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Effects of milking methods on test-day milk yield and milk quality traits in early lactating multiparous buffalo cows and the relationships among these parameters

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Abstract

Buffaloes are not fully adapted to machine milking because they are generally milked manually. Therefore, a comprehensive examination of the relationships between milking practices and productivity is critical to obtain high quality milk products. The aims of the present study were i) to investigate the effects of different milking methods on test-day milk yield (TDMY) and milk quality traits [dry matter (DM), fat, solids-not-fat (SNF), protein, fat-to-protein ratio (FPR), lactose, density, freezing point (FP), somatic cell count (SCC), pH and electrical conductivity (EC) in multiparous Anatolian buffalo cows and ii) to investigate the relationships among these milk quality traits. The study material consisted of 120 milk samples collected from 60 buffalo cows machine and hand milked in five barns at the same location on a family farm. The collected milk samples were analysed for their milk components (DM, fat, SNF, protein lactose, density and FP), SCC, pH value, and EC value using the following devices: i) a milk analyser, ii) a portable somatic cell counter, iii) a pH meter, and iv) an EC-meter equipped with a conductometric sensor. The statistical analyses were conducted using *t-test* and Pearson Correlation procedures. The quantitative (DM, fat, protein, density, SCC, EC, and pH)-qualitative (TDMY) milk parameters of hand-milked cows was found to be higher than that of machine-milked cows. Furthermore, a positive correlation was found between SCC and pH and EC values. It was determined that milk DM, SNF and lactose percentages decreased as TDMY value increased. The SCC and EC displayed a negative correlation with the milk fat percentages, and a positive correlation with milk mineral percentages. Although manual milking improves quantitative-qualitative milk parameters in buffalo cows, it may pose more risks to their udder health and milk hygiene. Therefore, the advantages and disadvantages of current milking practices should be carefully considered.

Key Words: *Buffalo cows, hand milking, machine milking, quantitative-qualitative milk parameters*

Introduction

It is evident that buffalo milk, which has high nutritional content, has recently attracted the consumers' interest because it can be transformed into high value-added products (Akdağ et al., 2024). Therefore, it is important to improve the quality and quantity traits of milk produced from buffalo cows (*Bubalus bubalis)*. The quantitative-qualitative milk parameters of buffalo cows (Okuyucu et al., 2024; Akdağ et al.,2024) can be influenced by i) resource-based factors (e.g. space allocation, barn facilities, soil and climatic factors), ii) management-based factors (e.g. handling strategies, breeding strategies, milking practices and health plan) and iii) animal-based factors (e.g. body condition, udder hygiene, lameness and milking temperament). Particularly, milking practices are important for buffalo cows because buffaloes are sensitive to non-routine practices and environmental stimuli (Napolitano et al., 2013). This process can cause considerable stress to buffalo cows (Erdem et al., 2022). As buffalo farming is predominantly extensive worldwide (Erdem et al., 2022), manual milking is a common practice. However, with increasing intensification, the use of mechanical milking on dairy farms has also increased. Therefore, the advantages and disadvantages of milking methods applied to buffalo cows should be carefully considered. Since manual milking is one of the most labour- and time-consuming practices, manual milkers may encounter health problems such as stiff shoulders, pain and weakness in the knee joints and spine (Khatri et al., 2021). In addition, contamination of the cow's teat in manual milking can result in an increase in microorganisms in milk. Although machine milking, which is another milking method, is beneficial in terms of milk hygiene and reducing labour use, the responsiveness of buffalo cows to machine milking may quantitative [dry matter (DM), fat, solids-not-fat (SNF), protein, fat-to-protein ratio (FPR), lactose, density, freezing point (FP), somatic cell count (SCC), pH and electrical conductivity (EC)]-qualitative [test-day milk yield (TDMY)] milk parameters (Erdem et al., 2022; Okuyucu et al., 2024; Akdağ et al.,2024). Therefore, milking cows under hygienic conditions with milking methods that provide optimum welfare conditions is the most critical management practice for quality milk production.

A large body of authors has reported that effective milk extraction plays an important role in milking techniques and procedures and can influence milk yield and its components (Argov-Argaman, 2019; Couvreur and Hurtaud, 2017; Palii et al.,2020). However, the literature on the effects of different milking methods on TDMY and milk components of buffalo cows is limited. The aims of this study were i) to investigate the effects of different milking methods on quantitative (DM, fat, SNF, protein, FPR, lactose, density, FP, SCC, pH and EC)-qualitative (TDMY) milk parameters and ii) to investigate the relationships among milk parameters.

Materials and Methods

This study was conducted in five barns at the same location on a family farm in Samsun, located in the Black Sea Region of Türkiye. The study material consisted of 120 milk samples collected from 60 multiparous buffalo cows machine and hand milked in barns (closed-tied-stall barns). The cows included in the study were cows of parity 2 (n= 10), 3 (n=10) and 4 (n=10). The data were collected during once per week visits to the farm between January 2025 and February 2025.All cows were not grazed on pasture and were fed ad libitum with a TMR in the barn. The TMR available consisted of wheat straw, maize silage and concentrate.

Routine practices on the farm were not changed throughout the study. The farm were visited and milk was collected twice during the study. Manual and machine milking practices were as shown in Table 1.

Table 1. Manual and mechanical milking processes

Milking methods	Milking processes		
	Milking processes	Pre-milking processes	Post-milking processes
Manual	Cows milked manually by two stockperson	Calves suckled their dams for approximately two minutes. Then, the cow's udders were washed with fresh water and dried with a clean cloth.	After the milking process no iodine teat dip was applied.
Machine	Each cow was milked individually with a portable milking machine (PLS-2/1; Sezer, Bursa, Türkiye).		

At the end of milking, the milk samples were weighed on an electronic scale and the TDMY values were determined. Approximately 50 ml of milk samples were then collected in plastic milk tubes. These samples were transported to the laboratory at 4°C to evaluate the content of milk components and physical traits. All analyses were performed when the milk was at 30°C to 32°C. The milk components (milk fat, protein, lactose, and mineral percentages), and physical traits [density (mg/mL), and the FP (°C)] of the milk samples were analyzed using an milk analyzer (Lactostar, Funke-Gerber, Germany). Furthermore, FPR were calculated as fat percentage/protein percentage. The milk EC (mS/cm) and pH were measured using an EC-meter equipped with a conductometric sensor (FiveEasy Plus, Mettler Toledo, Switzerland) and pH meter (Testo 205), respectively. The SCC value of the milk was determined using a portable cell counter (DeLaval, Tumba, Sweden).

The statistical analyses were conducted using the SPSS software program (version 21.0, SPSS Inc., Chicago, IL, USA). Prior to analysis, the normality and variance homogeneity of data from test-day records of quantitative (DM, fat, SNF, protein, lactose, mineral, density, FP, SCC, EC, and pH)-qualitative (milk yield) milk parameters were screened using the Kolmogorov-Smirnov and Levene's tests, respectively. The logarithmic transformation procedure was applied to the SCC data, which is one of the available milk quality traits, because the data did not show a normal distribution.

To determine the effects of milking practices on quantitative-qualitative milk parameters, *t-test* procedure was applied. To this end, the cows were allocated to ensure that an equal number of cows were represented in each group [machine (n=30) and manual (n=30) milking]. Correlation coefficients among these traits were calculated by applying Pearson correlation procedure.

Results and Discussion

In the present study, the mean values of quantitative-qualitative milk parameters of Anatolian buffalo cows in early lactation were as shown in Table 2. In previous studies on buffaloes, the mean TDMY (5.37 kg, Tripaldi et al., 2010; 5.7 kg, Şekerden, 2011; 3.96-4.53 kg, Sarıözkan et al., 2021) value was lower than the results obtained in the current study. However, the current results were higher than those (2.95 kg) reported by Özcan and Erdem, (2022). Furthermore, the milk DM percentage was higher than the findings reported in several studies (16.207-17.230%, Sahin et al., 2014; 14.18-15.67%, Sarıözkan et al., 2021; 17.29% for Nili-Ravi, 18.23% for Murrah, Abdel-Hamid et al., 2023), but lower than in other reported studies (20.18% for Mediterranean, Abdel-Hamid et al., 2023). The findings of this study demonstrated a congruence with the outcomes documented in several preceding studies, particularly with regard to the percentage of fat in milk (Özcan and Erdem, 2022; Yuan et al., 2022). Similarly, the milk protein percentage was also consistent with those reported in previous studies (4,515-4,955% for Anatolian Buffalo, Sahin et al., 2014; 4.38 % for Nili-Ravi, Abdel-Hamid et al., 2023). The percentage of milk lactose was higher than reported reported by Tripaldi et al. (2010). However, the percentage of lactose was lower than that reported in other studies (Özcan and Erdem, 2022; Yuan et al., 2022; Abdel-Hamid et al., 2023). Also, the milk mineral percentage was not in agreement with those reported by Özcan and Erdem, (2022).

The density value, which is one of the physical traits of milk, was consistent with those reported in previous studies (Sarıözkan et al., 2021; Özcan and Erdem, 2022). However, the milk FP value was not in agreement with those reported in previous studies (Özcan and Erdem, 2022; Okuyucu et al., 2024; Akdağ et al., 2024). The SCC value was lower than those reported in previous studies (Sarıözkan et al., 2021; Viana et al., 2024) but higher than those reported in others studies (Özcan and Erdem, 2022; Yuan et al., 2022). The milk pH value was consistent with those reported in previous studies (Şekerden and Avşar, 2008; Tripaldi et al., 2010). Similarly, the milk EC values were also in accordance with those reported in previous studies on Anatolian buffaloes (Okuyucu et al., 2024; Akdağ et al., 2024).

Table 2. Means test-day milk yield and milk quality traits of Anatolian buffalo cows

Variables	Number of records	Mminumum	Maximum	Mean	SE
Milk yield trait (kg)					
TDMY	120	2.00	4.78	3.38	0.066
Milk components (%)					
DM	120	14.99	23.46	18.62	0.241
Fat	120	6.10	12.35	8.48	0.169
SNF	120	7.13	13.39	10.14	0.160
Protein	120	3.00	6.09	4.40	0.093
FPR	120	1.19	4.00	1.98	0.060
Lactose	120	3.36	6.61	4.94	0.740
Mineral	120	0.45	0.98	0.65	0.013
Milk physical traits					
Density (mg/ml)	120	1.00	1.06	1.03	0.001
FP (˚C)	120	-0.42	-0.87	-0.60	0.014
SCC (x10^3 cells/ml)	120	10	347	76.4	7.333
logSCC	120	4.00	5.54	4.78	0.042
pH	120	6.23	7.00	6.59	0.027
EC (mS/cm)	120	3.00	5.30	3.50	0.070

TDMY: Test-day milk yield; DM: Dry matter; SNF: Solids-not-fat; FPR: Fat-to-protein ratio; SCC: Somatic cell count; FP: Freezing point; EC: Electrical conductivity

The TDMY obtained from machine milked cows was lower than those milked manually (*p*=0.018; Table 3). Similarly, the DM (*p*=0.028), fat (*p*=0.043) and protein (*p*=0.047) precentages and density (*p*=0.029) in the milk of machine milked cows was lower than that of hand milked cows. Although the design of this study was not suitable to prove the following hypotheses, the higher TDMY value, DM, fat and protein content in hand milked cows compared to machine milked cows may be related to i) negative effects of machine milking on oxytocin release mechanism of cows, ii) high reactivity (increase in behavioural responses) to machine milking and iii) machine milking equipment not being suitable for buffalo udder physiology (Okuyucu et al., 2024; Ahmet et al., 2024). It was a remarkable finding that milk SCC (*p*=0.049), pH (*p*=0.048) and EC (*p*=0.038) values, which are considered important indicators of udder health and milk quality, were higher in hand milked cows compared to machine milked cows. In a previous study on dairy cows, it was reported that the number of microorganisms (microorganisms x 1000/ml) in the milk of cows milked by hand was higher than that of cows milked by machine (Filipovic and Kokaj, 2009). In the same study, it was reported that the several milk components (fat and protein percentages) and SCC values of hand-milked cows were comparatively higher than those of machine-milked cows, although this difference was not statistically significant. Simlarly, milking methods (manual and machine milking) did not statistically affect milk components and SCC values (Sarıözkan et al., 2021; Li et al., 2024). The results obtained suggest that milk yield and the content of some milk components are positively influenced, whereas udder health (high SCC, pH and EC values) and milk hygiene may be negatively influenced.

Table 3. Means test-day milk yield and milk quality characteristics of cows according to milking practices

Variables	Milking methods		SEM	*p*-value
	Machine	Manual		
Milk yield trait (kg)				
TDMY	3.23	3.53	0.066	0.018
Milk components (%)				
DM	18.09	19.14	0.241	0.028
Fat	8.14	8.82	0.169	0.043
SNF	9.96	10.32	0.160	0.254
Protein	4.22	4.57	0.093	0.047
FPR	2.0	1.96	0.060	0.745
Lactose	4.91	4.98	0.740	0.629
Mineral	0.64	0.66	0.013	0.718
Milk physical traits				
Density (mg/ml)	1.02	1.03	0.001	0.029
FP (˚C)	-0.60	-0.61	0.014	0.838
SCC (x10^3 cells/ml)	61.5	91.2	7.333	0.049
pH	6.53	6.64	0.027	0.048
EC (mS/cm)	3.4	3.7	0.070	0.038

TDMY: Test-day milk yield; DM: Dry matter; SNF: Solids-not-fat; FPR: Fat-to-protein ratio; SCC: Somatic cell count; FP: Freezing point; EC: Electrical conductivity

The TDMY value of buffalo cows displayed a negative correlation with the milk DM (*p*=0.041), SNF (*p*=0.008), and protein percentages (*p*=0.005; Table 4).

Table 4. Correlations between the qualitative milk trait (TMDY) and quantitative milk traits examined

	DM	Fat	SNF	Protein	FPR	Lactose	Mineral	Density	FP
TDMY	-0.313 (*p*=0.041)	-0.040 (*p*=0.760)	-0.339 (*p*=0.008)	-0.201 (*p*=0.123)	0.093 (*p*=0.481)	-0.359 (*p*=0.005)	0.026 (*p*=0.843)	0.189 (*p*=0.148)	-0.144 (*p*=0.274)

TDMY: Test-day milk yield; DM: Dry matter; SNF: Solids-not-fat; FPR: Fat-to-protein ratio; SCC: Somatic cell count; FP: Freezing point; EC: Electrical conductivity

The SCC displayed a negative correlation with the milk fat percentages (*p*=0.030), and a positive correlation with milk mineral percentages *(p*=0.041; Table 5). Similarly, the EC values correlated positively with the mineral percentages (p=0.026) and negatively with the fat percentages (*p*= 0.003), FPR (*p*=0.024), and density values (*p*= 0.020). However, no statistically significant relationship was found between quantitative-qualitative milk parameters and pH (Table 5). High SCC in milk causes changes in milk enzymes. This may result in the breakdown of milk fat globules. The aforementioned statements elucidate the mechanism responsible for the observed decline in milk fat percentages concomitant with elevated SCC (Aytekin and Boztekin, 2014). Also, the SCC increases due to damage in the mammary tissue, resulting in increased Na+ and Cl concentrations (Aytekin and Boztekin, 2014). Thus, a high mineral content can lead to a significant increase in the EC value of milk.

Table 5. Correlations between milk components and SCC, pH and EC parameters

	SCC	pH	EC
TDMY	-0.068 (p=0.606)	-0.116 (p=0.375)	-0.030 (p=0.818)
DM	0.159 (p=0.225)	0.148 (p=0.258)	0.210 (p=0.107)
Fat	-0.280 (p=0.030)	-0.238 (p=0.067)	-0.383 (p=0.003)
SNF	-0.057 (p=0.668)	-0.028 (p=0.831)	-0.088 (p=0.506)
Protein	0.054 (p=0.684)	0.009 (p=0.944)	-0.048 (p=0.715)
FPR	-0.127 (p=0.335)	-0.119 (p=0.363)	-0.292 (p=0.024)
Lactose	-0.046 (p=0.729)	0.023 (p=0.864)	-0.012 (p=0.927)
Mineral	0.265 p=0.041	0.177 (p=0.177)	0.287 (p=0.026)
Density	-0.206 (p=0.115)	-0.063 (p=0.634)	-0.299 (p=0.020)
FP	0.018 (p=0.892)	-0.092 (p=0.484)	0.071 (p=0.591)

TDMY: Test-day milk yield; DM: Dry matter; SNF: Solids-not-fat; FPR: Fat-to-protein ratio; SCC: Somatic cell count; FP: Freezing point; EC: Electrical conductivity

A positive correlation was found between SCC and pH values (*p*<0.001). Furthermore, the highest correlation coefficient was calculated between SCC and EC (r = 0.710; *p*<0.001; Table 6). Similarly, in a study conducted on Mediterranean buffalo cows (Tripaldi et al., 2003), it was reported that milk pH increased as SCC increased. In a another study on Murrah cross buffaloes, a positive correlation was found between SCC and EC values (Dhakal et al., 2008). The findings were consistent with those reported in previous studies (Tripaldi et al., 2003; Dhakal et al., 2008). The present results indicate that pH and EC parameters can be reliably used to indirectly assess udder health and SCC.

Table 6. Correlations between SSC, ph and EC values

	pH	EC
SCC	0.511 (*p*<0.001)	0.710 (*p*<0.001)
pH		0.382 (*p*=0.003)

SCC: Somatic cell count; FP:Freezing point; EC: Electrical conductivity

In the present study, hand milked buffalo cows had higher milk yield and some milk components but higher SCC, which are considered important indicators of milk hygiene and udder health, compared to machine milked buffalo cows. Although available evidence suggests that manual milking improves milk yield and milk composition, it also poses a risk to milk hygiene and udder health. Also, manual milking, a common practice on buffalo dairy farms, is one of the most labour- and time consuming practices. In this regard, manual milkers may encounter health problems such stiff shoulder, pain in knee joints and backbone and weakness (Khatri et al.,2021). Therefore, the advantages and disadvantages of differences in the milking methods of buffalo cows should be carefully examined. Furthermore, the effects of these methods on both animal welfare and the quality of the human-animal interaction should be investigated in detail.

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