

## Effect of Supplementation of Minerals and Enzymes on Service Period and Postpartum Plasma Minerals Profile in Crossbred Cows

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### Abstract

Twenty freshly calved healthy triple crossbred (HF x J x K) cows were divided randomly into four groups each of five animals from the day of calving to observe the effect of supplementation of minerals + proteins-vitamins (Nutri-sacc power pack, Vetcare) and enzymes (Neozyme RU, Biocon India Ltd) on service period and fortnightly plasma profile of macro-micro minerals up to 105 days postpartum. The animals of Group-I (T0) served as control; of Group-II (T1) were given nutri-sacc powder (@100 g/d/h; while animals of Group-III (T2) and Group IV (T3) received neozyme supplementation @ 750 and 1000 g per ton of concentrate mixture, which was fed @ 1 kg for every 3 kg milk. The supplementation of minerals and enzymes did not influence service period significantly (147±13.69 days). The pooled plasma calcium, inorganic phosphorus and magnesium concentrations were 10.29±0.11, 4.43±0.10 and 3.40±0.09 mg %, respectively. Significant (P<0.05) differences were observed between groups and between periods for all the three traits. Calcium level was significantly higher in enzyme treatment (T3) as compared to the control group, while phosphorus showed inverse trend. The calcium level was 8.15±0.61 mg % on the day of calving, which increased significantly by day 15 postpartum and again by day 60 postpartum and remained more or less static thereafter. The level of phosphorus and magnesium increased significantly by day 30-45 postpartum. Highly significant differences (P<0.01) were observed between periods for plasma zinc, iron, copper and manganese concentrations with pooled values of 1.19±0.03, 1.53±0.04, 0.85±0.02 and 0.09±0.01 ppm, respectively. The zinc and iron levels increased from day 45 postpartum and remained high till day 105 postpartum, while the copper increased significantly by day 15 postpartum. Nutri-sacc and/or Neozyme supplementation did not influence the plasma trace minerals profile, except of copper.

**Keywords:** Crossbred cows, Postpartum period, Supplementation of minerals and enzymes, Plasma minerals profile, Service period.

### Introduction

The re-establishment of regular estrus cycle after parturition in bovine is delayed for a variable period of time for many reasons. The relationship between reproduction and minerals has been recognized long back. Essential minerals are required for reproduction because of their cellular roles in metabolism, maintenance and growth. However, these nutrients may also have specific role and requirement in reproductive process. Recent works in dairy cattle suggests that borderline nutrient deficiency may be manifested as impaired fertility before other clinical symptoms are apparent. Therefore, considerable

attention is being focused to identify specific reproductive and nutritional problems and thereby to augment bovine fertility. This study was undertaken to optimize inter-calving period in triple crossbred cows through supplementation of minerals and enzymes for a period of three months postpartum and to study its effect on service period and plasma profile of minerals.

### Materials and Methods

Twenty normal healthy freshly calved triple crossbred ( $\frac{1}{2}$ K x  $\frac{1}{4}$ HF x  $\frac{1}{4}$ J) cows maintained in loose housing system at Livestock Research Station of the University, Anand, were utilized. All these animals were

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vaccinated and dewormed against prevailing pathogens. They were fed green fodder, hay and concentrate as per the standard feeding schedule of the farm. Natural mating was followed for breeding.

The animals were divided randomly into four groups, each of 5 cows. The animals of group-I (T0) served as control, whereas animals of group-II (T1) were supplemented with minerals + proteins-vitamins (Nutri-sacc Power Pack, Vetcare India Ltd.) @100 g/d/h; and those of group-III (T2) and group IV (T3) received enzyme supplementation (Neozyme RU, Biocon India Ltd., Bangalore) @ 750 and 1000 g per ton of concentrate mixture, respectively, which was fed @ 1 kg for every 3 kg milk. The supplementation of Nutri-sacc and Neozymes was given from the day of calving till 105 days postpartum. The interval for fertile estrus (service period) was recorded for each cow.

Jugular blood samples were collected from all animals at fortnightly intervals from day 0, i.e. day of calving till 105 days postpartum into heparinized vials and centrifuged for 15 min @ 3000 rpm. The plasma was separated and stored in 2 ml plastic vials at -20°C till analysis with a drop of Merthiolate (0.01%). The levels of calcium, phosphorus and magnesium were estimated on semi-autoanalyser (Photometer, BT) using standard kits.

For micro minerals, the plasma samples (1 ml) were digested with diacid mixture (5 ml, Perchloric acid : Nitric acid, 1:4) on a hot plate (Krishna and Rajhan, 1980). The clear transparent residues were diluted in double glass-distilled water up to required volume (5 ml). These aliquots were then used for the estimation of trace minerals, viz. copper, iron, zinc and manganese using Atomic Absorption Spectro-Photometer (Model AAS 4141), as described by Oser (1976). The data was analyzed by using CRD and critical difference test (Snedecor and Cochran, 1994).

## Results and Discussion

The supplementation of vitamin-minerals and enzymes to crossbred cows right from calving for 105 days did not influence the interval to first estrus postpartum ( $31 \pm 0.60$  days) or service period ( $147 \pm 13.69$  days). Dabas et al. (1987) and Mathur et al. (2005), however, found beneficial effect of supplementation in anestrus cattle. No report on the influence of enzyme supplementation on reproductive performance could be seen in the literature reviewed.

### Macro-minerals Profile

The pooled mean plasma calcium, inorganic phosphorus and magnesium concentration in the animals under study was  $10.29 \pm 0.11$ ,  $4.43 \pm 0.10$  and  $3.40 \pm 0.09$  mg %, respectively. Significant differences were observed between groups ( $P < 0.05$ ) and between periods ( $P < 0.01$ ) for all the three traits. As compared to the control group, enzyme treatment (T3) @ 1 kg per

ton of feed significantly raised the plasma calcium level and dropped magnesium level, whereas mineral supplementation elevated phosphorus level (Table 1). The mean calcium level on the day of calving was  $8.15 \pm 0.61$  mg %, which increased significantly by day 15 postpartum and then again by day 60 postpartum and thereafter it remained more or less static in most groups. The levels of plasma phosphorus increased significantly by day 30-45 postpartum (Table 2).

Present findings on calcium level and its trend from calving through early postpartum till 15th week postpartum corroborated with the reports of Belyea et al. (1975) and Patel et al. (2006). Moddie and Robertson (1962) reported abrupt reduction in the plasma calcium levels around parturition, which recovered by 14th to 30th day postpartum in dairy cows. Deshpande et al. (1998) reported significant increase in calcium and inorganic phosphorus levels from day of calving to 7th, 14th and 21st day postpartum. Sane (1977) opined that infertility due to nutritional deficiencies particularly of phosphorus is usually characterized by failure of oestrus or cessation of estrous cycle. Singh and Vadnere (1987) recorded significantly higher plasma calcium and phosphorus levels at induced oestrus in anoestrus cows supplemented with minerals as compared to pre-treatment and control values. Asthana et al. (2007) reported that repeat breeding cows supplemented with mineral mixture revealed significant difference of serum inorganic phosphorus at different stages of observation i.e. day 0, 21 and 45. It also differed significantly with other treatment groups at day 45, but not on day 0 and day 21. No report could be seen in the literature to compare or deficit the present findings with enzyme supplement in postpartum cows.

The role of calcium in sensitizing tubular genitalia for action of hormones is well established (Moddie, 1965). The lower level of plasma calcium and phosphorus at parturition and subsequently at oestrus or early pregnancy was thought to be due to high oestrogen levels, as oestrogen may change appetite in cows, hence diminished calcium and phosphorus intake and absorption (Sahukar et al., 1984). Patel et al. (2006) did not find any variation in the overall means of plasma calcium, inorganic phosphorus and magnesium levels in fertile and infertile groups of cows. In the present study, the fortnightly means of plasma calcium and phosphorus fluctuated significantly in the normal physiological limits between different intervals postpartum.

The present findings on magnesium, however, did not agree to the reports of Moddie and Robertson (1962), Larson et al. (1980) and Deshpande et al. (1998), who could not find significant change in magnesium level over the postpartum period in cows. Belyea et al. (1975) recorded a gradual decrease in

Table 1: Average plasma minerals profile postpartum in crossbred cows under control and different supplement groups

Plasma minerals	Treatment groups				Mean±SE Overall
	T0	T1	T2	T3	
Calcium (mg %)*	9.76±0.46	10.24±0.56	10.37±0.43	10.80±0.34	10.29±0.11
Phosphorus (mg %)*	4.62±0.13	4.89±0.14	4.06±0.13	4.18±0.20	4.43±0.10
Magnesium (mg %)*	3.52±0.16	3.28±0.15	3.55±0.19	3.23±0.12	3.40±0.09
Zinc (ppm)	1.15±0.06	1.20±0.09	1.19±0.06	1.22±0.06	1.19±0.03
Iron (ppm)	1.49±0.08	1.53±0.07	1.53±0.06	1.55±0.05	1.53±0.04
Copper (ppm)*	0.88±0.05	0.90±0.03	0.76±0.02	0.86±0.04	0.85±0.02
Manganese (ppm)	0.09±0.02	0.08±0.01	0.08±0.01	0.10±0.02	0.09±0.01

\* P<0.05 between groups; T0 = Control Group; T1 = Nutri-sacc supplement 100 g/d/h; T2 & T3 = Neozyme RU supplement @ 750 g and 1000 g per tone of feed, respectively.

plasma magnesium levels for cows sampled at 4 days interval from parturition to 60 days postpartum. Our findings on plasma levels of magnesium, further coincided with the reported values of 3.14±0.12 and 2.41±0.15 mEq/L, respectively, during early postpartum period by Murtuza et al. (1979) and Jacob et al. (2003). It is postulated that all enzyme reactions, which are catalysed by ATP have an absolute requirement for magnesium. The magnesium and calcium has reciprocal relationship. Most evidences show that increased thyroid activity tends to decrease magnesium concentration in plasma (Simeson, 1970).

#### Micro-minerals Profile

Average plasma zinc, iron, copper and manganese levels in the experimental animals were 1.19±0.03, 1.53±0.04, 0.85±0.02 and 0.09±0.01 ppm, respectively. No significant influence of treatment/group was observed for these elements, except copper, but the period effect was significant for all the four elements (Table 1, 2). The value of calcium increased from 30-45 days postpartum and remained higher till 105 days postpartum. Jacob et al. (2003) also reported significant rise in zinc level at one month postpartum compared to level at calving. The present findings also corroborated well with the reports of Rupde et al. (1993) and Chauhan and Nderingo (1997), who stated that zinc level was not related to postpartum anoestrus period or fertility in cows. Further, the decreasing trend of zinc levels observed in early postpartum period also was in contrary to the findings of Mehere et al. (2002). Significantly lower levels of zinc observed during early postpartum period in the present study might be attributed to its more specific role as micronutrient, as it is a component of many enzyme systems including carbonic anhydrase. The present values of zinc were above the critical limit of 0.6 to 0.8 µg/ml suggested by Mc Dowell (1992). Setia et al. (1994) noted high levels during early to mid lactation as compared to values at calving (37 vs 24 mmole/L).

The iron level increased significantly by day 60

postpartum, whereas the copper level rose steeply from day 15 postpartum in all the groups. These findings closely corroborated with the reports of Bostedt et al. (1974). Further, the present values of iron match well with the report of Rupde et al. (1993). Larson et al. (1980) reported that copper level at day 14-21 and 38-45 postpartum was not related to any of the reproductive performance traits in dairy cows. Setia et al. (1994) reported identical serum levels of iron from calving till mid lactation in cows, but copper level was little lower during early lactation than at calving or late lactation. Mehere et al. (2002) and Jacob et al. (2003), however, found almost same values of serum iron level and rising level of copper from day of calving till 4-6 weeks postpartum. Like iron, the lower concentration of copper recorded at parturition followed by increasing trend after 4-5 weeks postpartum could be due to increased transfer of this nutrient across the placenta and haemodilution during late pregnancy and at calving, together with initiation of ovarian follicular activity postpartum, leading to high circulatory estrogens which stimulate binding of iron and copper with the proteins in liver and thereby increased concentration in plasma (Mehere et al., 2002). Like present findings, almost uniform level of plasma manganese concentration has been documented earlier from calving till mid lactation by Setia et al. (1994), whereas Mehere et al. (2002) observed 2 to 2.5 folds high value of serum manganese at 4 weeks postpartum than that at calving in crossbred cows. Manganese is one of the important micronutrients essential for reproductive rhythm and is an integral component of several enzymes involved in reproduction. Dabas et al. (1987) reported that supplementation of anoestrus cows with Nuvimin forte (Sarabhai chemicals) restored serum inorganic phosphorus, copper and other mineral concentrations and 45 out of 60 anoestrus cows returned to normal heat within one month.

Thus, Nutri-saac and/or Neozyme supplementation did not influence the plasma trace minerals

Table 2: Average plasma minerals profile at different intervals postpartum in crossbred cows

Groups	Postpartum interval (days)							
	0	15	30	45	60	75	90	105
Calcium (mg%)**	8.15 ±0.61	10.40 ±0.21	9.95 ±0.54	9.71 ±0.52	11.13 ±0.29	10.60 ±0.57	11.51 ±0.46	10.88 ±0.21
Phosphorus (mg%)*	4.12 ±0.31	4.39 ±0.43	4.59 ±0.22	4.61 ±0.31	4.22 ±0.15	4.32 ±0.24	4.33 ±0.13	4.93 ±0.22
Magnesium (mg%)*	2.94 ±0.19	3.21 ±0.23	3.38 ±0.11	3.25 ±0.18	3.47 ±0.22	3.44 ±0.11	3.64 ±0.20	3.83 ±0.32
Zinc (ppm)**	1.07 ±0.06	1.08 ±0.04	1.11 ±0.05	1.25 ±0.06	1.24 ±0.03	1.26 ±0.04	1.22 ±0.06	1.25 ±0.07
Iron (ppm)**	1.36 ±0.10	1.17 ±0.08	1.21 ±0.04	1.41 ±0.09	1.63 ±0.05	1.52 ±0.07	1.69 ±0.10	1.49 ±0.11
Copper (ppm)*	0.72 ±0.01	0.84 ±0.08	0.86 ±0.05	0.86 ±0.03	0.79 ±0.06	0.91 ±0.04	0.89 ±0.05	0.93 ±0.07
Manganese (Ppm)*	0.10 ±0.01	0.09 ±0.01	0.09 ±0.02	0.08 ±0.02	0.09 ±0.01	0.10 ±0.02	0.08 ±0.01	0.09 ±0.03

\* P&lt;0.05; \*\* P &lt;0.01 between intervals.

profile, except of copper, but the postpartum interval had significant influence on it.

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