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INFLUENCE OF MURRAH BUFFALO BEHAVIOR IN MILKING PARLORS ON PRODUCTION CHARACTERISTICS

Adriano Henrique do Nascimento Rangel[1], Juliana Paula Fernandes de Oliveira[1], Henrique Rocha de Medeiros[1], Viviane Maia de Araújo[1], Luciano Patto Novaes[1], Dorgival Morais de Lima Júnior[2]

[1] UFRN
[2] UFAL
Correspondência: Dorgival Morais de Lima Júnior: limajunior@zootecnista.com.br

ABSTRACT: The present study aimed to assess the influence of different behavioral scores in milking parlors on possible variations in the production characteristics of primiparous and multiparous Murrah buffaloes. Observations of behavioral response in the milking parlor environment were made with an ethogram for subsequent evaluation of behavioral scores. Milk production and somatic cell count (SSC) were measured, in addition to physicochemical analysis of milk. Results for different behavioral scores were assessed by analysis of variance and comparison of means. The Spearman correlation was used to analyze production characteristics. Primiparous buffaloes exhibited higher mean behavioral scores and lower mean milk production when compared with multiparous buffalo cows. Despite greater reactivity, primiparous cows showed no significant differences in milking time, milk let-down time and milk let-down rate in relation to multiparous buffalo cows. In general, more reactive cows display greater mean milk production. Higher behavioral means were recorded in the first week of observation. Behavior did not influence milk composition or SSC. Mean surface skin temperature was positively correlated to total fat and solids only within primiparous buffalo cows.
Key Words: ethology; parity; milk composition; milk quality

INFLUÊNCIA DO COMPORTAMENTO EM SALAS DE ORDENHA SOBRE AS CARACTERÍSTICAS DE PRODUÇÃO DE LEITE DE BÚFALOS DA RAÇA MURRAH

RESUMO: O presente estudo teve como objetivo avaliar a influência do escore de comportamento em salas de ordenha sobre as variações nas características de produção de búfalas da raça Murrah em diferentes ordens de parto. As observações de resposta comportamental no ambiente de sala de ordenha foram feitas com um etograma para posterior avaliação dos escores de comportamento. A produção de leite e contagem de células somáticas (CCS) foram medidas, bem como a análise físico-química do leite. Os resultados para os escores de comportamento foram avaliados por análise de variância e comparação de médias. A correlação de Spearman foi utilizada para analisar as características de produção. Búfalas primíparas apresentaram maior escore de comportamento e menor produção média de leite, quando comparado com búfalas multíparas. Apesar da maior reatividade, búfalas primíparas não apresentaram diferenças significativas no tempo de ordenha e taxa de descida do leite em relação às búfalas multíparas. Em geral, as búfalas mais reativas exibiram maior produção média de leite. Mudanças significativas no comportamento foram registradas na primeira semana de observação. A média de temperatura da superfície da pele foi positivamente correlacionada com a gordura e sólidos totais em búfalas primíparas. O comportamento não influenciou a composição do leite ou a CSS.
Palavras-chave: etologia, composição do leite, ordem de parto, qualidade do leite

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INTRODUCTION

Buffalo milk production in Brazil is expanding rapidly, mainly because buffalo milk exhibits characteristics allowing greater yields in dairy products, adding value to these products and favoring commercialization when compared with cow's milk in relation to farmers' income.

The buffalo, in addition to being a source of protein with high biological value, can be an economical enterprise, primarily due to its adaptability, early maturity and longevity (PETERS *et al*., 2007). However, its production like the other ones produces an active and routine interface between animal and man, whose effects on the animal performance can be positive or negative (PETERS *et al*., 2007). Assuming that animal's productivity, efficiency and comfort are interactive ones, it becomes a positive concern of consumers and society. Thus, an alternative is to invest in the education and training of labor in order to promote a good management of animals and favoring increased productivity and efficient production systems (GONÇALEZ *et al*., 2008). Therefore, the study of animal behavior can contribute to understanding the animal and man interactions, since it is related to animal's reaction which is related to feeding and/or management practices, and to use them as indicators of animal welfare or stress (ALBRIGHT, 1993).

The animal welfare can be understood as their condition in relation to attempts to adapt to the environment (Broom, 1988), and behavioral measurements are equally significant in assessing it (BROOM and MOLENTO, 2007). Furthermore, an animal avoiding or strongly evading from an object or event he provides information on their feelings and, consequently, their well-being. The stronger the evasive reaction the more discomfort the animal shows when faced with that object or event (MOLENTO, 2007).

In addition to the notion of welfare, another widely used concept to understand human-animal interaction is temperament. This has been used to establish a link between behavior and individual behavior (ROTHBART and DERRYBARRY, 1981). Selection of docile animals need to be developed and combined with studies of adequate management techniques in order to minimize problems and negative consequences for humans and animals (LE NEINDRE *et al*., 1996). Specifically, buffalo cows behavior in milking parlors deserves attention, given that it can directly affect milk production and hence the profitability of this farming (SINGH *et al*., 2010). Considering management tools, likewise separation of buffalo mothers from their offspring, may cause stress to both and resulting in inhibition or reduced milk ejection by the cow. Observing buffalo cows' behavior during milking is of importance, since it is on this parameter that the welfare and conditioning of these animals during milking can be measured, including to evaluate their temperament characteristics.

Buffaloes and dairy cows have a similar milk ejection reflex (THOMAS, 2004) and a complete removal of milk from the udder depends on elevated oxytocin concentrations release during the entire milking (BRUCKMAIER and BLUM, 1998). Milking difficulties is associated with buffaloes and it is associated with the morphological differences of buffalo udder which suggests some peculiarity of milk letdown and it needs to be considered to improve the efficiency of machine milking (BAVA *et al*., 2007). Dairy cows, sheeps and goats have a larger total cisternal area and milk cisternal fraction than buffaloes cows does. The cisternal fraction in buffaloes is, on average, only 5% of total milk, decreases during

lactation and increases with the age of the animal (Thomas, 2004). This author also suggests that the small volume of buffalo cistern require longer teat stimulation before teat cup attachment to guarantee oxytocin supply and a good milk letdown. Results from Bava *et al*. (2007) suggest that proper pre-milking stimulation and prompt cluster takeoff could improve milking efficiency, ensuring good milk letdown and protecting teat conditions.

This study aimed to assess the influence of different behavioral reactions in the milking parlor on possible changes in the milk production characteristics of primiparous and multiparous Murrah buffaloes.

MATERIAL AND METHODS

The experiment was conducted at Tapuio Agropecuária Ltd., in the county of Taipu in the Agreste region of Rio Grande do Norte state. It was carried out over a period of six weeks, from September 2 to October 8, 2010. Data collection was taken on two consecutive days in each week during afternoon milking.

Thirty Murrah buffalo cows were used, 15 primiparous and 15 multiparous. Days in lactation at the start of the experiment varied from 11 to 64 (30,93 ± 13,59) days and 3 to 32 (15,33 ± 9,38) days for primiparous and multiparous females, respectively. Milking interval was 10-14 hours, with the first milking at 5am and the second at 3pm; milking were made in a double 20 herringbone milking parlor with low level vacuum line. One month before their expected calving date first calving heifers were introduced into the milking parlor to adapt to milking procedures. Lactating animals were managed in a pasture of *Brachiaria brizantha* on a rotational grazing. After birth, calves suckled directly from their dams for seven consecutive days when they were separated from cows, permanently. During milking, each cow received a concentrated mixture of cottonseed, sunflower meal, urea and mineral salt at a rate of concentrate to milk of 1:3.

Prestimulation of cows to evoke milk ejection is very important to perform in dairy buffaloes since the milk volume in the udder cistern is very low compared to cows and goats. It was not used hormonal stimulation in buffaloes in this study. Knowing this fact, milking management consisted of the animals kept for resting in a shaded corral with natural ventilation and access to water before entering into the milking parlor. Once inside milking parlor pre-dipping, drying the teats, attaching milking cluster, removal of milking cluster, post-dipping and exit of the animals.

Behavioral observations in the milking parlor were performed twice a week by two trained observers during the six consecutive postpartum weeks, with events recorded on a previously compiled ethogram. The milking process was divided into pre-milking, consisting of animal entry and pre-dipping; milking, from attaching milking teat cups until milk flow stop; and post-milking, corresponding to the removal of milking teat cups , post-dipping and animals leaving the milking parlor. Behavioral responses were recorded throughout these three stages, as shown in Table 1.

Table 1 - Behavioral events in the milking parlor environment.

Event	Pre and Post-Milking
Displacement	Entrance and exit of animals
Kicking	Blows inflicted with the hind legs
Movement	Level of restlessness during milking procedures
Urination	At the end of urination
Defecation	On completion of defecation
Vocalization	Emitting vocal sounds
Removal of milking teat cups[1]	Level of restlessness during the removal of milking teat cups
Event	**Milking**
Attachment of milking lines	Level of restlessness while attaching milking lines
Milk let-down time	Elapsed time to milk let-down
Milking time	Duration of milking

Adapted from Plovesan (1998) and Bouchinhas (2008). [1] Specific to post-milking.

In accordance with records of behavioral events for primiparous and multiparous cows in the milking parlor, a behavioral score (BS) was defined consisting of four classes regarding

aversion or reaction to each stage of milking described above, displayed in ascending order:

1. Calm – no reluctance observed throughout the three milking phases;

2. Slightly impatient – exhibiting reluctance by attempting kicks, resisting placement/removal of milking teat cups and/or entrance/leaving the milking parlor;

3. Restless – moderate reaction during attachment/removal of milking teat cups, avoiding entry into the milking parlor, substantial movement during milking, attempted kicks, defecation/urination;

4. Agitated – extreme agitation, primarily during milking, executing several kicks, vocalization, avoiding attachment/removal of milking teat cups and during pre and post-dipping, significant movement or urination/defecation.

This was quantified individually, once a week, during morning and afternoon milking by a scale meter attached to each milking unit. The milk let-down was observed from the time the teats of the cows were attached to the milk cups until the appearance of the first milk flow in the suspension cup. The rate of milk flow was also evaluated by dividing the total milk yield by the total time spent in milking.

In the second and sixth weeks of the experiment, individual cow milk samples were collected during morning milking for analysis of composition and somatic cell count. Samples were taken by a collecting cup attached to each milking unit, homogenized, and transferred to 40 mL flasks containing Bronopol®. Following identification, they were sent to the Laboratory of the Management Program for Dairy Herds in the Northeast, in the Department of Animal Sciences at State Rural Federal University of Pernambuco. Analyses of total fat, protein and solids were conducted by mid infrared absorption using a Bentley 2000® instrument, in addition to somatic cell count (SCC) by flow cytometry with a Somacount 300 – Bentley®.

Skin surface temperature of the animals was measured once a week in four different regions of the body in order to establish individual mean temperature. Measurement was accomplished with an Instrutherm® digital infrared thermometer, positioned as close to the animal as possible, in areas close to skin of head, foreleg, udder and back, with the average of the four measurements being obtained.

Data analyzed were submitted to a normality test using the proc univariate and proc means procedures of SAS (2002). All data showed normal distribution, except milk flow rate; milk let-down time and milking duration, which were transferred to 10-logarithmic transformation. Analysis of variance (ANOVA) was performed for the variables: behavior, milk production, milk letdown rate and duration of milking considering the effects of parity and week of lactation, applying the GLM procedure (SAS, 2002). Means were, standard deviation, compared using Tukey at 5.0%. The means of the variables somatic cell count and milk production and composition to assess the effects of behavior and surface temperature on primiparous and multiparous cows were analyzed and compared using Pearson's correlation for the second and sixth weeks of lactation. Data of somatic cell count were transferred to a 10-logarithmic transformation.

RESULTS AND DISCUSSION

Means for behavior observed in the milking parlor and productive characteristics are displayed in Table 2 and 3.

Table 2 - Means of behavior and production aspects of buffaloes in the milking parlor.

Parity/ Variable	Behavior	Production	MFR*	Milk let-down*	Milking time[1]
Primiparous	1.75 ± 0.97 [a]	8.97 ± 3.12 [b]	2.95 ± 0.44 [a]	4.88 ± 0.95 [a]	5.71 ± 1.66 [a]
Multiparous	1.44 ± 0.76 [b]	10.07 ± 2.83 [a]	2.87 ± 0.45 [a]	4.93 ± 0.96 [a]	5.99 ± 1.56 [a]
P value	0.0005	0.0005	0.1199	0.6142	0.1206

Production = Milk production (liter); MFR = Milk flow rate (ml/sec); Milk let-down = Milk let-down time (seconds); Milking time = Duration of milking (seconds). Means followed by different letters, in the same column, are statistically different (P<0.05). [1] Values expressed in 10-log.

Parity influenced behavior in the milking parlor (P<0.05). Primiparous females exhibited higher mean behavioral scores when compared with multiparous cows, likely because multiparous buffaloes are more accustomed to the parlor environment than their primiparous counterparts. These results corroborate those of Cavallina *et al*. (2008), who evaluated the behavioral response of Murrah buffaloes submitted to mechanical milking. Higher frequencies of agitated behavior including kicks, defecation and urination and knocking off the milking cluster for attaching or detaching were more frequent among primiparous cows. Thus, the greater the occurrence of these behaviors, the higher was the mean behavioral score for the individual temperament. De Rosa *et al*. (2003) found that frequency of kicking is an indicator of agitation and aggressiveness, while Das and Das (2004) confirmed that urination frequency is also strictly related to temperament, as well as being an indirect measurement of fear.

In addition to displaying greater mean behavioral scores, primiparous buffaloes also produced less milk, emphasizing the score's negative effect on production (Table 3). This corroborates Oliveira *et al*. (2010), who evaluated Murrah buffalo behavior in the presence of strangers and recorded reduced milk production among animals that defecated and urinated in the milking parlor. Moreover, Peters *et al*. (2007). The authors observed that low reactivity during milking, primarily regarding the frequency of defecation and urination, resulted in higher milk production and quality levels. Oliveira *et al*. (2010) also showed that milk production varied significantly when milking occurred in the presence or absence of strangers, with greater production when animals were familiar with the people present.

Table 3 - Means of production characteristics as a function of behavioral scores for Murrah buffaloes cows.

Variable	Behavioral score				P value
	1	2	3	4	
Milk production	8.99 ± 3.05 [a]	10.11 ± 2.80 [b]	10.28 ± 2.74 [ab]	11.25 ± 2.81 [b]	0.0002
MFR*	2.89 ± 0.46 [a]	2.91 ± 0.43 [a]	2.93 ± 0.47 [a]	3.09 ± 0.37 [a]	0.3471
Milk let-down*	4.84 ± 0.89 [a]	4.99 ± 1.00 [a]	4.96 ± 1.04 [a]	5.16 ± 1.15 [a]	0.3112
Milking time[1]	5.77 ± 1.69 [a]	5.97 ± 1.48 [a]	6.31 ± 0.35 [a]	5.55 ± 2.16 [a]	0.2817

MFR = Milk flow rate (ml/sec); Milk let-down = Milk let-down time (seconds); Milking time = Milking Duration (seconds); Milk production (liter). Means followed by different letters, in the same line, are statistically different (P<0.05). [1] Values expressed in 10-log.

Although primiparous cows were more reactive and produced less milk, milking time, milk let-down and milk let-down rates did not vary in comparison with multiparous females (P>0.05). These findings agree with those of Fruchi *et al*. (2009), who also recorded superior milk production among multiparous buffaloes, but found that parity did not influence milking duration. It is important to note that higher milk production among multiparous cows may not be attributed to behavior, but also a result of physiological factors favoring productive performance when females reach maturity (COBUCI *et al*. 2000).

According to Bruckmaier *et al*. (1993) the central milk ejection where oxytocin inhibition from hipophyse occurs with frequency during various emotional stresses. Milking in unknown ambient resulted in milk ejection inhibition mainly in primiparous cows when first time milked (BRUCKMAIER *et al*., 1992) since it is a new experience for these cows; but, a gradual transferring of these animals to unknown ambient make the oxytocin release to reach a normal level (BRUCKMAIER *et al*., 1996).

When evaluating behavioral scores separately and in conjunction with production characteristics, we found that the most reactive buffaloes, that is,

those with higher scores, displayed greater mean milk production. However, Nayak and Mishra (1984), when comparing docile, agitated, nervous and aggressive cows, reported that docile females were easier to milk, handle and manage and produced more high-quality milk in relation to "nervous" or aggressive buffaloes. They also observed that docile females exhibited higher consumption of the concentrate, had lower time to reach milk let-down, shorter milking duration, larger milk flow rates and greater percentages of fat in their milk.

Although milk production demonstrated variations between different cows' behavior, there was no significant difference (P>0.05) between milking time and milk flow rate. Gonzaga and Lorenzo (2007) mention that the greater the milk production, the higher the milk flow rate is, therefore, more time is required for milking once they were positively correlated. The fact that milk letdown rate and milking time did not vary across the behavioral scores may be due to adequate animal handling in milking parlor. Table 4 presents behavioral scores means in the milking parlor and milk production characteristics of Murrah buffaloes in the first six weeks of lactation, showing increased milk production as lactation progresses. In the first third of lactation, milk production increases to a peak, declining as the lactation stage advances. The milk flow rate, milking time and behavior did not vary over the six weeks.

Regarding to behavioral score, the mean in the first week was expected to be higher than in the sixth, since cows were separated from their calves, management practice that influenced milk production for that week, which was the lowest in the study period. It was expected as lactation advanced would contribute to reducing behavioral scores due to cows' adaptation to the milking parlor environment. However, this did not occur, since buffaloes produced well even when displaying high behavioral scores. This may be explained by the good milking practices carried out in farm management practices.

Table 4 - Behavioral means in the milking parlor and production aspects for Murrah buffaloes in the first six weeks of lactation.

Variable	Week						P
	1	2	3	4	5	6	value
Milk production	8.13 ± 3.06 [a*]	9.40 ± 2.48 [a*b]	9.89 ± 3.15 [b]	9.89 ± 2.80 [b]	9.86 ± 3.07 [b]	9.94 ± 3.21 [b]	0.0050
MFR*	2.74 ± 0.54 [a]	2.98 ± 0.45 [a]	2.93 ± 0.45 [a]	2.94 ± 0.39 [a]	2.89 ± 0.40 [a]	2.98 ± 0.42 [a]	0.8766
Milk let-down[1]	4.89 ± 1.09 [a]	4.76 ± 0.82 [a]	4.82 ± 0.87 [a]	4.76 ± 1.13 [a]	5.09 ± 0.92 [a]	5.11 ± 0.81 [a]	0.0763
Milking time[1]	5.65 ± 1.90 [a]	5.97 ± 1.21 [a]	5.95 ± 1.51 [a]	5.87 ± 1.63 [a]	5.92 ± 1.63 [a]	5.72 ± 1.78 [a]	0.1364
Behavior	1.75 ± 1.07 [a]	1.58 ± 0.77 [a]	1.43 ± 0.81 [a]	1.65 ± 0.88 [a]	1.63 ± 0.84 [a]	1.53 ± 0.91 [a]	0.4826

MFR = Milk flow rate (kg/sec), Milk let-down = Milk let-down time (seconds), Milking time = Milking duration (seconds). Means followed by different letters, in the same line, are statistically different (P<0.05). [1]Values expressed in ln(x).

When exposed to stressors, dairy cattle exhibit disruptions in milk ejection (Van Reenen *et al*., 2002). According to Negrão and Marnet (2003), during the first time milking, particularly among primiparous buffaloes cows secret and release catecholamine and a failure in releasing of oxytocin occurs with a decrease in blood flow to the mammary glands.

Tables 5 and 6 show correlations regarding behavioral score, mean surface skin temperature and production characteristics of primiparous buffaloes in the second week of lactation. For multiparous cows, correlations were made between the same variables, however no significant difference was found. Regardless of calving order, behavior was not significantly correlated (P>0.05) with the other variables, likely due to the short study period or small number of observations. These results corroborate those of Peters (2008), who reported that stress caused by inadequate management practices did not influence milk composition or SSC. It is possible that the short duration of treatment, where the stressor is the milking parlor environment, was not sufficient to alter behavioral scores.

Table 5 - Correlation between behavior, temperature and production characteristics of primiparous buffaloes cows in the second week of lactation.

	Behavior	Mean Temperature	% Fat	% protein	% lactation	% TS	SCC	Production
Behavior	1.000	-0.053	0.241	0.062	0.065	0.152	0.319	0.320
Mean Temperature	-0.053	1.000	0.586*	-0.139	0.021	0.654* (p<0.01)	-0.270	-0.304

Production = Milk production, liter; Behavior, score; SCC, 10-log; Temperature, ºC.

With respect to mean surface temperature, a positive correlation was recorded for fat (P<0.05) and total solids (P<0.01) in primiparous females. These findings may be partially attributed to the fact that primiparous cows have lower milk production and, as a result, their milk contains higher concentrations of nutrients.

Table 6 - Correlation between behavior, temperature, production characteristics and milk yield (production) of multiparous buffaloes cows in the second week of lactations.

	Behavior	Mean Temperature	% Fat	% protein	% lactation	% TS	SCC	Production
Behavior	1.000	-0.020	0.172	-0.028	-0.080	0.131	-0.035	-0.060
Mean Temperature	-0.053	-0.020	1.000	-0.154	0.369	-0.211	-0.132	-0.193

Production = Milk production, liter; Behavior, score; SCC, 10-log; Temperature, ºC. Means are not significant (p>0.05).

These findings contrast those of Peters *et al*. (2007), who observed that low reactivity during milking and reduced defecation and urination resulted in higher milk production and superior quality milk. Oliveira *et al*. (2010) also emphasize that the presence of strangers in the parlor environment has a significant negative impact on milk production. Moreover, Porcionato *et al*. (2005) reported that initial milking are stressful, while Tancin *et al*. (2001) state that modifying the environment provides favorable behavioral changes during the milking period.

Tables 7 and 8 depict correlations between behavior, temperature and production characteristics of primiparous and multiparous females in the sixth week of lactation. As in the second week, there was no significant correlation between behavior and milk composition, indicating that under these study conditions, regardless of calving order, behavioral score did not affect this production characteristic (P>0.05). Among multiparous cows, a negative correlation (P<0.05) was observed with milk production, that is, the higher the surface body temperature, the lower the animal's milk production.

Table 7 - Correlation between behavior, temperature, production characteristics and milk yield (production) of multiparous buffaloes cows in the sixth week of lactation.

	Behavior	Mean Temperature	% Fat	% protein	% lactation	% TS	SCC	Production
Behavior	1.000	-0.105	0.121	0.027	-0.233	0.239	0.281	0.477
Mean temperature	-0.105	1.000	-0.199	0.318	0.052	-0.329	-0.049	-0.250

Prod. = Milk production (liters); Behavior (score); SCC (10-log); Temperature (ºC). Means are not significant (p>0.05).

Breuer *et al*. (2000) found that negative interactions resulted in greater reactivity during milking, although milk production remained unaffected. However, this outcome was not corroborated by Hemsworth *et al*. (2000), who observed that negative interaction during milking showed significant negative correlation with milk production and fat and protein content.

Table 8 - Correlation between behavior, temperature, production characteristics and milk yield (production) of multiparous buffaloes cows in the sixth week of lactation.

	Behavior	Mean Temperature	% Fat	% protein	% lactation	% TS	SCC	Production
Behavior	1,000	-0.349	-0.100	0.000	-0.142	-0.141	0.292	0.454
Mean Temperature	-0.349	1.000	0.132	0.130	0.091	0.154	-0.371	-0.545* (p<0.05)

Prod. = Milk production (liters); Behavior (score); SCC (10-log); Temperature (ºC).

Peters (2008) points out that negative attitude, such as shouting and hitting, may result in higher stress levels among the animals, compromising milk production and quality since it favors the accumulation of residual milk. The author also recorded no correlation between animal welfare indicators and the occurrence of mastitis, corroborated by findings in the present study. Nevertheless, milkers displaying aggression towards the animals may be negligent with regard to hygiene during milking, predisposing the herd to increased mastitis. However, Rosa (2004) argues that positive attitudes by themselves are not sufficient to improve behavioral indicators and animal well-being.

CONCLUSION

Primiparous buffaloes exhibit greater restlessness during milking than their multiparous counterparts.

Behavior did not influence milk composition or SSC. Mean surface temperature is positively correlated to fat percentage and total solids in primiparous buffaloes, and negatively correlated with milk production in multiparous females.

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