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Combining total and differential somatic cell count to screen for mastitis

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Somatic cell count (SCC) has been extensively used as indicator of udder health and milk quality. Recent developments in milk-testing technology have led to cell differentiation in milk in a high throughput manner. Information on the proportion of the different cell types in milk would represent a valuable asset for a more precise definition of udder health status. The aim of the present study was to apply receiver-operating characteristic curve analysis to define the most accurate thresholds of milk differential somatic cell count (DSCC), which represents the percentage of neutrophils plus lymphocytes in the total SCC. The dataset accounted for 117,482 test-day records of 60,009 Holstein Friesian, Brown Swiss and Simmental cows. Different thresholds were defined so that DSCC trends were analysed throughout the lactation, considering also the classification factors of breed and parity. Finally, cows were classified as healthy, susceptible, mastitic or chronic on the basis of their health status, which was defined combining the information of SCC (below or above 200,000 cells/mL) and DSCC (below or above the specific cut-off). Our findings offered new insights for a practical use of DSCC to screen for mastitis, in order to help farmers make decisions to reduce the use of antimicrobials in the herd.

Keywords: differential somatic cell count; receiver-operating characteristic (ROC) curve; cut-off; mastitis; cattle

1 Introduction

Over the last decades, somatic cell count (SCC) has been measured in routine milk recording systems in many countries and has been extensively used for monitoring udder health and improving milk quality at herd and individual cow levels (Schukken et al., 2003; Ruegg & Pantoja, 2013). In addition, alternative traits derived from milk SCC, like the presence of test-day SCC above specific thresholds in the lactation (Bobbo et al. 2018), as well as blood parameters (Cecchinato et al. 2018) have been evaluated to better assess udder health.

Milk somatic cells are mainly leukocytes, i.e. polymorphonuclear neutrophils, macrophages and lymphocytes. These three cell types play different roles in the immune response to mastitis and their proportion in milk varies according to the infection status of the mammary gland. For instance, macrophages are the predominant cell type in uninfected udders with low SCC (Lee et al., 1980; Schwarz et al., 2011), whereas high proportions of macrophages in milk with elevated SCC could indicate udders affected by chronic mastitis (Leitner et al., 2008). In infected glands, neutrophils, which play a defense role against invading pathogens, can increase up to 95% of total SCC (Kehrli & Shuster, 1994). Nevertheless, in the early stages of infection, neutrophils increase even faster than total SCC (Schwarz et al., 2011; Pilla et al., 2012).

Recent developments in milk-testing technology have led to cell differentiation in milk in a high throughput manner using flow cytometry (Damm et al., 2017). Milk differential somatic cell count (DSCC) is a novel indicator of udder health and it represents the percentage of neutrophils plus

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lymphocytes in the total SCC; therefore, the percentage of macrophages can be calculated as 100 - DSCC (Damm et al., 2017). The genetic background of DSCC has been recently investigated by Bobbo et al. (2019), who reported that this novel trait may be exploited in selection programs for mastitis resistance, as its heritability (0.08) was even higher than that of somatic cell score (SCS; 0.04). Moreover, information on the proportion of the different cell types in milk, in combination with total SCC, would represent a valuable asset for a more precise definition of udder health status (Pillai et al., 2001; Pilla et al., 2012; Pilla et al., 2013), and would help farmers make decisions to reduce the use of antimicrobials. In fact, the combined use of SCC and DSCC would help identify healthy animals (those with SCC and DSCC below thresholds to be defined), animals susceptible to mastitis (those where an immune response has begun, so that there is an increase of neutrophils, i.e. DSCC, but not yet of total SCC), animals with a mastitic event in progress (those with high SCC and DSCC) and animals with possible chronic inflammation (those with high SCC and low DSCC, as macrophages prevail). The information about udder health status may be used to develop mastitis pre-screening protocols, which would help veterinarians to identify the healthy cows and those to be eventually treated, especially at the end of lactation. In fact, antimicrobial therapy at dry off must be applied in a rational and targeted way, and not routinely on the whole herd, neither as a preventive measure nor to compensate for scarce hygiene or poor management.

To this purpose, receiver-operating characteristic (ROC) curve analysis allows to assess how well a marker can discriminate between diseased and healthy individuals (Kamarudin et al., 2017). In particular, the ROC procedure allows the evaluation of the accuracy of a continuous biomarker (in our case DSCC) along all the range of possible values it can assume. Furthermore, it allows to identify the optimal cut-off, i.e. the value of DSCC that maximizes the difference between true positives and false positives.

Therefore, the aim of the present study was to apply ROC analysis to define the most accurate thresholds of DSCC to be used in combination with SCC for a more precise identification of the stage of mammary gland inflammation in cows. Different DSCC thresholds were defined so that classification factors of breed, parity and lactation stage were taken into account. The estimated DSCC thresholds were then used in combination with SCC to classify cows according to their udder health status.

2 Material and methods

2.1 Data and editing

Test-day milk records collected from January 2018 to January 2019 within the national routine milk recording system were provided by the Breeders Association of Veneto Region (Padova, Italy). Data included milk yield (kg/day); fat, protein, casein and lactose percentages, pH, SCC (cells/mL) and DSCC (%) measured using the new Combifoss 7 DC (Foss Electric, Hillerød, Denmark). The original database was edited to select Holstein Friesian, Brown Swiss and Simmental cows from parity 1 to 9 and from 5 to 305 DIM; other breeds present in the original dataset were excluded due to few observations available. Records containing values of DSCC equal to zero [data not reliable due to accuracy and repeatability issues of the instrument, as reported by Damm et al. (2017)] were discarded from the dataset. After editing, 117,729 test-day records of 60,009 cows in 1,081 herds were available for subsequent analysis.

2.2 Statistical analysis

In order to define different DSCC threshold according to breed, parity and lactation stage, the dataset was split into six subsets, each corresponding to a combination of breed (Holstein Friesian, Brown Swiss and Simmental) and parity order (primiparous and pluriparous). Moreover, cows of each subset were grouped into 10 classes of 30 DIM each (from 5 to 305 DIM) with the aim to detect possible variation of DSCC thresholds during lactation. One test-day record per class of DIM and cow was taken into account. Therefore, statistical analysis was performed on a total of 117,482 test-day records using R 3.4.4 software (R Core Team, 2018). Linear mixed models were fitted for each of the subsets to estimate the contribution of the random effect of herd to the phenotypic variance of DSCC; this information was then included in the subsequent ROC analysis to adjust for herd effect. Somatic cell count was considered as the gold standard analysis and a value of 200,000 cells/mL was referred to as the threshold to identify presence or absence of mastitis (Zecconi et al., 2019). Whereas in the European Community a bulk milk SCC threshold of 400,000 cells/mL has been established for milk destined for human consumption, a threshold of SCC greater than 200,000 cells/mL is usually considered, at individual cow level, to identify animals with subclinical infection (Dohoo and Leslie, 1991).The ROC analysis was performed using the OptimalCutpoints R package (Lopez-Raton et al., 2014), which allowed to calculate optimal cut-offs in continuous diagnostic tests according to levels of given categorical variables. The ROC analysis was used to determine the optimal DSCC cut-off value at each DIM class, to be used in combination with SCC for a better definition of the stage of inflammation. The best DSCC cut-off is the value that maximizes specificity (true negative cases) and sensitivity of the test (true positive cases). Youden Index (Youden, 1950) was used to define the best cut-off and the area under the ROC curve (AUC), which is a measure of accuracy, was reported for each test. The AUC ranges from 0.5 (no diagnostic ability) to 1.0 (perfect diagnostic ability). For each breed and parity, the following udder health categories were defined:

- Healthy = cows with SCC ≤ 200,000 cells/mL and DSCC ≤ cut-off.
- Susceptible = cows with SCC ≤ 200,000 cells/mL and DSCC > cut-off.
- Chronic = cows with SCC > 200,000 cells/mL and DSCC ≤ cut-off.
- Mastitic = cows with SCC > 200,000 cells/mL and DSCC > cut-off.

3 Results and discussion

3.1 Descriptive statistics

In the present study, not all milk samples collected in the framework of the routine milk-recording procedures in Veneto Region could be analysed for DSCC using the new Combifoss 7 DC. Indeed, only one third of the milk samples could be randomly analysed for this new phenotype, while the other two thirds were analysed for milk quality only using MilkoScan FT6000. This explain why, although data were recorded over a 1-year period, the average number of test-days per cow with DSCC measurements was quite low: 1.95 ± 1.03 for Holstein Friesian, 1.98 ± 1.04 for Brown Swiss and 2.08 ± 1.12 for Simmental (Table 1). Descriptive statistics of milk yield, composition and pH (Table 1) were generally in agreement with findings reported for the same Italian breeds (Cassandro et al., 2008; Bobbo et al., 2016; Viale et al., 2017). As expected, Holstein Friesian produced more milk than Brown Swiss and Simmental (33.0 kg/day vs 27.2 kg/day and 24.9 kg/day, respectively). The greatest mean percentages of fat (4.06%), protein (3.64%) and casein (2.83%) were found in milk of Brown Swiss cattle. The average DSCC ranged from 65.2% (Brown Swiss) to 66.6% (Holstein Friesian; Table 1). Mean DSCC of Holstein Friesian observed in the present study was slightly higher than the value (62.07%) reported by Bobbo et al. (2019); the difference is likely related to the fact that, after an update of the software, DSCC values corresponding to SCC < 50,000 cells/mL were set to zero in the output of the instrument (data not included in the current study). As a consequence, the average values of SCC increased slightly (Table 1). Our results differed to some extent from those of Damm et al. (2017), who reported DSCC from 72.68% to 76.12%. Greater means can be explained by greater minimum value of DSCC: 20% in Damm et al. (2017) vs 2.5% in the present study.

Table 1 Mean and standard deviation (SD) of test-day milk yield, composition, pH, somatic cell count (SCC) and differential somatic cell count (DSCC) of three cattle breeds

	Holstein Friesian		Brown Swiss		Simmental	
Trait	Mean	SD	Mean	SD	Mean	SD
Milk yield (kg/day)	33.0	9.5	27.2	8.2	24.9	8.2
Milk composition (%)						
Fat	3.81	0.85	4.06	0.81	3.84	0.85
Protein	3.36	0.37	3.64	0.38	3.48	0.39
Casein	2.62	0.29	2.83	0.30	2.70	0.30
Lactose	4.82	0.21	4.79	0.22	4.76	0.24
pH	6.57	0.07	6.58	0.07	6.57	0.07
SCC (10^3/mL)	298	598	273	540	260	518
DSCC (%)	66.6	16.4	65.2	16.7	65.6	16.5
N. records		99,232		9,429		9,068
N. cows		50,884		4,766		4,359

3.2 Receiver-operating characteristic curves for repeated measures

Continuous biomarkers are often used to discriminate between healthy and diseased individuals. To this purpose, it is necessary to select a cut-off to define positive or negative results of the test. With the ROC procedure it is possible to evaluate the accuracy of a marker considering all the values it can assume, in order to identify the optimal cut-off. However, the standard approach of ROC analysis considers both the binary disease status and the continuous biomarker as fixed over time, which is appropriate for cross-sectional studies (e.g., when one single observation per individual is available). As reviewed by Kamarudin et al. (2017), different estimation methods of ROC curve as a function of time have been proposed, mostly dealing with survival analysis; nevertheless, application of these methodologies is still lacking. In repeated-measures designs (e.g. longitudinal studies), each individual has multiple observations and intra-individual correlation is present. The use of traditional cross-sectional approaches to fit ROC curves in presence of repeated measures (e.g. considering test-day records throughout the lactation) is not appropriate as it ignores intra-individual correlation and underestimates the AUC (Liu & Wu, 2003). Therefore, using generalized linear mixed models and a Wilcoxon non-parametric approach, Liu and Wu (2003) proposed a SAS macro (SAS Institute Inc., Cary, NC) to estimate ROC curves and their summary statistics in repeated measures designs. Nevertheless, cut-off value of the investigated marker (our major objective) could not be provided in the output of the SAS macro. Moreover, as mentioned by Michael et al. (2019), such approach does not account for the possible greater dependency between marker and binary outcome nearer in time (in our case between data measured in subsequent test-day records). To address these issues, Michael et al. (2019) proposed prediction rules with modelling based on Markov chain and autoregressive process to extend the ROC procedure to longitudinal studies. As mentioned by the authors, this approach was developed assuming that the marker was measured at regular time intervals (and this is not the case of milk recording system) and appropriate model checking analysis to adapt it to our data was not feasible. To deal with such statistical problems, we decided to use a simplified approach, with a more “practical” side for dairy farmers: the dataset was split to define different DSCC thresholds according to breed, parity order and stage of lactation, and to avoid intra-cow correlation we fit ROC curves taking into account only one record per cow within each subset and adjusting for possible herd effect.

To perform ROC analysis, a gold standard is required. Mastitis, which is an inflammation of the mammary gland, is mostly caused by an intramammary infection (IMI), i.e. by the presence of pathogens in the mammary gland. Although inflammation and infection often go together, there are no diagnostic tests able to define them both (Adkins & Middleton, 2018). Bacteriological analysis and PCR are considered the primary methods to diagnose IMI, whereas SCC is the diagnostic test commonly used to detect subclinical mastitis (Adkins & Middleton, 2018). Thus, indicators of inflammation (SCC) are often used to diagnose mastitis and, indirectly, to identify cows with IMI (Adkins & Middleton, 2018), although they present some limitations as diagnostic tests. Given that there is no true gold standard for the diagnosis of mastitis or IMI (Adkins & Middleton, 2018), we focused on inflammation rather than infection and considered SCC as a gold standard to perform ROC analysis, as previously done by Zecconi et al. (2019). Investigations on DSCC for the identification of IMI have been recently conducted by Kirkeby et al. (2019) and Schwarz et al. (2019), who concluded that DSCC can add significant information on IMI and that obtained results justify further studies on the combined use of DSCC and SCC to detect IMI.

3.3 Cut-off values in different breeds, parities and stages of lactation

Differential somatic cell count thresholds yielding optimal classification results for Holstein Friesian (Table 2), Browns Swiss (Table 3) and Simmental cows (Table 4) were reported according to parity (primiparous and pluriparous) and stage of lactation (10 classes of 30 d each, from 5 to 305 DIM). Within breed and parity, each of the 10 classes of DIM included 7% to 11% of the data. To the best of our knowledge, this is the first study in which cut-off values of DSCC were defined separately according to breed and parity order and their trends were analyzed throughout the lactation, avoiding within-cow correlation. Moreover, DSCC thresholds for Brown Swiss and Simmental cattle were provided for the first time. In Holstein Friesian cattle (Table 2), DSCC thresholds with the best performances ranged from 72.7% (DIM 6) to 77.7% (DIM 3) in primiparous cows and from 71.2% (DIM 5) to 74.5% (DIM 2) in pluriparous cows. The AUC associated to these thresholds were always greater than 0.85, meaning that the test had good accuracy. Parity-by-DIM-based thresholds of DSCC were identified also by Zecconi et al. (2019) in Italian Holstein cattle. Those authors considered two classes of DIM (≤100 DIM and >100 DIM) and parity (primiparous and pluriparous), and combined them to define four classes. Averaging the first three classes of DIM (5 to 95 DIM) and the last seven (96 to

305 DIM) and comparing our results with findings reported in the literature, we observed that the thresholds in our study were on average 5% to 8% higher than those of Zecconi et al. (2019): 76.9% vs 69.1% for primiparous in early lactation, 74.5% vs 69.4% for primiparous in mid-late lactation, 73.7% vs 66.3% for pluriparous in early lactation, and 71.8% vs 64.8% for pluriparous in mid-late lactation. Such differences were likely due to different approaches in performing ROC analysis (i.e. dealing or not with the repeated measures) and to differences in the origin of the data. Moreover, Zecconi et al. (2019) reported that the best test performance was observed for the DSCC thresholds calculated when 3 classes of DIM were considered (3 classes of 100 d, with the last class >200 DIM), without considering parity order. In Brown Swiss (Table 3), DSCC thresholds ranged from 68.9% (DIM 9) to 78.1% (DIM 2) in primiparous cows and from 68.3% (DIM 10) to 75.1% (DIM 8) in pluriparous cows, with AUC between 0.76 and 0.88. Similar results were obtained for Simmental cows (Table 4).

Table 2 Differential somatic cell count (DSCC) thresholds yielding optimal classification results for Holstein Friesian, according to parity (primiparous and pluriparous) and stage of lactation (10 classes of 30 days each, from 5 to 305 days in milk)

	DSCC cut-off (%)	Sensitivity	Specificity	AUC (95% CI)[1]
Primiparous				
DIM 1	76.25	0.69	0.80	0.81 (0.79-0.83)
DIM 2	76.77	0.82	0.77	0.87 (0.85-0.88)
DIM 3	77.71	0.77	0.82	0.87 (0.86-0.89)
DIM 4	74.50	0.83	0.78	0.87 (0.86-0.89)
DIM 5	73.02	0.82	0.75	0.86 (0.84-0.88)
DIM 6	72.72	0.84	0.74	0.87 (0.85-0.88)
DIM 7	73.84	0.81	0.77	0.87 (0.85-0.88)
DIM 8	75.30	0.77	0.79	0.86 (0.85-0.88)
DIM 9	75.69	0.76	0.80	0.85 (0.84-0.87)
DIM 10	76.18	0.75	0.82	0.87 (0.85-0.88)
Pluriparous				
DIM 1	72.73	0.76	0.77	0.84 (0.82-0.85)
DIM 2	74.52	0.83	0.76	0.87 (0.86-0.88)
DIM 3	73.98	0.82	0.79	0.88 (0.87-0.88)
DIM 4	71.86	0.83	0.76	0.88 (0.87-0.88)
DIM 5	71.24	0.82	0.75	0.86 (0.85-0.87)
DIM 6	72.60	0.76	0.79	0.86 (0.85-0.87)
DIM 7	71.97	0.75	0.79	0.84 (0.83-0.85)
DIM 8	71.97	0.74	0.72	0.83 (0.82-0.84)
DIM 9	71.28	0.72	0.76	0.81 (0.80-0.82)
DIM 10	71.82	0.70	0.79	0.81 (0.80-0.82)

[1] AUC = area under the curve with 95% confidence interval.

Table 3 Differential somatic cell count (DSCC) thresholds yielding optimal classification results for Brown Swiss, according to parity (primiparous and pluriparous) and stage of lactation (10 classes of 30 days each, from 5 to 305 days in milk)

	DSCC cut-off (%)	Sensitivity	Specificity	AUC (95% CI)[1]
Primiparous				
DIM 1	75.71	0.72	0.76	0.80 (0.74-0.87)
DIM 2	78.07	0.65	0.82	0.76 (0.67-0.85)
DIM 3	70.60	0.91	0.68	0.86 (0.80-0.92)
DIM 4	69.26	0.88	0.74	0.85 (0.71-0.93)
DIM 5	75.76	0.75	0.86	0.88 (0.84-0.93)
DIM 6	74.10	0.77	0.80	0.84 (0.77-0.91)
DIM 7	77.78	0.62	0.90	0.85 (0.79-0.90)
DIM 8	74.22	0.74	0.85	0.82 (0.76-0.89)
DIM 9	68.86	0.79	0.67	0.80 (0.73-0.87)
DIM 10	76.91	0.70	0.87	0.82 (0.74-0.89)
Pluriparous				
DIM 1	72.14	0.78	0.73	0.82 (0.79-0.85)
DIM 2	73.78	0.86	0.78	0.88 (0.85-0.91)
DIM 3	71.39	0.83	0.79	0.88 (0.85-0.91)
DIM 4	73.44	0.79	0.80	0.86 (0.83-0.89)
DIM 5	69.55	0.83	0.74	0.85 (0.82-0.88)
DIM 6	72.03	0.74	0.81	0.85 (0.82-0.88)
DIM 7	71.30	0.69	0.78	0.80 (0.77-0.84)
DIM 8	75.12	0.62	0.85	0.80 (0.77-0.84)
DIM 9	70.25	0.71	0.74	0.80 (0.76-0.83)
DIM 10	68.26	0.79	0.67	0.81 (0.77-0.85)

[1] AUC = area under the curve with 95% confidence interval

Classification of the cows on the basis of their health status, defined combining the information of SCC (below or above 200,000 cells/mL) and DSCC (below or above the specific cut-off), showed that 59 to 62% of primiparae were healthy, whereas the frequency of healthy pluriparae ranged from 50 to 52% (Table 5). The frequencies of susceptible and chronic animals were similar in all three breeds and both in primiparous and pluriparous cows. Particular attention should be payed to susceptible animals, which are cows where an immune response has begun and thus they could likely undergo a mastitis event. To note, classification of cow's udder health status could possibly be slightly affected by exclusion of records with DSCC = 0, underestimating healthy cows. In accordance to Wall et al. (2018), the combination of DSCC and SCC can be used for early detection of mastitis by revealing the increase in neutrophils at low total SCC levels. As expected, the percentage of mastitic cows, which include both clinical and subclinical cases, was slightly higher in Holstein Friesian compared with Brown Swiss and Simmental breeds. This classification represents an evolution of the grouping proposed by Wall et al. (2018), who indicated a possible DSCC threshold of 86% to differentiate between infected and uninfected quarters. Those authors suggested a combination of high SCC (> 200,000 cells/mL) and DSCC (lower or greater than 86%) to define the stage of mastitis (late or early stage of mastitis, respectively). Schwarz et al. (2019) investigated DSCC as a supplementary tool to SCC to identify IMI and tested classification systems based on different SCC (100,000 and 200,000 cells/mL) and DSCC illustrative thresholds (50, 60 and 70%) in cows at the end of lactation. The AUC for identification of IMI by major pathogens was greater for the combination of DSCC and SCC than for the two single traits alone. The best thresholds in terms of sensitivity to identify cows as infected were 200,000 cells/mL for SCC and 60% for DSCC. In the present study we also applied a SCC threshold of 200,000 cells/ml; nevertheless, specific cut-offs calculated by ROC analysis rather than illustrative ones were considered for DSCC.

Table 4 Differential somatic cell count (DSCC) thresholds yielding optimal classification results for Simmental, according to parity (primiparous and pluriparous) and stage of lactation (10 classes of 30 days each, from 5 to 305 days in milk)

	DSCC cut-off (%)	Sensitivity	Specificity	AUC (95% CI)[1]
Primiparous				
DIM 1	71.48	0.86	0.68	0.82 (0.77-0.88)
DIM 2	77.99	0.66	0.91	0.86 (0.80-0.91)
DIM 3	77.94	0.68	0.86	0.85 (0.79-0.91)
DIM 4	74.36	0.77	0.79	0.84 (0.79-0.90)
DIM 5	76.74	0.79	0.87	0.88 (0.82-0.94)
DIM 6	66.91	0.92	0.59	0.82 (0.76-0.89)
DIM 7	76.11	0.82	0.80	0.87 (0.82-0.93)
DIM 8	73.11	0.78	0.82	0.84 (0.77-0.90)
DIM 9	79.50	0.61	0.87	0.80 (0.72-0.87)
DIM 10	76.19	0.62	0.78	0.75 (0.66-0.83)
Pluriparous				
DIM 1	73.00	0.81	0.78	0.85 (0.82-0.88)
DIM 2	73.33	0.85	0.77	0.85 (0.82-0.88)
DIM 3	75.50	0.81	0.81	0.88 (0.85-0.91)
DIM 4	72.73	0.78	0.82	0.87 (0.84-0.90)
DIM 5	72.58	0.80	0.81	0.87 (0.84-0.90)
DIM 6	71.48	0.76	0.77	0.84 (0.80-0.87)
DIM 7	73.89	0.67	0.84	0.81 (0.78-0.85)
DIM 8	71.08	0.72	0.72	0.79 (0.76-0.83)
DIM 9	71.69	0.68	0.79	0.79 (0.75-0.83)
DIM 10	69.70	0.76	0.68	0.79 (0.74-0.83)

[1] AUC = area under the curve with 95% confidence interval

Table 5 Classification[1] of the cows' health status, combining the information of somatic cell count (SCC) and differential somatic cell count (DSCC), according to breed and parity

	Holstein Friesian		Brown Swiss		Simmental	
Classification[1]	Primiparous	Pluriparous	Primiparous	Pluriparous	Primiparous	Pluriparous
Healthy (%)	59.2	49.9	62.3	50.7	60.7	52.2
Susceptible (%)	19.0	15.2	18.7	15.5	19.1	15.5
Chronic (%)	4.0	7.3	4.7	8.0	4.8	7.9
Mastitic (%)	17.8	27.6	14.3	25.8	15.4	24.4
N. records	34,603	64,429	2,653	6,748	2,512	6,537

[1] Healthy = cows with SCC ≤ 200,000 cells/mL and DSCC ≤ cut-off identified by ROC analysis; Susceptible = cows with SCC ≤ 200,000 cells/mL and DSCC > cut-off identified by ROC analysis; Chronic = cows with SCC > 200,000 cells/mL and DSCC ≤ cut-off identified by ROC analysis; Mastitic = cows with SCC > 200,000 cells/mL and DSCC > cut-off identified by ROC analysis

4 Conclusions

The knowledge of the proportion of the different cell types in milk, rather than just the total count, could provide valuable information for a more precise definition of the health status of the udder and would possibly help farmers reduce the use of antimicrobials, although bacteriology is still the gold standard for definition of intramammary infection. In the present study we defined the most accurate thresholds of DSCC, a novel indicator of udder health, that can be used in combination with SCC for a more precise identification of the stage of udder inflammation. Rather than simply calculate one cut-off value for DSCC, different thresholds were defined so that the classification factors of breed, parity and lactation stage were taken into account.

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