Potential of lactoperoxidase to diagnose subclinical mastitis in goats

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Abstract

This study was conducted to determine the potential of lactoperoxidase (LP) for the diagnosis of subclinical mastitis in goats. The activities of LP and somatic cell counts (SCC) were determined in the milk of clinically healthy Saanen and South African Indigenous goats and the correlation between LP activity and SCC was measured. An increase in LP activity was observed with increasing number of somatic cells in the milk of the two goat breeds. A significantly ($p < 0.01$) positive correlation was observed between LP activity and SCC in the Indigenous ($r = 0.91$) and Saanen ($r = 0.95$) goat milk samples. The mean SCCs in Indigenous and Saanen goat milk samples were 2.5×10^4 and 2.0×10^6 cells/ml, respectively. The LP activity of Saanen goat milk ranged from 0.49 to 1.07 units/ml with a mean value of 0.80 units/ml. In the Indigenous goat milk, the LP activity ranged from 0.03 to 0.38 units/ml with a mean value of 0.26 units/ml. The positive correlation observed between SCC and LP activity suggests that LP may be used to detect subclinical mastitis in dairy goats.

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1. Introduction

Different methods have been suggested for detection of subclinical mastitis in dairy animals, such as the California Mastitis Test, White Side Test, presence of pathogens in milk, the somatic cell count (SCC), the determinations of *N*-acetyl-β-d-glucosaminidase (NAGase) activity and serum albumin (Mavrogenis et al., 1995).

The activity of many milk enzymes such as lipase, phosphatase, lactase dehydrogenase, lysozyme, plasmin, xanthine oxidase (Harmon, 1994), catalase, NAGase (Harmon, 1994 and Walstra et al., 1999), β-glucunoridase and lactoperoxidase (IDF, 1979) increases when SCC increases. High SCC is reported to increase the level of lactoperoxidase (LP) both in bovine and human milk (Reiter, 1985). Tenovuo (1985) reported that total colostrum peroxidase activity seemed to increase as a function of the number of cells. Measurement of the activity of some enzymes in milk such as catalase, NAGase (Harmon, 1994 and Walstra et al., 1999) and antitrypsin (Harmon, 1994) has been used to monitor udder health in dairy cows. Any disease condition that allows increased concentrations of leukocytes in milk will increase the activities of certain enzymes in milk (Jenness, 1985). Since LP is mainly synthesized by polymorphonuclear leukocytes (Korhonen, 1980), its activity is expected to increase with increase in SCC in milk and thus may be used to detect mastitis in dairy goats.

The enzyme lactoperoxidase (EC 1.11.1.7) is used as an index of pasteurization efficiency in milk (Griffiths, 1986) and its antimicrobial property makes it a proven alternative for the preservation of raw milk in areas with warm climate and where it is not possible to use the conventional cooling system for technical and/or economic reasons (IDF, 1988 and FAO, 1999). Application of the LP system to improve the quality and safety of goat milk (Seifu et al., 2004b) and goat milk cheese (Seifu et al., 2004a) has been indicated in recent reports. Apart from its antimicrobial effect and its use as an index of pasteurization efficiency of milk, the enzyme LP could possibly be used to detect subclinical mastitis in dairy goats. This study was, therefore, conducted to determine the levels of and the relationship between LP activity and SCC in Saanen and South African Indigenous goat milk.

2. Materials and methods

2.1. Experimental animals

Ten Saanen and 10 South African Indigenous goats were used in this experiment. The goats were kept at the Onderstepoort Teaching Animals Unit of the Faculty of Veterinary Science, University of Pretoria. Both the Indigenous (5–7 years) and Saanen (5 years) goats kidded in April 2001. These animals were permanently housed in the facilities of the Faculty of Veterinary Science and managed similarly. All the goats were fed on a similar ration ad libitum composed of 28.3% (w/w) of yellow maize meal, 19.84% (w/w) malt dust, 4.96% (w/w) sunflower oil cake, 7.93% (w/w) yeast, 29.76% (w/w) eragrostis, 11.9% (w/w) molasses, 0.5% (w/w) mono calcium phosphate, 0.5% (w/w) salt, 0.5% (w/w) limestone powder, 0.3% (w/w) premix and 0.025% (w/w) Romensin.

2.2. Milk sample collection

The goats were milked by a milking machine according to standard procedures. Prior to milking, the teats of the goats were washed with clean tap water and dried with single service paper towel. Before attaching the milking machine to the teats of the goats, the first three to four streams of milk from both teats of each goat were discarded onto a strip cup and examined for any sign of mastitis.

Only morning milk samples of both Saanen and Indigenous goats were used for analyses. Milk samples from both goat breeds were collected from June 2001 up to August 2001. Milk sample collection from individual goats of each breed repeated on the average every 5 days. Milk yield of each doe was recorded daily.

Milk samples (100 ml) for the SCC, LP activity and thiocyanate content determinations were taken from a graduated measuring cylinder attached to individual milking units, which gives a representative sample from each milking. The milk samples were stored at 4 °C in a refrigerator until analysis. Milk samples intended for the SCC determination were preserved by potassium dichromate (0.2%, w/v).

2.3. Sample analysis

Goat milk was analyzed within 24 h of collection at the Milk Laboratory of the Faculty of Veterinary Science, University of Pretoria. The somatic cell count was determined using the Fossomatic 90 instrument (Foss, Electric, Hillerød, Denmark) according to IDF (1984). Thiocyanate concentration (SCN^-) in raw milk was determined spectrophotometrically as described by IDF (1988). Lactoperoxidase activity of the milk samples was measured with one-step 2,2′-azino-bis-(3-ethyl-benzthiazoline-6-sulphonic acid) (ABTS) (Pierce Chemical Co., Rockford, USA) solution as substrate using the method described by Seifu et al. (2004b). The activity of LP expressed in units/ml was calculated according to Kumar and Bhatia (1999). All samples were analyzed in duplicate and an average value for each sample was used for statistical analysis.

2.4. Statistical analysis

The differences in yield, SCC, LP activity and SCN^- content between Saanen and South African Indigenous goat milk were analyzed by the analysis of variance technique. The data for SCC was transformed to logarithmic value ($\log_{10}$) prior to statistical analysis. The correlations between SCC, LP activity and SCN^- content were performed by the SPSS Statistical Package (SPSS 10.0 for Windows, 1999).

3. Results

All the milk samples from Indigenous goats had SCC < 5×10^5 cells/ml whereas 86.8% of Saanen goat milk samples had SCC > 5×10^5 cells/ml and 57.4% of the Saanen goat milk samples had SCC greater than the threshold value of 1.0×10^6 cells/ml (Table 1). Significant differences ($p < 0.05$) in SCN^- content, SCC, LP activity and yield were observed between Saanen and Indigenous goat milk (Table 2). In the Saanen goat milk, SCC ranged from 6.3×10^4 to 1.2×10^7 cells/ml with a mean value of 2.0×10^6 cells/ml (Table 2). In the Indigenous goat milk, SCC ranged from 5.0×10^3 to 7.4×10^4 cells/ml with a mean value of 2.5×10^4 cells/ml (Table 2). The LP activity of Saanen goat milk ranged from 0.49 to 1.07 units/ml with a mean value of 0.80 units/ml. In the Indigenous goat milk, the LP activity ranged from 0.03 to 0.38 units/ml with a mean value of 0.26 units/ml (Table 2). High SCC was almost always associated with high LP activity both in Saanen and in the Indigenous goat milk.

Table 1.
Distribution of somatic cell counts (SCC) in Saanen and South African Indigenous goat milk

Breed	**SCC (×1000 cells/ml)**			
	<500	**500–750**	**750–1000**	**>1000**
Saanen (*n* = 68)	9 (13.2%)	14 (20.6%)	6 (8.8%)	39 (57.4%)
SAIG (*n* = 89)	89 (100%)			

Values in the table indicate the number of samples with SCC in the respective range indicated and values in parentheses indicate percentage of the total count; SAIG: South African Indigenous goat.

Table 2.
Milk yield (ml/doe/day), thiocyanate content (ppm), somatic cell count ($\log_{10}$) and LP activity (U/ml) in Saanen and South African Indigenous goat milk

Parameter	Saanen			South African Indigenous goat		
	Mean ± S.D.	***n***	**Range**	**Mean ± S.D.**	***n***	**Range**
SCN^-	$2.784^a \pm 1.24$	68	1.30–4.61	$4.58^b \pm 1.98$	89	2.24–8.21
SCC	$6.08^a \pm 0.44$	68	4.80–7.10	$4.34^b \pm 0.26$	89	3.70–4.87
LP activity	$0.795^a \pm 0.18$	68	0.49–1.07	$0.26^b \pm 0.11$	89	0.03–0.38
Milk yield	$1378^a \pm 416.72$	68	700–2800	$186^b \pm 90.12$	89	50–600

SCC: $\log_{10}$ transformed somatic cell count; SCN^-: thiocyanate content; LP: lactoperoxidase; S.D.: standard deviation. Means with different superscript letters (a and b) in a row are different ($p < 0.05$).

The correlation analysis showed a significantly ($p < 0.01$) positive correlation between LP activity and SCC in Indigenous ($r = 0.91$) and Saanen ($r = 0.95$) goat milk samples (Table 3). No significant correlation was observed between SCN^- concentration and LP activity and between SCN^- concentration and SCC (Table 3).

Table 3.

Correlation coefficients (r) between somatic cell counts, lactoperoxidase activity and thiocyanate content of Saanen and South African Indigenous goat milk

	LP activity	**SCC**
SAIG		
SCN^-	0.36^{ns}	0.42^{ns}
LP activity		0.91^{**}
Saanen		
SCN^-	0.55^{ns}	0.43^{ns}
LP activity		0.95^{**}

ns: not significant; SCC: somatic cell count; SCN^-: thiocyanate content; LP: lactoperoxidase; SAIG: South African Indigenous goat.

** $p < 0.01$.

4. Discussion

The International Dairy Federation recommends classification of quarter cow milk as mastitic or non-mastitic using a SCC threshold of 5×10^5 cells/ml (IDF, 1979). However, because of the physiological difference in milk secretion between goats and cows, a threshold of 1×10^6 cells/ml can be used when classifying goat milk as either mastitic or non-mastitic (Poutrel and Lerondelle, 1983 and White and Hinckley, 1999). Based on this recommendation, the mean SCC (2.0×10^6 cells/ml) observed in this study for Saanen goat milk suggests that most of the lactating Saanen goats at the time of the experiment had subclinical mastitis. Over 57% of the Saanen goat milk samples examined had SCC above the 1.0 million threshold level recommended for goat milk. Whereas all the SCC values of the Indigenous goat milk were below 5×10^5 cells/ml and this suggests that the Indigenous goats were apparently healthy.

The results of the analysis of variance showed difference ($p < 0.05$) in LP activity and SCN^- content between Saanen and South African Indigenous goat milk. This is consistent with the finding of Zapico et al. (1991) who reported that the activity of LP and the SCN^- concentration in milk varies between breeds of goats. The high LP activity observed in Saanen goat milk may be attributed to the higher level of somatic cells in the Saanen goat milk at the time of the experiment. Since the number of somatic cells in goat milk increase with the occurrence of mastitis in goats and since LP is synthesized mainly by polymorphonuclear leukocytes (Korhonen, 1980), it is logical to expect that the LP activity increases with the increase in SCC and thus with the occurrence of subclinical mastitis in goats. The current study supports this claim. Saanen goat milk had higher LP activity and apparently there was high prevalence rate of subclinical mastitis in Saanen goats which was evident from the fact that 57.4% of the Saanen goat milk samples examined had SCC above the 1 million threshold value. In an experiment aimed at investigating the antibacterial property of the LP system against selected food-borne pathogens in Saanen and South African Indigenous goat milk, Seifu et al. (2004b) found

that activation of the LP system resulted in strong inhibition of (bactericidal effect) *Staphylococcus aureus* ATCC 25923 in Saanen goat milk while it only exhibited bacteriostatic effect against the same strain of *S. aureus* in the South African Indigenous goat milk. They attributed this difference to the high LP activity in Saanen than in the Indigenous goat milk.

Mastitis generally increases the enzymatic and biochemical activities in milk (Korhonen and Kaartinen, 1995). The activity of enzymes such as catalase, phosphatase, peroxidase, xanthine oxidase, lipoprotein lipase, plasmin, milk proteinase, lysozyme and NAGase is noticeably higher in mastitic milk than in normal milk (Korhonen and Kaartinen, 1995). The increased activity of some of these enzymes has been used to detect mastitis in dairy animals. Lysozomal enzymes such as NAGase increase during inflammation and have proven to be reliable inflammation indicators (Sandholm, 1995 and Walstra et al., 1999). NAGase activity in cow milk is associated with udder cell damage resulting from the growth of pathogenic bacteria (Kitchen, 1981) and the level of NAGase has been used as an indicator of the degree of inflammation of the udder in cows (Kitchen et al., 1978), ewes (Maisi et al., 1987), goats (Vihan, 1989 and Maisi, 1990) and camels (Abdurahman, 1996). NAGase activity and SCC showed significant correlation in cow ($r = 0.88$, $p < 0.01$) (Williams et al., 1991), ewe ($r = 0.85$) (Leitner et al., 2001) and camel ($r = 0.38$, $p < 0.0001$) (Abdurahman, 1996) milk and could be used to monitor mastitis in these animals.

Lactoperoxidase activity increased with the increase in the number of somatic cells in the goat milk samples used in the current study. This finding is consistent with that reported previously for cows' milk. The level of LP in cows' milk increases with mastitic infection (Sandholm and Korhonen, 1995) and there exists a highly positive correlation between the LP concentration and SCC in cows' milk (Korhonen, 1980). The positive correlation observed between LP activity and SCC in the current study suggests that the enzyme lactoperoxidase could be used as a useful screening test for diagnosis of subclinical mastitis in goats. Since the biological role of lactoperoxidase is to protect the lactating mammary gland from bacterial infection (Reiter, 1985), the increased activity of LP with increase in the number of somatic cells observed in this experiment is expected and suggests its involvement in the natural host defence system against invading

microorganisms. In vivo experiments in cows indicated that the LP system plays a role in protecting the lactating mammary gland from infection with *Streptococcus uberis* (Marshall et al., 1986).

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