Effects of Feeding Time, Breed, Parity, Stage of Lactation and Milking Methods on Somatic Cell Count and Milk Constituents in Dairy Cows

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Abstract

Purpose: This study investigated the effect of feeding time, parity, stages of lactation, milking methods, and breed on milk composition and somatic cell count (SCC) with the main objective of understanding how these factors influence the nutritional properties and udder health of dairy cow.
Methods: Twenty (Holstein Frisian, Jersey and Lulu) subclinical mastitis-free crossbred cows were used for a 180-day study on different feeding strategies (pre-milking, during milking, and post-milking feeding at varying intervals). The animals were categorized into three groups, each based on their parity (early, mid and late) and stages of lactation (early, mid and late), then their effect on different parameters were examined. The milk composition and SCC was analyzed (by transforming them at log10 base) fortnightly. The effect of feeding time, parity, stages of lactation, breed and milking methods on SCC and milk composition were worked out for ANOVA by General Linear Model (univariate) using SPSS statistical packages (version 25).
Results: Results revealed that feeding time significantly affected milk composition, with pre-milking feeding yielding in higher milk fat, while post-milking feeding strategies led to lower SCC. Parity and stages of lactation also showed notable effects on milk composition and SCC, with early parity and mid-lactation cows exhibiting higher SCC. Additionally, milking methods and breed influenced milk composition, with higher protein and lactose in machine milked cows, and Lulu producing milk with higher fat (numerical), protein and lactose.
Conclusions: Our research findings provided valued insights into the factors influencing milk quality and udder health in dairy cows, which could be inferred for a better management practice aimed at optimizing quality milk production.

Keywords: Day in milk, Lulu, Somatic cell count, Udder health

1 Introduction

The composition and functional properties of cow milk are crucial for dairy farmers, manufacturers and consumers (National Research Council, 1988). Somatic cell count (SCC) and milk constituents play important roles, not only as indicators of udder health but also as determinants of nutritional value (Fadillah et al., 2023). The average nutritional composition of cow milk includes 3.5% protein, 4.5% fat, 8.5% solid-not-fat, 4.6% lactose, and a freezing point of -0.55°C (Mishra et al., 2022). These milk properties may be influenced by various factors (Sarkar *et al.*, 2006) including seasonal variation (Bernabucci *et al.*, 2002, 2015), milk storage (Hanna *et al.*, 2004), nutritional levels, breeds (Mishra et al., 2022), parity (Shibru *et al.*, 2019; Shuiep *et al.*, 2016), milk SCC (Ramos *et al.*, 2015), lactation stage (Mishra et al., 2022; Shibru *et al.*, 2019; Shuiep *et al.*, 2016) and the geography (Alyaqoubi *et al.*, 2014). Feeding time has been identified as a significant factor affecting milk production and composition (Niu *et al.*, 2014; Vanbergue *et al.*, 2017). Indeed the timing of feed intake in relation to milking sessions can impact the cow's metabolic processes and consequently, the quality of the milk produced (Schütz *et al.*, 2023), both in terms of composition and SCC. Research findings suggested that feeding cows before milking may positively influence milk fat and protein while feeding during or after milking yields milk with low SCC (DeVries *et al.*, 2010). On the other hand, the parity and lactation stages of a dairy cow is known to affect composition and quality of milk (Ben Meir *et al.*, 2019; Gajbhiye et al., 2019; Gurmessa & Melaku, 2012; Kro *et al.*, 2020; Sabek *et al.*, 2021; Shibru *et al.*, 2019; Shuiep *et al.*, 2016; Walter *et al.*, 2022). Primiparous and multiparous cows may exhibit differences in lactation curves, influencing fat, protein, and lactose content (Wathes *et al.*, 2007). Thus, managing nutritional requirements based on parity and stages of lactation, emphasizing equally on SCC pictures, is critical for maximizing the quality and overall production efficiency.
Total milking days in a lactation period was reported to have a variable effect on the composition of milk. The

higher levels of fat, protein, casein, whey proteins, and sodium was observed in late-lactation milk (O'Brien *et al.*, 1999) but milk yield and lactose content decreased (Auldist *et al.*, 1998). This change in milk composition could be due to reduced milk volume at late lactation (Hinz *et al.*, 2012), resulting in the concentration of milk constituents within the udder. Conversely, early lactation is characterized by increased energy and nutrient demands, impacting fat and protein content (Gross, 2023). Despite facing a significant negative energy balance (NEB) during early lactation, cows continue to produce more till peak lactation, demanding higher energy and nutrient supply (Jozwik *et al.*, 2012). However, dry matter intake (DMI) decreases during this period and rises much slowly than the increase in production. Consequently, DMI fails to meet the necessary nutrient requirements, leading to a NEB characterized by body weight loss and reduction in protein, calcium, and other nutrients (Gross, 2023). Fat and milk protein percentages are similarly influenced by the lactation stage. Colostrum, obtained just after calving, contains the maximum concentration of fat and protein, dropping to their lowest point 25 to 50 days after calving and reaching their maximum at 250 days when milk production begins to decline (Amalfitano *et al.*, 2021).
Furthermore, the methods either hand or machine milking, for milking can affect milk composition and quality. Mishra *et al.* (2020) reported significant differences in milking time and milk flow rate between hand and machine milking methods but the effect on total yield and milk composition was non-significant. However, Dang & Anand (2007) found a significantly higher SCC in milk with lower lactose when cows were hand milked but found no effect in fat and proteins. It is well known that SCC influences the qualitative and technological properties of milk. Nevertheless, it has been recently reported that other traits such as lactose, pH and minerals might be used as udder health indicators (Macciotta *et al.*, 2012). Lactose concentration decreases during mastitis and its association with SCC has been widely studied (Kitchen, 1981). Breed, as a genetic factor, significantly affects the composition, quantity, and quality of milk. Bobbo *et al.* (2014) reported differences in milk composition, specifically in milk fat and protein in the study which enrolled six breeds of cows. The authors reported the highest milk protein content in Brown Swiss and the lowest in HF, while fat content was highest in Simmental and lowest in Rendena breeds.
The existing literature broadly covers various factors influencing the nutritional constituents and quality of cow milk in terms of SCC based on breed, lactation stages, parity, milking methods and feeding time in organized commercial herds. However, there is a notable knowledge gap in understanding the effects of these factors comprehensively on smaller herds reared on subsistence management, representing the characteristics Nepalese dairying. Therefore, this study is focused on examining the effects of feeding time, breed, parity, stages of lactation and milking methods on SCC and different milk constituents in the dairy cows.

2 Materials and methods

2.1 Experimental animals and location

This research was done by using subclinical mastitis (SCM) free Holstein Friesian (HF), and Jersey (J) crossbred and indigenous Lulu cows at National Cattle Research Program (NCRP), National Agriculture Research Council, Chitwan, Nepal. A total of 20 dairy cattle were randomly selected from milking herd of NCRP based on similar ranges of parity, stages of lactation, initial SCC and total milk production with no record of mastitis in past. Somaticell Kit (Intervet Schering Plough, Whitehouse, NJ) was used to screen and select the SCM free cows (with iSCC, 84 - 109 × 1000 cells/ml). Somaticell is an on-farm rapid test and is conducted as detailed by Langoni *et al.* (2012). The experiment was done during September, 2019 to March, 2020 for the period of 180 days in Completely Randomized Design with 5 treatments, each with 4 replications for measuring the effect of feeding strategies on milk composition, characteristics and SCC.
$\mathbf{T_1}$ (-10PMF): Feed offered 10 min before milking **(10 min pre- milking feeding)**
$\mathbf{T_2}$ (MFD): Feed offered during milking **(milking during feeding)**
$\mathbf{T_3}$ (+iPMF): Feed offered right after milking **(immediate post milking feeding)**
$\mathbf{T_4}$ (+15PMF): Feed offered 15 min after milking **(15 min PM feeding)**
$\mathbf{T_5}$ (+30PMF): Feed offered 30 min after milking **(30 min PM feeding)**
The study cows, machine, or hand-milked twice daily at 05.00- 07.00 morning and 16.00- 17.00 evening, were managed in tail-to-tail fashion under conventional housing system with assess to free grazing for 3- 4 hours daily at day time and standard housing space was provided according to national guidelines. All the daily farm management procedure, feeding management as well as the feed ingredients were kept similar for the herd. Commercial concentrates on the basis of production level and *ad libitum* green fodders were offered to address the nutritional requirements of the study cows.

2.2 Collection of milk samples and SCC analysis

Approximately 30 ml morning milk samples were collected aseptically in sterilized sampling bottles from each quarter of all focus dairy cows at fortnightly interval for 180 days. Udders and teats were washed with clean water and wiped off with towels soaked in antiseptic solution and allowed to dry. The first 2-3 streaks of foremilk were discarded before collecting milk samples to avoid the initial milks collected in teat canal. The collected samples were transported to the laboratory immediately in a cool box with maintained temperature of < 4°C and Lactoscan SCC (Milkotronic Ltd., Bulgaria; www.milkotronic.com) was used for analyzing SCC following the standard procedures; both the absolute (×1000 cells/ml) and log10SCC values were determined. The udder level SCC was calculated as a mean of four quarter SCC at each sampling.

2.3 Milk composition

The milk sample were analyzed by using automated Lactoscan milk analyzer (Milkotronic Ltd., Bulgaria; www.milkotronic.com) for milk fat, SNF, protein, lactose, electrical conductivity, pH, freezing point and daily milk production.

2.4 Statistical analysis

The animals were categorized into three groups each based on their parity (early = up to 3 calving, mid = 4 to 6 calvings and late = above 6 calvings) and stages of lactation (early = 0 to 100 DIM, 2 = 100 to 200 DIM and 3 = above 200 DIM), then parity and lactation stage effect on different parameters were examined. The teat level absolute SCC were first transformed at log10 base to achieve the normal distribution and minimize the heterogeneity of variance. The effect of feeding time, parity, stages of lactation, breed and milking methods on SCC and different milk composition parameters were worked out for analysis of variance by General Linear Model (univariate) using SPSS statistical packages (version 25) and expressed as mean and standard error of the mean. Mean separation was done by Duncan's Multiple Range Test (DMRT) for stratification of effect of feeding time on milk composition and SCC and by Least Significant Difference (LSD) test for partitioning the effect of parity, breed, stages of lactation and milking methods on milk composition and SCC. Mean differences were maintained at $p \leq 0.05$

3 Results

3.1 Milk composition, characteristics and SCC in different feeding strategies

Feeding strategies based on feed offer time affected the milk composition, characteristics and also reflected for log10 SCC (mean and SEM) in tie stall dairy cows (Table 1). The milk fat was higher ($p < 0.001$) in -10 PMF cows (5.96) among all the treatments; milk fat content in +iPMF (5.58) was also higher ($p < 0.001$) than MFD (4.59) and +30PMF (5.10), while the milk fat in MFD cows was lowest ($p < 0.001$) . The milk SNF was higher ($p < 0.001$)in +30PMF cows (9.10) than that in MFD (8.86) and +iPMF (8.80); the SNF content in +iPMF was lowest($p < 0.001$) , but was comparable with -10PMF and MFD. Reflecting the same trend, the milk protein and lactose content were highest ($p < 0.001$) in +30PMF (3.32, 4.99), and lowest in +iPMF (3.20, 4.832). However, milk electrical conductivity (mS/cm) was higher ($p < 0.001$) in MFD (4.61) than other treatments except +iPMF (4.53). The milk pH was observed on natural line with greater value ($p < 0.01$) in MFD (4.59) than all other groups. The milk freezing point (ºC) was found higher ($p < 0.001$) in +30PMF (0.54) among all treatments and the lowest in +30PMF (0.53). In contrast, +iPMF and +15PMF cows had significantly lower ($p < 0.001$) log10 SCC than -10PMF, MFD and +30PMF animals, clearly stating the observed lower risk of experiencing udder infections (Table 1).

Table 1: Milk composition, characteristics and $\log_{10}$ SCC (mean and SEM) in different feeding time-based treatments in tie stalls dairy cattle at Chitwan, Nepal

Factors	Fat	SNF	Protein	Lactose	EC	pH	FP	log10 SCC
	(%)				(mS/cm)		(-ºC)	
T1	5.96^{a}	8.95^{abc}	3.24^{bc}	4.89^{bc}	4.40^{bc}	6.42^{b}	0.537^{bc}	5.38^{a}
T2	4.59^{d}	8.86^{bc}	3.23^{bc}	4.88^{bc}	4.61^{a}	6.53^{a}	0.544^{a}	5.40^{a}
T3	5.58^{b}	8.80^{c}	3.20^{c}	4.83^{c}	4.53^{ab}	6.43^{b}	0.539^{b}	4.97^{b}
T4	4.92^{cd}	8.98^{ab}	3.28^{ab}	4.94^{ab}	4.35^{c}	6.44^{b}	0.536^{bc}	4.96^{b}
T5	5.10^{c}	9.10^{a}	3.32^{a}	4.99^{a}	4.40b^{c}	6.45^{b}	0.533^{c}	5.37^{a}
SEM	0.13	0.05	0.02	0.03	0.05	0.02	0.002	0.054
F- value	18.93	5.13	6.23	5.43	5.40	2.47	5.69	15.54
Significance level	***	***	***	***	***	**	***	***

Mean values within the same column with different superscript letters are different; (***)- $p < 0.001$; (**)- $p < 0.01$ SCC: Somatic cell count, SNF: Solid not fat, EC: Milk electrical conductivity, FP: Milk freezing point, SEM: Standard error of mean

3.2 Milk composition, characteristics and SCC in different parities

The milk composition, characteristics and log10 SCC (mean and SEM) in different parities in tie stall dairy cattle at Chitwan, Nepal is shown in Table 2. The milk fat, SNF, protein and lactose content did not differ significantly according to the level of parities, however, the milk electrical conductivity (mS/cm) was observed higher ($p < 0.001$) in early parity (4.52) than that in mid parity (4.34) but was similar during late parity (4.49). The same trend was evident in milk freezing point with significantly higher ($p < 0.001$) value during early parity (0.54) than that in mid one (0.53) but was comparable with the value during late parity (0.54). The milk SCC showed the increasing trend with advancement of parity and the results showed that cows in early parity (5.12) had significantly lower ($p < 0.001$) log10 SCC than at mid and late parities.

Table 2: Milk composition, characteristics and $\log_{10}$ SCC (mean and SEM) in different parities in tie stalls dairy cattle at Chitwan, Nepal

Factors	Fat	SNF	Protein	Lactose	EC	pH	FP	log10
	(%)				(mS/cm)		(-ºC)	SCC
Early (up to 3 calvings)	5.13	8.94	3.25	4.91	4.52^a	6.47	0.540^a	5.12^b
Mid (4-6 calvings)	5.23	8.98	3.26	4.92	4.34^b	6.45	0.533^b	5.33^a
Late (>6 calvings)	5.42	8.87	3.23	4.90	4.49^a	6.43	0.538^a	5.42^a
SEM	0.11	0.04	0.01	0.02	0.04	0.02	0.001	0.02
F- value	1.66	1.56	1.10	1.15	6.38	1.25	6.99	17.83
Significance level	ns	ns	ns	ns	***	ns	***	***

Mean values within the same row with different superscript letters are different; (***)- $p < 0.001$, (*)- $p < 0.05$ and ns- non significant $p > 0.05$; SCC: Somatic cell count, SNF: Solid not fat, EC: Milk electrical conductivity, FP: Milk freezing point, SEM: Standard error of mean

3.3 Milk composition, characteristics and SCC in different stages of lactation

The milk composition, characteristics and $\log_{10}$ SCC (mean and SEM) in different stages of lactation in tie stall dairy cattle at Chitwan, Nepal has been presented in Table 3. Accordingly, it was revealed that the SNF content of milk increased with advancement of lactation, and was higher ($p < 0.001$) during late lactation (9.05). The milk protein, lactose and ash content showed the comparable trend with SNF and was higher ($p < 0.001$) at late lactation (3.30, 4.97 and 0.73). Interestingly, the milk electrical conductivity (mS/cm) and freezing point (-ºC) was higher ($p < 0.001$) in late lactation (4.56) than that in early and mid-lactation. The differences in milk fat content were non-significant ($p > 0.05$) with respect to the stages of lactation, however it was evident that milk fat content increased with the advancement of lactation. The $\log_{10}$ SCC was significantly higher at mid lactation (5.38) and dropped to the comparable level as that of early lactation.

Table 3: Milk composition and characteristics at udder level (mean ±SE) in different stages of lactation in tie stalls dairy cattle at Chitwan, Nepal

Factors	Fat	SNF	Protein	Lactose	Ash	EC	pH	FP	log10
	(%)					(mS/cm)		(-ºC)	SCC
Initial (upto 100 DIM)	5.12	8.89^b	3.23^b	4.87^b	0.72^b	4.36^b	6.44^b	0.534^b	5.20_b
Mid (100- 200 DIM)	5.22	8.81^b	3.20^b	4.85^b	0.72^b	4.45^b	6.47^a	0.538^{ab}	5.38^a
Late (>200 DIM)	5.34	9.05^a	3.30^a	4.97^a	0.73^a	4.56^a	6.46^a	0.541^a	5.26^b
SEM	0.12	0.04	0.01	0.02	0.003	0.04	0.02	0.001	0.02
F- value	1.14	8.97	11.21	8.95	6.92	8.51	0.56	5.73	4.24
Significance level	ns	***	***	***	***	***	ns	***	*

Mean values within the same row with different superscript letters are different; (*)- $p < 0.05$, (***)- $p < 0.001$ and ns- non significant $p > 0.05$; SCC: Somatic Cell Count, SNF: Solid not fat, EC: Milk electrical conductivity, FP: Milk freezing point, DIM: days in milk, SEM: standard error of mean

3.4 Milk composition and characteristics as per milking methods

Milking methods followed had significant effect on some parameters of milk composition and characteristics (mean and SEM) in tie stall dairy cattle at Chitwan, Nepal (Table 4). The milk SNF (8.98), protein (3.27) and lactose (4.94) were significantly higher ($p < 0.001$) when milking is accomplished by using milking machine. In contrast, milk electrical conductivity (4.52 mS/cm) was higher ($p < 0.05$) in milk when hand milking is performed.

Table 4: Milk composition, characteristics and $\log_{10}$ SCC based on milking methods in tie stalls dairy cattle at Chitwan, Nepal

Factors	Hand milking	Machine milking	SEM	F-value	Sig. level	95% CI	
						Hand	Machine
Fat %	5.36	5.16	0.10	2.13	ns	-	-
SNF %	8.85^{b}	8.98^{a}	0.03	8.30	***	8.77- 8.92	8.77- 8.92
Protein %	3.21^{b}	3.27^{a}	0.01	14.75	***	3.18- 3.24	3.18- 3.24
Lactose%	4.84^{b}	4.94^{a}	0.02	15.23	***	4.80- 4.88	4.80- 4.88
EC	4.52^{a}	4.42^{b}	0.04	4.04	*	4.44- 4.59	4.44- 4.59
pH	6.42	6.47	0.02	3.45	ns	-	-
FP (-ºC)	0.536	0.539	0.001	2.02	ns	-	-
Log10 SCC	5.27	5.25	0.03	0.08	ns	-	-

Mean values within the same row with different superscript letters are different; (***)- $p < 0.001$, (*)- $p < 0.05$ and ns- non significant $p > 0.05$, SCC: Somatic Cell Count, SNF: Solid not fat, EC: Milk electrical conductivity, FP: Milk freezing point, SEM: standard error of mean, CI: confidence interval

3.5 Milk composition, characteristics and log10 SCC in different breeds of dairy cow

The milk composition, characteristics and $\log_{10}$ SCC (mean and SEM) in different breeds of dairy cattle in tie stall management at Chitwan, Nepal has been presented in Table 5. Accordingly, the milk SNF content was higher ($p < 0.05$) in Lulu cattle (9.11) than that in Jersey (8.94) and HF cattle (8.90); milk protein (3.33) and lactose content (5.01) too followed the same tendency and were significantly higher ($p < 0.001$) in Lulu cattle. However, milk EC was significantly higher ($p < 0.001$) in HF (4.54) than that in Lulu (4.01) but was similar with that of Jersey cow (4.47). The milk SCC was found significantly higher ($p < 0.001$) in Jersey cows (5.34) than both HF and Lulu and the lowest score was evident in Lulu cow milk (Table 5).

Table 5: Milk composition and characteristics at udder level (mean ±SE) in different breeds of dairy cattle in tie stall management at Chitwan, Nepal

Factors	Jersey	HF	Lulu	SEM	F-value	Sig.	95% CI		
							Jersey	HF	Lulu
Fat %	5.18	5.22	5.46	0.15	0.82	ns	-	-	-
SNF %	8.94^{b}	8.90^{b}	9.11^{a}	0.04	3.78	*	8.87- 9.02	8.83- 8.96	8.97- 9.26
Protein %	3.26^{b}	3.23^{b}	3.33^{a}	0.01	6.21	***	3.23- 3.28	3.21- 3.25	3.28- 3.38
Lactose%	4.92^{b}	4.88^{b}	5.01^{a}	0.02	5.13	***	4.88- 4.96	4.84-4.91	4.93- 5.09
EC	4.47^{a}	4.54^{a}	4.01^{b}	0.04	31.18	***	4.41- 4.53	4.49- 4.59	3.89- 4.13
pH	6.48	6.43	6.43	0.02	2.19	ns	-	-	-
FP (-ºC)	0.542	0.536	0.532	0.002	2.46	ns	-	-	-
Log10 SCC	5.34^{a}	5.22^{b}	5.11^{b}	0.03	5.56	***	5.27- 5.41	5.15- 5.28	4.97- 5.25

Mean values within the same row with different superscript letters are different; (***)- $p < 0.001$, (*)- $p < 0.05$ and ns- non significant $p > 0.05$, SCC: Somatic Cell Count, SNF: Solid not fat, EC: Milk electrical conductivity, FP: Milk freezing point, SEM: standard error of mean, CI: confidence interval

4 Discussion

Feed-offer-time based feeding strategies had affected the milk composition, characteristics and SCC in dairy cows in this research. Pre-milking feeding management produced the highest milk fat content while SNF, including milk protein and lactose were rich in late post-milking feeding management, i.e. 30 minutes post-milking feeding. Because timing of feed-offer is considered the common adjuster of circadian rhythms in dairy cow (Piccione *et al.*, 2007; Schütz *et al.*, 2023), feeding strategies along with numerous environmental signals might modify the rate of milk synthesis, thereon the composition and properties of milk (Rottman et al., 2014; Svennersten *et al.*, 1995). All three parameters associated with milk properties (EC, pH and FP) were within the normal range on all feed-offer based treatments, but during milking feeding and immediate post-milking feeding strategies well-tuned EC, pH and FP. However, Johansson *et al.* (1999) reported increased milk production, milk flow and milking duration when cows were fed during milking. The possible explanation of these results might be associated with the altered time of feeding that had remodeled feeding deeds of dairy cows and the post-feeding patterns of rumen pH, volatile fatty acids and ammonia production. Such changes in patterns of rumen digestion and nutrient delivery subsequently modify the release of metabolites and alter their peripheral blood concentrations

(Plaizier *et al.*, 2005; Robinson *et al.*, 2002), which ultimately influence nutrient utilization throughout the day regardless of feed delivery times (Sehgal, 2004). Finally, all these physiological turnovers that were clicked and tuned by the changes in feeding time of milking cow result in variations in nutrient composition and characteristics as well as in overall milk quality. Aharoni *et al.* (2005) reported that switching to the evening feeding, instead morning, can reduce heat stress and enhance both feed efficiency and lactation persistency in cows. This might be linked with delivery of fresh feed, coupled with resting period of night in view of ambient temperature, that prompts eating and influences daily eating patterns in lactating cows (DeVries et al., 2003).

The feeding strategies, particularly offering green fodders immediately after milking (+iPMF) or 15 minutes after milking (+15PMF), consistently resulted in lower SCC in this study, indicating a reduced risk of udder infections. This decrease in SCC is likely attributed to the post-milking feeding plans employed, which encouraged the study animals to spend more time eating and thus keep on foot for longer after milking. Previous studies have extensively documented how the provision of fresh green feeds stimulate cow feeding behavior and encourage longer standing times after milking (Bharti & Bhakat, 2019; DeVries *et al.*, 2010; Tyler *et al.*, 1997; Watters et al., 2013). These findings align with the study by Bharti & Bhakat (2019) which observed a correlation between higher post-milking standing durations and lower SCC, although they compared a 15-minute versus 90-minute post-milking feeding strategy to assess SCC and intramammary infections. Similarly, Watters et al. (2013) reported a connection between the standing period after milking and the incidence of elevated somatic cell count (eSCC), noting that cows lying down after 90 minutes post-milking for the first time had a reduced risk of developing a new eSCC compared to those lying down before 90 minutes post-milking. Additionally, DeVries *et al.* (2010) found that cows standing for 40 to 60 minutes after milking had a lower risk of acquiring a new intramammary infection. The lower SCC noticed in cows fed immediately or 15 minutes following milking in this study might be explained by the fact that cows stood longer after milking, providing extended period for phenomenal involution of teat orifices and canals, thereby reducing the chances of contact between the teat and associated structures with stall surface, which significantly reduced the risk of infection (Shultz, 1985).

Electrical conductivity (EC) and freezing point (FP) of milk were influenced remarkably by parity but milk fat and SNF (protein, lactose and ash) too with EC and FP were significant due to stage of lactation in this study. The milk EC and FP was higher in early parity, showed decreasing trend till sixth parities but peaked back to the comparable level as with early parities afterwards. However, both EC and FP increased with advancement of lactation and were higher in late lactation. These findings were comparable with the report by Syridion *et al.* (2012) as the authors described higher EC in cows later parity but they did not find any changes in milk composition due to stages of lactation. Matera *et al.* (2022) reported low EC in primiparous buffalo up to 3rd-parity for the complete lactation and described that EC increased across the lactation in multiparous buffalo from 90 DIM onwards. In addition, Paudyal *et al.* (2020) reported relationship of EC and number of parities in dairy cows and explained that the cows in early parity had highest EC and it is much lower in mid parity ($> fourth$). These results are obvious due to the fact that EC, a measure of milk's resistance to the flow of electricity, is influenced by concentration of sodium, potassium, and chloride ions (Panchal *et al.*, 2016; Paudyal *et al.*, 2020) and the levels of these ions were higher in terminal lactation and are more pronounced in primiparous animal. The changes of FP due to lactation stage was in partial agreement with Henno *et al.* (2008) who reported significant increase up to the third month and then decreased as the lactation progressed. The authors recorded the highest FP during the second- and third-months coupled with lowest level of milk protein.

The findings of lactation stage effect on milk composition were in accordance to Shuiep *et al.* (2016) who observed significant changes on fat, SNF and lactose coupled with higher fat in late stage of lactation. Mishra *et al.* (2022) and Gajbhiye *et al.* (2019) discussed stage of lactation as determinant on fat and protein content in Sahiwal and on fat and SNF in Kankrej but they did not observe changes in EC, pH and FP during different stages of lactation. In addition, higher protein and fat content (Parmar et al., 2020) as well as protein (Jozwik *et al.*, 2012) in Polish HF were reported in late lactation stage. However, Shibru *et al.* (2019) reported negligible changes in fat content, but a significant effect in protein content in HF crossbreds due to stages of lactation. Fat content during different lactation stages may vary due to energy deficit, particularly noticeable in early lactation and more pronounced towards the end (Vanbergue *et al.*, 2017). Milk composition, including lactose synthesis, is influenced by water influx, with consistent lactose and water secretion rates throughout lactation (Pollott, 2004). Late lactation stages exhibit higher nonfat components in milk SNF, attributed to increased minerals, proteins, and lactose, possibly due to changes in milk composition as volume decreases (Gurmessa & Melaku, 2012; Hickey et al., 2006).

The milk SCC increased with advancement of days in milk and parity, thus the cows in mid lactation and late parity had significantly higher log10 SCC. These findings are in perfect match with Stocco *et al.* (2023) as the authors documented the highest SCC or somatic cell traits in aged multiparous cows at the terminal stage of lactation. They observed the trend of SCC across the lactation and parities showing the greater risks of higher SCC in older cows towards the end of lactation. Syridion et al. (2012) described the linear relationship of parity and milk SCC with increasing SCC on advancing parity (1.31 in first and 1.76 in above fourth parity), but they observed numerically higher SCC in late lactation. In addition, Matera *et al.* (2022) recently opined that the progression of SCC (SCS, somatic cell score) is steady until mid-lactation stage for all parity and afterwards the rate of increase changed according to parity in buffalo. Laevens *et al.* (1997) also reported the significant effect of parity and stage of lactation on milk SCC in dairy cow and they pictured out that the SCC increases with advancing parity and is specifically much higher during mid lactation and above.

The milk SNF, protein and lactose were significantly higher on machine milked samples while milk EC was higher in hand milked one. Milking methods, hand or machine milking, were found to exert non-significant effect on other constituents (fat, SCC, FP). The fact that mechanism of work of milking machine mimics to that of hand milking for ejecting out milk from udder might be the reason for these results with almost similar characteristics of milk. Our results are in line with Biradar et al. (2018) as the authors had reported higher SNF and Protein in milk obtained by machine milking and larger SCC in hand milking but fat content was similar. Dang & Anand (2007) reported higher lactose and SCC in milk by hand milking but fat and protein content were non-significant. In contrast, Filipovic & Kokaj (2009) found negligible changes in milk constituents and mean SCC at different milking methods. In addition, Sinapis (2007) documented the similar findings in mountainous Greek ewes and reported similar composition but lower log SCC in machine milked groups.

The milk composition except fat, EC and SCC varied among three cattle breeds, Lulu cattle contained higher SNF, protein and lactose but the lowest EC. In contrast, milk SCC was found significantly higher in Jersey and the lowest was evident in Lulu cow. Mishra *et al.* (2022) demonstrated significant breed effects of fat and SNF but so in other milk parameters were indifferent which is partially in agreement with this finding. The significant changes in SNF in this study is in line with Bobbo et al. (2014), Falta *et al.* (2014) and Kedzierska-Matysek et al. (2011). However, Myburgh *et al.* (2012) found non-significant breed effect in protein content and remarkable alterations in lactose content as with this study while working with African cattle. The pictures of changes in milk EC among breeds might be associated with the udder health status of the cow as it is considered as a proxy indicator of SCC and is higher in Jersey. Stocco *et al.* (2020) discussed the higher influence of breed of cow on SCC and reported the higher SCC (SCS) in HF with clear facts of the highest SCC in aged multiparous cows at the terminal stage of lactation. The higher content of all the milk constituents, though fat content were numerically rich, and cleanest level of SCC were found in Lulu cows as expected, because of its indigenous genetic make-up and lower level of production. Surprisingly, Jersey cows experienced higher SCC than HF.

Conclusion

The feeding time, breed, parity, lactation stages, and milking methods altered various milk constituents and SCC in dairy cows. Feeding strategies, particularly during-milking feeding, immediate post-milking feeding resulted in lower SCC, and reduced the risk of intramammary infections. Parity and lactation stages influenced milk composition, with early parity cows exhibiting lower SCC compared to mid and late parities. Moreover, milk composition varied throughout lactation, with late lactation characterized by higher fat (numerical increment), protein and lactose but lower milk volume. Milking methods was indifferent in terms of SCC but use of machine milking yielded higher SNF, protein and lactose content compared to hand milking. Breed differences are evident, with Lulu cows exhibiting higher SNF, protein, and lactose compared to Jersey and HF cows. Our research findings thus provided valued insights into the factors influencing milk quality and udder health in dairy cows, which could be inferred for a better management practice aimed at optimizing quality milk production.

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Competing Interests

Authors have no competing interests.

Author Contributions

Conceptualization: DKC and NRD; methodology: DKC, NRD, HBB and DBK; investigation: DKC; formal analysis: DKC, NRD; data curation: DKC, NRD, HBB and DBK; writing original draft preparation: DKC; writing review and editing: DKC, NRD, HBB and DBK; supervision: NRD. All authors have reviewed and consented to the final version of submitted manuscript.

Ethical approval

The study did not involve approaches that challenge well-being and welfare of dairy cows; we, authors, hereby declare that Principles of laboratory animal care as well as specific national laws were followed, where applicable.

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