytes on the total SCC]), across investigated effects. THI = temperature-humidity index. Error bars represent SE. See the "Materials and Methods" section for a detailed description of thresholds used to define fixed effects levels (eff_level).

Table 3. Pearson product-moment correlations among the mastitis indicators,[1] based on the residuals extracted from the mixed models

Item	EC	SCS
SCS	0.33***	
DSCC	0.009^{NS}	0.54***

[1]EC = electrical conductivity; DSCC = differential SCC (i.e., the proportion of neutrophils and lymphocytes on the total SCC).

***$P < 0.001$.

The variation in mean EC, SCS, and DSCC observed in the selected herds can reflect differences in milking practices and hygienic conditions. Indeed, the relatively high prevalence and incidence of mastitis in dairy buffaloes' herds seem to suggest still insufficient hygiene (Fagiolo and Lai, 2007). In addition, the absence of an official SCC threshold to regulate the processing of buffaloes' bulk milk and the absence of penalties for selling milk with high SCC can lead to a scarce attention toward this disease (Costa et al., 2020b). Thus, the improvement of managerial strategies still represents a crucial starting point to improve mastitis prevention.

A decrease in SCC around the lactation peak and an increase in the subsequent months have been previously reported in the literature, both in IMB (Moroni et al., 2006), Murrah buffaloes (Cerón-Muñoz et al., 2002), and in dairy cattle (Zecconi et al., 2020b; Stocco et al., 2023). Indeed, in healthy animals, the plot of SCC across DIM represents the inverse of the lactation curve and the lowest or highest values reported at lactation peak or drying off are usually due to a concentration or dilution effect (Harmon, 1994). In addition, the rise of SCC at the end of lactation can be the results of higher occurrence of chronic mastitis (Zecconi et al., 2020b). However, the decrease of SCC at the lactation peak corresponded to increased DSCC levels, underlying a more stressful and inflammatory status when maximum yield was reached. Variation in EC, with greater values at the beginning and the end of lactation, were previously reported by Matera et al. (2022b), and can also be explained by variation in milk concentration and composition.

Average EC, SCS, and EC increased with increasing parity, especially moving from primiparae to pluriparae. Lower SCC in the first parity, in comparison to other parities, were previously observed both in IMB (Matera et al., 2022b), Murrah buffaloes (Cerón-Muñoz et al., 2002), and in dairy cattle (Stocco et al., 2023). The rise in SCC in older animals can be the result of the damage of the mammary gland due to previous infections (Bartlett et al., 1990). Such infections, as well as damages caused by the automatic milking, could potentially alter the ionic environment, and EC as a consequence (Matera et al., 2022b).

Variation of mastitis indicators, especially EC, across year-seasons of calving and of sampling can reflect changes in herd management strategies, as well as physiological changes in the animals (Cerón-Muñoz et al., 2002), although in our studies we adjusted for the effect of herd, DIM, and parity. In addition, the effect of the year-season of calving can be due to the strong seasonality of this species, which affect the distribution of calvings throughout the year. Indeed, in our study, most of the calvings from primiparae occurred between March and May, whereas pluriparae calved mainly between June and August (data not shown). Costa et al. (2020b) reported a peak of calvings in February for primiparae, which is in accordance with the natural behavior of buffaloes; multiparous animals reached the maximum in July, naturally an off-breeding period, to enhance milk production during summer and comply with the requirement of the Mozzarella cheese market (Zicarelli, 2010).

The effect of the sampling season on the variability of buffalo milk traits has been previously reported in literature only in few studies, mostly regarding milk production and quality (Pasquini et al., 2018; Costa et al., 2020c).

Low-producing animals (class 1) had greater milk EC and SCS, whereas a high milk production level (class 4) was associated with lower EC and SCS; this is the result of a concentration or dilution effect (Moroni et al., 2006; Stocco et al., 2023). Conversely, the concentration of milk did not affect DSCC, which is expressed as percentages (Stocco et al., 2023). Indeed, high-producing animals had slightly greater DSCC values in milk, indicating a greater susceptibility to mastitis (Moroni et al., 2006).

In livestock, THI, an index that combines the effects of T and RH, is commonly used to study heat stress (Hahn et al., 2003), which might affect production, reproduction, and the health of farm animals (Kadzere et al., 2002; Polsky and von Keyserlingk, 2017). According to a previous study (Bernabucci et al., 2014), the negative effect of heat stress on the performances of dairy cattle can start more than 4 d before the TD. For this reason, to compute THI calculation, we used the average T and RH of the 5 d before sampling. Given that buffaloes are generally considered more robust and heat tolerant than cattle, little is known about the effect of THI on milk traits. In the present study, animals that experienced greater T and RH values (classes 3 and 4 of THI) had slightly greater EC, SCS, and DSCC values in their milk, confirming the negative effect of heat stress on animal's health. Costa et al. (2020a) did not find SCS, measured on bulk milk of IMB, to be affected by THI. Conversely, Matera et al. (2022a) reported udder health to be affected by THI in dairy buffaloes.

The estimated correlation between EC and SCC was slightly higher than that reported by Costa et al. (2020a) using buffaloes' bulk milk data, 0.33 versus 0.24, respectively. The correlation between models' residuals confirmed a less-than-unity correlation between SCS and DSCC (0.54), which is similar to that reported in the literature for dairy cattle (Bobbo et al., 2019). These findings confirmed the added values of combining SCC and DSCC to gain a broader overview of the inflammatory status of the mammary gland.

CONCLUSIONS

Nongenetic sources of variation of EC, SCS, and DSCC were investigated in the IMB. The effects of herd, DIM, parity, year-season of calving and sampling, milk production level, and THI were notable for the investigated traits. Given the high economic value of the buffalo's milk used to produce the Mozzarella di Bufala PDO cheese, and considering the growing interest of the consumers for animal health and antibiotic usage, more effort should be placed to improve mastitis prevention and detection. The large amount of data collected in the frame of the monthly milk recording system, including EC, SCC, and DSCC, should be better exploited to this purpose, for example, including these traits as breeding objectives in the selection index.

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