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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 3390-3395

www.thepharmajournal.com
Received: 19-05-2022
Accepted: 22-06-2022

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Evaluation of milk constituents, electric conductivity and milk pH as a pre-indicator of mastitis in Sirohi goats

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Abstract
Somatic cell count (SCC) in milk has been shown to be an excellent marker for subclinical mastitis but very difficult to execute at farm level. Due to the damage to the udder tissue, concentrations of Milk Constituents Changed and concentrations of Na+ and Cl- increases in milk which results in increase in electric conductivity and pH. The present investigation was carried out to investigate the correlation of milk Constituents, pH and electric conductivity with somatic cell count and can be used as an indicator to detect subclinical mastitis (SCM) in 105 lactating Sirohi goats maintained under arid region of Rajasthan at Sheep Breeding Farm, Fatehpur, Sikar. Total 210 milk samples were screened for somatic cell count, electrical conductivity (EC), pH and milk Constituents by deluxe electric conductivity meter manufactured by Systronics India limited, Ahmadabad pH meter and ultrasonic milk analyzer manufactured by Rajasthan Electronics & Instrument Limited (REIL), Jaipur respectively. The result demonstrated statistically significant ($p<0.01$) increase in electric conductivity, pH and fat percentage while significant ($p<0.01$) decrease in milk lactose. From the results a conclusion can be made that electrical conductivity and milk lactose exhibits a high correlation with SCC scores and can be used as the tools to predict subclinical mastitis at farm level.

Keywords: Electric conductivity, milk lactose, somatic cell count, subclinical mastitis

Introduction

Goat milk production is a dynamic and growing industry that is fundamental to the well-being of millions of people worldwide and is an important part of the economy in many countries (Silanikove *et al*., 2010) [16]. Mastitis is a serious disease in dairy animals causing great economic losses due to reduction in milk yield as well as lowering its nutritive value. Somatic cell count is a major indicator of health status of mammary gland in dairy cattle (Sharma *et al*., 2016) [14]. Goat milk has higher SCC than cow milk and sheep milk. Milk secretion in goat is an apocrine process which results in presence of many cytoplasmic particles. This type of secretion is characterized by the detachment of the apical part of the epithelial cells from their base and release into the alveolar lumen at the end of the secretory phase (Makovicky *et al*., 2012) [7]. According to National Mastitis council, to differentiate between healthy and infected udder secretion the limit of SCC has been decided as 10,00,000 cells/ml. (Hinckley and Williams., 1981) [4].

Milk yield and composition are the main selection objective of dairy goat. Unlike milk production loss, there is a direct relationship between SCC and milk quality. An elevated SCC in milk has a negative influence on the quality of raw milk. A number of studies have been done to investigate relation of SCC with milk quality in cattle but information regarding goat are scanty. Somatic cell count (SCC) in milk has been shown to be an excellent marker for subclinical mastitis but very difficult to execute at farm level. Therefore the present investigation was carried out in native Sirohi Goats with the objective to evaluate the relationship between SCC and milk composition i.e. Milk Constituents, pH and electric conductivity for development of new investigating criteria for diagnosis of subclinical mastitis at farm level in Goats.

Materials and methods: Location

The study was conducted at Sheep breeding farm Fatehpur, Sikar goat paddock located in Fatehpur city of Rajasthan. A total of 210 apparently healthy, clinical mastitis free Udder half milk samples from 105 healthy Sirohi goats were collected in the study. All goats were housed under paddock system and milked twice daily by hand milking method.

Collection of samples and diagnosis of intra-mammary infection

Representative milk samples were collected from udder half of lactating goats. For this purpose, half were designated as Left half (LH) and Right Half (RH). About 30 ml of milk was collected aseptically in the sterile clean sampling bottles after discarding the first 5-6 streaks of fore milk from each teat. Prior to collection of milk, udder was washed with water and cleaning of teats with cotton soaked in 70% ethyl alcohol. The collected samples were kept in ice box at 40c and were brought to the laboratory immediately for further analysis. The analysis of milk Constituents, pH, electric conductivity and smear for SCC were performed within one hour of collection. The SCC in milk was done by manual microscope count method 12 which were slightly modified.

The test milk samples were thoroughly mixed by gentle shaking the vials and 10µl of milk was taken on the pre-drawn one cm square marked area over a grease free clean glass slide which was uniformly smeared with a standard sterilized bacteriological platinum loop. After drying the smears were fixed with alcohol, air dried and stained with the modified Newman's Lampert stain, by keeping the prepared slide in the staining solution for 1 to 2 minutes. Afterwards the smears were gently washed in tap water and dried. The dried stained smears were examined under the oil immersion lens of the microscope. Counting of cells in 30 different fields was done under oil immersion objective lens (100x) and the counting was repeated twice per smear to assess average number of somatic cells in 30 fields and average number of the cells per field. The average number of cells was multiplied by the multiplication factor of the microscope i.e. 393170 to obtain the number of cells per ml of the milk. The health status of individual quarters was defined on the basis of Somatic cell count of udder half milk samples which was predefined by National mastitis council. The udder half having somatic cell count <10, 00,000 cell/ml comes under category of healthy half, while udder half having somatic cell count >10, 00,000 cells/ml included in SCM infected half. Due to SCC not displaying a normal distribution, data of SCC were log transformed to base10. The Electric conductivity of milk samples were analysed through deluxe electric conductivity meter-model 720 manufactured by Systronics India limited, Ahmadabad and contents of milk and pH were analysed through Lactoscan ultra-sonic milk analysis-model LM2 manufactured by Rajasthan Electronics & Instrument Limited (REIL), Jaipur.

Statistical analysis: Due to SCC not displaying a normal distribution, data of SCC were log transformed to base 10. The data were examined by general linear model procedure of SPSS statistical package (ver. 16.0) software. Secondly, Pearson's correlation coefficients (r) were also established to determine the relationships between the various studied parameters. The results were considered significant if the associated P-value was <0.05. and highly significant if P-value was <0.01.

Results and Discussion

Milk Constituents, pH and electric conductivity of milk and milk somatic cell count of Sirohi Goats varied between healthy and sub-clinical mastitic animals. In present study all pooled and udder halves wise milk samples were subjected to SCC analysis. The arithmetic mean ± SE of SCC (absolute and logarithmic) of pooled milk sample were 8,22,009 ± 23415 cells/ml of milk and 5.894 ± 0.141 respectively. Range of SCC varied from 2,91,132 cells/ml to 13,74,733 cells/ml of milk samples collected from farm (Table 1). The SCC cells/ml converted into log scale to minimize the heterogeneity of variance. Udder half-wise means ± SE of Log10SCC has been presented in the Table 2 where highest values found in right udder half i.e., 5.899±0.045.

Table 1: Mean ± SE values and range of somatic cell counts

Number of observations	Mean ± SE (cells/ml)	Mean ± SE (Log10SCC)	Range (cells/ml)
105	8,22,009±23415	5.894 ± 0.141	2,91,132-13,74,733

Table 2: Udder half-wise Mean ± SE values of Log10SCC

Different Udder halves	Number of observations	Mean ± SE Log10SCC
Overall mean	210	5.893± 0.021
Left udder half	105	5.889±0.044
Right Udder half	105	5.899±0.045

Relationship of milk SCC with milk pH

Data of the present study revealed that the milk pH value was found to be higher in the SCM affected animals than the healthy animals. The average (±SE) value of pH was observed in healthy and SCM samples 6.58 ± 0.030 and 6.80 ± 0.040 respectively. The pH value of milk samples from SCM affected udder halves was distinctly higher to the alkaline side. Similar findings were reported by Kamal *et al.* (1998) [5], Ghosh *et al.*, (2004) [3] Samanta *et al.* (2006) [13] and Sharma *et al.* (2007) [15].

Significant ($P<0.05$) positive correlation was found between the milk pH and Log10SCC (Table 4). The mean pH value of healthy and SCM group of animals were significantly ($P<0.01$) differed from each other (Table 3).

The pH of SCM milk was higher than normal milk, which is in agreement with results of Wielgisz-Groth and Groth (2003) [19], Kamal *et al.* (1998) [5] and Garg and Singh (2008) [2], support the present findings.

Table 3: Mean ± SE values and range of pH of Milk and level of significance

Particulars	Number of observations	pH	
		Mean ± SE	Range
Healthy	72	6.584±0.030a	5.72 to 6.97
SCM	33	6.801±0.040b	6.14 to 7.27
Average	105	6.65±0.026	5.72 to 7.27

Mean with different superscript differ significantly from each other ($P<0.05$).

Table 4: Correlation coefficients of Log10SCC with Milk pH

Log10SCC			pH
Log10SCC	Pearson Correlation	1	0.222*
	Sig. (2-tailed)		0.023
	N	105	105
pH	Pearson Correlation	0.222*	1
	Sig. (2-tailed)	0.023	
	N	105	105

*Significant ($p<0.05$)

Relationship of milk SCC with milk electric conductivity

Data of the present study revealed that the average ±SE value of electric conductivity from healthy and SCM udder halves was observed to be 5.30 ± 0.055 and 5.987 ± 0.087 respectively (Table 5). It was observed that the milk electric conductivity value was higher in the SCM affected animals than the healthy animals. Similar findings were reported by Ying *et al.* (2004) [20], Chen *et al.* (2008) [1], Romero *et al.* (2012) [12] and Sharma *et al*. (2016) [16].

The correlation coefficient between the Log10SCC and milk EC has been presented in the Table 6 and found that the highly significant ($P<0.01$) positive correlation was found between the milk EC and Log10SCC. The mean value of EC in healthy and SCM group of animals was significantly ($P<0.01$) differed from each other (Table 5). Similar findings were also observed by Romero *et al.* (2012). EC of milk was significantly varied of Sirohi goats between the healthy and SCM animal groups because increase in concentration of Na+ and Cl-in the mastitic milk which tends to increased EC of milk from the SCM udder halves. (Kitchen, 1981).

Table 5: Mean ± SE values & range of Electric Conductivity of milk and level of significance

Particulars	Number of observations	EC	
		Mean ± SE	**Range**
Healthy	72	5.300±0.056a	4.20 to 6.50
SCM	33	5.987±0.087b	5.00 to 6.90
Average	105	5.516±0.056	4.20 to 6.90

Mean with different superscript differ significantly from each other ($P<0.05$).

Table 6: Correlation coefficients of Log10SCC with milk Electric Conductivity

Log10SCC			EC
Log10SCC	Pearson Correlation	1	0.285**
	Sig. (2-tailed)		0.003
	N	105	105
EC	Pearson Correlation	0.285**	1
	Sig. (2-tailed)	0.003	
	N	105	105

**Highly Significant ($p<0.01$).

Relationship of milk SCC with Milk Constituents

The mean ±SE value of milk constituents in healthy and SCM animals were 3.26±0.101 and 4.35±0.140 for fat percent, 3.04±0.039 and 3.30±0.0623 percent for protein, ±0.070 and 8.44±0.117 per cent for SNF, 4.63±0.0.34 and 4.23±0.0376 percent for lactose respectively (Table 7 and Fig. 1). The mean value of milk constituents were significantly differs in healthy and SCM group except SNF.

The results pertaining to correlation coefficient in Log10SCC and milk constituents have been depicted in Table 8. Fat percent was significantly positively correlated with the Log10SCC. Protein and SNF percent were positively but non-significantly correlated with the Log10SCC while lactose was negatively correlated with Log10SCC.

Leitner *et al*. (2004) [6] found the milk composition (fat, protein and lactose) varied among flocks, with lower mean total protein in uninfected halves than in infected ones and higher lactose in uninfected than healthy halves of 500 Israeli goats. Marija *et al.* (2003) [8] observed statistically highly significant ($p\leq0.01$) positive correlation between Log10SCC and fat content. The higher fat content (5.79 ± 1.62) was found in cow with higher somatic cell count by Ouedraogo *et al.* (2008) [10]. On the other hand significant negative correlation of fat with SCC was reported by Petlane *et al.* (2013) [11]. Fat percentage had very low and non-significant correlations with SCC (Orman *et al.*, 2011) [9].

Strzalkowska *et al.* (2002) [17], Petlane *et al.* (2013) [11] and Sharma *et al*., (2016) [16] found the statistically significant negative correlation between Lactose and Log10SCC which was similar to our study.

Negative correlation of SCC with lactose and SNF content indicated that as SCC increases, all these content decreases. Elevated SCC associated with a decrease in milk constituents because of reduced synthetic activity of the mammary tissue while increase in fat concentration because of reduction in milk production. The fat droplets are large relative to the gaps between the cells and are contained within the alveoli and consequently their concentration increases.

Table 7: Effect of SCM on Log10SCC and Milk Constituents

Traits	Healthy	SCM	Level of significance
Log10SCC	5.063±0.030	5.702±0.034	$P<0.01$
Fat %	3.26±0.101	4.35±0.140	$P<0.05$
Protein %	3.04±0.039	3.30±0.0623	$P<0.05$
SNF%	8.27±0.070	8.44±0.117	Non-Significant
Lactose%	4.63±0.0.34	4.23±0.0376	$P<0.05$

Table 8: Correlation coefficients of Log10SCC with Milk Constituents

		Log10 SCC	Fat	Protein	SNF	Lactose
Log10 SCC	Pearson Correlation	1	.399**	.105	.117	-.281**
	Sig. (2-tailed)		.000	.284	.235	.004
	N	105	105	105	105	105

**Highly Significant ($p<0.01$), *Significant ($p<0.05$)

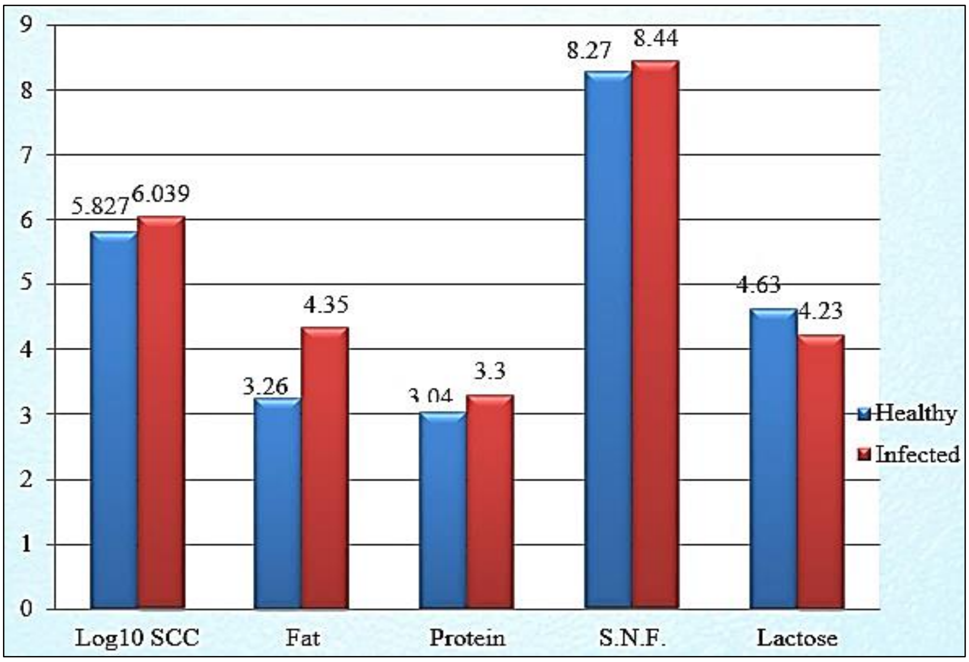

Fig 1: Mean Log10SCC, Fat, Protein, SNF and Lactose per cent in healthy and infected group

Table 9: Means (±SE) of Log10SCC, EC and milk lactose of healthy and sub-clinical mastitis infected cows

Characteristics	Healthy cows	SCM infected cows	significance
Log10SCC	5.083 ±0.011	5.812 ±0.020	**
Electric Conductivity (mS)	5.489 ±0.017	5.928 ±0.025	**
Milk Lactose %	4.544 ±0.016	4.186 ±0.031	**

**Highly significant (*P*<0.01)

Table 10: The Pearson correlation between SCC, EC and Milk Lactose percentage

	SCC	Milk Lactose	Milk E.C.
SCC (Cells/ml)	1.00	-0.459**	0.574**
Milk Lactose (%)	-0.459**	1.00	-0.498**
Milk E.C. (mS)	0.574**	-0.498**	1.00

**Highly significant (*P*<0.01)

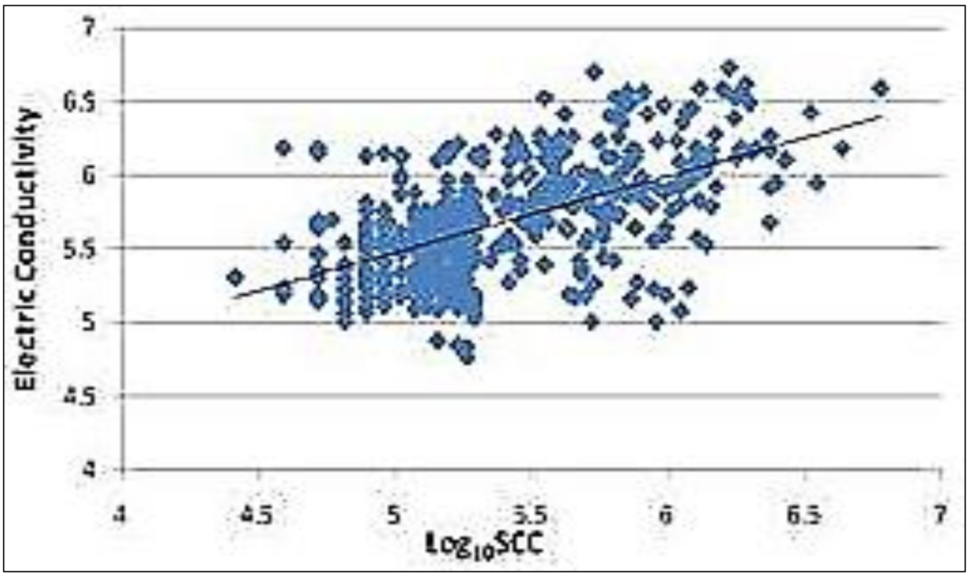

Fig 2: Positive correlation between Log10SCC and Electric Conductivity

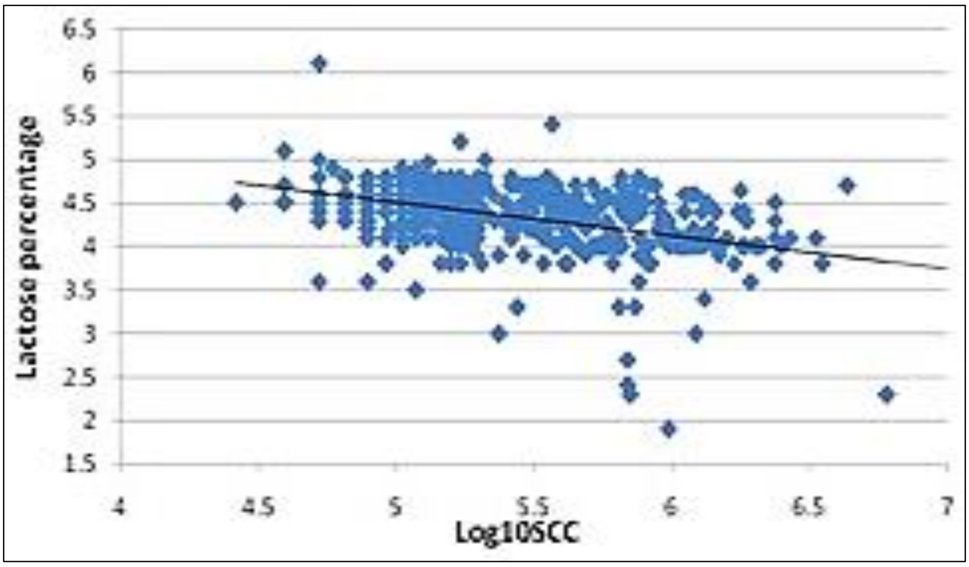

Fig : Negative correlation between Log10SCC and Milk Lactose

Conclusion
It is concluded that Milk pH, Electric conductivity, milk fat percentage and Lactose content showed similarity with somatic cell count in detection of subclinical mastitis. Our study showed that the Fat and Lactose content, pH and electric conductivity are highly significant with SCC. The result demonstrated statistically significant ($p<0.01$) increase in electric conductivity, pH and fat percentage while significant ($p<0.01$) decrease in milk lactose. From the results a conclusion can be made that electrical conductivity and milk lactose exhibits a high correlation with SCC scores and can be used as the tools to predict subclinical mastitis at farm level.

Acknowledgments
We are grateful to Dean PGIVER, Jaipur (RAJUVAS) for providing facilities, continuous encouragement and support during the study

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