Praca oryginalna *Original paper*

Effect of storage conditions and preservation with Bronopol on somatic cell count with the DeLaval cell counter in cow milk

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Summary

The aim of the examination was to evaluate the effect of the preservative and storage conditions of samples on the somatic cell count (SCC) with the DeLaval cell counter (DCC) and Fossomatic 90 (FS) in different kinds of cow milk. SCC was measured in 25 premilk samples from the healthy quarters (HQ) of 25 clinically healthy cows, in 25 premilk samples from the quarters with subclinical *mastitis* (SMQ) of other 25 clinically healthy cows, in 25 composite cow milk (CCM) samples of other 25 clinically healthy cows, and in 25 samples of bulk tank milk (BTM) from 25 farms. The somatic cell count determined with DCC immediately after sampling ranged from 1,000 to 156,000/ml in samples from HQ, from 142,000 to 1 672,000/ml in samples from SMQ, from 5,000 to l 442,000/ml in CCM and from 46,000 to 603 000/ml in BTM. It was established that DCC indicates almost an identical number of cells in milk immediately after sampling, and in the same samples cooled and stored for 1h, 24h, 72h or frozen and stored for 7 days, irrespective of preservation with Bronopol. Somatic cell counts in unpreserved and preserved milk samples determined with DCC were very close to cell counts established with Fossomatic 90. The correlation between data obtained with DCC and Fossomatic 90 was statistically significant ($p < 0.01$).

Keywords: cow milk, somatic cell count, preservative, storage, DeLaval cell counter

Udder inflammations are still the most frequent and costly diseases affecting dairy cows in the world (15, 30, 39). *Mastitis* alters both milk composition and production. Mammary gland inflammation is a consequence of the activity of a number of cell and soluble factors that function together to eliminate invading microorganisms (27, 29).

Milk from inflamed glands is characterized by a lower concentration of basic components (lactose, casein, vitamins, some minerals), and higher concentration or activity of cytokines, eicozanoids, some enzymes, minerals and proteins (8, 3, 14, 20, 25, 31, 41). The presence of higher concentrations of TNF-alpha and other cytokines, PGF2-alpha, IGF, BSA, histamine, bradykinin, thiocyanate, reactive oxygen species or lower concentrations of beta-casein, lactoalbumin-alpha, lactoglobulin-beta, vitamin E and C and triiodothyronine in fresh milk, that are connected with *mastitis*, can be potentially detrimental both to the health of calves and, above all, for humans (21).

Clinical udder inflammation is diagnosed by clinical methods and does not require additional equipment. Subclinical *mastitis* can be detected using a variety of direct and indirect laboratory tests but the primary definition is based upon the somatic cell count. In milk from the healthy quarters of healthy cows the number of somatic cells is lower than 100,000/ml (16, 36, 39, 41). The number of leucocytes, mostly PMNs, increases during the inflammation of the mammary gland. SCC in milk from subclinically infected and inflamed quarters fluctuates from 200,000 to 2,000,000/ml or even more, and in clinically sick quarters SCC often exceed 10 million/ml (9, 13, 19, 22, 36, 40).

Determination of SCC is the most widely used method for detecting and measuring the inflammatory process in the udder as well as milk losses both from the cow and at the herd level. SCC is also chosen and accepted as an indicator of bulk tank milk quality (9, 18, 29, 31, 33). In practice milk somatic cells are measured directly by the microscopic method or with highly sophisticated apparatuses (Fossomatic, Somacount, Somascope) in laboratories and with portable instruments such as the C-reader system (24) and DeLaval cell counter (34, 36), that are suitable for farmers and laboratories.

The aim of the examination was to evaluate the effects of the preservative, temperature and time of sample storage on the SCC measured with a DeLaval cell counter.

Material and methods

Somatic cell count was measured in 25 premilk samples from the healthy quarters (HQ) of 25 clinically healthy cows, in 25 premilk samples from quarters with subclinical *mastitis* (SMQ) of other 25 clinically healthy cows, in 25 composite cow milk samples (CCM) of 25 clinically healthy cows, and in 25 milk samples of bulk tank milk (BTM) from 25 farms.

Samples from the healthy quarters of 25 healthy cows were taken in farm Gr before the morning milking. Teats were cleaned, strict premilk was forstripped on strip plates and checked for clinical *mastitis* (visible changes) and then California Mastitis Test (CMT) was performed. Interpretation (scoring) of CMT (TOK in Poland): 1) negative (–), 2) questionable or slight (+/–), 3) slight-moderate (+), 4) moderate (++), 5) heavy (+++). From one chosen CMT-negative (–) quarter of each cow a 100 ml of premilk was collected manually into a sterile glass vial. Somatic cell count was measured immediately with a DeLaval cell counter (DCC) according to the manufacturer's instructions in three repetitions (examination I). The sample was then divided into 2 identical portions (halves). One half was treated with the preservative (Bronopol; 0.05%: according to Polish regulations – complete with the number of standard "(PN-EN ISO 13366-1:2000)" – and the second one was not treated. Both halves were then put into a portable cooler and cooled to 5-8°C. The samples were then transported (about 30-50 min.) to the Laboratory of National Veterinary Research Institute in Bydgoszcz. Immediately after delivery both preserved and unpreserved samples were heated to 42°C and examined with DCC and Fossomatic 90 (FS) in three repetitions. Preserved and unpreserved samples were then once more divided. One part was stored in a refrigerator (4°C) and the second part was frozen (minus 20°C). Examinations of the refrigerated samples (DCC, FS) were made after 24 hours (examination III) and next 48 hours, i.e. 72 hours after sampling (examination IV). The frozen milk samples were examined only once – in the 7th day after sampling (examination V).

Samples from the subclinically inflamed quarters (subclinical *mastitis* quarters; SMQ) of 25 clinically healthy cows were taken in farm Sc. Teats were cleaned, first streams of milk (foremilk) were checked for clinical *mastitis* and CMT was performed. From one chosen CMT-positive quarter (+: slight-moderate or ++: moderate) 100 ml of foremilk was taken manually into sterile glass vials. Examination I (with DCC), preservation, cooling, transportation of samples, and all measurements with DCC and FS (examinations II, III, IV, V) in the Laboratory were performed as in the case of samples from healthy quarters (HQ).

Composite cow milk (CCM) samples (cow milk, milk of individual cows) were taken in farm Gr immediately after the morning milking. Teats were cleaned; foremilk was stripped on strip plates and checked for clinical *mastitis*. After the sanitizing and drying of teats teat cups were attached and cows were milked to individual cans. Teat cups were removed when milking was ended and the teats were dipped. The sample (100 ml) for SCC examinations was taken after a slow stirring of milk from the middle part of the can and immediately checked with DCC (examination I). The preservation, cooling, transportation of samples and all measurements with DCC and FS (examinations II, III, IV, V) in the Laboratory were performed as already described (HQ, SMQ).

Bulk tank milk (BTM; farm milk) samples were taken from 25 bulk tanks belonging to 25 farms (milked from 10 to 50 cows) located near Bydgoszcz. Cows were prepared for milking as already described. The sample (100 ml) for SCC examinations was taken from the middle part of the tank and immediately checked with DCC (examination I). The preservation, cooling, transportation of samples, and all measurements with DCC and FS (examinations II, III, IV, V) in the Laboratory were performed as described in the case of HQ, SMQ and CCM.

In addition fresh milk samples from healthy quarters and from subclinically inflamed quarters were examined bacteriologically in the Laboratory.

During the examinations attention was also paid to the sensitivity and repeatability of cell count results obtained with a DeLaval cell counter in the same sample.

Statistical calculations included multifactorial variance analysis with the division of means into homogenous groups with Fischer test. The analysis was performed with Statistica® (Statsoft) software.

Results and discussion

SCC in fresh milk immediately after sampling. Somatic cell count (SCC) established with a DeLaval Cell Counter (DCC) in premilk from healthy quarters (HQ) ranged from 1,000 to 156,000/ml. Twenty-one samples (84%) had less than 100 000 cells /ml and only 4 samples (16%) had slightly more. In addition, all samples were bacteriologically negative. SCC in premilk from subclinical *mastitis* quarters (SMQ) ranged from 142,000 to 1672,000/ml. All samples were bacteriologically positive. SCC in fresh composite milk from individual cows (CCM, can milk) ranged from 5,000 to 1 442,000 and in fresh bulk tank milk (BTM) this count ranged from 46,000 to 603,000/ml.

Premilk from healthy quarters. The arithmetical means of SCC in samples from the HQ of healthy cows determined with DCC and FS are presented in table 1. Measurements of fresh milk with DCC at the farm immediately after sampling showed 35,533 +/– 48,159 cells in 1 ml of premilk. It can be observed that DCC and Fossomatic produced almost the same results if measurements were performed immediately after samples were delivered to the Laboratory (examination II). Results did not change if measurements were done after 24 h (examination III) and 72 h (examination IV) of storing milk in the refrigerator (plus 4 degrees C). Bronopol (BR) did not change SCC if measurements with DCC and Fossomatic were performed after the transportation of samples to the Laboratory (examination II). Preservation did not affect SCC in milk stored in the refrigerator for 24 and 72 hours. Somatic cell count in milk samples frozen for 7 days that was established with DCC and Fossomatic was lower, but not statistically, compared to results noted in samples cooled only and stored for 24 and 72 hour in the refrigerator.

Premilk from subclinically inflamed quarters. The arithmetical means of SCC in premilk from the SMQ of clinically healthy cows measured with DCC and FS are presented in table 2. DCC established 560,613 +/– 328,028 cells in 1 ml of fresh milk immediately after sampling at cow side (examination I). Mean counts obtained with DCC were higher (not statistically) when measurements of

Tab. 1. Arithmetical averages and standard deviations of somatic cell count (SCC/ml) measured with a DeLaval Cell Counter (DCC) and Fossomatic 90 (FS) in milk samples from the healthy quarters of healthy cows

No of examination	DCC (n = 25)		FS (n = 25)	
	Unpreserved	Preserved	Unpreserved	Preserved
I (at the farm)	35,533 ± 48,159^{A}	Not examined	Not examined	Not examined
II (1 h after sampling)	38,933 ± 48,390^{Aa}	41,760 ± 54,163^{Aa}	41,320 ± 54,033^{Aa}	41,160 ± 54,923^{Aa}
III (stored at 4°C for 4 h)	46,187 ± 45,842^{Aa}	47,520 ± 54,479^{Aa}	41,213 ± 55,916^{Aa}	39,080 ± 52,352^{Aa}
IV (stored at 4°C for 2 h)	44,707 ± 52,605^{Aa}	41,360 ± 54,880^{Aa}	45,093 ± 57,426^{Aa}	38,613 ± 51,545^{Aa}
V (frozen for 7 days)	33,813 ± 49,408^{Aa}	31,893 ± 43,292^{Aa}	28,293 ± 42,414^{Aa}	20,813 ± 30,891^{Aab}

Explanation: different lowercase letter – statistically significant difference in the row, different capital letter – statistically significant difference in the column, correlation coefficients for results obtained with DCC and FS after 1, 24, 72 hours and 7 days: R (unpreserved milk) = 0.9589, $p < 0.01$; R (preserved milk) = 0.9722, $p < 0.01$

Tab. 2. Arithmetical averages and standard deviations of somatic cell count (SCC/ml) measured with a DeLaval Cell Counter (DCC) and Fossomatic 90 (FS) in milk samples from the quarters with subclinical *mastitis* of clinically healthy cows

No of examination	DCC (n = 25)		FS (n = 25)	
	Unpreserved	Preserved	Unpreserved	Preserved
I (at the farm)	560,613 ± 328,028^{A}	Not examined	Not examined	Not examined
II (1 h after sampling)	621,028 ± 365,023^{Aa}	733,760 ± 404,134^{Aa}	825,267 ± 481,921^{Aa}	762,480 ± 440,230^{Aa}
III (stored at 4°C for 24 h)	631,533 ± 380,269^{Aab}	741,627 ± 408,149^{Aab}	780,720 ± 459,101^{ABa}	730,693 ± 413,831^{ABab}
IV (stored at 4°C for 72 h)	694,480 ± 403,274^{Aa}	752,853 ± 404,734^{Aa}	798,067 ± 454,439^{Aa}	739,013 ± 440,893^{ABa}
V (frozen for 7 days)	576,253 ± 349,291^{Aa}	596,480 ± 342,164^{Aa}	573,827 ± 373,923^{Ba}	533,87 ± 337,680^{Ba}

Explanation: different lowercase letter – statistically significant difference in the row, different capital letter – statistically significant difference in the column, correlation coefficients for results obtained with DCC and FS after 1, 24, 72 hours and 7 days: R (unpreserved milk) = 0.9451, $p < 0.01$; R (preserved milk) = 0.9619, $p < 0.01$

cooled samples were performed in the Laboratory (examination II, III and IV). Somatic cell numbers were slightly higher in samples preserved with Bronopol. Fossomatic 90 found more cells than a Delaval cell counter in unpreserved samples after their delivery to the laboratory (examination II). Results noted after 24 hours (examination III) with DCC and Fossomatic were close. Average somatic cell counts determined with DCC and FS in milk samples stored for 72 hours (examination IV) were almost identical. However, SCC established with FS in frozen samples were statistically lower if compared to counts established earlier in cooled samples, but they were almost identical if compared to SCC in frozen samples determined with DCC.

Cow composite milk. The arithmetical averages and standard deviations of SCC in the CCM samples of composite particular cows' milk assessed with DCC and FS are presented in table 3. Average SCCs measured with DCC (examination I) in fresh milk immediately after sampling were 179,333 +/– 280,137. The average somatic cell counts established with DCC during further examinations (II, III, IV) were almost the same, and they were slightly lower if cells were measured in frozen milk (examination V). The results of measuring SCC with Fossomatic in milk samples after their transportation to the laboratory (examination II) and after storing in the refrigerator (examinations III and IV) did not differ, but results obtained in examination V (fresh and preserved frozen milk) were slightly lower.

Bulk tank milk. The arithmetical averages determined with a DeLaval cell counter and Fossomatic 90 are presented in table 4. SCC measured with DCC at examination I was 258,347 +/– 139,527. The number of cells determined with DCC 1 hour after sampling as well after 24 hours, 72 hours (milk stored in the refrigerator), and 7 days (milk frozen) fluctuated slightly when compared to the results of the first examination in the cow-shed, irrespective of preservation. The results of the examinations of unpreserved and preserved milk with Fossomatic were almost the same.

Sensitivity and repeatability. During the examination it was also noted that DCC can detect from one thousand cells to 1 672,000 cells or even more in 1 ml of milk in particular samples. It proves that DCC is a highly sensitive apparatus. Repeatability of results was also high both in samples from the healthy quarters of healthy cows and in samples from subclinically inflamed quarters, as well as in composite cow milk and in bulk tank milk samples, especially in milk containing more than 50,000 SCC/ml. Data on the repeatability and correlations depending on SCC established with DCC and FS are presented in figure 1. It can be noted that a single outcome can differ by even more than 40% from the average value only in milks with a small number of somatic cells (20,000/ml or less). This error was smaller if SCC was higher. Single outcome differed from the average count by 15% in milk with 50,000 cells/ml, by 10% in milk with 100,000 cells/ml and by 3 to 7% in 1 ml of milk with more than 200,000 cells. It should be added that the errors of single outcomes in measurements with DCC were almost identical as those in measurements with FS.

Somatic cell count (SCC) is a fast and reliable analytical tool. It is related to the immunological status of the udder and increases in response to an inflammatory stimulus such as bacterial infections (19, 27, 28, 33). Different models of Fossomatic cell counters have been recommended by International Dairy Federation as one of the methods for SCC determination (17). The number of somatic cells determined with Fossomatic is also com-

Tab. 3. Arithmetical averages and standard deviations of somatic cell count (SCC/ml) measured with a DeLaval Cell Counter (DCC) and Fossomatic 90 (FS) in composite individual cow milk samples

No of examination	DCC (n = 25)		FS (n = 25)	
	Unpreserved	Preserved	Unpreserved	Preserved
I (at the farm)	179,333 ± 280,137^{A}	Not examined	Not examined	Not examined
II (1 h after sampling)	230,853 ± 125,120^{Aa}	246,160 ± 355,417^{Aa}	250,307 ± 417,980^{Aa}	223,413 ± 369,144^{Aa}
III (stored at 4°C for 24 h)	214,747 ± 336,183^{Aa}	230,347 ± 373,051^{Aa}	237,053 ± 394,962^{Aa}	223,160 ± 394,346^{Aa}
IV (stored at 4°C for 72 h)	195,347 ± 241,435^{Aa}	238,693 ± 392,748^{Aa}	254,640 ± 419,205^{Aa}	230,387 ± 390,043^{Aa}
V (frozen for 7 days)	141,573 ± 191,044^{Aa}	190,907 ± 326,823^{Aa}	160,107 ± 208,117^{Aa}	163,867 ± 301,277^{Aa}

Explanation: different lowercase letter – statistically significant difference in the row, different capital letter – statistically significant difference in the column, correlation coefficients for results obtained with DCC and FS after 1, 24, 72 hours and 7 days: R (unpreserved milk) = 0.9732, $p < 0.01$; R (preserved milk) = 0.9936, $p < 0.01$

Tab. 4. Arithmetical averages and standard deviations of somatic cell count (SCC/ml) measured with a DeLaval Cell Counter (DCC) and Fossomatic 90 (FS) in bulk tank milk samples

No of examination	DCC (n = 25)		FS (n = 25)	
	Unpreserved	Preserved	Unpreserved	Preserved
I (at the farm)	258,347 ± 139,527^{A}	Not examined	Not examined	Not examined
II (1 h after sampling)	239,880 ± 121,587^{Aa}	268,093 ± 133,943^{Aa}	226,107 ± 133,435^{Aa}	206,107 ± 121,362^{Aa}
III (stored at 4°C for 24 h)	293,040 ± 152,115^{Aa}	282,600 ± 149,721^{Aa}	247,800 ± 134,307^{Aab}	238,453 ± 133,371^{Aab}
IV (stored at 4°C for 72 h)	282,453 ± 147,139^{Aa}	257,933 ± 137,164^{Aa}	266,947 ± 154,229^{Aa}	249,573 ± 137,696^{Aa}
V (frozen for 7 days)	269,693 ± 148,608^{Aa}	277,240 ± 146,359^{Aa}	262,800 ± 146,027^{Aa}	239,547 ± 131,732^{Aa}

Explanation: different lowercase letter – statistically significant difference in the row, different capital letter – statistically significant difference in the column, correlation coefficients for results obtained with DCC and FS after 1, 24, 72 hours and 7 days: R (unpreserved milk) = 0.9060, $p < 0.01$; R (preserved milk) = 0.9358, $p < 0.01$

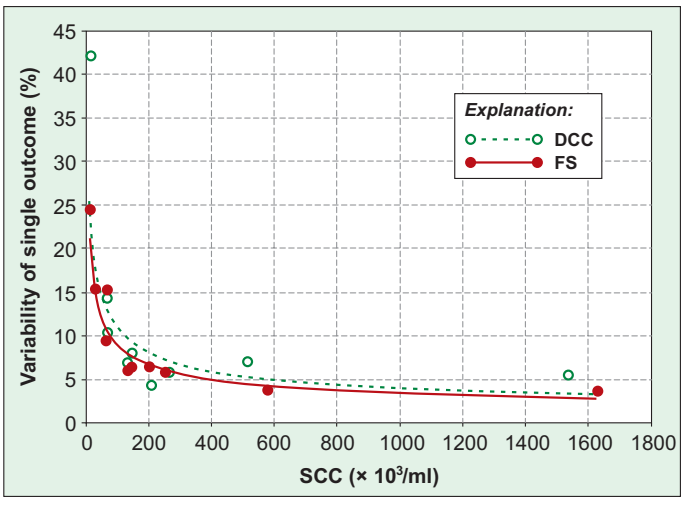

Fig. 1. Repeatability of a single outcome obtained with DCC and FS depending on the SCC/ml of milk

monly used as a reliable control in cases of other indirect and direct methods of evaluation (1, 5, 7, 24). The effect of preservatives, duration of cooled milk storage, temperature and conditions of heating and measurements on FS results are well documented (8, 35, 38). It was also proved that Bronopol does not affect SCC in milk (2, 11).

In the present examination somatic cell counts measured with a DeLaval cell counter in premilk from the healthy or subclinically inflamed quarters of clinically healthy cows as well as in composite cow milk and in bulk tank milk were very close to the numbers of cells established with Fossomatic 90. DCC showed the same numbers of cells in milk immediately after sampling, and in the same samples cooled to 5-8°C and stored for 1 h, refrigerated to 4°C and stored for 24 or 72 hours, irrespective of preservation with Bronopol. The correlation coefficients between outcomes both in unpreserved and preserved milk established with DCC and FS were very high and statistically significant. However, in some cases both DCC and especially FS counted less cells (not statistically) in samples frozen to minus 20°C and stored for 7 days when compared to results established in fresh or refrigerated milk. Other authors also reported a decrease in SCC after freezing and thawing of milk. Barkema et al. (4) reported a 10% decrease of SCC in cow milk frozen (minus 20°C) and stored for 28 days. Martinez et al. (23) also found lower SCC in ovine milk after freezing as compared to outcomes established after refrigeration. The decrease in SCC was probably connected with mistakes during freezing and thawing of samples.

Results achieved in our examinations are in agreement with other authors' data. Sarikaya and Bruckmaier (36) stated that DCC is a useful apparatus for the determination of disorders in the cow mammary gland. Van Werven et al. (42) underlined that DCC gives more precise results in comparison with the indirect method of counting cells. It enables diagnosing mastitic cows and selecting udders or quarters for bacteriological examination. Ruegg et al. (34) indicated a high level of agreements of SCC in cow milk measured with Fossomatic and DCC. The correlation between the Log_{10} FS (5.1) and Log_{10} DCC (5.1) was 0.92 ($p < 0.001$). When subclinical *mastitis* was defined based on a threshold of 250,000 cell/ml, there was a 95.6% agreement observed between data from FS and DCC. The high correlation between cell numbers determined by DCC, Fossomatic and the microscopic method in ovine milk was stated by Gonzalo et al. (12). It should be added that DCC was used as a precise instrument in scientific examinations of cows (36), ewes (11) and goats (6 milk samples).

In our examinations the repeatability of measurements was also satisfactory, especially in cases with higher SCC. This conclusion agrees with the results of Faust and Timms

(10). They stated that repeatability was higher for milk samples with SCC above 500,000/ml than for samples with a lower number of cells. The coefficient of variation for high SCC samples was 6.8% but it was ≥ 25% for samples with less than 500,000/ml. Our results are also close to the conclusions of Sarikaya and Bruckmayer (36), who stated that measuring SCC with DCC provides reliable and precise results particularly in quarters with a high SCC.

The authors' examinations enable them to state that DCC is highly useful both for field veterinarians and farmers. Veterinarians can diagnose subclinical *mastitis* in the cow shed and select cows (quarters) for bacteriological examinations. They can also evaluate progress in the treatment of *mastitis*. It is also possible to determine SCC in samples delivered to the clinic or laboratory. Farmers can measure SCC both in composite cow milk or bulk tank milk and in the milk of a cow that was treated. However, it should be taken into account that SCC changes in the course of lactation (7, 26), between the a.m. and p.m. milking (32), and in milk fractions during milking (25, 36). According to Riekering et al. (32), for accurate interpretations of SCC tests – whether by a laboratory, portable SCC device, or the CMT – veterinarians, researchers, and udder health advisors should take milk samples immediately before milking.

In conclusion, the DeLaval cell counter is a highly useful portable apparatus for accurate measurements of somatic cell count in milk from healthy and mastitic udder quarters. DCC is especially useful for the evaluation of SCC in bulk tank milk. DCC can measure the number of somatic cells both in fresh unpreserved and Bronopol preserved milk samples. Measurements of SCC with DCC can be performed also in milk samples stored at the temperature of 4°C for 24 or 72 hours as well as in milk samples frozen to minus 20°C and stored for 7 days.

References

1. *Akerstedt M., Person Waller K., Sternesjö A.*: Haptoglobin and serum amyloid A in relation to the somatic cell count in quarter, cow composite and bulk tank milk. J. Dairy Res. 2007, 74, 198-203.
2. *Ardo Y.*: Bronopol as a preservative in milk samples for the determination of cell content using Fossomatic. Milchwissenschaft 1982, 37, 139-142.
3. *Bansal B. K., Hamann J., Grabowski N. T., Singh K. B.*: Variation in the composition of selected milk fraction samples from healthy and mastitic quarters, and its significance for mastitis diagnosis. J. Dairy Res. 2005, 72, 144-152.
4. *Barkema H. W., van der Schaus J., Schukken Y. H., De See A. L. W., Lam T. J. G. M., Benedictus G.*: Effect of freezing on somatic cell count in quarter milk samples as determined by a Fossomatic electronic counter. J. Dairy Sci. 1997, 80, 422-426.
5. *Baro J. A., Roldan P., Carleos C. E., Grillo G. J., Perez M. A.*: Video microscopy as an alternative method for somatic cell count in milk. J. Dairy Sci. 2005, 72, 93-100.
6. *Berry E., Broughan J.*: Use of the DeLaval cell counter (DCC) on goats' milk. J. Dairy Res. 2004, 74, 345-348.
7. *Chagunda M. G., Larsen T., Bjorring M., Ingvartsen K. L.*: L-lactate dehydrogenase and N-acetyl-beta-D-glucosaminidase activities in bovine milk as indicators of nonspecific mastitis. J. Dairy Res. 2006, 73, 431-440.
8. *Clarke T., Evans M. E., Hepwoth G., Moate P. J., Stewart J. A.*: Mordant factors that affect the fluorescence and counting of somatic cells by instruments. J. Dairy Res. 1995, 62, 373-394.
9. *Djabri B., Bareille N., Beaudeau F., Seegers H.*: Quarter milk somatic cell count in infected dairy cows: a meta analysis. Vet. Res. 2002, 33, 335-357.
10. *Faus M. A., Timms L. L.*: Estimates of variability for somatic cell count measurements in Iowa dairy industry. J. Dairy Sci. 1995, 78, 546-551.
11. *Gonzalo C., Boixo J. C., Carriedo J. A., San Primitivo F.*: 2004 Evaluation of rapid somatic cell counters under different analytical conditions in ovine milk. J. Vet. Sci. 2004, 87, 2623-2628.
12. *Gonzalo C., Linage B., Carriedo J. A., de la Fuerte F., Primitivo F. S.*: Evaluation of the overall accuracy of the DeLaval cell counter for somatic cell counts in ovine milk. J. Dairy Sci. 2006, 89, 4613-4619.
13. *Green L. E., Schukken Y. H., Green M. J.*: On distinguishing 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. 2006, 76, 74-89.
14. *Grönlund U., Sandgren C. H., Persson Waller K.*: Haptoglobin and serum amyloid in milk from dairy cows with chronic subclinical mastitis. Vet. Res. 2005, 36, 191-198.
15. *Halasa T., Huijps K., Østeras O., Hogeeven H.*: Economic effects of bovine mastitis and mastitis management: A review. Vet. Quart. 2007, 29, 18-31.
16. *Hamann J.*: Diagnosis of mastitis and indicators of milk quality. Mastitis in dairy production. Current knowledge and future solution. Wageningen Academic Publishers 2005, 82-90.
17. International Dairy Federation 148-2: 2008 – Milk – Enumeration of somatic cells – Part 2: Guidance on the operation of fluoro-opto-electronic counters.
18. *Jayarao B. M., Pillai S. R., Sawant A. A., Wolgang D. R., Hegde N. V.*: Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. J. Dairy Sci. 2004, 87, 3561-3573.
19. *Leitner G., Shoshami E., Krfucks O., Chaffer M., Saran A.*: Milk leukocyte population patterns in bovine udder infection of different aetiology. J. Vet. Med. 2000, 47, 581-589.
20. *Liebe A., Schams.*: Growth factors in milk: interrelationships with somatic cell count. J. Dairy Res. 1998, 65, 93-100.
21. *Malinowski E., Kłossowska A., Smulski S.*: Changes in biologically active cows' milk components caused by mastitis. Medycyna Wet. 2008, 64, 14-19.
22. *Malinowski E., Lassa H., Kłossowska A., Markiewicz H., Smulski S.*: Relationship between mastitis agents and somatic cell count in foremilk samples. Bull. Vet. Inst. Pulawy 2006, 50, 349-352.
23. *Martinez J. R., Gonzalo C., Carriedo J. A., San Primitivo F.*: Effect of freezing on Fossomatic cell counting in ewe milk. J. Dairy Sci. 2003, 86, 2583-2587.
24. *Moon J. K., Koo H. C., Joo Y. S., Jeon S. H., Hur D. S., Chung C. J., Jo S. H., Park Y. H.*: Application of a new portable microscopic cell counter with disposable plastic chip for milk analysis. J. Dairy Sci. 2007, 90, 2253-2259.
25. *Nielsen N. J., Larsen T., Bjerring M., Ingvartsen K. L.*: Quarter health, milking interval, and sampling time during milking affect the concentration of milk constituents. J. Dairy Sci. 2005, 88, 3186-3200.
26. *Ostensson K.*: Variation during lactation in total and differential leucocyte counts, N-acetyl-beta-D-glucosaminidase, antirypsin and serum albumin in foremilk and residual milk from non-infected quarters in bovine. Acta Vet. Scand. 1993, 34, 83-93.
27. *Oviedo-Boyso J., Valdez-Alacôn J. J., Cajero-Juarez M., Ochoa-Zarzosa A., López-Meza J. E., Bravo-Patiño A., Baizabal-Aquirre V. M.*: Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. J. Infect. 2007, 54, 399-409.
28. *Paape M. J., Bannerman D. D., Zhao X., Lee J. W.*: The bovine neutrophil: Structure and function in blood and milk. Vet. Res. 2003, 34, 597-627.
29. *Paape M., Mehrzad J., Zhao X., Detilleux J., Burvenich C.*: Defense of the bovine mammary gland by polymorphonuclear neutrophil leukocytes. J. Mammary Gland Biology Neoplasia 2002, 7, 109-120.
30. *Petrovski K. R., Trajcev M., Buneski G.*: A review of the factors affecting the coasts of bovine mastitis. J. South African Vet. Assoc. 2006, 77, 52-60.
31. *Pyörälä S.*: Indicators of inflammation in the diagnosis of mastitis. Vet. Res. 2003, 34, 565-578.
32. *Riekerink R. G. M., Barkema H. W., Veenstra W., Berg F. E., Stryhn H., Zadoks R. N.*: Somatic cell count during and between milkings. J. Dairy Sci. 2007, 90, 3733-3741.
33. *Riollet C., Reinard P., Poutrel B.*: Cells and cytokines in inflammatory secretion of bovine mammary gland. Adv. Exp. Med. Biol. 2000, 480, 247-258.
34. *Ruegg P. L., Hulland C., Rieth B.*: Performance of the direct cell counter used on milk samples obtained from fresh cows. National Mastitis Council Annual Meeting Proc., Orlando, Florida 2005, p. 291-292.
35. *Sanchez A., Sierra D., Luengo C., Corrales J. C., Morales C. T., Contreras A., Gonzalo C.*: Influence of storage and preservation on Fossomatic cell count and composition of goat milk. J. Dairy Sci. 2005, 88, 3095-3100.
36. *Sarikaya H., Bruckmaier R. M.*: Importance of the samples milk fraction for the prediction of total quarter somatic cell count. J. Dairy Sci. 2006, 89, 4246-4250.
37. *Sarikaya H., Verner-Misof C., Atzkern M., Bruckmaier R. M.*: Distribution of leukocyte populations, and milk composition, milk fractions of healthy quarters in dairy cows. J. Dairy Sci. 2005, 88, 486-492.
38. *Schmidt-Madsen P.*: Influence of storage and preservation of milk samples on microscopic and Fossomatic somatic cell counts. Nordisk Vet. Med. 1979, 31, 449-454.
39. *Seegers H., Fourichon C., Beaudeau F.*: Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet. Res. 2003, 34, 475-491.
40. *Sølverød L., Simonsen S., Waldmann A., Østerås O., Ropstad E.*: Variation in somatic cell count and milk components in fraction collected quarter milk samples. Mastitis in dairy production. Current knowledge and future solutions. Wageningen Academic Publishers 2005, 462-466.
41. *Urech E., Puhan Z., Schällibaum M.*: Changes in milk protein as affected by subclinical mastitis. J. Dairy Sci. 1999, 82, 2402-2411.
42. *Werven T. Van, Nijhof C., Van Bussel T., Hogeveen H.*: Use of on-farm testing of somatic cell count for selection of udder quarters for bacteriological culturing. Mastitis in dairy production. Current knowledge and future solution. Wageningen Academic Publishers 2005, 481-486.

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