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Alterations in udder health indicators in goats with intramammary infection

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Abstract

A total of 397 udder halves foremilk samples from 200 lactating goats from 10 loose dairy goat flocks from different parts of Punjab were collected and subjected to bacteriological examination for assessing infected and non-infected udder halves. Further, the samples were analysed for California Mastitis Test (CMT), pH, Electrical Conductivity (EC), Somatic cell count (SCC), NAGase enzyme and milk biochemical parameters to observe the effect of intramammary infection. In the present study, the infected and non-infected udder halves were 28.97% (115/397) and 71.03% (282/397), respectively. The Mean values of SCC, Log_{10}SCC, CMT, EC, NAGase, Log_{10}NAGase and MBRT were significantly higher (p<0.01) in infected udder halves than that of non-infected udder halves. On comparing these parameters organism wise, the mean values of SCC and Log_{10}SCC were significantly (p<0.01) higher in udder halves positive for CNS (SCC=3481.89±403.45, Log_{10}SCC = 6.30±0.05) and CS (SCC = 3749.28±795.80, Log_{10}SCC = 6.31±0.12) than that of udder halves having no growth (SCC=1422.15±17, Log_{10}SCC =5.67±0.03). However, the difference was not significant between udder halves positive for CNS and CS. Similar trend was observed with CMT score, EC, MBRT, NAGase and Log_{10}NAGase activity among different organisms. The protein and lactose contents were significantly (p<0.01) lower in udder halves positive for CNS than that of udder halves having no growth.

Goat milk being sufficient in protein and calcium content, significantly contributes to human nutrition, with a higher availability and affordability than cow milk (Haenlein, 2001). Nutritional and health benefits of milk and dairy products from goats are important for people, especially those with medical problems such as cow milk allergy (Haenlein, 2004) and also recommended for use in dyspepsia, peptic ulcer and pyloric stenosis. People are also experiencing the benefit of its nutritional value which is giving impetus to goat milk business and making it a commercial success.

Milk quality in dairy goats is primarily affected by bacterial contamination of the mammary gland and exhibited in the form of clinical or subclinical mastitis (SCM) (Boscos *et al* 1996). Mastitis in the goat is mainly subclinical (McDougall *et al* 2002) that affects 5-30% goats (Contreras *et al* 2007) and results in decreased milk yield and quality in goat (Silanikove *et al* 2005). The primary bacterial causes of SCM in goats are *Staphylococcus* spp., *Streptococcus* spp. and *Micrococcus* spp. (Persson and Olofsson 2011). Mbindyo *et al* (2014) reported coagulase negative staphylococci (CNS) as the most prevalent (28.3%) pathogen, followed by *Staphylococcus aureus* and *Streptococcus* spp. IMI caused by CNS are associated with clinical mastitis, changes in milk composition, and reduced milk yield (Honkanen-Buzalski *et al* 1994); Koop *et al* (2012) observed an effect of persistent CNS infection on SCC and milk yield as compared to non-infected udder halves. The determination of bacteriological status of milk samples is regarded as a "Gold Standard" for the determination of the udder health status. It has impact on udder health indicators like somatic cell count (SCC), California Mastitis Test (CMT), electrical conductivity (EC), milk composition (fat, protein, lactose), Methylene blue reduction test (MBRT) and N-acetyl-β-D-glucosaminidase (NAGase) (Wakwoya *et al* 2006). Intramammary infection (IMI) raises milk SCC and reduces milk yield and milk quality in dairy cows and sheep but in goats this interrelationship is less clear (Sanchez *et al* 2002). So, in the light of above facts, the present study was envisaged with the objective to evaluate the effect of IMI on udder health indicators in dairy goats.

Materials and Methods

The flocks were visited during morning hours and the quarter foremilk (QFM) samples were collected. Proper cleanliness and dryness of teats was ensured prior to collection of milk sample. The teat orifice was scrubbed with a cotton wool wetted with 70% alcohol (spirit). First few streaks of milk were discarded and the individual udder halves samples (about 15 ml) were collected in sterilized labelled test tubes. Immediately after collection, milk samples were subjected to physical examination with naked

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eyes to detect any abnormalities in colour, odour, consistency, presence of blood and clot, flakes and any other visible abnormalities. The milk samples were then packed in ice and transferred immediately (within 1-2 hours of completion of sampling) to the laboratory for bacteriological examination, California mastitis test (CMT), Somatic Cell Count (SCC), pH, electrical conductivity (EC), NAGase activity and biochemical composition viz., fat, protein, lactose.

The isolation and identification of microbial organisms from milk samples was done as per standard microbial procedures of National Mastitis Council (1999) on 7% defibrinated sheep blood agar and McConkey agar plates. Organisms were stained with standard Gram's staining technique (Cruickshank *et al* 1982) and were differentiated into Gram positive and Gram negative. The individual bacterial colonies were picked up and subjected for identification. The analysis of milk samples for SCC was done using milk somatic cell counter from DELTA Instrument, BV Kelvinlaan 3, 9207 JB Drachten, The Netherland. Results were expressed in $\times 10^3$cells/ ml. The California Mastitis Test (CMT) was conducted by using CMT kit developed by the university. The reaction was interpreted as per standard method described by Pandit and Mehta (1969). The results were scored as 0, 0.5 (Trace), 1, 2 and 3 depending upon the degree of gel formation. The electrical conductivity was recorded with the help of Electrical Conductivity Meter (Mettler Toledo). The results were expressed in milli Siemens per cm (mS/ cm). The pH of milk was recorded with the help of digital pH meter (Mettler Toledo). The biochemical composition of the milk i.e. fat, protein, lactose was analyzed by using Milk analyser Lactoscan LA, serial no. 26824 from Milkotronic LTD, Bulgaria. The results were expressed: fat, protein, lactose (%). The NAGase activity was measured using the spectrophotometric method of Kitchen *et al* (1978) with some modifications.

Defining udder health status

The quarter health status was assessed and defined on the basis of bacteriology and SCC estimate (CMT score) of quarter foremilk samples as described below:

CMT score	Microbial pathogen	
	Not detected	Detected
< 1	Healthy	Latent infection
≥1	Non-specific mastitis	Specific mastitis

Data collection and statistical analysis

Structured data handling format was prepared and every important information (variable) associated with the overall objective of the investigation was properly gathered and recorded in Microsoft excel 2007 were analyzed by using SPSS (Statistical Package for the Social Sciences) version 16.0 software. The data pertaining to quarter and goat composite milk somatic cell count and NAGase activity were log transformed to obtain a normal distribution. The paired t-test was applied to determine significance.

Results and Discussion

In the present study, the bacteriologically positive and non-infected udder halves were 28.97% (115/397) and 71.03% (282/397), respectively. The mean values of SCC and Log_{10}SCC were significantly (p<0.01) higher in infected udder halves (SCC = 3619.63±35, Log_{10}SCC = 6.31±0.05) than that of non-infected udder halves (SCC = 1422.15±17, Log_{10}SCC = 5.67±0.03). Contreras *et al* (1999) also reported that uninfected udder halves had a lower foremilk SCC (1.3 and 1.0×10^6/ml, respectively) than the infected udder halves (1.74 and 1.66×10^6/ml), respectively. Similar pattern was also observed by Sanchez *et al* (1999). They further analyzed that among the samples from uninfected udder halves, 70% had SCC <500 × 10^3 cells/ ml, 83% had SCC <10^6 cell/ml and 89% had SCC <1.5 × 10^6 cells/ml. Aulrich and Barth (2008) also showed that infected udder halves had significantly higher SCC than non-infected udder halves.

The CMT was also significantly (p<0.01) higher in infected udder halves (1.79±0.11) than that of non-infected udder halves (0.75±0.06). The relationship between SCC, CMT and infection status was reviewed by many researchers (Kalogridou-Vassiliadou *et al* 1991, Contreras *et al* 1996, Schaeren and Maurer 2006 and Jendretzke 2009). It was proposed that low levels of CMT indicate an absence of mammary gland infection (Maisi 1990b). Within his study, using scores from 1 to 5, CMT scores of udder half samples lay at 1 or 2 throughout the lactation with the exception of the colostral period. Infected halves gave higher scores ranging from 3 to 5. Maisi (1990b) suggested the use of a threshold score of 4 for CMT as an indication of infection, but scores of 3 may already give a hint of an infection. In a summarising study, Haenlein (2002) concluded that CMT might be able to identify infected udder halves.

Table 1: Intramammary infection (IMI) vis-à-vis milk quality parameters (Mean±SE)

	Log_{10} SCC	CMT	Fat (%)	Protein (%)	Lactose (%)	pH	EC (mS/cm)	Log_{10} NAGase
NI	5.67±0.03[a] (N=282)	0.75±0.06[a] (N=282)	3.51±0.10[a] (N=266)	3.50±0.02[a] (N=272)	4.75±0.02[a] (N=272)	6.44±0.03[a] (N=273)	6.28±0.06[a] (N=275)	1.52±0.02[a] (N=282)
INF	6.31±0.05[b] (N=114)	1.79±0.11[b] (N=115)	3.41±0.15[a] (N=113)	3.43±0.02[a] (N=114)	4.72±0.04[a] (N=114)	6.49±0.05[a] (N=114)	6.59±0.08[b] (N=112)	1.80±0.03[b] (N=115)

NI: Non-infected; INF: Infected
Values within columns with different superscripts differ significantly, *p<0.01*

The EC of milk was significantly (p<0.01) higher in infected (6.59±0.08) than that of non-infected udder halves (6.28± 0.06). Romero *et al* (2014) also found that EC of healthy glands was significantly lower than that of infected glands at stripped milking. The higher value of EC in mastitic quarters might be due the changes in the electrolyte concentration in the mastitic milk. When there is intramammary infection, the capillary permeability of udder increases which allows the movement of electrolytes particularly sodium and chloride from blood into milk and account for higher EC.

The Log_{10}NAGase values were also significantly (p<0.01) higher in infected (1.80±0.03) than that of non-infected udder halves (50.66±3.67 vs. 1.52±0.02). Vihan (1989) also found significant differences in NAGase activity in two goat flocks between infected and non-infected halves regardless of the type of infection. SCC and the NAGase activity were significantly higher with CNS and *Mycoplasma agalactiae* compared to uninfected goats. The author supposed a correlation of the higher NAGase levels within CNS infected halves with increased secretion of epithelial cytoplasmic particles. Vihan (1989) concluded from his own studies and the literature that NAGase activity seems to be a sensitive method for detecting SCM in goats. Maisi (1990a) analysed milk samples from 39 goats over a whole lactation and found that infected udder halves showed higher values for NAGase along with higher CMT values as compared to healthy udder halves during the whole period of lactation, with the exception of the colostral period. In healthy udder halves average values for NAGase of 0.9 ± 1.5 units were measured in contrast to infected halves with 10.3 ± 6.3 units. Barth *et al* (2010) proposed that the infection status had a significant effect on log_{10} SCC ($F_{2,103} = 20.22$, $p < .001$) and log_{10} NAGase ($F_{2,103} = 12.06$, $p < .001$). Also he suggested that NoInf/Inf indicating that the infected halves did not influence their uninfected parallel half. For log10 NAGase this was different: infected halves differed significantly from NoInf/NoInf ($p < .01$) but not from NoInf/Inf which might be caused by a dependency of the udder halves.

No statistically significant (p>0.05) differences were observed in pH, fat, protein and lactose contents between infected udder halves and non-infected udder halves. Leitner *et al* (2004a) analysed the fat content in 25 crossbreed goats and found no differences between infected halves (3.88 % ± 0.12) and uninfected halves (3.89 % ± 0.11). In another study by Leitner *et al* (2004b), they did not find significant influence on fat content in infected (3.75 %) and uninfected halves (4.2 %) of goats. Min *et al* (2007) also could not find any significant interaction between infection status and milk fat in 35 mixed-age Alpine goats.

Organism *vis-à-vis* udder health parameters

Data illustrated in Table 11 shows the difference in milk quality parameters according to different organisms. The Log_{10}SCC was significantly higher (p<0.01) in udder halves positive for CNS (6.30±0.05) and CS (6.31±0.12) than that of udder halves having no growth (5.67±0.03). However, the difference was not significant between udder halves positive for CNS and CS. Aulrich and Barth (2008) also showed that infected udder halves had significantly higher SCC than non-infected udder halves. SCC levels in CNS induced IMI increased to > 10^6 cells/ ml (Leitner *et al* 2004a). CNS infections with *S. epidermidis* seem to show the highest values of the SCC (Deinhofer and Pernthaner 1995; Contreras *et al* 1996). In contrast to these findings, Moroni *et al* (2005) observed that SCC of infected udder halves was greater with *S.caprae* than with *S.epidermidis* and other CNS. In the study from Aulrich and Barth (2008) infections with *S. epidermidis* and *S. xylosus* showed a tendency to increase the SCC in infected udder halves when compared to non-infected

Table 2: Organism wise variation in milk quality parameters (Mean±SE)

Health	Log_{10} SCC	CMT	Fat (%)	Protein (%)	Lactose (%)	pH	EC (mS/cm)	Log_{10} NAGase
NG	5.67±0.03^{a} (N=282)	0.75±0.06^{a} (N=282)	3.51±0.10^{a} (N=266)	3.50±0.02^{a} (N=272)	4.75±0.02^{a} (N=272)	6.44±0.03^{a} (N=273)	6.28±0.06^{a} (N=275)	1.52±0.02^{a} (N=282)
CNS	6.30±0.05^{b} (N=87)	1.76±0.12^{b} (N=87)	3.45±0.18^{a} (N=86)	3.41±0.03^{b} (N=86)	4.70±0.05^{a} (N=86)	6.49±0.06^{a} (N=86)	6.66±0.08^{b} (N=86)	1.80±0.03^{b} (N=87)
CS	6.31±0.12^{bc} (N=21)	1.84±0.26^{bc} (N=22)	3.18±0.27^{bc} (N=22)	3.46±0.05^{a} (N=22)	4.80±0.12^{ab} (N=22)	6.47±0.11^{a} (N=22)	6.43±0.23^{ab} (N=21)	1.80±0.07^{bc} (N=22)

Values within columns with different superscripts differ significantly, $p<0.01$

halves. Sanchez *et al* (1999) reported that among infected halves, SCS was highest among those from which *S. aureus* was isolated.

Similar trend was observed with CMT score and Log_{10}NAGase activity among different organisms. Vihan (1989) also observed significantly higher NAGase activity in CNS affected goats as compared to uninfected goats.

The EC of milk was significantly higher (p<0.01) in udder halves positive for CNS than that of udder halves having no growth but did not differ significantly between CNS and CS positive udder halves. No literature related to this study could be found.

There was no significant (p > 0.01) difference in pH and fat values of different udder halves. Similarly, Leitner *et al* (2004b) found no effect of organism on fat concentrations. The protein and lactose contents were significantly lower (p<0.01) in udder halves positive for CNS (3.41±0.03) than that of udder halves having no growth (NG) (3.50±0.02). Similarly, Leitner *et al* (2004b) observed significant reduction in lactose content of infected glands. The reason may be decrease in lactose concentration related to microbial activity or to the effect of plasmin induced casein derived products on mammary epithelial cells (Shamay *et al* 2002). No significant differences were observed in left and right udder halves.

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