

## SERUM CONCENTRATIONS OF CALCIUM, PHOSPHORUS AND MAGNESIUM IN PREGNANT NILI-RAVI BUFFALOES WITH OR WITHOUT VAGINAL PROLAPSE IN IRRIGATED AND RAIN FED AREAS OF PUNJAB, PAKISTAN

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### ABSTRACT

The present study was planned to determine the macro-mineral status (calcium, phosphorus and magnesium) in riverine buffaloes suffering with prepartum vaginal prolapse (n=100) in comparison with control (n=100) buffaloes kept in irrigated and rain fed (Barani) agro-ecological zones of Punjab, Pakistan. Serum macro-mineral status was determined by spectrophotometer using commercially available kits. Mean serum calcium and phosphorus levels were lower in buffaloes suffering with prepartum vaginal prolapse compared with their healthy counterparts ( $P<0.01$ ), while reverse was true for serum magnesium concentration. Mean serum calcium was significantly higher ( $P<0.01$ ) and phosphorus was non-significantly lower in control and vaginal prolapse affected buffaloes kept in irrigated zone compared to their counterparts in rain fed zone. However, magnesium concentration was non-significantly higher in control buffaloes and significantly lower in vaginal prolapse affected buffaloes belonging to irrigated zone compared to their counterparts in rain fed zone. It may be inferred that deficiency of calcium and phosphorus and higher levels of magnesium may possibly be the contributing factors in causing prepartum vaginal prolapse in buffaloes. The levels of calcium, phosphorus and magnesium were, at least partially, zone dependent.

**Key words:** Buffaloes, vaginal prolapse, calcium, phosphorus, magnesium, serum.

### INTRODUCTION

Buffalo is contributing significantly towards the rural economy of Pakistan. It is reported to have low reproductive performance manifested by delayed age at first calving (Lundstorm *et al.*, 1982), late postpartum conception (Arora, 1987) and long calving intervals (Jainudeen *et al.*, 1983). Among prepartum reproductive disorders, vaginal prolapse is considered to be the major problem causing heavy economic losses to the farmers (Khan *et al.*, 1984). Samad *et al.* (1987) reported the incidence of prepartum vaginal prolapse as 42.9% in buffaloes presented for treatment of reproductive problems at the clinics of Department of Theriogenology, University of Agriculture, Faisalabad. Various etiological factors considered to be contributing to this condition include elevated steroid concentration, deficiency of serum minerals and faulty management conditions (Seitaridis and Papadopoulos, 1978; Nanda, 1979). Most of the studies reported earlier aimed at the stall fed animals and very little attention has been paid to animals maintained under field conditions.

Blood mineral contents in buffaloes and cows have been reported to differ from area to area (Bedi and Khan, 1984). Similarly, the mineral contents of plants have been reported to vary with variation in chemical

composition of soil which is largely affected by the climatic conditions, especially temperature, rain fall and drainage of water (McDowell and Arthington, 2005). It was hypothesized that the deficiency or excess of some macro minerals in the blood contribute to the occurrence of prepartum vaginal prolapse in buffaloes and this excess or deficiency is area dependent. Therefore, the present project was planned to investigate serum macro mineral contents in pregnant buffaloes suffering with prepartum vaginal prolapse in comparison with their pregnant healthy counterparts kept under irrigated and rain fed agro-ecological zones of Punjab, Pakistan.

### MATERIALS AND METHODS

#### Experimental area

Pakistan is predominantly an agricultural country with a semi-arid continental subtropical climate. It is located between latitude 23° and 36°N and longitude 60° and 75°E. The present study was conducted on Nili-Ravi buffaloes in district Bahawalpur (Irrigated zone) and Muzaffargarh (Rain fed zone). The district Bahawalpur is located between latitude 29° and 59°N, longitude 73° and 19°E, while the district Muzaffargarh is located between latitude 30° and 20°N, longitude 71° and 5°E.

### Experimental animals

A total of 100 buffaloes were included from each experimental zone, out of which 50 were suffering with prepartum vaginal prolapse (affected group) during their third trimester of pregnancy, while the remaining 50 were normal pregnant buffaloes (control group) in their third trimester of pregnancy. These animals were raised and maintained under the existing field management conditions. Blood samples were collected from door to door visit of the farmers.

### Collection of serum samples

Blood samples were collected during the months from May to September, 2006. About 20 ml of blood from each buffalo was collected in a clean sterilized glass test tube through jugular venipuncture using a sterile 16 gauge needle. Test tubes containing blood were placed in slanting position for one hour to let the serum ooze out, while those tubes in which serum did not ooze out were centrifuged at 3000 rpm for 10 minutes. The serum was aspirated carefully with a disposable glass pipette, placed in glass vials and stored at  $-20^{\circ}\text{C}$  until analyzed.

### Determination of macro-mineral contents

Serum calcium, phosphorus and magnesium concentrations were determined by using spectrophotometric method. Commercially available kits, for calcium (Wiener Lab.- Argentina, Lot. 606634), phosphorus (Dia Sys GmbH-Germany, Lot. 600074439) and magnesium (Centronic GmbH-Germany, Lot. 0512010) were used.

### Statistical analysis

The mean ( $\pm$  SE) values of calcium, phosphorus and magnesium of different experimental groups of buffaloes in the two experimental areas were calculated. The mean values were subjected to two factor completely randomized design (Steel *et al.*, 2006). The values were considered significant at  $P<0.01$ .

## RESULTS

Mean serum values ( $\pm$  SE) for calcium, phosphorus and magnesium in control and vaginal prolapse affected buffaloes of both irrigated and rain fed zones are presented in Table 1. In irrigated zone, mean serum calcium concentration in buffaloes suffering with vaginal prolapse was  $6.75 \pm 0.13$  mg/dl, while in healthy pregnant counterparts it was  $9.12 \pm 0.16$  mg/dl. Similarly, in rain fed zone, the mean calcium concentration in buffaloes suffering with vaginal prolapse was  $6.31 \pm 0.13$  mg/dl versus  $8.49 \pm 0.14$  mg/dl in healthy pregnant buffaloes. In both zones,

mean serum calcium concentration was significantly lower ( $P<0.01$ ) in buffaloes suffering with vaginal prolapse compared with that of healthy pregnant buffaloes. When the mean calcium concentrations of affected and healthy buffaloes between the two zones were compared, it was found that mean calcium concentration in affected and healthy buffaloes of irrigated zone was significantly higher ( $P<0.01$ ) compared with that of the affected and healthy buffaloes of rain fed zone.

In irrigated zone, mean ( $\pm$  SE) phosphorus concentration in the serum of buffaloes suffering with vaginal prolapse was  $3.02 \pm 0.09$  mg/dl, versus  $5.95 \pm 0.10$  mg/dl in healthy pregnant counterparts. Similarly, in rain fed zone, the mean ( $\pm$  SE) phosphorus concentration in the serum of buffaloes suffering with vaginal prolapse was  $3.07 \pm 0.09$  mg/dl and in healthy pregnant buffaloes it was  $6.20 \pm 0.11$  mg/dl. Serum phosphorus concentration differed significantly ( $P<0.01$ ) in affected buffaloes in comparison with healthy pregnant buffaloes in irrigated and rain fed zones. Comparison of serum phosphorus concentrations in affected and healthy buffaloes of two zones revealed that there was numerically lower mean serum phosphorus concentration in affected and healthy buffaloes of irrigated zone compared with that of the affected and healthy buffaloes of rain fed zone, the difference was, however, non-significant.

In irrigated zone, mean magnesium concentration in the serum of buffaloes suffering with vaginal prolapse was  $2.35 \pm 0.02$  mg/dl, while in healthy pregnant counterparts it was  $2.17 \pm 0.01$  mg/dl. In rain fed zone, the mean ( $\pm$  SE) magnesium concentration in buffaloes suffering with vaginal prolapse was  $2.44 \pm 0.01$  mg/dl versus  $2.13 \pm 0.01$  mg/dl in healthy pregnant buffaloes. In both the zones, magnesium concentration in affected buffaloes was significantly higher ( $P<0.01$ ) compared with the healthy pregnant buffaloes. When the mean magnesium concentrations of affected and healthy buffaloes between the two zones were compared, it was recorded that there was significantly lower ( $P<0.01$ ) mean serum magnesium concentration in affected buffaloes of irrigated zone compared with that of the affected buffaloes of rain fed zone, while healthy pregnant buffaloes of irrigated zone had numerically higher magnesium concentration compared with that of the healthy buffaloes of rain fed zone, the difference was, however, non-significant. It was revealed that serum calcium, phosphorus and magnesium levels in the serum of healthy buffaloes in the two agro-ecological zones were different, however reached significance in case of calcium but not for phosphorus and magnesium.

**Table 1: Mean values ( $\pm$  SE) of macro-minerals (mg/dl) in healthy and vaginal prolapse affected buffaloes raised under different agro-ecological zones**

Parameter	Irrigated zone		Rain fed zone	
	Prolapsed group <sup>a</sup> ◇	Control group <sup>a</sup> ◆	Prolapsed group <sup>a</sup> ◇	Control group <sup>a</sup> ◆
Calcium	6.75 $\pm$ 0.13 <sup>a</sup>	9.12 $\pm$ 0.16 <sup>b</sup>	6.31 $\pm$ 0.13 <sup>c</sup>	8.49 $\pm$ 0.14 <sup>b</sup>
Phosphorus	3.02 $\pm$ 0.09 <sup>a</sup>	5.95 $\pm$ 0.10 <sup>b</sup>	3.07 $\pm$ 0.09 <sup>a</sup>	6.20 $\pm$ 0.11 <sup>b</sup>
Magnesium	2.35 $\pm$ 0.02 <sup>b</sup>	2.17 $\pm$ 0.01 <sup>c</sup>	2.44 $\pm$ 0.01 <sup>a</sup>	2.13 $\pm$ 0.01 <sup>c</sup>

Values sharing different letters in a row differed significantly ( $P < 0.01$ ). Groups sharing similar symbols were compared. Each value is based on 50 samples ( $n=50$ ).

## DISCUSSION

In the present study, the mean serum calcium concentration in pregnant buffaloes suffering with vaginal prolapse was significantly lower compared to healthy pregnant buffaloes in both the agro-ecological zones. Similarly, Mandali *et al.* (2002) and Ahmed *et al.* (2005) reported lower calcium concentrations in buffaloes suffering from vaginal prolapse.

The mean serum calcium concentration recorded in control buffaloes was higher in both the agro-ecological zones than those reported by Jagatheesan *et al.* (2005), while it was lower than those reported by Mandal *et al.* (1996) and Das *et al.* (2002) for buffaloes. These differences may be due to difference in geographical areas because the blood mineral contents in buffaloes and cows have been reported to differ from area to area (Bedi and Khan, 1984).

In the present study, the mean phosphorus concentration in the serum of buffaloes suffering with vaginal prolapse was significantly lower compared to healthy pregnant buffaloes. These results corroborate with those reported by Nanda and Sharma (1982), Mandali *et al.* (2002) and Ahmed *et al.* (2005). However, Khan *et al.* (1984) and Singh (1998) reported non-significantly lower phosphorus concentration in buffaloes suffering with vaginal prolapse compared with that of healthy buffaloes.

The mean serum phosphorus concentration in control buffaloes was similar to that reported by Mandal *et al.* (1996), while higher values were reported by Das *et al.* (2002) and Kumar *et al.* (2001). In the present study, the mean serum phosphorus concentration in buffaloes of control group was lower than that reported by Pathak and Janakiraman (1987) and Jagatheesan *et al.* (2005). This may be due to different areas in which research was conducted as blood mineral contents in animals vary from area to area.

The mean serum magnesium concentration in buffaloes suffering with vaginal prolapse was significantly higher compared with healthy pregnant buffaloes. Similar results have been reported by Pandit *et al.* (1982), while non-significant differences in magnesium concentration between prolapse affected and healthy buffaloes were reported by Khan *et al.* (1984) and Vicenti *et al.* (1992) for cows. Contrary to

our findings, Ahmed *et al.* (2005) reported significantly lower serum magnesium concentration in buffaloes suffering with vaginal prolapse. The reason is still unclear. The mean serum magnesium concentration in control buffaloes was higher than that reported by Kumar *et al.* (2001), while lower values were reported by Pathak and Janakiraman (1987). Among micro minerals, serum copper and zinc were lower in prolapsed buffaloes compared to healthy animals (Bhatti *et al.*, 2006).

Calcium is necessary for neuromuscular excitability, cell membrane permeability, muscle contraction and nerve impulse transmission and its deficiency can lead to reduced vaginal and uterine muscle tone which predisposes the animals to vaginal prolapse (Roberts, 1986). A negative correlation between the serum calcium, phosphorus and estrogen levels has been documented (Jacono and Robertson, 1987). The increased level of estrogen during earlier part of third trimester of pregnancy may result in greater relaxation of pelvic structure and the situation is further accentuated by decreased levels of calcium, resulting in atony of the reproductive tract, thereby disposing the animal to vaginal prolapse, it is possible that a combination of these two factors may initiate the vaginal prolapse.

The serum levels of calcium, phosphorus and magnesium were not only different in affected buffaloes of both the zones but were also different in the healthy animals of the two zones under study, indicating the zone effect. It is possible that fodder grown in rain fed zones may have lower mineral contents, at least for some minerals, due to leaching of soil compared with fodders grown in irrigated zones and this deficiency is reflected in the serum mineral contents of animals raised in these areas. It has earlier been reported that mineral contents of plants vary with the soil chemical composition and is affected by the climatic influences like temperature, rain fall and water drainage in a given area (McDowell and Arthington, 2005). A study to develop a correlation between the soil, fodder and serum mineral contents may be more revealing.

Based on the information obtained from this study, it appears that deficiency of calcium, phosphorus and higher level of magnesium during pregnancy might be

the possible causes that predispose buffaloes to parturition vaginal prolapse.

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