Fossomatic Cell-Counting on Ewe Milk: Comparison with Direct Microscopy and Study of Variation Factors

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ABSTRACT

Using the Fossomatic method (FSCC) a total of 23,003 analytical SCC observations were carried out on 6400 aliquots taken from 80 individual ewe milk samples with the objective of studying the influence of 4 preservation procedures (without preservation, potassium dichromate, azidiol, and bronopol), 2 storage temperatures (ambient and refrigeration), 10 milk ages (3, 6, 12, and 24h, and 2, 3, 4, 5, 7, and 9d postcollection), and two analytical temperatures (40 and 60°C). In addition, each sample was analyzed with direct microscopic method (DMSCC), using 3 different stainings for each sample: methylene blue (MB), May-Grünwald-Giemsa (MGG) and Pyronin Y-methyl green (PGM). This allowed DMSCC and FSCC (at 24 h of age) to be compared.

The reference DMSCC from MB staining was a reliable method in ewe milk, though more specific stainings such as MGG and PMG slightly improve the residual standard deviation for repeated SCC. Between DMSCC and FSCC, the highest coefficients of correlation (0.972 to 0.996) corresponded to preserved and refrigerated milk, and the lowest (0.708 to 0.919) to unpreserved and ambient stored aliquots. Except for the unpreserved and ambient stored aliquots, SCC values were similar in all aliquots.

Under FSCC, preservation, storage and analytical temperature, milk age, and most of the interactions showed a significant effect on SCC variation. In preserved samples, logSCC values ranged between 5.67 (bronopol) and 5.62 (azidiol). The highest values (5.72) were for unpreserved milk, which showed false overestimation of SCC due to bacterial proliferation. LogSCC was higher at 60°C (5.68) than at 40°C (5.65). The interaction between age, preservation and storage temperature showed no cell degeneration in properly handled samples over the 9 d of study.

(**Key words:** ewe milk, somatic cell count, Fossomatic method, direct microscopic method)

Abbreviation key: AT = ambient temperature, AZ = azidiol, BR = bronopol, DMSCC = direct microscopic SCC method, FSCC = Fossomatic SCC method, MB = methylene blue, MGG = May-Grünwald-Giemsa, PD = potassium dichromate, PMG = pyronin Y-methyl green, RT = refrigeration temperature, SD ϵ = residual standard deviation, WP = without preservative.

INTRODUCTION

In cows, and more recently in dairy sheep (González-Rodríguez et al., 1995; Gonzalo et al., 2002), SCC is a useful predictor of IMI, and therefore, an important component of milk under aspects of quality, hygiene and mastitis control. Accuracy of milk SCC is very important to most dairy farmers and to the dairy industry. In fact, the most widely-used SCC methods, such as direct microscopic (DMSCC), Coulter counter, and Fossomatic (FSCC), have been completely standardized for cow milk (Schmidt-Madsen, 1975; IDF, 1995). However, there is very little information on its specific application in ewe milk (Gonzalo et al., 1993), which has a higher content of total solids than cow milk. Also, the presence of extracellular membranous matter, nuclear remains and cell fragments, described in ewe milk (Schalm et al., 1971; Lee and Outteridge, 1981) calls for the need for SCC methods to be widely contrasted in this species. The reference method recommended by the IDF (1995) for SCC is DMSCC by methylene blue (MB) staining, the only negative aspect being its lack of specificity between cells and cytoplasmic particles. This is why comparisons using dyes with higher tint specificity, such as May-Grünwald-Giemsa (MGG) or Pyronin Y-methyl green (PMG) (Gonzalo et al., 1988) would be of great use in ewe milk.

At present, DMSCC is used as a verification and contrast technique in automated somatic cell counters, the most widely used of which are based on Fossomatic. Basically, the Fossomatic counter is a fluorescence microscope. The ethidium bromide dye penetrates the cell

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Table 1. Study design from 80 ewe milk samples at 3, 6, 12, and 24 h, 2, 3, 4, 5, 7 and 9 d postcollection using a schematic diagram.

Preservation	Storage	Analytical temp. (°C)
20 Aliquots without preservative	10 at ambient temperature	40 and 60
	10 at refrigerated temperature	40 and 60
20 Aliquots with potassium dichromate	10 at ambient temperature	40 and 60
	10 at refrigerated temperature	40 and 60
20 Aliquots with azidiol	10 at ambient temperature	40 and 60
	10 at refrigerated temperature	40 and 60
20 Aliquots with bronopol	10 at ambient temperature	40 and 60
	10 at refrigerated temperature	40 and 60

and forms a fluorescent complex with the nuclear DNA. Each cell produces an electrical pulse, which is amplified and recorded. The influence of certain SCC variation factors, such as the type of preservative used (Schmidt-Madsen, 1979; Lee et al., 1986; Barcina, et al., 1987; Bertrand, 1996; Ubben et al., 1997), the analytical temperature (Miller et al., 1986), storage conditions (Lee et al., 1986; Barkema et al., 1997), or milk age (Kennedy et al., 1982), have enabled this method to be standardized for cow milk. However, optimal conditions for SCC have scarcely been specified for ewe milk (Gonzalo et al., 1993). Moreover, standardizing Fossomatic for ewe milk is essential in quality controls carried out in SCC laboratories and equipments in order to guarantee reproducibility of results. Further information on these SCC variation factors in ewe milk is necessary in order to know the most accurate methodology for SCC analysis.

The objectives of this study were to assess the performance of Fossomatic in ewe milk by comparing it on one hand with DMSCC using dyes of different specificity, and on the other by defining optimal analytical conditions, especially as far as the storage method, type of preservation, analytical temperature, and age of the milk samples are concerned.

MATERIALS AND METHODS

Fossomatic Cell Counts

A total of 80 individual 250-ml ewe milk samples, ranging between 10×10^3 and $20,000 \times 10^3$ cells/ml, were divided into 80 3-ml aliquots, which, in turn, were divided into 4 groups according to the preservative used: 20 without preservative (**WP**), 20 with potassium dichromate (**PD**) (0.1 g/100 ml), 20 with azidiol (**AZ**) (0.024 g sodium azide/100 ml) and 20 with bronopol (**BR**) (0.05 g/100 ml). As shown in the schematic diagram in Table 1, 10 aliquots out of each group were stored at ambient temperature (**AT**) (18 to 25°C) and the other 10 were stored at refrigeration temperature (**RT**) (4°C). The 10 aliquots in each batch were analyzed by FSCC 3, 6, and 12-h, and 1, 2, 3, 4, 5, 7, and 9-d

postcollection. The SCC was determined for each sample at 40 and 60°C. All the analyses were carried out in duplicate with a Fossomatic 90 (cytometry on disk) (A/S N Foss Electric, Hillerød, Denmark) using the previously described method (Gonzalo et al., 1993; IDF, 1995). The total number of SCC analytical observations came to 23,003.

FSCC variation factors were studied from this material (all aliquots), whereas only aliquots corresponding to age 24 h were used for comparison between DMSCC and FSCC.

Direct Microscopic SCC

SCC of each original milk sample was determined in duplicate by the 3 DMSCC variants, within 6 h postcollection. The milk was heated to 40°C in a water-bath held for 15 min at that temperature before being cooled to 20°C with careful stirring. 0.01 ml of milk was spread on a 1-cm² (0.5 × 2 cm) area of a degreased microscopic slide and was dried in a horizontal position. The slides were previously treated with poly-L-lysine to increase the adherence of the film of milk. Three different methods were used to stain the film: MB, according to IDF regulation 148A (IDF, 1995), MGG, and PMG staining, according to the methodology of Gonzalo et al. (1998). In short, for MGG, after drying overnight, duplicate smears were fixed with 96% ethyl alcohol (3 min), air dried, defatted with xylol (8 min) and rinsed smoothly with 60% ethyl alcohol, air dried, successively dyed with pure May-Grünwald (2 min), at 50% (2 min) and Giemsa solution (20 min), air dried, and dehydrated in an increasing series of alcohols and xylols. For PMG, after drying overnight, duplicate smears were fixed in Carnoy's solution (10 min), air dried, successively hydrated with 50% ethyl alcohol (2 min), at 30% (2 min) and distilled water (2 min), air dried, dyed with PMG stain (20 min), rinsed and mounted for microscopy. The working factor was 2.255 in all cases.

Statistical Analysis

Comparison of methods. Three DMSCC variants and 16 FSCC analytical conditions were compared.

These 16 conditions corresponded to preservation types (WP, PD, AZ, and BR), storage temperature (AT and RT), and analytical temperature (40 and 60°C) at 24 h of age. Denominations of all 19 analytical conditions were: MB, MGG, and PMG for DMSCC, and WP-AT-40°C, PD-AT-40°C, AZ-AT-40°C, BR-AT-40°C, WP-AT-60°C, PD-AT-60°C, AZ-AT-60°C, BR-AT-60°C, WP-RT-40°C, PD-RT-40°C, AZ-RT-40°C, BR-RT-40°C, WP-RT-60°C, PD-RT-60°C, AZ-RT-60°C, and BR-RT-60°C for FSCC.

These analytical conditions were compared according to three types of statistical studies: means comparison, standard deviation for repeated SCC, and coefficients of correlation.

Means comparison was carried out following the general linear model (GLM) procedure of SAS (SAS, 1992). In the statistical model used, ewe effect was considered random and the analytical condition effect fixed:

$$Y_{ijk} = \mu + A_i + E_j + e_{ijk}$$

where Y_{ijk} = dependent variable logSCC; μ = mean; A_i = effect of analytical condition (19 ones previously defined); and E_j = effect of ewe (80 ewes). For each ewe, 2 replicates were considered, as the analytical determinations were carried out in duplicate.

In the second statistical study, 19 analysis of variance were carried out for each one of the 19 conditions considered, following the model:

$$Y_{ij} = \mu + E_i + e_{ij}$$

where Y_{ij} = dependent variable SCC and logSCC, and E_i = ewe effect (80 levels). For each ewe, 2 replicates were taken into account. Residual standard deviation ($SD\epsilon$) and residual coefficient of variation ($CV\epsilon$) were estimated from this model. $SD\epsilon$ was the square root of the mean square error, and $CV\epsilon$ was calculated as a percentage by dividing $SD\epsilon \times 100$ by the arithmetic mean of SCC. These parameters were regarded as a measure of standard deviation for repeated SCC (repeatability). $SD\epsilon$ was estimated for SCC and logSCC variables. For an untransformed variable, $SD\epsilon$ could be quantified in cells/ml. However, from a statistical point of view, the use of a logSCC variable would be more suitable, as it complies more rigorously with the underlying hypotheses of analysis of variance (normal distribution of variables).

Finally, linear regression studies were performed to establish the relationship between each of the three DMSCC analytical variants, taken as reference methods, and each of the 16 FSCC analytical conditions considered. The corresponding correlation coefficients were estimated in all cases. As the analytical determinations

were carried out in duplicate, the arithmetic mean of the two replicates was determined beforehand. Estimation was carried out from 80 observations (pairs of data) for each regression straight line.

Variation factors of FSCC. The statistical study was carried out following the GLM procedure of SAS (SAS, 1992). In the model used for this study, the effect of ewe was random and the others were fixed:

$$Y_{ijklm} = \mu + E_i + A_j + S_k + P_l + T_m + AS_{jk} + AP_{jl} + AT_{jm} + SP_{kl} + ST_{km} + PT_{lm} + ASP_{jkl} + AST_{jkm} + APT_{jlm} + SPT_{klm} + e_{ijklm}$$

where:

Y_{ijklm} = Dependent variable log RCS,

μ = Mean,

E_i = Effect of ewe (80 levels),

A_j = Effect of milk age (10 levels corresponding to these ages: 3, 6, and 12 h, and 1, 2, 3 d, 4, 5, 7, and 9 d postmilk),

S_k = Effect of storage temperature (2 levels: AT and RT),

P_l = Effect of preservation (4 levels: WP, PD, AZ, and BR),

T_m = Effect of analytical temperature (2 levels: 40 and 60°C),

AS_{jk} = Age $\times$ storage interaction,

AP_{jl} = Age $\times$ preservation interaction,

AT_{jm} = Age $\times$ analytical temperature interaction,

SP_{kl} = Storage $\times$ preservation interaction,

ST_{km} = Storage $\times$ analytical temperature interaction,

PT_{lm} = Preservation $\times$ analytical temperature interaction,

ASP_{jkl} = Age $\times$ storage $\times$ preservation interaction,

AST_{jkm} = Age $\times$ storage $\times$ analytical temperature interaction,

APT_{jlm} = Age $\times$ preservation $\times$ analytical temperature interaction,

SPT_{klm} = Storage $\times$ preservation $\times$ analytical temperature interaction, and

e_{ijklm} = Random residual.

An experimental design of $80 \times 10 \times 2 \times 4 \times 2$ was followed for the ewe, age of milk, storage temperature, preservation, and analytical temperature factors, respectively. The analytical determinations were carried out in duplicate (replicates).

RESULTS AND DISCUSSION

Comparison Between DMSCC and FSCC

Table 2 shows the comparison of logSCC mean values obtained for the 3 DMSCC stainings and the 16 analyti-

Table 2. Least squares means of logSCC obtained by direct microscopic (DMSCC) and Fossomatic (FSCC) method, and residual standard deviation (SD_{ε}) of SCC and logSCC for each of 19 analytical conditions compared.

Aliquots	LSM ¹ (log SCC)	SCC ($\times 10^3$ cells/ml)	SD_{ε} (log SCC)	SD_{ε} ($\times 10^3$ cells/ml)	CV_{ε} ² (%)
FSCC-WP ³ -AT ⁷ -60°C ⁹	6.025 ^a	995	0.034	277	14.25
FSCC-WP-AT-40°C ¹⁰	5.808 ^b	612	0.049	224	14.81
FSCC-PD ⁴ -AT-60°C	5.721 ^c	527	0.033	145	10.04
FSCC-BR ⁵ -AT-40°C	5.707 ^{cd}	512	0.033	235	15.52
DMSCC-MGG ¹¹	5.696 ^{cde}	500	0.061	161	10.46
FSCC-PD-AT-40°C	5.693 ^{cde}	495	0.043	190	13.60
FSCC-BR-RT ⁸ -60°C	5.689 ^{cde}	491	0.032	350	22.29
FSCC-WP-RT-60°C	5.684 ^{def}	484	0.032	121	8.12
FSCC-PD-RT-60°C	5.681 ^{def}	481	0.033	116	7.48
FSCC-BR-AT-60°C	5.678 ^{def}	479	0.033	157	9.78
FSCC-BR-RT-40°C	5.676 ^{def}	475	0.028	40	2.76
FSCC-AZ ⁶ RT-60°C	5.670 ^{efg}	469	0.032	153	10.01
DMSCC-MB ¹²	5.665 ^{efg}	467	0.076	189	12.63
DMSCC-PMG ¹³	5.655 ^{fg}	455	0.059	108	7.32
FSCC-AZ-AT-60°C	5.654 ^{fg}	452	0.032	187	12.46
FSCC-PD-RT-40°C	5.641 ^{gh}	439	0.034	207	15.94
FSCC-WP-RT-40°C	5.618 ^{hi}	416	0.031	119	9.86
FSCC-AZ-RT-40°C	5.605 ⁱ	405	0.034	148	11.64
FSCC-AZ-AT-40°C	5.605 ⁱ	405	0.038	302	23.59

a,b,c,d,e,f,g,h,i Means in the same column with different superscripts differ ($P < 0.05$).

¹SEM of LSM = 0.010.

² CV_{ε} = Residual coefficient of variation.

³WP = Without preservative.

⁴PD = Potassium dichromate.

⁵BR = Bronopol.

⁶AZ = Azidol.

⁷AT = Ambient temperature.

⁸RT = Refrigeration temperature.

⁹60°C = Analyzed at 60°C.

¹⁰40°C = Analyzed at 40°C.

¹¹MGG = May-Grünwald-Giemsa staining.

¹²MB = Methylene blue staining.

¹³PMG = Pyronin Y-methyl green staining.

cal conditions assayed by FSCC (24 h postcollection). LogSCC for DMSCC varied between 5.655 and 5.696, depending on the staining method used, and between 5.605 and 6.025 for FSCC. Both AT-stored WP aliquots analyzed by FSCC showed means (5.808 and 6.025) much higher ($P < 0.0001$) than the remaining ones. Microscopic observation of these aliquots with high SCC, immediately after FSCC analysis, revealed the presence of large clumps of bacteria probably capable of causing pulses with gains similar to or even greater than those of the somatic cells. These impulses would obviously be counted by Fossomatic, thereby falsely increasing the SCC. Consequently, FSCC was not valid for the AT-stored WP milk. Excluding these conditions, the remaining FSCC aliquots showed very similar logSCC values to those of DMSCC. The lowest values were for the aliquots AZ preserved and analyzed at 40°C by FSCC (5.605).

As regards repeated SCC, Table 2 shows SD_{ε} of SCC and logSCC for the aliquots studied. For both variables,

MB staining in DMSCC showed slightly greater variability among the replicates (SD_{ε} : 189×10^3 cells/ml for SCC and 0.076 for logSCC) in comparison with the other two more specific ones, especially PMG (SD_{ε} : 108×10^3 cells/ml and 0.059). In logSCC variables, more rigorous from a statistical point of view, SD_{ε} for DMSCC aliquots were consistently higher than those for FSCC ones (0.028 to 0.049), in agreement with the higher repeatability of the electronic methods vs DMSCC demonstrated in cow milk (Schmidt-Madsen, 1975). However, this was not observed for SCC variables, which showed higher SD_{ε} values for FSCC in some cases. These results are interesting because they can be expressed in cells/ml or as a percentage; however, they are statistically less suitable due to the important bias of the non-normal distribution of the untransformed variable. Milk BR preserved, RT stored and analyzed at 40°C gave the lowest SD_{ε} (40×10^3 cells/ml for SCC and 0.028 for logSCC), and its SCC was therefore the more repeatable.

Table 3. Correlation coefficients between DMSCC and FSCC.

DMSCC	FSCC			
	WP-AT-40°C	PD-AT-40°C	AZ-AT-40°C	BR-AT-40°C
MB	0.895	0.977	0.975	0.981
MGG	0.919	0.992	0.992	0.993
PMG	0.894	0.988	0.984	0.988
	WP-AT-60°C	PD-AT-60°C	AZ-AT-60°C	BR-AT-60°C
MB	0.708	0.961	0.980	0.983
MGG	0.776	0.981	0.994	0.995
PMG	0.732	0.973	0.988	0.992
	WP-RT-40°C	PD-RT-40°C	AZ-RT-40°C	BR-RT-40°C
MB	0.957	0.978	0.972	0.980
MGG	0.978	0.993	0.991	0.993
PMG	0.967	0.988	0.983	0.989
	WP-RT-60°C	PD-RT-60°C	AZ-RT-60°C	BR-RT-60°C
MB	0.978	0.981	0.981	0.982
MGG	0.993	0.995	0.995	0.996
PMG	0.988	0.990	0.990	0.991

Correlations between MB staining and MGG and PMG stainings were 0.981 and 0.982, respectively. The correlation coefficient for MGG and PMG stainings was 0.990. The correlation coefficients between all three staining variants for DMSCC were therefore very high, close to 1.00.

Between DMSCC and FSCC (Table 3), the lowest correlation coefficients were established among the three DMSCC staining variants and AT-stored WP samples analyzed by FSCC at 40°C (0.894 to 0.919) and 60°C (0.708 to 0.776). Such low coefficients make these analytical conditions unadvisable in FSCC. In all the other cases, the correlation coefficients between the reference DMSCC variants and the different FSCC analytical conditions were consistently very high (0.957 to 0.996). These values coincided with those found in cow milk by other authors for different working factors (Grappin and Jeunet, 1974; Heeschen, 1975; Schmidt-Madsen, 1975; 1979; Heald et al., 1977). They were also similar to those obtained by Gonzalo et al. (1993) in ewe milk (0.986), using MGG staining and a working factor of 1.600.

DMSCC showed that cytoplasmic particles were constantly present in most of the processed ewe milk samples, though at a very small concentration (logarithmic means of 4.14, 4.22 and 4.24 for the MB, MGG and PMG stainings, respectively). These counts were much lower than those obtained for goat milk (Dulin et al., 1982) and have no apparently significant repercussion on the accuracy of SCC methods, though in some cases the particle concentration was as high as 95×10^3 /ml in the present experiment. Similar particles have also been described in cow milk by Paape and Tucker (1966), Brooker (1978), and Lee et al. (1980).

FSCC Variation Factors

Preservation, analytical temperature, age of sample, storage type, and most of the interactions of the previously mentioned effects contributed significantly to the SCC variation.

The effect of preservation on logSCC showed significant differences ($P < 0.001$) among 4 types studied. The highest values were for WP milk (5.72 ± 0.002), followed by milk preserved by BR (5.67 ± 0.001), PD (5.63 ± 0.001), and AZ (5.62 ± 0.001). These results coincided with those obtained in the previous experiment and showed a false overestimation of SCC in WP milk due to bacterial proliferation. Also, higher SCC found in BR preserved milk were in accordance with those obtained by Bertrand (1996) and Lee et al. (1986) in cow's milk.

The analytical temperature of the milk had a very significant effect ($P < 0.001$) on SCC. As in cow's milk, (Miller et al., 1986), logSCC was higher at 60°C (5.68 ± 0.001) than at 40°C (5.65 ± 0.001), probably because higher temperatures favor better penetration of the ethidium bromide dye in the cell, or because it disperses the fat in ewe milk better.

The effect of milk storage showed significantly higher logSCC ($P < 0.001$) at RT (5.67 ± 0.001) than AT (5.65 ± 0.002), probably because RT favored the maintenance of cell integrity. However, as can be seen in Table 4, the interaction between preservation, analytical temperature, and storage was statistically significant ($P < 0.001$) and enabled the previously mentioned effects to be studied with greater precision. BR-preserved, AT-stored, and PD-preserved samples (independently of the storage temperature), showed no differences in SCC with regard to the analytical temperature. The low SCC for AZ preserved milk analyzed at 40°C, coincided with

Table 4. Least squares mean of logSCC ($\pm$ SE) by storage $\times$ preservation $\times$ analytical temperature interaction.

Storage	Analytical temperature					
	40°C			60°C		
	LSM	SE	SCC ¹	LSM	SE	SCC ¹
Refrigeration						
WP	5.69 ^b	0.003	486	5.73 ^a	0.004	540
BR	5.68 ^b	0.003	475	5.69 ^a	0.003	485
PD	5.66	0.003	456	5.66	0.003	456
AZ	5.61 ^b	0.003	406	5.66 ^a	0.003	458
Ambient temperature						
WP	5.68 ^b	0.010	475	5.77 ^a	0.009	585
BR	5.66	0.003	456	5.65	0.003	449
PD	5.61	0.003	410	5.61	0.003	404
AZ	5.58 ^b	0.003	384	5.64 ^a	0.003	441

^{a,b} Means in the same row with different superscripts differ ($P < 0.05$).

¹SCC = Geometric means ($\times 10^3$ cells/ml).

the results found at 24 h (Table 2), and indicates the need to increase the analytical temperature of these milk samples to 60°C so as to improve SCC accuracy.

The effect of age was significantly affected ($P < 0.001$) by preservation and storage (Figures 1 and 2), especially due to the different behavior of WP samples. In the case of refrigerated WP milk, the SCC start to become abnormally high after d 2 of sample age, which considerably limits the analysis period for this type of sample (Figure 1). With regard to AT storage, the analytical period guaranteeing SCC quality and reliability would only be 12 h (Figure 2). These results

would be useful in the case of SCC and bacteriological analytical protocols, as WP samples noticeably limit the storage period of milk.

The interaction between age, preservation and storage temperature also demonstrated the suitability of refrigerating preserved milk; the SCC was more homogenous and accurate during the 9 study days in comparison with non-refrigerated milk. The relative decrease between the 3 h and 9 d postcollection of SCC geometric means for RT stored samples was 2.8, 6.9 and -0.5% for BR-, PD-, and AZ-preserved milk, respectively (Figure 1). At AT, SCC evolved more dispersedly, decreasing

Figure 1. Evolution of SCC through the period of study according to preservation type in milk stored at refrigeration temperature and FSCC analyzed (SEM: 0.007).

Figure 2. Evolution of SCC through the period of study according to preservation type in milk stored at ambient temperature and FSCC analyzed (SEM: 0.01).

much more noticeably throughout time; SCC dropped at between 3 h and 9 d postcollection by 31.3, 51.2, and 12.4% for BR-, PD-, and AZ-preserved samples, respectively (Figure 2). Therefore, RT storage offered ideal conditions for cellular integrity over the time.

CONCLUSIONS

The reference MDSCC (MB stain) (IDF 148A) was a valid method in ewe milk, even though more specific stainings such as MGG or PMG lowered the $SD\epsilon$ value slightly. Nevertheless, the correlation coefficients between the three DMSCC stainings were very high (almost 1.00), so they can all be considered FSCC reference methods. As a whole, the results obtained enabled us to demonstrate the efficacy of FSCC, with its different analytical variants, in ewe milk. The method was not valid for AT-stored WP milk.

With regard to routine sampling from test day or bulk tank milk recordings, variations brought about by such factors may not be that important in determining the health status of sheep, as long as the samples are preserved and refrigerated after collection. However, uniformity of preservation, storage, age of milk, and analytical temperature are essential for intra- and interlaboratory quality control tests, with the aim of guaranteeing reproducibility of results.

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