The Haryana Veterinarian (December, 2023) **62(2),** 68-71 ISSN 0033-4359 (P) **Research Article**

PREDICTING UDDER HEALTH IN GIR COWS USING MILK COMPONENTS: AN ROC APPROACH

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ABSTRACT

The aim of this study was to predict udder health using Receiver Operating Characteristics (ROC) analysis of milk components such as milk fat, solid not fat (SNF), protein, lactose and ash content. Quarter milk samples (n=1160) were collected from apparently healthy animals and screened for subclinical mastitis (SCM) using California Mastitis Test (CMT) and Somatic Cell Counts (SCC) as a gold standard test. Milk samples were analysed for milk components such as milk fat, SNF, protein, lactose and ash percent. Result showed 15.93, 15.63 and 16.05% SCM quaters in Gir cows during early, mid and late lactation phase, respectively. Significant reduction in milk SNF, protein and lactose were observed in SCM during early, mid and late lactation phase. The ROC analysis of milk components indicated that SCM of quarter could be predicted using milk components but with low accuracy. Among different milk components, the area under ROC curve to differentiate healthy and SCM quarters was highest for milk lactose (AUC=0.681, $p<0.001$) in early lactation. Taken together it may be concluded that milk lactose could be used as a biomarker and non-invasive tool to predict udder health status in Gir cows.

Keywords: Gir cows, Milk components, ROC analysis, Udder health

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Mastitis is considered as one of the important production diseases which causes huge economic losses to the dairy industry i.e. US$ 2.0 billion and 98.2 billion in USA and India, respectively (Krishnamoorthy *et al.*, 2021). Losses due to mastitis are comparatively higher in subclinical form than the clinical form as the former is difficult to detect and accounts 57.9% of the total economic loss due to mastitis (Panchal *et al.*, 2016). Major economic loss owing to subclinical mastitis (SCM) in dairy cattle occurs due to production loss (43.95%) followed by veterinary expenses (36.54%) (Sinha *et al.*, 2014). Krishnamoorthy *et al.* (2021) reported that global prevalence of SCM is 42% whereas in India is 45%, and it is higher in cattle than buffaloes (49 vs. 32%). The SCM also affects the raw milk constituents, which affects dairy farmers, producers and consumers as far as milk quality and price is concerned. Alteration of milk constituents may occur due to damage of mammary gland epithelium or blood milk barrier (Reis *et al.*, 2013) during inflammation. Lactose is considered as most variable milk constituent during udder inflammation compared to other constituents but there was inconsistent effect of SCM on other milk constituents like fat, protein, solid not fat (SNF), ash and total solid contents in dairy bovines (Bansal *et al.*, 2005; Reis *et al.*, 2013; Bagri *et al.*, 2018). Therefore, early prediction of subclinical mastitis in dairy animals could reduce economic losses and improve milk quality.

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Receiver operating characteristic (ROC) analysis is an important statistical tool used for evaluation of diagnostic biomarkers to classify healthy and diseased state of animals based on area under curve. The ROC analysis is also used to develop optimum threshold value of diagnostic biomarkers along with the corresponding sensitivity, sensitivity and likelihood ratio (Patbandha *et al.*, 2016). The present study aimed to predict udder health in Gir cows using ROC analysis of milk components such as milk fat, SNF, protein, lactose and ash content.

MATERIALS AND METHODS

The present study was conducted on Gir cows maintained under loose housing system at Cattle Breeding Farm, Junagadh, Gujarat, India. A subtropical dry to sub-humid climate prevails in the study area and receives average annual rainfall of 625-750 mm. Ambient temperature in the study area ranges from 10º C (winter) to 40º C (summer). All managerial practices were followed uniformly throughout the year to reduce the environmental effect. The cows were fed measured quantity of seasonal green (@10 kg/cow/head) and ad-lib dry fodder. Mixture of cottonseed cake, ground maize and Amul dan was offered during milking to fulfill the nutrient requirement as per Indian Council of Agricultural Research (ICAR) feeding standard (ICAR, 2013). Chopped green fodder was distributed twice a day (10-11 am during morning and 04-05 pm during afternoon). Cows were milked by hand

milking twice a day at 4.00 am and 4.00 pm and calves were allowed to suckle for milk let down.

Milk samples were collected from each quarter of apparently healthy animals at afternoon milking and milk compositions (fat, SNF, protein, lactose and ash) were analyzed immediately, using automatic milk analyzer 'Lactoscan' (New Dairy Engineering and Trading Company Pvt. Ltd., Delhi, India). Samples were also tested for SCM by California Mastitis Test (CMT) and Somatic Cell Counts (SCC) microscopically using modified Newman's stain. The milk samples which were CMT positive and had SCC between 2-5 lakh cells/ml (Dang *et al.*, 2008; Ebrahimie *et al.*, 2018) were considered as SCM.

Statistical Analysis

Milk composition of 1160 quarter milk samples (452, 384 and 324 samples, respectively from early, mid and late lactation) were used for analysis. Prevalence of SCM was carried out by chi-square test. The milk components from healthy and infected quarters' milk samples were analyzed by using t-test. The milk components that showed significant difference ($p \leq 0.05$) between normal and infected quarters were further analysed by Receiver Operating Characteristics (ROC) analysis to differentiate healthy and infected quarters and to develop optimum threshold values using Sigmaplot (version 11).

RESULTS AND DISCUSSION

Sub-clinical mastitis (SCM) incidence

In Gir cows, out of 1160 quarters, 184 (15.86%) were observed positive for subclinical mastitis (SCM). The prevalence of SCM was 15.93% (72/452), 15.63% (60/384) and 16.05% (52/324), respectively during early, mid and late lactation phase. However, the occurrence of SCM was observed similar throughout the lactation period ($\chi^2 = 0.026$; df = 2; p = 0.986). The overall prevalence of SCM (15.86%) observed in this study is contradictory to Modh *et al.* (2017), who reported 27.83% quarter affected with SCM in Gir cows in Gujarat. Higher quarter wise prevalence of SCM was also reported in HF crossbred (31.16%, Sharma *et al.*, 2018) and Jersey crossbred (54.65%, Pranay *et al.*, 2017). Moreover, in indigenous Kangayam cattle quarter wise prevalence of SCM was 20.5% SCM (Manokaran *et al.*, 2020). This indicates that indigenous cattle are less prone to udder infection. Gir cattle, is an important zebu cattle (*Bos indicus*), well known for heat tolerant and disease resistance capacity due to unique inherent physical characteristics (Patbandha *et al.*, 2020). The animals were also maintained under optimum nutritional and managemental practices; this might be attributed to lower prevalence of SCM in this study.

Milk components

The milk components of SCM and healthy milk samples during early, mid and late lactation are depicted in Table 1. In early lactation, there were significant reduction of milk SNF ($p=0.008$), protein ($p=0.041$), lactose ($p<0.001$) and ash ($p=0.007$) content in milk samples from SCM udder quarters as compared to healthy quarters. However, milk fat content was not affected by udder health status ($p=0.065$). A significant reduction of milk SNF ($p<0.001$), protein ($p=0.004$), lactose ($p<0.001$) and ash ($p=0.001$) percent was observed in mid lactation in SCM quarter milk samples. Further, significant reduction in milk SNF ($p=0.031$), protein ($p=0.017$) and lactose ($p=0.037$) percent was recorded in SCM milk samples during late lactation. Reis *et al.* (2013) observed significant effect of SCM on milk lactose, SNF and total solid contents in Brazilian Gir cows. However, milk fat and protein content were unaffected. In another study on Brazilian Gir cows by Araujo *et al.* (2018) reflected lower milk lactose in SCM condition than the healthy quarters; but other milk traits (fat, protein and total solids) remained similar. In present study, there were lower milk SNF, protein, lactose and ash content during early and mid lactation; whereas lower milk SNF, protein and lactose during late lactation in SCM quarters as compared to healthy quarters. In crossbred cows, Bagri *et al.* (2018) observed significant reduction of milk fat, protein, lactose, SNF and TS in lactating cows affected with SCM than healthy cows. Bansal *et al.* (2005) reported significant reduction of milk lactose in dairy cows affected with SCM compared to healthy cows, but such effect was not observed in other traits like fat and protein. This reflected that among different major milk constituents, lactose is the single trait that reduced markedly and consistently during udder infection in dairy cows. Thus lactose could be used as biomarker for identification of udder health in dairy cows.

Area Under Curve (AUC)

The area under curve (AUC, reflects the accuracy to differentiate healthy quarters from infected quarters) for the milk components are presented in Table 2. The AUC ranged from 0.614 to 0.681 and from 0.627 to 0.649, respectively for different milk components in early and mid lactation phase. Comparison of ROC curve area indicated that discrimination power of milk lactose was significantly higher than milk SNF ($\chi^2 = 7.228$; df=1; $p<0.001$), protein ($\chi^2 = 4.662$; df=1; $p<0.001$) and ash ($\chi^2 =$

Table 1. Milk composition of healthy and subclinical mastitic (SCM) udder quarters of Gir cows

Infection status	Fat (%)	SNF (%)	Protein (%)	Lactose (%)	Ash (%)
			Early lactation		
Healthy (n=380)	4.118±0.031	8.664±0.041	3.177±0.016	4.767±0.023	0.706±0.004
SCM (n=72)	3.975±0.074	8.382±0.116	3.090±0.048	4.521±0.064	0.682±0.009
p-value	0.065	0.008	0.041	<0.001	0.007
			Mid lactation		
Healthy (n=324)	4.034±0.034	8.599±0.050	3.138±0.018	4.705±0.024	0.704±0.004
SCM (n=60)	3.920±0.098	8.153±0.152	3.001±0.054	4.461±0.081	0.666±0.013
p-value	0.208	<0.001	0.004	<0.001	0.001
			Late lactation		
Healthy (n=272)	4.031±0.037	8.471±0.049	3.149±0.022	4.660±0.027	0.694±0.005
SCM (n=52)	3.963±0.085	8.202±0.127	3.020±0.046	4.514±0.076	0.675±0.011
p-value	0.468	0.031	0.017	0.037	0.096

Table 2. Area under curve, threshold value, sensitivity, specificity and likelihood ratio of milk components

Parameters	Area Under Curve	*P*-value	Threshold values	Sensitivity (%)	Specificity (%)	LR+
			Early lactation			
Milk SNF (%)	0.624±0.038 (0.548-0.698)	<0.001	< 8.615	69.44 (57.47-79.76)	58.42 (53.28-63.43)	1.67
Protein (%)	0.628±0.038 (0.552-0.704)	<0.001	< 3.135	66.67 (54.57-77.34)	60.53 (55.41-65.47)	1.68
Lactose (%)	0.681±0.037 (0.608-0.754)	<0.001	< 4.685	72.22 (60.41-82.14)	62.11 (57.02-67.00)	1.90
Ash (%)	0.614±0.037 (0.540-0.787)	0.002	< 0.705	69.44 (57.47-79.76)	56.84 (51.69-61.88)	1.61
			Mid lactation			
Milk SNF (%)	0.637±0.042 (0.553-0.719)	<0.001	< 8.515	66.67 (53.31-78.31)	54.94 (49.34-60.44)	1.47
Protein (%)	0.628±0.041 (0.547-0.709)	0.001	< 3.100	55.00 (41.61-67.88)	60.49 (54.94-65.85)	1.39
Lactose (%)	0.649±0.041 (0.568-0.730)	<0.001	< 4.540	51.67 (38.39-64.77)	69.14 (63.79-74.12)	1.67
Ash (%)	0.627±0.042 (0.545-0.709)	0.001	< 0.685	51.67 (38.39-64.77)	59.26 (53.69-64.66)	1.26
			Late lactation			
Milk SNF (%)	0.547±0.042 (0.464-0.629)	0.282	< 8.425	51.92 (37.63-65.99)	50.00 (43.90-56.10)	1.03
Protein (%)	0.567±0.042 (0.483-0.650)	0.125	< 3.125	59.62 (45.10-72.99)	46.32 (40.28-52.45)	1.11
Lactose (%)	0.541±0.041 (0.459-0.623)	0.344	< 4.598	44.23 (30.47-58.67)	56.62 (50.50-62.59)	1.02

LR = Likelihood ratio; Values within parenthesis indicates 95% Confidence Interval

7.963; df=1; p<0.001) content during early lactation. However, the discrimination power of different milk components did not vary during mid and late lactation phase (p>0.05). The AUC for milk lactose observed in Gir cows (AUC = 0.681, p<0.001 and 0.649, p<0.001, respectively during early and mid-lactation) is comparable with Patbandha *et al.* (2016) who reported AUC of milk lactose as 0.64-0.72 for classification of SCM quarters from healthy quarters in Jaffrabadi buffaloes. Previous studies also assessed the discrimination power of milk components particularly milk lactose and SNF to differentiate healthy and infected quarters in dairy cows (Pyorala, 2003; Bansal *et al.*, 2005) and buffaloes (Bansal *et al.*, 2007; Patbandha *et al.*, 2016). The discrimination power of milk lactose ranged from 73.9 to 80.85% in cows (Pyorala, 2003; Bansal *et al.*, 2005) and 64.0 to 83.7% in buffaloes (Bansal *et al.*, 2007; Patbandha *et al.*, 2016). In Gir cows, the discrimination power of milk lactose was comparatively higher than other traits (SNF, protein and ash), which agrees with Bansal *et al.* (2007). The AUC is considered as less accurate to classify healthy and diseased state if AUC is 0.5-0.7 (Patbandha *et al.*, 2016); hence in Gir cows, milk components (AUC = 0.614-0.681) were less accurate to differentiate infected quarters from healthy quarters.

Threshold value

The optimum threshold values of different milk components for identification of udder health are presented in Table 2. Milk lactose is considered as the most important negative indicator of mastitis, which decreases consistently with increase in severity of infection (Ebrahimie *et al.*, 2018). That's why majority of

researchers estimated threshold value of milk lactose for early identification of mastitis in dairy bovines. The threshold value of milk lactose has been reported to be 4.7g% for dairy cattle by several authors as a biomarker for SCM (Pyorala, 2003; Televicius *et al.*, 2021). However, Bansal *et al.* (2005) observed threshold value of milk lactose 4.85 g% in German Black Pied cows for identification of SCM. In Gir cows the optimum threshold observed was 4.68 g% (≈ 4.7g%) during early lactation, indicates that like other studies this could be used as biomarker for identification of udder health. On the other hand, in mid lactating cows, optimum threshold value of milk lactose was 4.54 g% comparatively lower than the other studies. Bansal *et al.* (2007) reported threshold value for milk SNF as 9.5 g% for SCM in buffaloes. In Gir cows, during early and late lactation the threshold values of SNF for SCM were 8.6 g% and 8.5 g%, respectively. In early lactating cows, udder quarters having milk lactose below 4.68% were 1.9 times more likely to be infected with SCM (LR+ = 1.9). But, in mid lactating cows, udder quarters having milk lactose below 4.54% were 1.67 times more likely to be affected with SCM (LR+ = 1.67). In a similar line, Televicius *et al.* (2021) reported that cows with milk lactose < 4.7% had 15 times higher chances of SCM than the cows with lactose concentration above this level (p = 0.001).

CONCLUSIONS

The results of the present study showed significant reduction in milk components particularly milk SNF, protein and lactose in SCM. The discrimination power was highest for milk lactose to differentiate healthy and SCM quarters compared to other milk components. Taken together it may be concluded that milk lactose could be used as a quick and non-invasive bio marker to predict udder health status in Gir cows.

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