EFFECT OF MINERALS AND ENZYMES SUPPLEMENTATION ON POSTPARTUM FERTILITY AND BLOOD PROFILE OF PROGESTERONE AND METABOLITES IN TRIPLE CROSSBRED COWS

K.K. Hadiya, H.J. Derashri And N.P. Sarvaiya

Dept. of Animal Reproduction, Gynaecology and Obstetrics
College of Veterinary Science and Animal Husbandry
Anand Agricultural University, Anand-388 001

ABSTRACT

Twenty healthy freshly calved 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 postpartum fertility and fortnightly blood plasma profile of hormone progesterone and certain metabolites from calving till 15th week postpartum. The animals of Group-I (T_0) served as control; of Group-II (T_1) were given nutri-sacc powder (@100 g/d/h; while animals of Group–III (T_2) and Group IV (T_3) 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 significantly the intervals of first oestrus postpartum (31±0.60 days) or service period (147±13.69 days). The pooled average plasma progesterone and glucose concentrations were 0.76±0.14 ng/ml and 69.37±2.84 mg %, respectively, with significant ($P<0.01$) effect of periods, but not of groups. The values for glucose were highest 60 days postpartum. Average plasma total protein (8.87±0.18 g %) varied neither among groups nor among periods. The average plasma levels of cholesterol and triglyceride were 143.37±6.41 and 160.62±9.28 mg %, respectively. These were also not influenced by the groups or periods. The results indicated that supplementation of minerals + protein–vitamins or enzymes at given level during postpartum period were not distinctly beneficial in triple crossbred cows.

KEY WORDS: Crossbred cows, Supplementation, Minerals, Enzymes, Postpartum fertility, Blood profile.

INTRODUCTION

Reproduction is an important consideration in the economics of the livestock production. Successful reproduction encompasses the desire and ability to mate, the capacity to conceive and nourish the embryo and deliver the viable young one at the end of the normal gestation period. In the absence of regular breeding and calving, the livestock enterprise can not be profitable. For every successful pregnancy and parturition a proper synergism between anabolic and catabolic reaction is essential (Martson *et al.,* 1972). Reduced efficiency of reproduction may be caused by various factors like underfeeding, hormonal imbalance, disease condition and carelessness either singly or in combination. Blood plasma profile of certain hormones and metabolites is a potential aid in characterizing nutritional status and reproductive performance of an animal (Larson *et al.,* 1980). It is useful to make up any deficit through supplementation of various kinds of minerals, vitamins, enzymes and other elements. 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 blood metabolites and progesterone profile.

MATERIALS AND METHODS

The study was conducted on 20 normal healthy freshly calved triple crossbred (½K × ¼HF × ¼J) cows maintained in loose housing system at Livestock Research Station of the University, Anand. All the animals were vaccinated and dewormed against prevailing pathogens. The animals were fed green fodder, hay and concentrate as per the standard feeding schedule of the farm. Natural mating was being followed for breeding.

The animals were divided randomly into four groups, each of 5 cows. The animals of group-I (T_0) served as control, whereas animals of group-II (T_1) were supplemented with minerals + proteins-vitamins (Nutri-sacc Power Pack, Vetcare India Ltd.) @100 g/d/head; and those of group–III (T_2) and group IV (T_3) 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, in addition to 1 kg for maintenance. The supplementation of Nutri-saac and Neozymes was given from the day of calving till 105 days postpartum. The intervals for first oestrus postpartum and service period were recorded for each cow.

Jugular blood samples were collected from all animals at 15 days interval 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%) as a preservative. Another 3-4 ml whole blood was also collected in a glass vial containing 0.5 ml of sodium fluoride (10%) as an anticoagulant and centrifuged as above for estimation of plasma glucose.

Plasma progesterone was estimated by RIA employing standard technique of Kubasik *et al.* (1984). Labelled antigen with I^{125}, antibody coated tubes and standards were procured from Diasiorin S.r.l. Salaggia (Vercelli), Italy. The sensitivity of the assay was 30 pg/ml. The coefficients of intra-assay and inter-assay variation were 5.8 and 9.0 %, respectively. Blood glucose, total protein, cholesterol and triglyceride were estimated by semi-autoanalyser (Photometer, BT) using standard kits. The data was analyzed by using CRD and critical difference test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Postpartum Oestrus Interval and Service Period

The triple crossbred animals under study were observed in oestrus by 31±0.60 days postpartum (12 out of 20 animals – 3 in each group) and had the fertile oestrus interval of 147± 13.69 days for 80 % of cows conceived. There was no significant difference among groups for calving to first oestrus interval or conception. Several workers have recorded a wide range for calving to first oestrus postpartum and service period in dairy bovines. McDonald (1980) stated that 1st postpartum ovulation occurs 25 to 35 days postpartum without any apparent signs of oestrus in cows. El-Hariri *et al.* (1980) found significant effect on fertility of buffalo heifers by supplementing 20 g of vitamineral and 100 g of iodinated casein over a 42-days period. In the present study, the interval from calving to first oestrus was optimum and interval from calving to conception was pretty long. Mineral and enzyme supplementation had no significant effect on first or fertile oestrus postpartum although calving to conception interval was less (99.00±13.69 days) in group-II (T_1) having mineral supplements.

Plasma Progesterone Profile

The average plasma progesterone concentration (ng/ml) in cows under study was 0.76±0.14 ng/ml. No significant effect was observed due to treatment, however, significant difference ($P<0.05$) was observed among periods (Table 1). These findings are in agreement with the report of Mahapatra *et al.* (1990). Tegegne *et al.* (1993[a]) reported plasma progesterone levels to be < 1 ng/ml in all African Zebu cows until week 12 postpartum. In the present study, P_4 rise was found earlier (2-3 weeks postpartum) but with short or irregular cycles and values of P_4 were much lower than those of above report. The comparatively higher P_4 levels observed in earlier study might be due to variation in species, breed, environment, season, nutrition and assay technique. Smolders *et al.* (1996) also reported first increase in progesterone by about 28 days after calving and in only 28 per cent of the cows first oestrous cycle was normal with luteal phase of 12-17 days. The delayed return to oestrus after AI indicates early embryonic mortality.

Plasma Glucose Concentration

Plasma glucose concentration in cows under study was 69.37±2.84 mg per cent. No significant difference was observed among groups, but the period effect was highly significant ($P<0.01$). The values for glucose increased at 30 and 60 days postpartum (Table 2). The present values of mean glucose in different groups were within the normal physiological range. Setia *et al.* (1992), however, recorded gradual and significant rise in blood glucose level from calving till 12th week postpartum, while Wahbi *et al.* (1981) noted a reverse trend with values comparable to our findings. The low blood glucose level noted at early postpartum period might be due to temporary decrease in plasma triglycerides (Ghosh *et al.*, 1991). Jain and Pandita (1995) recorded mean blood glucose level of 37.75±3.50 mg/dl before $PGF_2\alpha$ treatment and 57.57±5.05 mg/dl at induced oestrus in cows, which favoured the present findings of rise in glucose level from 4th to 15th week

postpartum in neozyme treated group compared to the control.

Table 1. Postpartum plasma progesterone (ng/dl) profile in treatment and control group of cows

Groups	Days Postpartum								Overall
	0	15	30	45	60	75	90	105	
T_0	0.41 ±0.29	0.82 ±0.45	0.19 ±0.08	0.70 ±0.38	0.49 ±0.29	0.58 ±0.27	0.67 ±0.39	1.12 ±0.42	0.62 ±0.32
T_1	0.28 ±0.11	0.28 ±0.11	0.79 ±0.36	0.79 ±0.36	0.28 ±0.11	0.79 ±0.36	1.45 ±0.46	1.46 ±0.37	0.76 ±0.28
T_2	0.52 ±0.28	0.85 ±0.31	0.97 ±0.39	0.70 ±0.37	0.83 ±0.36	1.00 ±0.37	1.51 ±0.27	1.51 ±0.26	0.98 ±0.32
T_3	0.26 ±0.09	0.26 ±0.09	0.31 ±0.08	0.26 ±0.09	0.89 ±0.34	0.31 ±0.08	1.34 ±0.35	1.68 ±0.16	0.66 ±0.16
Overall	0.37 ±0.12^{a}	0.55 ± 0.18^{a}	0.57 ±0.17^{ab}	0.61 ± 0.21^{b}	0.62 ± 0.23^{b}	0.67 ± 0.19^{b}	1.24 ± 0.31^{c}	1.44 ± 0.27^{c}	0.76 ± 0.14

T_0 = Control Group; T_1 = Nutri-sacc supplement 100 g/d/h;

T_2 & T_3 = Neozyme RU supplement @ 750 g and 1000 g per tonne of feed, respectively.

Means with different superscripts differ significantly among periods.

Table 2. Postpartum blood glucose (mg%) profile in treatment and control group of cows

Groups	Days Postpartum								Over all
	0	15	30	45	60	75	90	105	
T_0	65.66 ±3.90	60.90 ±2.24	67.28 ±4.84	67.67 ±3.86	70.27 ±5.17	67.81 ±4.65	69.80 ±4.75	68.13 ±3.01	69.44 ±4.04
T_1	67.23 ±2.03	70.21 ±5.65	72.08 ±5.95	62.18 ±0.71	74.16 ±7.81	75.17 ±2.7	77.33 ±8.25	69.14 ±6.76	70.93 ±4.50
T_2	45.28 ±3.96	46.01 ±5.68	63.25 ±2.91	66.66 ±3.58	81.56 ±3.85	80.92 ±5.4	77.74 ±2.69	82.93 ±3.41	69.97 ±3.93
T_3	65.43 ±1.88	64.83 ±2.40	68.81 ±5.33	67.90 ±4.44	76.0 ±4.18	65.30 ±3.81	64.08 ±9.15	64.64 ±8.39	67.12 ±4.86
Overall	60.91 ±2.09^{a}	60.48 ±3.12^{a}	67.86 ±3.47^{bc}	66.10 ±2.89^{b}	75.49 ±4.07^{c}	72.30 ±3.83^{c}	74.24 ±4.28^{c}	73.71 ±3.73^{c}	69.37 ±2.84^{c}

T_0 = Control Group; T_1 = Nutri-sacc supplement 100 g/d/h;

T_2 & T_3 = Neozyme RU supplement @ 750 g and 1000 g per tonne of feed, respectively.

Means with different superscripts differ significantly among periods.

Plasma Total Protein

Average total protein in crossbred cows was 8.87±0.18 g per cent. No significant difference was observed among groups or periods for this trait. The present level of protein compared with that of Tegegne *et al.* (1993[b]), who could neither see any consistent level in plasma proteins during first 33 weeks postpartum nor any influence of cyclic and acyclic nature of cows. Jordon and Swanson (1979) documented a linear decrease in serum total protein levels in non-suckled dairy cows from 4th day to 4th week postpartum and

then a constant low level for next 10 weeks postpartum. Wahbi *et al.* (1981), however, recorded a gradual and significant increase in plasma total protein during the first two months postpartum in primipara cows. The present findings with respect to mean values compared well with the report of Vohra *et al.* (1997).

Table 3. Postpartum plasma total protein (g%) profile in treatment and control group of cows

Groups	Days Postpartum								Overall
	0	15	30	45	60	75	90	105	
T_0	9.16 ±0.85	9.14 ±0.89	9.16 ±0.4	9.79 ±0.38	8.03 ±0.20	8.46 ±0.10	9.51 ±0.8	9.70 ±0.01	9.16 ±0.4
T_1	8.93 ±0.22	8.68 ±0.22	9.01 ±0.04	8.75 ±0.32	8.80 ±0.33	10.25 ±0.98	8.81 ±0.25	9.09 ±0.18	8.93 ±0.16
T_2	8.35 ±0.01	8.55 ±0.01	8.43 ±0.57	8.61 ±0.54	9.19 ±0.20	8.32 ±0.48	7.64 ±0.59	7.75 ±0.41	8.35 ±0.6
T_3	9.05 ±0.79	9.29 ±0.79	8.91 ±0.94	8.68 ±0.99	8.93 ±0.55	8.60 ±0.97	9.52 ±0.15	9.38 ±0.39	9.05 ±0.76
Overall	8.87 ±0.35	8.92 ±0.38	8.68 ±0.47	8.96 ±0.53	8.74 ±0.31	8.91 ±0.65	8.87 ±0.49	8.98 ±0.32	8.87 ±0.18

T_0 = Control Group; T_1 = Nutri-sacc supplement 100 g/d/h;

T_2 & T_3 = Neozyme RU supplement @ 750 g and 1000 g per tonne of feed, respectively.

Plasma Cholesterol Profile

Average plasma cholesterol (mg %) in the experimental animals was 143.37±6.41 mg per cent. No significant difference was observed among groups and among periods for plasma cholesterol concentration. The increasing trend of plasma total cholesterol concentration seen from the day of calving to subsequent weeks postpartum, was associated with the initiation of ovarian activity and establishment of oestrus cyclicity postpartum. Rowlands *et al.* (1980) observed 2.5 fold increase in cholesterol levels during first 8 weeks postpartum in non-suckled HF cows, but it had no relation with conception rate. Setia *et al.* (1992) and Guedon *et al.* (1999) recorded gradual and significant rise in serum cholesterol level till 10th week postpartum and then decline (Table 4) .

Prakash and Tandon (1979) observed a significant drop in serum cholesterol concentration at calving as compared to gestational values and then a steep rise leading to 2-3 fold increase by second month of lactation. This may be attributed to drop in its circulatory levels through increased coupling with oestrogen and thyroxine after parturition, which normally inhibit cholesterogenesis. Lactation probably also affects the level of serum cholesterol, which acts as a fatty acid carrier in the form of cholesterol ester for milk synthesis, as a result there is gradual increase in serum cholesterol level with advancing lactation. These reports and our findings clearly proved that plasma total cholesterol, being precursor of steroid hormones, is closely associated with physiological status of the animal and reproduction in particular. Patel and Dhami (2005) recorded significant and liner increase in postpartum plasma cholesterol concentration in HF cows irrespective of GnRH or $PGF_2\alpha$ treatment effect.

Table 4. Postpartum plasma cholesterol (mg%) profile in treatment and control group of cows

Groups	Days Postpartum								Over all
	0	15	30	45	60	75	90	105	
T_0	126.20 ±10.08	147.80 ±14.60	114.56 ±5.16	141.60 ±15.07	154.20 ±11.52	146.40 ±10.70	165.40 ±12.00	145.6 ±14.90	143.97 ±9.88
T_1	153.60 ±7.65	134.40 ±12.95	153.80 ±18.98	155.80 ±13.50	164.61 ±11.61	144.00 ±18.65	129.60 ±10.67	147.80 ±12.19	149.20 ±13.27
T_2	149.62 ±13.95	142.60 ±8.81	152.80 ±12.03	145.21 ±13.75	140.01 ±14.73	132.40 ±11.51	139.60 ±12.62	145.80 ±11.83	143.52 ±11.87
T_3	140.80 ±16.47	138.60 ±16.67	151.20 ±16.57	155.60 ±16.51	162.20 ±7.79	139.21 ±13.93	124.60 ±10.18	136.02 ±12.20	144.78 ±13.52
Overall	142.55 ±7.27	140.85 ±8.91	143.09 ±12.09	149.55 ±13.34	152.75 ±8.18	140.50 ±12.06	139.80 ±9.98	143.81 ±9.63	143.37 ±6.41

T_0 = Control Group; T_1 = Nutri-sacc supplement 100 g/d/h;

T_2 & T_3 = Neozyme RU supplement @ 750 g and 1000 g per tonne of feed, respectively.

Plasma Triglyceride Profile

Plasma triglyceride (mg %) in the animals under study was 160.62±9.28 mg per cent. No significant difference was observed among groups and among periods. The present almost constant weekly profile of triglyceride did not agree with the results of Marques and Castillo (1996), who reported decrease in triglyceride levels at the beginning of lactation in Holstein cows. Guedon *et al.* (1999) observed that the plasma triglyceride level was influenced by physiological status of animal and was higher during last 10 weeks of pregnancy than at or after calving. Prakash and Tandon (1979) found lowest level of total lipids at calving in crossbred cows, and the values rose steeply reaching to 2-3 folds hike by 6th week postpartum. This rapid increase in the triglycerides during early postpartum/lactation was attributed to increased demand of the udder for fatty acids synthesis for milk fat, and also to lowest level of circulatory oestrogen and thyroxine profile, which influence the lipid metabolism. Our observations of slight rise in triglyceride levels at 4-8 week postpartum compared to early or late phase to some extent goes with this opinion (Table 5).

Table 5. Postpartum plasma triglyceride (mg%) profile in treatment and control group of cows

Grp	Days Postpartum								Overall
	0	15	30	45	60	75	90	105	
T_0	141.06 ±8.08	161.06 ±19.65	145.42 ±2.50	154.72 ±20.05	175.02 ±16.38	171.18 ±24.36	145.06 ±19.75	155.98 ±18.35	159.56 ±16.20
T_1	169.88 ±21.8[1]	149.88 ±21.99	176.86 ±21.00	178.76 ±19.39	153.76 ±19.31	160.32 ±14.55	165.94 ±20.50	134.16 ±12.05	161.19 ±16.00
T_2	148.96 ±24.68	148.96 ±18.20	173.58 ±18.00	166.80 ±21.21	155.06 ±16.17	139.14 ±10.00	164.24 ±19.03	158.64 ±19.50	159.42 ±18.37
T_3	134.80 ±5.32	154.80 ±18.09	157.54 ±13.57	170.94 ±17.14	173.46 ±21.07	152.68 ±18.02	155.68 ±16.55	169.90 ±21.89	162.47 ±16.75
Overall	148.68 ±12.82	151.91 ±13.11	163.33 ±14.76	167.80 ±13.15	165.81 ±14.09	155.73 ±14.53	157.72 ±15.39	156.67 ±16.84	160.62 ±9.28

T_0 = Control Group; T_1 = Nutri-sacc supplement 100 g/d/h;

T_2 & T_3 = Neozyme RU supplement @ 750 g and 1000 g per tonne of feed, respectively.

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