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EFFECTS OF BEHAVIOUR DURING MILKING ON PRODUCTION AND REPRODUCTION INDICATORS IN ROMANIAN WATER BUFFALOES

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Abstract

The aim of this study was to evaluate the effects that behaviour during milking has on production, reproduction and welfare indicators in water buffalo cows reared under loose housing system. The study was conducted in a private farm, on 102 dairy buffalo cows (6.4±0.1 lactations) at the beginning of their lactation (100 days in milk, DIM). The behaviour during milking was evaluated using a 5-point subjective scale (1 – calm to 5 – nervous), using two individual trained observers placed at 0.5-1 m behind the animals, using an 8x2 herringbone milking parlour. The animals were grouped based on their behaviour as 'calm' (scores 1, 2 and 3; n = 76) or 'nervous' (scores 4 and 5; n = 26). Milk yield and milking speed were significantly influenced by the behaviour during milking (p>0.05), with calmer buffaloes outperforming their nervous counterparts. No significant influence of the behaviour during milking was found on body condition score, calving interval, age at first calving or animal-based welfare indicators. Current findings suggest that incorporating behavioural assessments during milking into management practices could enhance productivity in dairy water buffaloes.

***Key words**: animal-based indicators, animal welfare, milking behaviour, water buffaloes.*

INTRODUCTION

Comparative neuroanatomical analyses and studies of emergent cognitive–behavioural functions in human ancestors suggest that temperament is linked to associative conditioning, a trait highly conserved across all species (Cloninger et al., 2009). Studying temperament (behavioural reactivity) provides a direct insight into the adaptive mechanisms that evolved in animals, which allowed them to quickly and effectively respond to external stimuli, being essential for their health and survival (Cloninger et al., 2019).

Different temperaments evolved to help animals adapt to naturally occurring events, with variations in reactions to both external and internal stimuli being documented. These variations were essential regulation of the immune and inflammatory response and energy production maintenance, along with processes that mediate habit learning, emotional reactivity, cognitive flexibility, sensory sensitivity and circadian rhythmicity (Mendoza et al., 2011; Steelman et al., 2011; Dyson et al., 2015).

Livestock behaviour during handling is influenced by both genetics and past experiences, with recent research suggesting that fear is just one of the many temperament traits and proper (gentle) habituation from a young age, especially with feed rewards, can promote calmer animals (Grandin et al., 2024). For large domestic ruminants like cattle and water buffaloes, the social environment plays a crucial role for their well-being (De Freslon et al., 2020), as there is evidence that these species can exhibit aspects of social complexity, such as social learning (Cooke et al., 2021). Water buffaloes (*Bubalus bubalis*) are predominantly reared in traditional production systems world-wide, nonetheless modern intensive systems and mechanization are expanding rapidly in some European countries, such as Italy, Romania and Bulgaria (Borghese et al., 2010). This shift has inevitably displaced water buffaloes from their natural habitats and brought changes to various aspects of intensive farming, such as feeding, milking machines, handling and housing conditions (De Rosa et al., 2005; Napolitano et al., 2019). While buffaloes are becoming more prevalent in milk production, their temperament and strong bond with their calves make the entire milking process challenging (Erdem et al., 2022). Like all animals, water buffaloes rely on associative conditioning for learning and adapting to their environment, particularly in response to handling, milking and human interactions. However, unlike higher primates and humans, their ability for intentional self-control and self-awareness is limited, meaning that their behaviour is primarily driven by learned associations, sensory experiences and innate temperament traits (Zwir et al., 2020).

Frequent human-animal interactions in dairy species (particularly during milking routine) are closely associated with productive and welfare indicators (Simitzis et al., 2021), with strong evidence (especially in dairy cows), that calmer and less reactive animals outperform their reactive counterparts when milk attributes are taken into consideration (Haskell et al., 2014). In general, temperament describes an animal's individual response to human handling and, when heritable, this trait holds potential for improving welfare through the selective breeding of animals that are less reactive to handling. In dairy water buffaloes, behaviour during milking refers to the animal's response throughout the entire milking process. This behaviour is commonly assessed using visual scoring systems that evaluate the frequency and intensity of movements, which in turn reflect levels of fear and overall responsiveness (Hedlund et al., 2015). Lactating water buffalo cows exhibit a heightened sensitivity to handling during milking in comparison to dairy cattle, a difference attributed to their relatively limited genetic selection for traits associated with milking performance (Thomas et al., 2005; Bidarimath and Aggarwal, 2007). Previous research has emphasized that adequate pre-milking stimulation, together with stressors mitigation during milking routine, are essential for triggering the ejection of the alveolar milk fraction, which constitutes approximately 90–95% of the total milk yield in this species (Costa et al., 2020). In buffalo cows, exposure to even minor environmental or procedural changes during milking can elicit a stress response characterized by elevated adrenaline secretion. This, in turn, suppresses endogenous oxytocin release, thereby impairing the milk ejection reflex (Borghese et al., 2007). Given the limited success of genetic selection in enhancing spontaneous milk ejection in buffaloes, exogenous oxytocin administration has become a widespread practice in large-scale production systems to facilitate milk let-down (Napolitano et al., 2019; Shahid et al., 2021). However, repeated administration of oxytocin has been proved to adversely affect the mammary gland secretory function and to inhibit the physiological milk ejection mechanism (Mustafa et al., 2008). Moreover, it has been associated with detrimental effects on reproductive performance, delaying the onset of a new ovarian cycle and contributing to reproductive disorders such as poor estrus expression, low conception rates, longer calving intervals and increased embryonic mortality in buffalo cows (Faraz et al., 2020).

Given the limited research on the implications of temperament in this species, the overall objective of this study was to evaluate the effects that behaviour during milking has on production, reproduction and welfare indicators in water buffalo cows reared under loose housing system (Noce et al., 2021; Vidu et al., 2013). The hypothesis was that calmer and less excitable buffalo cows would outperform their more reactive and nervous counterparts, when taking into account the production variables, reproduction outputs and welfare indicators.

MATERIALS AND METHODS

Animal management and milking reactivity assessment

The research was conducted in a commercial farm (46°11'77''N 25°1'33''E) in Mesendorf, Romania, (altitude of site 460 m), on 102 (6.4 ± 0.2 lactations) dairy water-buffalo cows. The study focused on animals at the beginning of lactation (first 100 days of lactation, 100 DIM) and was carried out between May and August 2022. Buffaloes were managed under identical feeding and housing conditions, with a loose housing system and individual resting cubicles, with a space allocation of 10 m² per animal in the resting and movement area. *Ad libitum* access to water, mineral blocks and outdoor paddocks was provided. The feeding system at the farm was not seasonally differentiated, with the fodder being ensured from the farm's own resources. Feed was administered as a total mixed ration (TMR) twice a day in the paddock feeding line, with a feeding space of 90-100 cm per animal. The daily ration per buffalo included 7 kg alfalfa hay, 7 kg pasture hay, 4 kg corn silage and 2.5 kg concentrates. Animals were kept in groups of 50 to 60 animals, depending on their lactation stage and productivity. Reproduction was conducted through natural mating (one bull for each 20-25 buffalo cows), while inbreeding was avoided by replacing the bulls every 24 months. The animals were milked twice daily (starting at 5:30 AM and 5:30 PM) in a "herring-bone" milking parlour (2 x 8 units). The milking parlour was equipped with GEA DemaTron 70 software and hardware and the milking machines were fitted with individual milk meters (MKV Milk Meter). Milking reactivity (MR) was assessed according to the protocol described by Mincu et al. (2022), with two trained observers positioned in the main alley of the milking parlour throughout the entire milking process. Each buffalo cow was scored on a 5-point scale (score 1 = calm response to the milking procedure to score 5 = highly reactive response, in increments of 1). Based on the milking reactivity scores assigned during milking, the buffaloes were classified as either "calm" (scores 1, 2 and 3; n=76) or "nervous" (scores 4 and 5; n=26).

Data collection and welfare assessment

A total of 12 parameters per buffalo were considered to evaluate the effects of behaviour during milking (milking reactivity) on production, reproduction, and welfare indicators. The identification number, milk yield per milking session (kg), and milking duration (minutes) were recorded directly during MR assessment. The milking speed (kg/min) for each animal was obtained by dividing the milk yield by the milking duration. Additional production data were collected, including milk yield per 100 days in milk and daily milk yield per buffalo (kg/day), which was measured twice during the data collection period, following standardized guidelines (ICAR, 2012). The reproductive performance of the studied buffalo cows, including age at first calving and calving interval, was recorded by the farm veterinarian and technicians. Additional welfare-related data were collected during MR assessment day, including body condition score (BCS), cleanliness of udder, rump and hind legs, excessive hoof growth, skin lesions incidence, hairless patches, nasal, ocular and vulvar discharges incidence, and tarsal joint lesions. The BCS of the buffaloes was assessed using the 9-point scale method (where score 1 was severely emaciated, with no presence of fat either visible or palpable, and physically weak, and score 9 was severely obese with typical 'fat pads'), in increments of 1, according to a previously developed scale by Negretti et al. (2008). Tarsal joint and skin lesion incidence were assessed during the behavioural evaluation of the animals. Claw overgrowth, considered an indicator of lameness, was categorized into three levels: 0 - mild growth, 1 - moderate growth, and 2 - severe growth, following the method described by Whay et al. (2003). Integumentary alterations, including hairless patches, were scored as follows: 0 - no hairless patches, 1 - presence of at least one hairless patch, and 2 - multiple hairless patches. Additionally, nasal, ocular, and vulvar discharges were evaluated individually based on the Welfare Quality® (WQ) protocol for dairy breeds, with a score of 0 indicating no visible discharge and 2 indicating the presence of discharge.

Statistical analysis

The data were statistically analysed to estimate the effects that behaviour during milking has on production, reproduction and welfare indicators in water buffaloes raised in an intensive system. The influence of behaviour during milking (calm vs. nervous) on production, welfare and reproductive indicators in buffaloes was analyzed using both descriptive and inferential statistics. Mean and Standard Error of the Mean (SEM) were calculated for all continuous variables. The distribution of Body Condition Scores (BCS) was compared between reactivity groups using Fisher's Exact Test to assess whether behaviour during milking influenced the proportion of animals in different BCS categories. Logistic regression was applied to evaluate the association between milking reactivity and welfare indicators, with odds ratios (OR) used to quantify the likelihood of poor conditions in nervous animals relative to calm ones. Reproductive traits, including calving interval and age at first calving, were analyzed using the Mann-Whitney U test due to non-normal data distribution. Differences in milking speed and milk yield were assessed using the Wilcoxon rank sum test, providing insights into the impact of milking reactivity on milk production efficiency. Results were visualized using violin plots for reproductive parameters and bar plots with mean ± SEM for BCS distribution and milking traits. Statistical significance was set at $p < 0.05$ and all analyses were conducted using R® software.

RESULTS AND DISCUSSIONS

Significant differences in productive indicators were observed between calm and nervous buffalo dairy cows.

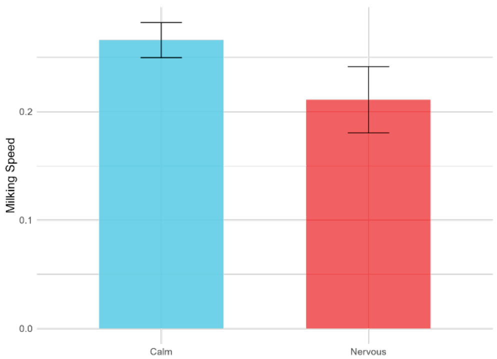

Figure 1. Milking speed (kg/min) for calm and nervous buffaloes (mean ± SEM)

For milking speed, calm buffalos were more efficient, with an average speed of 0.266 kg per minute and lower variability (SEM = 0.0162), compared to 0.211 kg per minute in nervous buffalos, which exhibited higher variability (SEM = 0.0305). The difference was statistically significant ($p = 0.0436$), indicating that calm animals are better suited to the milking process, likely due to reduced stress and better disposition during milking (figure 1). In contrast, the slower milking speed in nervous buffalos might prolong the milking routine, increase labour costs and reduce the overall efficiency.

Milk yield for 100 DIM has showed even more significant diferences. Calm animals produced an average of 399 liters per lactation, compared to 316 liters in nervous cows, with a substantial difference of 83 liters. This disparity, also statistically significant ($p = 0.0436$, Figure 2), has serious economic implications. With current milk prices, such losses could significantly impact farm's profitability, emphasizing the need to address temperament-related traits through improved handling and animal selection practices.

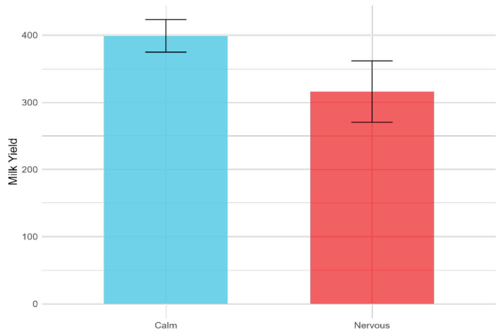

Figure 2. Milk yield (kg/100DIM) for calm and nervous buffaloes (mean ± SEM)

These findings suggest that temperament is a key factor influencing productivity in dairy water-buffalo farming. Calm animals consistently outperformed nervous buffalos, demonstrating higher milking speed and greater milk yields. Current results are in accordance with Erdem et al. (2022), which found that higher milking temperament scores in Anatolian buffaloes were significantly correlated with increased frequencies of stress-related behaviours such as vocalizing, kicking, stepping, defecating, urinating and pulling the teat cup off. Additionally, higher temperament scores negatively impacted milk yield per milking and milk flow rate, while increasing milking time, with primiparous buffalo cows showing higher temperament scores, though these decreased over time as they got habituated to the milking procedures.

The impact of behaviour during milking (calm vs. nervous animals) on reproductive indicators was evaluated by comparing the calving interval and age at first calving between the two groups. Descriptive statistics showed that the mean calving interval was 465 ± 17.9 days in the calm group and 468 ± 26.6 days in the nervous cows. Similarly, the mean age at first calving was 71.0 ± 4.23 months in calm buffaloes and 67.3 ± 6.49 months in nervous animals. The Mann-Whitney U test was used to assess statistical differences between temperament groups for both traits. No significant difference was observed for the calving interval ($W = 887$, $p > 0.05$), indicating that milking reactivity does not play a major role in this reproductive parameter (Figure 3). Likewise, age at first calving did not differ significantly between the two groups ($W = 1055$, $p > 0.05$), suggesting that milking reactivity does not have a measurable effect on the timing of reproductive maturity (Figure 4). These findings indicate that reproductive efficiency in dairy buffaloes is not strongly influenced by temperament related traits.

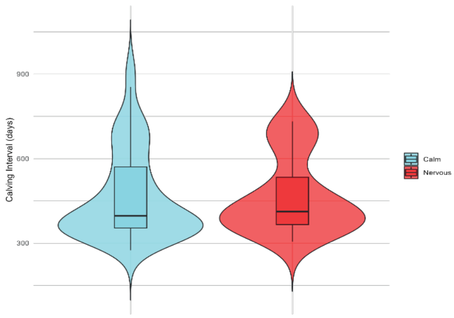

Figure 3. Descriptive statistics (violin plots) for calving interval in calm and nervous buffaloes (*Violin plots display the full distribution of the data through a rotated kernel density plot, where the width of each violin represents the frequency (density) of the data points. The black box inside each violin indicates the interquartile range (IQR), while the horizontal line shows the median value)*

In contrast to the findings of the present study, Khan et al. (2007) reported that calm-tempered buffaloes had shorter calving intervals and higher conception rates, compared to their more reactive counterparts.

The discrepancies between the two studies might be attributed to several factors, the most relevant being the use of different breeds (with the Romanian Buffalo being a rustic breed, not selected for reproductive precocity), environmental conditions (such as climate and altitude), as well as the different rearing and feeding systems.

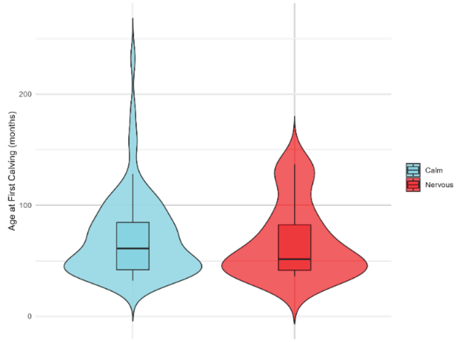

Figure 4. Descriptive statistics (violin plots) for age at first calving in calm and nervous buffaloes

There was no statistically significant difference in the distribution of BCS categories between the calm and nervous groups (Fisher's Exact Test, $p = 0.4741$). Body Condition Score (BCS) was analyzed to assess differences between temperament groups, expressed as the percentage of individuals within each group falling into specific BCS categories. Additionally, Figure 5 illustrates the mean ± SEM for each BCS value across the two groups, providing a clearer picture of the internal variability. In the calm group, BCS values ranged from 4 to 9. The distribution was relatively broad, with a higher concentration in the upper BCS categories. The highest percentage of animals was observed at BCS 8 (34.2%), followed by BCS 7 and 9 (21.1% each). Lower BCS scores were rare: BCS 5 accounted for 6.58%, and BCS 4 was recorded in only 1.32% of the animals. In contrast, the nervous group showed a more concentrated distribution skewed towards higher BCS values. The most frequent score was BCS 9 (30.8%), followed by BCS 6 and 7 (23.1% each). Only 7.69% of nervous animals had a BCS of 5, and none had a BCS of 4. A general increasing trend was observed in the calm group, with the proportion of animals rising progressively from lower to higher BCS values, peaking at BCS 8. However, this trend declined slightly at BCS 9, suggesting a partial crescendo pattern. On the other hand, the nervous group displayed a bimodal distribution, with peaks at BCS 6/7 and BCS 9, indicating a less consistent pattern but a strong tendency toward higher BCS values. When comparing the two groups, calm animals exhibited a more evenly distributed BCS pattern, with a marked peak at BCS 8, while nervous animals showed a stronger concentration in the highest categories, particularly BCS 9. These differences might suggest a potential association between temperament and body condition, where nervous individuals tend to have higher BCS, possibly due to stress-related alterations in energy metabolism or feeding behaviour. The overlay of mean ± SEM values further support this pattern.

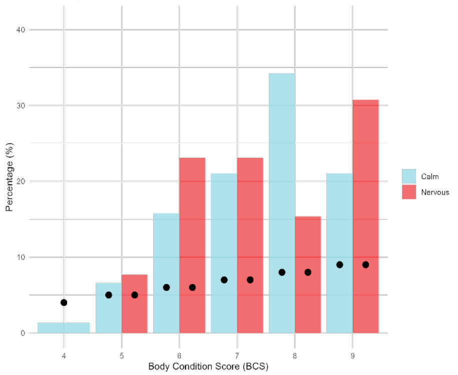

Figure 5. BCS percentage and mean ± SEM for individual score groups in calm and nervous buffaloes

In most BCS categories, nervous animals showed higher mean scores, although with greater variability, especially in the upper BCS range. This was reflected by wider SEM bars, particularly in the nervous group.

The frequency of dirty rumps was observed in 30.3% of the calm group compared to 23.1% of the nervous group, while the frequency of dirty udders was recorded in 44.7% of calm animals and 34.6% of nervous animals. The highest levels of dirtiness conditions were seen in cleanliness of the hind legs, affecting 88.2% of the calm group and 84.6% of the nervous group.

Regarding tarsal joint lesions, these were present in 52.6% of calm animals and 42.3% of nervous animals. Similarly, claw overgrowth was prevalent in both groups, with 78.9% of calm animals and 92.3% of nervous animals being affected (Figure 6). These findings indicate that while both groups face substantial welfare challenges, coat dirtiness conditions were slightly more frequent in the calm group for cleanliness of the rump and cleanliness of the udder, whereas claw overgrowth was more pronounced in the nervous group.

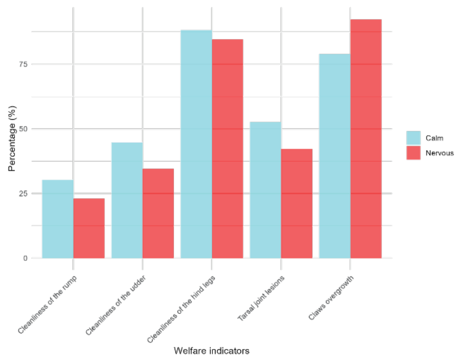

Figure 6. Percentage of poor conditions (score = 2) for welfare indicator in calm and nervous buffaloes

The odds of dirty rumps in the nervous group were 31% lower compared to the calm group (OR = 0.69, 95% CI: 0.23-1.87). However, this difference was not statistically significant (p = 0.485). Animals in the nervous group were slightly less likely to have dirty udders compared to the calm group (OR = 0.65, 95% CI: 0.25-1.62). The difference did not reach statistical significance (p = 0.369). The odds of dirty hind legs were marginally lower in the nervous group compared to the calm group (OR = 0.74, 95% CI: 0.22-2.94), though this result was not statistically significant (p = 0.641). The likelihood of tarsal joint lesions in the nervous group was 34% lower than in the calm group (OR = 0.66, 95% CI: 0.26-1.61). This difference was also not statistically significant (p = 0.365). Animals in the nervous group were 3.2 times more likely to exhibit claws overgrowth compared to the calm group (OR = 3.20, 95% CI: 0.82-21.22). While suggestive of a trend, this result was not statistically significant (p = 0.140, Table 1).

Surprisingly, the present study observed a higher number of animals with soiled body areas among calm buffalo cows compared to their nervous counterparts. The initial working hypothesis assumed that nervous buffaloes would alternate more frequently between standing and lying positions, leading to increased soiling of the evaluated anatomical regions. A possible explanation for the current findings is that nervous buffaloes, being more excitable, might occupy dominant positions within the social hierarchy, thus gaining preferential access to cleaner resting areas compared to lower-ranking individuals.

Table 1. Logistic regression results comparing welfare indicators between calm and nervous buffaloes (calm group = reference)

Welfare Indicator	Odds Ratio	95% CI Lower	95% CI Upper	p-value
Clean. of rump	0.69	0.23	1.87	0.485
Clean. of udder	0.65	0.25	1.62	0.369
Clean. of hind legs	0.74	0.22	2.94	0.641
Tarsal lesions	0.66	0.26	1.61	0.365
Claws overgrowth	3.20	0.82	21.22	0.140

*CI=confidence interval

CONCLUSIONS

The results of this study suggest that buffalo cows with a calm response during milking exhibit superior performance in terms of milk yield and milking speed compared to their more nervous counterparts.

Although no statistically significant differences were observed in reproductive traits or most welfare indicators, some patterns emerged that may indicate subtle temperament-related effects on body condition and coat cleanliness.

Overall, behavioural responses during milking might serve as a practical and non-invasive indicator for identifying individuals with higher productive potential and could be considered in future management and selection strategies to enhance farm efficiency.

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