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COMPARISON OF SOMATIC CELL COUNTING DEVICES IN COWS' MILK: EVALUATING ACCURACY AND RELIABILITY OF DELAVAL CELL COUNTER, AND LACTOSCAN SCC WITH FOSSOMATIC METHODS

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ABSTRACT

This study aimed to compare three devices (Delaval cell counter / DCC, Lactoscan SCC / LSc SCC (somatic cell count), and Fossomatic FC / FSCC) used to determine the number of somatic cells in cow's milk. The agreement, sensitivity, and specificity of DCC and LSc SCC were evaluated against Fossomatic FC from the accredited laboratory of the Breeding Services of the Slovak Republic. The SCC results obtained from the Fossomatic FC are widely regarded as the benchmark or reference standard. The threshold of somatic cells for subclinical mastitis was 200,000 SC/mL. Mixed milk samples (a sample from all quarters) form of 144 Holstein-Friesian dairy cows were used in the study. Results were analyzed statistically using the Pearson correlation test. Subclinical mastitis was detected in 62 cows (43.1%) by FSCC, in 63 cows (43.8%) by DCC, and in 50 cows (34.7%) by LSc SCC. DCC matched with FSCC in 91.0% of samples, and LSc SCC matched in 86.8%. The sensitivity and specificity for DCC were 91.1% and 92.1%, respectively, and for LSc SCC, they were 79.5% and 94.3%. The correlation coefficient between FSCC and DCC reached 0.91 ($P < 0.001$), and between FSCC and LSc SCC, it was 0.73 ($P < 0.001$). The results obtained in the present study point to a conclusion that tested methods prove their different quality to somatic cell counting. Compared to Delaval Cell counter, Lactoscan SCC has a lower agreement with Fossomatic SCC, but also a lower detection of subclinical mastitis than other methods. In addition to these results, each of the two analyzers also has certain benefits. DCC provides easy and quick measurements of SCC on the farm. LSc SCC is cheaper, but it is less accurate.

Keywords: Cow, Milk, Indirect method, Somatic cell count, Mastitis

INTRODUCTION

Bovine mastitis is recognized worldwide as one of the most common diseases affecting dairy cattle herds. Its prevalence varies in different parts of the world, influenced by numerous risk factors such as environmental, host and pathogen factors (**Cheng and Han, 2020**). Environmental factors encompass a wide range, including nutrition, housing methods, bedding type, stand duration, environmental temperature and humidity, airflow speed in cattle shed, season, herd size, grazing conditions, housing hygiene, flooring and box types, milking technique, and milking equipment. Host factors include individual characteristics such as breed, lactation phase, number of lactations, milk leakage between milkings, peripartum-related diseases, cow cleanliness, and lameness. Mammary gland factors involve teat condition (influenced by milking hygiene), teat injuries, hyperkeratosis of teat ends, teat deposits, or previous bacterial infections (**Neja et al., 2016; Cheng and Han, 2020**).
The economic impact of mastitis results in widespread financial losses, including reductions in milk production and quality (**Awale et al., 2012; Azooz et al., 2020**). Additionally, mastitis negatively impacts the productive and reproductive performance of dairy cows (**Gunay & Gunay, 2008**). On a global scale, the average cost of mastitis failure is $131 per cow per year (**Hogeveen et al., 2019**). For clinical mastitis, losses range from $179 to $518 (**Bar et al., 2008; Rollin et al., 2015**). Subclinical forms, although not detectable by visible changes in milk appearance, occur 15-40 times more frequently than clinical cases (**Shearer & Harris, 2003; Seegers et al., 2003**). Subclinical mastitis (SCM) is characterized by production losses, reduced milk quality, and elevated SCC (**Gruet et al., 2001; Cobirka et al., 2020**). Somatic cell count (SCC) is used as a key indicator of milk quality. In the European Union, China, New Zealand, Australia, Switzerland, and Canada, the legal bulk milk SCC (BMSCC) limit is $3\text{-}4\times10^5$ cells/mL; in South Africa, India, and Brazil, it is 5×10^5 cells/mL; while in the USA, the limit is 7.5×10^5 cells/mL (**Alhussien & Dang, 2018**).
However, in raw milk threshold of somatic cell counts (SCC) is 200,000 cells/ml, whereby in higher SCC (over 200,000) the loss per dairy cow in early lactation is $170 (**Rodriguez et al., 2024**). With the increase in SCC, the decline in milk production begins. According to Tongel and Mihin's study (**2005**), milk losses reached 12% at an SCC of 1,000,000 cells/mL. According to research form 1999 the loss per dairy cow with somatic cell counts (SCC) above 400,000 cells/mL was $ 275 (**Ott, 1999**). When considering factors like cow culling, illness and calf mortality, the total impact of subclinical mastitis is likely to be even greater. Cost of the culled cow on Dutch dairy farms is €480 (**van Soest et al., 2016**).
To mitigate economic losses, simple tests and instruments are employed directly on farms. This includes both direct and indirect methods for diagnosing subclinical mastitis, which are routine on farms. Recently, new devices for determining somatic cell counts in cow's milk have been introduced into practice. However the research lacks a more detailed analysis of these new commercially produced analyzers, especially a comparison the results of these counters with long-term used instruments in accredited laboratories. Manufacturers claimed that these commercial methods show agreement with the commonly used laboratory ones. Therefore, the study focused on comparison agreement, sensitivity and specificity of two new devices (Delaval Cell Counter / DCC, Lactoscan SCC / LSc SCC) with Fossomatic FC (FSCC).

MATERIAL AND METHODS

Dairy Cow Production, Housing, and Milking

The monitoring was conducted on a dairy farm in eastern Slovakia, specifically in the district of Kosice. Holstein dairy herds were accommodated on the farm in a free-stall system with solid manure bedding. From these herds, 144 cows unaffected by the clinical form of mastitis were randomly selected for comparison of detection methods during the spring and summer months. The average milk yield was 9,000 kg. The cows were milked twice daily in a herringbone milking parlour (Agromont, Slovakia) equipped with twelve fixation boxes arranged in two rows opposite each other. Prefoam+ (Hypred S.A., Dinard, France) was used for udder hygiene before milking and was applied as foam. After foaming, the teats were mechanically cleaned with dry paper wipes intended for cleaning the entire udder and for fore-stripping. The milking vacuum was adjusted to 42 kPa, with a pulsation ratio of 60:40 and a pulsation rate of 52 cycles per minute. Milking ceased automatically once the milk flow decreased to 0.2 liters per minute. After

milking, teat disinfection was carried out using HM-VIR-Film (Agromont Nitra, SK) through teat dipping. The collected milk was kept in refrigerated tanks at +4 °C and was collected daily at approximately 11:30 a.m.

Milk collection

Upon the cows' arrival in the parlour, a clinical examination of the udder and a sensory examination of the milk were performed. Milk without any changes, taken during milking from the tank container, was collected into two tubes. The milk sample from the first tube was used for SCC analysis using simple analysers, and the milk from the second tube was used for laboratory somatic cell counting.

Somatic cell counting

Within one hour after milking, the determination of SCC in milk was performed using a DeLaval cell counter (DCC, DeLaval International AB, Tumba, Sweden) and a LactoScan SCC (LCs SCC, Milkotronic Ltd., Nova Zagora, Bulgaria). Somatic cell counting by the Fossomatic FC (FSCC, Foss, Hilleroed, Denmark) was carried out in an accredited laboratory (Certified Milk Quality Assessment Centre of the Breeding Services of the Slovak Republic s.r.o. in Žilina) the following day.

DeLaval Cell Counter

The DeLaval cell counter is a portable, battery-operated optical instrument designed to detect SCC in milk within one minute. After the milk is added, a cartridge with a small quantity of reagent (propidium iodide) is inserted into the device, where it is exposed to light. The somatic cells in the milk that come into contact with the reagent emit fluorescent signals, and the device counts the somatic cells based on the intensity of the fluorescence. The cost of the analysis is 3 EUR per sample.

LactoScan SCC

It is an advanced SCC device featuring direct fluorescence, a low-magnification microscope with quick autofocus, and cell counting software for rapid, precise, and reliable somatic cell analysis of cow, sheep, goat, buffalo, and human milk. It is designed to detect subclinical mastitis with the lowest cost per test for somatic cell count. The price of the analysis is 0.09 EUR per sample.

Statistical analysis

The SCC results obtained using the commercially available DCC device and LactoScan SCC analyzer were compared with those determined by the FSCC. The SCC measured by the Fossomatic FC was considered the gold standard. The milk was categorized based on SCC as follows: cows that were healthy or had already recovered from mastitis had an SCC below 200,000 cells/mL, while subclinical mastitis (SCM) was indicated by an SCC above 200,000 cells/mL. Agreement of the methods (%) was assessed as follows: DCC vs. FSCC and LactoScan SCC vs. FSCC. Other methods of statistical evaluation included sensitivity and specificity tests. Correlations with the FSCC (Pearson's coefficient) were also calculated for the DCC and LCs SCC (Microsoft excel, '2016 Edition, MicroSoft®, Inc., USA).

RESULTS AND DISCUSSION

The mean somatic cell count of all milk samples was the highest by FSCC (471.7 x10^3 SC/mL), followed by DCC (352.0 x10^3 SC/mL) and by LSc SCC (270.0 x10^3 SC/mL). Of the total number of dairy cows (n = 144), the subclinical mastitis (SCC over 200,000 SC/ml) was detected by FSCC in 62 cows (43.1%), with the mean SCC being 1002.6 x10^3 SC/mL; by DCC in 63 cows (43.8%) it was 721.7 x10^3 SC/mL; and in 50 cows (34.7%), it was 677.4 x10^3 SC/mL by LSc SCC (Table 1). The overall agreement of DCC matched with FSCC in 91.0% of samples and LSc SCC in 86.8%. The sensitivity and specificity for DCC was 91.1% and 92.1%, for LSc SCC 79.5% and 94.3%, respectively (Table 2).

Table 1 Determination of SCC by the three methods

	Fossomatic FC n / x	**DeLaval Cell Counter n / x**	**Lactoscan n / x**
0 - 400 x10^3	82 / 69.8 x10^3	81 / 71.9 x10^3	94 / 59.3 x10^3
>400 x10^3	62 / 1002.6 x10^3	63 / 721.7 x10^3	50 / 677.4 x10^3

Legend: n – number of cows, x – average

Table 2 Interpretation of comparison between two analysers with Fossomatic FC

	Agreement	**Sensitivity**	**Specificity**
Delaval Cell Counter *vs.* Fossomatic FC	91.0%	91.1%	92.1%
Lactocan *vs.* Fossomatic FC	86.8 %	79.5%	94.3%

When assessing the correlation between FSCC and DCC, the coefficient reached 0.91 ($P < 0.001$, Fig. 2), and between FSCC and LSc SCC, it was 0.73 ($P < 0.001$, Fig. 2).

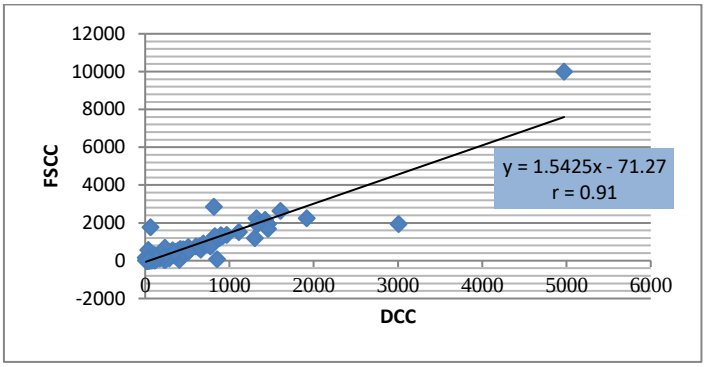

Figure 1 Correlation analysis comparing the SCC results of the DeLaval Cell counter with Fossomatic FC
Note: FSCC – Fossomatic FC , DCC – Deleval Cell counter

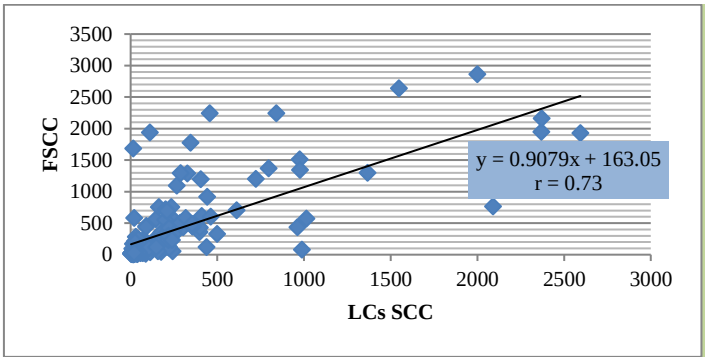

Figure 2 Correlation analysis comparing the SCC results of the LactoScan SCC with Fossomatic FC
Note: FSCC – Fossomatic FC, LCs SCC – LactoScan SCC

Indirect diagnosis of subclinical mastitis is a routine method on farms. In the past, simple semi-quantitative tests were used to detect somatic cell counts using ranges alone, providing inaccurate numerical results. However, recently, new devices for determining somatic cell numbers in milk have entered the market, suitable for use directly on farms. One such device evaluated here is the DeLaval Cell Counter (DCC), specifically developed for measuring SCC $\leq 4 \times 10^6$ cells/mL of milk from bulk tanks or individual cows (**Kawai et al., 2013**). Several studies have used DCC as the gold standard, comparing the results of simpler tests with those determined by DCC (**Malinowski et al., 2008; Kandeel et al., 2018; Arifa et al., 2022**). Our goal was to compare this instrument with a laboratory analyzer in an accredited laboratory and to assess its benefits and limitations in SCC determination under farm conditions. The correlation coefficient of SCC determined by DCC compared to FSCC was r = 0.91 ($P < 0.001$).

In a study by Kawai et al. (**2013**), DCC and Fossomatic devices recorded a correlation coefficient of r = 0.96 ($P < 0.001$), consistent with our results. Similarly, in experiments with sheep's milk, high correlation values of r = 0.99 and regression of 0.91 to 1.01 with Fossomatic were found (**Gonzalo et al., 2008**). In a Czech research comparing SCC determined in milk samples from several livestock species (cows, sheep, and goats) using the DeLaval cell counter (DCC) and Fossomatic 90 (FSC), the highest correlation between DCC and FSC was observed in cow milk, where the coefficient reached 0.99 (**Hanuš et al., 2011**). Another study evaluating somatic cell quantification in lactating cows found correlation coefficients of 0.81 and 0.88 for DCC and FSCC, respectively (**Enger et al., 2020**). Additionally, in a comparison with direct microscope methods, the correlation coefficient for DCC was 0.95 (**Berry et al., 2007**). In a previous Slovak study from 2023, the agreement for SCC up to 200,000 SC/mL between DCC and FSCC was 98.3%. At higher SCC values above 200,000 SC/mL in dairy cow milk, the agreement values were significantly lower. The correlation coefficient for DCC was 0.917 (**Hisira et al., 2023**). In a Turkish study analyzing nine different subclinical mastitis screening methods, practical tests like CMT, LE, EC, and highly reliable tests like DCC ranked high for large-scale dairy farms (**Akköse, & Polat, 2024**). In a U.S. study comparing various devices for measuring somatic cell count (SCC) in individual Holstein mammary quarters with a reference standard,the Fourier Transform Spectrometer 600 Combi System, both the RT-10 (Platinum microscope SCC testing device for the iPhone – camera of iPhone is used to count SCC) and DSCC showed high sensitivity and specificity for identifying quarter SCC above and below 200,000 cells/mL (**Jacobsen et al., 2023**). The next research focused on the assessment of several analyzers using quarter samples from 658 individual cows and 100 bulk-tank samples to measure

either SCC or individual quarter SCC. The RT-10 showed the highest coefficient of determination ($R^2 = 0.86$), the lowest mean square prediction error (MSPE), and the highest proportion of MSPE attributed to random variation (96%). Both the RT-10 and DSCC exhibited the highest sensitivity and specificity in distinguishing quarter SCC levels above and below 200,000 cells/mL. For bulk-tank SCC, the DSCC demonstrated the highest coefficient of determination ($R^2 = 0.45$), the lowest MSPE, and the greatest proportion of MSPE due to random variation (80%). The use of these devices (RT-10 and DSCC), which can determine the SCC in a very short time, can also contribute to reducing the administration of ATB during dry periods (**Jacobsen et al., 2023**).

In addition to DCC, the LactoScan SCC laboratory instrument was evaluated in more detail for the first time. The results showed slightly lower compliance (86.8%) and correlation with FSCC (0.73) compared to DCC. In a Bulgarian study, the coefficient of variation of SCC determined with the LSc SCC device was evaluated against microscopic somatic cell counting, with the device showing lower values in all groups (**Chengolova et al., 2019**).

CONCLUSION

The results of the present study indicate that the tested methods demonstrate varying quality in somatic cell counting, each with its own benefits. DCC provides easy and quick measurements of SCC on the farm and allows measurement of SCC of cooled bulk tank milk. However, it cannot count SCC over four million cells/mL and is relatively expensive. LSc SCC shows reasonable accuracy and a reasonable price, but it is less accurate and requires longer sample preparation time before analysis. In the recommnedation both instruments can be used in the monitoring of udder health on dairy farms and enable rapid decisions on hygienic or therapeutic treatment to be made. It enables earlier detection and intervention of subclinical mastitis, preventing economic losses and more severe disease development. Prevention is always better than treatment.

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