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EFFECT OF DIFFERENT REARING SYSTEMS ON BEHAVIOURAL RESPONSES TO NOVEL STIMULI IN MURRAH BUFFALO CALVES

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ABSTRACT

The study reported aimed to assess the effect of different rearing systems on behavioural responses to novel stimuli in Murrah buffalo calves. Calves in the experiment were grouped in three treatments in accordance with differed maternal interaction: dam-reared through fenceline (FMC; n = 8), restricted maternal contact (RMC; n = 8); no maternal contact (NMC; n = 8). FMC calves had permanent access to their mothers for first 5 days of birth and thereafter were housed in groups with fence line separation from their mothers and suckled twice a day. RMC calves had access to their mother only twice a day during the suckling hours. NMC calves were separated from their mothers within 24 h after birth and fed via bottle with nipple. Behavioral response of each calf toward novel object (a bright coloured ball) was evaluated on exposure during the test for three consecutive days across three months. The findings showed that buffalo calves that were given fence line contact with their mothers performed significantly ($P<0.05$) the best across all treatment groups in terms of response to a novel stimuli. Whereas the performance of calves with restricted contact with their mothers was superior to calves weaned at birth from their mothers, it was not on par with calves with full time fence line contact with their mothers, calves weaned at birth with no provision of maternal contact performed poorly in terms of behavioural response to novel stimuli.

Keywords: *Bubalus bubalis*, buffaloes, behaviour, calves, Murrah, novel

INTRODUCTION

Dam-calf contact for an extended period of time is believed to improve the social skills of the calves (Santo *et al*., 2020). Early separation of the calf from its dam, constitutes an important animal welfare dilemma in the organic dairy system, as it prevents certain natural behaviours being expressed in calf (Wenker *et al*., 2021). Separating calves from their mothers within 24 h after birth has raised public attention and concern (Busch *et al*., 2017). Traits like being fearful, bold, exploratory, social, ability to cope with stressful conditions are related individual characteristics (Forkman *et al*., 2007; Foris *et al*., 2018; Neave *et*

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al., 2020; Lecorps *et al*., 2018; Van Reenen *et al*., 2005). Alternative systems, which allows dam-calf contact is receiving increased attention (Brombin *et al*., 2019). Rearing with dams has shown to reduce fearfulness, improve social competence, increase vigilant behaviour and is believed to have long lasting effect on submissive behaviour of calves (Wagner *et al*., 2012; Wagner *et al*., 2013; Buchli *et al*., 2017). Also suckling of dams is another confounding factor that has calming effects on calves (Hänninen *et al*., 2008). The ability to characterize animals based on personality has many practical applications in animal production such as management ease, genetic selection, and welfare implications for animals like enhanced locomotor activity is an indicator of stress (Koolhaas and Van Reenen, 2016; Pérez *et al*., 2017). The individual differences in traits can impact how calves handle early transitions and stressors that they experience on a commercial dairy farm and relate to overall performance. However, there has been little research into the influence of growing buffalo calves with their dams on calves' behavior. As a result, the purpose of this study was to investigate the influence of the mother rearing system on calf behaviour, which is generally not observed when calves are weaned from their mothers.

MATERIALS AND METHODS

The Institutional Animal Ethics Committee accepted this work, which was carried out in accordance with IAEC established criteria as per Article Number 13 of the Government of India's Committee for the Control and Supervision of Experiments on Animals (CPCSEA) laws. The research was carried out at the ICAR - National Dairy Research Institute's Livestock Research Centre in Karnal, Haryana, India (29°42' N; 76°58' E, altitude = 227 m above mean sea level). Summer temperatures range from 40 to 43 degrees Celsius, while winter temperatures range from 2 to 15 degrees Celsius. The experiment began in September 2019 and concluded in November 2019.

Design of experiment

Following successful parturition, a total of 24 calves (31.4±1.2 kg, 29.4±1.3 kg, and 30.3±1.1 kg) were individually assigned to one of three treatments (n = 8 each).

The calves in the first group were kept apart from their mothers (NMC = No Mother-Calf Contact). The calves were taken from their dams immediately after birth and put in a neonatal calf shed, where they had no social contact with their dams. For the first 5 days, calves were given colostrum, and then whole milk from their mothers twice daily at 6.00 and 18.00 h, using plastic nipple bottles at a rate of 10% of body weight until nutritional weaning.

Calves in the second experimental group were only allowed to see their mothers on a limited basis (RMC). After parturition, the calves were permitted to lick by their dams and were housed in the same pen until the first colostrum suckling, at which point they were separated from the dams and group-housed in the calf pen. They were granted 30 minutes of contact with their mothers twice a day, at 6.00 to 6.30 h and 18.00 to 18.30 h and were allowed to nurse colostrum (10% of body weight) for up to 5 days. Calves were separated from their mothers after the sixth day and allowed to nurse milk (10% of body weight) twice daily (6.00 and 18.00 h) in the milking parlour. The third set of calves experienced calf-mother contact along the fence line (FMC). Calf-mother pairing in this study were kept together in the same calving pen

for the first 5 days after parturition, providing for complete physical contact and voluntary access to calves to suckle colostrum. Calves and dams were divided by a fence line composed of galvanised iron pipe railings with a 5-foot height and a wire mesh starting on the sixth day. Furthermore, these calves were provided 30 minutes of physical contact and sucking of milk at roughly 10% of their body weight until nutritional weaning twice daily at each milking (6.00 to 6.30 and 18.00 to 18.30).

Calf housing and feeding

The experimental calves were kept in a loose housing arrangement with a covered shed and an adjoining open pasture with a total floor space of 3 m^2 per calf and a common feeder and drinker via a fence line feed barrier. Floor space and feeding space were provided in accordance with Bureau of Indian Standards for buffaloes in loose housing systems (BIS: 1223-1987). The concrete floors of the calves' sheds had grooves under the covered and open areas. Calves from the RMC and NMC groups remained in the calf enclosure, which had a covered shed adjacent to a gate and an open yard. The fence line group calves were maintained in a loose shed, with mothers having a covered area with an open paddock connecting them.

Calves were fed full buffalo milk twice a day at 10% of their body weight until they were three months old. Chopped maize green fodder and calf concentrate were given ad libitum beginning in the second week of life, along with clean water and a salt lick block. The calf concentrate mixture had 35% maize, 20% wheat bran, 10% grams, 32% groundnut cake, 2% mineral mixture, and 1% common salt. Ad libitum access to clean, fresh water with a salt lick was provided.

Calf's reaction to an unfamiliar object

A novel object test was done inside the training pen to assess the amount of fear and anxiety in calves for three treatment groups, according to Meagher *et al.* (2015). A brilliantly coloured ball was chosen as the novel object for the experiment as represented in Figure 1. The tests lasted 10 minutes each for each calf. The animal was acclimated to the novel object for around 5 minutes prior to the test. The calves were then withdrawn from the novel object's touch. After a few minutes, the calf was returned into the test arena of a novel object for 10 minutes, and the behavioural reactions recorded are listed in Table 1.

Statistical analyses

The response of each calf to the novel object was recorded for each treatment group and were compared using a mixed model (IBM SPSS Statistic 22.0 computer software). Treatment, time, and their interaction were included as fixed effects, and the individual calves were included as random effects. Differences were considered statistically significant when $P<0.05$. Results are presented as LS means ± SEM.

RESULTS AND DISCUSSIONS

The results of the calves' behavioural responses in the novel object test are summarized across months in Table 2, for three consecutive days in Table 3, and overall reaction of calves to novel object exposure in Table 4. Following an evaluation of calves' behavioural responses to novel objects on exposure to novel object test, it was determined that calves when reared with their mother perform better than calves who have been

restricted or complete separated from their mothers during their early days of life.

Contact with the novel object by calves

Calves from the FMC group had a greater overall mean percentage of calves that made contact with the object (P<0.05 for all), followed by the RMC and NMC groups. Also, it has been reported that calves when raised beside their dams are able to detect novel feed sooner than calves kept separately (Costa *et al*., 2014). Contact with the novel object by calves in the FMC group was higher (P<0.05) during the three months, but calves in the RMC group made considerably more contact with the novel object during the second and third months of the trial. During the three months, the calves in the NMC group had much less interaction with the object. A significant difference in the contact with the novel object was not observed in a comparison of tests done on day 1 in various treatment groups. On day 2 and 3 of the trial, contact with the novel object was (P<0.05) higher in the FMC group, followed by the RMC group, and finally in the NMC group.

Number of retreats by calves from novel object

To evaluate fear latency in approaching a novel item is often used, where more latency indicates a higher fear level and vice versa (Van Reenen *et al*., 2004). The overall mean number of retreats from novel objects, as well as the number of retreats across months, were higher (P<0.05) in NMC calves, followed by RMC, and lowest (P<0.05) in FMC calves. On days 1, 2, and 3, there was a significant difference in the number of calves retreating in various treatment groups, with the NMC group having a greater number of retreats (P<0.05), followed by the RMC group, and lastly the FMC group. The study's higher frequency of retreats from novel objects could be linked to impaired judgement following maternal separation. Fearful reactions to any novelty in a dairy farm are a major concern in animal welfare. The more number of retreats from novel object in NMC calves can be indicative of the impact of early maternal separation which may have provoked fearfulness behaviour in these calves. Whereas in the RMC and FMC groups, calves had limited and full-time contact with their mothers, making them more sociable and attentive to novel environments, implying that early social interaction diminishes behavioural and physiological response to environmental novelty (Vieira *et al*., 2012).

Time spent in exploring novel object

The overall mean time (second) spent exploring novel object was higher (P<0.05) in FMC calves, followed by RMC, and lowest in the NMC group. The current findings support the hypothesis that calves with natural suckling spend more time exploring than calves fed artificially in buckets (682.5258.5 vs. 192.8112.9 minutes) (Chen *et al*., 2015). Calves in the FMC group spent more time exploring the novel object (P<0.05) over the three months, but calves in the RMC group made significantly more contact with the novel object during the first and second months of the study. The calves in the NMC group spent the least amount of time exploring the object over the course of three months. On days 1, 2, and 3, there was a significant (P<0.05) difference in time spent examining novel objects between treatment groups, with the FMC group spending more time exploring the object than the NMC group. Also, it has been reported in study that response to the novel screen is lower after maternal separation compared to before separation (623.6 vs. 723.6%) (Daros *et al*., 2014). Calves weaned for one day explore more than

calves weaned for one month (Lensink *et al*., 2006), emphasising the importance of calves' contact with their mothers. The ability to explore has long been seen as an essential component of animal welfare (Ohl and van der Staay, 2012). A decrease in this behaviour and a decreased interest in novelty (Hart, 1988; Haba *et al*., 2012) has been viewed as a behavioural alteration when an animal is ill, which in the current study could be a rationale for lower exploratory behaviour in the NMC group. Reduced exploratory behaviour is an indicator of difficulty in adapting to new managemental techniques in farms such as changes in pen, feed, and so on, which may impede with receiving essential nutrients with animals being latent in situations (Cramer and Stanton, 2015).

Time spent standing in the test arena

The overall mean time (second) spent standing and time spent standing over the three months in the test arena were higher ($P<0.05$) for calves with FMC compared to the RMC and NMC groups but did not differ significantly between the RMC and NMC groups. During the experiment, there was a significant difference in the time spent standing for calves in the FMC group against the RMC and NMC groups on day 1, day 2, and day 3.

Time spent running in the test arena

The overall mean time (second) spent running, time spent running across the months, and time spent running on day 1, day 2, and day 3 during the trial in the test arena were lower ($P<0.05$) in FMC calves compared to RMC and NMC groups but did not differ between the two groups. Longer running times in the NMC and RMC groups may be linked to the consequences of early life deprivation, which is a rather permanent impact generated by environment-induced change. This reduced or absence of sensory input to calves with RMC and NMC may have resulted in calves being less adaptable to novel situations since altered sensations make the brain susceptible to rapid stimulation and early handling may leave significant modifications within the neuroendocrine system (Denenberg *et al*., 1967).

Number of escape attempts from test arena

The mean number of escape attempts from the test arena, across the months and on day 1, day 2 and day 3 during the trial were ($P<0.05$) higher for calves with NMC compared to RMC and NMC groups, with an insignificant difference between RMC and FMC groups.

Time spent in exploring objects other than novel object in the test arena

The mean time spent in the test arena exploring objects other than novel objects and across months was ($P<0.05$) higher for calves with NMC compared to RMC and NMC groups but did not differ between RMC and FMC groups. On day 1, day 2, and day 3 of the study, the NMC group spent ($P<0.05$) more time examining objects other than novel objects in the test arena than the other Treatment groups. The presence of a novel object was thought to elicit a more fearful response in NMC calves than in RMC and FMC calves, causing them to become latent towards the unfamiliar novel object and more explorer of familiar objects in the test arena, ultimately leading to more exploration of other test arena objects (gate, walls, manger, etc.) to find a way out of the arena. This behaviour in the current study is easily described by the notion that exploration can be extrinsic (for food or water) or intrinsic (for a stimulus that has no biological meaning) (Berlyne, 1960; Wood-Gush and Vestergaard, 1989).

Table 1. During the experiment trial, different behavioural responses that were observed in Murrah buffalo calves when exposed to novel object.

No.	Behaviour	Description
1	Contact with the novel object	Whether the calf made contact with the novel object during the 10 minutes trial period was indicated by allocating 100 points for touch and 0 points for no contact with the novel object
2	Number of retreats from object	When the calf attempted to walk away while oriented to the object
3	Novel object exploring time	The time spent by the calf sniffing, licking, and pushing the object during the trial
4	Standing time	Time when the calf's weight was supported by at least one hoof and there was no translocation of the body as a result of exposure to the novel object
5	Running time	A four-beat gait with forward or sideways movement lasting more than one second
6	Number of escapes from the test arena	The calf's attempts to flee from all potential exit places of the arena
7	Exploration of other items in pen	The time spent by the calf sniffing and pushing objects present in the test arena.
8	Defecation	Passing out excreta in reaction to a novel condition during the trial. The calf that passed excreta during the trial received 100 points, while the calf that did not pass excreta during the 10-minute testing time received 0 points
9	Number of vocalizations	Sounds made by the calf on being exposed to a novel situation during the trial period

Table 2. Response of calves to novel object exposure in different treatment groups across months.

Parameter	First month			Second month			Third month		
	FMC	RMC	NMC	FMC	RMC	NMC	FMC	RMC	NMC
Contact with the object (%)	58.33^{b}±12.19	45.83^{ab}±8.76	20.83^{a}±8.76	66.60^{b}±8.9	54.10^{b}±8.76	25.0^{a}±8.30	79.16^{b}±8.70	41.66^{b}±13.70	12.50^{a}±8.70
No. of retreats from object	18.58^{a}±0.60	26.50^{b}±1.13	39.8^{c}±1.87	19.40^{a}±0.96	27.70^{b}±0.87	38.37^{c}±1.77	18.70^{a}±0.67	27.20^{b}±1.20	41.50^{c}±1.60
Novel object exploring time (sec)	79.25^{b}±11.15	57.83^{b}±11.89	14.54^{a}±5.72	75.90^{b}±10.7	61.40^{b}±10.20	17.20^{a}±5.60	92.10^{b}±10.20	43.40^{a}±17.10	13.80^{a}±6.90
Standing time (sec)	235.30^{b}±10.70	128.10^{a}±11.70	117.90^{a}±8.50	247.20^{b}±15.20	125.60^{a}±8.20	119.40^{a}±9.60	245.90^{b}±8.60	122.25^{a}±8.60	107.50^{a}±6.80
Running time (sec)	364.60^{a}±10.70	471.80^{b}±11.70	482.04^{b}±8.50	352.50^{a}±15.10	474.30^{b}±8.20	480.50^{b}±9.60	354.08^{a}±10.80	477.70^{b}±8.60	492.40^{b}±6.80
No. of escape attempt from test arena	30.90^{a}±2.09	31.50^{a}±1.60	41.60^{b}±3.00	25.90^{a}±2.00	30.70^{a}±1.90	40.50^{b}±2.70	27.70^{a}±1.63	32.08^{a}±1.50	41.60^{b}±1.86
Exploring of other objects in pen (sec)	353.00^{ab}±14.10	333.10^{a}±18.80	395.90^{b}±17.60	305.50^{a}±16.40	322.50±16.70	393.20^{b}±17.90	316.70^{a}±14.40	328.30^{a}±18.50	407.20^{b}±22.30
Defecation (%)	33.33±14.00	45.80±15.30	45.80±15.30	12.50^{a}±6.00	29.10^{a}b±9.80	54.10^{b}±12.50	29.30±13.2	33.30±10.90	58.30±15.10

The values are Mean ± SE of observations on eight animals in each group.

Values with different superscripts a, b differ significantly ($P<0.05$) in a row.

Table 3. Response of calves on exposure to novel object for three consecutive days in different groups.

Parameter	Day 1			Day 2			Day 3		
	FMC	RMC	NMC	FMC	RMC	NMC	FMC	RMC	NMC
Contact with the object (%)	58.33±13.70	45.80±12.50	25.00±10.40	79.16±10.70	54.10^{ab}±12.50	25.00^{b}±10.40	70.80^{b}±13.20	41.60^{ab}±12.10	8.30^{a}±5.40
No. of retreats from object	19.50^{a}±0.62	27.370^{b}±1.10	39.20^{c}±2.40	18.30^{a}±0.80	26.75^{b}±1.60	39.90^{c}±1.80	18.80^{a}±0.90	27.30^{b}±1.30	40.50^{c}±1.30
Novel object exploring time (sec)	73.20^{b} ±12.20	52.04^{ab}±16.10	20.30^{a}±6.80	93.70^{b}±13.70	62.75^{b}±14.50	17.70^{a}±7.60	80.25^{b}±16.00	47.80^{ab}±15.80	7.40^{a}±4.90
Standing time (sec)	235.30^{b}±10.70	128.10^{a}±11.70	117.90^{a}±8.50	247.20^{b}±15.20	125.60^{a}±8.20	119.40^{a}±9.60	245.90^{b}±10.80	122.25^{a}±8.60	107.50^{a}±6.80
Running time (sec)	364.60^{a}±10.70	471.80^{b}±11.70	482.04^{b}±8.50	352.50^{a}±15.10	474.30^{b}±8.20	480.50^{b}±9.60	354.08^{a}±10.80	477.70^{b}±8.60	492.40^{b}±6.80
No. of escape attempts from test arena	30.90^{a}±2.09	31.50^{a}±1.30	41.60^{b}±3.00	25.90^{a}±2.00	30.70^{a}±1.90	40.50^{b}±2.70	27.70^{a}±1.63	32.08^{a}±1.50	41.60^{b}±1.86
Exploring of other objects in pen (sec)	353.00ab±14.10	333.10±18.80	395.90^{b}±17.60	305.50^{a}±16.40	322.50^{a}±16.70	393.20^{b}±17.90	316.70^{a}±14.40	328.30^{a}±18.50	407.20^{b}±22.30
Defecation (%)	33.33±14.00	45.80±15.30	45.80±13.90	12.50^{a}±6.00	29.10^{ab}±9.80	54.10^{b}±12.50	29.30±13.2	33.30±10.90	58.30±15.10
No. of vocalization	30.60±0.70	42.30^{b}±1.50	110.40^{c}±4.60	28.70^{a}±1.60	38.70^{b}±1.50	101.80^{c}±2.50	29.30^{a}±1.00	38.70^{b}±1.20	102.90^{c}±4.20

The values are Mean ± SE of observations on eight animals in each group.

Values with different superscripts a,b differ significantly ($P<0.05$) in a row.

Table 4. Overall total response on exposure to novel object in different groups.

Parameter	Fenceline mother contact	Restricted mother contact	No mother contact
Contact with the object (%)	$68.06^{c}\pm7.40$	$47.22^{b}\pm4.06$	$19.44^{a}\pm5.40$
No. of retreats from object	$18.92^{a}\pm0.40$	$27.17^{b}\pm0.70$	$39.90^{c}\pm1.50$
Novel object exploring time (sec)	$82.44^{c}\pm8.80$	$54.22^{b}\pm7.20$	$15.21^{a}\pm2.92$
Standing time (sec)	$242.85^{b}\pm5.80$	$125.36^{a}\pm6.60$	$114.90^{a}\pm7.40$
Running time (sec)	$357.08^{a}\pm5.80$	$474.60^{b}\pm6.60$	$485.01^{b}\pm7.40$
No. of escape attempts from test arena	$28.21^{a}\pm1.24$	$31.46^{a}\pm1.38$	$41.31^{b}\pm2.13$
Exploring of other objects in pen (sec)	$325.10^{a}\pm11.20$	$328.04^{a}\pm12.80$	$398.80^{b}\pm17.60$
Defecation (%)	$25.00^{a}\pm7.40$	$36.10^{ab}\pm5.40$	$52.70^{b}\pm9.30$
No. of vocalization	$29.60^{a}\pm0.80$	$39.90^{b}\pm1.25$	$105.08^{c}\pm3.50$

The values are Mean ± SE of observations on eight animals in each group.

Values with different superscripts a,b differ significantly ($P<0.05$) in a row.

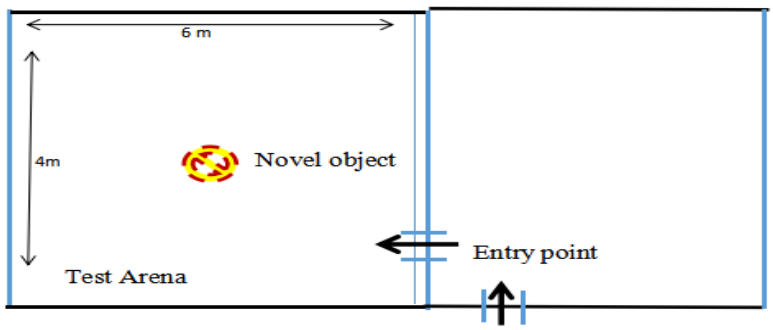

Figure 1. Diagram showing the arrangement of the test arena during the experimental trail.

Defecation by calves in the test arena

The mean proportion of calves that passed out faeces in the test arena was higher ($P<0.05$) in the NMC group compared to the FMC group but did not differ significantly in the RMC group. During the trial, the calves in the NMC group defecated ($P<0.05$) more than the calves in the FMC group during in the first month. In the second and third month, no significant difference in defecation was seen between the Treatment groups. On day 2 of the study, there was a significant ($P<0.05$) difference with calves in the NMC group defecating more. The response of calves defecating in the arena may be linked to a higher level of fear in the NMC group compared to the RMC and FMC groups, as the number of retreats and escape attempts from the test arena were higher in the NMC group compared to the RMC and FMC groups.

Vocalization by calves

The mean number of vocalisations during the trial and on days 1, 2, and 3 was higher ($P<0.05$) in calves with NMC, followed by RMC, and lowest in the FMC group. On day 3 of the trial, there was a significant ($P<0.05$) difference in vocalisation between calves in the NMC group and those in the RMC and FMC Treatment groups. Furthermore, frequent vocalisation in the NMC group indicates a high level of fear and a strong desire to leave the test environment.

CONCLUSION

Based on the results of the current experiment and subsequent discussion, buffalo calves that were given fence line contact with their mothers fared the best across all Treatment groups in terms of response to a novel stimuli. Whereas the performance of calves with restricted contact with their mothers was superior to calves weaned at birth from their mothers, it was not on par with calves with full time fence line contact with their mothers, calves weaned at birth with no provision of maternal contact performed poorly in terms of behavioural response to novel stimuli.

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