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INFLUENCE OF PERIPARTUM NUTRITIONAL SUPPLEMENTATION ON PLASMA MACRO-MICRO MINERALS PROFILE AND POSTPARTUM FERTILITY IN CROSSBRED COWS

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Abstract: Twenty healthy advanced pregnant crossbred cows of 2-4 parity were selected and equally divided from 2 wks prepartum to 8 wks postpartum into control (routine farm feeding) and treatment (RFF + ASMM @ 50 g/h/d and bypass fat @ 100-200 g/h/d) groups to evaluate their plasma minerals profile and postpartum fertility. Heparinized blood samples were collected on days -14, -3, 0, 3, 14, 28 and 42 of calving. The mean plasma calcium levels decreased significantly (p<0.01) from day 14 prepartum to day of calving, and then gradually increased in days postpartum with the overall higher mean value in treatment than control group (8.84±0.24 vs 8.18±0.23 mg/dl, P<0.05). The drop in phosphorus levels was however non-significant. The inorganic phosphorus levels fluctuated non-significantly between different intervals peripartum with significantly (p<0.01) higher mean value in treatment than control group (5.90± 0.30 vs 5.40±0.29 mg/dl). The mean magnesium concentration decreased significantly (p<0.05) at 3 days postpartum, and thereafter increased to reach values comparable to the prepartum, without group difference (3.16±0.09 vs 3.11±0.07 mg/dl). The highest mean copper values were found on day 3 prepartum and on day of calving. The iron concentration showed a consistent increasing trend from day 3 prepartum to postpartum period. The zinc values fluctuated between the pre- and postpartum days with the lowest on the day of calving. The mean cobalt values showed no distinct changes in the periods studied. No significant differences were observed for micro-minerals profile between treatment and control groups. The periods for occurrence of first estrus postpartum (38.00±1.95 vs. 42.32±4.14 days) and service period (85.22±7.17 vs 100.67±5.60 days) were significantly shorter in treated than control group with higher conception rate (80 vs 60%). It was concluded that peripartum supplementation of bypass fat and min mix to crossbred cows had no significant effect on uterine involution, but had a beneficial effect on certain plasma mineral constituents and postpartum fertility in terms of early occurrence of postpartum first and fertile estrus.
Keywords: Crossbred cows, Transition period, Nutritional management, Mineral profile, Fertility.

Introduction

Early postpartum phase exerts biological and physiological stress on the dam. Fats in the diet can influence reproduction positively by altering both ovarian follicle and corpus luteum function via improved energy status and by increasing precursors for the synthesis of reproductive hormones such as steroids and prostaglandins (Rahbar *et al.,* 2014). The days

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required for complete uterine involution and postpartum first ovulation were reduced and conception rates were improved after dietary fat and vitamin-mineral supplementation in cattle (Khalil *et al.,* 2012; Theodore *et al.,* 2016) and buffaloes (Hussein *et al*., 2013; Modi *et al.,* 2016). Therefore, this study was planned to assess the effect of incorporating minerals as well as bypass fat in the ration of transitional cows on their plasma macro-micro-minerals profile and postpartum fertility.

Materials and Methods

This investigation was carried out at University Farm in Anand during November 2014 to May 2015 on 20 advanced pregnant HF crossbred cows of 2^{nd} to 4^{th} parity. The experiment was initiated at two weeks prepartum by randomly dividing the animals equally into control and treatment groups. The cows approaching parturition were segregated in calving pen, and calving events were monitored closely. The cows of control group (n=10) were maintained on routine farm feeding schedule (green fodder, hay and compounded concentrate mixture @ 18-20, 4-5 and 3.0-3.5 kg, respectively, with 50 g of min. Mix.) during last two months of pregnancy and early postpartum. After calving the level of concentrate fed was @ 40 per cent of milk produced, while those of treatment group (n=10) in addition to routine farm feeding, received daily extra 50 g of chelated ASMM and 100 g of bypass fat (Sunegry, Polchem) with compound concentrate mixture (Amul brand) for 2 weeks each before and after calving. The level of bypass fat was then increased as per the milk production @ 15 g per litre of milk produced until 60 days postpartum limiting to maximum of 200 g/day.

Blood samples were collected from all the cows by jugular veinipuncture in heparinised vacutainers on days -14, -3, 0, 3, 14, 28 and 42 of calving. The plasma was separated out by centrifugation and stored at -20°C with a drop of merthiolate until analyzed. The plasma levels of macro-minerals (Ca, P, Mg) were estimated by using standard procedures and assay kits of Coral Clinical System, Goa on chemistry analyser (Nova 2021, Analytical Technologies Pvt Ltd). The levels of plasma micro-minerals (Zn, Fe, Cu, Co) were estimated on Atomic Absorption Spectrophotometer (Model AAS 4141, ECI Ltd) using samples diluted with Milli-Q water @ 1:2 without wet digestion. Cows were palpated per rectum at weekly intervals from day 15 postpartum onward to assess uterine involution. The occurrence of first estrus and fertile estrus postpartum and conception rates were also recorded and compared by ‘t’ test. Cows showing estrus 60 days after calving were inseminated and pregnancy was confirmed 45 days after last AI. The data on plasma minerals profile within group was analyzed using ANOVA and DNMRT, and between groups by 't' test for each trait employing SPSS software version 20.00.

Results and discussion

Plasma Macro-Minerals Profile

The mean plasma calcium levels, in the cows under both the groups, decreased significantly ($p<0.01$) from day 14 to 3 prepartum to reach the lowest value on the day of calving. These values then gradually increased in the subsequent days postpartum. The mean values in treatment group were observed to be higher than control group, with significant difference in overall pooled values (Table 1). This may be attributed to peripartum ASMM supplemented. The trend of changes in calcium levels observed during pre- and postpartum periods closely corroborated with the observations of Desmukh *et al.* (2001), Yokus *et al.* (2010) and Patel (2014). The mean plasma calcium concentration (8.51±0.23 mg/dl) found was comparable with the reports of Yokus *et al.* (2010) and Patel (2014). However, as the calcium levels were found to be within the physiological range (8-11 mg/dl), the differences in calcium concentration did not elicit any effect on the period of uterine involution. On the other hand, the occurrence of first estrus postpartum (38.00±1.95 vs. 42.32±4.14 days) and service period was found to be significantly shorter in the treated animals (85.22±7.17 vs 100.67±5.60 days) with comparatively higher conception rate (80 vs 60%) indicating the positive effect of periparum nutritional supplementation on postpartum fertility.

Table 1: Mean plasma calcium, inorganic phosphorus and magnesium (mg/dl) levels during peripartum periods in crossbred cows under control and treatment groups

Days pre- and Post-partum	Calcium (mg/dl)		Inorganic Phosphorus (mg/dl)		Magnesium (mg/dl)	
	Control (n=10)	Treatment (n=10)	Control (n=10)	Treatment (n=10)	Control (n=10)	Treatment (n=10)
-14	8.80±0.27^{c}	9.80±0.27^{cd}	5.45±0.20	6.29±0.35	3.29±0.08^{b}	3.22±0.05^{ab}
-3	7.84±0.25^{b}	9.07±0.27^{c}	5.34±0.21	6.21±0.28	3.30±0.05^{b}	3.09±0.08^{ab}
0	6.51±0.18^{a}	7.16±0.32^{a}	5.12±0.17	5.93±0.34	3.19±0.05^{b}	3.09±0.07^{ab}
3	7.74±0.32^{b}	8.33±0.25^{b}	5.01±0.29	5.56±0.34	2.89±0.11^{a}	2.81±0.19^{a}
14	7.75±0.32^{b}	8.12±0.24^{b}	5.57±0.44	5.59±0.36	3.09±0.10^{ab}	3.15±0.04^{ab}
28	8.94±0.15^{cd}	9.29±0.10^{c}	5.85±0.46	6.22±0.19	3.12±0.11^{ab}	3.18±0.02^{ab}
42	9.67±0.15^{d}	10.13±0.19^{e}	5.45±0.25	5.51±0.24	3.23±0.12^{b}	3.23±0.04^{ab}
Overall	8.18±0.23*	8.84±0.24	5.40±0.29 **	5.90±0.30	3.16±0.09	3.11±0.07

Means bearing different superscripts (a,b,c,d) within column differ ($p<0.01$) between intervals; *($p<0.05$) between groups; **($p<0.01$) between groups.

The mean plasma inorganic phosphorus levels fluctuated non-significantly between different time intervals within the group. The overall mean plasma inorganic phosphorus value in treatment group (5.90±0.30 mg/dl) was significantly ($p<0.01$) higher as compared to control group (5.40±0.29 mg/dl), which can be attributed to the effect of peripartum nutrient supplementation in that group. The trend of mean plasma inorganic phosphorus levels obtained from calving to early postpartum period concurred well with the findings of Dhami *et al*. (2006), Ram (2006), Sutaria (2010) and Patel (2014). Larson *et al.* (1980) found that the inorganic phosphorus had no consistent relationship to reproductive performance in dairy cattle. The overall pooled mean phosphorus value corroborated with the findings of Shende *et al.* (2006) and Patel (2014). However, Patel *et al.* (2005) and Benzaquen *et al.* (2015) found relatively higher values than the present one.

The mean magnesium concentrations were found to be reduced significantly ($p<0.05$) at 3 days postpartum with the lowest value of 2.89±0.11 and 2.81±0.19 mg/dl in control and treatment groups, respectively. The values, thereafter, increased with subsequent postpartum days to reach prepartum levels. The overall mean plasma magnesium values in cows under treatment and control groups (3.11±0.07 and 3.16±0.09 mg/dl) were almost same and within the physiological limits. The peripartum trend of fall and rise in plasma magnesium levels observed corroborated with the findings of Melendez *et al*. (2002). However, Hadiya *et al.* (2010) and Piccione *et al.* (2012) reported rise in the plasma magnesium concentration from the day of calving with increasing days of lactation. The overall pooled mean plasma magnesium concentration was in agreement with the findings of Hadiya *et al*. (2010) and Patel (2014). However, Yokus *et al.* (2010) found relatively higher plasma magnesium values, while others (Piccione *et al.,* 2012) found comparatively lower values.

Plasma Trace Minerals Profile

The mean plasma iron concentration showed a gradual increasing trend ($p<0.05$) in both the groups from day 14 prepartum to day 28-42 postpartum, however, the group difference was not significant overall or at any of the intervals. The iron levels found in the present study were in agreement with the findings of Mehere *et al*. (2002), Patel *et al.* (2006) and Sutaria (2010). However, relatively lower (Patel *et al.,* 2006) and higher (Ram, 2006; Mishra *et al.,* 2008) values have been reported by others. Iron is abundantly present in all the feed and fodder; hence a deficiency in adult ruminant seems improbable. But in some instances very low availability of iron in some roughages or its impaired absorption at intestinal level affect the ruminant reproduction. The low level of iron could possibly result into improper tissue oxygenation to the uterus resulting in impaired nutrition to the uterus, thereby affecting the process of involution of uterus. Existence of positive correlation between the concentration of iron in the blood serum and the fertility status of dairy cows has also been reported by Hidiroglou (1979).

The mean prepartum plasma zinc levels of cows under both control and treatment groups decreased significantly on the day of calving and then showed an abrupt rise on day 3 postpartum followed by a gradual declining trend particularly in treatment group during postpartum period. Statistically, the period effect was however significant only in control group. The present trend of findings is in accordance with the observations made by Ram (2006) and Sutaria (2010). Further, the present values are comparatively higher than those reported by some workers (Patel *et al.,* 2006), while others documented lower mean plasma zinc values (Mehere *et al.,* 2002; Mishra *et al.* 2008). Reproductive failure in female is a manifestation of zinc deficiency, as it is an integral part of certain enzyme systems in body.

Table 2: Mean plasma iron and zinc (ppm) levels during peripartum periods in crossbred cows under control and nutrient supplemented (treatment) groups

Days pre- and Postpartum	Iron (ppm)		Zinc (ppm)	
	Control (n=10)	Treatment (n=10)	Control (n=10)	Treatment (n=10)
-14	1.95±0.11[a]	1.99±0.13[a]	1.64±0.14[b]	1.76±0.21
-3	2.10±0.08[ab]	2.19±0.04[ab]	1.46±0.15[ab]	1.43±0.09
0	2.12±0.10[ab]	2.24±0.09[b]	1.27±0.17[a]	1.35±0.11
3	2.20±0.04[ab]	2.24±0.10[b]	1.57±0.06[ab]	1.75±0.12
14	2.30±0.10[b]	2.33±0.15[b]	1.28±0.13[a]	1.67±0.13
28	2.41±0.07[b]	2.39±0.07[b]	1.41±0.18[ab]	1.59±0.13
42	2.41±0.12[b]	2.30±0.12[b]	1.47±0.16[ab]	1.40±0.18
Overall	2.21±0.09	2.24±0.10	1.44±0.14	1.57±0.14

The means bearing different superscripts in a column differ significantly ($p<0.05$) between intervals.

Table 3: Mean plasma copper and cobalt (ppm) levels during peripartum periods in crossbred cows under control and nutrient supplemented (treatment) groups

Days pre- and Postpartum	Copper (ppm)		Cobalt (ppm)	
	Control (n=10)	Treatment (n=10)	Control (n=10)	Treatment (n=10)
-14	0.83±0.06[a]	0.76±0.04[ab]	0.29±0.03	0.32±0.04
-3	1.13±0.05[b]	1.12±0.10[b]	0.32±0.02	0.33±0.03
0	1.18±0.09[b]	1.02±0.08[ab]	0.33±0.04	0.34±0.03
3	0.98±0.10[ab]	0.84±0.09[ab]	0.33±0.03	0.33±0.03
14	0.95±0.07[a]	0.89±0.08[ab]	0.34±0.02	0.35±0.03
28	0.77±0.14[a]	0.95±0.16[ab]	0.37±0.03	0.36±0.03
42	0.77±0.08[a]	0.83±0.12[ab]	0.30±0.04	0.37±0.04
Overall	0.95±0.09	0.92±0.09	0.33±0.03	0.34±0.03

The means bearing different superscripts in a columns differ significantly ($p<0.01$) between the time intervals.

The mean prepartum plasma copper levels of cows in control group increased significantly on the day of calving, dropped within next 3 days and then showed more or less consistent trend till day 42 postpartum, and similar was the trend in treatment group, however no such variation was noticed in plasma cobalt content (Table 3). The present findings on mean plasma copper levels and a fluctuating trend observed in peripartum period in cows of both the groups compared well with the findings of Patel *et al.* (2006), Ram (2006), Mishra *et al.* (2008), Sutaria (2010). Some workers however documented relatively higher plasma copper values (Parikh, 2009).

Copper levels in peripheral blood circulation appear to be influenced by hormones of reproduction and its concentration in blood serves as an indicator of gonadal hormones and pituitary gonadotrophins. Copper deficiency leads to reproductive disturbances. Various minerals (Cu, Co, Se, Mn, Zn, I) can influence reproductive performance of ruminants (Hidiroglou, 1979). Cobalt is required to ensure fertility in ruminants. Field experience suggests that cobalt deficiency impairs breeding performance in cattle. The most common manifestation of cobalt deficiency is a marked reduction in conception rate. Reproductive failure may be induced by deficiencies of single or combination of trace elements and by imbalances. According to Larson *et al.,* (1980), blood copper level at 14 to 21 and 38 to 45 days postpartum was not related to any of the postpartum reproductive performance in non-suckled dairy cows. The present findings thus corroborates with this report. Moreover, the difference observed in the concentrations of macro and micro-minerals might be due to variation in nutritional status, breed, climatic zone and mineral status of soil as these factors seem to exert an influence on the concentration of these elements in the circulating blood.

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