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Factors associated with milking-to-milking variability in somatic cell counts from healthy cows in an automatic milking system

Håvard Nørstebø[a,b,*], Gunnar Dalen[a,b], Amira Rachah[a], Bjørg Heringstad[c], Anne Cathrine Whist[b], Ane Nødtvedt[a], Olav Reksen[a]

[a] Norwegian University of Life Sciences, Department of Production Animal Clinical Sciences, PO Box 369 Sentrum, N-0102, Oslo, Norway
[b] TINE SA, P.O. Box 58, N-1430, Ås, Norway
[c] Norwegian University of Life Sciences, Department of Animal and Aquacultural Sciences, Ås, Norway

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ABSTRACT

Fully automated on-line analysis equipment is available for analysis of somatic cell count (SCC) at every milking in automatic milking systems. In addition to results from on-line cell counters (OCC), an array of additional cow-level and quarter-level factors considered important for udder health are recorded in these systems. However, the amount of variability in SCC that can be explained by available data is unknown, and so is the proportion of the variability that may be due to physiological or normal variability. Our aim was to increase our knowledge on OCC as an indicator for disturbances in udder health by assessing the variability in OCC in cows free from clinical mastitis. The first objective was to evaluate how much of the variability in OCC could be explained by different potential sources of variability, including intramammary infection (IMI) status (assessed by bacterial culture of quarter milk samples). The second objective was to evaluate the repeatability of the OCC sensor used in our study and the agreement between OCC values and SCC measured in a dairy herd improvement (DHI) laboratory. A longitudinal study was performed in the research herd of the Norwegian University of Life Sciences from January 5th 2016 to May 22nd 2017. Data from 62,471 milkings from 173 lactations in 129 cows were analyzed. We used ln-transformed OCC values (in 1000 cells/ml) as the outcome (lnOCC) in linear mixed models, with random intercepts at cow-level and lactation-level within cow. We were able to explain 15.0% of the variability in lnOCC with the following fixed effects: lactation stage, parity, milk yield, OCC in residual milk from the previous milking, inter-quarter difference between the highest and lowest conductivity, season, IMI status, and genetic lineage. When including the random intercepts, the degree of explanation was 55.2%. The individual variables explained only a small part of the total variability in lnOCC. We concluded that physiological or normal variability is probably responsible for a large part of the overall variability in OCC in cows without clinical mastitis. This is important to consider when using OCC data for research purposes or in decision-support tools. Sensor repeatability was evaluated by analyzing milk from the same sample multiple times. The coefficient of variation was 0.11 at an OCC level relevant for detection of subclinical mastitis. The agreement study showed a concordance correlation coefficient of 0.82 when comparing results from the OCC with results from a DHI laboratory.

1. Introduction

Management of udder health is essential for maintaining an efficient and sustainable dairy production. Somatic cell count (SCC) is a widely used indicator of udder health status in dairy cows, and is used both at quarter level, cow level, and bulk-tank level (Schukken et al., 2003). Dairy herd improvement (**DHI**) programs commonly include monthly or bimonthly measurements of cow-level SCC for assessing udder health and implementing selective dry cow therapy strategies (Østerås et al., 1999; Torres et al., 2008). In automatic milking systems (**AMS**), fully automated on-line analysis equipment is available for on-farm analysis of SCC at every milking (Sørensen et al., 2016). This represents a substantial increase in the amount of data containing information, e.g., for udder health management, which may also serve as phenotypes for breeding programs. In addition to frequent measurements of SCC, a whole array of additional cow-level and quarter-level factors considered of importance for udder health are recorded in the AMS at every milking (Hogeveen et al., 2010). This raises the question regarding the

* Corresponding author at: Norwegian University of Life Sciences, Department of Production Animal Clinical Sciences, PO Box 369 Sentrum, N-0102, Oslo, Norway.
E-mail address: havard.norstebo@nmbu.no (H. Nørstebø).

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extent to which the variability in SCC can be explained by different explanatory factors, and the proportion of the variability that may be due to physiological variation within and between cows. Therefore, it is important that the relevance of using such frequent measurements is evaluated against known biological states, and that sources of variability are studied within and between animals before a conclusion on a given animal's health status is reached.

A literature review estimated that the geometric mean SCC level in uninfected quarters was 68,000 cells/mL (Djabri et al., 2002). However, SCC in milk can increase by tenfold or more during an intramammary infection (**IMI**) (de Haas et al., 2002). An IMI caused by bacteria is considered to be the most common cause of elevated SCC in dairy cows (Schepers et al., 1997; IDF, 2013). Other reasons for fluctuations in SCC include, among other causes: systemic disease, trauma to the udder, lactation stage, parity, and seasonal variation (IDF, 2013). However, a large proportion of the variability in SCC remains unexplained, even when accounting for these factors (Schepers et al., 1997). The milking-to-milking variability in milk composition, including SCC, has been investigated in previous studies (Quist et al., 2008; Forsbäck et al., 2010). However, in these studies, data on bacteriological udder health status were either not included at all (Quist et al., 2008) or only sparsely (Forsbäck et al., 2010). Both these studies were of short duration, being only five and 21 days, respectively. Hence, milking-to-milking variability in SCC over more prolonged periods in cows with known bacteriological udder health status has, to our knowledge, not previously been described.

Although the detection of clinical mastitis in AMS still receives substantial attention, implementation of preventive measures should be preferable to reduce production losses, to reduce the use of antimicrobial drugs, and to improve animal welfare. Detection of subclinical mastitis by SCC plays an important role in mastitis prevention programs. A recent study investigated the performance of results from an on-line somatic cell counter (**OCC**) as an indicator for subclinical mastitis (Dalen et al., 2019). Although the sensitivity and specificity for detection of subclinical mastitis were reported to be better than those of traditional DHI systems (Reksen et al., 2008; Dalen et al., 2019), the amount of false positive alerts remains challenging. Increasing our knowledge on potential sources of variability in OCC and determining how much of the variability can be attributed to specific measurable factors, might help improve future decision-support tools for udder health management.

Sensor performance can be described by repeatability (the variation in the results when the same sample is measured repeatedly) and the agreement between one method and a reference method (also called reproducibility) (Dohoo et al., 2009). The agreement between OCC measurements and SCC measurements from a DHI system has previously been evaluated in commercial Holstein and Jersey herds (Sørensen et al., 2016). However, the repeatability of the OCC sensor has yet to be reported. As variability caused by suboptimal sensor performance will be incorporated in the total variability in the frequent OCC measurements, this needs to be evaluated separately in order to assess how the precision might influence overall variability.

The aim of this study was to increase our knowledge on OCC as an indicator for disturbances in udder health by assessing the variability in frequently measured OCC in cows free from clinical mastitis. Our first objective was to evaluate how much of the variability between frequently measured OCC could be explained by potential explanatory factors, including subclinical IMI status as determined by bacterial culture in quarter milk samples (QMS), variability between cows, and variability between milkings in the same cow, among other factors. To assess the sensor as a potential source of variability in our data, a second objective was to evaluate the repeatability of the OCC sensor used in this study, and the agreement between OCC results and SCC measured in a DHI laboratory.

2. Material and methods

2.1. Milking-to-milking variability in OCC

2.1.1. Field study and data collection

This study used data collected at the research herd at the Norwegian University of Life Sciences in a study previously described by Dalen et al. (2019). Cows in two lactation pens, each holding approximately 50 cows, were investigated over 17 months from January 5th 2016 to May 22nd 2017. Each lactation pen was equipped with one AMS (DeLaval VMS, DeLaval International AB, Tumba, Sweden) and an On-line Cell Counter (DeLaval International AB, Tumba, Sweden) that recorded cow-level OCC at every milking. Both AMS were adjusted to minimize the amount of residual milk in the system after milking to reduce the effect of carryover of milk from the previous cow. First, when a milking had started, the milk pump was run for a short period to replace the residual milk in the pump with milk from the current milking. Secondly, instead of mixing the milk in the receiver jar before sampling, small pulses of milk were collected during pumping the entire milk volume. The AMS recorded milking interval, electrical conductivity, average milk-flow rate, and milk yield at quarter level in every milking. These data were obtained from the DelPro management system (DeLaval International AB, Tumba, Sweden). Because OCC is reported at cow level, a variable describing the average milk-flow rate per milking (kg/min) at cow level was calculated as the average value for quarters with non-missing values. Conductivity was also reported per quarter, and, to convert this into a cow-level indicator of disturbances in udder health, the difference between the highest and lowest conductivity among the four quarters (inter-quarter difference) was calculated (Sheldrake et al., 1983; Nielen et al., 1992). Milk yield per milking (kg) was reported at cow level. Most cows in the study herd belonged to one of two genetic groups of Norwegian Red cattle, one selected for high milk yield and the other for low incidence of clinical mastitis (Heringstad et al., 2007). Differences in SCC have previously been reported between these two genetic groups (Heringstad et al., 2008).

Aseptic QMS were collected monthly from all lactating cows, and were frozen after collection and during transport to the laboratory for microbiological analyses (Hogan et al., 1999). From the cultured bacteria, species identification was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) microflex LT (Bruker Corporation, Billerica, USA) (Cheuzeville, 2015). Samples with culture results indicating more than 2 morphologically different colony types were treated as contaminated and excluded from further analyses.

2.1.2. IMI status

The culture results from the QMS were used to assign a subclinical IMI status for each cow throughout the study period. Dalen et al. (2019) described the methodology in detail. In short, pathogens were divided into 2 groups; the group of pathogens from which a high cell count would be expected during an IMI episode was named Pat 1, while known mastitis pathogens that were not included in Pat 1, were in the Pat 2 category. Positive culture results were considered to be associated with an episode of subclinical IMI when fulfilling at least one of the following three criteria: (1) $\geq$ 1000 cfu/mL of a single mastitis pathogen were cultured from a single sample in at least 1 quarter, (2) $\geq$ 500 cfu/mL of a mastitis pathogen were cultured from 2 out of 3 consecutive milk samples from the same quarter, or (3) $\geq$ 100 cfu/mL of a mastitis pathogen were cultured from 3 consecutive milk samples from the same quarter. These definitions were adapted from Zadoks et al. (2002). Cows with positive milk cultures that did not meet any of the above criteria were classified as being transiently colonized (Reksen et al., 2012). To assign an IMI status to every milking based on the monthly QMS, we used the mid-point estimation method previously described by Zadoks et al. (2002), assuming that a shift from one udder health status to another happened midway between two sampling

occasions. Furthermore, because the OCC is recorded at the cow level, the udder health status at quarter level were aggregated into cow-level diagnoses. When assigning the IMI statuses, we implemented a hierarchical order in the classification such that a cow could only be assigned to the Pat 2 IMI group when there was no simultaneous diagnosis of a Pat 1 IMI in the same cow during the same period. Based on this set of criteria, cows were assigned one of the following four udder health statuses for every milking: No IMI, Pat 1 IMI, Pat 2 IMI, or transient colonization.

Details on the results from the microbiological analyses performed on the QMS can be found in Dalen et al. (2019). Briefly, mastitis pathogens were cultured from 1222 out of 5330 QMS, and the pathogens detected most frequently were *Staphylococcus epidermidis* (n = 234), *Corynebacterium bovis* (n = 225), *Staphylococcus chromogens* (n = 167), *Staphylococcus aureus* (n = 119), and *Staphylococcus haemolyticus* (n = 116).

2.1.3. Inclusion and exclusion criteria

A total of 96,524 milkings were performed in the two AMS during the study period. This included data from 257 full or partial lactations in 173 cows. Observations fulfilling the following criteria were included in the analysis: days in milk (DIM) from 5 to 305, milking interval of 4–24 h, and milk yield of $\geq$ 3.5 kg per milking. Furthermore, observations with missing or zero OCC values, observations with missing OCC from the previous milking in the same AMS, and lactations with data from fewer than 100 days were omitted. All data from lactations where a case of clinical mastitis had been recorded were excluded from the analysis.

2.1.4. Statistical analysis

The dataset used in the statistical analyses contained 62,471 milkings from 173 lactations in 129 cows; 85 cows contributed with one lactation, and 44 cows with two lactations. At lactation level, the distribution among parities were as follows: 81 first parity, 42 second parity, and 50 third or higher parities.

We used OCC (in 1000 cells/mL) transformed to a logarithmic scale (lnOCC) as the outcome variable in linear mixed models (Schepers et al., 1997; Reksen et al., 2008). The explanatory variables evaluated are described below and summarized in Table 1. We included the lnOCC value from the previous milking in the same AMS to adjust for the carryover effect due to residual milk from the previous cow, as suggested by Løvendahl and Bjerring (2006). Milk yield per milking (kg) was included to account for the dilution effect of milk from healthy quarters in the same cow (Green et al., 2006) and differences in milk production between cows.

To adjust for possible differences between the two sensors used in the study, a categorical variable, distinguishing between the two milking stations, was included in the analysis. The maximum inter-quarter difference in conductivity per milking was included as an indicator of pathological processes in one or more quarters (Sheldrake et al., 1983; Nielen et al., 1992). Previous research has shown that average milk-flow rate is associated with SCC (Berry et al., 2013), and the average milk-flow rate per milking was therefore included. Because both the milk yield and the milk-flow rate per milking are associated with time since last milking, our models adjusted for this by including the milking interval in hours (Hogeveen et al., 2001). To account for changes in SCC related to stage of lactation, DIM and lnDIM were included in the model (Reksen et al., 2008). Cows with different parities differ in SCC level (Laevens et al., 1997), and therefore our model included a categorical variable distinguishing between first, second, and third or later parities. To account for variability in OCC due to genetic differences between cows, a categorical cow-level variable accounting for differences between cows according to genetic group ("low mastitis"; "high yield"; "unknown") was included in our model, with "low mastitis" set as the baseline level (Heringstad et al., 2008). Seasonal variability was accounted for by including a categorical variable distinguishing between winter (Dec., Jan., Feb.), spring (Mar., Apr., May), summer (Jun., Jul., Aug.), and autumn (Sep., Oct., Nov.). Finally, the IMI status (No IMI, Pat1 IMI, Pat2 IMI, or transient colonization) was also included in the analysis as a categorical variable.

Due to the lack of independence between repeated OCC measurements within cows and lactations, we used a multi-level modeling approach. Random intercepts were specified at cow level and lactation level within cows. The significance of the random intercept terms was evaluated against a model with a fixed intercept using the likelihood ratio test. A variance component model was used for calculating the intraclass correlation coefficient to describe how much of the overall variability resided at the cow level and at within-cow lactation level. To model the dependency between the residual error terms within cow and lactation, the following correlation structures were evaluated: exponential, compound symmetry, and no within-lactation correlation between the error terms. The exponential and compound symmetry correlation structures were specified with the same grouping variables as the random intercepts, and data was sorted by milking number within lactations. First-order autocorrelation was also considered, but was not used due to unequal time intervals between observations. The model resulting in the lowest Akaike information criterion (AIC) value was selected. Subsequently, a backwards variable selection procedure was applied, and statistical significance was considered at P-value $<$ 0.05. The regression modeling was performed in the package 'nlme' in the statistical software R, version 3.6.1 (R Core Team, 2019).

Goodness-of-fit was evaluated by calculating the marginal and conditional coefficient of determination (Nakagawa and Schielzeth, 2013), which describes the variance explained by the fixed factors only and the combination of the random and fixed factors, respectively. The estimates were calculated using the package 'MuMin' in the statistical software R, version 3.6.1 (R Core Team, 2019) based on parameter estimates from the final model. To evaluate the approximate

Table 1
Summary of explanatory variables evaluated in the study.

Variable	Brief description[a]
Carryover	ln-transformed OCC (in 1000 cells/mL) from the previous cow milked in the same AMS.
Milking station	Categorical variable distinguishing between the two OCC sensors used in the study.
Milk yield (kg)	Adjustment for dilution effect on SCC from healthy quarters and differences in milk production between cows.
Conductivity	Difference between highest and lowest conductivity among the four quarters.
Milk flow rate (kg/min)	Average milk flow rate from quarters with registered milk flow.
Milking interval (hours)	Time since previous milking for the same cow.
Lactation curve	A lactation curve described by DIM and the natural logarithm of DIM accounting for changes in OCC related to lactation stage.
Parity	Categorical variable for first, second, and third or later lactation.
Lineage	Categorical variable distinguishing between different genetic lineages; low mastitis, high milk yield, and unknown.
Seasonal variability	Categorical variable; winter, spring, summer, autumn.
IMI status	Categorical variable; No IMI, Pat 1 IMI, Pat 2 IMI, transient colonization.

[a] OCC = on-line somatic cell count; AMS = automatic milking system; SCC = somatic cell count; DIM = days in milk; IMI = intramammary infection; Pat 1 IMI = IMI with mastitis pathogens from which a high somatic cell count would be expected; Pat 2 IMI = IMI with other known mastitis pathogens.

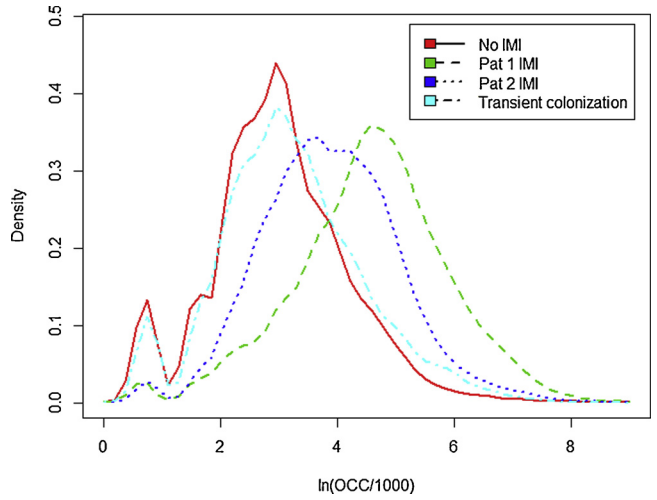

Fig. 1. Smoothed density plot showing the distribution of ln-transformed Online Cell Count (OCC) values (in 1000 cells/mL) in periods of 1) no intramammary infection (No IMI), 2) IMI with known mastitis pathogens from which a high somatic cell count would be expected (Pat 1 IMI), IMI with other known mastitis pathogens (Pat 2 IMI), and 3) Transient colonization.

contribution of the individual variables to the overall fit for the final model, we used the difference in marginal coefficient of determination between the final model and models where one term at a time was omitted. The two parameters of the lactation curve (DIM and lnDIM) were included simultaneously in all models.

Residual diagnostics were performed by graphical assessment of the distribution of the residuals calculated for the individual observations and for the random intercepts, respectively.

To compare the variability in OCC between periods of different IMI status (no IMI, Pat 1 IMI, Pat 2 IMI, or transient colonization), the distribution of lnOCC in periods of different IMI statuses was evaluated graphically using smoothed density curves. In addition, coefficients of variation were calculated for each of the four IMI statuses, assuming a log-normal distribution in OCC.

2.2. Agreement between OCC and SCC

To evaluate the agreement between results from the OCC sensor used in the current study and SCC measurements from a laboratory accredited by the International Committee for Animal Recording (ICAR), additional composite milk samples were collected at 16 occasions over 5 weeks for a subset of milkings in one of the two milking stations. The 64 cows present in one of the two lactation pens were sampled multiple times. The samples were collected with an automated milk sampler (DeLaval, Tumba, Sweden), conserved with bronopol (2-bromo-2-nitropropane-1,3-diol) and shipped refrigerated to the ICAR-accredited laboratory used for routine milk analyses by the Norwegian Dairy Herd Recording System. The samples were analyzed in a Bentley Somacount FCM (Bentley Instruments Inc., Chaska, MN). The dataset included 1661 OCC values with corresponding SCC measurements from 64 cows.

Both SCC and OCC values were transformed to the natural logarithmic scale. Because neither of the methods could be considered a gold standard due to differences in sampling equipment, the concordance correlation coefficient (**CCC**) was chosen for the statistical analysis. A version of CCC modified to account for repeated measurements within cow was used. The analysis was performed in the package 'cccrm' in the statistical software R, version 3.6.1 (R Core Team, 2019). In addition, a scatterplot with a superimposed 45 ° line (representing perfect agreement) was used for graphical assessment of the data.

2.3. Repeatability

To evaluate the repeatability of the OCC sensors, a sample of bulk tank milk (5 L) was collected. The milk was mixed gently, but thoroughly, to ensure an even distribution of the milk constituents, before drawing a number of consecutive 5 mL samples in syringes. These samples were subsequently injected directly in the OCC apparatus, which was operated in manual mode. The process was repeated as many times as possible in the available time slot (n = 62) for both OCC sensors used in the study. The mean OCC value, standard deviation and coefficient of variation (CV) were calculated for both sensors.

3. Results

3.1. Milking-to-milking variability in OCC

3.1.1. Descriptive results

The arithmetic and geometric mean OCC value in the final dataset was 96,629 cells/mL and 35,279 cells/mL, respectively. The lowest OCC value was 1000 cells/mL (detection limit) and the highest was 7,474,000 cells/mL.

The intraclass correlation coefficient calculated from the variance component model was 0.155 at the cow level, and 0.536 at the lactation level. Hence, in our data, 15.5% of the variability in lnOCC could be attributed to differences between cows, and 53.6% to differences between lactations (within cows). Consequently, 46.4% of the variability could be attributed to milking-to-milking differences within lactation.

Smoothed density curves showing the distribution of lnOCC values in periods of no IMI, Pat 1 IMI, Pat 2 IMI and transient colonization are presented in Fig. 1. The no IMI-group has the highest density between lnOCC of 2 and 3 (7400 and 20,000 cells/mL, respectively), whereas the periods of Pat 1 IMI and Pat 2 IMI had their highest densities at an lnOCC value of around 5 and 4, respectively (148,400 cells/mL and 54,600 cells/mL). Periods classified as transient colonization showed a similar distribution as periods of No IMI. There was, however, a large overlap between the lnOCC values between the groups. Supplementing the graphical assessment in Fig. 1, the coefficients of variation for OCC in periods of No IMI, Pat 1 IMI, Pat 2 IMI, and transient colonization were 1.67, 2.13, 1.70, and 1.89, respectively.

3.1.2. Multivariable model

The multivariable linear mixed model, using an exponential correlation structure, was selected based on the lowest AIC. The likelihood ratio test showed that the random intercept terms of "cow" and "lactation" within cow contributed significantly to a better model fit ($P < 0.001$). The estimates from the final model are presented in Table 2. The model showed that, compared with culture-negative periods, the lnOCC increased on average by 0.43 units in periods of subclinical Pat 1 IMI, and by 0.29 units in periods of subclinical Pat 2 IMI. At an SCC of 100,000 cells/mL this corresponds to an increase of 54,000 and 33,000 cells, respectively. The regression coefficients for DIM and lnDIM describe a lactation curve where lnOCC decreases rapidly in early lactation, reaches a minimum around 70 DIM, and slowly increases towards the initial level during the rest of the 305-d lactation. Cows belonging to the genetic group selected for high milk yield had higher lnOCC values than cows in the low mastitis group. The relationship between lnOCC and milk yield was negative; hence higher milk yield was associated with lower lnOCC. The carryover effect showed a positive relationship between the lnOCC in a given milking and the OCC measured in the residual milk from the previous cow milked in the same AMS. No difference was found between lnOCC in the two milking stations, and the variable distinguishing between the two milking stations was omitted from the final model. Only minor changes in the estimates for the other variables were seen after this omission.

Random effect estimates for the final model, reported as standard deviations (95% CI), were 0.41 (0.31 – 0.53) for cow, and 0.72 (0.64 –

Table 2
Parameter estimates from the final multivariable model describing ln-transformed on-line somatic cell count measured by DeLaval on-line cell counter in a Norwegian Red dairy herd. The model included random intercepts at cow- and lactation-level (within cow), and an exponential correlation structure.

Variable[a]	Coefficient	Standard error	P-value	R-squared[b] (%)
Lactation curve:				1.3
Days in milk (DIM)	0.005	0.0003	< 0.001	
lnDIM	−0.358	0.023	< 0.001	
Yield at cow level in the current milking (kg)	−0.038	0.002	< 0.001	1.0
Carryover	0.141	0.002	< 0.001	2.4
Parity:				
First (reference)	–	–	–	–
Second	0.303	0.148	0.047	
Third or later	0.463	0.156	0.005	
Udder health status:				2.9
No IMI (reference)	–	–	–	
Pat 1 IMI	0.434	0.031	< 0.001	
Pat 2 IMI	0.278	0.024	< 0.001	
Transient colonization	0.081	0.024	< 0.001	
Conductivity (inter-quarter difference), mSv	0.381	0.011	< 0.001	1.4
Genetic lineage:				2.7
Low mastitis incidence (reference)	–	–	–	
High milk yield	0.371	0.137	0.008	
Unknown	0.372	0.374	0.322	
Milking interval (hours)	−0.033	0.002	< 0.001	−0.5
Average milk flow rate (kg/min)	0.480	0.052	< 0.001	0.8
Season:				0.1
Winter (reference)	–	–	–	
Spring	−0.051	0.017	0.003	
Summer	−0.117	0.029	< 0.001	
Autumn	−0.048	0.024	0.047	
Intercept	3.787	0.143	< 0.001	

[a] Carryover = lnOCC from the previous cow milked in the same AMS; IMI = intramammary infection; Pat 1 IMI = IMI with mastitis pathogens from which a high somatic cell count would be expected; Pat 2 IMI = IMI with other known mastitis pathogens.

[b] R-squared = the change in marginal coefficient of determination (Nakagawa and Schielzeth, 2013) when a variable was added to a model already containing all other variables in the final model.

0.80) for lactation within cow. Within group standard error (95% CI) was 0.87 (0.86 – 0.88). The correlation structure parameter ρ^2 (95% CI) was 1.81 (1.76–1.85).

The marginal and conditional coefficients of determination showed that the fixed effects in the final model described 15.0% of the variability in lnOCC, while the fixed and random effects together described 55.2% of the milking-to-milking variability of lnOCC in clinically healthy udders. The approximate contributions of the individual variables to the overall marginal coefficient of determination are reported in Table 2.

3.2. Agreement between OCC and SCC

The CCC between the results from the OCC and the DHI laboratory, estimated on ln-transformed data, was 0.82 (95% CI: 0.78 - 0.85). The CCC has a maximum value of 1, representing the situation of perfect agreement between the two methods.

The agreement between OCC and SCC is displayed in Fig. 2. Although most observations was clustered around the superimposed line of perfect agreement, it appears that the agreement increases by increasing lnSCC values.

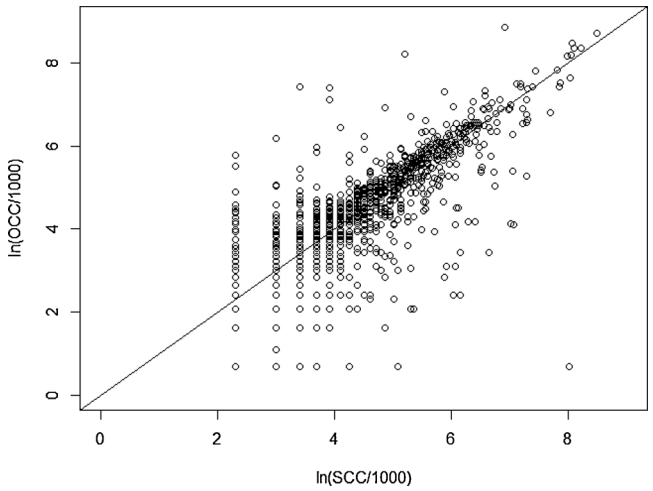

Fig. 2. On-line Cell Count (OCC) results plotted against Somatic Cell Count (SCC) measured in a DHI laboratory. Scatterplot including 1661 observations with corresponding OCC and SCC results with a superimposed 45° line representing the situation of perfect agreement between the two methods.

3.3. Repeatability

Results from the repeatability study showed nearly identical results for the two OCC sensors used in the current study. The 62 analyses performed on OCC 1 resulted in a mean OCC value (in 1000 cells/mL) of 112, a standard deviation of 12.8, and consequently a CV of 0.11. The 62 analyses performed on OCC 2 resulted in a mean OCC value (1000 cells/mL) of 117.9, a standard deviation of 12.7, also resulting in a CV of 0.11.

4. Discussion

To the authors' knowledge, this is the first presentation of the basic characteristics of frequently measured OCC relative to known IMI status. Only lactations with no records of clinical mastitis were included in the analyses. Our findings contribute to a better understanding of the normal variability in OCC; this is important for further improving the use of OCC for research, for udder health management in AMS herds, and for breeding programs.

SCC data are often used in research studies investigating how different aspects of dairy production (e.g., housing, milking routines, treatment protocols, etc.) might affect udder health (Bielfeldt et al., 2004; Erdem et al., 2007; Bhutto et al., 2010). The underlying assumption is that a risk factor affects udder health, which, in turn, results in changes in SCC. A major strength of our study is the close monitoring of IMI status by monthly QMS bacterial cultures together with detailed data recorded by the OCC and AMS at every milking. This enables us to evaluate factors of importance for lnOCC and to assess the variability in lnOCC obtained at every milking in clinically healthy cows. An important finding is that inclusion of subclinical IMI status in our model increased the degree of explanation by only 2.9 percentage points, from 12.1% to 15.0%. This is, however, a conservative estimate because the effect of IMI on OCC is adjusted by other variables included in the model. The IMI status used in this study describes persistent infections with known udder pathogens, which are recognized to be the most important cause of elevated SCC (IDF, 2013). It is therefore relevant to discuss some possible explanations why our study resulted in a relatively low degree of explanation attributed to udder health status: Firstly, this study focused on clinically healthy udders, and lactations with clinical mastitis were excluded from the analysis. By doing so, the range of IMI statuses was restricted to subclinical mastitis, and it is likely that including cases of clinical mastitis would have increased the degree of explanation in our model. Secondly, quarter milk samples

were collected monthly. It is possible that a higher sampling frequency would also have increased the degree of explanation between the subclinical mastitis cases as defined in our study and the OCC values obtained at every milking.

As for all biological variables, some degree of normal or physiological variation should be expected in SCC. Our results obtained from a herd of Norwegian Red cows show that the normal variation is likely to be much higher than can be explained through close monitoring of clinically healthy cows in sensor systems commonly used in AMS. This is underlined by the graphical assessment of the distribution of lnOCC, which shows a large extent of overlap in lnOCC values between periods without IMI and periods with either IMI or transient colonization. Nevertheless, the use of SCC in udder health management has contributed to substantial improvements in dairy production by identifying cows in need of closer attention, e.g., when implementing selective dry cow therapy (Østerås et al., 1999; Zecconi et al., 2018a; Lipkens et al., 2019).

In one of the few reports on variability in SCC, Schepers et al. (1997) estimated variance components for factors affecting SCC at quarter level from data recorded at approximately monthly intervals and reported that their model explained 50.2% of the variation in ln-transformed SCC. The model of Schepers et al. (1997) included herd and cow within herd, in addition to season, bacterial diagnoses, stage of lactation, parity, and clinical mastitis. In their data from seven herds, cow within herd explained 11% of the overall variability, while herd explained only 0.6%. In contrast to Schepers et al. (1997), the present study used OCC data at cow level, and data were recorded at every milking. It is possible that this difference has introduced additional variability to our data. In addition to the monthly QMS, our model used conductivity data measured at every milking as an indicator of changes in udder health status; this is a possible explanation for reaching a similar overall degree of explanation as that of Schepers et al. (1997).

Mastitis has been included in the breeding program for the Norwegian Red breed since 1978, resulting in genetic improvement (Heringstad and Østerås, 2013). More recently, geometric mean SCC over 305-day lactations have been included in the genetic evaluation for Norwegian Red (Interbull, 2012). SCC have also been evaluated as an alternative trait in the absence of reliable data on clinical mastitis, and a genetic correlation of 0.7 between these two traits shows not only that SCC is a relevant indicator for clinical mastitis, but also that SCC and clinical mastitis are genetically different traits (Ødegård et al., 2003). In our study, clinically healthy cows of the genetic group for high milk yield had higher lnOCC values than cows bred for low mastitis risk, also after adjustment for differences in milk yield. Hence, the effect of genetic lineage on lnOCC in our final models is likely to be a true effect of genetic differences in mastitis resistance, rather than a correlated response of differences in production level. This is in agreement with previous research results in the same breed (Heringstad et al., 2008).

The AMS used in our study were adjusted to reduce the amount of residual milk in the system after each milking (carryover effect). Nevertheless, our statistical adjustment for the carryover effect was significant in the multivariable models, and increased the marginal coefficient of determination by 2.4%. Løvendahl and Bjerring (2006) and Løvendal et al. (2010) reported up to 20% carryover in various types of AMS, showing that the impact of carryover, and the need for adjustment, is pronounced in commercial herds for which the sampling equipment has not been optimized. As pointed out by Sørensen et al. (2016), correction of carryover effect is also relevant for DHI samples collected in AMS. In this case, the carryover effect will not only affect the SCC measurement, but also the other milk constituents measured in the same sample. These results show that further improvements in the sampling equipment are necessary. Furthermore, by obtaining data on the sampling order, it might be possible for DHI systems to adjust for the carryover effect and provide more precise estimates for SCC and other milk constituents.

With the high degree of normal variability in OCC and the large extent of overlap in OCC in periods with and without IMI, it seems likely that identification of new biomarkers or combinations of biomarkers that are better at distinguishing pathological from physiological processes in the udder would be of benefit to the dairy industry. The difference in electrical conductivity between the quarter with the highest and lowest value was significantly related to OCC, which is in agreement with previous research (Nielen et al., 1992); a higher difference was associated with increasing OCC. Like SCC, electrical conductivity is used as an indicator of ongoing inflammatory processes in the udder. However, conductivity has been shown to have poor diagnostic test properties for the detection of subclinical mastitis (Norberg et al., 2004). Although the combination of electrical conductivity and SCC has been found to improve detection of clinical mastitis (Kamphuis et al., 2008), it is not known whether this is also the case for subclinical mastitis. A number of alternative biomarkers, such as L-lactate dehydrogenase, N-acetyl-β-D-glucosaminidase activity, and milk amyloid A, have been evaluated for the detection of clinical mastitis (Chagunda et al., 2006; Gerardi et al., 2009). The use of these on commercial farms is limited, and only L-lactate dehydrogenase has been implemented in on-farm systems (DeLaval Herd Navigator; DeLaval, Tumba, Sweden). Furthermore, the concentration of these biomarkers in milk is related to a compromised blood-milk barrier, and they are therefore less suitable for detection of subclinical mastitis. Methods differentiating between cell types in milk have recently been developed for the use in DHI laboratories (Damm et al., 2017), but it is still unclear how much useful information this adds over traditional SCC measurements (Zecconi et al., 2018b). Another aspect is the dilution effect of milk from healthy quarters, which represents an important limitation of using composite milk samples for detection of changes in milk arising in one quarter. Forsbäck et al. (2010) studied the variability in milk constituents at quarter level and argued that repeated measurements at quarter level provides more accurate information on udder health than cow-level data.

Sørensen et al. (2016) evaluated the agreement between OCC results and SCC results from a DHI laboratory in seven commercial herds, and reported generally good agreement between the two methods (mean $R^2 = 0.86$), although their results differed between herds and breeds. In line with Sørensen et al. (2016) the results from the current study indicates that the agreement between the two methods was reasonably good (CCC = 0.82) also in this herd of Norwegian red cows. However, the graphical assessment revealed that the results differed substantially between methods in some cases, and that this trend was more pronounced at low lnSCC values. This needs to be taken into consideration when operating at low thresholds for defining subclinical mastitis.

The repeatability of the OCC sensor, as evaluated by coefficient of variation, was identical for the two devices used in our study (CV = 0.11) at an OCC-level comparable to threshold values for the detection of IMI (e.g. 132.000 cells/mL at Sp = 90% for the detection of Pat 1 IMI; Dalen et al., 2019). For comparison, the manufacturer of the Bentley Somacount, which was used at the DHI lab, reports a CV ≤ 0.06 at 100,000 cells/mL (Bentley Instruments Inc., Chaska, MN). However, the present study was performed by manually injecting the milk sample into the apparatus, hence any additional variability caused by the sampling method could not be quantified. It should also be noted that the current study evaluated the repeatability at one OCC level only, and that data for other OCC levels is needed before concluding on the repeatability for the whole range of possible OCC values.

Milking interval was included in the final model and showed a significant relationship with the outcome variable. Nevertheless, with the chosen method for evaluating the contribution of the individual variables to the overall degree of explanation, milking interval apparently had a negative impact. This can be interpreted as an artifact arising from the combination of milking interval as a fixed effect and the correlation structure included in our model to account for the dependency between residual error terms within cow and lactation.

Because milking interval can be considered a measure of the temporal proximity between two observations, including this variable in the model will affect the correlation structure parameters. This way, when removing milking interval from the model, a larger proportion of the variance was accounted for by the random effects, resulting in the situation where omitting milking interval as a fixed effect apparently led to a higher marginal degree of explanation. In lack of a more sophisticated method, we acknowledge that the results should be interpreted as approximate contributions to the overall model fit.

We recognize that our study has some limitations that should be considered when interpreting the results. The data were obtained from a single Norwegian Red herd, and although the herd, including management practices, housing, milking procedure etc., is comparable to herds on commercial Norwegian farms of the same size, extrapolation to other herds and other breeds should be done with caution.

5. Conclusion

This study identified several factors associated with fluctuations in frequently measured OCC values in clinically healthy cows in an AMS herd. However, these factors only explained a small proportion of the overall variability in the data, and a large degree of the overall variability remain unexplained despite close monitoring of the IMI status by monthly quarter milk samples. The unexplained variability likely represents physiological fluctuations in OCC, which is important to consider when using frequently measured OCC in research or for herd management purposes.

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References

Berry, D.P., Coughlan, B., Enright, B., Coughlan, S., Burke, M., 2013. Factors associated with milking characteristics in dairy cows. J. Dairy Sci. 96, 5943–5953. https://doi.org/10.3168/jds.2012-6162.

Bhutto, A.L., Murray, R.D., Woldehiwet, Z., 2010. Udder shape and teat-end lesions as potential risk factors for high somatic cell counts and intra-mammary infections in dairy cows. Vet. J. 183, 63–67. https://doi.org/10.1016/j.tvjl.2008.08.024.

Bielfeldt, J.C., Badertscher, R., Tölle, K.-H., Krieter, J., 2004. Factors influencing somatic cell score in Swiss dairy production systems. Schweiz. Arch. Tierheilk. 146 (12), 555–560. https://doi.org/10.1024/0036-7281.146.12.555.

Chagunda, M.G.G., Larsen, T., Bjerring, M., Ingvartsen, K.L., 2006. L-lactate dehydrogenase and N-acetyl-β-D-glucosaminidase activities in bovine milk as indicators of non-specific mastitis. J. Dairy Res. 73, 431–440. https://doi.org/10.1017/S0022029906001956.

Cheuzeville, L., 2015. Rapid Detection of Microorganisms in the Dairy Value Chain by MALDI-TOF MS. MS Thesis. Faculty of Science and Technology, University of Stavanger, Stavanger, Norway.

Dalen, G., Rachah, A., Nørstebø, H., Schukken, Y.H., Reksen, O., 2019. The detection of intramammary infections using online somatic cell counts. J. Dairy Sci. https://doi.org/10.3168/jds.2018-15295.

Damm, M., Holm, C., Blaabjerg, M., Bro, M.N., Schwarz, D., 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 Haas, Y., Barkema, H.W., Veerkamp, R.F., 2002. The effect of pathogen-specific clinical mastitis on the lactation curve for somatic cell count. J. Dairy Sci. 85, 1314–1323. https://doi.org/10.3168/jds.S0022-0302(02)74196-9.

Djabri, B., Bareille, N., Beaudeau, F., Seegers, H., 2002. Quarter milk somatic cell count in infected dairy cows: a meta-analysis. Vet. Res. 33, 335–357. https://doi.org/10.1051/vetres:2002021.

Dohoo, I.R., Martin, W., Stryhn, H., 2009. Veterinary Epidemiologic Research, 2nd. edition. VER Inc., Charlottetown, PEI, Canada.

Erdem, H., Atasever, S., Kul, E., 2007. Some environmental factors affecting somatic cell count of Holstein cows. J. Appl. Anim. Res. 32, 173–176. https://doi.org/10.1080/09712119.2007.9706871.

Forsbäck, L., Lindmark-Månsson, H., Andrén, A., Åkerstedt, M., Andrée, L., Svennersten-Sjaunja, K., 2010. Day-to-day variation in milk yield and milk composition at the udder-quarter level. J. Dairy Sci. 93, 3569–3577. https://doi.org/10.3168/jds.2009-3015.

Gerardi, G., Bernardini, D., Azzurra Elia, C., Ferrari, V., Iob, L., Segato, S., 2009. Use of serum amyloid A and milk amyloid A in the diagnosis of subclinical mastitis in dairy cows. J. Dairy Res. 76, 411–417. https://doi.org/10.1017/S0022029909990057.

Green, L.E., Schukken, Y.H., Green, M.J., 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.

Heringstad, B., Klemetsdal, G., Steine, T., 2007. Selection responses for disease resistance in two selection experiments with Norwegian Red cows. J. Dairy Sci. 90, 2419–2426. https://doi.org/10.3168/jds.2006-805.

Heringstad, B., Sehested, E., Steine, T., 2008. Correlated selection responses in somatic cell count from selection against clinical mastitis. J. Dairy Sci. 91, 4437–4439. https://doi.org/10.3168/jds.2008-1330.

Heringstad, B., Østerås, O., 2013. More than 30 years of health recording in Norway. In: ICAR 2013 Health Data Conference: Challenges and Benefits of Health Data Recording in the Context of Food Chain Quality, Management and Breeding. May 30-31, 2013, Århus, Denmark. pp. 39–45. http://www.icar.org/Documents/technical_series/tec_series_17_Aarhus.pdf.

J. S. Hogan, R. N. Gonzalez, R. J. Harmon, S. C. Nickerson, S. P. Oliver, J. W. Pankey, K. L. Smith. Laboratory Handbook on Bovine Mastitis. Revised Edition 1999 National Mastitis Council Madison, WI.

Hogeveen, H., Kamphuis, C., Steeneveld, W., Mollenhorst, H., 2010. Sensors and clinical mastitis — the quest for the perfect alert. Sensors 10, 7991. https://doi.org/10.3390/s100907991.

Hogeveen, H., Ouweltjes, W., de Koning, C.J.A.M., Stelwagen, K., 2001. Milking interval, milk production and milk flow-rate in an automatic milking system. Livest. Prod. Sci. 72, 157–167. https://doi.org/10.1016/S0301-6226(01)00276-7.

IDF, 2013. Guidelines for the use and interpretation of bovine somatic cell counts (SCC) in the dairy industry. Bull. Int. Dairy Fed. Int. Dairy Fed Brussels, Belgium.

Interbull, 2012. International Bull Evaluation Service Official Website. Accessed Mar. 14, 2019. http://www.interbull.org.

Løvendahl, P., Bjerring, M., Larsen, T., 2010. In: Determination of carry-over in automated milking, recording and sampling systems using fluorescent tracers. In: Proc. ICAR 37th Annual Meeting, Riga, Latvia from 31 May to 4 June. pp. 147–152.

Kamphuis, C., Sherlock, R., Jago, J., Mein, G., Hogeveen, H., 2008. Automatic detection of clinical mastitis is improved by in-line monitoring of somatic cell count. J. Dairy Sci. 91, 4560–4570. https://doi.org/10.3168/jds.2008-1160.

Laevens, H., Deluyker, H., Schukken, Y.H., De Meulemeester, L., Vandermeersch, R., De Muêlenaere, E., De Kruif, A., 1997. Influence of parity and stage of lactation on the somatic cell count in bacteriologically negative dairy cows. J. Dairy Sci. 80, 3219–3226. https://doi.org/10.3168/jds.S0022-0302(97)76295-7.

Lipkens, Z., Piepers, S., Verbeke, J., De Vliegher, S., 2019. Infection dynamics across the dry period using Dairy Herd Improvement somatic cell count data and its effect on cow performance in the subsequent lactation. J. Dairy Sci. 102, 640–651. https://doi.org/10.3168/jds.2018-15130.

Løvendahl, P., Bjerring, M.A., 2006. Detection of carryover in automated milk sampling equipment. J. Dairy Sci. 89, 3645–3652. https://doi.org/10.3168/jds.S0022-0302(06)72404-3.

Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4, 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x.

Nielen, M., Deluyker, H., Schukken, Y.H., Brand, A., 1992. Electrical conductivity of milk: measurement, modifiers, and meta analysis of mastitis detection performance. J. Dairy Sci. 75, 606–614. https://doi.org/10.3168/jds.S0022-0302(92)77798-4.

Norberg, E., Hogeveen, H., Korsgaard, I.R., Friggens, N.C., Sloth, K.H.M.N., Løvendahl, P., 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.

Ødegård, J., Klemetsdal, G., Heringstad, B., 2003. Genetic improvement of mastitis resistance: validation of somatic cell score and clinical mastitis as selection criteria. J. Dairy Sci. 86, 4129–4136. https://doi.org/10.3168/jds.S0022-0302(03)74027-2.

Østerås, O., Edge, V.L., Martin, S.W., 1999. Determinants of success or failure in the elimination of major mastitis pathogens in selective dry cow therapy. J. Dairy Sci. 82, 1221–1231. https://doi.org/10.3168/jds.S0022-0302(99)75345-2.

Quist, M.A., LeBlanc, S.J., Hand, K.J., Lazenby, D., Miglior, F., Kelton, D.F., 2008. Milking-to-milking variability for milk yield, fat and protein percentage, and somatic cell count. J. Dairy Sci. 91, 3412–3423. https://doi.org/10.3168/jds.2007-0184.

Reksen, O., Gröhn, Y.T., Barlow, J.W., Schukken, Y.H., 2012. Transmission dynamics of intramammary infections with coagulase-negative staphylococci. J. Dairy Sci. 95, 4899–4910. https://doi.org/10.3168/jds.2011-4675.

Reksen, O., Sølverød, L., Østerås, O., 2008. Relationships between milk culture results and composite milk somatic cell counts in Norwegian dairy cattle. J. Dairy Sci. 91, 3102–3113. https://doi.org/10.3168/jds.2008-1006.

Core Team, R., 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/.

Schepers, A.J., Lam, T.J.G.M., Schukken, Y.H., Wilmink, J.B.M., Hanekamp, W.J.A., 1997. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. J. Dairy Sci. 80, 1833–1840. https://doi.org/10.3168/jds.S0022-0302(97)76118-6.

Schukken, Y.H., Wilson, D.J., Welcome, F., Garrison-Tikofsky, L., Gonzalez, R.N., 2003. Monitoring udder health and milk quality using somatic cell counts. Vet. Res. 34,

579–596. https://doi.org/10.1051/vetres:2003028.
Sheldrake, R.F., Hoare, R.J.T., McGregor, G.D., 1983. Lactation stage, parity, and infection affecting somatic cells, electrical conductivity, and serum albumin in milk. J. Dairy Sci. 66, 542–547. https://doi.org/10.3168/jds.S0022-0302(83)81823-2.
Sørensen, L.P., Bjerring, M., Løvendahl, P., 2016. Monitoring individual cow udder health in automated milking systems using online somatic cell counts. J. Dairy Sci. 99, 608–620. https://doi.org/10.3168/jds.2014-8823.
Torres, A.H., Rajala-Schultz, P.J., DeGraves, F.J., Hoblet, K.H., 2008. Using dairy herd improvement records and clinical mastitis history to identify subclinical mastitis infections at dry-off. J. Dairy Res. 75, 240–247. https://doi.org/10.1017/S0022029908003257.
Zadoks, R.N., Allore, H.G., Hagenaars, T.J., Barkema, H.W., Schukken, Y.H., 2002. A mathematical model of Staphylococcus aureus control in dairy herds. Epidemiol. Infect. 129, 397–416. https://doi.org/10.1017/S0950268802007483.
Zecconi, A., Sesana, G., Vairani, D., Cipolla, M., Rizzi, N., Zanini, L., 2018a. Somatic cell count as a decision tool for selective dry cow therapy in Italy. Ital. J. Anim. Sci. https://doi.org/10.1080/1828051X.2018.1532328.
Zecconi, A., Sesana, G., Vairani, D., Cipolla, M., Rizzi, N., Zanini, L., 2018b. Assessment of subclinical mastitis diagnostic accuracy by differential cell count in individual cow milk. Ital. J. Anim. Sci. https://doi.org/10.1080/1828051X.2018.1533391.