PLASMA MINERAL STATUS OF ANOESTRUS DAIRY ANIMALS IN CENTRAL ZONE OF PUNJAB AND EFFECT OF AREA SPECIFIC MINERAL MIXTURE FEEDING ON REPRODUCTIVE PERFORMANCE OF ANOESTRUS ANIMALS

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ABSTRACT

The aim of the present study was to compare plasma mineral status of anoestrus and normally cyclic cattle and buffaloes of central zone of Punjab and to evaluate the use of area specific mineral mixture as a treatment of anoestrus in dairy animals. Thirty two non-cyclic animals (16 cattle, 16 buffaloes) and 32 cyclic animals (16 cattle, 16 buffaloes) in the 2nd to 3rd lactation were selected for the study on basis of rectal examination and plasma progesterone levels at 0, 7 and 14 days by Radio Immuno Assay (RIA) technique. Blood samples from these animals were analyzed for various minerals *viz*., Ca, Mg, P, Cu, Zn, Fe and Mn. Anoestrus dairy animals had significantly lower levels of calcium, phosphorus and copper as compared to cyclic animals. For therapeutic trial, anoestrus animals (10 cattle and 10 buffaloes) kept as treatment group were fed area specific mineral mixture (50 gm/animal/day) for 2 months. In total, 17 animals out of 20 (85%) regained cyclicity i.e. had at least one value of plasma progesterone level above 0.5 ng/ml out of 3 weekly samples collected. Average time taken for animals to come into heat after area specific mineral mixture supplementation was found to be 53.4±2.7 and 48.9±2.1 days in buffaloes and cattle, respectively

Keywords: *Bubalus bubalis*, buffaloes, anoestrus, area specific mineral mixture, cattle, progestrone.

INTRODUCTION

Anoestrus is a major infertility problem of dairy animals and affects the profitability of dairy business in India. Mineral levels have been cited as a major limiting factor for livestock production (Judson and McFarlane, 1998). Excess or deficiency of minerals produces a detrimental effect on the reproductive performance of livestock (Akhtar *et al*., 2009). Workers have reported a difference in mineral levels of non-cyclic (anoestrus) and cyclic cows and an improvement in their condition after area specific mineral mixture supplementation (Mohapatra *et al*., 2012). The present study was conducted to compare plasma mineral status of anoestrus and normally cyclic cattle and buffaloes of central zone of Punjab and to evaluate the use of area specifc mineral mixture as a treatment of anoestrus in dairy animals.

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MATERIALS AND METHODS

Thirty two non-cyclic animals (16 cattle, 16 buffaloes) and thirty two cyclic animals (16 cattle, 16 buffaloes) were selected from 14 villages of central Zone of Punjab (Ludhiana, Nawanshahr and Fatehgarh Sahib). All the animals selected were in the 2nd to 3rd lactation. Selection of animals was based on basis of owner's history, rectal examination and plasma progesterone levels at 0, 7 and 14 days by Radio Immuno Assay (RIA) technique. Animals having plasma progesterone levels below 0.5 ng/ml were diagnosed as being non-cyclic, anoestrus animals and animals having plasma progesterone levels more than 0.5 ng/ml for at least one sampling were considered to be cyclic.

For mineral estimation, 2 ml of each of the plasma sample was digested with 10 mL of double distilled nitric acid over a hot plate and heated below 80ºC till digestion, followed by one cycle of hydrogen peroxide AR (2.0 ml of 30%), until volume reduced to 1 to 2 ml. The digested samples were diluted with double glass distilled water and the volume of the digestate was made 10 ml. Samples were processed and levels of Ca, Mg, Cu, Zn, Fe and Mn were estimated as given by Sharma *et al.* (2003). Plasma inorganic Phosphorus (Pi) was determined using method given by Tausky and Shorr (1953).

For therapeutic trial, anoestrus animals were divided into 2 groups; with 10 cattle and 10 buffaloes kept as treatment group and 6 cattle and 6 buffaloes kept as control group. Animals kept as treatment group were fed area specific mineral mixture (50 gm/animal/day) for 2 months. Sampling of blood was done after treatment at 0, 7 and 14 days at the end of trial period; and progesterone levels were estimated by RIA and animals were classified as anoestrus or cyclic as mentioned above. Management and feeding practices were not changed during the trial period.

Mean mineral levels of anoestrus and cyclic animals were compared by t-test using SPSS for Windows (version 16.0; Microsoft). Fischer's exact test was used to compare the difference between treatment and control groups after feeding trial.

RESULTS AND DISCUSSION

Mean plasma mineral levels in both cyclic and anoestrus animals were above the critical limits as given by Radostits *et al.* (2007), except for Pi in anoestrus cattle and Cu in anoestrus cattle and buffaloes which were lower than the critical limits. Plasma Ca and Pi levels were found to be significantly lower ($P<0.01$) in anoestrus animals than cyclic animals (Table 2 and 3). Similarly, Dutta *et al.* (1988); Sharma *et al.* (1999) reported lower plasma Ca and Pi, respectively in anoestrus buffaloes as compared to cyclic buffaloes. Singh and Singh (2005) reported lower plasma Ca and Pi levels in anoestrus cows as compared to cyclic cows. The Ca level in the serum might be one of the reasons of lower fertility status of the animals as Ca dependant mechanisms are involved in steroid biosynthesis in testes, adrenal gland and ovaries (Bodesta *et al*., 1980). Phosphorus is essential for transfer and utilization of energy, phospholipid metabolism and large number co-enzyme activation (Hurley and Doane, 1989) and its deficiency may be cause of anoestrus in dairy animals.

Plasma Cu levels were found to be significantly lower ($P<0.05$) in anoestrus animals than cyclic animals (Table 2 and 3). Similarly, Eltohamy *et al.* (1989); Tabrizi (2012) reported lower plasma Cu levels in anoestrus buffaloes

and cows, respectively as compared to normal, cyclic animals. Copper is cited as a component of enzyme systems and a catalyst involved in steroidogenesis and prostaglandin synthesis (Smith and Akinbamijo, 2000).

Out of the 10 buffaloes that were fed area specific mineral mixture, 9 regained cyclicity i.e. had at least one value of plasma progesterone level above 0.5 ng/ml out of 3 weekly samples collected. In control group, 2 out of 6 buffaloes regained cyclicity without any feeding of area specific mineral mixture. Overall the comparison between treatments on the basis of Fisher's exact test was found to be significant ($P<0.05$).Out of 10 cattle, 8 regained cyclicity after supplementation whereas 2 remained non-cyclic. One out of 6 control cattle became cyclic during the period of trial. Overall the comparison between treatments on the basis of Fisher's exact test was found to be significant ($P<0.05$).

In total, 17 animals out of 20 (85%) were cured of anoestrus in the treatment group, whereas 3 animals out of 12 (25%) in control group regained cyclicity. Average time taken for animals to come into heat after area specific mineral mixture supplementation was found to be 53.4±2.7 and 48.9±2.1 days in buffaloes and cattle, respectively. Similarly, Newar *et al.* (2000); Srivastava (2008) observed return to cyclicity in 80% and 93.33% in anoestrus buffaloes and cows, respectively. However, Mohapatra *et al.* (2012) reported return to cyclicity in 57.5% of anoestrus animals after area specific mineral mixture supplementation, which was less than the observations of the present study.

In this study, it was concluded that anoestrus dairy animals had significantly lower levels of certain minerals such as calcium, phosphorus and copper as compared to cyclic animals and a significant improvement in reproductive performance was observed in anoestrus animals after area specific mineral mixture supplementation for two months.

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Table 1. Composition of area specific mineral mixture (per 100 Kg).

Source	Weight (in Kg)
Dicalcium phosphate	66.66
Limestone	17.15
Magnesium oxide	6.66
Potassium iodate	0.067
Copper sulphate	0.549
Cobalt sulphate	0.0363
Manganese sulphate	0.462
Zinc oxide	1.245
Sodium sulphate	7.171

Table 2. Plasma mineral status in buffaloes (Mean ± SE).

Group	Ca (mmol/l)	Mg (mmol/l)	Pi (mmol/l)	Fe (µmol/l)	Cu (µmol/l)	Zn (µmol/l)	Mn (µmol/l)
Anoestrus (N=16)	2.09±0.17**	1.06±0.08	1.47±0.09**	131.43±4.61	8.96±0.35*	16.11±0.39	1.17±0.05
Cyclic (N= 16)	2.45±0.06**	1.09±0.08	1.71±0.10**	131.86±5.12	11.11±0.39*	17.2±0.47	1.21±0.07

Figures in a column having '*' as superscripts are significantly different (P<0.05)

Figures in a column having '**' as superscript are significantly different (P<0.01)

Table 3. Plasma mineral status in cattle (Mean ± SE).

Group	Ca (mmol/l)	Mg (mmol/l)	Pi (mmol/l)	Fe (µmol/l)	Cu (µmol/l)	Zn (µmol/l)	Mn (µmol/l)
Anoestrus (N=16)	2.05±0.13**	1.02±0.07	1.43±0.09**	118.36±4.81	8.72±0.36*	15.69±0.44	0.96±0.06
Cyclic (N= 16)	2.39±0.07**	1.02±0.04	1.74±0.05**	115.69±4.41	10.88±0.42*	15.85±0.55	1.03±0.08

Figures in a column having '*' as superscripts are significantly different (P<0.05).

Figures in a column having '**' as superscript are significantly different (P<0.01).

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REFERENCES

Akhtar, M.S., A.A. Farooq and M. Mushtaq. 2009. Serum trace minerals variation during pre and post-partum period in Nili-Ravi buffaloes. *J. Anim. Plant Sci*., **19**(4): 182-184.

Bodesta, E.J., A. Milani, H. Steffen and R. Nehr. 1980. Steriogenic action of body condition score changes and reproductive performance in isolated adrenocortical cell. *Biochem. J*., **185**: 391.

Dutta, J.C., R.N. Baruah, L. Dutta and S.C. Talukdar. 1988. Blood biochemical studies in anoestrus and normal cyclic cattle. *Indian Vet. J*., **65**: 239-241.

Eltohamy, M.M., M. Younis, H.A. Salem, A. Afar, H. Shawky and A.A. Farakat. 1989. Role of some micro-and macro-elements in inducing repeat breeding in buffaloes. *Indian J. Anim. Sci*., **59**: 1406-1409.

Hurley, W.L. and R.M. Doane. 1989. Recent developments in the roles of vitamins and minerals in reproduction. *J. Dairy Sci*., **72**: 784-804.

Judson, G.J. and J.D. McFarlane. 1998. Mineral disorders in grazing livestock and the usefulness of soil and plant analysis in the assessment of these disorders. *Aust. J. Exp. Agri*., **38**: 707-723.

Mohapatra, P., R.K. Swain, S.K. Mishra, G. Sahoo and K.K. Rout. 2012. Effect of supplementation of area specific mineral mixture on reproductive performance of the cows. *Indian J. Anim. Sci*., **82**(12): 1558-1563.

Newar, S., K.K. Baruah, B. Baruah, A Baruah and D. Bhuyan. 2000. Studies on role of mineral imbalance in causation of postpartum anoestrus in Swamp buffaloes. *Asian Austral. J. Anim*., **13**: 545.

Radostits, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff. 2007. *Veterinary Medicine: A Textbook of Disease of Cattle, Sheep, Pig, Goat and Horses*. WB Sanders Harcourt Publishers Ltd., London, UK.

Sharma, K.B., S. Nayyar, V.S. Malik, R. Singh and S.P.S. Sodhi. 1999. Levels of hormones and minerals in cyclic, anestrus and subestrus buffalo heifers. *Indian J. Anim. Sci*., **69**(4): 214-216.

Singh, A.S. and N. Singh. 2005. Assessment of haematological and biochemical parameters in crossbred heifers with anoestrus or oestrus *Indian J. Anim. Sci*., **75**(12): 1372-1376.

Smith, O.B. and O. Akinbamijo. 2000. Micronutrients and reproduction in farm animals. *Anim. Reprod. Sci*., **60-61**: 549-560.

Srivastava, S.K. 2008. Effect of mineral supplement on oestrus induction and conception in anoestrus crossbred heifers. *Indian J. Anim. Sci*., **78**(3): 275-276.

Tabrizi, A.D. 2012. Comparative survey of serum concentration of copper in cyclic and anoestrus dairy cows. *Annals of Biological Research*, **3**(1): 390-394.