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Short Paper

Milking reactivity influences daily yield and electrical resistance of milk in Jaffarabadi buffaloes

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Abstract

Background: Buffalo reactivity during milking affects milking procedures, milk yield, and quality. **Aims:** This study evaluated the influence of milking reactivity on the yield, composition, somatic cell count, pH, and electrical resistance of milk in Jaffarabadi buffaloes. **Methods:** A 1-4 point scale, based on leg movement, was used to assess the milking reactivity of buffaloes (n=40). Based on the milking reactivity score, animals were classified into four groups: reactivity score-1 (RS1), reactivity score-2 (RS2), reactivity score-3 (RS3), and reactivity score-4 (RS4). The influence of milking reactivity on yield, composition, somatic cell count, pH, and electrical resistance of milk was observed. **Results:** Buffaloes with RS1 and RS2 produced significantly ($P \leq 0.001$) higher daily milk yield, 6% fat-corrected yield, solid-corrected yield, and energy-corrected yield than the RS3+4 group. Milking reactivity score did not influence milk fat, protein, lactose, ash, solid-not-fat, total solids content, and the fat: protein ratio. However, daily yield of milk fat ($P<0.001$), protein ($P=0.001$), lactose ($P=0.001$), ash ($P=0.002$), solid-not-fat ($P=0.001$), and total solids ($P<0.001$) were significantly higher in buffaloes in the RS1 and RS2 groups than in the RS3+4 group. Milk somatic cell count and somatic cell score were not influenced by milking reactivity score ($P>0.05$). Milk density and pH did not differ significantly ($P>0.05$) between groups. However, the electrical resistance of milk in the RS1 group was significantly ($P<0.05$) higher than in the RS2 and RS3+4 groups. **Conclusion:** Milking reactivity influences daily milk yield, milk component yield, and electrical resistance, but not milk composition in Jaffarabadi buffaloes.

Key words: Electrical resistance, Jaffarabadi buffaloes, Milking reactivity, Milk yields

Introduction

Contribution of water buffalo to global milk production has increased significantly in the last few decades (Zicarelli, 2020). The major share comes from well-established breeds of water buffalo, namely Murrah, Nili-Ravi, Bhadawari, Surti, Mehsana and Jaffarabadi. Response or reactivity of animals to surrounding environment is called temperament, and farmers are keen to have animals with good temperament which enables safe and easy handling (Broucek *et al*., 2008). Water buffaloes possess semi-wild-type behaviour and are more sensitive to minor alterations of the surrounding micro-environment particularly during milking and handling (Borghese *et al*., 2007). Furthermore, the milking reactivity score is used to assess the temperament of animals during milking. Animal temperament has been reported to influence productivity and milk composition (Carvalhal *et al*., 2017). Hence, milking reactivity scores can be used for selection of animals for breeding to augment productivity.

A previous report highlighted that those cattle exhibiting calm responses at milking possess better milk yield and lower residual milk (Haskell *et al*., 2014). Similarly, milking reactivity has been reported to interfere with milk yield in various water buffalo breeds (Singh *et al*., 2016; Choudhary *et al*., 2017). However, few reports have been published on the association between milking temperament and milk quality, and the literature lack a consensus. Carvalhal *et al*. (2017) reported significant influence of temperament on milk fat content and somatic cell count (SCC) in purebred Murrah buffaloes and Murrah × Jaffarabadi crossbred buffaloes. However, Singh *et al*. (2016) reported a significant effect of temperament on SCC but a non-significant effect on milk fat content in Murrah buffaloes. Breed probably has an effect of on temperament and milking reactivity in water buffaloes.

The Jaffarabadi buffalo is a high-yielding (milk yield of approximately 2500 L per lactation; Savsani *et al.* (2015)) native breed of the Gir forest region of Gujarat state in India and possesses different temperaments compared with other domesticated breeds of water buffaloes. Limited literature is available on the temperament or milking reactivity of Jaffarabadi buffalo and no report has been published on the influence of milking reactivity on milk yield and quality in Jaffarabadi buffaloes. Hence, this study was designed to evaluate the effect of milking reactivity on yield, composition, SCC, pH, and electrical resistance of milk in Jaffarabadi buffaloes. This study may help in designing selection criteria of animals with docile temperament to augment the productive performance of Jaffarabadi buffalo herd.

Materials and Methods

The present study was conducted on Jaffarabadi buffaloes (n=40) at the Cattle Breeding Farm, Junagadh. Mean values of average air temperature ranged from 29.6 to 32.4°C and relative humidity ranged from 32.2 to 68.3% during the study period (1st March to 30th June 2022). The minimum temperature ranged from 13.1 to 37.6°C, while maximum temperature ranged from to 29.6 to 43.6°C across different months during the same period. The temperature humidity index [THI = (1.8 × air temperature + 32) – {(0.55 - 0.0055 × relative humidity) × (1.8 × air temperature - 26.8)}] varied between 76.3 and 83.3. The mean parity of the experimental buffaloes was 2.45 ± 0.18, and the mean number of days in milk (DIM) was 113.25 ± 6.70. Buffaloes used in the study had an average to good body condition score and moderate to good milk production performance (5-15 L/day). Animals were maintained under a loose housing system, and artificial insemination was performed to breed the animals. Animals were fed seasonal green fodder (10 kg per animal per day) and *ad libitum* dry fodder. However, concentrate mixture was offered inside the milking parlour according to their production. Water was splashed at 11 am and 3 pm to reduce heat stress. Buffaloes were milked twice a day (4 am and 4 pm) by hand in milking parlor. Animals were checked for udder health status using California mastitis test at the start of milking and only apparently healthy animals free from clinical or subclinical mastitis were included in the study. Milking reactivity score (RS) of buffaloes was assessed based on leg movement (Carvalhal *et al*., 2017) by a trained person, fortnightly. Buffaloes having no movement of hind limbs were assigned reactivity score 1 (RS1), slight movement of hind limbs were assigned reactivity score 2 (RS2), frequent hind limbs movement were assigned reactivity score 3 (RS3), and reactivity score 4 (RS4) was assigned to animals with very frequent hind limbs movement or when hind limbs had to tie by rope to restrain animals.

The daily milk yield of individual buffaloes was recorded by adding morning and evening milk yields. A total of 320 composite milk samples from 40 animals (8 samples from each animal) were collected during afternoon milking at fortnightly intervals. Twenty milliliter milk samples were immediately transported to milk analysis laboratory at Veterinary College, Junagadh, Gujarat, India for estimation of milk composition, somatic cell count (SCC), pH, and electrical resistance. The density of milk and other compositional parameters such as fat, solid not fat (SNF), protein, lactose, and ash were estimated using the "Lactoscan MCC combo" (Milkotronic Ltd., Bulgaria). Milk total solids content was calculated by summing fat and SNF content of milk. Milk SCC was estimated using a 4-channel disposable lactochip and read using the "Lactoscan MCC combo" machine. Milk electrical resistance and pH were measured using a Draminski mastitis detector (Draminski, Gietrzwald, Poland) and Thermo Scientific Orion Star A111 pH meter (Thermo Fisher Scientific, Inc., USA), respectively. Daily 6% fat-corrected milk yield (FCM), solid-corrected milk yield (SCM), and energy-corrected milk yield (ECM) were calculated using standard formulae (Sharma *et al*., 2022).

6% FCM yield (kg) = [((0.4 × milk yield in kg) + (15 × fat yield in kg))/(1.3)]

SCM yield (kg) = [(12.3 × fat yield in kg) + (6.56 × solid-not-fat yield in kg) - (0.0752 × milk yield in kg)]

ECM yield (kg) = [(0.3246 × milk yield in kg) + (12.86 × fat yield in kg) + (7.04 × protein yield in kg)]

Milk SCC values were further log-transformed to obtained somatic cell score (SCS) using the standard formula [milk SCS = $\log_2$ (SCC/100, 000) + 3] given by Matera *et al*. (2022). The SCS values resulted normal distribution of data set and homogeneity of variance. Daily fat, protein, lactose, ash, SNF, and total solid yield of individual animals were calculated by simply multiplying daily milk yield with the respective content of the components.

Statistical analysis

Animals were classified based on reactivity score. As the sample numbers of RS3 and RS4 groups were small, both groups were combined to form three groups: RS1, RS2, and RS3+4 for statistical analysis. The data sets were checked for normality before analysis. The means of milk yield, composition, SCC, pH and electrical resistance among the groups were compared by one-way analysis of variance. The pairwise significant difference between the groups were compared by 'Duncan' post-hoc test. Statistical analyses were carried out using statistical package for the social sciences (SPSS) version 16.

Results

Daily milk yield (kg/day) and corrected yields (kg/day)

Daily milk yield and corrected yields (6% FCM,

Table 1: Milking reactivity scores (RS) and mean daily milk yield, 6% FCM, SCM, ECM, fat, protein, lactose, ash, SNF, and total solids yields of Jaffarabadi buffaloes

Milk traits	RS: 1 (n=126)	RS: 2 (n=168)	RS: 3+4 (n=26)	Overall (n=320)	F-value	P-value
Milk yield (kg/day)***	8.54±0.27^a	7.67±0.25^a	6.15±0.56^b	7.89±0.18	7.085	0.001
FCM yield (kg/day)***	11.15±0.35^a	9.79±0.29^a	7.75±0.67^b	10.17±0.22	9.983	<0.001
SCM yield (kg/day)***	13.89±0.43^a	12.28±0.38^a	9.62±0.84^b	12.69±0.27	9.761	<0.001
ECM yield (kg/day)***	14.40±0.44^a	12.70±0.39^a	10.03±0.87^b	13.15±0.28	9.715	<0.001
Fat yield (g/day)***	739.18±23.79^a	644.58±19.66^a	507.83±44.20^b	670.72±14.82	10.514	<0.001
Protein yield (g/day)***	301.63±9.91^a	272.93±9.59^a	212.70±20.07^b	279.34±6.69	6.683	0.001
Lactose yield (g/day)***	455.77±14.91^a	411.05±14.45^a	321.31±30.25^b	421.37±10.08	6.804	0.001
Ash yield (g/day)**	63.49±2.13^a	57.45±2.08^a	44.85±4.32^b	58.80±1.44	6.305	0.002
SNF yield (g/day)***	830.44±27.11^a	751.86±26.27^a	585.04±55.08^b	769.25±18.33	6.766	0.001
Total solids yield (g/day)***	1569.60±48.26^a	1396.80±44.28^a	1092.90±96.30^b	1440.10±31.81	9.024	<0.001

n: Number of observations, ** P≤0.01, and *** P≤0.001. Means with different superscript (^{a}, ^{b}) in a row differ significantly (P≤0.05)

Table 2: Milking reactivity scores (RS) and milk compositions (fat, protein, lactose, ash, SNF, total solids content, fat: protein ratio, SCC, SCS, density, electrical resistance, and pH) of Jaffarabadi buffaloes

Milk traits	RS: 1 (n=126)	RS: 2 (n=168)	RS: 3+4 (n=26)	Overall (n=320)	F-value	P-value
Fat (%)	8.77±0.12	8.68±0.12	8.56±0.29	8.71±0.08	0.263	0.769
Protein (%)	3.53±0.03	3.51±0.02	3.44±0.05	3.51±0.01	0.968	0.381
Lactose (%)	5.33±0.04	5.29±0.04	5.20±0.07	5.30±0.02	1.086	0.339
Ash (%)	0.742±0.006	0.737±0.006	0.722±0.012	0.737±0.004	0.702	0.496
SNF (%)	9.72±0.07	9.70±0.06	9.46±0.12	9.69±0.04	1.235	0.292
Total solids (%)	18.49±0.09	18.39±0.10	18.02±0.24	18.40±0.06	1.644	0.195
Fat: Protein ratio	2.53±0.06	2.51±0.05	2.52±0.11	2.52±0.03	0.052	0.949
SCC ($\times 10^3$ cells/ml)	103.56±11.57	88.64±8.45	108.92±17.41	96.16±6.51	0.758	0.469
Somatic cell score (SCS)	2.13±0.15	2.02±0.12	2.43±0.34	2.09±0.09	0.740	0.478
Density (g/cm^3)	1.027±0.000	1.027±0.000	1.026±0.001	1.027±0.000	0.337	0.714
Electrical resistance (unit)***	648.73±5.46^a	622.02±5.62^b	598.08±16.01^b	630.59±3.96	8.468	<0.001
pH	6.62±0.01	6.63±0.01	6.60±0.02	6.62±0.007	0.499	0.608

SCC: Somatic cell count, n: Number of observations, and *** P≤0.001. Means with different superscript (^{a}, ^{b}) in a row differ significantly (P≤0.05)

SCM, and ECM) of Jaffarabadi buffaloes are depicted in Table 1. Results showed significant (P≤0.001) influence of milking reactivity score (RS) on daily milk yield, 6% FCM, SCM, and ECM. Difference in means of daily milk yield, 6% FCM, SCM, and ECM were non-significant (P>0.05) in animals having RS1 and RS2, but was highly significant (P<0.001) in RS3+4.

Milk components (%) and daily yield (g/day)

Animals with milking RS1, RS2, and RS3+4 did not show significant (P>0.05) differences in means of fat, protein, lactose, ash, SNF, total solids content, and fat: protein ratio (Table 2). However, milking reactivity had significant influence on the daily yield of milk fat (P≤0.001), protein (P≤0.001), lactose (P≤0.001), ash (P=0.002), SNF (P≤0.001), and total solids (P≤0.001). Daily fat yield of buffaloes with milking RS3+4 was 231 and 137 g/day lower than RS1 and RS2 groups, respectively (Table 1). Similarly, buffaloes with milking RS3+4 had 89 and 60 g/day lower protein yield, 134 and 90 g/day lower lactose yield, 18.6 and 12.6 g/day lower ash yield than the RS1 and RS2 groups, respectively. Furthermore, the SNF yield of buffaloes having milking RS1 and RS2 was, respectively 245 and 167 g/day higher than RS3+4 group whereas the total solid yield was, respectively 477 and 304 g/day, higher than RS3+4 group.

Milk somatic cell count, density, pH and electrical resistance

Milk somatic cell count (SCC) and somatic cell score (SCS) values were not affected by the milking reactivity scores of Jaffarabadi buffaloes. Mean milk density and pH values among buffaloes with different reactivity score did not differ significantly (P>0.05). However, electrical resistance of milk in RS1 group was significantly (P≤0.05) higher compared to RS2 and RS3+4 groups (Table 2).

Discussion

Milk yield (kg/day) and corrected yields (kg/day)

The influence of temperament on milk yield has been reported in water buffaloes such as Murrah (Singh *et al.*, 2016; Choudhary *et al.*, 2017), Surti (Singh *et al.*, 2019), Romanian buffaloes (Mincu *et al.*, 2022), and Anatolian buffaloes (Erdem *et al.*, 2022). These studies reported that buffaloes with docile temperament had higher daily milk yields than those with poor temperament (aggressive and nervous). The daily milk yields of Jaffarabadi buffaloes with milking RS1 and RS2 were significantly higher than that of RS3+4. However, Carvalhal *et al.* (2017) observed higher daily milk yields in water buffaloes (purebred Murrah and crossbred Murrah×Jaffarabadi) with RS1 than with RS2 and

RS3+4. Additionally, we observed that the milking reactivity score influenced 6% FCM, SCM, and ECM in the Jaffarabadi buffaloes.

Higher daily milk yield in docile buffaloes might be attributed to differences in the secretion of circulating hormones particularly prolactin, growth hormone, oxytocin, and cortisol during milking. Singh *et al.* (2016) observed lower level of circulating prolactin and higher level of growth hormone and oxytocin during 2-3 min of milking in docile buffaloes than non-docile buffaloes. Furthermore, in buffaloes with docile temperament, the prolactin hormone remains at a lower level up to 10-11 min, while growth hormone and oxytocin levels were elevated for up to 5-7 min. In nervous or aggressive buffaloes, the cortisol level is also elevated which may prevent milk let-down and increase residual milk, resulting in a lower yield (Singh *et al.*, 2016; Carvalhal *et al.*, 2017). Thus, buffaloes which are more reactive to milking process may release more stress hormones such as adrenaline that hold up milk and prevent it from falling (Borghese *et al.*, 2007). Higher circulating stress hormones like cortisol also alter energy metabolism and may negatively affect milk yield (Carvalhal *et al.*, 2017). The more reactive buffaloes are also non-cooperatives to the milkers and are not completely milked, resulting in lower daily yields. Proper handling and management before calving reduces fear in highly reactive buffaloes (Das *et al.*, 2020). Although, the heritability of milking temperament is low to moderate (heritability range: 0.07-0.53) in dairy bovines (Haskell *et al.*, 2014), careful selection of calm temperamental buffaloes for breeding along with proper management practices might improve dairy productivity.

Milk components (%) and daily yield (g/day)

It is well established fact that temperament affects milk yield but scanty reports are available on the influence of RS on milk composition in water buffaloes. We did not find any significant influence of RS on milk composition (fat, SNF, protein, lactose, ash and total solids) in Jaffarabadi buffaloes. Similarly, Singh *et al.* (2016) reported a non-significant effect of temperament on milk fat, SNF, protein, and lactose percent in Murrah buffaloes. However, Carvalhal *et al.* (2017) reported a significant effect of temperament on milk fat content but not on other components in water buffaloes. Among different milk components, fat is considered the most variable, followed by protein and lactose (Patbandha *et al.*, 2016). It is interesting to note that milk let-down is affected in buffaloes with poor temperament and the udder is not emptied completely, because during stressful condition buffaloes produce adrenaline that inhibits oxytocin circulation (Borghese *et al.*, 2007). Milk composition at the end of milking is 2.5 to 5 times richer in fat content than milk removed at the beginning of a single milking (Lollivier *et al.*, 2002). Hence, incomplete milking may result in variable milk fat content. There is a complex relationship between the behavioural response and milk production in dairy cattle, depending on the criteria used to measure behaviour and productivity, including the breed genetic (Hedlund and Lovlie, 2015). This might be the reason for the variation in the results on the association of buffaloes RS with milk composition in different studies.

Milk somatic cell count, density, pH, and electrical resistance

Milking reactivity did not influence milk SCC and SCS in Jaffarabadi buffaloes. These results are contrary to those of Singh *et al.* (2016), who reported a significant ($P \leq 0.05$) effect of milking temperament on milk SCC in Murrah buffaloes (milk SCC of 1.24×10^5 and 1.81×10^5 cells/ml, respectively in docile and nervous buffaloes). Similarly, Carvalhal *et al.* (2017) found a significantly ($P \leq 0.05$) higher linear SCS in water buffaloes with milking RS3+4 than in the RS2 group. In this study, we screened animals for clinical and sub-clinical mastitis using CMT, and only apparently healthy buffaloes were included. Animals with clinical mastitis tend to show restlessness especially during milking, along with a high SCC. This might be the reason; we did not observe influence of milking reactivity on SCC and SCS.

Our result showed a non-significant ($P > 0.05$) effect of RS on mean milk density and pH. The overall milk density (1.027 g/cm^3) observed in this study was slightly lower than the normal range (1.028-1.033 g/cm^3; Sahin *et al.*, 2016). In healthy buffaloes, fresh milk pH usually ranges from 6.7 to 6.9 (Mejares *et al.*, 2022), however, a slightly lower pH (pH 6.62) was observed in this study. Milk samples were collected during March and June, which are considered summer season, in the study area. The minimum air temperature ranged between 13.1 and 37.6°C and the maximum air temperature ranged between 29.6 and 43.6°C. The higher air temperature in the study area may explain the lower milk pH. A previous study also reported that milk pH is negatively correlated with ambient temperature (Mejares *et al.*, 2022). Unlike density and pH, the milk electrical resistance differed significantly ($P < 0.001$) among the different groups. Buffalo cows without leg movement (RS1) exhibited significantly ($P \leq 0.05$) higher milk electrical resistance than the other two groups (RS2 and RS3+4). Irrespective of RS, the milk electrical resistance value observed in Jaffarabadi buffaloes is comparable to the result observed by Ali and Dahl (2022), who reported a buffalo milk electrical resistance as 300-700 units. According to the Draminski mastitis detector manual, a value above 300 units indicates mammary gland infections in cattle. However, using this threshold (electrical resistance = 300 units), Kala *et al.* (2021) observed lower accuracy in predicting sub-clinical mastitis as compared to CMT, SCC, and milk pH (77.3% vs. 89.8, 92.7% and 79.6%, respectively). Ali and Dahl (2022), observed threshold value for mammary gland infection of 515 units in Iraqi buffaloes. Milk electrical resistance is regulated by Na+, Cl+, K+, and lactose concentrations in the milk. Milk electrical resistance decreases with an increase in Na+ and Cl+ and a decrease in K+ and lactose concentrations. Furthermore, milk fat content positively affects electrical resistance (Ali and Dahl, 2022). The reactivity of buffaloes during milking procedure affects their performance by altering circulating hormonal profiles (Singh *et al*., 2016). Stressful condition stimulates cortisol secretion in blood stream that has an association with mammary gland tight junctions. This may alter electrolyte content, density, and milk pH (Napolitano *et al*., 2022). The temperament of the animals is more aggravated under stressful situations, and if they fail to cope with the prevailing stressful situations, their safeties, performances, and welfare are compromised (Friedrich *et al*., 2015).

The milking reactivity influences the daily milk yield, milk component yields, and electrical resistance but not milk composition in Jaffarabadi buffaloes. Milking reactivity score can be used as a selection marker for breeding of Jaffarabadi buffaloes for better milk production.

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Conflict of interest

The authors declare that there was no conflict of interest.

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