



# Buffalo behavioural response to machine milking in early lactation

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## ABSTRACT

Buffalo farming in Italy is traditionally oriented towards mozzarella cheese production and over the last decade it has been rapidly increased. As a result, intensive techniques and mechanisation of farm activities have been introduced. Those sudden changes in rearing techniques have aroused a general concern about buffaloes welfare and its possible consequences on products quality. Human-animal interactions are recognized to have an impact on productivity, behaviour and welfare, particularly in dairy farms, where the milking process involves a close interaction with a human handler. Focusing on the first month of lactation, this preliminary study aimed at evidencing buffalo behavioural responses to machine milking. Relationship between behaviour and oxytocin administrations, often performed to allow milk let down, has been also investigated. The experiment included 8 multiparous and 6 primiparous buffaloes, calving in the same period. Starting from the first entrance in the milking parlour, the animals were followed two days/week during the morning milking for the first 5 weeks of lactation. Behaviour observation was performed following a "focal animal sampling" (continuous recording) technique. Proportional frequencies of the following behaviours were calculated: kicking, stepping, defecating, urinating, vocalizing, pulling the teat cup off the teats. The exogenous oxytocin administration at milking was recorded. Pearson Chi-Square test was used to verify the presence of differences between primiparous and multiparous cows' behaviour at milking. Cochran's Q test was used to assess the variability of behaviour over time and a binomial regression was performed in order to verify the correlations between animal behaviours and the need to administer oxytocin. Considering lactation number, every behavioural pattern in primiparous cows, except for stepping, resulted to be more frequently performed (36.67% vs 24.36% for kicking; 5% vs 2.56% for defecating; 11.67% vs 5.13% for pulling the teat cup off the teats). The behaviour of urinating (48.33% vs 11.54%) proved to have a significantly higher mean frequency of occurrence compared to the multiparous one ( $P < 0.001$ ). The frequency of oxytocin administration showed a high variability over time ( $P < 0.001$ ), soon decreasing, while the selected behaviours proved to be more stable in time, except for kicking and urinating ( $P < 0.05$ ). Some correlations between animal behaviour at milking and oxytocin administration were found; particularly kicking, stepping and urinating appeared to be significantly related to the requirement of exogenous molecule ( $P < 0.001$ ). The occurrence of behaviours considered as indicators of acute stress and their correlation with oxytocin administration are a clear proof of the machine milking impact on buffaloes welfare and productivity.

*Key words:* *Bubalus bubalis*, Machine milking, Behaviour, Oxytocin.

## RIASSUNTO

RISPOSTA COMPORTAMENTALE DELLA BUFALA DURANTE  
LA MUNGITURA MECCANICA NELLE PRIME FASI DELLA LATTAZIONE

*L'allevamento della Bufala Mediterranea Italiana è tradizionalmente finalizzato alla produzione della mozzarella. Il recente successo della filiera bufalina ha determinato un forte impulso alla produzione, che ha portato alla meccanizzazione e all'adozione di tecniche intensive per lo più mutuata dall'allevamento bovino da latte. Gli improvvisi cambiamenti introdotti nelle tecniche di allevamento della bufala, hanno portato all'attenzione generale la problematica del benessere e della qualità delle produzioni in questa specie, fino a ieri allevata in maniera estensiva in aree marginali. Il rapporto uomo-animale si è rivelato in grado di determinare importanti ricadute sulla produttività, sul comportamento e sul benessere degli animali, soprattutto nelle aziende da latte, in virtù dell'elevato numero di situazioni che comportano un'interazione con gli addetti al bestiame, fra cui in particolar modo la mungitura. Quest'ultima può risultare stressante specialmente in soggetti di primo parto, in cui l'effetto novità del luogo e della situazione è massimo. Il presente lavoro si è incentrato sul primo mese di lattazione allo scopo di evidenziare la risposta comportamentale della bufala all'avvio alla mungitura meccanica, valutando, inoltre, la correlazione con la somministrazione di ossitocina. La prova ha riguardato 8 pluripare e 6 primipare che sono state seguite durante due mungiture mattutine alla settimana, a partire dalla prima entrata in sala di mungitura fino alla quinta settimana di lattazione. Le osservazioni comportamentali sono state eseguite secondo il metodo dell'animale focale in continuous recording per la registrazione di comportamenti preselezionati, comprendenti: scalcio, sollevamento dell'arto posteriore, defecazione, minzione, vocalizzazione, distacco del gruppo di mungitura e somministrazione di ossitocina esogena. Per ogni comportamento sono state calcolate le frequenze relative, utilizzando il test chi quadrato di Pearson per verificare la presenza di differenze fra primipare e pluripare. Il test Q di Cochran ha verificato la variabilità dei comportamenti nel tempo e la regressione binomiale è stata impiegata per individuare correlazioni fra i comportamenti manifestati e la somministrazione di ossitocina. Considerando l'ordine di lattazione, le primipare, fatta eccezione per il sollevamento dei posteriori, hanno mostrato maggiori frequenze per tutti i comportamenti (36,67% vs 24,36% per lo scalcio; 5% vs 2,56% per la defecazione; 11,67% vs 5,13% per il distacco del gruppo di mungitura), e in particolare la minzione è risultata significativamente ( $P < 0,001$ ) maggiore rispetto allo stesso parametro delle pluripare (48,33% vs 11,54%). La somministrazione di ossitocina si è rivelata l'unico parametro significativamente variabile nel tempo ( $P < 0,001$ ), caratterizzato da un rapido declino rispetto ad una maggior stabilità dei parametri comportamentali, fatta eccezione per scalcio e minzione ( $P < 0,05$ ). La somministrazione di ossitocina è risultata correlata ad alcuni comportamenti: scalcio, sollevamento dell'arto posteriore e minzione ( $P < 0,001$ ). La manifestazione di comportamenti indicativi di stress acuto e la loro correlazione con la somministrazione di ossitocina, sottolinea l'impatto che ha la mungitura meccanica sul benessere e sulla produttività della Bufala Mediterranea Italiana.*

Parole chiave: *Bubalus bubalis, Mungitura meccanica, Comportamento, Ossitocina.*

**Introduction**

Italian buffalo breeding is mainly oriented towards milk production for the manufacturing of mozzarella cheese. Over the last decade the Italian buffalo population strongly increased. At the same time, buffalo milk production has greatly improved, reaching an increase of 177.8% in milk yield which is most striking especially when compared to the world trend that showed an increase

of only 50.7% (ANASB, 2004). The Italian dairy buffalo production is rapidly increasing for various reasons: the demand for this typical product is rising both on the national and international market, the registration as "Mozzarella di Bufala Campana" PDO (Protected Designation of Origin) has increased production value and last but not least, the bovine milk quotas on surplus imposed by the EU are not applied to buffalo milk. Such a development involved a growth

either with regards to the number of buffalo farms in the country or with the number of heads per farm. As a result, to improve the production, intensive techniques and mechanisation of farm activities have been introduced, mostly inspired to dairy cattle farming. Those changes implied rapid modifications in buffalo management introducing high energy level and protein concentration in total mixed ration, loose housing system, machine milking etc.. In a species suddenly exposed to rearing techniques intensification with environmental and management changes, welfare and products quality are of particular concern. Machine milking can be considered as a critical point, such process being repeated at least twice a day and involving physical and psychological stressors: machine settings and maintenance, new environment, calf separation and close human contact, that represent potential source of chronic stress (Van Reenen *et al.*, 2002; Saltalamacchia *et al.*, 2007). Rushen *et al.* (2001) found that dairy cows milked alone in an unfamiliar room registered higher plasma cortisol concentration and heart rates and gave less milk due to higher residual milk and reduced oxytocin secretion. The same study proved that human contact, with a familiar person, can reduce behavioural signs but not the endocrine response to stress. In a previous work by Rushen *et al.* (1999), the presence of a gentle handler, has been shown as affecting milk yield or residual milk, while a rough one increased residual milk by 70%. The negative effects on bovine productions have sometimes been denied (Willis, 1983), but certainly milking process can affect the animal behaviour and fearful animals are often more difficult to handle and manage, time consuming, as well as dangerous for themselves and the stockpersons. This is particularly true in buffaloes, that are sensitive even to small changes in the milking routine, influencing both milking related release of

oxytocin and milk yield (Thomas *et al.*, 2005a, 2005b). In fact the small udder cistern causes almost 95% of buffalo milk to be stored in the alveolar compartment (Thomas *et al.*, 2003), where it can be removed only by oxytocin. In buffaloes, a longer pre-milking stimulation is required, when compared to cows (Costa and Reinemann, 2004). In some countries, where milking is predominantly performed by hand, stimulation is still carried out using calves, while when machine milking is available, it is allowed by milking routine. The latter consists in a series of actions conditioning the milk let down, including important procedures to keep the udder clean such as the washing of the udder. Washing has been proven as a factor of great influence on milk hygiene but most of all on milk release by buffaloes (Thomas *et al.*, 2005a). To shorten the time taken to milk and to improve the amount of milk obtained, the conditioned "let down" response should be reinforced by the same pattern at each milking and the handling of the udder should be the last part before milking starts (Gilmour, 1999). In addition, the milkers should be familiar with the milking machine and the buffalo behaviour. The buffalo species is characterised by milk let down difficulties consequent to pre-milking treatments. Such a condition gets to the absence of endogenous oxytocin peaks and leads to an incomplete udder emptying. (Thomas *et al.*, 2005a). If buffaloes feel uncomfortable or scared, they can withhold milk thus reducing the milk yield (Hogberg and Lind, 2003). Those problems are usually overcome in farms with exogenous oxytocin administration. De Rosa *et al.* (2005) identified the prevalence of oxytocin injection at milking as a possible welfare indicator related to human-animal interactions in the buffalo species. Even though some behaviours at milking, like the stepping that can be an expression of anxiety, proved a good repeatability (De Rosa *et al.*, 2003), they are influ-

enced by many unpredictable variables (insects presence, social interactions, lameness, milking machine maintenance). On the other hand, Saltalamachia *et al.* (2005) found that the prevalence of oxytocin injection is correlated to stepping and kicking and highly reliable when re-tested after five months. These authors, aiming at monitoring buffalo behaviour at milking and oxytocin injection administration in the whole herd, considered all the animals in the various stages of lactation (0-90 days, 91-180 days and more than 180 days) and found a higher milk let down difficulty in the primiparous cows. The present study is focused on the first month of lactation considering multiparous and primiparous cows calving in the same period. Starting from the first entrance in the milking parlour and during the following weeks, the objectives of this preliminary experiment were to evidence behavioural changes during the time as a sign of adaptation. Moreover the work aimed at finding differences between multiparous and primiparous cows and correlations between animal behaviour and the need to administer oxytocin. These informations could be useful in order to obtain indications for future studies on buffalo training for machine milking.

### **Material and methods**

The study took place in a commercial buffalo farm located in the production area of the "Mozzarella di Bufala Campana". The total herd size amounts to about 250 heads. All the animals are kept in loose housing system, without bedding and pasture, and the total mixed ration (TMR) is released in the trough twice a day. The waiting area in front of the milking stall is sheltered and the machine milking is performed twice a day (05:00 and 17:00) in a herring bone parlour with 6+6 batches without automatic cluster remover system, nor feed troughs.

As a consequence, during milking no food is administered to the animals. In every lactation they enter the milking parlour for the first time four days after calving. Until this moment, the calves are kept with their mothers. The mean milk yield per head in 270 days of lactation is about 2200 kg. The milking machine maintenance is regularly performed every six months setting up an operational vacuum of 50 kPa, 70 pulsation cycles/min at a ratio of 65:35. The two milking operators, both of Indian origin, follow a pre-stimulation routine during each milking consisting of washing the teats with tap water. It lasts for approximately 5 sec; if no milk ejection occurs within 2 min of machine milking, 10 IU oxytocin are administered intramuscularly.

In February, 14 buffaloes at calving were randomly selected to enter the study so as to reach a number of 6 primiparous and 8 multiparous cows to be followed starting from the first entrance in the milking parlour. The observations took place in two consecutive days/week during the morning milking for 5 weeks, performing a total of 10 observations/animal. The observations during the afternoon milking were performed only in the case it represented the first entrance of the animal in the milking parlour. The selected primiparous cows were all 3 year old, while the multiparous ranged from 3 year and a half to 14 year old, with a mean of  $6\pm 3$  years. The study lasted until May but the possibility of animal behaviour changes in spring, due to the presence of insects during milking, especially for the kicking or stepping behaviour, was prevented by mosquito-nets and fly killers.

Observations took place during the time of milking, including pre-milking (from the time the buffalo entered the milking parlour until the milking unit was attached), milking (from the time the milking unit was attached until it was removed after milk flow ceased), and post-milking (until the animal

left the milking parlour). The mean duration was 15 min.

A focal animal continuous recording method was used to describe buffaloes behaviour. The observed behaviours were selected among those widely considered as indicators of acute stress or fear in cattle and buffaloes at milking (Willis, 1983; Hemsworth *et al.*, 1987, 1989, 2000; Rushen *et al.*, 1999; Breuer *et al.*, 2000; Munksgaard *et al.*, 2001; Waiblinger *et al.*, 2002; De Rosa *et al.*, 2005; Saltalamacchia *et al.*, 2005):

kicking, stepping, defecating, urinating, vocalizing, and pulling the teat cup off the teats; oxytocin administration at milking was also recorded.

In order to avoid the influence of a stranger presence on animal behaviour and productivity, the observations were always performed by the same trained observer, familiar to all the animals. The observer recorded the different behaviour frequencies on a printed form. For the differentiation between kick and step for the hind legs, we adopted the definitions provided by Rushen *et al.* (2001), Hemsworth *et al.* (2002), Van Reenen *et al.* (2002) and De Rosa *et al.* (2003): a step is a hindlimb movement in which the hoof is lifted less than 15 cm off the ground, while in the kick it is raised at least 15 cm off the ground at the mammary gland level or towards the stockperson.

Data were statistically analysed using STATA statistics package. Presence/absence of every behaviour in every observation ( $n=10$ ) were calculated. Pearson Chi-Square test was applied to verify the differences between primiparous and multiparous cows' behaviour at milking. A non parametric test for testing the hypothesis that dichotomous variables measured several times on the same subjects have the same means (Cochran's test) was used to assess the variability of recorded behaviours over time. Finally a binomial regression was performed

in order to verify the correlations between animals' behaviours and the need to administer them oxytocin.

## Results and discussion

During the behavioural observations no vocalizing has ever been registered in the selected buffaloes ( $n=14$ ). The behaviours recorded during the first month of lactation are reported in Table 1.

Comparing primiparous and multiparous cows' behaviour (Table 2), we can see how the behaviours considered as indicators of stress were performed more frequently by primiparous, except for stepping, probably due to the fact that the leg movements are expressed mostly as kicking by the primiparous. Only urinating behaviour proved to have a significantly higher frequency of occurrence in primiparous cows (chi-square,  $P<0.001$ ).

We verified how the recorded behaviours varied over time. The frequency of oxytocin administration proved a significant variation ( $Q=44.2$ ,  $P<0.001$ ) soon decreasing in the first three weeks, as for kicking and urinating ( $Q=6.14$ ,  $P<0.05$  and  $Q=7.17$ ,  $P<0.05$  respectively). Figure 1 gives a visual representation of the temporal trend of oxytocin administra-

Table 1. Proportional frequencies of occurrence of the selected behaviours and of oxytocin administration.

Behaviour	%
Kicking	29.7
Stepping	9.42
Defecating	3.62
Urinating	27.5
Vocalizing	0
Pulling the teat cup off	7.97
Oxytocin administration	36.2

Table 2. Frequency of occurrence (%) for the selected behaviours in primiparous and pluriparous cows.

Behaviour	Primiparous	Pluriparous
Kicking	36.7	24.4
Stepping	6.67	11.5
Defecating	5.00	2.56
Urinating	48.3**	11.5**
Pulling the teat cup off	11.7	5.13
Oxytocin administration	41.7	32.1

\*\*  $P < 0.001$  within row.

Figure 1. Proportional frequency of occurrence of kicking, urinating and oxytocin administration over time.

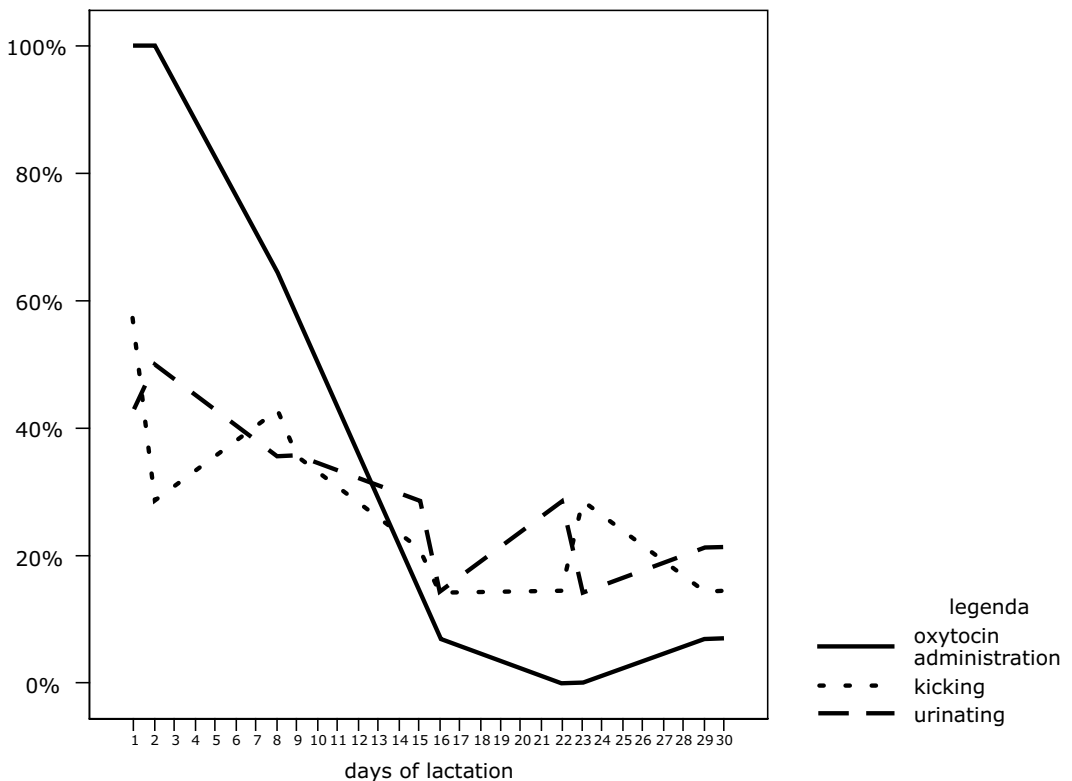


Table 3. Frequency of occurrence (%) for the performed behaviours in case of oxytocin administration or not.

Behaviour	No oxytocin administration	Oxytocin administration
Kicking	31.7**	68.3**
Stepping	23.1**	76.9**
Defecating	100	0
Urinating	36.8**	63.2**
Pulling the teat cup off	34.7	65.4

\*\*  $P < 0.001$  within row.

tion, kicking and urinating. Considering separately primiparous and multiparous cows, both showed a significant decrease in oxytocin administration ( $Q=20.7$ ,  $P < 0.001$  and  $Q=24.2$ ,  $P < 0.001$  respectively), while only the primiparous significantly reduced kicking progressing in milkings ( $Q=7.71$ ,  $P < 0.05$ ). These results confirm that the leg movements are expressed mostly as kicking by the primiparous during the first milkings.

The relationship between oxytocin administration and milking behaviour was considered and the results are shown in Table 3. The behaviours that appeared to be significantly related to the requirement of exogenous molecule are kicking, stepping and urinating ( $P < 0.001$ ). Kicking and stepping have previously been considered as indicators of agitation and aggressiveness (De Rosa *et al.*, 2003); eliminative frequency (urination) seems to be closely related to temperament in bovine cows, and is considered an indirect measure of fear (Das and Das, 2004).

## Conclusions

This study is based on a simple but strict observation protocol, that could be easily applied on farm surveys and during daily farm practices, as it is rapid and does not require a specific training for the observer. These preliminary results have proved the occurrence of behaviours considered as indica-

tors of acute stress in buffaloes starting the lactation with a machine milking system. It is noteworthy that it has evidenced the relationship between kicking, stepping and urinating occurrence and the need to administer oxytocin. In addition, kicking and urinating resulted associated to the animal milking experience level. They revealed, indeed, a significant reduction over time and characterised the primiparous behavioural pattern. In fact, in this group of animals, kicking significantly decreased, progressing in milkings, and urinating was significantly higher respect to the multiparous. As kicking and eliminative behaviours are considered indicators of agitation and fear, it can be assumed that the stressful moment represented by milking, is enhanced in primiparous cows. In this particular farm, no training for the heifers is provided and moreover the first milking occurs contemporaneously to separation from calf and introduction in a new group of animals. These findings suggest the need for more investigations on the training of the animals and management practices for milking, that can be very different from farm to farm. Kicking and urinating could be applied as valid indicators in buffalo milking welfare assessment. Oxytocin administration, correlated to those behaviours, confirm the oxytocin prevalence value as an objective welfare index in buffalo breeding.

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