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# Studies on serum macro and micro minerals status in repeat breeder and normal cyclic Nili-Ravi buffaloes and their treatment strategies

**Muhammad Saleem Akhtar[1]*, Abdul AsimFarooq[1], Laeeq AkbarLodhi[2], SayyedAun Muhammad[3], M. MazharAyaz[1], Mushtaq Hussain Lashari[1], Saeed Murtaza[1], Irtaza Hussain[1], Muhammad Irshad[1], Maqbool Hussain[1] and Muhammad AsifRaza[4]**

[1]Faculty of Veterinary Sciences, BahauddinZakariya University, Multan, Pakistan.
[2]University of Agriculture, Faisalabad, Pakistan.
[3]College of Veterinary and Animal Sciences, Jhang, Pakistan.
[4]Animal Husbandry in the Tropics and Subtropics, University of Kassel-Witzenhausen, Germany.

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**The present study was carried out with the objective to know the calcium (Ca), inorganic phosphorus (P), magnesium (Mg), copper (Cu), iron (Fe) and zinc (Zn) concentrations in serum of repeat breeder and normal cyclic buffaloes during oestrus. On the day of estrus, blood samples were collected from 35 buffaloes with a history of repeat breeding (RB) and 35 normal cycling (NC) buffaloes for mineral estimation. In the second part of the study, 35 repeat breeder (RBS) buffaloes were treated with a mineral mixture given orally for 10 days at the dosage rate of 150 g per day whereas other 35 repeat breeder buffaloes were given no mineral mixture (RBC). The overall pregnancy rate as well as 1st, 2nd and 3rd service pregnancy rate was calculated. The serum calcium, inorganic phosphorus, magnesium, copper, iron and zinc concentrations were significantly lower (P<0.01) in RB buffaloes as compared to NC buffaloes. Sodium concentrations differed non-significantly between repeat breeder and normal cyclic buffaloes. Repeat breeder buffaloes (RBS) when fed orally 150 g per day of the mineral mixture for 10 days, the 1st, 2nd and 3rd service pregnancy rates were 42, 25 and 20%, while, overall pregnancy rate in these animals was 87%; whereas in repeat breeder control buffaloes, the overall pregnancy rate was 21%. In conclusion, the concentrations of macro and micro minerals were significantly lower in repeat breeder buffaloes and mineral mixtures should be added in the food stuff to improve reproductive efficiency of repeat breeder buffaloes.**

**Key words:**Buffalo, repeat breeder, minerals, pregnancy rate.

## INTRODUCTION

Livestock is an important sector of agriculture in Pakistan contributing about 55.1% of the agricultural value added as well as 11.5% to gross domestic product. Repeat breeding is one of the most important reproductive problem in buffalo which anguish fertility and results to massive economic losses to buffalo farmers. Typical repeat breeding is defined as the animal that did not conceive after three or more consecutive inseminations, despite it comes normally in heat and shows clear estrus signs with no clinical detectable reproductive disorders

*Corresponding author. E-mail: drsaleem46@hotmail.com. Tel: 0092314-7890797.

(Yusuf et al., 2010).

Augmented competence for milk production has been related through a decreased fertility in lactating dairy cows (Butler, 2000) by alterations in reproductive physiology (Wiltbank et al., 2006), causing more number of services per conception (Lucy, 2001). Consequently, the incidence of repeat breeding has increased (Dochi et al., 2008). The incidence varies from 15 to 32% and is higher in adult buffaloes up to the third parity (Noakes et al., 2009). The different risk factors associated with repeat breeding includes abnormal recommencement of postpartum ovarian cycles, lower parity and shorter days in milk at first artificial insemination (AI) (Yusuf et al., 2010).

Normal levels of several minerals may affect the reproductive performance of ruminants. Differences in the level of trace minerals are related with different reproductive disorders (Jain, 1993). Excess or deficiency of minerals can cause repeat breeding in cattle (Das et al., 2002). The blood picture may have potential in describing the problematic situation and diagnosing deficiency conditions.

At present, there is scanty evidence on blood mineral levels during the oestrus in repeat breeding Nili-Ravi buffaloes. We speculated that variations in levels of different minerals in blood may contribute on the occurrence of repeat breeding in buffaloes. The current study was carried out with the objective to know the calcium (Ca), inorganic phosphorus (P), magnesium (Mg), copper (Cu), iron (Fe) and zinc (Zn) concentrations in serum of repeat breeder and normal cyclic Nili-Ravi buffaloes during oestrus and the application of some field treatment trials.

## MATERIALS AND METHODS

### Animals

The current study was completed on Nili-Ravi buffaloes selected from private farms located around Multan district during the period from September 2010 to March 2011. A total of 35 buffaloes with a history of repeat breeding (RB) and 35 normal cycling (NC) buffaloes were utilized. The ages of experimental animals were between five to nine years with body weight from 400 to 550 kg. The animals were raised under stall-fed conditions, fed sufficient green forages and given *ad libitum* water. Before the start of experiment, palpation of the reproductive tract per rectum was conducted and buffaloes that had been bred three or more times without conception and free from any abnormalities in their ovaries and uteri on palpation were diagnosed as repeat breeder.

### Blood sampling for mineral estimation

Blood samples were collected from repeat breeder (RB) and normal cyclic (NC) buffaloes on the day of estrus. The serum was stored at -20°C till analyses. The spectrophotometric method was adopted for the determination of calcium, magnesium, phosphorus and sodium. The micro minerals (Cu, Fe and Zn) were measured with the help of atomic absorption spectrophotometer (AA-5).

### Treatment, insemination and pregnancy diagnosis

In the second part of the study, 35 repeat breeder (RBS) buffaloes were treated with a mineral mixture given orally for 10 before breeding days at the dosage rate of 150 g per day whereas other 35 repeat breeder buffaloes were given no treatment and served as repeat breeder control (RBC). Each 1000 g of mineral mixture contained calcium (155 g), phosphorus (135 g), magnesium (55 g), sodium (45 g), iron (1000 mg), zinc (3000 mg), manganese (2000 mg), copper (600 mg), cobalt (10 mg), iodine (40 mg) and selenium (3 mg) (L.S. Minerals, Nawan Laboratories Pvt Ltd., Karachi-Pakistan).

Estrus detection of RBS and RBC buffaloes was done two times everyday (morning and evening) through visual observation by means of a vasectomized teaser buffalo bull. Buffalo which stood to be mounted by teaser bull was considered to be in estrus. The artificial insemination (AI) was done almost 12 h subsequently to the beginning of estrus with frozen thawed semen in 0.50 ml straw having 30 million spermatozoa. Animals which returned to estrus were again inseminated at the following estrus. Pregnancy diagnosis was done after 60 days of insemination in all those buffaloes that do not come back to estrus. The overall pregnancy rate as well as $1^{st}$, $2^{nd}$ and $3^{rd}$ service pregnancy rate was calculated for RBS and RBC buffaloes.

### Statistical analysis

The mean (± SE) for different tmacro (calcium, phosphorus, magnesium, sodium) and micro (copper, iron and zinc) minerals of two experimental groups of buffaloes were computed. Completely randomized design (Steel et al., 2006) was applied with $P<0.01$ level of significance.

## RESULTS

The serum means (± SE) for calcium, inorganic phosphorus, magnesium, sodium, copper, iron and zinc in repeat breeder and normal cyclic buffaloes are presented in Table 1.

The serum calcium, concentrations were significantly lower ($P<0.01$) in repeat breeder (RB) buffaloes as compared to normal cyclic (NC) buffaloes. Similarly, mean serum inorganic phosphorus and magnesium concentrations were also significantly lower in RB buffaloes as compared with NC buffaloes. Sodium concentrations differed non-significantly between repeat breeder and normal cyclic buffaloes. Among micro-minerals, the concentrations of copper, iron and zinc were significantly lower ($P<0.01$) in RB buffaloes when compared with NC buffaloes.

Thirty five (35) repeat breeder buffaloes were given orally as 150 g per day of the mineral mixture for 10 days and then at estrus, these animals were artificially inseminated with semen of a fertile bull. In repeat breeder (RBS) buffaloes treated with mineral mixture, the first service pregnancy rate was 42% while the second and third service pregnancy rates were 25 and 20%, whereas in repeat breeder control (RBC) buffaloes, the first service pregnancy rate was 6% while the second and third service pregnancy rates were 6 and 9%. The overall

**Table 1.** Mean (±SE) values for different minerals in the serum of repeat breeder and normal cyclic buffaloes.

| Group | Calcium (mg/dl) | Inorganic phosphorus (mg/dl) | Magnesium (mg/dl) | Sodium (mmol/l) | Copper (µg/dl) | Iron (µg/dl) | Zinc (µg/dl) |
|---|---|---|---|---|---|---|---|
| Repeat breeder (n=35) | $7.46^{a}$±0.29 | $3.71^{a}$±0.19 | $2.13^{a}$±0.03 | 138.2±1.22 | $64.3^{a}$±1.09 | $353.81^{a}$±1.96 | $139.82^{a}$±1.67 |
| Normal cyclic (n=35) | $9.22^{b}$±0.33 | $5.56^{b}$±0.11 | $2.18^{b}$±0.04 | 139.9±1.69 | $72.5^{b}$±1.35 | $367.49^{b}$±1.31 | $172.19^{b}$±1.46 |

Values sharing different superscripts in the same column differed significantly (P<0.01).

pregnancy rate in repeat breeder buffaloes treated with mineral mixture was 87% and it was 21% in repeat breeder control buffaloes.

## DISCUSSION

Repeat breeding is among reproductive disorders which hinder favourable productivity in buffaloes (Sah and Nakao, 2006). In the present study, mean calcium concentrations were significantly lower (P<0.01) in RB buffaloes than NC buffaloes. These findings are in agreement with the results of many other workers (Chaurasia et al., 2010; Singh and Pant 1998; Chandrakar 1999). El-Shahata and Maatyb (2010), also reported that calcium plays a key part in improving the number and size of ovarian preovulatory follicles, and the ovulation rate.

In comparison with NC buffaloes, the concentrations of serum inorganic phosphorus were significantly lower (P<0.01) in RB buffaloes. Lower inorganic phosphorus concentration in repeat breeder buffalo have also been reported in many other studies (Chaurasia et al., 2010). Burleet al. (1995) pointed out significantly lower inorganic phosphorus concentrations in repeat breeder crossbred cows.

In this study, repeat breeder Nili-Ravi buffaloes had significantly lower (P<0.01) serum magnesium concentrations when compared with normal cyclic buffaloes. Magnesium deficiency in repeat breeder buffalo have also been reported earlier (Das et al., 2009). Though magnesium do not play any unwavering function in reproduction, yet it is involved in numerous enzymatic reactions catalysed by ATP linked enzymes. Moreover, magnesium affects the absorption of calcium and phosphorus (Sharma et al., 2004). Thus, magnesium imbalance can affect reproduction secondarily. The results of the present study specified that the lower calcium, inorganic phosphorus and magnesium concentrations might be associated to the ovulatory disturbances in repeat breeder buffaloes.

Serum copper, iron and zinc concentrations were significantly (P< 0.01) lower in repeat breeder buffalos compared to normal cyclic animals. Ahmad et al. (2010) also reported lower micro-minerals (Cu, Fe and Zn) in repeat breeder buffaloes. The lower concentrations of copper, iron and zinc found in this study coincide with the findings of other scientists (Das et al., 2009; Ceylan et al., 2008) working on repeat breeders buffalo cows.

The lower levels of serum iron (Fe) have been associated with altered usual gonadal activity by decreasing follicular growth and fertility (Ceylan et al., 2008). The lower concentrations of micro minerals in the present study might be associated to the ovulatory disturbances in repeat breeder buffaloes.

Repeat breeder buffaloes (RBS) of the present study when fed orally 150 g per day of the mineral mixture for 10 days, the 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ service pregnancy rates were 42, 25 and 20%, respectively while the overall pregnancy rate in these animals was 87%. Improvements in pregnancy rates after mineral mixture supplementation have been reported in Egyptian buffalo (Ahmed et al. 2010). The increase in pregnancy rate in our findings is also in accordance with those of Sah and Nakao (2006) who reported that 64.6% of repeat breeder buffaloes came to estrus and 58.4% conceived within one month after supplementation with vitamin/mineral mixture for three weeks.

In conclusion, this study demonstrates that the concentrations of macro (Ca, P and Mg) and micro (Cu, Fe and Zn) minerals were significantly lower in repeat breeder buffaloes which are perhaps essential for the normal ovulatory processes. Also, mineral mixtures should be added in the food stuff to improve reproductive efficiency of repeat breeder buffaloes.

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