



## Blood biochemical profiles and physical-activity parameters in neonatal buffalo calves under normal and forced calving

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### ABSTRACT

The evaluation of metabolic homeostasis in difficult parturition is vital to clinical recognition of underlying disorders to provide appropriate therapeutic measures. The biochemical status of buffalo calves delivered normally (n=17) and through manipulation and forced extraction (n=17) was studied over a period of 5 h immediately after calving. Plasma aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase, blood glucose and total protein were estimated within 1 h after sampling. Different physical-activity parameters like time from birth till first rise of head, time from birth till sternal recumbancy, time from birth till first attempt to stand-up, duration of fetus in womb after appearance of water bag, duration of fetal extraction/expulsion and time from birth until recovery from neonatal stress were also noted and correlated with metabolic changes. Alterations in the biochemical status were suggestive of metabolic changes with some compensation immediately after delivery in normally delivered (ND) calves. The calves delivered through forced extraction (FD) had elevated enzymatic profiles, which remained elevated up to 4 h post-delivery but improved over the period of time (up to 4 h) after calving. Furthermore, wider differences were recorded in the blood glucose values in the two set of calves revealing informative significance regarding the level of stress in the FD group. An alternative diagnostic tool, which appeared in estimating the condition of a newborn calf, was different physical-activity parameters. Thus, blood biochemical estimation should be used as a matter of intensive care and corrective measures adopted in neonates.

**Key words:** Biochemical profile, Buffalo, Calf, Calving, Forced calving, Normal calving

Changes in specific biochemical molecules are the early manifestation of many diseases in domestic animals (Gemelli 2003). Metabolic profiles are extensively used in the recent past in the veterinary clinical practice and its breaches are well established in adult animals (Cetin *et al.* 2002). Knowing the reference values of metabolic profiles in domestic animals would be helpful in correct valuation and interpretation to develop effective therapeutic measures against a particular disease (Angelov *et al.* 2009). The reference values in neonatal calves are obscure and this makes it difficult to do the interpretation. Furthermore, various physical-activity parameters like time from birth till first rise of head, time from birth till sternal recumbancy, time from birth till first attempt to stand-up, duration of fetus in womb after

appearance of water bag, duration of fetal extraction/expulsion and time from birth until recovery from neonatal stress during the perinatal period are not well documented in buffalo calves. The limited literature of blood metabolic profile and physical-activity parameters in the neonatal buffalo calves prompted us to carry out present research work.

### MATERIALS AND METHODS

**Selection of animals:** The study included equal numbers of calves delivered normally (ND) (17); and calves (17) delivered following correction of malpresentation and forced traction (FD). Dystociac buffaloes had been in labor for 4 to 8 h. The dams of all the calves were either in their first or second parity.

**Blood collection:** Blood samples were collected from all the calves in lateral recumbancy by jugular veinupuncture under anaerobic conditions immediately at birth and subsequently at 1, 2, 3, 4 and 5 h post-delivery in heparinized vials (10 IU heparin/ml blood). Blood samples (5 ml) were capped, placed on ice and immediately transferred to laboratory so as to avoid alterations in biochemical profile. All the samples were analyzed within 1 h of sampling. Plasma

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aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were estimated using dry test slides. Blood glucose and total protein were determined using dry test slides.

Various physical-activity parameters, viz. time from birth till first rise of head (T-RH), time from birth till sternal recumbancy (T-SR), time from birth till first attempt to stand-up (T-UP), duration of fetus in womb after appearance of water bag (DFW), duration of fetal extraction/expulsion (DFE) and time from birth until recovery from neonatal stress (T-RS) were determined through close observations on neonates as well as history from the owner of the animals. DFW was referred to the time from appearance of water bag until the extraction/expulsion of a viable fetus. DFE included the time from the start of manipulation, until the calf was born. Follow-up inquiries were made about vitality, whether the calves survived the first week of life, else when they died during the first week.

**Statistical analysis:** The data were analyzed by the use of Statistical Package for Social Sciences program (SPSS 16.0). The differences between ND and FD for biochemical changes were tested using 2×2 analysis of variance (ANOVA) with significant interaction at 5% level. Differences in mean  $\bar{X}$  ( $\pm$ SEM) biochemical changes in the 2 groups with unequal variances were tested with students 't' test of independence with Bonferroni correction. The significant interactions were tested using Duncan's multiple range test. Correlation coefficients were calculated according to Spearman as per the methods described previously (Zar 2008).

## RESULTS AND DISCUSSION

Changes in plasma enzyme activity and metabolic profile are shown in Table 1. Any fluctuation in the blood

biochemical parameters seemed to indicate the dynamic changes occurring due to impending calving.

**Aspartate Aminotransferase (AST):** Plasma AST was significantly ( $P < 0.05$ ) higher in FD than in ND calves, although both the groups exhibited a decreasing trend after calving till the end of observations. Marked changes in plasma AST activity reflect acute cellular injury (Zanker *et al.* 2001). This stands true for calves delivered forcefully which might have resulted in myocyte injury of skeletal muscle due to manipulations applied in the process of delivery in present study. Plasma AST has a longer half-life, and therefore, it would be expected to have increased sensitivity during the recovery process (Palmisano *et al.* 1995). This could be evident from the unstable values in the FD group even till 5 h after calving. Eventually, short-term period of observations might not be sufficient to alleviate the values, if any, in the current set of examination.

**Alanine aminotransferase (ALT):** A significantly ( $P < 0.05$ ) higher value of ALT was noticed in FD as compared to ND calves during the period of sampling. While the enzymatic activity tend to remain within the physiological limit immediately after calving in the latter group, the levels were not stabilized even up to 5 h after calving in the former. A relative acute increase in plasma ALT activity is suggestive of hypoxia (Perino *et al.* 1993). Dystocia is a stressful event often associated with higher degree of anxiety, muscular activity and pain and often results in hypoxia of the tissue in calves (Gunter *et al.* 2003). Therefore, calves delivered through a calf puller had shown a distinct shift of ALT towards increase (Pearson *et al.* 2005). Eventually, in the current investigation, the neonates suffered from hypoxia in the FD group in the cascade of events due to obstetrical intervention as well (results not shown here). Oxygen diffusion abnormalities leading to hampered oxygen utilization by the

Table 1. Blood biochemical profile of neonatal buffalo calves delivered normally and forcefully (mean $\pm$ SEM)

| Parameter     | Unit  | Group | Hours after calving           |                                |                                |                                |                                 |                                 | Mean $\pm$ SEM                |
|---------------|-------|-------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|---------------------------------|---------------------------------|-------------------------------|
|               |       |       | 0                             | 1                              | 2                              | 3                              | 4                               | 5                               |                               |
| AST           | U/L   | ND    | 87.8 $\pm$ 10.5 <sup>a</sup>  | 78.2 $\pm$ 10.4 <sup>a</sup>   | 71.6 $\pm$ 10.1 <sup>a</sup>   | 68.8 $\pm$ 9.0 <sup>a</sup>    | 63.2 $\pm$ 9.1 <sup>a</sup>     | 67.4 $\pm$ 9.2 <sup>a</sup>     | 72.8 $\pm$ 3.6 <sup>a</sup>   |
|               |       | FD    | 111.8 $\pm$ 11.0 <sup>*</sup> | 105.6 $\pm$ 11.2 <sup>b*</sup> | 93.4 $\pm$ 10.8 <sup>b</sup>   | 87.6 $\pm$ 9.1 <sup>b</sup>    | 84.0 $\pm$ 9.3 <sup>b</sup>     | 74.0 $\pm$ 10.2 <sup>ab</sup>   | 92.7 $\pm$ 5.7 <sup>b</sup>   |
| ALT           | U/L   | ND    | 34.0 $\pm$ 2.5 <sup>a*</sup>  | 31.0 $\pm$ 2.2 <sup>a</sup>    | 25.8 $\pm$ 2.7 <sup>b</sup>    | 28.4 $\pm$ 2.5 <sup>ab</sup>   | 24.6 $\pm$ 2.4 <sup>b</sup>     | 26.8 $\pm$ 2.6 <sup>b</sup>     | 28.4 $\pm$ 1.4 <sup>b</sup>   |
|               |       | FD    | 52.4 $\pm$ 7.0 <sup>*</sup>   | 47.4 $\pm$ 7.6 <sup>c*</sup>   | 41.6 $\pm$ 5.6 <sup>c#</sup>   | 38.4 $\pm$ 5.2 <sup>c#</sup>   | 33.6 $\pm$ 4.0 <sup>#</sup>     | 38.3 $\pm$ 6.3 <sup>c#</sup>    | 42.0 $\pm$ 2.8 <sup>c</sup>   |
| ALP           | U/L   | ND    | 188.6 $\pm$ 31.6 <sup>a</sup> | 161.6 $\pm$ 40.5 <sup>a</sup>  | 136.6 $\pm$ 31.5 <sup>a</sup>  | 145.8 $\pm$ 31.9 <sup>a</sup>  | 143.4 $\pm$ 34.9 <sup>a</sup>   | 139.3 $\pm$ 32.0 <sup>a</sup>   | 152.6 $\pm$ 16.1 <sup>a</sup> |
|               |       | FD    | 340.6 $\pm$ 55.5 <sup>*</sup> | 309.4 $\pm$ 51.9 <sup>b*</sup> | 265.2 $\pm$ 57.8 <sup>b#</sup> | 232.6 $\pm$ 45.3 <sup>b#</sup> | 249.8 $\pm$ 46.0 <sup>b*#</sup> | 194.3 $\pm$ 41.8 <sup>ab#</sup> | 265.3 $\pm$ 21.6 <sup>b</sup> |
| ALT:ALP       | —     | ND    | 1:5.5                         | 1:5.2                          | 1:5.3                          | 1:5.1                          | 1:5.8                           | 1:5.2                           | 1:5.4                         |
|               |       | FD    | 1:6.5                         | 1:6.5                          | 1:6.4                          | 1:6.1                          | 1:7.4                           | 1:5.1                           | 1:6.2                         |
| Glucose       | mg/dl | ND    | 67.8 $\pm$ 7.0                | 65.8 $\pm$ 6.7                 | 62.0 $\pm$ 5.2                 | 63.8 $\pm$ 6.5                 | 62.2 $\pm$ 5.9                  | 69.5 $\pm$ 6.1                  | 65.2 $\pm$ 2.5 <sup>a</sup>   |
|               |       | FD    | 75.6 $\pm$ 5.9                | 73.2 $\pm$ 5.0                 | 79.0 $\pm$ 9.1                 | 74.7 $\pm$ 7.9                 | 76.6 $\pm$ 7.9                  | 68.8 $\pm$ 9.9                  | 74.7 $\pm$ 4.1 <sup>b</sup>   |
| Total protein | g/dl  | ND    | 6.48 $\pm$ 0.22               | 6.70 $\pm$ 0.16                | 6.62 $\pm$ 0.11                | 6.62 $\pm$ 0.15                | 6.68 $\pm$ 0.23                 | 6.70 $\pm$ 0.17                 | 6.63 $\pm$ 0.10               |
|               |       | FD    | 6.76 $\pm$ 0.47               | 6.98 $\pm$ 0.44                | 6.90 $\pm$ 0.50                | 6.98 $\pm$ 0.35                | 6.82 $\pm$ 0.35                 | 6.60 $\pm$ 0.44                 | 6.84 $\pm$ 0.20               |

Values in same row and column with different superscripts of a parameter are significantly different ( $P < 0.05$ )

ND, normally delivered; FD, forcefully delivered.

tissues might have led to elevated enzymatic profile. Nevertheless, a significant ( $P < 0.05$ ) difference was observed within the FD group immediately after calving and till the end of the study period indicating the process of recovery of calves from the neonatal tissue hypoxia.

**Alkaline phosphatase (ALP):** The average enzymatic value was significantly ( $P < 0.05$ ) higher in FD calves than in their ND counterparts (Table 1.). Plasma ALP has been recognized as a marker of stress in animals (Xu *et al.* 2002). The calves delivered forcefully generally suffer from acute stress and this might have resulted in increased level of the enzyme. The process of parturition, though physiological, is a stressful event and abnormal parturition (dystocia) further adds to the normal stress of calving (Prabhakar *et al.* 1999). Thus, ALT and ALP can give an indication about stress associated-hypoxemia and can be used as marker under stressful condition like dystocia. Therefore, stress of parturition and dystocia might have predisposed the neonates to such enzymatic derangements.

**Alanine Aminotransferase: Alkaline Phosphatase (ALT: ALP):** Although it is only a general type of interpretation, ratio of plasma ALT to ALP, can have a conclusive clinical significance (Horney *et al.* 2003). Normal ALT: ALP is 1: 5. A high ratio is indicative of muscular activity (Vanveen *et al.* 2005). Despite being nonsignificant ( $P < 0.05$ ) a high ratio was always observed in FD calves indicating the neonates suffering from stress attenuated tissue apprehension. The highest ratio recorded in the calves delivered with strong forceful extraction was due to dystocia-associated muscular activity (Naokes *et al.* 2001). Five h after delivery, the drop in the ratio was observed in FD calves signifying the process of homeostasis in the current set of observations (Table 1).

**Blood glucose:** Although dams with normal parturition were fed under standard feeding regime, plasma glucose was lower ( $P < 0.05$ ) in calves delivered by them. Contrarily, the overall plasma glucose concentrations were significantly ( $P < 0.05$ ) higher in the FD group (Table 1.). A persistent acute hyperglycemia as observed in the FD group is the single most important diagnostic criteria of stress evolution (Hoffman 2008). The secretion of plasma cortisol from the fetal adrenals increases, rises markedly and compete with action of progesterone in the regulation of the corticotrophin releasing hormone (CRH) gene (Karalis *et al.* 1996). The effect of cortisol hormone tends to raise blood glucose profile far beyond the physiological limit. Glucose utilization in the peripheral tissue decreases while the hepatic glucose production increases due to gluconeogenesis. Excessive fetal exposure to increased amounts of glucocorticoids inactivates the intracellular enzyme 11 $\beta$  hydroxysteroid dehydrogenase localized in the syncytiotrophoblast, the site of maternal-fetal exchange due to inflammation and tissue damage (common outcomes of dystocia) prevent rapid decline of cortisol in animals under stress and may induce pathways of aerobic energy production associated with parturition

resulting in increased blood glucose profiles (Yang 1997).

**Total protein:** No significant ( $P < 0.05$ ) variation in the levels of plasma protein was observed in both the groups (Table 1.). A normalcy in total protein value may reflect a normal rate of protein catabolism resulting in continuous turnover of protein and indicating liver functioning status immediately at birth. The findings are in agreement with the observations of Walsh (2002). Although non-significant, a slight decrease in the protein concentration was seen in the calves delivered forcefully. This is truly indicative of acute phase inflammatory response as a result of acute inflammatory disease owing to high concentration of fetal cortisol as a result of dystocia (Skinner 2001). Once the FD calves were delivered, the protein level almost reached nearly similar to that of ND calves.

**Physical-activity parameters:** Various physical-activity parameters in two groups are presented in table 2. Observations revealed that calves delivered forcefully were retained in womb for significantly ( $P < 0.05$ ) longer duration. They recorded significantly ( $P < 0.05$ ) higher time from birth to experience their first rise of head (T-RH), time from birth till sternal recumbancy (T-SR), time from birth till first attempt to stand-up (T-UP) and hence time from birth until recovery from neonatal stress (T-RS) than that of corresponding values recorded in ND calves. None of the FD calf experienced its T-SR within 10 min of delivery as compared to 100% ND calves showing their T-SR in less than 10 minutes of calving ( $6.3 \pm 2.6$  min vs  $14.6 \pm 4.2$  min). Similar studies (Schuijt and Taverne 1992) in cows also showed that type of delivery adversely affects the onset of sternal recumbancy ( $4.0 \pm 2.2$  min in normal parturition vs.  $9.0 \pm 3.3$  min in powerful extraction calves). Most farmers and veterinarians use a 10 min period of observation for vitality of the newborn calf. Determination of T-SR appeared to be easy since, to a large extent it is independent of external factors such as the condition of the floor and influence of animal's behavior (Kovalcik *et al.* 1980). Eventually, in the current investigation an extra-effort applied on the calves delivered forcefully could have exhausted the energy resources in neonates as well, thereby causing delay in attainment of steady posture. Under normal calving, a calf is expected to show its first rise of head by 45 seconds and first attempt to stand by 25 min after parturition (Williams *et al.* 1996). In the present study, a longer time observed for both the parameters could be a species difference. Within groups, the FD calves took significantly ( $P < 0.05$ ) longer duration for both the above-mentioned parameters as compared to ND calves thereby signifying the extended duration of neonatal stress in the former. These findings are in consonance with the observations of Fraser (1989) who reported that both first rise in head and first attempt to stand was prolonged in the calves delivered with forced traction that ultimately affected neonatal stress. Both T-RH and T-UP are largely influenced by the kind of floor, type of animal as well as stimulation

Table 2. Physical-activity parameters and their correlation with plasma enzymatic values in neonatal buffalo calves delivered normally (ND) and forcefully (FD) (mean±SEM)

| Physical-activity parameter                                   | Unit    | Type of | Distribution-delivery time for various parameters | Correlation coefficient |          |          |
|---------------------------------------------------------------|---------|---------|---------------------------------------------------|-------------------------|----------|----------|
|                                                               |         |         |                                                   | AST                     | ALT      | ALP      |
| Time from birth till first rise of head (T-RH)                | Seconds | ND      | 56.4±6.8 <sup>a</sup>                             | -0.56*                  | +0.21*   | +0.17*   |
|                                                               |         | FD      | 76.7±7.7 <sup>b</sup>                             | -0.52*                  | +0.39*   | +0.35**  |
| Time from birth till sternal recumbancy (T-SR)                | Minutes | ND      | 6.3±2.6 <sup>a</sup>                              | -0.68*                  | +0.46*   | +0.52*   |
|                                                               |         | FD      | 14.6±4.2 <sup>b</sup>                             | -0.53***                | +0.77*** | +0.81*** |
| Time from birth till first attempt to stand-up (T-UP)         | Minutes | ND      | 31.7±5.4 <sup>a</sup>                             | -0.57*                  | +0.36*   | +0.32*   |
|                                                               |         | FD      | 55.2±9.6 <sup>b</sup>                             | -0.72*                  | +0.52*** | +0.73*** |
| Duration of fetus in womb after appearance of water bag (DFW) | Minutes | ND      | 112.9±12.5 <sup>a</sup>                           | -0.51*                  | +0.32*   | +0.25*   |
|                                                               |         | FD      | 328.2±37.2 <sup>b</sup>                           | -0.82*                  | +0.55**  | +0.67**  |
| Duration of fetal extraction/expulsion (DFE)                  | Seconds | ND      | 204.7±17.1 <sup>a</sup>                           | -0.47*                  | +0.19*   | +0.26*   |
|                                                               |         | FD      | 1694.1±112.0 <sup>b</sup>                         | -0.63**                 | +0.61**  | +0.49**  |
| Time from birth until recovery from neonatal stress (T-RS)    | Minutes | ND      | 132.6±26.6 <sup>a</sup>                           | -0.34*                  | +0.27*   | +0.17*   |
|                                                               |         | FD      | 228.9±37.4 <sup>b</sup>                           | -0.19*                  | +0.68**  | +0.52**  |
| Effect of logic sign                                          | —       | —       | —                                                 | Inverse                 | Direct   | Direct   |

Values in same column with different superscripts for a parameter are significantly different ( $P < 0.05$ ); Significance: \* =  $P < 0.05$ , \*\* =  $P < 0.02$ , \*\*\* =  $P < 0.01$ ; ND = normally delivered; FD = forcefully delivered.

from intensive licking by the dams and requires continuous attention (Yavas and Watton 2000). However, attempts were made to minimize the differences between the two types of calving, if any, through deliveries on similar type of floor (concrete), same breed (Murrah) and parity (first and second) and through close observations on the calf delivered in the current investigation. A significantly ( $P < 0.05$ ) longer time taken by FD calves in womb (DFW) as well as in their extraction (DFE) as compared to their normally delivered counterparts is in order with the findings of Szenci *et al.* (1988) in cows. The calves delivered by forceful extractions showed substantial stress and longer duration in womb aggravated the condition. This might result from intrauterine hypoxia, hampered respiration and/or less effective neonatal respiration associated with stress (Hoyer *et al.* 1990). Prolonged duration of fetus in womb after initiation of labor leads to acute respiratory distress that may hamper the fetal life (Owens and Edey 1985). Therefore, the type of delivery seemed to be the most probable cause to influence the physical-activity parameters. Together, a significantly ( $P < 0.05$ ) longer time taken in T-RH, T-SR, T-UP, DFW and DFE accounted for prolonged duration neonatal stress in the calves delivered forcefully, in the present study.

**Correlation of physical-activity parameters and enzymatic profiles with respect to the type of delivery:** Significant ( $P < 0.05$ ), moderately negative correlations existed between various parameters and plasma AST values in both the groups (Table 2). Positive correlations were found between the physical-activity parameters and plasma ALT and ALP in ND and FD calves. Significantly ( $P < 0.01$ ) high correlation between the above-mentioned parameters and enzymes in the forcefully delivered calves indicates stress in neonatal

calves. The relationship between enzymatic profile and physical activity parameters according to the type of delivery, as found in the present study agrees with the findings of Szenci *et al.* (1988). Amongst different parameters, the level of significance was most pronounced for T-SR, DFE in both the groups. Nevertheless, the positive correlation of T-RH, T-UP and DFW with plasma ALT and ALP renders their contribution toward neonatal stress along with T-SR. This means that T-RH, T-SR, T-UP, DFW and DFE criterion of minutes had a high diagnostic value and can be obtained within a practicable time. The strong correlation between physical- activity parameters and T-RS, as found in the present study, may be explained by type of delivery, duration of neonate in womb and the physical efforts applied in the process of calving which may all influence the recovery from stress and hypoxia during the time that the calf tries to stand. Certainly, blood biochemical estimations must remain the cornerstone of peri-natal research because it is quantitative information which can give us better understanding of the underlying metabolic derangements. However, it is unrealistic to expect any single measurement or observation to provide a complete insight into the condition of the newborn calf. Close attention for initiation of respiration and typical symptoms of serious stressful conditions during the first few minutes of life is always needed. It is, therefore, recommended to monitor physical-activity parameters in conjunction with the blood biochemical parameters as a matter of intensive and critical care.

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