

Influence of Maternal Contact on Hormonal, Growth, and Behavioral Responses of Murrah Buffalo Calves in Winter

ABSTRACT

The study was conducted to reveal the effect of calf-mother contact and ameliorative measures on cortisol, growth rate, and behaviour of Murrah Buffalo calves during the winter season in Livestock Research Centre (LRC) of ICAR-NDRI, Karnal, India and between November 2021 to February 2022, a total of 21 advanced-pregnant Murrah buffaloes were carefully chosen and the buffalo-calf pairs were divided into three treatment groups, each consisting of 7 pairs. In the first group (T0), the Murrah buffalo mothers were permitted to nurse their calves with colostrum and later milk twice a day. In the second group (T1), the buffalo mothers had direct contact with their calves during the first five days, allowing free-choice colostrum suckling. Afterward, they were housed near their calves, separated by a fence line. These buffalo mothers were allowed to nurse their calves twice daily, following a routine similar to the T0 group. In the third group (T2), all conditions were similar to those of the T1 group, a notable distinction was that the calves in the T2 group were provided with halogen lights in the shed. Results show Minimum temperature in T2 shed was significantly ($p < 0.05$) higher than that in T0 and T1 shed. The mean cortisol level of T1 (6.64 ± 0.71 ng/mL) and T2 (6.21 ± 0.67 ng/mL) was significantly lower ($P < 0.05$) (as compared to T0) (7.88 ± 0.70 ng/mL) (group of calves). The findings indicated that within the experimental calf groups, both T2 and T1 exhibited higher ($P < 0.05$) average daily gain (ADG) compared to the T0 group on a weekly basis every week. Moreover, calves in T2 and T1 spent significantly ($P < 0.05$) more time resting, eating, and engaging in rumination compared to the calves in the T0 group. The findings show that offering fence-line mother-calf contact can reduce stress and enhance the growth, behavior, and welfare of calves more effectively than calves with restricted contact during winter season.

Keywords: Murrah calves, climate change, cold stress, mother-calf contact

INTRODUCTION

Calf-mother contact, a fundamental aspect of animal husbandry, plays a pivotal role in the well-being and development of young Murrah buffalo calves. This interaction encompasses the bonding, nurturing, and physical proximity between a calf and its mother during their early stages of life [1]. The quality and extent of calf-mother contact can significantly impact the health, growth, and overall welfare of these young animals. This close connection includes behaviors such as nursing, grooming, and the establishment of a secure emotional attachment, which can have profound effects on the calf's physical and emotional development [2]. This intricate connection between maternal care and the well-being of Murrah buffalo calves carries implications not only for the individual animals but also for the overall productivity and sustainability of the dairy industry. In comparison to complete separation, enabling interaction between a mother and her offspring through a barrier diminishes the behavioral response to weaning Price [3].

Calves are particularly susceptible to cold stress at birth due to their relatively large surface area compared to adults, the absence of heat generated from rumen fermentation, and their wet condition resulting from amniotic fluid [4]. This vulnerability becomes even more pronounced in regions with harsh winter climates, such as the Northern part of the country, where ambient temperatures can plummet to as low as $1-2^{\circ}\text{C}$. The consequences are dire, leading to increased calf mortality and hindered growth rates, translating into substantial economic losses for dairy farmers. Therefore, safeguarding buffalo calves from the rigors of cold weather is imperative to unlock their full genetic potential in terms of growth. Scientifically, the thermal comfort zone for calves typically falls within the range of $15-25^{\circ}\text{C}$, with the lower critical temperature spanning from 9 to 15°C during the first two weeks of life [5]. It is evident that innovative strategies are required within the livestock sector to maintain and enhance production potential under changing climatic conditions. Climate change may lead to more extreme weather conditions, making winter seasons even harsher. Understanding how to manage calf-mother contact during such periods can contribute to the resilience of the livestock industry in changing environmental conditions.

The rearing of healthy and thriving calves is a cornerstone of sustainable dairy farming, as these young animals represent the future of the herd. Maximizing their survival rates until weaning is not only a matter of animal welfare but also of paramount economic significance. In the contemporary dairy industry, effective calf management assumes a pivotal role in refreshing the herd with productive animals and ultimately enhancing the farm's economic viability. Postnatal calf management, especially during the early stages of life, significantly influences growth rates and feed conversion efficiency [6]. It is directly linked to the long-term productivity of the animals. Losses incurred during the critical neonatal period can substantially impact the economic sustainability of cow/calf operations. The challenges imposed by extreme temperature fluctuations, especially during harsh winters, can exert a profound influence on calf growth and development. Prolonged exposure to these adverse conditions can lead to growth stunting and a compromised immune status in vulnerable young calves.

Various effective measures have been employed to combat cold stress, including the use of various bedding materials, calf jackets, hot boxes, warm water bath and various types of light like halogen and infrared [7]. Hence, the present study was conducted to investigate the role of calf mother contact and winter ameliorative measures in mitigating cold stress and promoting the well-being and growth of Murrah buffalo calves during the winter months. This study aims to explore the multifaceted relationship between maternal interactions and the physiological and behavioral responses of Murrah buffalo calves in the context of winter, shedding light on the broader implications for animal welfare and production efficiency in the dairy industry.

1. MATERIAL AND METHODS

1.1. LOCATION OF EXPERIMENT AND CLIMATIC CONDITION

The research was conducted at the Livestock Research Centre (LRC) of ICAR-NDRI in Karnal. This facility is situated at geographical coordinates of approximately $29^{\circ}42'20''$ North Latitude and $76^{\circ}58'52.5''$ East Longitude, with an elevation of approximately 247 meters above sea level. During the summer season, the highest recorded temperatures in this location typically range between 42 and 46°C , while

in winter, they range between 1 and 5°C, exhibiting a daily temperature fluctuation of 16–22°C. The annual average rainfall in this region typically amounts to around 650 mm.

1.2. EXPERIMENTAL ANIMALS

To conduct the study, a total of 21 advanced-pregnant Murrah buffaloes were carefully chosen from the general buffalo herd maintained at the institute. The research spanned from November to February. These buffaloes were transferred to maternity pens 15 days prior to their expected calving dates. Following successful calving, the buffalo-calf pairs were divided into three treatment groups, each consisting of seven pairs. These groups were made homogeneous in terms of buffalo parity, with an average of 2.42 ± 0.53 across all three treatments.

In the first group (T0), the Murrah buffalo mothers were permitted to nurse their calves with colostrum, and subsequently, they were milked twice a day. After milking, they were separated from their calves and placed in a shelter without any additional measures to protect them from the cold. In the second group (T1), the buffalo mothers had direct contact with their calves during the first five days, allowing free-choice colostrum suckling. Afterward, they were housed in close proximity to their calves, separated only by a fence line. These buffalo mothers were allowed to nurse their calves twice a day, following a similar routine as the first group. However, like the T0 group, these buffaloes were also housed in a shelter without specific measures to alleviate the effects of cold stress. In the third group (T2), all conditions mirrored those of the T1 group. The buffalo mothers had direct contact with their calves during the initial five days, followed by proximity through a fence line. They were also permitted to nurse their calves twice a day after milking sessions. However, a notable distinction was that these buffalo calves in the T2 group were provided with halogen lights to shield them from the impacts of cold stress.

1.3. HOUSING AND FEEDING OF EXPERIMENTAL ANIMALS

1.3.1. HOUSING

The experimental calves were accommodated in a communal facility comprising a loose house with both a sheltered section and an adjacent open paddock, providing each calf with a total floor space of 3 square meters. These calves shared a common feeding area facilitated by a fence-line feed barrier and also had access to a shared drinker. The allocation of floor space and feeding area adhered to the specifications outlined by the Bureau of Indian Standards for buffaloes in loose housing systems (BIS: 1223–1987). The flooring in the enclosures of the calves was constructed with concrete and included grooves, both in the sheltered and open sections. Calves belonging to the T0 group were housed separately, following the standard farm practices. During the winter nights, these calves remained within an enclosed area. The calves in the fence-line group were sheltered in a shed, with their mothers having access to a covered area, which was connected to the open paddock. During the winter, the fence-line group calf shed was enclosed with curtains on three sides, leaving one side open to provide separation from their mother buffaloes. In the covered area designated for the T2 group windows and doors were covered with curtains made up of tarpaulin. Straw bedding was provided along with extra provision of halogen lights in the shed.

1.3.2. FEEDING

The calf's dietary regimen was adjusted in accordance with the nutritional guidelines established by ICAR-2013 for the proper nourishment of growing buffalo calves. Initially, calves were provided with buffalo milk twice daily, equating to 10% of their body weight, until they reached the age of 3 to 4 months. Commencing from the second week of their lives, they were introduced to chopped maize green fodder without any dietary restrictions. Additionally, they had access to clean water and a salt lick block. From the second week onward, the calves were also introduced to calf starter feed, provided at a rate of 1% of their body weight, and they were allowed unrestricted access to chopped green fodders. The calf starter feed composition included maize (33%), wheat bran (21%), gram (10%), groundnut cake (33%), mineral mixture (2%), and common salt (1%). This feed formulation was designed to encompass 22% digestible crude protein, an energy content of 2572 kcal/g, and 70% of total digestible nutrients.

1.4. RECORDING OF CLIMATIC VARIABLES

Maximum and minimum temperature was recorded using maximum-minimum thermometer (HTC, Made in China (installed in all the three sheds. Thermometers were hung 2 feet above the animal head :one in the center and one on each side wall)longitudinal (of the shed, to minimize errors.

1.5. BODY WEIGHT OF CALVES

The body weight of calves was recorded immediately after birth and then at weekly intervals .The weighing of the calves was done in the morning before suckling and feeding using an electronic weighing machine with a precision of 200 g.

1.6. AVERAGE DAILY GAIN

The live weight of the experimental calves was recorded on weekly basis throughout the experimental period. Weights of all calves were recorded in the morning between 6:00 a.m. and 6:30 a.m., before animals were offered with milk feed or water, using an electronic weighing machine with a precision of 200 g. The average daily gain was expressed in grams/day and calculated using the following formula:

$$\text{ADG (g)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{weekly intervals and expressed in grams/day.}}$$

1.7. RECORDING OF BEHAVIOUR OF CALVES

All the behaviour parameters were recorded by digital video recording done by CCTV outdoor cameras (CP Plus). The camera had 8x digital zoom for closer viewing. The cameras were enabled with array infrared technology for best night vision. Cameras were installed at different places and different angles in the experimental shed so that whole shed was covered in viewing angle. All the parameters were recorded in hours: minutes format initially which were later changed to minutes according to needs of parameters.

1.8. ELISA FOR PLASMA CORTISOL

Blood samples were collected on day 6, 15, 30, 45, 60 and 90 after birth for estimation of plasma cortisol. Plasma cortisol was estimated using 'Bovine Cortisol ELISA Kit' and 'Bovine Prolactin ELISA Kit' supplied by Bioassay Technology Laboratory, 205 5/F 2 Bidg, 501 Changsheng S Rd, Nanhudist, Jiaxing, Zhejiang, China. The detection range and sensitivity of cortisol were 0.5-200ng/mL and 0.02 ng/mL, respectively.

1.9. STATISTICAL ANALYSIS

To compare cortisol levels, calf body weight, average daily gain (ADG), and different behaviors, statistical analyses were performed using IBM SPSS version 28.0.1.1 software. This analysis employed a one-way analysis of variance (ANOVA) and univariate general linear models (GLM). The model integrated treatment and time as fixed factors, along with their interaction. Differences were considered statistically significant when $p < .05$. Results are presented as LS means \pm SE.

2. RESULTS AND DISCUSSION

2.1. ENVIRONMENTAL VARIABLES

Data on fortnightly average minimum and maximum temperature during winter season are presented in the table 1. Average minimum temperature ranged from 5.66 to 12.28, 5.56 to 12.18 and 9.96 to 15.53 in T0, T1 and T2 sheds respectively. Minimum temperature in T2 shed was higher ($P < 0.05$) as compared to the T1 and T0 shed. The higher temperature in T2 shed might be due to extra provision of cold stress amelioration. Similarly higher temperature in the shed provided with infrared lamps was reported by Bhatt et al [7].

Table 1. Fortnightly mean maximum and minimum temperature ($^{\circ}\text{C}$) inside the sheds during winter season

Fortnight	Minimum Temperature ($^{\circ}\text{C}$)			Maximum temperature ($^{\circ}\text{C}$)
	T0 shed	T1 shed	T2 shed	
1	12.28 ^a \pm 0.46	12.18 ^a \pm 0.32	15.53 ^b \pm 0.38	28.61 \pm 0.15
2	9.51 ^a \pm 0.18	9.57 ^a \pm 0.11	13.61 ^b \pm 0.18	26.21 \pm 0.21
3	9.70 ^a \pm 0.58	9.75 ^a \pm 0.50	13.56 ^b \pm 0.50	23.04 \pm 0.61
4	5.66 ^a \pm 0.44	5.56 ^a \pm 0.41	9.96 ^b \pm 0.40	19.39 \pm 0.67
5	8.96 ^a \pm 0.84	8.76 ^a \pm 0.82	13.65 ^b \pm 0.72	16.47 \pm 0.71
6	7.38 ^a \pm 0.43	7.18 ^a \pm 0.42	12.13 ^b \pm 0.44	14.27 \pm 0.67
7	7.23 ^a \pm 0.54	7.33 ^a \pm 0.51	10.21 ^b \pm 0.34	19.03 \pm 0.83
8	9.10 ^a \pm 0.67	9.12 ^a \pm 0.65	11.62 ^b \pm 0.38	23.40 \pm 0.33
Overall	8.69^a\pm0.25	8.59^a\pm0.21	12.52^b\pm0.22	21.19\pm0.47

Data are presented as LS means \pm SEM .a, b, c indicate differences between the mean values of different groups . Differences at all points for each parameter were considered at $P < 0.05$.

2.2. Blood plasma cortisol

The mean \pm S.E. of blood plasma cortisol levels during winter season recorded on the sampling days is presented in the table 2. There was no significant difference in mean cortisol levels (ng/mL) among the three groups on day 6 and 90 but these levels on rest of sampling days in T1 and T2 groups of calves were significantly ($P < 0.05$) lower than T0 group of calves. Overall mean cortisol levels (ng/mL) of T1 (6.64 \pm 0.71 ng/mL) and T2 (6.21 \pm 0.67 ng/mL) was significantly lower ($P < 0.05$) as compared to T0 (7.88 \pm 0.70ng/mL) group of calves. Time, group and time x group interactions were significant ($P < 0.05$) in case of plasma cortisol. As the age progressed significant decrease in cortisol levels was observed in all the groups. The lower cortisol levels in T2 and T1 groups compared to T0 group in winter season indicated that the calves in these group were in lower stress which might have been due to fence line contact with their mothers. The findings of lower cortisol in calves provided with cold stress amelioration during winter season are in agreement with findings of Bhat et al [7] and Maurya et al [8] in buffalo calves.

Table 2. Mean cortisol (ng/mL) concentration in blood serum of different groups of calves at different intervals during winter season

Day after birth	Cortisol (ng/mL)		
	T0	T1	T2
6	15.30 ^F \pm 0.30	14.54 ^E \pm 0.84	13.22 ^C \pm 0.99
15	10.32 ^{bE} \pm 0.26	8.38 ^{aD} \pm 0.54	8.23 ^{aB} \pm 0.34
30	8.19 ^{bD} \pm 0.33	6.25 ^{aCD} \pm 0.60	6.68 ^{aB} \pm 0.45
45	6.06 ^{bC} \pm 0.28	4.74 ^{abBC} \pm 0.38	4.08 ^{aA} \pm 0.60
60	4.58 ^{bb} \pm 0.33	3.54 ^{abAB} \pm 0.34	2.85 ^{aA} \pm 0.32
90	2.81 ^A \pm 0.44	2.42 ^A \pm 0.14	2.23 ^A \pm 0.35
Overall	7.88^b\pm0.7	6.64^a\pm0.71	6.21^a\pm0.67

Data are presented as LS means \pm SEM .a, b, c indicate differences between the mean values of different groups .Where, A, B, C, D, E, F indicate differences in the same group at different time intervals. Differences at all points for each parameter were considered at $P < 0.05$.

2.3. Body weight and growth rate

The mean body weight (kg) recorded at weekly interval during winter season is presented in table 3. The average body weight of all calves at birth had no significant differences. The significant ($P<0.05$) difference in calves' body weight was seen from second week onwards and continued till the 12th week of study. At the end of 12 weeks the average body weight of T2 (76.07 ± 1.15 kg) and T1 (75.57 ± 1.41 kg) calves was significantly higher ($P<0.05$) than that of T0 (68.71 ± 1.08 kg) calves. The data on mean ADG during winter season are presented in the table 4. The average ADG was higher ($P<0.05$) in T2 and T1 group as compared to T0 group of calves from first week to third week. After third week there was no significant difference in ADG among the three groups of calves. The overall mean ADG was significantly ($P<0.05$) higher in T2 and T1 groups as compared to T0 group.

Table 3. Body weight (kg) of different groups of calves recorded at weekly intervals during winter season

Week after birth	Body weight (kg)		
	T0	T1	T2
At birth	29.14 \pm 1.26	30.43 \pm 1.00	30.57 \pm 2.15
1	31.36 \pm 1.43	34.14 \pm 1.12	35.07 \pm 2.08
2	32.51 ^a \pm 1.44	36.64 ^{ab} \pm 1.26	38.63 ^b \pm 2.03
3	34.74 ^a \pm 1.41	39.89 ^{ab} \pm 1.22	41.94 ^b \pm 1.91
4	37.86 ^a \pm 1.26	43.50 ^b \pm 1.22	45.71 ^b \pm 1.97
5	41.17 ^a \pm 1.23	47.29 ^b \pm 1.32	49.60 ^b \pm 1.91
6	44.79 ^a \pm 1.18	51.14 ^b \pm 1.55	53.71 ^b \pm 1.67
7	48.90 ^a \pm 1.42	55.01 ^b \pm 1.65	57.66 ^b \pm 1.71
8	53.14 ^a \pm 1.39	58.84 ^b \pm 1.87	61.29 ^b \pm 1.71
9	57.00 ^a \pm 1.33	63.86 ^b \pm 1.53	65.00 ^b \pm 1.43
10	60.00 ^a \pm 1.38	67.86 ^b \pm 1.44	68.29 ^b \pm 1.44
11	64.50 ^a \pm 1.19	71.14 ^b \pm 1.44	71.86 ^b \pm 1.32
12	68.71^a\pm1.08	75.57^b\pm1.41	76.07^b\pm1.15

Data are presented as LS means \pm SE. a, b indicate differences between the mean values of different groups. Differences at all points for each parameter were considered at $P < 0.05$.

Higher average body weight reported in T2 and T1 group calves compared to T0 group might be due to direct contact and free choice colostrum suckling during first five days after birth followed by fence line contact. T2 and T1 groups had no differences in body weight which shows that only fence line contact has effect on calves' body weight while cold stress had no much impact on calves' body weight. The initial higher ADG in T1 and T2 calves during first 3 weeks after birth may have resulted owing to availability of free choice colostrum suckling during first five days after birth in these calves. There was no significant difference in ADG between T1 and T2 calves indicating that winter had no impact on the growth rate of calves. It may be therefore deduced that the buffalo calves do not require elaborate winter protection if offered adequate nourishment in the form of free choice colostrum and mother contact for reducing social stress. These findings are in agreement with Singh et al [9] in buffalo calves who reported no change in ADG of calves when kept in conventional closed barn and in the loose house. However, Bhatt et al [7] in Vrindavani new born calves reported higher ADG in calves provided with infrared lamps in the individual calf shed and Sorathiya et al [10] in buffalo calves (2 month old) reported higher ADG in calves provided with straw bedding than calves with no bedding. The inconsistent results in these studies may be due to individual housing provided with infra red lamp in the study by Bhat et al [11] while we had group housing in our study and due to presence and absence of bedding in the study of Sorathiya et al [10] while in present study bedding was provided in all calves shed.

2.4. Behaviour of calves

2.4.1. Resting time (min/day) of calves

The daily average resting time spent by the calves during winter season is presented in the table 4. No significant difference in resting time among the three groups on day 10 and 30 were observed but resting time on rest days of recordings were significantly ($P<0.05$) higher in T2 and T1 groups of calves than T0 group of calves. The overall mean resting time of T1 and T2 was significantly ($P<0.05$) higher than T0 group of calves. The mean time spent on resting showed declining trend from first of age till 12th week of age in all the three groups of calves (Fig 1).

The higher resting time in T2 and T1 compared to T0 group showed that the calves when in fence line contact with mothers rest more while calves which have only restricted contact are restless and spend more time standing. These findings are consistent with those of Price et al [3] and Haley et al [12], who found that when mother and calf were in close proximity they rested more. The findings of Jensen [13], Calvo-Lorenzo et al [14] and Chaudhary et al [15], who reported that calves spend 16–19 hours per day lying down between the ages of 21 and 70 days, also support for the findings of the current study.

Table 4. Average time spent on resting (min/d) by the calves during winter season

Day after birth	Resting time (min/day)		
	T0	T1	T2
6	1088.59 ^a \pm 2.4	1255.79 ^b \pm 1.78	1249.23 ^b \pm 1.47
10	1074.27 \pm 3.16	1247.15 \pm 4.02	1238.86 \pm 4.24
15	1056.53 ^a \pm 3.19	1080.09 ^b \pm 3.81	1069.71 ^b \pm 2.49
30	1024.44 \pm 2.58	1017.29 \pm 8.55	1012.58 \pm 5.50
45	966.95 ^a \pm 7.97	916.08 ^b \pm 6.18	923.10 ^b \pm 4.68
60	926.27 ^a \pm 4.93	904.50 ^b \pm 4.40	910.13 ^b \pm 5.72
75	913.56 ^a \pm 5.06	890.52 ^b \pm 4.22	890.47 ^b \pm 2.69
90	903.46 ^a \pm 2.48	887.78 ^b \pm 3.02	894.52 ^b \pm 4.57
120	873.57 ^a \pm 4.03	890.37 ^b \pm 5.49	882.25 ^b \pm 3.78
Overall	980.85^a\pm10.64	1009.95^b\pm19.76	1007.87^b\pm19.17

Data are presented as LS means \pm SEM. a, b indicate differences between the mean values of different groups. Differences at all points for each parameter were considered at $P < 0.05$.

2.4.2. Standing time (min/day) of calves

The data on average daily standing time spent by the calves during winter season is presented in the table 5. No significant differences in mean standing time among the three groups of calves on day 30 was observed, but the standing time on day 6, 10, 15, 45, 60, 75, 90 and 120 was significantly ($P < 0.05$) lower in T2 and T1 groups of calves than T0 group of calves. The overall mean standing time of T1 and T2 groups of calves were significantly ($P < 0.05$) lower than T0 group of calves. The mean time spent on standing showed increasing trend from first week of age till 12th week of age in all the three groups of calves (Fig 2).

The lower standing time in T2 and T1 groups compared to T0 group showed that the calves when in fence line contact with mothers rest more while calves which have only restricted contact are restless and spend more time standing. The lower standing time in T2 and T1 group during summer season might be due to their mothers' continued olfactory, auditory, and visual contact with the calves. This suggested that fence line interaction helped the calves cope with their new environment and minimize the separation anxiety. Fence line housed calves (T1 and T2) spent less time standing than calves that were allowed restricted contact (T0). Our findings are consistent with the findings of Loberg *et al.* (2008)[16], Enriquez *et al.* (2010)[17] and Chaudhary *et al.* (2022)[15].

Table 5. Average time spent on standing (min/d) by the of calves during winter season

Day after birth	Standing time)min/day(
	T0	T1	T2
6	351.41 ^a ±2.4	184.21 ^b ±1.78	190.77 ^b ±1.47
10	365.73 ^a ±3.16	192.85 ^b ±4.02	201.14 ^b ±4.24
15	383.47 ^a ±3.19	359.91 ^b ±3.81	370.29 ^b ±2.49
30	415.56±2.58	422.71±8.55	427.42±5.50
45	473.05 ^a ±7.97	523.92 ^b ±6.18	516.90 ^b ±4.68
60	513.73 ^a ±4.93	535.50 ^b ±4.40	529.87 ^b ±5.72
75	526.44 ^a ±5.06	549.49 ^b ±4.22	549.53 ^b ±2.69
90	536.54 ^a ±2.48	552.22 ^b ±3.02	545.48 ^b ±4.57
120	566.43 ^a ±4.03	549.63 ^b ±5.49	557.75 ^{ab} ±3.78
Overall	459.15^a±10.64	430.05^b±19.76	432.13^b±19.17
Data are presented as LS means ± SEM .a, b indicate differences between the mean values of different groups. Differences at all points for each parameter were considered at $P < 0.05$.			

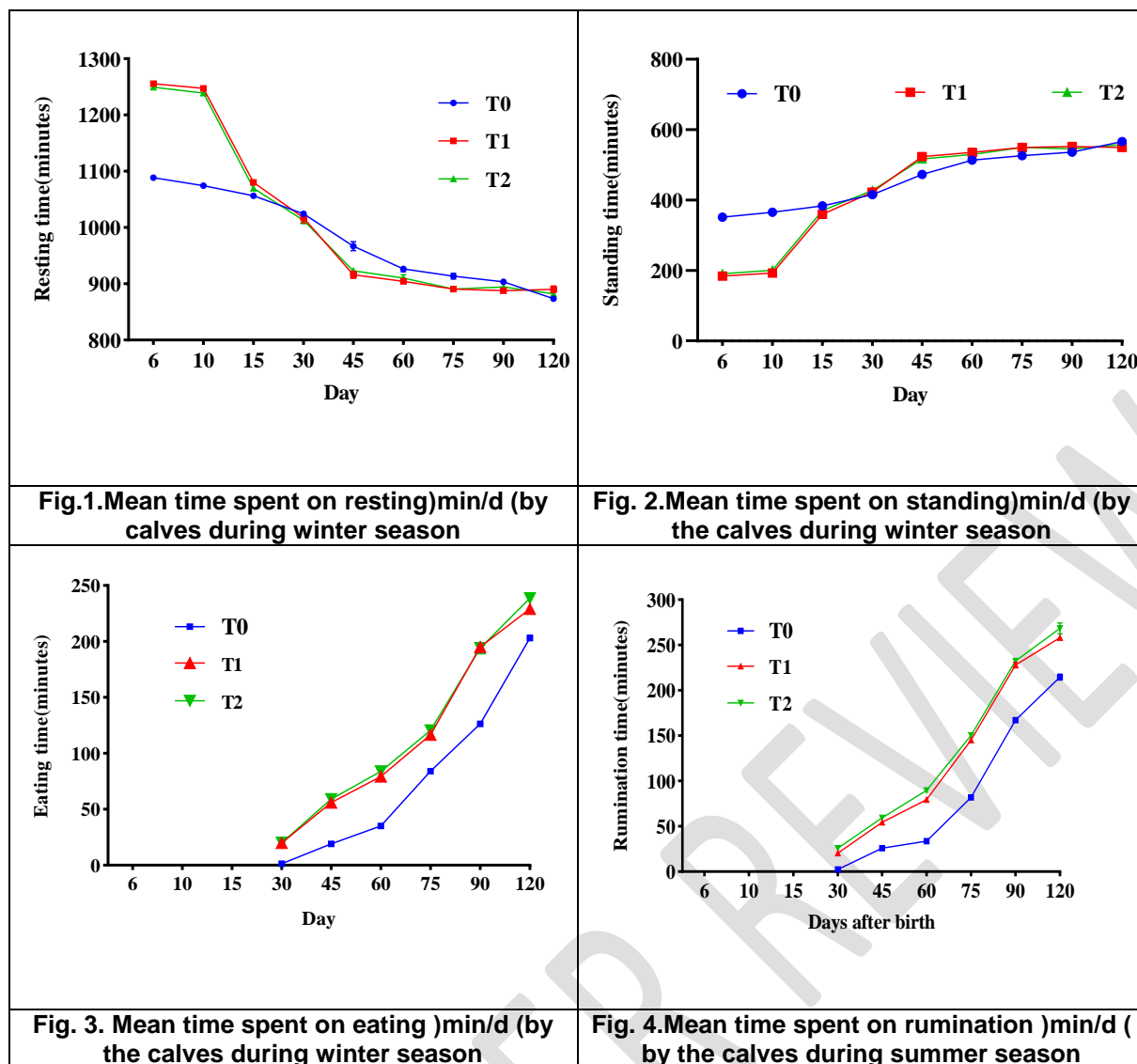
2.4.3. Eating time (min/day) of calves

The average daily time spent eating by the calves during winter season is presented in the table 6. The T2 and T1 groups of calves had significantly ($P < 0.05$) higher eating time than T0 group of calves. The mean time spent on eating showed an increasing trend from recording day till 12th week of age in all the three groups of calves (Fig 3).

It was seen that fence line calves (T1 and T2) spent more time eating than restricted contact calves (T0). This might be due to the free choice suckling in T2 and T1 group calves during the first five days after birth. It has already been reported that consuming more colostrum has significant impacts on GIT development and alters GI hormones and digestive enzymes [18]. Additionally, this might be due to transition from free-choice colostrum suckling to limited suckling and larger body weight, which led to earlier solid feed nibbling and longer feeding times in fence line group calves (T1 and T2). These results are in accordance with those reported by Price *et al* [3], Haley [12], Loberg *et al* [16] and Parashar [18] who found that calves that had fence line contact with their mothers ate for longer periods of time than calves those did not. Due to restricted suckling and restricted contact with mothers soon after birth might have led to reduced eating time in restricted contact calves (T0). The highest eating time in T2 group might be due to the both free choice suckling followed by fence line calf contact and the heat stress ameliorative measures provided in the T2 shed. The cold stress ameliorative measures provided in the T2 shed during winter season did not affect the eating time in calves.

Table 6. Average time spent on eating (min/d) by the of calves during winter season

Day after birth	Eating time)min/day(
	T0	T1	T2
30	1.41 ^a ±0.09	19.83 ^b ±0.67	20.10 ^b ±0.78
45	19.04 ^a ±0.7	55.93 ^b ±1.39	58.99 ^b ±1.59
60	35.04 ^a ±1.5	79.22 ^b ±0.95	83.78 ^b ±1.27
75	83.98 ^a ±1.67	116.59 ^b ±0.9	120.52 ^b ±2.97
90	126.42 ^a ±0.69	195.06 ^b ±2.97	193.99 ^b ±4.88
120	203.11^a±2.47	229.03^b±2.89	238.61^b±4.23
Data are presented as LS means ± SEM .a, b, c indicate differences between the mean values of different groups. Differences at all points for each parameter were considered at $P < 0.05$.			



2.4.4. Rumination time (min/day) of calves

The average daily time spent ruminating by the calves during winter season is presented in the table 7. The T2 and T1 groups of calves had significantly ($P < 0.05$) higher rumination time than T0 group of calves. The mean time spent on rumination showed an increasing trend from recording day till 12th week of age in all the three groups of calves (Fig 4).

In the current study, newborn calves did not engage in rumination until 15 to 20 days after birth. Higher feed intake and feeding duration may have contributed longer rumination times in fenceline calves (T1 and T2) from day 30 to 120 in our study. Similar findings were reported by Enriquez et al [17] and Chaudhary et al [15], who noted that fenceline-housed calves spent more time ruminating than the calves that were separated. The additional measures to reduce cold stress in the T2 shed during winter did not impact the calves' rumination time.

Table 7. Average time spent on rumination (min/day) by the calves during winter season

Day after birth	Rumination time \bar{X} (min/day) \bar{X}		
	T0	T1	T2
30	2.66 ^a ±0.26	15.94 ^b ±2.29	17.73 ^b ±1.99
45	30.48 ^a ±1.52	61.28 ^b ±0.65	65.52 ^b ±1.22
60	52.58 ^a ±1.81	89.28 ^b ±3.21	92.73 ^b ±2.69
75	109.81 ^a ±3.02	161.66 ^b ±2.40	163.39 ^b ±4.13
90	192.14 ^a ±3.76	229.22 ^b ±3.00	235.84 ^b ±3.14
120	227.49^a±1.28	279.88^b±2.60	283.02^b±1.36

Data are presented as LS means ± SEM .a, b, c indicate differences between the mean values of different groups. Differences at all points for each parameter were considered at $P < 0.05$.

3. CONCLUSION

The research demonstrated a significant effect of maternal contact on hormonal responses, as evidenced by lower blood plasma cortisol levels in calves that had access to maternal contact. This indicates that the provision of maternal contact plays a crucial role in reducing stress levels in buffalo calves during the winter season. The study revealed a substantial influence of maternal contact on growth parameters. Calves with maternal contact exhibited higher average body weights and growth rates, particularly in the early weeks of life, likely due to free-choice colostrum suckling and fenceline contact. This underscores the importance of early-life maternal interactions in promoting healthier growth in buffalo calves. The behavioural responses of the calves were also significantly affected by maternal contact.

Calves with maternal contact spent more time resting, less time standing, and increased time on eating and ruminating. The study underscores the critical role of maternal contact in promoting hormonal balance, healthier growth, and improved behaviour in Murrah buffalo calves during the challenging winter season. These findings highlight the practical importance of implementing maternal contact and stress-reduction measures in buffalo calf management practices, ultimately enhancing their well-being and growth potential.

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