Udder and Teat Morphometry in Association to Sub-Clinical Mastitis in Dairy Cattle and Buffaloes

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ABSTRACT

Dairy farming is an essential part of Pakistan's economy contributing significantly to the country's total milk production. Abundant cattle milk production has made it the most widely consumed milk around the World. However, mastitis is the inflammation in which the parenchymal cells of the mammary gland are involved. Visual assessment of udder and teat morphological characteristics is a valuable tool in selecting animals with desirable traits for mastitis-free conditions and optimal milk production. In this study udder and teat morphological traits were measured and recorded. Additional factors like breed, age, parity, and lactation stage were assessed to find their relationship with subclinical mastitis. Similarly milk composition analysis, milk minerals (calcium and magnesium) and standard plate was also determined to determine their concentration in healthy and subclinical mastitis cow. Pendulous shaped udder, globular shaped udder, pear shaped teats and funnel shaped teats showed higher level of somatic cell counts. Pearson correlation was utilized to study the impact of udder morphometry on clinical mastitis. Statistical analysis showed a strong positive correlation of subclinical mastitis with udder length, teat length, teat diameter, parity, age and standard plate count. Whereas moderate to weak positive correlation was seen in udder width, udder depth, difference between left and right teat, difference between front and rear teat, and lactation stage. Whereas milk production showed a strong negative correlation with subclinical mastitis. The result of milk analysis shows that the milk fat, lactose, calcium level, and magnesium level had a strong negative correlation with subclinical mastitis. Whereas SNF

(solid not fat) showed a weak negative correlation with subclinical mastitis. Protein level showed a non-significant positive correlation with subclinical mastitis.

Introduction

Milk has developed over time to fulfill the nutritional and physiological requirements of various living organisms. Since prehistoric times, humans have incorporated cattle (*Bos taurus*) and buffalo (*Bubalus bubalis*) milk into their diets (Roy *et al.*, 2020). Milk has been considered an essential part of a healthy diet especially during childhood and adolescence (Zhao and Agellon, 2017). On the other hand, the production of high-quality milk faces several obstacles in today's dairy industry. Mastitis harms the quality of milk, resulting in changes in milk composition due to many reasons like duration of infection, severity, disturbed cells of the epithelium, and other biological variations (Huma *et al.*, 2022). Mastitis is responsible for significant changes in milk composition as well as a reduction in udder health and milk supply. Subclinical infected cows often exhibit an increase in somatic cell count, indicating the presence of immune cells as a response to infection. This elevation in somatic cell count not only affects the visual appearance of milk but also negatively impacts its processing properties and shelf life, hindering the production of high-quality dairy products. (Halasa *et al.*, 2007).

Several diagnostic tests exist for mastitis but they have differences with respect to cost, specificity, and sensitivity (Viguier *et al.*, 2009). The California mastitis test (CMT) is an efficient, cheap, and responsive tool for precisely estimating somatic cell counts in milk samples from individual quarters. The California mastitis test qualitatively estimates the level of somatic cells in milk. This is useful because the concentration of white blood cells (WBC) and DNA in milk is directly corelated (Moroni *et al.*, 2018). The somatic cell count (SCC) can be used as an indicator of udder health. Healthy cows that have already recovered from mastitis should have an SCC < 200,000 cells/mL, and buffaloes with counts over 300,000 cells/mL should be considered to have an intramammary infection(Cobirka *et al.*, 2020).

Udder plays a significant role in dairy cattle which connects it to the body strongly. Besides pathological causes, Udder and teat morphological characteristics also increase the risk of infections such as mastitis (Haghkhah *et al.*, 2011). The structural characteristics of the udder and teat can be used to estimate milk production. The teat and udder morphometry are significant factors affecting milk quality, alongside environmental and physiological causes. However, changes in milk quality and udder infections are also associated with variations in the teats and udder morphology (Sinha *et al.*, 2022). The morphology of the udder is strongly correlated with mastitis in dairy animals, while teat serves as the first line of defense preventing mastitis. The entry of pathogens occurs through the teat orifice, and the occurrence of teat injuries plays a crucial role in the development of mastitis. (Chrystal *et al.*, 2001). Various factors, like teat length, udder length, teat diameter, udder diameter, teat position, and udder depth, are considerably related (Kaur *et al.*, 2018). Researchers use visual appraisal to classify teat shapes as steep, round, and pointed, and udder structures as oval, flat, and goat-like udders, all of which have a direct relationship to milk production. The physiological characteristics of the udder and teat traits contribute to suitable milk quality and buffalo production (Sinha *et al*., 2022). Moreover, their conformation has a significant impact on milk production and udder soundness in animals(Okkema and Grandin, 2021). The most variable component of milk in healthy cows and buffaloes is the milk fat, followed by protein and lactose. However, milk lactose is the most variable in cases of intra-mammary infections, followed by protein and fat. The components of milk, including fat, protein, lactose, and minerals, have changed in milk with a higher SCC, a consequence of udder infection. This alteration results in poor-quality of milk and milk products(Abd El-Salam and El-Shibiny, 2011). An inflammatory condition of the udder results in changes in milk composition, including decreased levels of carbohydrates, albumin, protein, and fat content, as well as decreased milk production and increased proteolysis. (Lima *et al*., 2018). Limited research has been conducted in Pakistan to assess the influence of subclinical mastitis on milk components such as lactose and SCC of dairy buffaloes. There have been studies on the influence of mastitis severity on the protein and fat content of cattle and buffalo milk (Ullah *et al*., 2020). The existence of mastitis leads to disruption of the blood-milk barrier, leading to a decrease in the synthesis and secretion of the udder epithelial cells. Which changes the mineral profile of milk, with altered calcium, copper, and zinc levels being observed in milk with varying severity and duration of the infection. Even subclinical mastitis can cause significant alterations in the mineral profiles of milk, leading to decreased levels of calcium, copper, and zinc (Singh *et al.*, 2017). As a result of this process, the levels of calcium, phosphorus, and potassium are significantly reduced in cases of subclinical mastitis.(Batavani *et al.*, 2007).

MATERIAL AND METHOD

Sampling population

The present study was conducted on a comprehensive sample size of 150 lactating animals, comprising 75 cattle and 75 buffaloes. The study included buffaloes and cattle of different parities, lactation stages, and different age groups. Different farms were selected for sample collection. A total n=150 milk samples of healthy and sub-clinical mastitis were collected aseptically

California mastitis test

California Mastitis Test (CMT) was employed for the assessment of subclinical mastitis by using the California Mastitis Test (CMT) Kit. CMT paddle cups were labelled as A, B, C, and D to check the quarters from which the milk will be collected, in a corresponding cup of the plastic paddle, one to two strips of milk approximately 2ml were taken from each teat and the same quantity of CMT solution will be added to each cup of the paddle which was rotated circularly for 10 seconds to mix the milk and CMT reagent. The visible reaction was read after 20 seconds and the grading of milk samples for subclinical mastitis was based on gel formation (Wang *et al.*, 2023).

Udder and teat morphometry

The udder and teat parameters measured were udder length, udder depth, udder width, teat length, teat diameter, distance between front teats, distance between the rear teats, and distance between the fore and the hind teats. All the parameters apart from teat diameter were measured with measuring tape in centimeters.

Udder length (UL): Measured as the distance between the front and rear attachment of the udder (cm).

Udder depth (UD): Was measured as the distance from the base to the lowest point of the udder at the place of attachment of the teats (cm).

Udder width (UW): Was measured at the widest point of the udder (cm).

Teat length (TL): Measured as the distance from the teat insertion base to the teat orifice (cm).

Teat diameter (TD): Defined as the distance between the widest points of the teat's circumference (cm). It was measured with the Vernier caliper at the middle point of the teat to the nearest 0.01 cm.

Distance between fore teats (DBFT): Measured as the distance between the two fore (front) teats at the tip of the teats (cm). Distance between hind teats

Distance between Hind teats (DBHT): Measured as the distance between two hind (rear) teats (cm). Distance between fore and hind teats

Distance between fore and hind teats(DBFHT): This was measured as the distance from one of the fore teats to the hind teat of the same side (cm).

Milk composition analysis

The milk samples collected for this study underwent thorough analysis to determine their composition. The analysis was carried out at the laboratory of the National Institute of Food Sciences and Technology, University of Agriculture, Faisalabad. To assess the milk composition, a specialized instrument called the Lactoscan milk analyzer was employed. Using the Lactoscan milk analyzer, various parameters of milk composition were measured, including lactose, protein, fat, and solid not-fat (SNF) values. Both healthy milk samples and those affected by subclinical mastitis were subjected to this analysis (Ullah *et al.*, 2020).

Milk somatic cell count

A somatic cell count, often known as an SCC, is a cell count that is performed on somatic cells that are found in a fluid specimen, most commonly milk. In the dairy industry, the specific coliform count (SCC) is a measure of the quality of milk, more precisely the low likelihood that the milk would contain dangerous bacteria and, as a result, its high level of food safety. Most of the somatic cells under consideration are white blood cells, often known as leukocytes. In response to pathogenic bacteria such as *Staphylococcus aureus*, a common cause of mastitis, the number of somatic cells in the affected area will rise. Quantification of the SCC is done in terms of cells per milliliter. There is widespread consensus that a reference range should be set at fewer than 100,000 cells/mL for buffaloes who have not been infected and larger than 280,000 cells/mL for buffaloes who have been infected with considerable amounts of pathogen. A cow-side measurement of somatic cell count is provided by a few assays, such as the Porta SCC milk test and the California mastitis test (Schalm *et al.*, 1971).

RESULTS AND DISCUSSION

The result of udder morphometry shows the visual assessment of both cattle and buffaloes from a total population of 140 animals consisting of 70 buffaloes and 70 cattle. Uder shapes were categorized as bowl, goaty, pendulous, round, and globular shaped. In cattle round shaped udders were found in around 50 % of the population and the least udder shapes were found of the goaty and pendulous shaped udders respectively 8.57%, whereas in buffaloes bowl shaped udders were mostly found in the population around 28.6% and least udder shapes were found of pendulous shaped udder around 11.4%. Teat shapes in cattle and buffalo were classified as cylindrical, bottle, funnel pear, and conical-shaped teats. Teat shapes evaluation shows that cylindrical teat shapes were common in most of the cattle around 67% and the least teat shapes were found of bottle-shaped around 4.3% in a total population of cattle, whereas in buffaloes similar cylindrical shaped teats were more prevalent around 29% and funnel-shaped teat were least around 12.6% from the total population of the buffaloes. Frequencies and percentage distribution of different udder and teat shapes are shown in table 1. Morphometric assessment of udder and teat morphometric traits for all the cattle and buffaloes was included in the study. The morphometric trait of the udder includes the udder length, width, and depth in different udder shapes. Upon analysis, the mean udder length was found to be highest in pendulous-shaped udder in both cattle and buffalo, whereas the least udder length was observed in goaty-shaped udder in cattle and globular shaped udder in the case of buffalo. Similarly, udder width was found to be highest in pendulous-shaped udder in both cattle and buffalo, whereas the least udder width was found in bowl shaped udder in cattle and globular shaped udder in case of buffalo. Mean with standard error of different udder shapes morphometric traits are shown in table 2. Teat length and teat diameter were also observed for all the different teat shapes. Teat length was classified as left front teat length, left hind teat length, right front teat length, and right hind teat length. Teat diameter was classified as left front teat diameter, left hind teat diameter, right front teat diameter, and right hind teat diameter. Similarly, teat distances were also observed for the distance between the front and right teat, the distance between front teats, and distance between hind teats. The mean value of teat morphometric traits of cattle shows that the shows that highest teat length was observed in the funnel-shaped teats and minimum teat lengths were observed in pear-shaped teats. The highest teat diameter was found in the conical-shaped shaped teat and the minimum teat diameter was observed in the cylindrical shaped teat, whereas the side teat distance was highest in the pear-shaped teats. Teat morphometric traits in buffalo show that the maximum teat length was observed in the funnel-shaped teat length and minimum teat length was observed in the cylindrical-shaped teats, teat diameter was maximum in the cylindrical shaped teats and minimum teat diameter was observed in the funnel-shaped teat whereas side teat distance was highest in the bottle shaped teats in buffaloes.

Table 1: Frequencies and percentages distributions of different udder and teat shapes

Parameter	**Cattle**		**Buffalo**	
Udder shape	Frequency	Percentage (%)	Frequency	Percentage (%)
BW	12	17.14 %	20	28.6
GT	6	8.57 %	12	17.1
PE	6	8.57 %	8	11.4
RR	35	50 %	16	22.9
GB	11	15.71 %	14	20
Teat shape				
CY	47	67.14 %	20	28.6
BT	3	4.29 %	12	17.1
FU	5	7.14 %	9	12.6
PR	14	20 %	15	21.4
CN	1	1.43 %	14	20

BW= Bowl, GT= Goaty, PE= Pendulous, RR= Round, GB= Globular, CY= Cylindrical, BT= Bottle, FU=Funnel, PR=Pear, CN= Conical.

Table 2: Morphometric traits of various udder dimensions (cm) in buffalo and cattle according to udder shapes

Udder shapes	**UL (cm)**	**UW (cm)**	**UD (cm)**
Cattle			
PE	44.3 ± 0.97	32.33 ± 1.089	18.67 ± 1.36
RR	42.11 ± 0.45	29.65 ± 0.66	17.63 ± 0.48
BW	42.79 ± 1.07	24.62 ± 1.06	16.93 ± 0.73
GB	43.64 ± 0.77	31.14 ± 0.97	17.98 ± 0.85
GT	40.33 ± 0.97	29.33 ± 1.09	17.67 ± 1.36
Buffalo			
PE	51.7 ± 0.7	46.6 ± 0.5	13.9 ± 0.3
RR	48.5 ± 0.6	43.8 ± 0.6	11.0 ± 0.3
BW	49.6 ± 0.5	45.8 ± 0.5	12.7 ± 0.2
GB	42.64 ± 0.77	31.17 ± 0.87	15.98 ± 0.75
GT	46.1 ± 0.9	42.09 ± 0.9	10.9 ± 0.4

BW=Bowl, GT=Goaty, PE=Pendulous, RR=Round, GB=Globular, UL=Udder length, UW=Udder width, UD=Udder depth.

Table 3: Means ± SE of various teat measurements in according to their shapes.

Teat shape	DBFHT	DBFT	DBHT	LFTD	LFTL	LHTD	LHTL	RFTD	RFTL	RHTD	RHTL
CY[a]	4.5 ± 0.5	4.3 ± 0.2	4.3 ± 0.1	3.7 ± 0.1	6.9 ± 0.1	3.6 ± 0.1	6.1 ± 0.1	3.9 ± 0.1	6.5 ± 0.2	3.8 ± 0.1	6.1 ± 0.1
PR[a]	4.3 ± 0.9	4.6 ± 0.3	4.5 ± 0.4	3.9 ± 0.1	6.0 ± 0.3	3.9 ± 0.1	5.3 ± 0.2	4.2 ± 0.2	6.0 ± 0.3	4.7 ± 0.1	5.5 ± 0.2
FU[a]	4.4 ± 0.7	4.2 ± 0.6	4.4 ± 0.4	3.7 ± 0.2	7.7 ± 0.9	3.6 ± 0.3	6.4 ± 1.0	3.8 ± 0.2	7.0 ± 0.9	4.3 ± 0.1	6.4 ± 1.0
BT[a]	4.3 ± 0.5	4.2 ± 0.3	4.6 ± 0.2	4.2 ± 0.2	6.5 ± 0.3	4.0 ± 0.3	6.7 ± 0.6	4.1 ± 0.3	6.4 ± 0.3	4.2 ± 0.3	6.7 ± 0.6
CN[a]	4.1	4.6	4.6	4.3	7.6	4.3	6.6	4.2	7.4	4.3	6.6
CY[b]	5.7 ± 0.3	6.1 ± 0.3	4.7 ± 0.5	3.5 ±0.08	7.5 ± 0.2	5.2 ± 1.4	8.2 ± 0.3	3.6 ± 0.8	7.4 ± 0.3	3.7 ± 0.1	8.4 ± 0.3
PR[b]	3.8 ± 0.3	4.2 ± 0.3	4.8 ± 0.5	3.7 ± 0.1	7.9 ± 0.3	3.9 ± 0.1	8.3 ±0.3	3.7 ± 0.9	7.7 ± 0.4	3.9 ± 0.1	8.6 ± 0.4
FU[b]	5.7 ± 0.3	6.1 ± 0.3	5.4 ± 0.3	2.9 ±0.1	8.4 ± 0.4	3.4 ± 0.1	9.3 ±0.4	3.1 ± 0.1	8.2 ± 0.4	3.2 ± 0.1	9.1 ± 0.4
BT[b]	5.9 ± 0.7	6.1 ± 0.7	5.6 ± 0.5	3.4 ±0.1	7.5 ± 0.3	3.5 ± 0.2	8.3 ± 0.5	3.5 ± 0.2	7.4 ± 0.3	3.6 ± 0.1	8.2 ± 0.4
CNN	4.9 ± 0.3	5.1 ± 0.4	4.6 ± 0.7	3.2± 0.2	8.1 ± 0.6	3.7 ± 0.1	9.02 ± 0.6	3.3 ± 0.2	8.2 ± 0.6	3.6 ± 0.2	8.9 ± 0.5

a= cattle, b= buffalo, DBHFT= Distance between fore and hind teat, DBFT=Distance between fore teats, DBHT=Distance between hind teats, LFTD=Left for teat diameter, LFTL=Left for teat length, LHTD=Left hind teat diameter, LHTL=Left hind teat length, RFTD=Right for teat diameter, RFTL=Right for teat length.

Table 4: Milk composition analysis of healthy and subclinical mastitis milk

Component (%)	Cattle		Buffalo	
	Healthy No.=20	**Mastitis No.=20**	**Healthy No.=20**	**Mastitis No.=20**
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Fat	4.2 ± 0.05	3.6 ± 0.05	5.8 ± 0.05	5.2 ± 0.4
SNF	7.05 ± 0.04	6.5 ± 0.04	8.05 ± 0.04	7.97 ± 0.1
Protein	3.5 ± 0.05	3.0 ± 0.05	3.1 ± 0.05	2.6 ± 0.05
Lactose	4.5 ± 0.07	3.98 ± 0.07	4.1 ± 0.07	4.03 ± 0.1

Milk composition analysis performed by the lactometer suggests that the fat, lactose, and protein levels vary greatly in the case of healthy and mastitis milk, whereas the level of SNF did not vary to much degree in healthy and mastitis milk in the case of both cattle and buffalo milk. The mean difference between health and mastitis milk is shown in table 4.

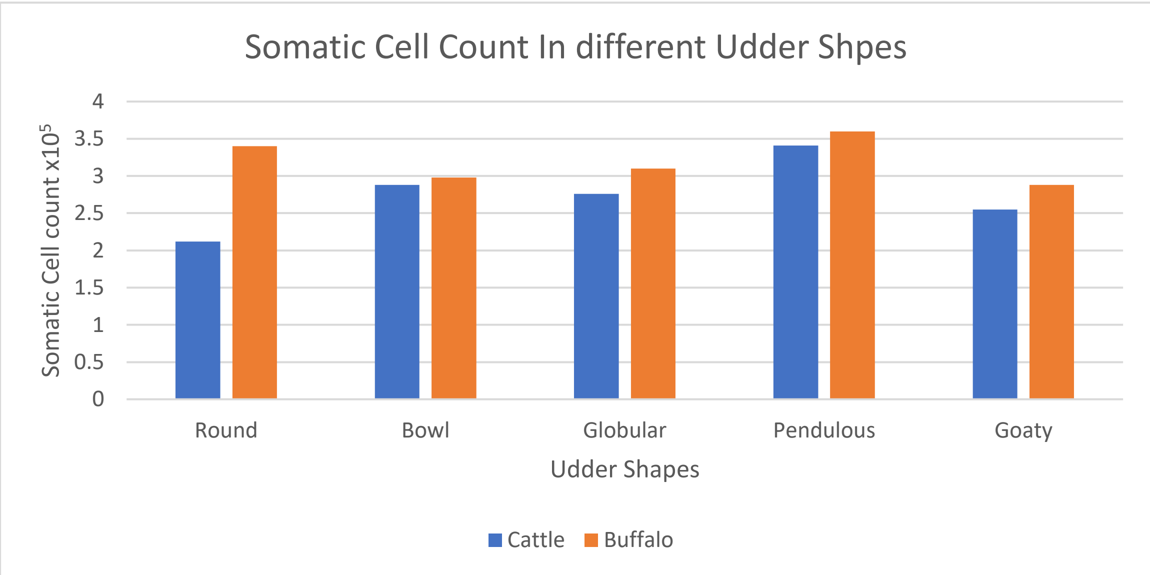

Fig 1: Somatic cell count in different udder shapes

The somatic cell count analysis in the subclinical mastitis regarding udder shapes showed that higher levels of the somatic cell counts were shown in the round and pendulous shaped udder in the case of buffalo while globular, bowl and pendulous shaped udder shows the greater level of somatic cells counts in their milk as shown in figure 1.

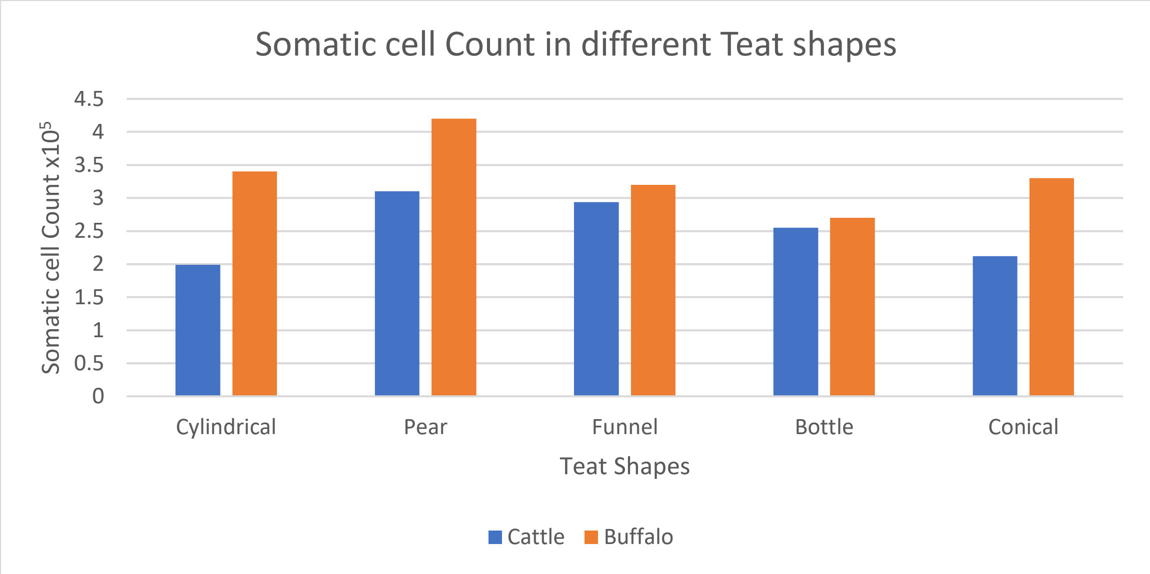

Fig 2: Somatic cell count in different teat shapes

The somatic cell count analysis in the subclinical mastitis regarding teat shapes showed that higher levels of the somatic cell counts were shown in the pear, conical, and cylindrical shaped teats in the case of buffalo while pear and funnel-shaped teats shows the greater level of somatic cells counts in their milk as shown in figure 2.

		Udder			Teat Length				Teat diameter				Milk Composition					Minerals		SCC
		UL	UW	UD	RF	LF	RR	LR	RF	LF	RR	LR	Yield	Protein	fat	lactose	SNF	Ca	Mg	
UL		1	0.35	0.39	0.06	0.03	0.02	0.01	0.1	0.35	0.03	0.10	-0.24*	-0.13	0.21*	-0.11	-.08	-0.1*	0.2*	0.2*
		1	0.43	0.29	0.06	0.07	0.04	0.05	0.2	0.52	0.05	0.24	-0.31*	-0.15	-0.31	-.021	-0.05	-0.2	-0.26	0.8
UW			1	0.46	0.22	0.01	0.24	0.31	0.34	0.27	0.09	0.133	-0.61*	-0.019	0.102*	-0.027	-0.08	-0.02	-0.10	0.12
			1	0.42	0.32	0.03	0.34	0.33	0.42	0.11	0.05	0.	-0.053	-0.023	-0.021	-0.032	-0.02	-0.04	-0.23	0.6
UD				1	0.18	0.01	0.01	0.07	0.01	0.00	0.10	0.15	-0.60*	-0.169	0.107	-0.122	-0.08	0.19*	0.09	0.2*
				1	0.46	0.22	0.01	0.24	0.31	0.34	0.27	0.09	-0.133	-0.61*	-0.019	0.102*	-0.02	-0.08	-0.02	-0.10
RFTL					1	0.31	0.103	0558	0.72	0.416	0.05	-0.20	-0.097	-0.165	-0.49	0.181	0.149	-0.01	-0.18	0.23*
					1	0.42	0.672	0.10	0.26	0.31	-0.26	-0.06	-0.083	-0.06	0.114	0.21	0.132	-0.06	-0.2	0.5
LFTL						1	0.452	0.672	0.10	0.266	0.31	-0.26	-0.064	-0.083	-0.06	0.114	0.083	0.04	-0.14	0.26*
						1	0.73	0.73	0.62	0.41	0.21	0.221	-0.063	-0.04	0.116	0.181	0.149	-0.07	-0.4	0.43
RRTL							1	0.262	0.05	0.194	0.013	0.315	-0.32	-0.032	-0.207	0.047	0.016	0.20	0.12	0.06
							1	0.145	0.02	0.11	0.085	0.106	-0.435	-0.015	-0.154	-0.169	-0.05	-0.07	-0.2	0.23
LRTL								1	0.11	0.085	0.106	0.435	-0.015	-0.154	-0.169	-0.05	-0.07	0.27	0.21*	-0.13
								1	0.32	0.078	-0.06	-0.04	-0.044	-0.05	0.22	0.17	0.04	-0.07	-0.2	0.32
Teat	RF								1	0.012	0.059	-0.31	-0.231	-0.063	-0.054	-0.05	-0.1	0.09	0.04	0.02
Diameter									1	0.021	-0.06	-0.04	-0.044	-0.05	0.22	0.17	-0.05	-0.08	-0.32	0.31
	LF									1	0.129	-0.31	-0.13	-0.014	-0.032	0.024	0.08	-0.09	-0.09	0.13
										1	0.072	0.061	-0.032	-0.056	0.047	0.086	0.02	-0.06	-0.23	0.42
	RR										1	0.322	-0.172	-0.06	-0.041	0.044	0.005	0.22	0.17	-0.16
											1	0.129	-0.312	-0.13	0.014	-0.032	0.024	-0.03	-0.43	0.38
	LR											1	-0.192	-0.04	-0.141	0.056	0.014	0.11	0.08	0.11
												1	-0.067	-0.031	-0.042	0.061	0.03	-0.03	0.05	0.21

Table 5: Pearson correlation coefficient of various factors with udder and that morphometric traits.

The person correlation was utilized to determine the correlation of various factors with the udder and teat morphometry. The values range from +1 to -1 showing from the strong positive to strong negative correlation. Whereas * shows the non-significant correlation

CONCLUSION

Udder and teat measurements could be used as a reliable criterion in the selection of buffaloes for milk production at the field level. The results of the present study suggest that the chances of mastitis are higher in buffaloes and cattle with udder-shaped bases are pendulous, bowl, round, and goaty respectively. The results of the present study suggest that the chances of mastitis are higher in buffaloes and cattle with teat-shaped bases are conical, pear, funnel, and cylindrical respectively. Some udder morphology traits had a positive correlation with milk yield and can be adapted for genetic improvement in the breeding programs of animals. This ultimately improves the milk production, milking ability, and udder health of dairy Buffaloes and cattle.

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