

Influence of Udder Morphological Traits on Milk Parameters in Healthy Sahiwal and Crossbred Cattle

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ABSTRACT

Ten each healthy lactating Sahiwal and crossbred cows were selected from the University Instructional Dairy Farm. Milk samples were collected without any preservative from 3 lactation stages as early (30-90±15 days), mid (100-180±15 days) and late (200-280±15 days). Milk composition was determined by using Ultrascan milch analyser, and enumeration of milk somatic cell and differential leucocyte counts by modified May-Grünwald and Giemsa staining techniques. Udder morphological traits of mammary teat shape (cylindrical, conical, bottle) and udder shape (round, bowl, trough) were done before milking. Test day milk yield was recorded highest in round shaped followed by bowl and least in trough shape udder. Round udder and cylindrical teat shape had significantly ($p<0.05$) higher milk somatic cell count and least in trough udder and conical teat shape. Cylindrical teat shape had significantly ($p<0.05$) higher milk neutrophil % in both cattle, while higher milk lymphocyte % in bottle teat shape of Sahiwal and conical teat shape of crossbred cows. Significant ($p<0.05$, $p<0.01$) positive correlations were observed between udder circumference, width, depth and teat apex, mid diameter with milk SCC; udder circumference, width, depth and teat length, apex, mid, base with milk neutrophil; udder height from the ground with milk lymphocyte in crossbred cattle. In Sahiwal cattle, significant positive correlations were noted only between udder circumference, depth and teat base with milk SCC. It was concluded that udder morphology has a significant impact on protection of mammary gland against intra-mammary infections and maintain udder health in lactating dairy cows.

Key words: Dairy cattle, Lactation, Milk, Udder morphology

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INTRODUCTION

Dairy cattle provide a livelihood to dairy farmers in terms of milk yield and milk products, and also as security to dairy farmers at times of natural calamities and crop failure. Crossbred cattle are known for higher milk yield and shorter calving interval than indigenous milch breed (Wakchaure *et al.* 2015). Sahiwal cattle originated from Sahiwal district of Punjab province of Pakistan, possess excellent endurance to hot climate of tropics and subtropics, resistance to tropical diseases, requires lesser inputs, and have better feed conversion efficiency (Khan and Mirza, 2014).

The major components of milk are proteins and peptides, lipids, and complex carbohydrates, somatic cells consisting mostly of blood leucocytes and few epithelial cells, which slough off from the lining of the mammary alveoli during milking. Milk somatic cell count indicates milk quality (Sharif and Muhammad, 2008), normal udder health (Huozha *et al.* 2022) and mammary gland infection causing mastitis (Barkema *et al.* 1999). Udder morphology is said to affect milk composition of lactating dairy cow. In view of the above facts, this study was undertaken to investigate the udder morphological traits influencing the milk parameters in healthy Sahiwal and crossbred cattle.

MATERIALS AND METHODS

The present study was carried out following approval by Institutional Animal Ethical Committee (IAEC/CVASc/

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VPB/433) in the Instructional Dairy Farm (IDF), Nagla of College of Veterinary and Animal Sciences, Pantnagar, GBPUAT, Udham Singh Nagar, Uttarakhand, India. Geographically the area is lying in Northern upper Gangetic plains of Tarai region in the foothills of Himalayas (344 m above the mean sea level, 79°E longitude, 29°N latitude). The climatic condition during summer to rainy seasons is hot-humid with short spring season.

Selection of Experimental Animals

The animals for this study were selected based on the records maintained in the institute farm. Ten each healthy lactating Sahiwal (300±10 kg BW) and crossbred (HF x Sahiwal cross, 350±10 kg BW) cows in lactation order of second and third were selected from the herd. The average lactation length was about 300±15 days in Sahiwal and 380±15 days in crossbred cattle. Milk samples were collected from these cows according to their lactation in early (30-90±15 days), mid (100-180±15 days) and late (190-280±15 days) stages. The animals were kept in open semi-intensive system, allowed *ad libitum* water supply and green fodders with concentrates as normal dietary regime followed for lactating cows.

Milk Collection and Analysis

Selected Sahiwal and crossbred lactating cows were milked twice (morning and evening) during each lactation stage. Approximately 30 mL milk was collected from all the four quarters in a sterile centrifuge tube (50 mL) without any preservative added and with due hygienic measures. All the milk parameters were analysed within 4 h after collection. Milk composition was measured by using Ultra Scan Milch Analyser machine (SWIFT, EU), where milk total solid (%) was calculated by using formula,

$$TS (\%) = \frac{CLR}{4} + 1.21F + 0.36$$

Where, F is the fat content of the milk, CLR is corrected lactometer reading.

Enumeration of Milk Somatic and Differential Leucocyte Cells

Each milk sample was smeared in duplicate on clean micro-slide. Milk somatic cell count (SCC) was enumerated microscopically according to the method described by Dang *et al.* (2008).

Milk differential leucocyte count (DLC) was done in the same smears in 50 microscopic fields. One hundred leucocytes (macrophages, neutrophils and lymphocytes) were counted (Dang *et al.*, 2008), along with few epithelial cells which were identified as large, non-nucleated, irregularly shaped and homogeneously lighter blue stained (Dosogne *et al.*, 2003). Each differential leukocyte cell was expressed in percentage.

DLC of a specific cell type (%) = (No of that specific cell / Total no of cells) × 100

Udder Morphology of Lactating Cows

Initially the mammary glands were screened for any abnormal gross lesions. After visual examinations, morphological traits for both udder and teat were measured before milking in each lactating cow. Each cow was put to standing position on level ground during measurements and observations were recorded once in the mid of each lactation stage using measuring tape by the same person to avoid between-recorder effects.

Udder morphological traits were recorded as per the method of Bhutto *et al.* (2010). Teat shapes included pointed, cylindrical, round and flat; while udder shape included cup, round, bowl and trough as depicted in Fig.1. Teat length and teat diameter were measured using Digital Vernier calliper (Aero space) for each quarter. Shortest distance from front and rear teat end to floor was measured using measuring tape.

Statistical Analysis

One-way analysis of variance (ANOVA) was applied for milk composition, somatic cells and differential leucocyte counts during lactation stages, to know the effect of udder morphology. Pearson's correlations between udder morphological traits and milk parameters were performed. All these analyses were done using IBM® SPSS® Version 20 Software.



Fig. 1: Various udder shapes of Sahiwal and crossbred cattle

RESULTS AND DISCUSSION

In our institute farm herd, the average milk yield recorded per lactation for Sahiwal cows was 1665 kg with an average lactation length of 300±15 days and for crossbred cows 4266 kg with an average lactation length 380±15 days. Peak average milk yield recorded was 11.6 kg/day in Sahiwal and 16.5 kg/day in crossbred cattle. Generally, cow milk yield followed a typical lactation curve which provides valuable information of persistency in milk yield (Grossman *et al.* 1999) and biological efficiency of each lactating cow (Scott *et al.* 1996). Mean test-day milk yield (TDMY) recorded during lactation in Sahiwal cows was 5.573±0.28 kg, and in crossbreds 10.920±0.67 kg, which differed significantly ($p<0.05$). The lactation stage-wise TDMY of Sahiwal cows was statistically similar, while in crossbreds it gradually and significantly ($P<0.001$) decreased from early, mid to late lactation stage (Table 1). Present TDMY recorded compared well with Sahib *et al.* (2019) and Bahiram *et al.* (2020) for both the cattle breeds.

Milk Composition during Lactation Stages

Sahiwal cattle had significantly ($p<0.05$) higher milk fat and total solids percent than that of crossbred cattle. Milk fat and TS percent significantly increased in late lactation stage as compared to early and/or mid lactation stage in both pure and crossbred cattle (Table 1). These findings concurred with the reports of Bhoite and Padekar (2002) and Talukdar *et al.* (2013). SNF, protein and lactose content of Sahiwal milk showed a gradual and significant rise from early to late lactation stage, but no such trend was noted in crossbreds. Besides breed differences, external factors and normal physiological state during lactation period may influence the milk synthesis and composition in dairy cows.

Milk Somatic Cell and Differential Leucocyte Counts

Milk somatic cell count ($\times 10^5/\text{ml}$) of lactating cows was significantly ($p<0.05$) higher in crossbreds than Sahiwal cattle, and it was also significantly higher in early than mid lactation in both the breeds (Table 1). Our findings agreed with Mukherjee *et al.* (2013) and Rizwana *et al.* (2021), who also opined that somatic cells are normally present as minor part of milk components. Cow's milk SCC numbers varied with milk yield and onset of lactation, and was linked to innate immune response during calving and lactation (Mukherjee *et al.*, 2013). Milk SCC is excellent indicator for both intra-mammary infections (Bansal *et al.*, 2007) and udder health (Huozha *et al.*, 2022). Milk SCC in normal range is used as international standard in measuring milk quality (Sharif and Muhammad, 2008).

Amongst the milk differential leucocytes, the neutrophils were significantly ($p<0.05$) higher and lymphocytes were lower in Sahiwal than the crossbred cows, and there was a trend of significant decrease in neutrophil count, and increase in lymphocyte count in both the breeds. Further, the macrophage showed a significant rise from early to mid lactation only in Sahiwal cows, but not in crossbred cows (Table 1). In an earlier study, similar predominant milk leucocytes, *viz.*, lymphocyte, macrophage and neutrophil percents were enumerated in Karan Fries crossbred cows (Alhussien *et al.*, 2016). Moreover, the results of Sahiwal cattle agreed to Swain *et al.* (2015) though their neutrophil percent was lower. These milk leucocytes are involved in immune response and performed active phagocytic activities against micro-organisms in lactating cows (Klaas *et al.*, 2004).

Effect of Udder & Teat Morphology on various Milk Parameters

Udder and teat shapes observed in Sahiwal and crossbred cattle along with their milk parameters are depicted in

Table 1: Milk parameters during different lactation stages in Sahiwal and crossbred cows

Milk parameters	Breed and Lactation stages							
	Sahiwal cattle			P Value	Crossbred cattle			
	Early	Mid	Late		Early	Mid	Late	P Value
TDMY (kg/day)	5.624 ±0.57	5.336 ±0.58	4.860 ±0.24	NS	13.60 ±0.86 ^c	9.600 ±0.74 ^b	6.560 ±0.52 ^a	<0.001
Fat (%)	4.570 ±0.24 ^a	4.800 ±0.21 ^{ab}	5.340 ±0.17 ^b	0.04	3.810 ±0.22 ^a	3.990 ±0.17 ^a	4.955 ±0.28 ^b	0.028
SNF (%)	8.540 ±0.10 ^a	8.900 ±0.07 ^b	9.030 ±0.07 ^b	0.001	8.930 ±0.21	8.750 ±0.14	8.889 ±0.12	NS
Protein (%)	3.130 ±0.04 ^a	3.240 ±0.04 ^{ab}	3.320 ±0.03 ^b	0.005	3.270 ±0.08	3.210 ±0.05	3.273 ±0.05	NS
Lactose (%)	4.690 ±0.05 ^a	4.840 ±0.05 ^{ab}	4.960 ±0.05 ^b	0.004	4.910 ±0.12	4.790 ±0.08	4.839 ±0.06	NS
Total Solids (%)	13.06 ±0.37 ^a	13.63 ±0.27 ^{ab}	14.52 ±0.26 ^b	0.003	12.70 ±0.29 ^a	12.73 ±0.27 ^a	13.97 ±0.35 ^b	0.009
Milk SCC ($\times 10^5/\text{ml}$)	1.334 ±0.09 ^a	0.802 ±0.15 ^b	1.119 ±0.11 ^{ab}	0.012	1.970 ±0.09 ^a	1.445 ±0.18 ^b	1.796 ±0.11 ^{ab}	0.029
Neutrophil (%)	42.89 ±1.0 ^{0a}	31.04 ±1.2 ^{0b}	33.17 ±0.96 ^b	<0.001	27.87 ±0.8 ^{6a}	27.02 ±1.3 ^{1ab}	23.55 ±1.33 ^b	0.029
Macrophage (%)	14.83 ±0.5 ^{0a}	24.20 ±1.2 ^{4b}	19.07 ±1.06 ^c	<0.001	19.85 ±0.88	17.70 ±1.15	16.45 ±1.56	NS
Lymphocyte (%)	42.25 ±0.9 ^{6a}	44.75 ±1.2 ^{8ab}	47.76 ±2.05 ^b	0.039	52.27 ±1.6 ^{6a}	55.47 ±1.7 ^{8ab}	59.99 ±2.51 ^b	0.027

*Mean ± SE values with alphabetical superscript (a, b) in a row within breed differ significantly ($p<0.05$).

TDMY-test day milk yield, SNF-solid-non-fat, SCC-somatic cell count.



Table 2 and 3. TDMY varied significantly ($p < 0.05$) due to different udder shapes in Sahiwal and crossbred cattle, being significantly higher in round and bowl shapes than in trough shape udder for both the cattle breeds. Udder shape, however, did not affect milk composition, *i.e.*, fat, SNF, protein and lactose content (Table 2). Milk SCC was significantly ($p < 0.05$) higher in round and bowl than trough shaped udder. Higher prevalence of mastitis has been documented in cattle with pendulous rather than round and bowl shaped udder (Hussain *et al.*, 2012). Milk DLC in different udder shapes were significantly ($p < 0.05$) affected, neutrophil percent was higher with round shape in both the cattle breeds, while lymphocyte percent was higher in trough shape udder of Sahiwal cattle than crossbred cattle (Table 2).

With respect to teat shape, significantly higher number of milk SCC was noted in cylindrical rather than conical teat shape in both the breeds (Table 3), which agreed with Bhutto *et al.* (2010). Conical teat shape had lower milk SCC, but yielded more milk yield and encountered less mastitis in

Friesians crossbreds (Mukherjee *et al.*, 2013). It is hypothesized that funnel-shaped (conical) teat offered greater resistance being drawn into the mammary teat cup and milk drained out more completely. Cylindrical and bottle shape teats have chances of mammary teat cup occlusion at the orifice, leading to decreased milk flow followed by complete blockage.

Milk leucocytes were significantly ($p < 0.05$) affected by teat shapes as there was significantly higher number of neutrophil percent in cylindrical teat shape, while higher lymphocyte percent in conical and bottle shape teats of Sahiwal and conical shape teat of crossbred cattle. Macrophage percent was however not influenced by the teat morphology in either of the breeds (Table 3).

Correlations of Udder Morphological Traits with Milk Parameters

Pearson’s correlations of udder morphological traits showed highly significant ($p < 0.05$, $p < 0.01$) positive correlations of udder length, udder width and udder depth in both the

Table 2: Udder shape and milk parameters in healthy Sahiwal and crossbred cows

Udder Shape	Milk parameters	TDMY (kg/day)	Fat %	SNF (%)	Protein (%)	Lactose (%)	Milk SCC (x10 ⁵ /ml)	Neutrophil (%)	Macrophage (%)	Lymphocyte (%)
Sahiwal	Trough	1.640±0.22 ^a	4.180±0.52	8.820±0.16	3.200 ±0.14	4.820±0.86	0.635±0.10 ^a	30.46±1.52 ^a	12.23±1.87	57.31±2.81 ^b
	Bowl	2.917 ±0.27 ^b	4.600 ±0.36	8.867 ±0.21	3.242 ±0.08	4.867 ±0.12	1.238 ±0.16 ^b	31.32±2.09 ^{ab}	17.55 ±1.28	51.13 ±3.03 ^{ab}
	Round	3.158 ±0.23 ^b	4.442 ±0.32	8.858 ±0.07	3.250 ±0.03	4.858 ±0.03	1.388 ±0.10 ^b	39.29 ±2.24 ^b	15.11 ±1.37	45.59 ±2.20 ^a
	<i>p</i> Value	0.003	NS	NS	NS	NS	0.02	0.02	NS	0.01
Crossbred	Trough	3.900 ±0.47 ^a	3.960 ±0.18	8.600 ±0.17	3.180 ±0.06	4.740 ±0.07	0.408 ±0.14 ^a	27.82±2.34 ^{ab}	13.80 ±1.24	58.38 ±2.87
	Bowl	5.957 ±0.42 ^b	3.950 ±0.32	8.825 ±0.07	3.233 ±0.03	4.858 ±0.04	1.109 ±0.19 ^{ab}	25.18 ±1.78 ^a	15.81 ±2.25	57.34 ±2.84
	Round	7.146 ±0.45 ^b	3.758 ±0.25	8.783 ±0.11	3.217 ±0.05	4.808 ±0.06	1.180 ±0.14 ^b	32.86 ±2.60 ^b	20.14 ±2.54	51.17 ±3.15
	<i>p</i> Value	0.001	NS	NS	NS	NS	0.035	0.04	NS	NS

*Mean ± SE values with alphabetical superscript (a, b) in column within breed differ significantly ($p < 0.05$).
 # TDMY-test day milk yield, SNF-solid-non-fat, SCC-somatic cell count.

Table 3: Effect of mammary teat shape on milk SCC and milk leucocytes in healthy Sahiwal and crossbred cows

Cattle breed	Teat shape	Milk leucocytes			
		Milk SCC (x10 ⁵ /ml)	Neutrophil (%)	Macrophage (%)	Lymphocyte (%)
Sahiwal	Cylindrical	1.581±0.08 ^b	38.63±1.12 ^b	13.95±1.02	45.84±2.02
	Conical	0.758±0.14 ^a	31.62±2.39 ^a	13.44±1.98	54.92±4.16
	Bottle	1.140±0.15 ^{ab}	30.46±2.59 ^a	12.46±2.15	57.07±3.53
	<i>p</i> Value	0.012	0.003	NS	NS
Crossbred	Cylindrical	1.287±0.11 ^b	33.83±1.23 ^b	15.57±1.01	50.61±1.72 ^a
	Conical	0.627±0.18 ^a	27.27±1.96 ^a	13.06±1.09	59.67±2.55 ^b
	<i>p</i> Value	0.011	0.028	NS	0.028

Mean ± SE values with alphabetical superscript (a, b) in column within breed differ significantly ($p < 0.05$)
 Bottle teat shape was not observed in crossbred cattle in this study.

Sahiwal ($r= 0.414$ to 0.699) and crossbred ($r= 0.377$ to 0.873) cattle, indicating its close interrelationship. These findings corroborated with other studies (Ahlawat *et al.*, 2010; Singhai *et al.*, 2013). Significant ($p<0.05$) negative correlations were found for milk fat percent with udder length ($r= -0.438$) and width ($r= -0.413$) in crossbred cattle, whereas it was non-significant in Sahiwal cattle. Hence, larger the udder volume lesser is the milk fat percent.

Significant positive correlations were found between milk SCC and udder circumference ($r= 0.407$), width ($r= 0.428$) and depth ($r= 0.605$); milk SCC with teat apex ($r= 0.504$) and mid ($r= 0.406$) diameters in crossbred cattle. Likewise, significant positive correlations were noted of milk SCC with udder circumference ($r= 0.465$), depth ($r= 0.590$) and teat base ($r= 0.644$) diameter in Sahiwal cattle. These findings correlated well with studies of Sharma *et al.* (2017a) and Bharti *et al.* (2015) and explained interaction between teat length during milking with occurrence of mastitis (Slettbackk *et al.*, 1990). There was significant negative correlation between milk SCC and udder height from the ground in crossbred ($r= -0.436$) and Sahiwal ($r= -0.578$) cattle. These observations agreed with other studies too (Bhutto *et al.*, 2010; Singh *et al.*, 2013).

In crossbred cows, milk neutrophil percent showed significant positive correlations with udder circumference ($r= 0.373$), width ($r= 0.386$), depth ($r= 0.379$), teat length ($r= 0.466$), apex ($r= 0.365$), mid ($r= 0.382$), base ($r= 0.366$) diameters, and negative correlation with udder height ($r= -0.377$) from the ground. Also milk lymphocyte percent showed significant positive correlation with udder height ($r= 0.449$) from the ground and negative correlations with udder circumference ($r= -0.368$), depth ($r= -0.441$), teat length ($r= -0.451$), apex ($r= -0.487$) and mid ($r= -0.392$) diameters, while no such correlations were observed in Sahiwal cattle.

Milk SCC showed significant positive correlation with milk neutrophil ($r= 0.410$), monocyte ($r= 0.400$) and negative correlation with lymphocyte ($r= -0.597$) percent in crossbred cattle, whereas there was significant and positive correlation with milk monocyte ($r= 0.375$) percent in Sahiwal cattle. These findings agreed with Sharma *et al.* (2017a,b).

CONCLUSIONS

Sahiwal cattle milk was found superior than crossbred cattle in milk fat percent and better immunity as indicated by milk leucocytes, though the milk yield was lesser. Pre-dominant milk leucocytes enumerated were neutrophil, lymphocyte and macrophage cells which changes with lactation stages. Udder and teat morphological traits significantly influenced the milk composition and leucocytes, which maintain a unique relationship in both lactating Sahiwal and crossbred cattle. Thus, udder morphological traits are involved in protecting the mammary gland against intra-mammary infections and maintain the udder health in lactating dairy cows.

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