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Relationship of Blood Metabolites with Reproductive Parameters during Various Seasons in Murrah Buffaloes

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ABSTRACT : Peri-partum metabolic profile was evaluated in winter and summer calving, with 15 Murrah buffaloes in each seasonal group. In summer calvers, significantly lower values were observed for blood plasma urea nitrogen (BUN) at day 30 pre-partum ($p<0.05$), on calving day ($p<0.05$) and at all other stages ($p<0.01$); plasma non-esterified fatty acids (NEFA) values were significantly lower on day 30 pre-partum ($p<0.01$) and on day 60 post-partum ($p<0.05$). This was associated with significant reduction in days to first service (DFS) and service per conception (SPC) and an overall better reproductive performance in terms of service period, risk to first service on days 60, 90 and >90, and pregnancy risk to first service up to days 60 and 90. This may be attributed to better pre-partum nutritional status. Cervical and uterine involution were completed in fewer days, involutional changes took place at a faster pace and there were a lower number of abnormal involutional changes in winter compared to summer season. This may be attributed to better post-partum nutrition and less environmental stress. However, validation requires further targeted cohort investigation with a large sample size. (**Key Words :** Murrah Buffalo, Reproduction, Metabolic Profile, Peripartum)

INTRODUCTION

Murrah is the most important and efficient producer of milk among the Indian breeds of buffalo. Bulls of this breed are used extensively for up-grading of inferior stock. The average milk yield in a lactation is 1,500 to 2,500 kg. Buffaloes have long been considered as sluggish breeders because of their inherent long calving interval (450 to 500 days), service periods (170.32±4.67 days) and seasonality in their breeding despite being regular breeders (Prakash et al., 2005; Khan et al., 2009). Infertility and sub-fertility due to sub-estrous, anoestrus, repeat breeding and longer post-partum interval (PPI) and service period remain the predominant issues and challenge to researchers, scientific community and veterinarians in general and the farming community in particular.

Photoperiod, nutritional and management factors and other unknown causes mainly affect seasonality in buffalo breeding. Among these, imbalanced or deficient nutrition alone contributes more than 80% of the reproductive problems. Besides general nutritional status, deficiency or imbalance of critical factors or specific nutrients have either immediate effects on health; productive and reproductive processes or the effects may be concealed and recognized after a prolonged period. The effects depend on the nature of the factor, extent of deficiency or imbalance, duration and physiological status of the animal. During the dry period, short or long term deficiencies cause impaired health in buffaloes in the subsequent lactation (Campanile et al., 1997).

In general, mineral deficiencies are associated with altered metabolic profile leading to most peri-parturient disorders and altered immune function in buffaloes. During advanced pregnancy and the early post-partum period, buffaloes are highly prone to the stress of heavy nutrient demand and drain, which could probably be prevented by addressing the basic etiology through balanced feeding and mineral supplementation (Mandali et al., 2002). Further impact of general health disorders on reproductive disorders is an important but covert factor. Therefore, nutritional

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management, in general, and reproductive health, in particular, should be considered of paramount importance (Mulligan et al., 2006). The factors responsible for low fertility in buffalo due to reproductive disorders should be better studied to mitigate them through greater understanding of the effects of better seasonal nutrition, improved management and markers for logical breeding programmes (Nanda et al., 2003).

Management involves decision on facts and sets targets for achievement of preset goals. In the face of targets not being achieved, evaluation of the herd status in terms of metabolic profiles, which is an indicator of nutritional adequacy or inadequacy, may provide a better insight of seasonality in buffaloes for better reproductive management and further decisions for improving the herd performance. This study was conceived to get an overall view of the herd reproductive status in two different seasons of calving by taking into account blood metabolite status in the pre- and post-partum period and by monitoring for reproductive success during the post-partum period.

MATERIALS AND METHODS

The present study was conducted on 30 pregnant dry Murrah buffaloes maintained at the Cattle Yard of the National Dairy Research Institute (NDRI), Karnal and divided into two groups each of 15 animals according to expected day of calving in winter (January to March) and summer (April to July) season. The herd was kept under loose housing and a group management system following standard management practices. The nutrient requirements of all the animals were mostly met through limited concentrates (1.5 kg per day for body maintenance) and *ad libitum* green fodder (Table 1). All the experimental buffaloes were monitored regularly for estrus by visual observation and by parading of a vasectomized bull in the morning and evening hours. Animal were confirmed for heat by rectal palpation and inseminated with frozen semen twice at 12 h intervals. Buffaloes not returning to estrus after 21 days of insemination were examined per rectum on the 45th day for pregnancy confirmation.

Blood metabolic profile examination

Blood samples were collected, early in the morning before feeding, from the jugular vein into heparinized (20 IU heparin/ml blood) tubes from all experimental animals at fortnightly intervals from 60 days pre-partum to 60 days post-partum. Immediately after sampling, the blood was centrifuged at 3,000 rpm for 15 to 20 minutes and the plasma was separated and stored under frozen conditions (-20°C) until analyzed. Plasma urea (BUN) was estimated according to the procedure described by Rahmatulla and Boyde (1980). Glucose in plasma was estimated by the end-point o-toluidine method. The copper soap extraction method as modified by Shipe et al. (1980) was adopted for the determination of plasma NEFA. Absorbance was measured at 525 nm for BUN; 620 nm for glucose and 440 nm for NEFA using a Specord 50 analyser.

Table 1. Fodder available for feeding of buffaloes during different months

Fodder	Months	CP % of DM
Berseem	Nov. to April	16.3 to 22.7%
Oats	Jan. to April	10.5 to 11.3%
Mustard	Nov. to Dec.	20 to 21%
Maize	May to Oct.	8.7 to 14%

Post-partum examination of reproductive organs

During the post-partum period, the reproductive organs were palpated trans-rectally on days 7, 14, 21, 28, 35 and 42 for inspection of cervical and uterine involutional changes, and abnormal discharges, if any, as per the score card of Sheldon and Noakes (1998) with slight modifications.

Reproductive performance of buffaloes

Reproductive performance of the buffaloes in two different seasons of calving was evaluated on the basis of PDD, uterine involution, days to first service, service period, risk to first service on days 60, 90 and >90, pregnancy risk to first service and up to days 60 and 90, and number of services per conception. Risk to first service on days 60, 90 and >90 was calculated as *per cent* animals receiving first service by days 60, 90 and >90 out of the total number of experimental animals. Pregnancy risk to first service and up to days 60 and 90 was estimated as *per cent* animals pregnant to first service and up to days 60 and 90 out of the total number of experimental animals.

Data analysis

Effect of season of calving on metabolic status and reproductive performance traits was calculated by t test, using the Systat 6 software package.

RESULTS

Metabolic profile reflects the nutritional and physiological status of the animal. In the present study the metabolic profile of the animals in terms of plasma glucose, BUN and NEFA were evaluated to delineate their effects on reproduction performance in summer and winter season calvers. The metabolic profile of the buffaloes which calved in the summer and winter season is presented in Table 2 for ease of interpretation.

Seasonal effect on metabolic profile of buffaloes

Plasma glucose : The concentration of plasma glucose

Table 2. Effect of calving season on metabolic status of buffaloes (Mean±SE)

Parameters	Season	Pre-partum		Post-partum		
		-60 d	-30 d	0 d	30 d	60 d
Glucose (mg/dl)	Winter	61.03±2.34	54.77±3.15	56.40±2.45	68.27±2.87	65.57±1.62
	Summer	61.64±5.30	58.71±1.65	65.02±3.69	74.91±3.91	66.31±3.22
BUN (mg/dl)	Winter	28.26±1.93**	24.49±1.97*	16.71±1.56*	31.34±2.00**	32.28±1.92**
	Summer	16.13±0.86**	18.39±0.94*	23.56±2.11*	20.27±1.95**	22.57±2.20**
NEFA (μmol/L)	Winter	333.97±9.45	348.99±10.92**	365.44±14.25	381.31±10.15	395.64±9.22*
	Summer	335.89±9.82	310.32±10.92**	357.93±11.98	367.04±11.98	361.04±12.09*

*, ** Signifies significance across the rows at p<0.05 and p<0.01, respectively.

did not differ significantly among the two seasons during both the pre- and post-partum period.

Plasma urea (BUN) : The plasma BUN values were lower in the pre-partum than post-partum period for both seasons. Plasma BUN decreased during the pre-partum period and increased during the post-partum period in winter season calvers whereas it followed an increasing trend except on day 30 post-partum in summer season calvers (Table 2). Also, on the day of calving, summer calvers had an increased value which may be attributed to increased protein supplied by feed and fodder to these buffaloes during the pre-partum period. Plasma urea nitrogen showed a significant difference (p<0.05) at day 30 pre-partum and calving day, and a highly significant difference (p<0.01) at all other stages. However, significantly lower concentrations were evident at all stages in summer season than winter season calvers, except on the day of calving, reflecting better availability of feeds and fodders supplying sufficient nutrients for optimum performance. Plasma urea concentrations were close to the normal range in summer-calved buffaloes but higher in the winter-calved buffaloes during the post-partum period.

Plasma NEFA : Plasma NEFA showed an increasing trend in winter season calvers, whereas it decreased up to day 30 pre-partum, then increased up to 30 d post-partum and slightly decreased thereafter in summer season calvers. The values obtained were statistically different significantly (p<0.05) on day 60 post-partum and highly significantly (p<0.01) on day 30 pre-partum. However, the differences in concentration at all stages showed lower levels in the summer than the winter season, reflecting better availability of fodders supplying sufficient nutrients for optimum performance. During the post-partum period, an increasing trend signifies slight negative energy balance, but the values were much lower than in cattle which may be attributed to mobilization of body reserves to fulfill the needs of lactation.

Post-partum examination of reproductive organs

During the post-partum period, the reproductive organs were palpated trans-rectally on days 7, 14, 21, 28, 35 and 42 for inspection of cervical and uterine involutional changes and any abnormal discharges which are presented in Table 3.

Table 3. Effect of season of calving on involutional changes as per score card

Days	Winter	Summer
7	4.73	6.64
14	3.4	4.18
21	1.47	1.6
28	0.06	0.6
35	0	0.3
42	0	0

Seasonal effect on uterine and cervical involution : There was no significant difference in days to completion of cervical and uterine involution. However, cervical and uterine involution was complete in fewer days (Table 3) in the winter compared to summer season, signifying the role of environment.

In winter season calvers, involutional changes took place at a faster pace than in the summer season (Table 4), and there were more abnormal involutional changes in the summer season calvers (Table 4), which may be attributed to a larger number of RFM cases, possibly due to sanitary and hygiene problems, environmental stress and other unknown causes during this season. The buffaloes with abnormal uterine involutional changes, particularly those having foul smelling discharges or purulent discharge (puerperal metritis) and RFM cases, were treated as soon as detected and were declared free of disease as per the score card after completion of uterine involution.

Table 4. Effect of season on abnormal uterine involutional changes as per score card

Days	Winter (%)	Summer (%)
7	13	33
14	0	13
21	6	0
28	0	20
35	0	13
42	0	0

Reproductive performance of buffaloes

Reproductive performance of the buffaloes in two different seasons of calving, as evaluated on the basis of PDD, uterine involution, days to first service, service period, risk to first service on days 60, 90 and >90, pregnancy risk to first service and up to days 60 and 90, and number of services per conception is shown in Table 5.

Seasonal effect on reproductive performance : Except for PDD, RFM and uterine involution, which could be attributed to seasonal effect in terms of environmental and other unknown causes, the summer season calvers showed better reproductive performance in all other reproductive parameters. Season of calving had a significant effect (p<0.05) on service period and services per conception. The better performance of the summer calvers could be attributed to availability of better nutrition providing sufficient nutrients for optimum function, favourable environmental effects after PPI and other unknown causes. As already discussed, metabolic status substantiates the seasonal effects on reproductive performance observed in the study.

DISCUSSION

Plasma glucose

In consonance, Qureshi et al. (2002) reported higher values in high breeding season as compared to low breeding season calvers. Mandali et al. (2002) and Prajapati et al. (2005) reported slightly lower values of glucose than the present findings. In productive buffaloes and buffalo heifers glucose level was 72.00-74.88 mg/dl and 75.60 mg/dl, respectively (Borghese, 2005). Serum glucose levels in buffaloes were very constant during the dry and lactation period and there was low incidence of metabolic disorders in buffaloes compared to other ruminants in lactation. It was clearly evident that circulating glucose levels depend on nutritional status (Campanile et al., 1997; Montemurro et al., 1997). The destination of glucose is regulated by various hormones such as insulin, cortisol, glucagon, somatotropin and adrenalin. The level of blood glucose depends on the nutritive value of the diet, social or environmental stress, and physiological status of the animal (Borghese, 2005).

Plasma BUN

Similar significant seasonal variations were obtained in blood urea concentration in dairy cows by Dhali et al. (2006) who also observed that milk urea was significantly associated with CP content of the forage rather than the concentrate. Serum urea levels in normal breeding season (NBS) calvers have been found to be lower than those of the low breeding season (LBS) calvers (31.69 vs. 39.42 mg/100 ml), and a positive correlation of serum urea levels with post-partum estrous interval (PEI) (r = 0.28, p<0.01) and post-partum ovulation interval (POI) (r = 0.30, p<0.01) has been reported. Higher BUN in winter calvers during the post-partum period indicates a deficiency in energy as sufficient quantity of leguminous fodder is supplied during this period. Anoestrus buffaloes, when compared with those resuming oestrus and from one month pre-partum to six months post-partum, showed higher serum urea concentration (46.0 mg/100 ml) (Qureshi et al., 2002). In buffaloes, serum urea levels rose from a basal value of 5.48 mmol/L (before calving) and of 5.15 mmol/L (at 45 days of lactation) to a peak of 10.29 mmol/L (at 160 days of lactation) (Montemurro et al., 1997). Besides renal function, other physiological and external factors, viz. days in milk (Campanile et al., 1997; Grasso et al., 2004), diet (Campanile et al., 1997; Dhali et al., 2006) and season (Qureshi et al., 2002; Dhali et al., 2006), influence serum blood urea levels. Urea concentration is an indicator of

Table 5. Effect of season on reproductive parameters

Parameter	Winter	Summer
PDD (h)	4.92±0.56	7.17±1.13
RFM (%)	13.3	20
Uterine involution (days)	27.07±0.93	30.33±1.48
DFS	85.62±11.54	81.07±10.56
Service period (days)	163±22.42*	101.6±11.66*
Service per conception	2.08±0.24*	1.4±0.16*
Risk to first service <60 days (%)	23.07	46.67
Risk to first service <90 days (%)	76.92	60
Risk to first service >90 days (%)	23.07	40
Pregnancy risk to first service (%)	30.77	66.67
Pregnancy risk up to 60 days (%)	0	20
Pregnancy risk up to 90 days (%)	46.15	66.67
Pregnancy risk up to 120 days (%)	46.15	80

* Significant (p<0.05).

PDD = Placental delivery duration; RFM = Retention of foetal membranes; DFS = Days to first service.

energy protein balance (Campanile et al., 1998; Dhali, 2001) and is typically increased in cows deficient in energy. Cows fed a high rumen-degradable-protein diet also lost more body weight and remained in negative nutrient balance during early lactation, were less likely to conceive at first service, and had a prolonged interval from calving to conception (Elrod and Butler, 1993; Westwood et al., 2002). High protein diets fed to pre-partum cows cause high plasma concentrations of urea nitrogen pre-partum and of β-hydroxybutyrate post-partum (Campanile et al., 1998; Doepel et al., 2002), and when fed to post-partum cows cause increased blood urea and a reduction in fertility (Canfield et al., 1990; Roche et al., 2000; Dhali, 2001; Roy et al., 2003). Higher protein supply should be avoided in cows whose protein requirements has already been met, as it triggers a more intense gluconeogenesis as depicted by higher glucose levels. Adequate supply of protein was positively correlated with milk production, but there was loss of body condition score, due to relative energy deficiency in animals with increased β-hydroxybutyrate, if not backed up by an adequate dietary energy level (Campanile et al., 1998). Milk urea concentrations can be used as a potential management tool to monitor the protein and energy feeding efficiency and reproductive performance of the herd (Campanile et al., 1998; Dhali, 2001; Roy et al., 2003).

Plasma NEFA

Slightly lower NEFA concentrations were reported by Mishra et al. (2007) in high- and low-yielder buffaloes. Energy balance of the animal, approaching parturitional stress, and previous nutritional status influence plasma NEFA concentration, and, among these, energy balance is the main determinant. During early lactation, plasma concentrations of NEFA have been found to be high, although lower than observed in cattle, due to utilization of body fat as a source of energy because higher energy requirements during this period are not fulfilled by dietary intake. Fat mobilization begins towards the end of gestation in buffalo (Campanile et al., 1997; Grasso et al., 2004). In post-partum buffalo cows, plasma NEFA concentration was highest at d 20 (0.48 mmol/L), then decreased and returned to the levels of the dry milk period (0.17 mmol/L) at about d 110.

There is greater loss of body condition score during the early lactation period leading to a higher percentage of anoestrous cows in the herd due to deleterious effects on the follicle or the corpus luteum (CL) by decreasing steroidogenesis (Roche et al., 2000). Increase in NEFA concentration during NEB has an inhibitory effect on proliferation of theca and granulosa cells, negatively influencing folliculogenesis during the early post-partum period in high-yielding dairy cows (Vanholder et al., 2006). NEB has been found to affect the ovary directly and ovarian function indirectly through decreases in LH-pulsatility and circulating IGF-1, insulin and glucose concentrations (Beam and Butler, 1999).

A holistic nutritional management approach should be taken into account to support large metabolic adaptations of glucose, fatty acid, and mineral metabolism during the transition and lactation period and thereby avoid metabolic dysfunction. Various workers have evaluated different nutritional strategies and suggested that increasing nutrient supply during the late dry period, rather than during the early dry period, facilitates metabolic adaptation to lactation (Contreras et al., 2004; Overton and Waldron, 2004) and improves reproductive efficiency (Roche et al., 2000).

Post-partum examination of reproductive organs

The cervical and uterine involutional changes and any abnormal discharges observed on days 7, 14, 21, 28, 35 and 42 post-partum are presented in Table 3. Cervical and uterine involution was complete in fewer days in winter compared to the summer season, signifying the role of environment. In winter season calvers, involutional changes took place faster than in the summer season (Table 4), and there were more abnormal involutional changes in the summer season calvers.

Seasonal effect on uterine and cervical involution

Chauhan et al. (1977) also reported less time for uterine involution in winter and spring calvers than in summer and autumn calvers. Qureshi and Ahmad (2008) reported a mean post-partum uterine involution time of 34.30±1.33 days, which corroborates the present findings. Improved nutrition of the late-gestation cow has been postulated to reduce the incidence of some of these disorders (Goff, 2006).

Reproductive performance of buffaloes

Except for PDD, RFM and uterine involution, which could be attributed to seasonal effect in terms of environmental and other unknown causes, summer season calvers showed better reproductive performance in all other reproductive parameters.

Seasonal effect on reproductive performance

Contrary to present findings, Capitan and Takkar (1988) reported better overall reproductive performance of buffaloes calving through Dec to Feb (winter) than of buffaloes calving through May to August (summer), except for service period and first post-partum estrus which corroborated the present findings. Serum urea levels in NBS calvers, have been found to be lower than those of LBS calvers (31.69 vs. 39.42 mg/100 ml,) and a positive correlation of serum urea levels with PEI (r = 0.28, p<0.01) and POI (r = 0.30, p<0.01) has been reported. Furthermore, higher serum urea concentrations have been observed in anoestrus buffaloes (46.0 mg/100 ml) when compared with those resuming oestrus, and the levels remained higher from one month pre-partum to six months post-partum (Qureshi et al., 2002). Cows fed a high rumen-degradable-protein diet also lost more body weight during early lactation, were less likely to conceive at first service and had a prolonged interval from calving to conception (Elrod and Butler, 1993; Westwood et al., 2002). Increased protein intake pre-partum has been shown to cause elevated plasma urea nitrogen concentrations (Campanile et al., 1998; Doepel et al., 2002), while high protein diets fed to post-partum cows caused increased blood urea and reduced fertility (Canfield et al., 1990; Roche et al., 2000; Dhali, 2001). Urea is also believed to aggravate the severity of NEB and its effect on fertility by preventing or delaying the start of cyclicity. Besides, urea has been shown to lower the pH of uterine fluid, giving rise to disturbances in follicular development and embryonic growth (Tamminga, 2006). Qureshi et al. (2002) propounded that low energy intake associated with poor body condition of buffaloes may be a key factor in low reproductive efficiency.

It has been emphasised that the amount of micronutrients needed for optimal immune function may exceed that which will prevent more classical signs of deficiency. In general, mineral deficiencies have been associated with altered metabolic profile leading to most peri-parturient disorders in buffaloes. Thus, such disorders could probably be prevented by addressing the basic etiology through balanced feeding and mineral supplementation during advanced pregnancy and the early post-partum period, when the animals are highly prone to the stress of heavy nutrient demand and drain (Mandali et al., 2002). Besides general nutritional status, deficiency or imbalance with respect to specific nutrients has been found to have a drastic effect on various determinants of reproductive performance. Further, the impact of such disturbances on general health, including insidious sub-clinical diseases/disorders, has been recognized as the most important but covert factor with deleterious consequences for reproductive performance. In this perspective, nutritional management for general and reproductive health has been considered to be of paramount importance (Mulligan et al., 2006). Greater understanding about the effects of better year-round nutrition, improved management and markers for logical breeding programmes are essential to curtail incidence of the reproductive disorders that reduce buffalo fertility (Nanda et al., 2003).

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