



Effect of season, lactation, parity and milk production on milk inflammatory parameters in healthy and mastitis infected Sahiwal cows

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ABSTRACT

The present investigation was undertaken to study the effect of different season, stages of lactation, parity and milk production on milk inflammatory parameters in healthy and mastitis infected Sahiwal cows. Three parameters, viz. pH, electrical conductivity (EC) and somatic cell count (SCC) were taken to assess the quality of milk. The study was conducted on 100 selected Sahiwal cows maintained at livestock farm at National Dairy Research Institute, Karnal, Haryana. Significant higher values of pH, EC and SCC were found in milk samples during rainy season, followed by winter and summer. Effect of parity, lactation and milk production on inflammatory parameters was non-significant. The pH, EC and SCC also differed significantly in healthy, subclinical and clinical stages of mastitis. A significant positive coefficient of correlation was observed between SCC and EC ($r=0.679$) whereas moderate ($r=0.526$) but significant positive correlation was found in pH and EC. In view of above results, it can be deduced that the elevated value of pH, EC and SCC indicates inflammation into the udder of the animals which leads to reduced quality of milk. Hence it is imperative that pH, EC and SCC need to be considered along with effective management strategies.

Keywords: Electrical conductivity, Mastitis, pH, Sahiwal, Somatic cell count

Increasing trend for greater milk production and mechanized milking systems impose unnatural stress on the bovine udder resulting in the possibility of intra-mammary infection into the udders of dairy cattle. Somatic cell count increases in milk above the recommended normal value of 2 lakhs in case of udder infection. In most of the dairy farms, somatic cell count (SCC) is taken as a raw milk quality indicator and also indicates hygienic conditions of milk production (Sharma *et al.* 2011, Petzer *et al.* 2017). Mastitis is a major problem for dairy farmers. In India, annual economic loss has been estimated as ₹7,165.51 crores, of which, 57.93% was because of subclinical mastitis (Bogni *et al.* 2011, Srivastava *et al.* 2015, Panchal *et al.* 2016a, Gaddi *et al.* 2016). Mastitis not only affects animal's health and milk quality but also affects economy of dairy industries (Jingar *et al.* 2014).

Normally, in big automated dairy farms, SCC is taken as an indicator of milk quality which is also affected by many other factors like system of management, season, breed and environment. Besides SCC, other tests like pH and electrical conductivity (EC) of milk are also commonly

used for milk quality assessment (Syridion *et al.* 2014). But, pH and EC are also affected by temperature, stage of lactation, fat percentage, milking interval, and breed (Sundhan and Sharma 2010, Panchal *et al.* 2016a). Hence, it is imperative to understand the limitations of these tests and how different physiological factors such as season, stage of lactation, parity and milk yield have effect on milk quality parameters. The available information regarding effect of physiological factors on milk quality parameters is scanty and particularly in indigenous breed like Sahiwal cows. Further, combination of above parameters would give more precise information in diagnosis of intra-mammary infection. Therefore, the present investigation was undertaken to see the effect of season, stage of lactation, parity and milk yield on pH, electrical conductivity and SCC for accessing the milk quality as well as diagnosis of intra-mammary infection in Sahiwal cows.

MATERIALS AND METHODS

The investigations were carried out on 100 lactating Sahiwal cows maintained by Livestock Research Centre at ICAR–National Dairy Research Institute, Karnal, India for a period of 1 year, i.e. March 2013–February 2014. The samples were collected twice a day, i.e. in the forenoon and in the evening from the animals covering all three seasons, viz. summer, rainy and winter. A total of 670 samples in triplicate were collected and screened for mastitis

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in this experiment. Somatic cell was counted as per the method described by Mammadova and Keskin (2015). Samples having SCC below 2 lakhs per ml were categorized as healthy animals, whereas, SCC in the range of 2–5 lakh per ml were considered to be subclinical mastitis animals, remaining animals with milk SCC more than 5 lakh per ml were positioned in clinical mastitis category (Alhussien and Dang 2018). Precision handheld pH meter of Haana instrument and EC meter of Oriental Instrument Ltd. Japan were used to check the pH and EC, respectively. Milk yield was recorded at the time of sampling collection using weighing balance. Data of stage of lactation, parity were taken from record room of dairy farm, NDRI, Karnal.

Three seasons, viz. summer (March–June), rainy (July–September), winter (November–February) were selected for data collection. The experimental animals were divided into three stages of lactation like early stage of lactation (15 to 100 days), mid stage of lactation (101 to 200 days) and late stage of lactation (above 200 days). Like the stages of lactation, animals parity were divided into four categories, viz. 1, 2, 3 and >4 parity. To study the relationship between milk production and milk quality parameters, the animals were classified into three different categories, based on their milk yield, viz. 0–4 litres, 5–10 litres, and >10 litres per day.

Statistical analysis: Analysis of the experimental data by one way ANOVA was performed using SAS 9.3 software package to determine the mean and standard error (SE). Pearson's correlation at $P<0.05$ was determined with the help of STATA13.

RESULTS AND DISCUSSION

Mastitis in dairy cattle leads to economic losses and it affects the future productivity of dairy animals. The subclinical mastitis lacks visible inflammatory changes. The sub-clinically affected animals can act as continuous source of infection to other animals also (Manoj and Singh 2020, Singh *et al.* 2021). Therefore, there is an urgent need to identify certain diagnostic tools to detect mastitis at its earliest stage. The changes in the ionic concentration of milk induce some variations in the electrochemical properties and have the potential to be used as an effective technique for early prediction of mastitis. The SCC varies with many external and internal factors associated with the cow (Deshapriya *et al.* 2019, Halasa and Kirkeby 2020). The different factors like season, stage of lactation, parity and milk yield on pH, EC and SCC were studied and results are discussed below.

Effect of season: Among different seasons, the values of milk pH and EC varied from 6.41 ± 0.07 to 7.04 ± 0.04 and 5.79 ± 0.10 to 4.26 ± 0.04 respectively (Table 1). Though the variation was not significant, but comparatively higher values of milk pH and EC were observed in rainy season than summer and winter which may be due to more stress experienced by the animals during hot humid rainy season. These results are in agreement with Syridion *et al.* (2014). The values of milk pH and EC was significantly ($P<0.05$) decreased in healthy cows than subclinical and clinical

Table 1. Effect of different season on milk inflammatory parameters in healthy and mastitis infected Sahiwal cows

Season	Healthy	Subclinical	Clinical
<i>Milk pH</i>			
Winter	$6.41^a\pm0.07$	$6.64^b\pm0.09$	$6.90^c\pm0.07$
Summer	$6.65^a\pm0.02$	$6.80^b\pm0.07$	$7.01^c\pm0.02$
Rainy	$6.75^a\pm0.02$	$6.91^b\pm0.01$	$7.04^c\pm0.04$
<i>Electrical Conductivity (mS/cm)</i>			
Winter	$4.26^a\pm0.04$	$4.70^b\pm0.05$	$4.85^b\pm0.13$
Summer	$4.30^a\pm0.02$	$4.93^b\pm0.10$	$5.74^c\pm0.19$
Rainy	$4.48^a\pm0.06$	$5.01^b\pm0.06$	$5.79^c\pm0.10$
<i>Somatic cell counts ($\times 10^5$)</i>			
Winter	$0.50^{aA}\pm0.02$	$3.50^{bA}\pm0.1$	$13.14^{cA}\pm4.20$
Summer	$1.23^{aB}\pm0.09$	$3.52^{bA}\pm0.17$	$17.28^{cB}\pm4.05$
Rainy	$1.52^{aC}\pm0.06$	$3.98^{bB}\pm0.32$	$19.04^{cC}\pm1.18$

Superscripts in small letters within rows differ significantly from each other ($P<0.05$). Superscripts in capital letters within column differ significantly from each other ($P<0.05$).

mastitis cows which may be due intra-mammary infection. Milk SCC values ranged from 0.50 ± 0.02 to 19.04 ± 1.18 lakhs among all the seasons (Table 1). Milk SCC increased significantly ($P<0.05$) from winter to summer season followed by rainy season. The results are also in consonance of Khate and Yadav (2010), Sharma *et al.* (2012), Purohit *et al.* (2014) who reported that the somatic cells are generally higher in rainy season followed by summer season and found lowest during the winter season. The higher values of SCC in summer in their experiment may be due to the increased number of environmental bacteria in the bedding material because of favorable temperature and humidity which might have contributed to an increased infection rate in summer thereby causing increased SCC than other seasons.

Effect of stages of lactation: Among different stages of lactation, the value of milk pH ranged between 6.45 ± 0.04 to 7.05 ± 0.02 (Table 2). Higher values of milk pH was found

Table 2. Effect of stages of lactation on milk inflammatory parameters in healthy and mastitis infected Sahiwal cows

Lactation	Healthy	Subclinical	Clinical
<i>pH</i>			
Early	$6.62^a\pm0.06$	$6.78^b\pm0.05$	$6.99^c\pm0.02$
Mid	$6.45^a\pm0.04$	$6.77^b\pm0.04$	$7.01^c\pm0.02$
Late	$6.66^a\pm0.02$	$6.88^b\pm0.02$	$7.05^c\pm0.02$
<i>Electrical conductivity (mS/cm)</i>			
Early	$4.34^a\pm0.04$	$4.87^b\pm0.07$	$5.49^c\pm0.08$
Mid	$4.31^a\pm0.05$	$4.98^b\pm0.08$	$5.66^c\pm0.11$
Late	$4.49^a\pm0.05$	$4.92^b\pm0.05$	$5.98^c\pm0.13$
<i>Somatic cell counts ($\times 10^5$)</i>			
Early	$0.08^a\pm0.09$	$3.27^b\pm0.19$	$18.50^c\pm3.37$
Mid	$1.32^a\pm0.12$	$3.62^b\pm0.13$	$17.81^{cA}\pm2.12$
Late	$1.48^a\pm0.07$	$3.84^b\pm0.11$	$27.51^{cB}\pm1.35$

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in late lactation than early and mid-lactation in healthy and infected Sahiwal cows. These differences were statistically non-significant. On the other hand, pH significantly ($P<0.05$) increased from healthy to subclinical and clinical mastitis. This change in pH might be due to reduced ability of mammary gland to produce bicarbonate ions which provides hydroxyl ions to absorb hydrogen ions (Aurelia *et al.* 2009). The value of EC ranged between 4.34 ± 0.04 to 5.66 ± 0.11 in different stages of lactation (Table 2). In early and mid-lactation, there was no specific pattern found but higher values were found in late lactation. The result of SCC were in similar tune with EC and varied significantly ($P<0.05$) between healthy (0.08 ± 0.09) and clinical mastitis (27.51 ± 1.35) irrespective of stage of lactation (Table 2). During late lactation, higher values were obtained in all the groups than early and mid-lactation. Sharma *et al.* (2011) reported that milk SCC increases with progressing lactation regardless of whether the cow is infected or not. The results of the present study also showed a similar pattern of SCC with the change in the stage of lactation (De and Mukherjee 2009)

Effect of parities: Among different parities, values of milk pH and EC ranged from 6.27 ± 0.07 to 7.08 ± 0.03 to 4.28 ± 0.11 to 6.40 ± 0.24 respectively (Table 3). Although no significant trend was found for milk pH and EC but higher values were found in 3rd parity among all the parities. The values of pH and EC were significantly ($P<0.05$) lower in healthy and subclinical cases than clinical cases in different parities. Reports from earlier studies also showed that, there is an increase in the EC as the advancement in the stage of lactation (Boas *et al.* 2017). In our study, older cows (≥ 4 parity) showed similar results which may be due to a higher frequency of infection in older cows. The values of SCC ranged between 1.29 ± 0.09 to 23.81 ± 1.72 lakhs

Table 3. Effect of different parity on milk inflammatory parameters in healthy and mastitis infected Sahiwal cows

Parities	Healthy	Subclinical	Clinical
<i>pH</i>			
Primiparous	$6.68^a\pm 0.06$	$6.85^b\pm 0.02$	$6.98^c\pm 0.05$
2 nd	$6.56^a\pm 0.02$	$6.76^b\pm 0.05$	$6.94^c\pm 0.04$
3 rd	$6.71^a\pm 0.01$	$6.91^b\pm 0.05$	$7.08^c\pm 0.03$
4 th and above	$6.27^a\pm 0.07$	$6.76^b\pm 0.05$	$7.01^c\pm 0.01$
<i>Electrical conductivity (mS/cm)</i>			
Primiparous	$4.28^a\pm 0.11$	$4.79^b\pm 0.05$	$5.27^c\pm 0.15$
2 nd	$4.31^a\pm 0.06$	$4.77^b\pm 0.10$	$5.25^c\pm 0.13$
3 rd	$4.44^a\pm 0.03$	$5.31^b\pm 0.22$	$6.40^c\pm 0.24$
4 th and above	$4.39^a\pm 0.05$	$4.93^b\pm 0.04$	$5.71^c\pm 0.07$
<i>Somatic cell counts ($\times 10^5$)</i>			
Primiparous	$1.29^a\pm 0.09$	$3.33^b\pm 0.13$	$10.44^c\pm 1.07$
2 nd	$1.30^a\pm 0.11$	$3.48^b\pm 0.18$	$21.55^c\pm 2.24$
3 rd	$1.46^a\pm 0.08$	$3.46^b\pm 0.17$	$29.76^b\pm 4.0$
4 th and above	$1.49^a\pm 0.06$	$3.71^a\pm 0.11$	$23.81^b\pm 1.72$

Superscripts in small letters within rows differ significantly from each other ($P<0.05$). Superscripts in capital letters within column differ significantly from each other ($P<0.05$).

Table 4. Effect of milk production on milk inflammatory parameters in healthy and mastitis infected Sahiwal cows

Milk yield (kg)	Healthy	Subclinical	Clinical
<i>pH</i>			
0–4	$6.60^a\pm 0.03$	$6.81^b\pm 0.04$	$6.99^c\pm 0.02$
5–10	$6.40^a\pm 0.07$	$6.78^b\pm 0.04$	$7.02^c\pm 0.02$
>10	$6.51^a\pm 0.09$	$6.86^b\pm 0.07$	$7.05^c\pm 0.03$
<i>Electrical conductivity (mS/cm)</i>			
0–4	$4.52^a\pm 0.02$	$5.00^b\pm 0.09$	$5.91^c\pm 0.10$
5–10	$4.41^a\pm 0.03$	$4.92^b\pm 0.06$	$5.45^c\pm 0.08$
>10	$4.27^a\pm 0.05$	$4.82^b\pm 0.05$	$5.47^c\pm 0.09$
<i>Somatic cell counts ($\times 10^5$)</i>			
0–4	$1.75^a\pm 0.06$	$3.71^a\pm 0.17$	$26.79^b\pm 1.94$
5–10	$1.47^a\pm 0.06$	$3.40^a\pm 0.08$	$19.69^b\pm 1.41$
>10	$1.22^a\pm 0.08$	$3.71^a\pm 0.25$	$14.70^b\pm 1.30$

Superscripts in small letters within rows differ significantly from each other ($P<0.05$). Superscripts in capital letters within column differ significantly from each other ($P<0.05$).

among different parities (Table 3). Higher values were noticed at clinical stage rather than subclinical and healthy Sahiwal cows but no significant difference found in milk SCC among different parities. Similar results were observed by Kavitha *et al.* (2009) also.

Effect of milk production: The effect of milk production on the milk pH, EC and SCC is presented in Table 4. The value of milk pH and EC ranged between 6.40 ± 0.07 to 7.05 ± 0.03 and 4.27 ± 0.05 to 5.91 ± 0.10 respectively among all milk production groups. Milk yield did not have a statistically significant influence on the milk pH and EC in healthy, subclinical and clinical mastitis. In all the levels of milk production, the clinical mastitis group showed a marked increase in milk pH over subclinical group. The alteration in milk pH can be explained by altered bicarbonate concentration which occurs in animals showing different levels of milk production. The altered bicarbonate concentration is somehow related to the metabolic activity of the cow and thus related to the production level of the cow. The finding in the present study is in agreement with Boas *et al.* (2017).

The results of SCC (1.22 ± 0.08 to 26.79 ± 1.94) varied significantly among the healthy, subclinical and clinical mastitis cases irrespective of milk production. Higher SCC concentrations were associated with lower milk production both in healthy, subclinical and clinical cases. In the lowest producing group, the SCC was highest in healthy and clinical cases than other milk yield group. The results of SCC are consistent with the results of similar previous studies concerning a negative relationship between SCC and milk yield during lactation (Vida *et al.* 2004). The marginal but significant increase of SCC in subclinical mastitis and significant increase in clinical mastitis compared to non-infected animals; suggests that diagnosis of subclinical mastitis using SCC will give more accurate results provided the SCC data of clinical mastitis accounted

for analysis (Hagnestam-Nielsen *et al.* 2009). Moreover, to rule out the physiological role for increasing SCC from subclinical mastitis, there is need for strict threshold for SCC based on Indian condition in order to use as diagnostic indicator.

Correlation between milk quality parameters: In the present study, the coefficient of correlation between pH, electrical conductivity and somatic cell counts was determined. A significant positive correlation ($P < 0.01$) was observed between SCC and EC ($r = 0.679$). A moderate ($r = 0.526$) but significant correlation was observed in pH and EC. The association between pH and SCC was low ($r = 0.411$) in present study. Similar results were reported by Ogola *et al.* (2007), Daunoras and Knys (2008) and Panchal *et al.* (2016b).

In view of the above results, it can be concluded that that pH, electrical conductivity and somatic cell counts significantly increased from healthy to subclinical and clinical mastitis. SCC is significantly influenced by season, stages of lactation and milk yield whereas parity does not have any effect on SCC. Positive correlation between milk pH, EC and SCC has the potential of combined usage in diagnosis of inflammation in the udders of Sahiwal cows. Therefore, these parameters need to be considered along with effective management strategies to improve the quality of milk.

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