



# Effect of Social Isolation on Behavioural Response of Murrah Buffalo Calves Towards Novelty

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## ABSTRACT

**Background:** During the production cycle, dairy animals in intensive production systems are exposed to various challenging events including novel stimuli (e.g., handlers, food and group-mates). The welfare of an individual animal depends on the ability to cope with environmental challenges. Novelty is generally associated with negative emotions however; individual behavioural and physiological responses toward challenging events can be very variable. So, studying behavioural response of calves towards novelty can help us address the welfare needs of the buffalo calves in a holistic manner.

**Methods:** For this study, 24 Murrah buffalo calves were selected at birth and randomly allotted to four groups (six calves each) comprising of SG group (restricted suckling and group housed), SI group (restricted suckling and individually housed), WG group (weaned at birth and group housed) and WI group (weaned at birth and individually housed) for a period of three months. All calves were trained with bottle feeding from day old of age. At 9<sup>th</sup> week of age, the behavioural response of the calves to novelty was tested in two different novel situations i.e. novel object test and social novelty test using CCTV camera.

**Result:** The results suggest that buffalo calves separated from mother and housed individually showed increased behavioural responses characterized by increased time in decision making, higher standing idle time and more fearful to an unfamiliar calf compared to other groups. It can be concluded that social isolation of the buffalo calves increases fearfulness to novelty hence reducing their welfare in future.

**Key words:** Housing, Murrah buffalo calves, Novel object test, Social novelty test, Weaning.

## INTRODUCTION

In India, most of the commercial dairy farms as well as organized farms, the calves are weaned from their mothers at birth and artificially fed and reared individually or in groups. Maternal care plays a primary function in development of social skills, psychological welfare and cognitive faculties in the calves. Research in cattle has shown that social isolation increases anxiety and fear-related behaviors. Isolated calves exhibit higher levels of stress-related biomarkers, such as cortisol and show avoidance behaviors when faced with unfamiliar objects or environments (Boissy *et al.*, 2007). In dairy calves, isolation impairs social learning, reduces exploratory behaviors and increases fearfulness in novel situations (Jensen *et al.*, 1997). Dairy cows are generally exposed to novel stimuli including changing diets, housing and social groups. Novelty tests, such as the novel object test and social novelty test, are widely used to measure fear and stress responses in animals (Forkman *et al.*, 2007). These tests provide insight into the underlying emotional states of animals when exposed to any unfamiliar stimuli. In cattle, novel object tests have demonstrated that calves raised in social isolation exhibit heightened avoidance and fear-related behaviors and reduced ability to cope with social challenges (Wagner *et al.*, 2012). Prolonged isolation not only increases fearfulness but also leads to poor growth, reduced immune function and compromised productivity (Hemsworth *et al.*, 2000). The welfare of animals in early life is closely linked to their long-term

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performance and adaptability and the ability of animals to cope with different environments through behavioural attributes (Kumar *et al.*, 2017). While these findings are valuable for cattle, very less work have focused on measuring behavioural response towards novelty in Murrah buffalo calves, leaving a knowledge gap on how social isolation along with weaning practices affects their behavioural response to novelty. Addressing these gaps is essential for developing management strategies that can optimize the welfare and productivity of Murrah buffaloes in commercial farming systems.

## MATERIALS AND METHODS

For this study, 24 Murrah buffalo calves were selected at birth and randomly allotted to four groups (six calves each) comprising of SG group (restricted suckling and group housed), SI group (restricted suckling and individually

housed), WG group (weaned at birth and group housed) and WI group (weaned at birth and individually housed) for a period of three months. The suckled group of calves had access to their mothers for total 2 hours for milking in morning and evening milking shifts for let-down of milk (restricted suckling) daily at milking barn but fed milk from bottle only. All the calves were trained with bottle feeding from day old of age.

At 9<sup>th</sup> week of age, the behavioural response of the calves to novelty was tested in two different novel situations i.e. novel object test and social novelty test. In novel object test the calves were presented with a novel object i.e. a red pyramid (15×15×30 cm). In social novelty test, the behavioural response of the experimental calves to social novelty was tested with an unfamiliar calf (approximately similar age and body weight was chosen). After completion of novel object test the calves were rested for two days before the start of social novelty test with an unfamiliar calf, in order to avoid any biasness. The calves were tested daily for 3 consecutive days (3 sessions) for both the novelty tests. The duration of the tests was of 10 min per session for each novelty test.

One calf was tested at one time. The calf was gently guided into the test arena. After that the novel object was placed in the center of the test arena by the experimenter and the door was closed. Similarly, during the social novelty test, after the experimental calf had been headed into the test arena, the unfamiliar calf was then introduced to the test arena and door was closed immediately. The behavioral response of the calves during each novelty test was recorded by CCTV camera (COSMIC HD camera, CP PLUS). Vocalizations (frequency per session) were recorded manually for each calf per session. Urine and faeces excreted was cleaned after each session. The ethogram of different behavior recorded for novel object test and social novelty test are described in Table 1.

## Statistical analysis

All the data were subjected to statistically analysis using SPSS statistical software (version 21.0). Normal distribution of all variables was assessed using the Shapiro-Wilk test. The P-value of <0.05 was considered significant.

## RESULTS AND DISCUSSION

### Novel object test

Behavioural response of calves towards fear to novelty was evaluated in a novel object test by presenting the calves with a red coloured pyramid. The results of behavioural response of the calves obtained in the novel object test are summarized in Table 2.

### Latency to touch novel object

The WI calves took longer ( $P < 0.001$ ) latency to touch the novel object compared to other groups. Across the groups, the SG calves touched the novel object much faster followed by the WG calves and SI calves. However, the latency to touch the novel object was statistically insignificant among SG, WG and SI groups. One of the primary concerns of livestock welfare is: the fear to novelty. As reviewed by Van Reenen *et al.* (2004) the latency to approach a novel object has been used as a reference measurement of fear in poultry, pigs and cattle, with long latencies putatively indicating high levels of fear and vice versa. The longer latency to approach the novel object by the WI calves indicates the impact of early maternal separation and social isolation from conspecifics may have provoked fearfulness behaviour in these calves. Moreover as these calves were housed individually, they were less acquainted with novel situations compared to calves of other groups. Studies have found that dairy calves weaned from their mothers have shown low growth performance, lowered

**Table 1:** Ethogram of behaviours recorded during novel object test (NOT), social novelty test (SNT) or both.

Behaviour	Test	Description
Latency of touch novel object <sup>a</sup>	NOT	Time duration from entering the test arena upto/until touching the novel object.
Latency to initiate social interaction <sup>a</sup>	SNT	Time duration from entering the test arena until initiating social contact with the unfamiliar calf.
Interaction with novel object <sup>a</sup>	NOT	Time duration of sniffing and licking or displacing/pushing the novel object with head.
Social interaction with unfamiliar calf <sup>a</sup>	SNT	Time spent on sniffing and licking the unfamiliar calf while standing or walking.
Exploring test arena <sup>a</sup>	Both	Time duration of sniffing and licking the walls, floor <i>etc.</i> of test arena other than the novel object or unfamiliar calf while standing or walking.
Standing Idle <sup>a</sup>	Both	Time spent standing doing no activity.
Attention directed towards exit door <sup>a</sup>	NOT	Time duration looking at the exit door.
Locomotion <sup>a</sup>	Both	Time duration when animal is walking slowly, looking in front or around.
Vocalization <sup>b</sup>	Both	Number of vocalizations per session.

<sup>a</sup> The time duration (sec) of the behaviour was recorded.

<sup>b</sup> The frequency of the behaviour was recorded.

immunity and altered oral behaviours as compared to suckled calves (Singh *et al.*, 2019; Bharti and Kamboj, 2024). Our findings are in agreement with previous studies (Meagher *et al.*, 2015; Gaillard *et al.*, 2014) who reported longer contact latency to novel object in individually reared calves than in calves housed in groups or pairs. The reduced fear towards the novel object in our experimental calves (SG, WG and SI groups) are consistent with other studies suggesting early social contact reduces behavioral and physiological reactivity to environmental novelty (De Paula Vieira *et al.*, 2012). Dairy calves reared in complex social environments were reported to taste novel food faster than the calves reared in socially isolated conditions (Costa *et al.*, 2014).

#### Interaction with novel object

Across the group, the SG calves interacted significantly ( $P<0.001$ ) more duration of time with the novel object however, no significant difference was found among SI, WG and WI groups. The longer duration of interaction with the novel object by the SG calves can be attributed to the social privileges of both mother and peers received by these calves over other groups, who received either one of them (SI and WG groups) or neither of them (WI group). Maternal contact of calves provides opportunities for social transmission of information from a mother to her young such as information about food sources and predators (Thorhallsdottir *et al.*, 1990; Mateo and Holmes, 1997). The present findings are in line with Chen *et al.* (2015) who reported that naturally suckled calves (2 months) spent significantly more time ( $682.5\pm258.5$  vs.  $192.8\pm112.9$  min,  $P<0.05$ ) in exploring the novel object (novel decoy of a Holstein calf) compared to calves fed artificially from buckets. Though the SI and WG groups in the present study received the social contact of either mother or peers, but were reluctant or showed less interest to interact with the novel object, highlighting the importance of mother as well as peer contact essential for the overall development of calves. Similarly, we assume that the detrimental effect of maternal and social deprivation in our WI calves induced more fear in approaching and interacting with the novel object.

#### Exploring test arena

The time spent on exploring the maze was lowest ( $P<0.001$ ) in WI calves compared to other groups. The SI calves were more active and spent more time exploring followed by SG calves and WG calves. However, there was no significant difference among SG, SI and WG groups. The presence of the novel object (red pyramid) was thought to provoke fear response in the WI calves taking longer latency to touch and less time interacting with the novel object, ultimately leading to less interest in exploration of the test arena.

#### Standing idle

The time spent on standing idle differed ( $P<0.001$ ) across the groups. The WI calves spent significantly more time standing idle followed by SI, WG and SG calves. The individually housed calves (WI and SI groups) preferred standing idle for more duration of time compared to group reared calves (SG and WG groups). The WI calves were inactive and preferred simply standing idle compared to other groups. The higher time spent on standing idle is indicative of fear and less interest towards the novel object (red pyramid). Similar studies have also suggested that individually housed calves are more fearful than group-housed calves, when introduced to new social situations or when isolated in a novel arena (Jensen *et al.*, 1997).

#### Attention directed towards exit door

The results revealed that SG calves looked towards the door for more ( $P<0.001$ ) duration of time followed by WG, WI and SI calves. The SG and WG groups differed significantly to each other however; the duration of time looking toward the exit door was statistically insignificant between SI and WI groups. This suggests that the group housed calves (SG and WG groups) looked towards the exit door more often compared to the individually housed calves (SI and WI groups). The results obtained in our study share similarities with findings of Wagner *et al.* (2013) who observed more escape attempts in calves reared with their mothers in an isolation test as compared to artificially reared calves at 43 days of age. Similar to the escape attempts, looking towards gate as evidenced in our calves indicates a strong social bonding between the peer calf

**Table 2:** Behavioural responses of buffalo calves in novel object test with a red pyramid.

Behaviour	SG calves (Suckled and group housed)	SI calves (Suckled and individually housed)	WG calves (Weaned and group housed)	WI calves (Weaned and Individually housed)
Latency to touch novel object (sec)	45.95 <sup>b</sup> ±4.99	163.73 <sup>ab</sup> ±44.55	120.67 <sup>ab</sup> ±35.81	248.78 <sup>a</sup> ±39.34
Interaction with novel object (sec)	118.17 <sup>a</sup> ±8.47	15.95 <sup>b</sup> ±2.35	31.11 <sup>b</sup> ±5.15	15.22 <sup>b</sup> ±2.43
Exploring test arena (sec)	129.72 <sup>a</sup> ±7.75	143.33 <sup>a</sup> ±8.13	110.89 <sup>a</sup> ±19.09	37.95 <sup>b</sup> ±11.34
Standing idle (sec)	48.17 <sup>d</sup> ±13.00	192.83 <sup>b</sup> ±13.66	103.11 <sup>c</sup> ±7.24	327.78 <sup>a</sup> ±17.93
Attention directed towards exit door (sec)	164.39 <sup>b</sup> ±9.99	86.17 <sup>c</sup> ±8.24	199.11 <sup>a</sup> ±9.25	70.39 <sup>c</sup> ±5.54
Locomotion (sec)	139.61±4.81	161.72±5.97	155.89±23.84	148.67±9.12
Vocalization (freq.)	15.39 <sup>a</sup> ±1.88	4.00 <sup>c</sup> ±0.67	10.78 <sup>b</sup> ±0.83	5.36 <sup>c</sup> ±0.57

\*Values bearing different superscript in row differ significantly from each other ( $P<0.001$ ).

group and the eagerness to unite with their social mates. This idea is also supported by earlier findings by Napolitano *et al.* (2008) and Sevi *et al.* (1999) in lambs.

### Locomotion

The time spent on locomotion was highest in SI group followed by WG group, SG group and WI group. However, there was no significant difference among the groups for the time spent on locomotion. These findings are in line with De Paula Vieira *et al.* (2012) who observed no difference in locomotion time in the calves irrespective of different housing systems during novel object test.

### Vocalization

Perusal of the results revealed significant ( $P<0.001$ ) differences across the groups for vocalizations. The SG calves vocalized more frequently followed by WG, WI and SI. The group housed calves (SG and WG groups) vocalized more frequently as compared to individually housed calves (SI and WI groups). Between the group housed calves, the vocalization was more ( $P<0.001$ ) frequent in SG calves when compared to WG calves. However, no statistical difference was observed between SI and WI calves. The frequent vocalizations observed in the group housed calves (SG and WG groups) may be due to the separation responses and stronger social bonds shared by the group mate with their social companions, corroborating with similar findings (De Paula Viera *et al.*, 2012; Jensen and Larsen, 2014) reporting higher vocalizations in pair housed calves as compared to individually reared calves. Moreover, frequent vocalizations also indicating a higher motivation of the group house calves to meet and unite with their social partners. In contrast, the SI and WI groups were socially deprived and were reared in individual pens with no access to peers, so were expected to have limited social connection resulting in less frequent vocalization.

### Social novelty test

The social novelty test was performed to evaluate the calf's behavioural response, especially the "fear response" when confronted with an unfamiliar calf of about similar age group. The behavioural response of the experimental calves toward an unfamiliar calf is summarized in Table 3.

### Latency to initiate social interaction

Similar to the novel object test, the WI calves took significantly ( $P<0.001$ ) longer latency to initiate social interaction with the unfamiliar calf as compared to other groups. These results clearly suggest that the WI calves were less interested to initiate social interaction with an unfamiliar calf compared to other groups. These findings corroborates with previous studies reporting deficits in social behavior for calves reared individually (De Paula Vieira *et al.*, 2012; Veisser *et al.*, 1994; Jensen *et al.*, 1997). The calves of SG, SI and WG groups in the present study had access to some kind of social contact either with mother or peers which may have helped to develop social skills in these calves. Calves reared with their mother have intense social behaviour towards unfamiliar calves (Flower and Weary, 2001). Our findings support the idea that social interaction is a cardinal prerequisite in early stages of calf's life.

### Social interaction with unfamiliar calf

There was a significant effect of treatment on social interaction with an unfamiliar calf. The SG calves were more socially active spending more duration of time in social interaction, when confronted with an unfamiliar calf followed by the WG and SI calves. Across the groups, the WI calves were least interested ( $P<0.001$ ) to interact with the unfamiliar calf. However, the duration of social interaction was statistically insignificant among SG, SI and WG calves. Our results are in agreement with previous studies (Jensen *et al.*, 1997) which suggests that calves that have been reared individually appear to be more fearful of unfamiliar calves. It is also reported that individual housing of dairy heifers could lead to serious implications like reduced ability to cope within groups later on in the future (Broom and Leaver, 1978). However, according to Dube and Jensen, (2012) cows housed with full social contact with a peer are more socially active than calves housed individually, with limited contact through bars. Rearing of calves with mother for unrestricted or restricted contact increases the opportunities for social experiences (Arnold and Taborsky, 2010), which might have led to increased social interaction with the unfamiliar calf. This explains the social activeness of our experimental calves of SG, SI and WG groups compared to WI group of calves.

**Table 3:** Behavioural responses of buffalo calves towards an unfamiliar calf in social novelty test.

Behaviour	SG calves (Suckled and group housed)	SI calves (Suckled and individually housed)	WG calves (Weaned and group housed)	WI calves (Weaned and individually housed)
Latency to initiate social interaction (sec)	16.83 <sup>b</sup> ±3.36	23.39 <sup>b</sup> ±4.67	38.33 <sup>b</sup> ±17.04	222.50 <sup>a</sup> ±32.93
Social interaction with unfamiliar calf (sec)	175.94 <sup>a</sup> ±20.29	136.33 <sup>a</sup> ±12.47	171.94 <sup>a</sup> ±30.74	37.17 <sup>b</sup> ±17.92
Exploring test arena (sec)	163.94±27.98	185.11±13.84	181.33±10.42	165.33±13.08
Standing idle (sec)	156.33 <sup>b</sup> ±14.72	133.39 <sup>b</sup> ±8.57	130.83 <sup>b</sup> ±32.71	255.00 <sup>a</sup> ±32.00
Locomotion (sec)	103.78±12.89	145.17±15.82	115.89±8.21	142.50±15.47
Vocalization (freq.)	17.33 <sup>a</sup> ±1.70	6.78 <sup>b</sup> ±0.68	19.22 <sup>a</sup> ±4.44	6.28 <sup>b</sup> ±0.71

\*Values bearing different superscript in row differ significantly from each other ( $P<0.001$ ).



### Exploring test arena

The duration of time spent on exploring the test arena did not differ significantly across the groups. However, it was observed that the calves housed individually (SI and WI) spent longer duration of time exploring the test arena than the calves housed in groups (SG and WG). The results obtained in the present study can be explained by the fact that, the space constriction in the individually housed groups (SI and WI groups) may have restricted them to investigate and explore their surroundings. However, when provided access to a bigger area these calves were more eager to explore the arena in the form of sniffing and licking the floors and wall of the test arena rather than establishing social connection with the unfamiliar calf. This clearly demonstrates the negative impact of individual housing system on the development of early social behaviours in the calves which can even have detrimental consequences in the future for example; socially isolated calves are more submissive when mixed in groups later in life compared to calves reared with their conspecifics (Veissier *et al.*, 1994).

### Standing idle

Across the groups the WI calves spent significantly ( $P < 0.001$ ) more duration of time standing idle. The duration of time standing idle did not differ significantly among other groups. The WI calves spending approximately/about double ( $1.8 \times \sim 2 \times$ ) the time on simply standing idle without any activity when compared to other groups provides evidence that the calves housed in individual pens are fearful to new environments and may take more time in adjusting to novel surroundings. The results are in agreement with De Paula Vieira *et al.* (2012) who reported that individually housed calves spent more time standing inactive than did the calves housed in pairs (mean 302.20 sec vs. 232.20 sec SEM 30.20, respectively). The calves having access to mother or social companions are thought to learn about potential threats and develop agonistic behaviours, which is of paramount importance when calves are mixed in herds in future. Earlier studies have demonstrated that mother reared calves showed more submissive behaviour in order to reduce the risk of being attacked during integration into the cow herd compared with heifers reared without mothers contact (Wagner *et al.*, 2012). Our results are suggestive of the fact that individually housed calves may not develop the social skills necessary to cope with group living later on, being either more or insufficiently aggressive, or more fearful of other calves.

### Locomotion

The time spent on locomotion was found to be non-significant across the groups. However, the time spent locomotion was highest in SI group followed by WI, WG and SG. This clearly suggests that the calves reared in individual housing (SI and WI calves) showed more locomotory behaviour compared to calves reared in group housing (SG and WG). The results corroborate with findings

of De Paula Vieira *et al.* (2012) who reported individually housed calves showed more locomotory behaviour in the form of running compared to the paired housed calves ( $83.2 \pm 19.1$  vs.  $57.3 \pm 19.1$  sec). Similar explanations as suggested by the De Paula Vieira *et al.* (2012) explains our results that, housing of calves in individual pens limits the expression of calf's innate play behaviour and access to large test arena might have been rewarding for these calves.

### Vocalization

The effect of housing showed significant effect on vocalizations. The group housed calves (SG and WG) vocalized more frequently ( $P < 0.001$ ) compared to the individually housed calves (SI and WI). Similar trend was also observed in novel object test when calves were presented with a red pyramid. Similar explanations as explained in novel object test supports the findings in the present study. Moreover in cattle, the presence of conspecifics is known to reduce behavioral reactions to social separation (Boissy and Le Neindre, 1997; Piller *et al.*, 1999). For example, calves vocalize less in a novel arena when in companionship with familiar calves compared with when they are placed with unfamiliar calves (Faerevik *et al.*, 2006). Similarly, in the current study the strong social attachment between the group mates led to more frequent vocalizations in the group housed calves.

### CONCLUSION

The two novelty tests, the novel object test and the social novelty test were shown to reflect underlying traits that also affect buffalo calf's behaviour in the test arena. It can be concluded that social isolation along with weaning of Murrah buffalo calves elevate fear toward novel situations affecting their overall welfare. Continued future research can provide insights into the the emotional states of buffalo calves in unfamiliar situations, strengthening our knowledge on their welfare.

### Conflict of interest

All authors declare that they have no conflict of interest.

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