

Somatic cell count in goat's milk as an indication of mastitis

N.H. Robertson¹ and C.J.C. Muller²

¹ARC, Animal Nutrition and Products Institute, P.O. Box 65, Elsenburg 7607, South Africa
E-mail: robertsonn@arc.agric.za

²Institute for Animal Production, Private Bag X1, Elsenburg 7607, South Africa
E-mail: carelm@elsenburg.com

Introduction

The somatic cell count (SCC), which greatly increases in milk due to the shedding of large numbers of leucocytes from an infected udder, is an accepted quantitative index for mastitis conditions of the bovine mammary gland. It is used both to evaluate the quality of milk and to predict udder infection (Poutrel & Rainard, 1981).

In the case of goat's milk, however, a considerable controversy exists as to the relationship between SCC and mastitic infection. The reason for this controversy is that milk secretion in the cow differs from that of the goat. In the cow, milk is squeezed out of the alveoli, while in the goat the alveoli actually burst open. Due to this apocrine secretion, large numbers of cytoplasmic particles occur in normal goat's milk. These non-leucocytic cell particles do not contain deoxyribonucleic acid (DNA) or a nucleus as leucocytes do. They occur normally in the milk and their presence not only masks, but also complicates the interpretation of the leucocyte response to inflammation. Due to the presence of these cells the total SCC in goat's milk does not correlate well with the leucocyte count in the milk. It is not uncommon to find high SCC's in goat's milk when actual leucocyte numbers are relatively low (Roguinsky *et al.*, 1971; Schalm *et al.*, 1971 and Kapture, 1980).

Cow's milk on the other hand, because of the merocrine milk secretion process, contains a relatively low number of epithelial cells and therefore the SCC is a far better indication of the number of leucocytes in the milk and thus the clinical state of the cow's udder.

Estimating SCC in goat's milk

In goat's milk, a large number of non-DNA containing particles occurs in the milk. Somatic cell counting methods based on the presence of DNA such as the Fossomatic or Bentley methods will give significantly lower counts than Coulter or direct microscopic procedures, using a non-specific stain. Estimates determined by means of the Coulter counter may be about twice as high as those obtained with the Fossomatic counter (Kalogridou-Vassliadou *et al.*, 1992).

Therefore, only counting methods specific for DNA should be employed for estimating the SCC in goat's milk. Such methods include the Fossomatic and Bentley methods in which ethidium bromide dye is used for the specific staining of the DNA in the cell wall.

Ideally, SCC expresses the number of leucocytes in milk. Although ethidium bromide staining eliminates the inclusion of non-DNA containing particles in the SCC, as determined by means of Fossomatic and Bentley procedures, these methods do not completely distinguish epithelial cells from leucocytes. Current instrumentation has a limit as to discriminator level in the degree of reflecting light or electronic signals which can identify epithelial cells relative to leucocytes, and therefore more technically advanced methods may be required to assay SCC in goat's milk accurately.

The above mentioned characteristics of goat's milk can create errors when SCC determination is done on the milk by means of Fossomatic- or Bentley analysers, calibrated against cow milk standards. Under these circumstances, researchers have found an overestimation of 27.3% (Zeng, 1996). To overcome this, the American Food and Drug Administration recommends that calibration of these analyzers should be done against goat's milk standards stained with pyronine y-methyl green. Pyronine Y-methyl green differentiates between leucocytes and cytoplasmic particles, thus excluding the latter from the total SCC (Pachard *et al.*, 1992).

Somatic cell counts in goat's milk

In healthy goat milk, the SCC is extremely variable and generally much higher than in cow milk (Hunter, 1984). A mean SCC in samples obtained from uninfected goats' udders and determined by methods based on the staining of DNA by ethidium bromide, may range from 270 000, to 360 000 cells/mL (Dulin *et*

al., 1982; Kalogridou-Vassliadou *et al.*, 1992). When counting is done by means of non-specific methods, e.g. the Coulter counter or direct microscopic procedures with a non-specific stain, the mean may vary from 680 000 to 880 000 (Okada, 1960; Nesbakken, 1976; Perez & Schultz, 1979; Petterson, 1981). In the milk from infected udders, mean Fossomatic SCC's were found to vary from as low as 550 000 to as high as 4 800 000/mL (Poutrel & Lerondelle, 1983; Kalogridou-Vassliadou *et al.*, 1992, Zeng, 1996; Kozacinski *et al.*, 2002). Mean SCC's as high as 6 800 000/mL were found when determined by a Coulter counter (Lerondelle & Poutrel, 1984).

During an examination of 1 408 goat milk samples in Croatia, by means of the CMT test and the Fossomatic counter, an average SCC of 1.3 million/mL with a geometric mean of 470 000/mL was found. A positive CMT reaction of 1-3 was observed in 46.2% and mastitis agents were found in 27.1% of these samples. The Croatian workers concluded that an increase in SCC is not the only, and therefore not a very good indication of mammary gland infection in dairy goats (Kozacinski *et al.*, 2002).

In the USA it was found that out of 2 582 bulk tank goat milk samples analyzed, 22% exceeded the legal limit of 1 million/mL and up to 51% of the samples collected between October and December had SCC's in excess of the legal limit (Zeng *et al.*, 1999).

Due to the uncertainty regarding the normal level and significance of the SCC in goat milk, the EU Directives, up to the year 2000, have not established threshold values for this parameter. The outcome of studies in progress, particularly those relating to the European Research Program, FAIR 1 CT 95-0881 in which France, Italy and Spain are taking part, may, however, lead to the laying down of such thresholds. The SCC in bulk tank goat's milk varies greatly both between different countries and between regions of the same country, e.g. in France between 1 200 000 and 1 500 000 cells/mL in different areas of production, in the Castilla-La Mancha area of Spain it is 1 600 000/mL and in Italy 1 753 000/mL.

The above indicates that the limit for the somatic cell count in goat's milk may exceed 1 000 000 cells/mL (IDF, 2000).

Comparison with other mastitis detection tests

The non-specific nature of the methods currently being used to determine the SCC in goat's milk creates varying degrees of doubt on the validity of this criterion as an indicator of mastitis in the udder of the goat. The important influence of the method used for determining SCC's, namely specific or general staining, and the type of calibration standards being used, namely goat's milk stained with pyronine y-methyl green, *versus* that used for cow's milk have already been pointed out.

The California Mastitis test (CMT), which is based on DNA and does not react with cytoplasmic particles, can be successfully implemented under practical farm situations to indicate intra-mammary inflammation in the udder of the goat. The significant positive relationships between SCC from CMT and Fossomatic test methods were found to be similar for goat's- and cow's milk and are confirmed by various studies (Poutrel & Lerondelle, 1983; Kalogridou-Vassliadou *et al.*, 1992; Haenlein, 2002).

The CMT score of 2 or 3 indicates the significance of this relationship in goat's milk, where 81% and 65% of udders were infected by major and minor pathogens, respectively (Kalogridou-Vassliadou *et al.*, 1992). It was found that mean cell counts for milk with CMT scores of traces, 1, 2 and 3 were 320 000, 650 000, 1 700 000 and 23 000 000/mL, respectively, and that the mean cell count of 37 samples that were CMT negative, and did not contain mastitis pathogens, was 90 000/mL. The method by which these SCC's were determined, is, however, not known (Venugopal & Paily, 1979).

In conclusion, it can be accepted that the CMT provides a means to identify inflammatory infections, but positive findings should be confirmed by bacteriological examination (Kozacinski *et al.*, 2002).

The beta- glucuronidase test was standardized for goat's milk. This test, which is based on the beta-glucuronidase activity of the leucocytes, has proven to be a reliable method of counting only the somatic enzyme cells in the milk and thus overcomes the interference encountered with the SCC method (Oliszewski *et al.*, 2002).

No significant correlation could be found between the electrical conductivity and the SCC in the foremilk or strippings from healthy goats, nor from the milk of goats with mastitis - before and after treatment (Park & Nuti, 1985).

Influence of SCC on milk composition

In goat's milk, the SCC correlates positively ($P < 0.01$) with the protein percentage (Park & Humphrey, 1986; Ying *et al.*, 2002), fat percentage (Park & Humphrey, 1986), pH, Koesler number and the

percentage chloride, non-casein, nitrogen and ash (Hamed *et al.*, 1993) and negatively ($P < 0.01$) with viscosity, casein number, the percentage acidity, total solids, fat, total protein and lactose (Hamed *et al.*, 1993).

The cytoplasmic particles, of which there is a high concentration in goat's milk, contain protein and may contribute to the positive relationship between SCC and protein content (Park & Humphrey, 1986; Kalogridou-Vassliadou *et al.*, 1992). Another reason for some of these correlations may be that, as milk production decreases with time in lactation, the solids content of milk increases. Uninfected and infected halves on the same udders of dairy goats were compared (Leitner *et al.*, 2004) and the following correlations between SCC and milk constituents were found (Table 1).

Table 1 Effects of mammary gland infection on milk production and composition using half-udder design with 25 Israeli goats tested 2 or 3 times at 10- to 20 –day intervals

Parameter	Bacteriological status		Significance P-value
	Uninfected	Infected	
SCC \pm s.e. ($\times 10^3$)	417 \pm 72	1 750 \pm 197	< 0.0001
Milk (kg/milking)	0.98 \pm 0.04	0.69 \pm 0.04	< 0.0001
Fat (g/L)	38.9 \pm 1.1	38.8 \pm 1.2	NS ¹
Protein (g/L)	34.2 \pm 0.5	35.0 \pm 0.5	0.07
Lactose (g/L)	47.0 \pm 1.0	41.7 \pm 1.3	0.004
Casein (mg/mL)	28.1 \pm 0.7	28.2 \pm 0.8	NS ¹
Albumin (μ g/mL)	279.9 \pm 22.2	471.8 \pm 49.8	0.003
Plasmin (activity units/mL)	20.32 \pm 2.4	39.81 \pm 6.1	0.0003
Ca ²⁺ -activity (mmol)	1.89 \pm 0.1	1.62 \pm 0.1	0.002
Ca ²⁺ -concentration (mmol)	4.80 \pm 0.4	5.05 \pm 0.3	NS ¹
Protease peptones (mg/mL)	0.35 \pm 0.05	0.53 \pm 0.05	0.0005
Curd yield (g/L)	231.6 \pm 2.9	207.8 \pm 2.7	< 0.001
Rennet clotting time (sec)	167 \pm 18.6	295 \pm 43.4	< 0.02

¹NS: $P > 0.1$

Intra-mammary infection reduces ($P < 0.0001$) milk yield as well as lactose ($P < 0.004$) concentration, calcium activity ($P < 0.002$) and curd yield ($P < 0.001$). The albumin ($P < 0.003$) and proteose-peptone concentrations ($P < 0.0005$), plasmin activity ($P < 0.0003$) and rennet clotting time were increased ($P < 0.02$), whereas fat and casein concentration did not differ and the protein concentration only tended ($P < 0.07$) to increase because of a higher SCC. Other results showed that an increase in SCC was accompanied by highly significant ($P < 0.01$) increases in protein and fat concentration (Park & Humphrey, 1986 and Ying *et al.*, 2002).

Bulgarian workers concluded that the SCC's ranging from below 400 000 to above 1 000 000/mL in the milk of Saanen, Toggenburg and Anglo-Nubian goats do not significantly influence ($P < 0.05$) fat, protein, solids-non-fat or total solids concentrations in mid-lactation milk, neither milk yield, protein or fat concentrations in late lactation milk. As the SCC increased, however, from below 400 000 to above 3,700 000/mL, the lactose levels decreased from 4.09 to 3.88% (Petrova *et al.*, 1996; Petrova, 1997).

Casein concentration tends to stay the same but whey protein, albumin, proteose-peptone and plasmin concentrations increase significantly in the milk from infected glands (Leitner *et al.*, 2004). Elevated SCC's are positively ($P < 0.01$) associated with a decrease in milk yield, lactose content, calcium activity (although the calcium concentration stays the same) and curd yield with an increase in the rennet clotting time (Leitner *et al.*, 2004).

Factors affecting the SCC in goat's milk

(i) Stage of lactation

In general, it is accepted that the cell count is related to the stage of lactation and that the SCC, except during the colostrum period, increases with the number of days in milk.

In the first week of lactation, SCC is normally fairly high, e.g. 1 000 000/mL. During the following two to three months, the count decreases to as low as 500 000 at the stage of maximum

milk yield. As lactation continues and milk yield declines, the SCC steadily increases to as high as 2 to 7 million/mL at the end of lactation (Cullen, 1968; Hickley & Williams, 1981; Sheldrake *et al.*, 1981; Rota *et al.*, 1993; Dankow *et al.*, 2003).

The observation that the SCC in uninfected milk increased from 140 400/mL in early lactation to 614 000/mL in mid lactation milk, confirms the general theory that the SCC increases with the advancement of lactation (Poutrel & Lerondelle, 1983).

(ii) Oestrus

Oestrus causes an increase in SCC in goat's milk. The increase is independent of the decline in milk volume at oestrus (McDougall & Voermans, 2002).

(iii) Method of milking

Machine milking tends to increase the SCC in goats milk. In one study, the SCC in the milk obtained by hand milking was 295 000/mL lower than in milk obtained by machine-milking (Dankow *et al.*, 2003).

(iv) Season

Available data indicate that the SCC is highest during autumn and winter and lowest during spring (Dankow *et al.*, 2003).

(v) Breed

Various reports indicated differences in the SCC in the milk of different breeds. The following are examples of SCC's compared by means of Fossomatic procedure:

- Milk from Alpine goats had a slightly higher SCC and a bigger range than Nubian milk, i.e. 48 000 to 6 200 000 cells/mL vs. 78 000 to 2 800 000 cells/mL (Poutrel & Rainard, 1981; Park & Humphrey, 1986; Kalogridou-Vassliadou *et al.*, 1992).

- Milk from Alpine goats had a lower SCC than Anglo- Nubian goat's milk (Park & Nuti, 1985).

- In Bulgaria it was found that for goat's milk with a SCC of above 1 000 000 cells/mL, the mean was 2.5, 3.3 and 3.7 million cells/mL for Toggenberg, Saanen and Anglo-Nubian breeds, respectively (Petrova, 1997).

However, it is not clear to what extent environmental differences may be responsible for these differences between breeds.

(vi) Lactation number

In general, an increase in lactation number is accompanied by a significant increase in the SCC in the milk (Dulin *et al.*, 1983; Rota *et al.*, 1993).

Summary

Due to the apocrine nature of milk secretion, somatic cell counts in goat's milk naturally include a high percentage of non-DNA containing cytoplasmic particles that largely distort the well-accepted relationship between SCC and the level of udder infection that occurs in cow's milk. In goat's milk the SCC is extremely variable and generally much higher than in cow's milk. Factors such as stage of lactation, oestrus, method of milking, season, breed and lactation number may influence the SCC.

To eliminate as far as possible the inclusion of non-DNA containing particles, counting procedures that are specifically based on the staining of the DNA present in leucocytes, such as the Fossomatic procedure, Bentley and direct microscopic counting by means of pyronine y-methyl green staining, is recommended to determine the SCC in goat's milk. If somatic cell counting on goat's milk is done by means of Fossomatic- or Bentley analyzers, which are standardized against cow's milk standards, the counts may be almost 30% too high. To overcome this over-estimation, standardization against standards prepared from goat's milk and stained with pyronine y-methyl green should be used instead.

The CMT provides a practical means to identify inflammatory infections in goat's milk but positive findings should be confirmed by bacterial examination. The beta-glucuronidase test, which is based on the beta-glucuronidase activity of the leucocytes has proven to be a reliable method of counting only the somatic enzyme cells in the milk and thus overcomes the interference encountered with the SCC method.

An increase in SCC is accompanied by an increase in albumin, proteose-peptone concentration, plasmin activity, rennet clotting time and a decrease in milk yield, calcium activity, curd yield and lactose level, while the influence of SCC on total nitrogen, fat and total solids is variable.

Recommendation

As the average SCC in bulk goat's milk varies from 1 200 000 to 1 750 000 cells/mL, the recommendation that counts above 1 million may justify penalization, needs reconsideration and further investigation.

References

- Cullen, G.A., 1968. Cell counts throughout lactation. Physiological variation in the cell count of cow's milk during lactation. *Veterinary Record*. 83, 125-128.
- Dankow, R., Cais-Sokolinska, D., Pikul, J. & Wojtowski, J., 2003. Cytological quality of goat's milk. *Medycyna-Weterynaryjna*. 59 (1), 77-80.
- Dulin, A.M., Paape, M.J., Schultze, W.D. & Weinland, B.J., 1983. Effect of parity, stage of lactation, and intramammary infection on concentration of somatic cells and cytoplasmic particles in goat milk. *J. Dairy Sci.* 66, 2426-2433.
- Dulin, A.M., Paape, M.J. & Wergin, W.P., 1982. Differentiation and enumeration of somatic cells in goat milk. *J. Food Protection*. 45, 435-439.
- Haenlein, G.F.W., 2002. Producing quality goat milk. <http://goatconnection.com/articles>. University of Delaware.
- Hamed, A.I., Abou-Zeid, N. A., Keary, K.M.K. & Radwan, A.A., 1993. Physical and chemical properties of subclinical mastitic sheep's and goat's milk. *Egyptian J. Dairy Sci.* 21 (1), 133-149.
- Hinckley, L.S. & Williams, L.F., 1981. Diagnosis of mastitis in goats. *Veterinary Medicine / Small Animal Clinician*. 76, 711-712.
- Hunter, A.C., 1984. Microflora and somatic cell content of goat milk. *Vet. Record*. 114, 318-320.
- International Dairy Federation. Bulletin 354/2000. Proc. Development Strategy for the Sheep and Goat Dairy Sector. Brussels, Belgium.
- Kalogridou-Vassliadou, D., Manolkidis, K. & Tsigoda, A., 1992. Somatic cell counts in relation to infection status of the goat udder. *J. Dairy Res.* 59, 21-28.
- Kapture, J., 1980. Somatic counts don't tell whole mastitis story with goat milk. *Dairy Goat Guide*. Dec. 3, 9.
- Kozacinski, L., Hadziosmanovic, M., Majic, T., Karadjole, I. & Cvrtila, Z., 2002. Relationships between the results of mastitis tests, somatic cell counts and the detection of mastitis in goat's milk. *Tierarztliche-Umschau*. 57 (5), 255-260.
- Leitner, G., Merin, U. & Silanikova, N., 2004. Changes in milk composition as affected by subclinical mastitis in goats. *J. Dairy Sci.* 87, 1719-1726.
- Lerondelle, C. & Poutrel, B., 1984. Characteristics of non-clinical mammary infections of goats. *Annales de Recherches Vétérinaires*. 15, 105-112.
- McDougall, S. & Voermans, M., 2002. Influence of oestrus on somatic cell count in dairy goats. *J. Dairy Sci.* 85, 378-383.
- Nesbakken, T., 1976. [The cell count in milk of goats.] *Diagnosis Nordisk Veterinormedici*. 28, 550-556.
- Okada M., 1960. Histology of the mammary gland, VII. Histological and histochemical studies of cells in the milk of domestic animals. *Tohoku. J. Agric. Res.* 11, 31.
- Oliszewski, R., Nunex de Kairuz, M.S., Gonzalezde-Elias, S.N., Oliver, G. & de Elias, S.N., 2002. Assessment of beta-glucuronidase levels in goat's milk as an indicator of mastitis: comparison with other mastitis detection methods. *J. Food Protection*. 65 (5), 864-866.
- Pachard, V.S., Tanni, S., Fugun, R., Heady, J. & Gilman, C., 1992. Direct microscopic methods for bacterial or somatic cells. *Standards methods for the examination of dairy products*. A.P.H.O. Washington D.C. p. 309.
- Park, Y.W. & Humphrey, R. D., 1986. Bacterial cell counts in goat milk and their correlations with somatic cell counts, percentage fat, and protein. *J. Dairy Sci.* 69, 32-37.
- Park, Y.W. & Nuti, L.C., 1985. Electrical conductivity of goat milk in evaluating subclinical mastitis. *Proc. 3rd AAAP Animal Sci. Congr.* May 6-10, 1985. Seoul. Korean Rep. 2, 1272-1274.

- Perez, M. & Schultz, I.L.H., 1979. Somatic cells in goat milk. Proc. Annual Meeting Nat. Mastitis Council. 18, 44-49.
- Petrova, N., 1997. Effect of the different somatic cell count on the macro-components in goat's milk. Bulgarian J. Agric. Sci. 3 (6), 783-787.
- Petrova, N., Todorova, D., Dochevski, D. & Stancheva, N., 1996. Effect of different somatic cell count levels on the production, fat and protein in goat's milk. Bulgarian J. Agric. Sci. 2 (5), 633-638.
- Petterson, K.E., 1981. Cell content in goat's milk. Acta Vet. Scand. 22, 226-237.
- Poutrel, B. & Lerondelle, C., 1983. Cell content of goat milk. J. Dairy Sci. 66, 2575-2579.
- Poutrel, B. & Rainard, P., 1981. California Mastitis Test guide of selective dry cow therapy. J. Dairy Sci. 64, 241-248.
- Roguinsky, M. Redon, J.F. & Le Mons, P., 1971. Causes and diagnostics of goat mastitis. La Chevre. 68, 43-45.
- Rota, A.M., Gonzalo, C., Rodriguez, P.I., Rojas, A.I., Martin, L. & Tovar, J.J., 1993. Somatic cell types in goat's milk in relation to total cell count, stage and number of lactation. Small Rumin. Res. 12, 211-219.
- Schalm, O.W., Carrol, B.S. & Jain, N.C., 1971. Bovine Mastitis. Lea and Febiger, Philadelphia, USA.
- Sheldrake, R.F., Hoare, R.J. & Woodhouse, V.E., 1981. Relationship of somatic cell count and cell volume analysis of goat's milk to intramammary infection with coagulase-negative staphylococci. J. Dairy Res. 48, 393-403
- Venugopal, K. & Paily, E.P., 1979. Cell counts in goat's milk. Kerala J. Vet. Sci. 10 (2), 306-308.
- Ying, C., Wanf, H., Hsu, J. & Ying, C., 2002. Relationship of somatic cell count, physical, chemical and enzymatic properties to the bacterial standard plate count in dairy goat milk. Livest. Prod. Sci. 74 (1), 63-77.
- Zeng, S.S., 1996. Comparison of goat milk standards with cow standards for analyses of somatic cell count, fat and protein in goat milk. Small Rumin. Res. 21, 221-225.
- Zeng, S.S., Popham, T. & Escobar, E.N., 1999. Seasonal variation of somatic cell count and chemical composition in bulk tank goat milk. Dairy, Food Environmental Sanitation 19 (10), 658-690.
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