



Expression of parlour behaviour, temperament, welfare and approach test in postpartum Surti buffaloes (*Bubalus bubalis*)

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ABSTRACT

The present study was carried out at Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat to investigate the effect of parity on milking parlour behaviour, temperament and welfare of postpartum Surti buffaloes. Fourteen postpartum Surti buffaloes were selected and grouped based on parity as primiparous and multiparous (2nd to 5th lactation). The milking behaviour of experimental animals was observed on the day of calving, 7th, 15th, 30th, 45th, 60th and 80th day post-calving keeping in view the peak production of buffaloes. The primiparous animals showed higher milk let down time and milking time, and lower rate of milk flow as compared to multiparous animals. The frequency of incidences of urination, defecation, kicking and stepping were significantly higher in primiparous group. The primiparous animals exhibited more avoidance behaviour when approached by a test person, indicative of more agitation. During milking and in paddock, primiparous animals were more aggressive and restless as compared to multiparous animals which exhibited calmer and docile temperament. From the present study, it could be conferred that parity significantly influences the parlour behaviour, temperament and welfare in postpartum Surti buffaloes.

Keywords: Milking behaviour, Parity, Surti buffaloes, Temperament, Welfare

Behaviour of animals in milking barn is affected by several factors such as parlour environment, milking routine, type of milking and parity. Parity significantly influences the temperament and milking behaviour in buffaloes. Primiparous animals show higher mean behavioural scores compared with their multiparous counterparts, likely because the multiparous animals are more accustomed to the parlour environment than the former ones (Rangel *et al.* 2014). Higher incidence of agitated behaviour is common in primiparous animals which include kicks, defecation and urination, and knocking off the milking cluster.

Buffalo cows behaviour in milking parlour needs attention and it can directly affect the milk yield and hence the profitability of the farm. The temperament of animal is very important in the handling of animals and improving their productive and reproductive abilities (Patel *et al.* 2017a). Milking behaviour, temperament and milk yield are inter-related, and any changes in either milking behaviour or temperament affects the milk yield (Prasad and Laxmi 2014). Docile buffaloes are good milk yielders and on an average have longer lactation period (Patel *et al.* 2017a).

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Behavioural studies are pertinent to determine the importance of behaviours such as rumination, defecation, urination, and reaction to milking, as these are closely related to animal welfare. Behavioural studies are helpful in selective breeding and also targeted mating. So it is necessary to evaluate a set of behavioural elements which enables the assessment of animal welfare and its effects on productivity. Limited reports are available on the temperament and milking behaviour in buffaloes, and also very few studies have been conducted on welfare of buffaloes *vis-à-vis* parity. In view of the need to address aforesaid issues, the present study was conducted to critically assess the changes in milking behaviour, temperament and welfare in primiparous and multiparous Surti buffaloes and to select buffaloes on the basis of calm milking behaviour even at farmer doorstep.

MATERIALS AND METHODS

Location of the study, climate and selection of animals: The present study was conducted in Livestock Research Station, Navsari Agricultural University, Navsari which is located at 20°51'0" North latitude and 72°55'0" East longitude at an elevation of 11.89 m above mean sea level from September, 2017 to May, 2018. The climate of the location of study forms a part of the coastal and tropical areas. Generally, the winter (November to February) remains fairly cold and dry, while the summer (March to June) is moderately hot and humid and rainy

season (July to September) is warm and extremely humid. For this study, 14 postpartum Surti buffaloes were considered and grouped based on parity as primiparous and multiparous (from 2nd to 5th lactation). Similar management practices were followed for all the animals under study. The animals were milked twice, i.e. during morning and evening hours.

Parlour behaviour, temperament and approach test: Milking behaviour attributes such as milk letdown time (sec), milking time (min), rate of milk flow (kg/min), kicking (lifting of the hind foot by at least 10 cm off the ground and moving it quickly forward) and stepping (weight shifting from one hind foot to the other with the foot less than 10 cm off the ground) incidences (%), butting by calf (%) and resistance to entry into parlour were observed during milking in the parlour on the day of calving, 7th, 15th, 30th, 45th, 60th and 80th day post calving. Simultaneously, milk yield was measured using an electronic weighing balance and temperament of experimental animals in parlour during milking was observed. The paddock temperament was also observed from the day of calving to 60th day post calving at fortnight interval. The temperament scoring of buffaloes was performed as per the score card presented in the Supplementary Table 1 (Dogra *et al.* 2002). The animals were approached by a test person other than herdsman (human approach test) and their behavioural responses were noted down up to 60 days post calving at fortnight interval. Based on their response to the unknown approaching person, the animals were scored as per the score card (Supplementary Table 2) given by Rousing *et al.* (2004).

Observation of welfare indices: Furthermore, welfare indices such as teat lesion, tick infestation, lameness, urination and defecation incidences, and cleanliness (as per the score card given by Rousing *et al.* 2004) were observed during the course of experimentation. Besides, the postpartum uterine discharge was observed up to 4 days of calving starting from the day of parturition. The post-parturient uterine status was scored based on colour as 0 (Clear to light brown watery fluid without off smell), 1 (Clear to yellowish white mucoid fluid with some off smell), 2 (Thick white pus with pronounced foul smell), and 3 (Dirty brownish watery to purulent with pronounced foul smell).

From the experimental animals, approximately 5 ml blood sample was collected on the day of calving, 15th, 30th and 60th day post calving for serum separation. The serum separated by centrifugation was stored in deep freezer (-20°C) until further analysis. Serum cortisol concentration (µg/dl) was measured by standard Enzyme Linked Immuno Sorbent Assay (ELISA) technique using assay kit (Calbiotech, Inc. California) based on the principle of solid phase competitive ELISA.

Statistical analysis: The data collected during the study period was analyzed using t-test and chi-square test for investigating the effect of parity on different traits under consideration. The obtained means within the groups

were compared using DMRT as per standard statistical procedures (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Milking behaviour: Milk let down in buffaloes is influenced by many factors like anatomy of teat and udder, physiological stage of the animal, parity, stage of lactation and environment. In the present study, overall mean milk let down time of primiparous group was higher ($P<0.01$) as compared to multiparous group (Table 1). Similar findings were reported in Murrah, Nili-ravi and non-descript buffalo by Kumar *et al.* (2006) and in Mehsana buffalo by Patel *et al.* (2017b). The possible reason might be familiarization ability of multiparous animals to the parlour environment as compared to primiparous animals and the peak oxytocin level is attained earlier in multiparous animals than their primiparous counterparts. In contrary to the present findings, Dash *et al.* (1976) reported least milk let down time in first parity and an increasing trend in milk let down time with the parity till 5th lactation. In Murrah buffaloes, Rangel *et al.* (2014) reported non-significant difference in milk let down time between primiparous and multiparous group. Maximum milk let down time was observed on the day of calving in primiparous group and found to be decreasing with increase of days in milk (Supplementary Fig. 1a). This might be due to optimisation of oxytocin level

Table 1. Overall effect of parity on milking behavioural attributes in postpartum Surti buffaloes

Milking behavioural attribute	Primiparous (n=49)	Multiparous (n=49)	t-value/ χ^2 value
Milk let down time (sec)	63.49±0.80	53.49±0.59	10.025**
Milking time (min)	4.57±0.08	4.25±0.11	2.311*
Rate of milk flow (kg/min)	0.27±0.01	0.39±0.01	-6.432**
Milking temperament score (1-5)	2.12±0.11	1.27±0.06	6.672**
Kicking incidence (%)			
Absent	65.3	100.0	20.568**
Occurs once	26.5	0.0	
More than once	8.2	0.0	
Stepping incidence (%)			
Absent	22.4	69.4	30.228**
Occurs once	34.7	28.6	
More than once	28.6	2.0	
Butting by calf (%)			
Absent	79.6	85.7	0.644
Occurs once	14.3	10.2	
More than once	6.1	4.1	
Resistance to entry into parlour	Absent	Absent	-

* and ** indicates significance at $P<0.05$ and $P<0.01$, respectively across rows.

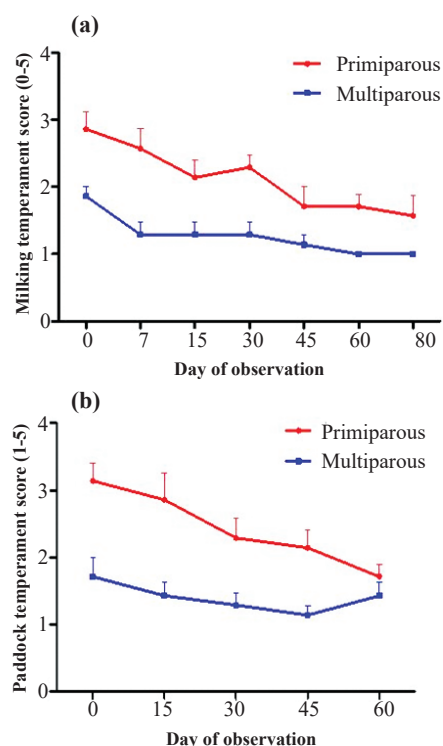


Fig. 1. Milking temperament score (a) and paddock temperament score (b) in primiparous and multiparous postpartum Surti buffaloes.

with familiarisation of parlour environment in primiparous animals as the days in lactation increases (Kumar *et al.* 2006). However, a definite trend was not observed in multiparous animals.

Overall milking time was higher ($P < 0.05$) in primiparous animals as compared to multiparous animals (Table 1). More milking time in primiparous animals might be due to time elapse in let down and anxiety related to milking. In contrary to present findings, Dash *et al.* (1976) and Patel *et al.* (2017b) reported an increase in milking time with increase in parity and Rangel *et al.* (2014) reported that milking time was not affected by parity in buffaloes. The change in milking time with increase of days in lactation is given in Supplementary Fig. 1b.

Milk flow rate was significantly lower ($P < 0.01$) in primiparous as compared to multiparous animals (Table 1). The rate of milk showed an increasing trend up to 30 days postpartum, and thereafter it did not show a definite trend in both the groups (Supplementary Fig. 1c). Similarly, Patel *et al.* (2016) reported an increase in rate of milk flow with lactation number which might be due to slower milk ejection and lower degree of udder filling in primiparous animals compared to multiparous animals. Contrarily, Rangel *et al.* (2014) reported that parity doesn't have any significant effect on rate of milk flow in buffaloes.

Overall 34.7% animals displayed kicking behaviour during the time of milking in primiparous group out of which 26.5% animals kicked once and 8.2% animals showed kicking more than once at the time of milking. However, in multiparous group, none of the animals

under study exhibited kicking at the time of milking on different test days of observation. Similarly, Cavallina *et al.* (2008) and Rangel *et al.* (2014) reported higher frequency of kicking among primiparous animals owing to more agitated behaviour of primiparous animals during milking as compared to their multiparous counterparts. Multiparous group displayed less ($P < 0.01$) stepping incidence as compared to primiparous group at the time of milking (Table 1). Experiencing more discomfort during milking leads to exhibition of more stepping behaviour in primiparous animals (Rousing *et al.* 2004). In primiparous group, butting by calf was observed in 20.4% animals, and in multiparous, butting by calf was observed in 14.3% animals. The higher incidences of butting by calf in primiparous group might be due to the lower mothering ability and milk flow in comparison to their multiparous counterparts. The frequency of stepping incidences and butting by calves during different days of observation is given in Supplementary Fig. 2. None of the animals under trial showed resistance to enter into parlour due to the fact that these animals were fed with concentrates during last trimester of their pregnancy in the milking parlour including primiparous ones.

The overall test day milk yield was lower ($P < 0.01$) in primiparous animals (2.40 ± 0.13 kg) as compared to multiparous group (3.14 ± 0.15 kg). The test day milk yield was minimum on the day of calving and it showed an increasing trend in both the groups with increase of days in lactation (Supplementary Fig. 1d). Similar to present findings, Yadav *et al.* (2013) also reported lower ($P < 0.05$) daily milk yield in first parity as compared to other parities in buffaloes, respectively. Contrarily, Sarkar *et al.* (2006) reported non-significant ($P > 0.05$) variation between different parities in test day milk yield although it was lower in first and second parities as compared to other parities.

Temperament during milking and in paddock: The result pertaining to temperament of animals during milking is given in Table 2. In the present study, among primiparous group, 51% animals were slightly restless followed by restless (24.5%), docile (20.4%) and aggressive (4.1%) during milking. In multiparous group, 73.5% animals were docile and 26.5% animals were slightly restless at the time of milking. Perusal of present findings showed that more number of animals exhibited docile temperament during milking. Similarly, Prasad and Laxmi (2014), Patel *et al.* (2016) and Choudhary *et al.* (2017) reported higher number of animals showing docile temperament during milking. Multiparous cows were calmer during udder preparation than primiparous cows, whereas cows of different parities behaved similarly during milking (Szenteleki *et al.* 2015).

The overall mean temperament score during milking was significantly ($P < 0.01$) higher in primiparous as compared to multiparous animals (Table 1). The novel parlour environment and milking procedures which acts as stressors for the primiparous animals might be the

Table 2. Effect of parity on milking temperament and paddock temperament in postpartum Surti buffaloes

Temperament of animals		Primiparous (n=49)	Multiparous (n=49)	χ^2 value
Milking temperament (% incidence)	Docile	20.4	73.5	32.485**
	Slightly restless	51.0	26.5	
	Restless	24.5	0.0	
	Aggressive	4.1	0.0	
	Nervous	0.0	0.0	
Paddock temperament (% incidence)	Docile	14.3	62.9	24.165**
	Slightly restless	40.0	34.3	
	Restless	34.3	2.9	
	Aggressive	11.4	0.0	
	Nervous	0.0	0.0	

* and ** indicates significance at $P < 0.05$ and $P < 0.01$, respectively across rows.

reason for higher temperament score in primiparous group (Rangel *et al.* 2014). Mean temperament score was higher on the day of calving and gradually decreased as the days in milk increased in both primiparous and multiparous group of animals (Fig. 1a).

The result of temperament of animals in paddock is given in Table 2. Among primiparous group, 14.3% animals showed docile temperament followed by slightly restless (40%), restless (34.3%) and aggressive (11.4%) in the paddock. In multiparous group, 62.9% animals were docile, 34.3% slightly restless and 2.9% restless in the paddock. Multiparous buffaloes were more adapted to regular handling procedures as compared to their primiparous counterparts (Choudhary *et al.* 2017). Mean paddock temperament score of primiparous animals (2.43 ± 0.15) was higher as compared to multiparous animals (1.40 ± 0.09) and it decreases gradually as the days in lactation progresses (Fig. 1b). Paddock temperament was higher in primiparous animals, as they were new to the lactating animal shed and to their herd mates. The mean paddock temperament score decreases as animals gradually accustomed to their environment and herd mates.

The animals of lower parity tend to show more avoidance and pessimistic behaviour as compared to multiparous animals, owing to their lower social order in the group (Clarke *et al.* 2018). Primiparous animals displayed more agitation and avoidance behaviour as compared to multiparous animals when approached by a test person. The overall mean human approach test score was lower ($P < 0.01$) in primiparous animals (1.34 ± 0.09) in comparison to their multiparous counterparts (1.89 ± 0.17). Familiarization of multiparous animals to the regular handling procedures makes them more approachable in paddock as compared to primiparous animals (Rousing *et al.* 2004). The change in human approach test score on different test days of observation has been presented in Supplementary Fig. 3a.

Welfare indices: Parity is one among the several factors that influence the welfare of animals. Urination and defecation frequency is related to temperament and in-turn welfare of animals; and it can be used as an indirect measure of fear (Das and Das 2004). In present study, overall mean

urination incidences were higher ($P < 0.05$) in primiparous animals (46.9%) as compared to multiparous animals (26.5%) (Supplementary Table 3). The change in urination incidences on different test days of observation has been presented in Supplementary Fig. 3b. In primiparous group, defecation incidence was 22.4%; whereas, defecation was not observed in multiparous animals during milking (Supplementary Table 3). Similarly, Cavallina *et al.* (2008) and Rangel *et al.* (2014) reported higher frequencies of urination and defecation in primiparous animals due to fear and anxiety experienced by them which indicates higher level of stress during milking as they were new to the parlour environment and milking procedure.

Concentration of cortisol in body fluids, hair and faeces can be used as an indicator of poor animal welfare (Barrell 2019). In the present study, serum cortisol concentration was higher in primiparous (3.50 ± 0.36 $\mu\text{g/dl}$) as compared to multiparous animals (2.91 ± 0.23 $\mu\text{g/dl}$), albeit it was not statistically significant (Supplementary Table 4). Similarly, Roelofs *et al.* (2019) reported non-significant difference in cortisol concentration among primiparous and multiparous sows as the acute stress response was comparable in both the groups. The higher serum cortisol concentration in primiparous animals might be attributed to their more agitated behaviour and less tolerance due to inexperience of routine handling as compared to multiparous animals (Mohammad and Abdel-Rahman 2013). Similarly, Singh *et al.* (2016) reported significantly higher serum cortisol concentration in nervous buffaloes in comparison to docile buffaloes as primiparous animals were more aggressive.

Post-partum uterine health is an important factor that influences the fertility and consequently duration of calving interval in dairy animals (Hay *et al.* 2019). Presently, only one animal under the study from primiparous group showed thick white pus discharge with pronounced foul smell. The results of uterine health status in primiparous and multiparous group of animals are presented in Supplementary Table 5. Parity did not significantly influence the uterine health status in postpartum Surti buffaloes. In contrary, multiparous animals are more susceptible to uterine infections due to age related

changes in uterus that delays the involution process (de Rezende *et al.* 2020).

Teat lesions, tick infestation and lameness were absent among primiparous and multiparous animals under study. Mean dirtiness score did not vary significantly between primiparous (2.29 ± 0.08) and multiparous buffaloes (2.26 ± 0.07). This is indicative of better management practices including cleanliness, adoption of vaccination, deworming protocols and precaution at the time of milking in the location of study.

The outcomes of the present study suggest that the multiparous animals exhibited better milking behaviour in comparison to their primiparous counterparts, and maintained a calmer temperament during milking and in the paddock. A significant difference in incidences of urination and defecation along with variation in serum cortisol concentration is suggestive of parity influencing the overall welfare of buffaloes. We recommend preferential rearing of multiparous buffaloes at doorstep of farmers with respect to production, milking behaviour and welfare status. Further studies are necessary to evaluate the influence of other on-farm stressors and the design of animal facilities on behaviour and temperament during milking to achieve better animal productivity and welfare.

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