SUPPLEMENTARY FIGURES AVAILABLE ONLINE

Indian Journal of Animal Sciences **92** (1): 89–95, January 2022/Article
https://doi.org/10.56093/ijans.v92i1.120931

Effect of mother contact and voluntary colostrum suckling on growth, health and stress of neonatal buffalo calves

SANJAY CHOUDHARY[1]✉, M L KAMBOJ[1], SHWETAMBRI JAMWAL[1], PRASANNA PAL[1], DEVAN ARORA[1], VINAYAK INGLE[1], PAWAN SINGH[1] and S S LATHWAL[1]

ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

Received: 20 July 2021; Accepted: 1 November 2021

ABSTRACT

The aim of present study was to investigate the effect of full mother contact and voluntary colostrum suckling on the health, stress and growth performance of neonatal Murrah buffalo calves. For this, two experiments (exp.) were conducted involving exp.1, 24 mother-calf pairs were divided into three equal groups (eight pairs/each group). In no mother contact (NC) group, weaning was allowed immediately after birth. In restricted mother contact (RC) group, restricted contact was allowed twice daily. In fence-line mother contact (FC) group, calves were in full-time mother contact from birth to day (d) 5 and allowed voluntary colostrum suckling and thereafter fence-line contact. In exp.2, another 24 mother-calf pairs were selected and divided into two groups (12 pairs in each group), with the same conditions as the RC and FC groups in exp.1. Average daily gain, total protein and total Ig concentrations differed between different groups in both experiments. Cortisol levels were lower in FC followed by RC and lowest in NC calves, and similar results were observed in exp.2. It was concluded that the provision of full mother contact along with free choice colostrum suckling from birth to 5 days followed by fenceline mother contact and twice daily suckling at milking time abated the separation and weaning stress and remarkably improved the growth, immune status and health of Murrah buffalo calves as compared to the calves which were allowed only limited contact twice daily or the calves which were weaned at birth.

Keywords: Buffalo, Fenceline contact, Full mother contact, Health, Separation, Stress

In buffalo dairy farming systems, buffalo mother-calf pairs are kept in dual-purpose systems where calves are allowed to suckle directly from their mothers before or after milking, then calves are weaned at 6–12 months of age (López *et al.* 2008). More recently, buffalo farming has changed to intensive systems employing artificial rearing of calves by separating them from mothers immediately after birth (de Rosa *et al.* 2009, Masucci *et al.* 2015). Previously, various workers have reported that in dairy and beef cattle the separation of mother and calf within the first few days after birth has significant negative effects on the immunity, health and growth performance of the calves (Alvez *et al.* 2016) with more pronounced stress responses leading to neutrophilia following weaning (Lynch *et al.* 2010, O'Loughlin *et al.* 2014). Similarly, in buffalo's early separation of calves is associated with a high cortisol level, suboptimal growth rate and higher mortality rate in neonatal calves (Aref *et al.* 2016, Singh *et al.* 2017). Furthermore, it has been reported that separation of mother and calf immediately after birth, or partial separation allowing restricted contact during milking, lead to severe hypogammaglobulinemia, possibly because of inadequate consumption of colostrum in calves at birth or later (Vale Echeto *et al.* 2002), as buffalo calves are born agammaglobulemic at birth (Bharti *et al.* 2015, Hedegaard and Heegaard 2016) and are slow learners in bottle feeding (Smijisha and Kamboj 2012). Unfortunately, poor colostrum management and weaning continues to be a problem on dairy farms, with approximately 31% and 52% mortality in cattle and buffalo calves (Pasha, 2013) and lower weight gain in the first 3 week of life (Donovan *et al.* 2007). In addition, it has been stated that buffalo calves are highly social animals with strong instincts and close bonds with their mothers, and therefore are more vulnerable to stress than cattle calves when separated from the mothers (Mustafa *et al.* 2010).

Previously, various weaning strategies have been tried in beef and exotic dairy cattle breeds. Recently, a fence-line mother-calf contact system (FMCC) in dairy cattle and buffaloes has been reported to be beneficial in reducing stress (Loberg *et al.* 2008), and further improving the health and growth of the calves (Price *et al.* 2003, Johnsen *et al.* 2016, Hassan *et al.* 2019). FMCC is also described as partial cow-calf contact, which is defined as limited physical contact between the dam and her own calf (Sirovnik *et al.* 2020). It has been reported that presence of the dam, especially during early part of calf's life, has a positive effect

Present address: [1]Livestock Production Management Division, ICAR-National Dairy Research Institute, Karnal, Haryana. ✉Corresponding author email: rajsaya07@gmail.com

on immunoglobulins absorption and stimulates urination, defecation and digestion in the calf (Paranhos da Costa *et al.* 2006). To the best of our knowledge there is no available information on the effect of full mother contact and voluntary colostrum suckling after birth to d 5 and thereafter fence-line housing of mother-calf pairs.

Therefore, the aim of present study was to investigate the effect of full mother contact and voluntary colostrum suckling after birth to d 5 and thereafter fence-line housing of mother and calf on the immunity, haematocrit level, health, stress and growth performance of neonatal calves.

MATERIALS AND METHODS

The experiments were approved by the Institutional Animal Ethics Committee (IAEC, registration no. 46-IAEC-20-8) and were conducted at the Livestock Research Centre, National Dairy Research Institute, Karnal, Haryana, India (29°42′ N; 76°58′ E, altitude = 227 m above the mean sea level). The maximum environmental temperature range is approximately 40 to 43°C in summer, and 2 to 15°C in winter. Two experiments (exp.) were conducted: exp.1 from September 2019 to November 2019, and exp. 2 from September 2020 to November 2020. Experiment two was repeated again to assess the difference between the FC and RC calves with greater number of calves in each group.

Experimental treatments

Exp.1: For exp. 1, a total of 24 calves were selected and were divided into three treatment groups of equal number (eight in each group; NC, RC, FC) based on parity (1.8±0.3, 1.8±0.3, 1.8±0.3), last lactation yield (2487.8±72.7, 2423.5±85.1, 2478.2±88.1 kg) of mothers and birth weight of calves (31.4±1.2, 29.4±1.3, 30.3±1.1 kg). In the no mother contact (NC) group, calves and mothers were separated immediately after birth and offered colostrum and milk twice a day by bottle (1/10th of body weight). In the restricted mother contact (RC) group, calves were separated from their mothers after first suckling at birth and were allowed restricted contact twice daily by allowing suckling of colostrum and milk (1/10th of body weight). In the fence-line mother contact (FC) group, calves were in full-time mother contact from birth to d 5 and allowed voluntary colostrum suckling. From d 6 onwards the mother-calf pair were kept in fence-line contact and allowed restricted suckling twice daily (1/10th of body weight).

Exp. 2: For exp. 2, a total of 24 calves were selected and divided into two equal groups (RC and FC) of 12 mother-calf pairs in each group based on parity (1.6±0.2, 1.9±0.2), last lactation yield (2506.4±98.7, 2525.5±74.5 kg) and birth weight of calves (29.5±1.2 and 30.6±0.8 kg). In the restricted mother contact (RC) group, calves were separated from their mothers after first suckling at birth and allowed restricted contact twice daily by allowing suckling of colostrum and milk (1/10th of body weight).

Housing of calves and feeding: Calves were given similar housing and feeding during both experiments. Calves in NC group were housed in a separate calf pen with a covered shed and an adjoining open paddock with a total floor space of 1 m^2 per calf under covered area and 2 m^2 per calf in open area, with a common fence-line feed barrier and a drinking trough. Calves in RC group were housed in a same calf section in different pen with same specifications. While, Fenceline mother-calf contact was provided by housing in adjacent sheds only, separated by the wire mesh 5-foot-high fence where auditory, olfactory, visual and tactile contact was possible. Calving occurred in individual indoor maternity pens bedded with straw or paddy straw, and mother-calf pairs were kept together in these pens for first 5 days. After d 6, calves and mothers were separated by the fence and housed in groups with a covered shed and an adjoining open paddock with a total floor space as given above in earlier two groups per calf, with a common feeder through a fence-line feed barrier and a drinking trough. Mothers and calves were allowed twice daily direct contact at the time of milking in milk parlour.

NC calves were given measured amount of colostrum (first 5 days after birth) and milk at 10% of the body weight and RC calves were offered colostrum and milk by allowing natural suckling twice daily at 10 % of the body weight. While, FC calves were allowed free choice colostrum suckling for 5 days after birth and after day 6 calves were allowed to suckle their mother twice daily at 10% of the body weight. At the second week of age, chopped maize green fodder and calf concentrate were offered *ad lib.* with clean water and salt lick block until weaning. Calf concentrate was composed of maize 35%, wheat bran 20%, gram 10%, Ground nut cake 32%, mineral mixture 2% and salt 1%. Calf's ration was provided after weaning, formulated according to ICAR-2013 recommendations for growing buffalo calves. Calves were offered concentrate at 1% per 100 kg body weight and *ad lib.* chopped green fodders. Clean and fresh drinking water with salt lick was offered *ad lib*.

Recording of body weight: The body weight of the experimental calves was recorded immediately after birth and then at weekly weighing of the calves before suckling using an electronic weighing machine with a precision of 200 g. Average daily gain (ADG) was calculated in grams (g) as final body weight-Initial body weight/ 7 days.

Physiological parameters: Health status was evaluated daily during the first week and thereafter on d 10, 15 and 30. Rectal temperature and heart rate were measured on d 1, 6, 10, 15 and 30 before and after colostrum and milk feeding time and their average values are presented. Eye temperature was taken using Flair infrared camera at the distance of 1 meter from the calves.

Blood collection: Calves were blood sampled via jugular venipuncture on d 0, 3, 6, 15 and 30 after birth. Complete blood count (CBC) was determined from EDTA anti-coagulated blood (2 mL) using an automated haematology analyzer equipped with software for bovine blood. Blood serum was separated to determine concentrations of glucose, total protein, calcium (Ca) and phosphorus (P) and were transported to the laboratory, stored at ambient temperature and processed within 3.5 h using ERBA Mannheim biochemical analyzer. For estimating total immunoglobulin (total Ig) and cortisol hormone, blood samples were immediately placed on ice, and serum was separated by centrifugation at 3,000 rpm for 15 min. Blood serum samples were stored at –20°C until concentrations of total immunoglobulin (total Ig) and cortisol hormone were quantified. These were measured with specific Elisa kits for bovine total Ig and bovine cortisol (Wuhan Fine Biological Technology Co. Ltd., Jiaxing, Zhejiang, China).

Statistical analysis: Values of haematocrit, physiological parameters, concentrations of total Ig and cortisol as well as ADG data, are expressed as mean±standard error of the means in both exp.1 and exp.2. For the time and treatment and group differences, ADG and concentration of haematocrit, physiological parameters, concentrations of total Ig and cortisol were evaluated using the RANDOM and REPEATED methods of the MIXED procedure of SAS (PROC MIXED, version 9.3; SAS University Edition, SAS Institute Inc., Cary, 164 NC). Treatment and time were used as fixed effects and the individual calves were used as random effects. For analyses of difference in time pattern between groups, the interactions (treatment and time) were included in the model. Differences were considered statistically significant when P<0.05 (in both experiments).

RESULTS AND DISCUSSION

Colostrum intake: The average colostrum intake by NC, RC and FC calves in exp.1, differed significantly (P<0.05) between the three groups of calves on d 1, 3 and 5, respectively. Colostrum intake was significantly (P<0.05) greater in FC (3.1±0.2, 3.9±0.2 and 4.7±0.2 kg), followed by RC (2.1±0.2, 3.2±0.2 and 3.7±0.1 kg) and the smallest amount was observed in NC (1.1±0.1, 2.2±0.1, and 2.4±0.1 kg) calves. Similar results were observed in exp. 2 with significantly (P<0.05) higher colostrum intake in FC (3.6±0.1, 4.3±0.1 and 4.9±0.2 kg) than RC (2.6±0.1, 3.5±0.1 and 4.0±0.2 kg) calves on d 1, 3 and 5, respectively. Higher colostrum intake in FC calves in both experiments may be due to direct contact between mother and calf, and the voluntary natural suckling of colostrum from their own mother. Further, it also reveals the importance of social stimulation of the calf by the mother. The mother’s stimulation of the calf may have caused it to be more active and to drink more (Krohn *et al.* 1999). Previously, various authors had also reported lower intake of colostrum and milk by Murrah buffalo calves in artificial rearing systems (Smijisha and Kamboj 2012). The lower colostrum intake in NC calves may attributed to the fact that buffalo calves have a strong maternal instinct and are considered slow learners as compared to the crossbred cattle calves, thus take more time to learn to drink from the nipple bottle under artificial feeding conditions, which led to decreased colostrum intake.

Average daily gain and body weight: Average daily gain during the colostrum period in exp.1 was significantly higher in FC (660.4±19.1 g) followed by RC (455.4±13.6 g) and lowest was in NC (357.5±13.4 g) calves, while similar results were observed during exp.2 in FC vs RC (908.7±43.5 g *vs* 475.0±28.9 g, P<0.05). Similarly, the overall ADG in exp. 1 differs significantly (P<0.05) between the three groups of calves (NC, RC, FC; (445.0±14.1 g, 482.7±8.3 g, 646.8±9.6 g) and during exp.2 it was significantly (P<0.05) higher in FC (701.9±22.8 g) than the RC (471.2±13.9 g). The overall mean body weight was significantly higher (P < 0.05) in FC than RC and NC calves during exp.1 (41.5±1.0 kg vs 37.7±0.9 kg and 38.7±0.8 kg, respectively) and during exp.2 FC calves had significantly higher body weight than RC calves (43.1±0.8 kg *vs* 36.7±0.9 kg; P<0.05).

Social interaction between cow and calf during the colostrum period had a positive effect on the daily gain of the calf (Krohn *et al.* 1999). Similarly, in the present study the calves who suckled their mothers on voluntary basis during birth to first week of life achieved a higher ADG than calves separated from their mothers at birth, probably due to the higher colostrum intake of the naturally reared calves. These results are in agreement with the finding of Odde *et al.* (1985), Egger and Kessler (1994) in exotic dairy and beef breeds of cattle. In exp.1 the restricted calf contacts with dam in RC group had higher body weight and average daily gain than artificial reared calves (NC), which demonstrated the importance of natural suckling of colostrum than bottle feeding of calves. Further, the superiority of fence-line group calves in body weight and ADG in the present study could be due to free choice colostrum suckling during the first 5 days after birth coupled with lower level of stress due to social stimulation provided by mother contact through provision of fenceline contact, where calves and mothers have the opportunity of social interaction. Our results are in agreement with Hassan *et al.* (2019) in buffalo calves; Price *et al.* (2003) and Kišac *et al.* (2011) in cattle calves.

Concentration of total protein and total immunoglobulin (Ig): There was a significant effect of voluntary colostrum suckling on the total protein, total Ig glucose, calcium and phosphorus in the calves. The data on mean total protein concentrations of exp. 1 are graphically represented in the (Fig. 1A). In the exp.1 statistical analysis revealed the total protein concentrations differed significantly (P<0.05) on d 3, 6, 10 and d 15 among FC, RC and NC calves, respectively. The mean total protein concentrations were significantly (P<0.05) higher in FC than in RC and NC calves on d 30. Further, in exp.2, The total mean protein concentrations were significantly higher in FC calves than RC calves on d 3, 6, 10, 15 and 30, respectively (Fig. 1A). Data pertaining to total immunoglobulin (Ig) concentrations of exp.1 and 2 are graphically presented in the Fig. 1B. There was an effect of time (P<0.01) group (P<0.01) and an interaction between group and time (P<0.01) on the calves total Ig concentrations. The total Ig concentrations on d 6 after birth differed significantly (P<0.05) among the three groups of calves, with the highest concentrations of total Ig in FC followed by RC and NC calves. While, on d

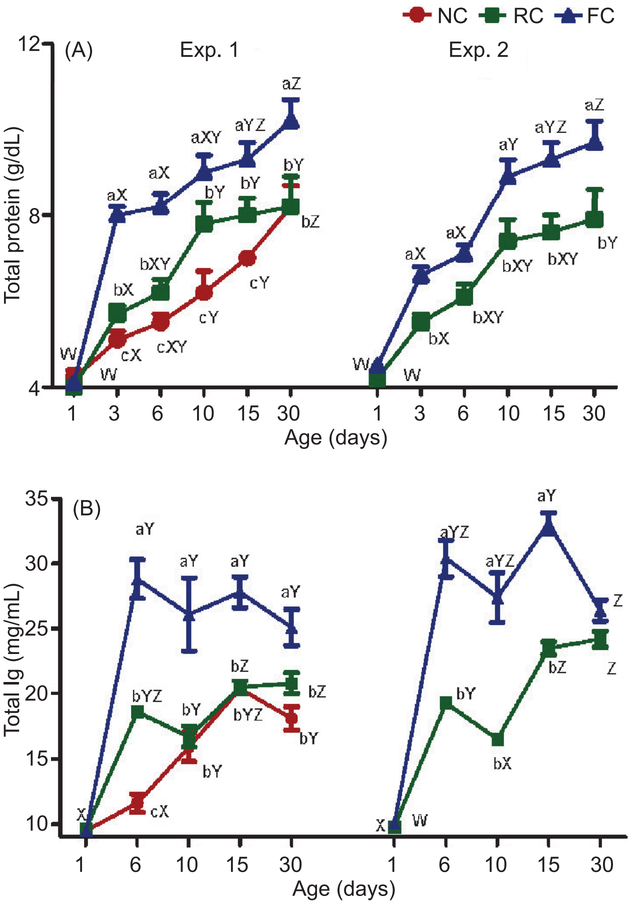

Fig. 1. Mean total protein (A) and total immunoglobulin (B) concentration in blood serum of the Murrah buffalo calves in exp. 1 and exp. 2.

10, 15 and 30, the mean total Ig concentrations were significantly (P<0.05) higher in FC than in RC and NC calves, respectively. In exp.2, the mean total Ig concentrations on d 6, d 10 and d 15 were higher in FC than RC calves (P<0.05 at all those days), and on d 30 it was statistically similar between the two groups of calves.

Buffalo calves are born agammaglobulemic and thus are highly dependent on mothers for development of passive immunity (Bharti *et al.* 2015, Hedegaard and Heegaard 2016). Similar results were observed in the present study in all the calves during both experiment when total Ig estimated before first suckling of the colostrum. Therefore, achieving early and adequate intake of high-quality colostrum is extensively documented as the single most important management factor in determining health and survival of the neonatal calf (Mcguirk and Collins 2015). Therefore, this shows the importance of direct mother-calf physical contact and opportunity for the voluntary choice of suckling over the restricted and artificial feeding system in buffalo calves. Results of the exp. 1 and 2 revealed higher total protein and total Ig in the FC calves, which might be due to repeated intake of colostrum on voluntary choice basis during first 24 h after the birth. Thus, repeated colostrum supply had a positive effect on total protein and IgG concentrations, as previously shown by Hammon and Blum (1998). In exp.1, it was observed that there was a slow rise in total protein and total Ig level in the blood serum of calves in the restricted suckled (RC) and bottle-fed calves (NC) and only in restricted suckled (RC) in exp. 2 than free choice colostrum fed group calves (FC). This might be due the fact that in order to establish a passive immunity in calves separated from their mothers after birth, they should receive 4–6 L of colostrum during the first 6 h (Hammon and Blum 1998). However, restricted suckled calves in the present study were separated from their mothers just after first suckling and second suckling was allowed after 12 h interval. This might be the reason behind the slow development of the passive immunity. While, further more slow development of passive immunity in the artificial raised calves was observed in the present study, which may be attributed to lesser colostrum intake on the first 24–36 h, possibly because of slow learning of bottle feeding by buffalo calves.

Concentration of blood serum glucose, Calcium (ca) and Phosphorus (P): During exp.1 as well as exp. 2, the serum glucose concentrations increased in all the groups of calves up to d 10 and d 15 (Fig. 2A). In exp. 1, the treatment, sampling time and their interaction was significant (P<0.01). The mean glucose concentration on d 3, d 6 d 10 and d 15 were significantly different among FC, RC and NC calves. In exp.2 also, the statistical analysis revealed the treatment, sampling time and their interactions were significant (P<0.01) on the calves' glucose concentrations. The analysis of variance of glucose concentrations on d 3, 6, 10 and 15 indicated a significant difference between FC and RC calves, respectively (Fig. 2A).

The mean calcium and phosphorus concentrations were lower at birth in all calves of both experiments and increased during colostrum suckling period (Fig. 2B and 2C). The mean concentrations of Ca and P during exp.1 differed significantly (P<0.05) among FC, RC and NC on d 3 and 6, respectively. While, these were significantly (P<0.05) higher in FC than in RC and NC calves, on d 10. Whereas, mean Ca and P concentrations were statistically similar among three groups of calve on 15 and 30. In exp. 2 also, the mean Ca and P concentrations were significantly (P<0.05) higher in FC than in RC calves on d 3 and 6, respectively. Whereas, these were statistically similar between RC and FC calves on d 10, 15 and 30. The quantity and timing of colostrum intake has been found to affect plasma glucose in neonatal calves (Hammon *et al.* 2000). In the present study on similar lines higher serum glucose concentrations in the full mother contact calves than the other groups were observed. The serum calcium and phosphorus concentrations in the present study were affected by the amount and timing of colostrum intake, however, these results contrary to the results of (Zanker *et al.* 2000).

Physiological parameters: The mean heart rate (beats/ min) (HR) on d 1 and 10 differs significantly (P<0.05) among three groups of calves, with significantly (P<0.05) lower HR in FC calves followed by RC and lowest was

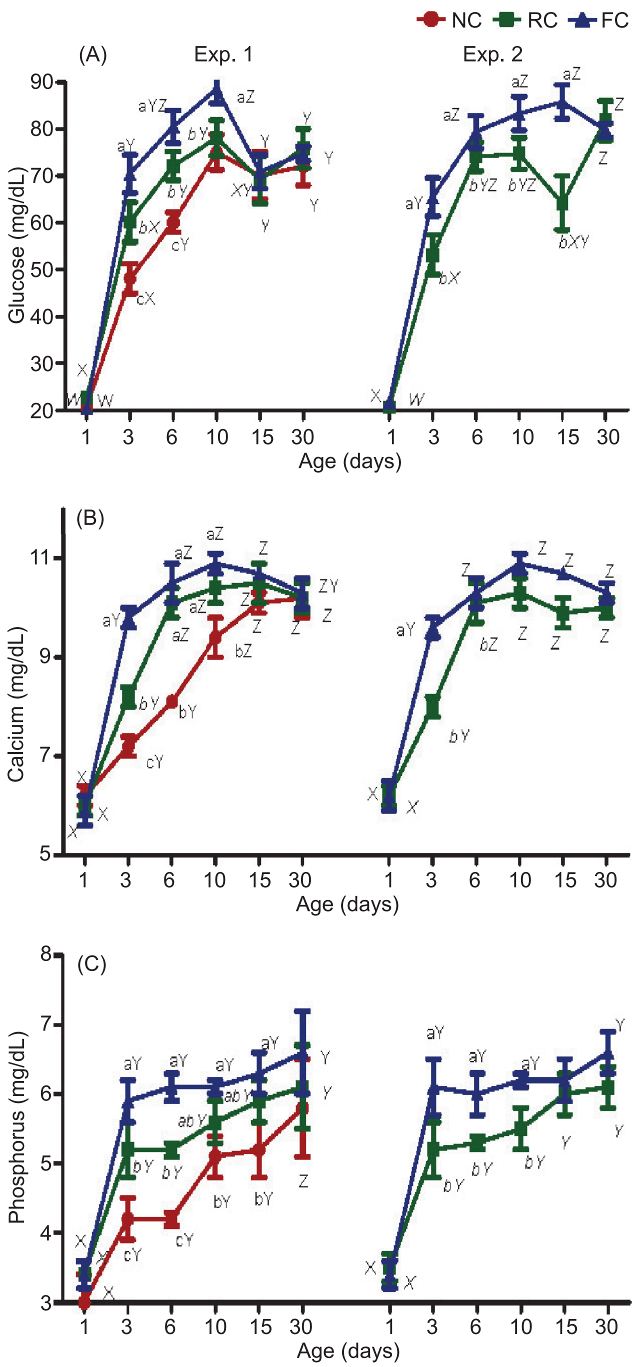

Fig. 2. Mean glucose (A), calcium (B) and phosphorus (C) concentration in blood serum of the Murrah buffalo calves in exp. 1 and exp. 2.

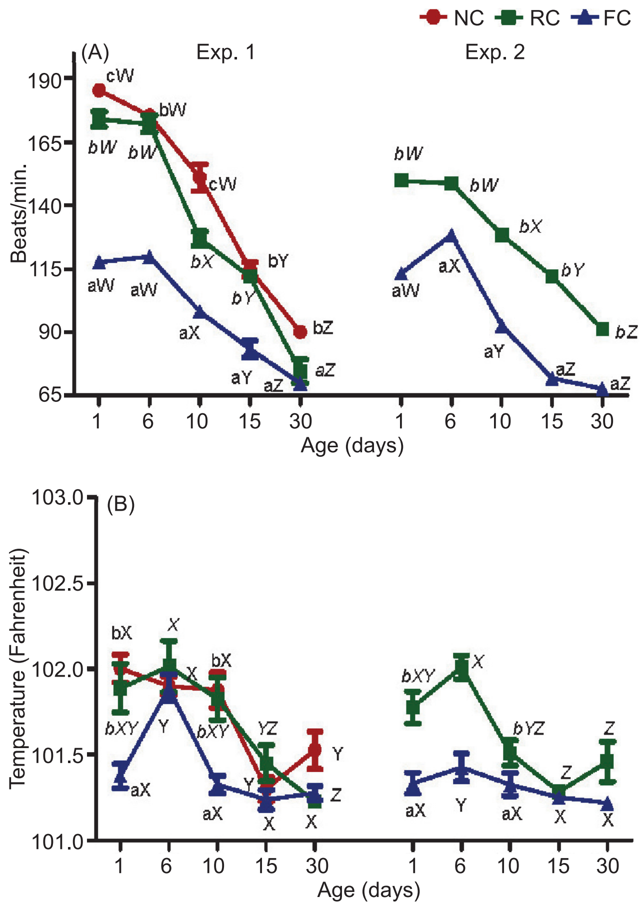

Fig. 3. Mean heart beats (A) and rectal temperature (B) of the Murrah buffalo calves in in exp. 1 and exp. 2.

observed in NC calves (Exp.1) (Fig. 3A). The HR was significantly (P<0.05) lower in FC than RC and NC calves on d 6, 15 and 30. There was an effect of time (P<0.01) group (P<0.01) and an interaction between group and time (P<0.01) on the calves' heart rate. Similar results were observed in the exp.2 in FC and RC calves (Fig. 3A).

Data pertaining to mean rectal temperature (RT) of calves in exp.1 are graphically represented in the Fig 3 B. The mean values of the vital signs demonstrated few differences between the FC, RC and NC calves. The mean Rectal temperature (RT) in FC (101.3±0.1°F and 101.3±0.1°F) was significantly lower than RC (101.8±0.1°F and 101.8±0.1°F) and NC (102.0±0.1°F and 101.8±0.1°F) on d 1 and 10 (P<0.05 on both days). Significant increase in RT was observed in FC calves on d 6 due to separation from their mothers. Mean RT values became normal on d 15 and 30 in all the three groups of calves (NC, RC and FC, respectively). On similar line, the mean RT in exp. 2 was significantly lower (P<0.05) in FC (101.3±0.1 and 101.3±0.1) than RC (101.7±0.1 and 101.5±0.1) calves on d 1 and 10, with similar observations on d 6, d 15 and 30 as were observed in exp. 1 (Fig. 3B). There was significant (P<0.05) treatment and time interaction for rectal temperature.

The mean eye temperature (ET) was significantly lower (P<0.05) in FC (97.4±0.3 and 97.1±0.2°F) than RC (99.1±0.1 and 98.1±0.3°F) and NC (99.1±0.1 and 98.2±0.2°F) calves on d 1 and 10. Mean ET for each group did not differ among three groups on d 6, 15 and 30. There was a statistically similar trend in mean ET between the two groups on all those days during exp. 2.

Physical separation of mother and calf, interruption of milk feeding, and an alteration in living environment are reported to result in both behavioural and physiological stress in relation to the weaning in beef calves (Stookey *et al.* 1997). Earlier, physiological research had supported the hypothesis that weaning and separation represents a stressful experience for both mother and young (Lupoli *et al.* 2001). In the present study also, the calves in restricted contact (RC) and no contact groups (NC) reacted to separation with an obvious increase in rectal temperature, heart rate and eye temperature. These findings agree with findings of Acevedo *et al.* (2005), Lynch *et al.* (2010) and O'Loughlin *et al.* (2014).

Hematology variables: The WBC count differed (P<0.05) between the three groups in exp.1 and between the two groups in exp.2, being greater (P<0.05) in NC and RC calves than FC calves and in RC *vs* FC (exp. 2) on d 3, 10, 15 and 30, respectively (Supplementary Fig. 1C). There were no significant differences between the treatment groups on d 1 and d 6 during both experiments. Similarly, there was a treatment and sampling time interaction (P<0.05) for neutrophil and lymphocytes. The percent neutrophil (Supplementary Fig. 1D) and lymphocytes (Supplementary Fig. 1E) were greater (P<0.05) in NC and RC than FC (exp. 1) and in RC than FC (exp. 2) on d 3, 10, 15 and 30. Differential assessment of the three groups in exp. 1 and two groups in exp. 2 yielded no significant difference in % neutrophil and lymphocytes on d 1 and 6. Mean RBC number and HGB concentration followed a similar trend for each group and varied only slightly during the period from birth to 30 days of age in both exp. 1 and exp.2.

In the present study, immediate separation and restricted contact have resulted in an increased neutrophils percentage. This increase is consistent with previous reports of Lynch *et al.* (2010) and O'Loughlin *et al.* (2014). In the present study, in FC calves, when calves shifted from full contact to fence-line contact on d 6, an increased neutrophils count was noticed. However, this increase became normal on the further days of sampling. Our results are supported by Price *et al.* (2003) and Lynch *et al.* (2010). While lymphocytes are usually regarded as being an adjunct to neutrophils in the detection of the bovine stress response which is also significant in the present study, there was a decrease in the lymphocytes count in NC calves following separation. This is consistent with previous studies of Lynch *et al.* (2010) and O'Loughlin *et al.* (2011).

Concentration of blood serum cortisol: The data of mean cortisol concentrations of exp. 1 are graphically presented in Supplementary Fig. 2. The mean cortisol levels (ng/mL) on d 1, 10, 15 and 30 differed significantly (P<0.05) among three groups (NC, RC and FC) of calves in exp. 1. The mean concentrations of cortisol were significantly lower (P<0.05) in FC on all days of sampling than in RC and NC calves. The cortisol levels on d 6 were significantly lower (P<0.05) in FC and RC calves than NC calves. Similar results were observed in exp. 2 with mean cortisol levels (ng/mL) on d 1, 10, 15 and 30 differed significantly (P<0.05) between two groups of calves (FC *vs* RC). Mean concentrations of cortisol were significantly lower (P<0.05) in FC on all days of blood sampling than in RC. While, the cortisol concentration on d 6 was, however, statistically similar in both the groups of calves (FC and RC) (Supplementary Fig. 2).

On separation, buffalo calves are more vulnerable to stress than cattle calves because they are highly social animals with a strong maternal instinct (Mustafa *et al.* 2010, Aref *et al.* 2016). In the present study, we observed significantly higher cortisol levels in weaned calves on different days of sampling than RC and FC calves. These results are in agreement with previous studies by O'Loughlin, *et al.* (2014) and Moggy *et al.* (2017). Stress increases, if separation occurs after the establishment of mother-calf bonding (Rhim 2012), and similar results were observed in present study during both experiments. The mean cortisol levels in restricted contact calves were higher than in FC calves which may be due to repeated reunion during morning and evening milking followed by separation from their mothers.

Disease incidences: The frequency of diarrhoea during exp.1 differed significantly (P< 0.05) between FC (3 and 10) RC (8 and 17) and NC (14 and 28) groups of calves from d 0 to 5 and d 6 to 30. The percentage cases of diarrhoea were significantly higher (P< 0.05) in NC (87.5 and 87.5%) followed by RC (75 and 62.5%) and least was observed in FC (25 and 37.5%) calves on d 0, d 5 and d 6, d 30. In exp. 2, the frequency of diarrhoea differed significantly (P<0.05) between FC (1 and 3) and RC (8 and 12) groups of calves from d 0 to 5 and d 6 to 30. The percentage cases of diarrhoea were significantly higher (P<0.05) in RC (46.6 and 58.3%) and least was observed in FC (8.3 and 16.6%) calves on d 0–5 and d 6–30. During exp. 1 we observed no fever cases in the FC calves, while fever was detected in NC (50 and 62.5%) and RC (25 and 25%) calves, and similar trends were observed in exp. 2. Respiratory distress during d 0 to 5 and d 6 to 30 was detected more in NC (37.5 and 50%) and RC (12.5 and 25%) calves, while none of the calves in FC suffered from respiratory distress. Incidences of eye infection were detected only in NC calves throughout the study period and similar trends were observed in exp. 2.

Prevalence of digestive disorder, fever, respiratory disorder and eye infection are major issues in buffalo calves (Singh *et al.* 2017). The incidence of these problems was lower in FC calves than in NC and RC during exp. 1 and then in RC during exp. 2. This may be attributed to the fact that FC calves had full mother contact and free choice colostrum suckling opportunity which promoted temporary protection to newborn calves due to passive transfer of immune factors such as immunoglobulins, leukocytes, hormones and cytokines and thus reducing the risk of outbreak of diseases, such as diarrhea. These results are in agreement with the findings of Razzaque *et al.* (2009) and Silva *et al.* (2013). Further, we assumed that voluntary colostrum suckling and mother contact had resulted in more frequent and small meals of colostrum which was absorbed rapidly, with improved digestion. Boland *et al.* (2008) reported that fenceline calves responded well to the separation by the fence line, likely because physical separation stress was moderated by visual and auditory interaction with dams. Restricted suckled calves encountered higher disease incidence due to lower level of colostrum suckling during first 6–12 h after birth which may have led to delay in development of passive immunity. Moreover, these calves were allowed to suckle colostrum and milk at the interval of 12 h twice daily, which may have led to an increased desire for satiety, and those calves suckled a greater amount of colostrum and milk which may have resulted in more incidence of undigested diarrhoea in RC than FC calves.

In conclusion, the provision of full mother contact along with free choice colostrum suckling from birth to 5 days followed by fenceline mother contact and twice daily suckling at milking time abated the separation and weaning stress and remarkably improved the growth, immune status and health of Murrah buffalo calves as compared to the calves which were allowed only limited contact twice daily during suckling at milking time or the calves which were weaned at birth and offered colostrum/milk through a nipple bottle.

ACKNOWLEDGEMENTS

The authors appreciatively acknowledge the Director, National Dairy Research Institute, Karnal for providing the necessary funds and facilities to conduct this research. We thankfully acknowledge Ashleigh F Brown for her help in correcting the manuscript for English language.

REFERENCES

Aref N E M, El-Sebaie A and Hammad H Z. 2016. New insights on ill-thriftiness in early-weaned buffalo calves. *Veterinary World* **9**(6): 579–86.

Bharti P K, Dutt T, Pandey H O, Patel B H M, Mahendran K, Kaswan S, Biswas P and Upadhyay V K. 2015. Effect of weaning age on health of Murrah buffalo calves. *Indian Journal of Animal Sciences* **85**(12): 1370–74.

de Rosa G, Grasso F, Pacelli C, Napolitano F and Winckler C. 2009. The welfare of dairy buffalo. *Italian Journal of Animal Science* **8**(1): 103–16.

Donovan D C, Reber A J, Gabbard J D, Aceves-Avila M, Galland K L, Holbert K A, Ely L O and Hurley D J. 2007. Effect of maternal cells transferred with colostrum on cellular responses to pathogen antigens in neonatal calves. *American Journal of Veterinary Research* **68**(7): 778–82.

Hammon H M, Zanker I A and Blum J W. 2000. Delayed colostrum feeding affects insulin-like growth factor I and insulin plasma concentrations in neonatal calves. *Journal of Dairy Science* **83**: 85–92.

Hassan T M M, Mahmoud M S H, Soliman A S M, El-Mahdy M R and Hassan H Z. 2019. Effect of fence-line weaning on egyptian buffaloes' milk. *Slovak Journal of Animal Science* **52**(3): 134–46.

Hedegaard C J and Heegaard P M H. 2016. Passive immunisation, an old idea revisited: Basic principles and application to modern animal production systems. *Veterinary Immunology and Immunopathology* **174**: 50–63.

Kamboj M L and Ajesh K. 2013. Effect of weaning on performance and behaviour of calves and their dams in dairy cows-A review. *Indian Journal of Animal Sciences* **83**(10): 991–97.

Kišac P, Brouèek J, Uhrinèat M and Hanus A. 2011. Effect of weaning calves from mother at different ages on their growth and milk yield of mothers. *Czech Journal of Animal Science* **56**(6): 261–68.

Loberg J M, Hernandez C E, Thierfelder T, Jensen M B, Berg C and Lidfors L. 2008. Weaning and separation in two steps-A way to decrease stress in dairy calves suckled by foster cows. *Applied Animal Behaviour Science* **111**(3–4): 222–34.

López J R, Elías A and Delgado D. 2008. The feeding system of buffalo calves. Its influence on the species efficiency. *Cuban Journal of Agricultural Science* **42**(3): 235–40.

Lynch E M, Earley B, McGee M and Doyle S. 2010. Effect of abrupt weaning at housing on leukocyte distribution, functional activity of neutrophils, and acute phase protein response of beef calves. *BMC Veterinary Research* **6**: 6–39

Masucci F, De Rosa G, Barone C M A, Napolitano F, Grasso F, Uzun P and Di Francia A. 2015. Effect of group size and maize silage dietary levels on behaviour, health, carcass and meat quality of Mediterranean buffaloes. *Animal* **10**(3): 531–38.

McGuirk S M and Collins M. 2004. Managing the production, storage and delivery of colostrum. *Veterinary Clinics of North America: Food Animal Practice* **20**(3): 593–603.

Mustafa M Y, Shahid M and Mehmood B. 2010. Management practices and health care of buffalo calves in Sheikhupura district, Pakistan. *Buffalo Bulletin* **29**(3): 217–23.

O'Loughlin A, McGee M, Waters S M, Doyle S and Earley B. 2011. Examination of the bovine leukocyte environment using immunogenetic biomarkers to assess immunocompetence following exposure to weaning stress. *BMC Veterinary Research* **7**(1): 45.

O'Loughlin A, McGee M, Doyle S and Earley B. 2014. Biomarker responses to weaning stress in beef calves. *Research in Veterinary Science* **97**(2): 458–63.

Paranhos da Costa M J R, Albuquerque L G, Eler J P and Augusto II de Vasconcelos Silva J. 2006. Suckling behaviour of Nelore, Gir and Caracu calves and their crosses. *Applied Animal Behaviour Science* **101**(3–4): 276–87.

Pasha T N. 2013. Prospect of nutrition and feeding for sustainable buffalo production. *Buffalo Bulletin* **32**(Spec. Issue 1): 91–110.

Price E O, Harris J E, Borgwardt R E, Sween M L and Connor J M. 2003. Fenceline contact of beef calves with their dams at weaning reduces the negative effects of separation on behavior and growth rate. *Journal of Animal Science* **81**(1): 116–21.

Rhim, S J. 2012. Vocalization and behavior of Holstein cows and calves after partial and complete separation. *Revista Colombiana de Ciencias Pecuarias* **26**(1): 24–29

Singh P K, Kamboj M L, Chandra S and Singh R K. 2017. Effect of calf suckling dummy calf used and weaning on milk ejection stimuli and milk yield of Murrah buffaloes (*Bubalus bubalis*). *Journal of Pharmacognosy and Phytochemistry* **6**: 1012–15.

Sirovnik J, Barth K, De Oliveira D, Ferneborg S, Haskell M J, Hillmann E, Jensen M B, Mejdell C M, Napolitano F, Vaarst M, Verwer C M, Waiblinger S, Zipp K A and Johnsen J F. 2020. Methodological terminology and definitions for research and discussion of cow-calf contact systems. *Journal of Dairy Research* **87**(S1): 108–14.

Smijisha A S and Kamboj M L. 2012. Colostrum intake of weaned Murrah buffalo calves reared under different management practices. *Tamilnadu Journal of Veterinary and Animal Sciences* **8**(1): 42–44.

Zanker I A, Hammon H M and Blum J W. 2000. Plasma amino acid pattern during the first month of life in calves fed the first colostrum at 0–2 or at 24–25 hours after birth. *Journal of Veterinary Medicine Series A* **46**: 101–21.