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Effect of calf suckling dummy calf used and weaning on milk ejection stimuli and milk yield of Murrah buffaloes (*Bubalus bubalis*)

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Abstract
Present study to investigate the effect of suckling, weaning and dummy calf on milk ejection stimuli and milk yield of Murrah buffaloes. Twenty four late pregnant Murrah buffaloes were assigned to three treatment groups: weaning, suckling and dummy calf group. Mean letdown time and means milking temperament score were significantly (P<0.01) lower in suckled buffaloes in compared to dummy used buffaloes and weaned buffaloes. The mean of daily milk yield were significantly (P<0.01) higher in suckled buffaloes as compared to dummy used buffaloes and weaned buffaloes. The mean milk flow rates were significantly (P<0.05) higher in suckled buffaloes as compared to weaned. The mean plasma cortisol levels were lower in suckled and dummy used buffaloes groups as compared to waned buffaloes. It was concluded that daily milk yield improved in buffaloes allowed natural suckling and dummy used buffaloes as compared to weaned group buffaloes.

Keywords: weaning, natural suckling, synthetic dummy calf, milking temperament

Introduction
Buffalo is considered as black gold of India. Presently, India is producing 162.7 million tons of milk from which buffalo is contributing 56% of total milk production. Buffalo population in India is 108.70 million (Livestock Census, 2012) [20] and almost all of the buffaloes in India are reared by small and marginal farmers. Under traditional management system of buffaloes as prevalent under village conditions, the calves are allowed to suckle their dams for their milk feeding as well as for the letdown of milk. However, at most of organized buffalo farms and at many of commercial buffalo farms in the country the calves are weaned from their mothers at birth and artificially fed and reared. The most common reasons for adopting this practice are the accurate recording of milk yield, the economical and scientific feeding of the calves, the facilitation of the mechanization and automation of farm operations such as machine milking, the saving of labour cost and ensuring clean milk production etc.

There are several adverse consequences of weaning of calves on the dams. Experience has shown that about 25 % of buffaloes do not adapt to weaning as they do not let down milk without being suckled and there is greater amount of residual milk as the let-down of milk is partial. The incidence of buffaloes letting down milk only once daily takes place shortly after calving reducing their milk yields. The buffaloes whose calves have been weaned have poorer milking temperament and their lactation especially in first calvers are shorter lowering their lactation production. When the calf is suckling the residual milk after milking, the increased degree of udder emptying enhances production (Sandoval-Castro *et al*., 2000) [15]. The problem in milk let down in buffalo is due to buffalo have small udder cistern and almost 95% of the milk is stored in the alveolar compartment (Fig.1). As a result, pre-milking stimulation is of extreme importance for optimal milk ejection response in buffalos as compare to other dairy animals.

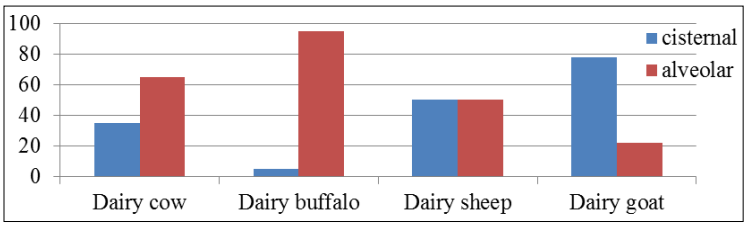

Fig 1: Milk occurrence in cistern and alveoli in different species of dairy animals

Therefore there is a need to evolve some alternative practice which may allow for weaning of calves and at the same time ensure proper let down in buffaloes. The use of a dummy calf may simulate a let-down stimulus as good as provided by natural suckling and may ward-off the use of oxytocin and at the same time it may allow for mechanization of milking operation and may improve the efficiency of milking. The present study is therefore planned with effect of calf weaning, natural suckling and use of dummy calf for milk let-down on milking behaviour, performance of Murrah buffaloes.

Materials and Methods

The present study was conducted on Murrah buffaloes reared under stall feeding conditions at Livestock Research Centre of NDRI, Karnal. All the buffalos were kept in loose housing system with brick flooring and managed as per the practices followed in the institute's herd. For the present study, 24 late pregnant Murrah buffaloes were selected from the herd Livestock Research Centre of NDRI, Karnal and were divided into three groups of 8 animals in each on the basis of their parity and last lactation yield i.e. suckled group, dummy calf used buffaloes and weaned group. The guidelines for animal experiments outlined by the Institutional Animal Ethics Committee which approved this study, and the ethical guidelines of the National Dairy Research Institute, Karnal (Haryana) were followed during all the animal experimentations. The selected late pregnant animals were shifted to calving pens 10-15 days before the expected date of calving. The advance pregnant animals were kept in a separate paddock from the general herd. They were offered green fodder and roughage *ad lib* and concentrate mixture was offered @ 1.5 to 2.00 kg per animal during advance stage of pregnancy. After calving the animal received *ad lib* supply of green fodder and roughages. A calculated amount of concentrate mixture based of the milk yield was offered at the time of milking. Free choice fresh water was available round the clock. From days after the parturition, buffaloes were shifted to the milking herd and maintained under standard feeding and management practices. The experiment was conducted from for a period of 6 month from the date of calving.

Dummy calf: Dummy calf was made by using a basal framework made of strong wooden frame. This framework was covered with the paddy straw mixed with clay. This structure was covered with black colour velvet cloth to give the appearance of the buffalo calf. Black colour velvet cloth provided the skin coat appearance and texture. Original hooves were used in the buffaloes dummy calf to make it look like as close as a natural buffalo calf. For the calf vocalization used the play recorded calf's voice at the time of milking. The voice recorded of the about one week old age calf before feeding.

Different practices used for bonding of dam with dummy calf

1. Replacement of calf with dummy calf immediately at the time of parturition- in this method the animal was observed during the parturition and immediately after parturition the calf was removed without making its any contact to the dam and dummy calf was placed in front of the dam and body of dummy calf was smeared with the amniotic fluid so that it may lead to the bonding between the dam and dummy calf. The bonding may take place due the fact that during the parturition calf while passing through the birth canal causes the vagino-cervical stimulation which leads to the release oxytocin from the hypothalamus and oxytocin acts on olfactory bulb and causes the release of dopamine, which initiates the sensitive period during which animal identify the calf.

3. Smearing of freshly drown milk on the body of the dummy calf- in this method milk of dam was smeared on the body of dummy calf and presented to the dam, dam licked the dummy calf and started identification of dummy calf. The licking of its own milk on the body of dummy calf may initiate the milk let-down. During the time of let-down the oxytocin is released from the pituitary which may sensitive olfactory bulb and causes the release of dopamine, which initiates the sensitive period.

4. Smearing of urine on the body of the dummy calf- in this method urine of the dam smeared on the body of dummy calf and presented to the dam.

5. Application of placental membranes on the body of the dummy calf- in this method the placenta of the dam is rubbing on the body of the dummy calf after 12 hours of parturition and then presented to the dam.

6. Use of recorded calf vocalization- Vocal communications plays an important role in the social relationships in many animal species. Calling to one another is particularly important between mothers and their infants. These vocalizations may be used to locate and maintain contact between mothers and their offspring. Vocalizations are often used by hungry youngsters to signal to their mothers that it is time to eat.

Parameters like milk let-down time was recorded as the time interval between the provision of milking stimulus and full engorgement of teats, milk yield was the amount of milk yielded in 24 hours obtained by milking the animals following complete letdown, the milk flow rate calculated by dividing the total milk yield (kg) by the milking time. It is calculated by formula given below-

MFR= Yield per milking (kg)/ Total milking time (min)

The milking temperament of experimental buffaloes were studied and scores were awarded according to the following five pint scale of milking temperament scores (Table: 1) (Tulloh, 1961) [18]. Blood was drawn in sterile heparinized vacutainer tubes from jugular vein puncture, posing minimum disturbance to the animal during collection, on days 0, 5, 7 and 15 (before and after milking) of parturition day. Blood samples were collected from all animals during the scheduled days. The samples were centrifuged at 3000 RPM for 20 min to separate the plasma. The plasma was aspirated, welled suitable aliquots and stored at -20^{0}C temperature till further analysis.

Statistical analysis

All data obtained in the study were subjected to the statistical analysis as per procedure described by the Snedecor and Cochran, (1994) [16]. The significance of the differences between the mean values of various parameters studied was tested by employing SPSS computer software.

Results and Discussion

Milk let-down time (min): The means milk letdown time of

experimental buffaloes are presented in table 1. The mean milk letdown time was significantly (P<0.05) lower in naturally suckled buffaloes than dummy calf used buffaloes and weaned group and also dummy calf used buffaloes was significantly (P<0.01) lower than the weaned group. The lowest milk let down time obtained in the calf suckled buffaloes may be attributed to the instant milk ejection stimulus provided by calf suckling by visual, tactile and olfactory as well as vocal stimuli and the manipulation of teat end never cells by the calf suckling which are very sensitive to touch. In case of dummy calf used buffaloes the stimulus was provided by the visual, olfactory and vocal cues and the tactile stimulus by way of teat manipulation was absent which may have caused a little delay in effecting the milk election reflex in these buffaloes. In weaned group of buffaloes the milk ejection stimulus was provide by offering of concentrate mixture and manual teat massage and the other important stimuli of olfaction, visual and vocal cues were absent which may have resulted buffaloes in this group taking longer time to perceive the sensory stimuli for milk ejection. The findings of present study are also supported by the findings of Dash *et al*., (1976) [3] who reported lower milk letdown time in suckled buffaloes (100 sec) as compared to weaned buffaloes (109 sec).

Daily milk yield (kg): The means daily milk yield of experimental buffaloes are presented in table 1. The mean daily milk yield of suckled buffaloes was significantly higher than the dummy calf used buffaloes (P<0.05) and weaned buffaloes (P<0.001). The highest daily milk yield among the three groups of buffaloes obtained in the suckled buffaloes might be due to the better milk let-down and its efficient removal from the udder. Another reason for this could be that when the calves suckled their mothers, the posterior pituitary of the buffaloes was stimulated and oxytocin continued to be secreted and affected the milk ejection. Mc Cowan *et al*., (2002) [10] suggested that the hormonal mechanism mediating this increase in milk production is likely growth hormone and may be prolactin, which mediate milk production, rather than oxytocin, which mediates milk let-down. Similar to present findings Kamboj *et al*., (2011) [6] reported the mean daily milk yield in natural suckled and organically managed and weaned and conventionally managed buffaloes group were 7.94 and 7.29 kg/day respectively. Similar finding were also reported in cows by Combellas *et al.,* (2003), Yilma *et al.,* (2006), Kaskous *et al*., (2006), Froberg *et al.,* (2007), Tesorero *et al.,* (2006) and Mendoza *et al.,* (2010) [2, 19, 7, 4, 17, 11] as they have shown the higher value of daily milk yield in suckled cows as compared to weaning cows. This higher milk production in restricted suckled cows was probably due to the effect of more frequent udder emptying because when the calf is suckling the residual milk after milking, increased degree of udder emptying enhances production (Sandoval- Castro *et al*., 2000) [14].

Milk flow rate (kg/min): The mean milk flow rate of experimental buffaloes are presented in table 1. The mean milk flow rate was significantly higher in suckled buffalo as compared to dummy calf used buffalo (P<0.01) and weaned buffalo (P<0.001). The mean milk flow rate is higher in suckled buffaloes which might be due to better letdown stimuli in presence of natural suckling by calf and dummy calf in comparison to slower or intermittent let-down stimuli in absence of calf in weaned buffalo group. Present study is also in agreement with the finding of Mendoza *et al*., (2010) [11] also reported the milk flow rate higher in RS group (1.76 kg/min) as compared to AR (1.35 kg/min). Bava *et al.,* (2007) [1] reported that the average milk flow rate in suckled buffaloes were higher than the weaned buffaloes (1.42±0.60 v/s 0.92±0.37 kg/min). Similarly several studies conducted by Roshanfekr *et al.,* (2010) [13], Kumar *et al.,* (2006) [8] and Dash *et al.,* (1976) [3] also supported the present finding as higher milk flow rate in suckling group as compared to weaning.

Milking temperament: The mean milking temperament of experimental buffaloes are presented in 1able 1. The mean milking temperament score was significantly (P<0.001) higher in weaned buffaloes compared to suckled buffaloes and dummy calf buffaloes used buffaloes. These temperament scores during first week after calving were 4.31±0.16, 2.63±0.13 and 3.44±0.20 in the weaned, suckled buffaloes and dummy calf used buffaloes groups respectively, which declined gradually in all groups as the lactation progressed. The lesser values of mean milking temperament scores in suckled buffaloes and dummy calf used buffaloes which might be attributed to the presence of their calf with the suckled buffaloes and synthetic dummy calf in dummy calf used buffaloes during the milking, maternal bonding and mothering instinct. Another reason further corroborated by the fact that the suckled buffaloes and dummy calf used buffaloes were comparatively under lower level of stress as revealed from the mean blood plasma cortisol concentration as compared to weaned buffaloes. Ramasamy and Singh (2004) found that the milking temperament score had deceased as number of days in lactation advanced. Our finding is also supported by Dash *et al.,* (1976) [3] whose reported that the un-weaned buffaloes were more docile (1.58) than weaned buffaloes (1.90). Gupta *et al.,* (1985) [4] found that the dairy temperament of 262 buffaloes ranged from docile to slightly restless with an average temperament score of 1.72±0.08.

Plasma cortisol level (ng/mL) in Murrah buffalo: The mean plasma cortisol level of experimental buffaloes are presented in table 2. There was significant (P<0.05) lower mean plasma cortisol levels in suckled and dummy used buffalo as compared to weaned buffalo on the first day, pre and post milking in Murrah buffaloes. These variations in cortisol level before and after milking shows a continuous decline trend with the advancement of lactation in experimental buffaloes. Lefcourt and Elsassel (1995) [9] reported Cortisol. On day 1, cortisol concentrations increased in control calves (0.97 ng/mL, P < 0.05), separated dams (1.02 ng/mL, P < 0.05), and control dams (0.93 ng/ml, P< 0.05) following the time of separation compared with initial concentrations. On day 2, concentrations for separated dams increased (1.33 ng/mL, P<0.0l) with time.

Table 1: Mean ± SE of milk letdown time, daily milk yield, milk flow rate (kg/min) and milking temperament of Murrah buffaloes in suckled, dummy calf used and weaned buffalo

Parameters	Suckled buffaloes	Dummy calf used buffaloes	Weaned buffaloes
Milk letdown time (min)	1.87^{b} ±0.13	2.40^{c} ±0.18*	3.09^{a} ±0.26**
Daily milk yield (kg):	9.11^{b} ±0.16	8.29^{c} ±0.46*	6.74^{a} ±0.40***
Milk flow rate (kg/min)	1.058^{b} ±0.08	0.948^{c} ±0.09**	0.816^{a} ±0.09***
Milking temperament	1.38^{b} ±0.13	2.04^{c} ±0.23***	2.69^{a} ±0.17***

Means bearing different superscripts differ significantly (P <0.05)*, (P<0.01)**, (P<0.001)***

Table 2: Mean ± S.E. of plasma cortisol levels (ng/mL) in suckled, dummy calf used and weaned buffalo

Day after calving	Weaned buffaloes		Suckled buffaloes		Dummy calf used buffaloes	
	Before milking	After milking	Before milking	After milking	Before milking	After milking
0	$3.93^{a} \pm 0.31$	$5.07^{A} \pm 0.19$	$2.45^{b} \pm 0.15$	$3.82^{B} \pm 0.15$	$2.81^{b} \pm 0.18$	$4.80^{C} \pm 0.10$
5^{th}	$3.28^{a} \pm 0.24$	$4.36^{A} \pm 0.11$	$1.56^{b} \pm 0.14$	$2.85^{B} \pm 0.10$	$2.05^{b} \pm 0.05$	$3.21^{B} \pm 0.05$
7^{th}	$2.41^{a} \pm 0.13$	$3.58^{A} \pm 0.20$	$1.45^{b} \pm 0.12$	$2.27^{B} \pm 0.13$	$1.68^{b} \pm 0.18$	$2.08^{B} \pm 0.02$
15^{th}	$2.11^{a} \pm 0.03$	$2.91^{A} \pm 0.13$	$0.88^{b} \pm 0.13$	$1.62^{B} \pm 0.13$	$1.34^{b} \pm 0.17$	$2.65^{B} \pm 0.14$
Overall	$2.93^{a} \pm 0.18$	$3.98^{A} \pm 0.16$	$1.59^{b} \pm 0.13$	$2.64^{B} \pm 0.13$	$1.97^{b} \pm 0.15$	$2.86^{B} \pm 0.08$

Means bearing different superscripts differ significantly ($P < 0.05$)

Conclusion: The present study reflects the importance natural suckling and dummy calf in buffalo in which they shows lower milk letdown time, and better milk flow rate and daily milk yield in natural suckling buffalo and dummy calf used buffalo as compared to weaned buffalo.

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