

COMPARATIVE STUDIES OF CERTAIN ENZYME ASSAY DURING VARIOUS REPRODUCTIVE STATES IN BUFFALOES

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ABSTRACT

Thirty healthy Murrah buffaloes of 5-9 years of age with second to fifth lactation were randomly chosen from well organized dairies located at Jabalpur to elucidate alteration in concentration of some enzymes viz. serum alkaline phosphatase (ALP), acid phosphatase (ACP), alanine transferase (ALT), and aspartate amino transferase (AST), in serum of normal cyclic, repeat breeder and anoestrus Murrah buffaloes. Gynecological examinations were employed for the diagnosis of reproductive status of animals. The selected 30 Murrah buffaloes were divided into three groups and each group comprised of 10 animals for the generation of experimental data. Blood samples were collected from animals of different groups on the day of estrus from normal cyclic, repeat breeder and anoestrus buffaloes on the same day for gynaeco-clinical examination. The serum was prepared following routine procedure. The serum enzymatic activities was monitored immediately for serum alkaline and acid phosphatase, alanine amino transferase and aspartate amino transferase. The serum alkaline phosphatase and acid phosphatase values were 9.190 and 1.357 ; 19.778 and 2.667; 14.064 and 2.100 KA units /100 ml in anoestrus , repeat breeder and normal cyclic groups, respectively. The serum alkaline phosphatase and acid phosphatases values were

significantly ($P < 0.01$) different among the groups. It was recorded to be highest in repeat breeder followed by normal cyclic and lowest in anoestrus buffaloes. The serum alanine aminotransferase and aspartate aminotransferase values were 26.402 and 69.124; 32.035 and 104.435; 34.007 and 85.093 U/L in anoestrus , repeat breeder and normal cyclic groups, respectively. The values of serum alanine aminotransferase and aspartate aminotransferase of repeat breeder was significantly ($P < 0.01$) higher than normal cyclic and lowest in anoestrus group.

Keywords: Serum alkaline phosphatase, Serum acid phosphatase, Alanine amino transferase and Aspartate amino transferase

INTRODUCTION

In India, estimated populations of buffaloes are 105.3 million among which female buffaloes consists of 54.5 million (NDDDB, 2012). These animals play an important role in Indian livestock economy. The success of dairy cattle and buffalo economy lies in proper and optimal reproductive rhythm of each individual cow and buffalo in the herd, within normal physiological range (Dhaliwal, 2005). Any deviation or prolongation in the breeding rhythm results in a progressive economic loss due to widening of dry period reduced calvings and

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lactations during the life span of the animal (Singh *et al.*, 2006). Barren or infertile buffaloes means a direct loss in milk production, whereas reduced calf crops hamper the selection efficiency in long term dairy herd improvement (Baghel, 2006). About 25 percent dairy animals. However, repeatability and anoestrus conditions are recognized as the serious problem in increasing the calving interval, therefore by subjecting the farmer into heavy economic loss. In general incidence of anestrus has been reported between 9.09-82.50 percent in buffalo (Thakor and Patel, 2013).

Blood profile might be potential in characterizing the problem and diagnosing a deficient condition (Eltohamy *et al.*, 1989; Jain, *et al.*, 2003). The present investigation was designed to study the biochemical changes during different reproductive states (Normal cyclic, repeat breeder and anoestrus) in Murrah buffaloes. The study was aimed to elucidate alteration in concentration of some enzymes in serum of normal cyclic, repeat breeder and anoestrus Murrah buffaloes.

MATERIALS AND METHODS

In the present investigation, healthy Murrah buffaloes (30) were randomly chosen from well organized dairies located at Jabalpur. These animals were in 5-9 years of age group and within second to fifth lactation. These animals were screened as per the approved technical program. Gynecological examinations were employed for the diagnosis of reproductive states of animals. The selected 30 Murrah buffaloes were divided into three groups and each group comprised of 10 animals for the generation of experimental data. Blood samples were collected from animals of different group on the day of estrus from normal

cyclic buffaloes.

Samples from repeat breeder and anoestrus buffaloes were taken on the same day for gynaecological examination. The serum was prepared following routine procedure. Separated serum was centrifuged at 3000 rpm for 10 minutes. Serum was used immediately for monitoring assay of enzyme activities serum alkaline phosphatase (ALP) and acid phosphatase (ACP), alanine amino transferase (ALT) and aspartate amino transferase (AST). Serum alkaline phosphate was measured as per Tietz, (1976). Acid phosphatase was estimated by the calorimetrically method of King and Jagatheesan, (1959), alanine aminotransferase and aspartate aminotransferase as per Henry, (1974). The data were analyzed statistically using analysis of variance technique (ANOVA) and the differences between means were compared using critical difference (Snedecor and Cochran, 1996).

RESULTS AND DISCUSSION

Concentration of serum enzymes in different reproductive states in Murrah buffaloes have been given in Table 1.

1. The serum alkaline phosphatase (ALP) activity was significantly ($P < 0.01$) increased in repeat breeder ($P < 0.01$) followed by that in normal cyclic and lowest in anestrus condition of buffaloes. The trend of higher ALP activity in repeat breeder was in agreement with the findings reported by Sharma *et al.*, (1986). They found higher serum ALP activity in primary infertile Kankrej heifers than normal cyclic heifers. Mehta *et al.* (1989) also recorded similar results on comparison between repeat breeder and normal cyclic cows. Gandotra *et al.* (1993) reported higher level of ALP activity in repeat breeder cattle and

buffaloes as compared to normal cyclic cattle and buffaloes. Chandrakar (1999) found serum activity concentration to be significantly higher in repeat breeder than normal fertile cows. Yaqub *et al.* (2013) reported that Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) concentration non significantly fluctuated during the estrous cycle in Red Sokoto goats.

In this study ALP activity during anoestrus condition was significantly lower than the levels in normal cyclic Murrah buffaloes. It might be due to malnutrition. The result can be correlated with the findings of Kalnath *et al.* (2007) who reported higher Alkaline Phosphatase (ALP) activity during follicular development. However, Derashri *et al.* (1984) reported the ALP levels in anoestrus condition to be higher as compared to the animals in oestrus but the difference was non-significant. In the light of present results it could be hypothesised that decreased level of AKP activity in normal cyclic as compared to Repeat breeders might be due to enhanced folliculogenesis resulting in increased

pace of conception while reverse is true in repeat breeder (Sharma *et al.*, 1986).

2. Serum acid phosphatase (ACP) activity showed a similar trend as ALP which was significantly ($P < 0.01$) highest in repeat breeder followed by reduced in normal cyclic and lowest in anoestrus condition. The result correlates with the findings of Gandotra *et al.* (1993). They recorded significantly higher values of ACP activity in repeat breeder than normal cyclic buffaloes. The result was also similar to Ganguly (2013) who reported that increase in Acid phosphate concentration decreases with increase in follicular size. It can be inferred that higher concentration of acid phosphate concentration increases follicular activity resulting in repeat breeding. On the contrary, Sharma *et al.* (1986) reported that mean values of ACP were significantly ($P < 0.01$) higher in normal cyclic Kankrej heifers. Increased activity of ACP might be helpful in hydrolysing the organic phosphomonoesters and thus provide energy in the form of phosphates in normal cyclic animals.

Table 1. Concentration of serum enzymes in different reproductive states in murrah buffaloes.

Attributes (KA unit/dl)	Normal Cyclic	Repeat Breeder	Anoestrus
Serum alkaline phosphatase (ALP)	14.064 \pm 0.58 ^b	19.778 \pm 0.385 ^c	9.190 \pm 0.48 ^a
Serum acid phosphatase (ACP)	2.100 \pm 0.234 ^b	2.667 \pm 0.187 ^c	1.357 \pm 0.133 ^a
Serum alanine aminotransferase (ALT) (U/L)	34.007 \pm 1.366 ^c	32.035 \pm 1.423 ^b	26.402 \pm 0.654 ^a
Serum aspartate aminotransferase (AST) (U/L)	85.093 \pm 3.070 ^c	104.435 \pm 2.416 ^b	69.124 \pm 2.608 ^a

Note: 1. Mean \pm SE values with different superscripts in row are highly significantly different ($P < 0.01$).

2. The enzyme unit (U) is a unit for the amount of a particular enzyme.

3. The enzyme activity unit (KA) is expressed in the term of KA/100 ml.

The result indicating significantly higher ACP activity in normal cyclic as compared to anoestrus buffaloes was in contrary to the findings of Derashri *et al.* (1984) who observed ACP activity level higher in anoestrus surti buffaloes than the normal oestrus buffaloes. The serum ACP activity level might be influenced by physiological conditions. Its activity levels may be used as an index in assessing the estrogen level in buffaloes.

3. The serum ALT activity level was significantly ($P<0.01$) increased in normal cyclic, followed by repeat breeder. The level of ALT was significantly ($P<0.01$) lower in anoestrus buffaloes as compared to other two groups of buffaloes. The findings of result were in agreement with the observation of Derashri *et al.* (1984). He reported the same trend of ALT activity in oestrus and normal cyclic condition and suggested that possible involvement of hormonal levels prevailing during normal oestrus/normal cyclic helps in regulation of ALT activity levels. Sharma *et al.* (1986) recorded significantly lower activity of ALT in infertile group of heifers than normal group. Pal *et al.* (1991) found level of ALT activity in the cyclic heifers and cows to be comparatively significantly higher than non cycling ones. In the present study the level of ALT was significantly lower in repeat breeder than normal cyclic buffaloes. These findings were again in agreement with Gandotra *et al.* (1993). They recorded that the ALT activity level was higher in normal cyclic cattle and buffaloes than the repeat breeder cattle and buffaloes. Higher level of serum ALT activity might be due to increase in metabolic activity mediated by physiological activity during oestrus cycle condition.

4. The serum AST activity level was significantly ($P<0.01$) increased in repeat breeder compared to normal cyclic and lowest in anestrus group. In the present result AST activity being

significantly lower in normal cyclic as compared to repeat breeders buffaloes, supported the findings of Pal *et al.* (1991). They reported that the AST activity in cyclic heifers and cows were comparatively higher and statistically significant ($P<0.01$) than the non-cyclic heifers. This enzyme activity was indicative of increased physiological activity and pathological condition of the tissue. In the present study AST activity was higher in repeat breeder than normal cyclic buffaloes and differences between two groups were significantly different. This was in agreement with Gandotra *et al.* (1993). They reported that AST activity level in repeat breeder cows and buffaloes were significantly higher than the normal cyclic cows and buffaloes. The possible cause of increased AST activity level may be uterine tissue damage in repeat breeder cows & buffaloes. The present result was in contrary to Sharma *et al.* (1986) who found AST activity to be higher in primary infertile Kankrej heifers as compared to normal cyclic heifers, however, the difference was found to be non significant. Sarwar *et al.* (2002) found that AST and ALT were significantly higher in endometritis in Nili-Ravi buffalo which can be correlated with the result of repeat breeder. However, result is dissimilar to those of findings of Yaqub *et al.* (2013) who reported ALT and AST to non significantly fluctuate during estrus cycle in goat. This might be because goat is a seasonal breeder whereas buffalo is a regular breeder.

Estrus cycle may produce measurable stress during estrus phase resulting in increased physiological activity which increases the AST activity in blood. But in case of repeat breeder higher values might be due to tissue damage.

CONCLUSION

Serum alkaline phosphatase and acid phosphatase activities in anoestrus group were significantly lower than normal cyclic and repeat breeders, Alanine amino transferase and aspartate amino transferase activities in normal cyclic were also significantly higher than anoestrus buffaloes.

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