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Effect of Mineral Supplementation on Post Partum Ovarian Activity in Nili-Ravi Buffaloes (*Bubalus bubalis*)

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Abstract.- We investigated the effect of mineral supplementation on ovarian activity of post partum buffaloes in different season. Ten buffaloes were divided in two groups of five buffaloes each. The mineral supplemented group (MSG), was offered minerals 10% more than NRC recommended Ca, P, Zn and Mn levels. The non-mineral supplemented group (NMS) was kept as control. Progesterone (P4), oestradiol (E2), Ca, P, Zn and Mn were analyzed in blood serum of the buffaloes. The first post partum rise in P4 in NMS and MSG was observed on day 91.0±29.7 and 54.8±16.0 ($P>0.05$), respectively. The first post partum rise in E2 in NMS and MSG was on day 24.0±4.43 and 30.6±6.33 ($P>0.05$), respectively. Post partum ovarian activity resumed in 80% (n=4) in both groups. The ovarian activity lasted for 86.0±23.8 and 132.0±23.6 days and then the animals underwent anoestrus period for 174.0±18.3 and 111.0±38.6 days ($P>0.05$) in MSG and NMS, respectively. Blood mineral levels did not differ except for Zn, which was higher ($P<0.05$) in MSG in 2 out of 12 samples. In conclusion, mineral supplementation did not affect the post partum ovarian activity in buffaloes, in different seasons.

Key words: Post partum, buffalo; anoestrus, minerals, progesterone, oestradiol.

INTRODUCTION

Buffalo is the main dairy animal in Pakistan. Of the 29,474,000 metric tons total milk production during the year 2005 in Pakistan, 19,700,000 (66%) was contributed by buffaloes (FAO, 2006). The buffalo milk is relished in this country due to its taste and high milk fat. Average milk production of Nili-Ravi buffalo is 1811 liters in 281 days of lactation (Cady *et al.*, 1983). However, buffalo is known for its poor reproductive efficiency in terms of late maturity and longer calving interval (Ahmed and Irfan, 1983). Among the factors reducing fertility in female water buffalo, post partum anoestrus is the main anomaly (Rao and Sreemannarayana, 1982; Samad *et al.*, 1984).

About 35% of parturating buffaloes experienced anoestrus during the breeding season from October to December (Anwar *et al.*, 2003) hampering their productivity by increasing the service period. A seasonal breeding of water buffaloes has also been reported in many studies (Sheth *et al.*, 1978; Janakiraman *et al.*, 1980; Kaker *et al.*, 1981). Efforts, however, have been made to breed the buffaloes throughout the year by using different hormones (Rajamahendran and Thamotharam, 1983; Singh, 2003), but with limited success. Under-nutrition and environmental stress have been recognized as causes of long anoestrous periods in buffaloes (Kaur and Arora, 1984).

It is well established that minerals play an intermediate role in the action of hormones and enzymes at cellular level which ultimately affect the reproductive performance of female (Bearden *et al.*, 2004). It is suggested that delay in post-partum ovarian activity in ruminants is related to lower level of minerals in blood (Krop, 1993; Manspeaker and Robl, 1993). Deficiency of mineral elements like phosphorus (P), copper (Cu) and zinc (Zn) are associated with subnormal fertility and anoestrous conditions in cows (Moddie, 1965; Campbell *et al.*, 1999). Moreover, Ca, P, Zn and manganese (Mn) have been found affecting post partum reproduction in cattle (Hidiroglou, 1979). To our knowledge there is no information available on the role of mineral supplementation on post partum ovarian activity in

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buffaloes in different seasons. Therefore, present study was designed to monitor the effect of mineral (Ca, P, Zn and Mn) supplementation on post partum ovarian activity in the four seasons of year in Nili-Ravi buffaloes.

MATERIALS AND METHODS

Mineral supplementation

Ten pluriparous pregnant Nili-Ravi buffaloes in their 3rd to 5th lactation were included in the experiment conducted at National Agricultural Research Centre, Islamabad, Pakistan, from August 2003 to September 2004.

The buffaloes were divided into two groups of five each. Starting from one month before parturition, all buffaloes were offered nutrients through concentrate and seasonal green fodder (NRC, 1978). Ten to twelve kg concentrate was offered to each buffalo according to pregnancy maintenance requirement before parturition and of lactation after parturition. The composition of the concentrate was 35% wheat straw, 35% cotton seed cake, 15% wheat bran and 15% ground maize. The concentrate contained 90% dry matter (DM), 10.45% crude protein (CP) and 59% total digestible nutrients (TDN) along with 0.42% Ca, 0.5% P, 68.8 ppm Zn and 13.9 ppm Mn. Ten kg mott grass and millet green fodders were fed to each buffalo during winter and summer, respectively.

Mineral supplemented group (MSG) of five buffaloes was given mineral supplementation comprising dicalcium phosphate ($CaHPO_4.2H_2O$ 50 g; Ca 11.6 gm and P 9 g), zinc sulphate ($ZnSO_4$ 1 g; Zn 400 mg) and manganese sulphate ($MnSO_4$ 1 g; Mn 364 mg) to each buffalo, orally daily, and the non-mineral supplemented group (NMS) was kept without supplementation as control. The animals were kept on this feeding regimen for one year after parturition and they were not mated over this period. The average milk production of the mineral supplemented and non-mineral supplemented buffaloes ranged 4.7 – 7.6 and 4.4 – 9.0 l per day, respectively.

Analysis of hormones

The buffaloes were studied for their post partum ovarian activity over one year by taking twice weekly samples, measuring blood serum P4 and E2 levels. The blood samples (15ml) from each experimental buffalo were collected by the jugular venipuncture according to the standards of Animal Experimentation Ethics Committee constituted at National Agricultural Research Centre, Islamabad, Pakistan.

Blood serum P4 and E2 were measured by using radioimmunoassay (RIA) kits (Immunotech, France). The assays for each sample were conducted in duplicate. The performance characteristics of the assays are presented in Table I. The onset of luteal activity in cattle has been identified by progesterone concentrations $\geq$1 ng/ml (Campbell *et al*., 1999). The hormone profile during the buffalo oestrous cycle is similar to that observed in cattle, but hormone levels are lower and the difference between the two species is greater in summer than during winter (Gordon, 2004). Therefore, a minimum E2 level $\geq$ 3.00 pg/ml was considered to be indicative of follicular activity and P4 level $\geq$ 0.3 ng/ml was considered to be indicative of luteal activity.

Table I.- The performance characteristics of the progesterone (P4) and oestradiol (E2) assays.

Performance characteristic	P4	E2
Analytical sensitivity	0.05 ng/ml	<6 pg/ml
Specificity	100%	100%
Intra assay precision (CV)	5.8%	12.1%
Inter assay precision (CV)	9.0%	11.2%

Blood mineral concentration

Blood samples collected during first week of each month were used for mineral analysis. Ca, Mn and Zn concentration in the blood serum of buffaloes were determined using atomic absorption spectrophotometer (Thermo Electric Elemental Corporation, UK); P was determined with kits (AMP Diagnostics, Austria).

Measurement of uterine involution

Uterine involution was monitored by rectal palpation twice weekly after parturition. Length and width of cervix and uterus were measured to assess gradual decrease in their size. The involution was assumed to have completed when these

measurements were constant for three continuous observations.

Statistical analysis

The data on day and blood serum concentrations of first post partum P4 and E2 peaks in the two groups of experimental buffaloes were analysed using t test. Mean values of the blood serum concentrations of Zn, Mn, Ca and P in experimental buffaloes were also compared using t test (Excel, MS Office, 2003).

RESULTS

Post partum ovarian activity

The data on the effect of mineral supplementation on ovarian activity and number of cycles observed in Nili-Ravi buffaloes within 6 months post calving based on blood serum progesterone concentration is given in Table II. The data on P4 and E2 levels in blood serum of NMS and MSG buffaloes is given in Table III.

Table II.- Effect of mineral supplementation on ovarian activity and number of cycles observed in Nili-Ravi buffaloes within 6 months post calving based on blood serum progesterone concentration.

Group	Tag number	Day post calving on which ovarian activity started and ceased in cyclic buffaloes		Total oestrous cycles
		Started	Ceased	
NMS	786	99	194	5
	929	79	135	3
	931	18	43	1
	941	0	0	0
	977	0	0	0
	*Mean	65	124	3
MSG	566	51	203	7
	636	95	181	4
	710	51	132	4
	864	0	0	0
	972	39	50	1
	*Mean	59	141.5	4

NMS = non mineral supplementation group
MSG = mineral supplementation group
*Means of only cyclic buffaloes are given

Blood serum concentrations of Ca, P, Zn and Mn

The blood serum mineral (Ca, P, Zn, Mn) concentrations of NMS and MSG buffaloes is given in Table IV. The blood Ca, P and Mn concentrations remained non-significantly different ($P>0.05$) between the two groups of buffaloes. The blood serum Zn concentrations (Mean±SE) in NMS and MSG buffaloes remained non-significantly different during the months of October, November, December, January, February, March, April, May, June, July and August. However, these concentrations were significantly higher ($P<0.05$) in MSG than in NMS buffaloes during September (0.95±0.04 vs. 0.75±0.03 ppm, respectively) and April (1.05±0.05 vs. 0.81±0.06 ppm, respectively).

Involution of uterus

The uterus of the parturating buffaloes involuted in NMS and MSG buffaloes at 30.6±3.9 days and 29.2±4.0 days post-partum, respectively. No significant effect of mineral supplementation was observed on involution of uterus ($P>0.05$).

DISCUSSION

Ovarian hormones (P4 and E2) have been measured to gauge reproductive functions during gestation, after parturition and during oestrous cycle in buffaloes (Kaur and Arora, 1984; Avenell *et al.*, 1985; Sharma and Kaker, 1990; Eissa *et al.*, 1995). During this study, post partum ovarian cyclicity was monitored by estimating P4 and E2 hormones, for one year, in non-mineral supplemented and mineral supplemented Nili-Ravi buffaloes. The effect of additional minerals (Ca, P, Zn and Mn) than NRC recommendations along with optimum nutrition (NRC recommendation) was noted on ovarian cyclicity. Reproductive efficiency, resumption of post partum ovarian activity and oestrous cycle in buffaloes has been reported to be affected by season and by reproductive maturity of the animals (Esposito *et al.*, 1992; Campo *et al.*, 2002). During the current study, a pattern of summer anoestrus was observed in buffaloes maintained even on better nutrition and management as reported in other studies (Perera *et al.*, 1982; Kaur and Arora, 1984). In the mineral supplemented group, 20% buffaloes did not resume post partum ovarian activity and

Table III.- Effect of mineral supplementation on day and concentration of first post partum peak of progesterone (P4) and oestradiol (E2) in Nili-Ravi buffaloes.

Treatment*	Day of first post partum		Concentration of first post partum	
	P4 peak (n=5)	E2 peak (n=5)	P4 peak, ng/ml (n=4)	E2 peak, pg/ml (n=4
NMS	91.00 ±29.70	24.00±4.43	0.48 ±0.05	14.27 ±2.81
MSG	54.8 ±16.0	30.60±6.33	0.41 ±0.06	15.86 ±1.49

Means (± SE) in same column did not differ (P>0.05).
For other abbreviations see Table II.

Table IV.- Effect of mineral supplementation on blood serum mineral concentrations in Nili-Ravi buffaloes (*n*=5) during 12 months post-partum.

Months post partum	Zinc (ppm)		Manganese (ppm)		Calcium (mg/dl)		Phosphorus (mg/dl)	
	NMS	MSG	NMS	MSG	NMS	MSG	NMS	MSG
Pr-Part	0.62±0.07	0.90±0.06	0.080±0.020	0.062±0.012	14.05±1.09	15.18±0.42	6.0±0.3	6.5±0.7
September	0.75±0.03^{b}	0.95±0.04^{a}	0.076±0.021	0.069±0.006	12.05±1.99	16.49±1.57	6.6±1.0	6.5±0.8
October	0.69±0.05	0.86±0.07	0.078±0.019	0.064±0.009	11.09±1.35	12.56±1.14	4.6±0.6	5.2±0.4
November	0.77±0.12	0.84±0.03	0.100±0.017	0.069±0.007	14.88±1.35	9.90±2.15	6.6±0.5	5.2±0.6
December	0.67±0.09	0.87±0.08	0.087±0.021	0.118±0.049	10.53±1.25	10.28±0.93	4.9±0.5	4.5±1.2
January	0.73±0.09	0.91±0.06	0.103±0.017	0.080±0.009	12.87±1.14	12.52±1.69	5.6±0.9	4.9±0.3
February	0.84±0.08	1.01±0.06	0.096±0.020	0.071±0.007	11.09±1.84	12.54±4.09	6.1±0.8	5.0±0.7
March	0.82±0.15	1.15±0.17	0.125±0.026	0.138±0.041	14.74±1.23	12.18±1.78	6.1±0.8	5.0±0.8
April	0.81±0.06^{d}	1.05±0.05^{c}	0.095±0.025	0.060±0.004	13.23±1.30	16.13±1.63	6.4±0.5	6.3±0.7
May	0.80±0.05	1.05±0.10	0.095±0.024	0.085±0.011	12.62±1.84	10.78±2.80	6.2±1.0	6.3±0.6
June	0.77±0.04	0.83±0.05	0.104±0.026	0.071±0.010	12.83±0.93	16.13±1.54	6.7±0.5	5.6±1.0
July	0.84±0.07	0.94±0.17	0.091±0.025	0.073±0.006	13.46±0.87	12.38±2.08	5.9±0.7	4.6±0.9
August	1.09±0.21	0.85±0.07	0.083±0.035	0.075±0.008	14.19±1.05	15.04±1.31	5.3±0.4	5.4±0.9

The means (± SE) of Zn in the same row with different superscripts differ (P<0.05).
For other abbreviations see Table II.

showed anoestrus for one year, whereas, rest of the 80% resumed post partum ovarian activity but then underwent anoestrus during summer months (April to August). Although under nutrition and high environmental temperatures have been ascribed to be the causes of long anoestrus period in buffaloes (Kaur and Arora, 1984), a portion of buffaloes undergoing long anoestrus periods along with 80% initially behaving normal and then undergoing short anoestrus periods suggests that factors other than under nutrition and temperature are involved in causing such a condition. Since phenomenon of anoestrus has been observed mostly during the months of April to August which have longer duration of the day light, it can be imagined that the increasing day light affects the oestrus behaviour in Nili-Ravi buffaloes as well. According to Zicarelli (1995) buffalo seem to be a short day species like sheep maintaining high melatonin values two hours from sunset. So a constant elimination of light sensitive subjects and ideal management may improve the reproductive efficiency. Data from Egypt, India and Pakistan indicated that only 34–49% of buffaloes showed oestrus during the first 90 days after calving and 31–42% remained anoestrus for more than 150 days (reviewed by El Wishy, 2007). These figures are in compliance with the findings of present study where first P4 peak detected post partum was 91.0±29.7 days in non-mineral supplemented and 54.8±16.0 days in mineral supplemented buffaloes.

Dietary mineral elements are known to affect the physiological function in general and reproduction in particular (Hidiroglou, 1979). Lall *et al.* (2004) reported that high plane of nutrition with proper mineral supplementation brought majority of

buffaloes back in oestrous after anoestrus. Amongst the major minerals, Ca is known to influence the animal's ability to use other trace elements. Its influence on certain enzyme systems may be mediated via disruption of reproductive efficiency. The view that Ca may directly or indirectly be related to reproductive function is also supported by the observation of Bansal *et al.*, (1978). Deficiency of P influences the level of hypophysis and ovaries and thereby produces aberrations in the normal reproductive rhythm. This hypothesis is further supported by the fact that supplementation of this mineral, either in the form of bone meal (Bansal *et al.*, 1978) or P compounds, has improved the reproductive performance of treated animals. The purpose of providing extra minerals to the buffaloes in ration in the present study was that such a provision might affect/overcome the anoestrus behaviour of buffaloes after parturition. However, the blood serum levels of calcium and phosphorus did not differ between non-mineral supplemented and mineral supplemented buffaloes, suggesting, thereby, no effect of the provision of extra Ca and P in ration for the improvement of post partum ovarian cyclicity pattern of Nili-Ravi buffaloes fed NRC recommended ration. The blood Ca, P and Zn levels observed in experimental buffaloes of our study were a bit higher than observed by Lall *et al.* (2004) in Indian buffaloes. It is suggested that the increased levels of minerals were due to the supplementation which have not reached to a significant level.

CONCLUSION

In conclusion, mineral supplementation did not affect the seasonal reproduction in buffaloes.

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