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Effect of Peripartum Nutritional Supplementation on Plasma Profiles of Macro Minerals and Postpartum Fertility in Jaffarabadi Buffaloes

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Abstract

This investigation was undertaken on 40 transitional Jaffarabadi buffaloes with the objective to evaluate the effect of peripartum nutritional (multiminerals and bypass fat) supplementation without and with micro-minerals (Inj. Stimvet) and oral ecbolic (Boli Exapar) on uterine involution and postpartum fertility including plasma minerals profile. The animals of treatment group (n=20) received additional oral supplements daily with 50 g of chelated mineral mixture and 150 g of bypass fat along with concentrates for 6 weeks prepartum and 2 weeks postpartum over the control farm fed group (n=20). Ten buffaloes in each control and treatment group further received Inj. Stimvet 5 ml (micro-minerals) around day 45 prepartum and on day of calving. Half of these Stimvet treated and control subgroups also received Exapar (n=5) 2 boli/day for 4 consecutive days postpartum. Blood samples were obtained on day –45, –30, –7 and 0 (day of calving), 7, 15, 30, 45, 60 peripartum for estimation of minerals profile. Animals were followed for puerperal events, uterine involution and intervals for postpartum first estrus and conception. The feeding of bypass fat and chelated minerals had significant effect on the time required for expulsion of placenta (3.93±0.24 vs 7.18±0.72 hrs; $p<0.01$), uterine involution (32.75±0.57 vs 37.00±0.56 days; $p<0.05$), intervals for first estrus postpartum (79.05±3.82 vs 100.55±3.47 days; $p<0.05$) and service period (107.10±4.43 vs 133.65±6.04 days; $p<0.05$). The prepartum mean plasma calcium and inorganic phosphorus levels in the buffaloes of both control and treatment groups decreased significantly on the day of calving, and then gradually increased during the days postpartum, again reaching at par with prepartum levels at around day 15-30 postpartum. The mean plasma calcium levels were observed to be higher in treatment than control group at most of the intervals. The mean plasma inorganic phosphorus values on periods closer to calving were apparently higher in treatment group than the control group. Further, the levels during close peripartum period were observed to be apparently higher in untreated subgroup as compared to Stimvet and Exapar alone or its combination subgroups. The mean plasma magnesium concentrations were found to be almost consistent during entire peripartum period studied in both the groups with apparently higher values in treatment than control group at most of the intervals. Very similar trend was also found in subgroups treated with Stimvet and oral Exapar alone or in combination, being little higher in Stimvet injected subgroup.

Introduction

A number of reproductive problems in buffaloes such as late maturity, seasonal variation in fertility, poor estrus expression, anestrus and long calving intervals are mainly due to our poor knowledge of the basic reproductive physiology of the female (Hemeida, 1988; Janakiraman, 1988). It is further established that, apart from energy status, macro and micro-nutrients play an important role in animal reproduction because they form components such as metallo-enzymes and enzyme co-factors. Some of these minerals are the components of hormones and thus directly regulate endocrine activity. Due to its involvement in carbohydrate, protein and nucleic acid metabolism, any change in the level may alter the production of reproductive and other hormones, thereby affecting postpartum fertility (Kumar *et al.*, 2011). Minerals play an important role in the regulation of reproduction and production of animals. Minerals like calcium, phosphorus and magnesium influence the ability of the animal to utilize other micro-minerals. Lack of minerals especially calcium and phosphorus upset the proper functioning of the genital organs. Lower concentration of circulatory minerals results in impaired reproductive function leading to cessation of cyclic activity (Martson *et al.*, 1972). Infertility due to nutritional deficiency is usually characterized by a failure of estrus or a cessation of estrous cycle, where mineral deficiency mainly includes phosphorus than any other trace minerals. Hence this study was aimed to evaluate the effect of peripartum supplementation of chelated mineral mixture and bypass fat on plasma minerals profile and postpartum fertility in Jaffarabadi buffaloes.

Materials and Methods

The prior approval from the Institutional Animal Ethics Committee was obtained for the use of farm animals in this study. In all 40 advanced pregnant Jaffarabadi buffaloes of 2-4 parity were selected from Cattle Breeding Farm of JAU, Junagadh. All buffaloes were maintained in well ventilated hygienic sheds and were fed green fodder, hay and compounded concentrate, as per standard feeding schedule followed on the farm. The buffaloes were divided into two equal groups, viz., control and treatment groups (n=20 each). The control animals were maintained on standard routine farm feeding schedule and the animals of treatment group were given additional oral supplements daily with 50 g of chelated mineral mixture and 150 g of bypass fat along with concentrates for 6 weeks prepartum and 2 weeks postpartum, and then bypass fat was given @ 15 g/lit of milk limiting to 200 g/day/head till 60 days postpartum. Both the groups were again subdivided equally into two subgroups each of 10 animals to evaluate the effect of Inj. Stimvet 5 ml (containing Se, Zn, Cu, Mn; 25, 200, 75 and 50 mg, respectively) around day 45 prepartum and on day of calving, keeping rests as Stimvet controls. They were further subgrouped and one of them received Boli Exapar (n=5) 2/day for 4 consecutive days postpartum. Blood was collected from each animal by jugular vein puncture in heparinized vacutainers on days –45, –30, –7, 0, 7, 15, 30, 45 and 60 (day 0 is day of parturition). The plasma was separated immediately after the collection of blood by centrifugation of samples for 10 minutes at 3000 rpm. The plasma was stored at -80°C with a drop of merthiolate until analyzed. Plasma calcium, inorganic phosphorus and magnesium concentrations were estimated by OCPC method (Bagainski, 1973), Molybdate UV method (Fiske and Subbarow, 1925) and Calmagite method (Gindler, 1971), respectively, using standard procedures and assay kits procured from Crest Bio-systems, a division of Coral Clinical Systems, Goa, with the help of Chemistry Analyzer (Nova 2021, Analytical Technologies Pvt Ltd, Vadodara). The puerperal invents and periods for uterine involution, first estrus postpartum and conception were recorded. The data on mineral profile were analysed statistically using online SPSS software version 20.0 for ANOVA, DMRT and 't' test for group and period effects.

Results and Discussion

The feeding of bypass fat and chelated minerals had significant effect on the time required for expulsion of placenta (3.93±0.24 vs 7.18±0.72 hrs; $p<0.01$) in Jaffarabadi buffaloes. The mean period required for the involution of uterus as assessed by per-rectal palpation was found to be significantly ($p<0.05$) shorter (32.75±0.57 vs 37.00±0.56 days; $p<0.05$) in buffaloes of supplemented group. The mean intervals for first estrus postpartum and service period observed were also significantly ($p<0.05$) shorter (79.05±3.82 vs 100.55±3.47 days and 107.10±4.43 vs 133.65±6.04 days) in nutrients supplemented group. These findings corroborated well with the reports of Mavi *et al.* (2006) for vitamin E and Se supplemented buffaloes. Similarly, Khan *et al.* (2015), Mane *et al.* (2016), and Kalasariya *et al.* (2017) found beneficial effect of peripartum nutritional supplementation on uterine involution and postpartum fertility in cattle and buffaloes. These observations clearly indicated that there was a positive effect of peripartum nutrient supplementation in buffaloes so far as uterine involution and onset of postpartum ovarian activity and fertility is concerned. The earlier resumption of cyclicity in the buffaloes under treatment group could be attributed to the effect of minerals and fat supplementation in the diet.

No significant effect of Stimvet injection and Exapar boli either alone or in combination was observed over control group on the traits studied. However, the conception rate was apparently higher in Stimvet plus Exapar treated group as compared to individual treatment groups. Dhakal (1999) recorded expulsion of placenta in 100 per cent animals with conception rate of 72 vs. 40 % in buffaloes and 55 vs. 25 % in cows following use of Exapar. Similarly, a significant positive effect was seen on expulsion of placenta, cessation of lochial discharge and uterine involution in diary animals following use of Exapar (Gautam *et al.,* 2005; Thakur *et al.,* 2013). Uterine ecbolics are safe and mainly act as an uterine cleaner, and reduce lochial discharge, increase uterine contractibility and tone during postpartum period in healthy bovines (Ravi and Bhagvat, 2007). However, in all these studies no other nutrients supplements were provided to the animals. In the present study, the non-significant influence of Stimvet injection and Exapar bolus alone or in combination could be attributed to the oral supplementation of chelated minerals and bypass fat to all the animals on the farm, which might have optimized the circulatory nutrients requirements of animals.

The prepartum mean plasma calcium levels in buffaloes of both control and treatment groups decreased significantly ($p<0.01$) on the day of calving. These values then gradually increased in the subsequent days postpartum again reaching at par with prepartum levels at around day 15-30 postpartum (Table 1). The trend in the changes of calcium levels during transitional periods in the study corroborated well with the observations of Kumar *et al*. (2000), Ashmawy (2015), Khan *et al.* (2015), Patel *et al.* (2015), Theodore *et al*. (2016) and Kalasariya *et al*. (2017). The mean plasma calcium levels were observed to be higher in Treatment than Control group at most of the intervals, which may be attributed to peripartum ASMM supplementation. The injection Stimvet and oral Exapar alone or in combination did not influence significantly the plasma calcium concentrations at any of the peripartum intervals, overall or among Control and nutrients Treatment groups (Table 1).

The mean plasma inorganic phosphorus levels followed the trend of plasma calcium, with significantly lower values on the day of calving in both Control and Treatment groups (Table 2). However the levels fluctuated non-significantly between different time intervals before and after 1 week peripartum within each group. Moderate reduction in the levels of phosphorus at and after calving might be due to the necessity of it for the colostrum synthesis and enhanced carbohydrate metabolism. Moreover, the overall ratio of calcium : phosphorus observed in the present study was 2.2 : 1.0, which is well within the recommended one for better fertility of animals. The overall pooled mean plasma inorganic phosphorus value (5.39±0.04 mg/dl) corroborated with the findings of Patel *et al*. (2015), Parikh *et al.* (2016) and Theodore *et al*. (2016). The mean plasma inorganic phosphorus levels obtained from calving to early postpartum period corroborated well with the findings of Ashmawy (2015), Parikh *et al.* (2016) and Theodore *et al*. (2016). The plasma inorganic phosphorus values on periods closer to calving were apparently higher in treatment group than the control group (5.50±0.06 vs. 5.29±0.05 mg/dl, $p<0.05$), which may be due to the effect of peripartum mineral supplementation in treatment group of buffaloes. Similar results were reported by Khan *et al.* (2015), Patel *et al.* (2015) and Theodore *et al.* (2016). Further, the levels during close peripartum period were observed to be apparently higher in untreated subgroup as compared to Stimvet and Exapar alone or its

Table 1: Plasma Calcium concentrations (mg/dl) in Jafarabadi buffaloes given peripartum nutrients supplementation and treatment with Stimvet and Exapar alone or in combination

Peri-partum Period	Overall nutrient groups			Treatment subgroups			
	Control (N=20)	Supplemented (N=20)	Pooled (N=40)	Stimvet (N=10)	Exapar (N=10)	S+E (N=10)	None (N=10)
-45 Day	11.96 ± 0.30^{abc}	12.83 ± 0.26^{bc}	12.40 ± 0.21^{bcd}	11.88±0.39	12.67±0.40	12.12±0.50	12.90±0.35
-30 Day	12.48 ± 0.27^{c}	13.15 ± 0.24^{c}	12.81 ± 0.18^{d}	12.26±0.45	13.24±0.35	12.67±0.34	13.09±0.29
-7 Day	12.25 ± 0.28^{bc}	12.89 ± 0.23^{bc}	12.57 ± 0.18^{cd}	12.16±0.32	12.84±0.32	12.78±0.43	12.50±0.41
0 Day	11.41 ± 0.25^{a}	11.76 ± 0.21^{a}	11.58 ± 0.16^{a}	11.33±0.31	11.73±0.30	11.80±0.25	11.48±0.45
7 Day	11.71 ± 0.21^{ab}	12.27 ± 0.23^{ab}	11.99 ± 0.16^{ab}	12.21±0.23	12.15±0.36	12.05±0.33	11.55±0.36
15 Day	11.98 ± 0.20^{abc}	12.29 ± 0.27^{ab}	12.14 ± 0.17^{bc}	12.07±0.28	12.25±0.25	11.99±0.27	12.24±0.50
30 Day	12.42 ± 0.21^{bc}	12.38 ± 0.23^{ab}	12.40 ± 0.15^{bcd}	12.22±0.36	12.54±0.36	12.54±0.20	12.31±0.32
45 Day	12.03 ± 0.17^{abc}	12.59 ± 0.13^{bc}	12.31 ± 0.11^{bcd}	11.95±0.23	12.34±0.23	12.59±0.27	12.37±0.15
60 Day	12.24 ± 0.16^{bc}	12.80 ± 0.19^{bc}	12.52 ± 0.13^{cd}	12.02±0.33	12.68±0.23	12.67±0.27	12.71±0.16
Overall	**12.05±0.08**	***12.55±0.08**	**12.30±0.06**	**12.01±0.11**	**12.49±0.11**	**12.36±0.11**	**12.35±0.12**

N= Number of animals; *P<0.05 between control and supplemented groups.
Means bearing uncommon superscripts within the column differ significantly between periods (p<0.05).

Table 2: Plasma Inorganic phosphorus concentrations (mg/dl) in Jafarabadi buffaloes given peripartum nutrients supplementation and treatment with Stimvet and Exapar alone or in combination

Peri-partum Period	Overall nutrient groups			Treatment subgroups			
	Control (N=20)	Supplemented (N=20)	Pooled (N=40)	Stimvet (N=10)	Exapar (N=10)	S+E (N=10)	None (N=10)
-45 Day	5.26 ± 0.20^{abc}	5.72 ± 0.21^{bcd}	5.49 ± 0.15^{bc}	5.27±0.31	5.59±0.40	5.31±0.24	5.79±0.23
-30 Day	5.68 ± 0.15^{c}	6.06 ± 0.19^{d}	5.87 ± 0.12^{d}	5.77±0.26	5.82±0.24	5.59±0.28	6.31±0.18
-7 Day	5.57 ± 0.11^{bc}	5.85 ± 0.20^{cd}	5.71 ± 0.11^{cd}	5.66±0.33	5.76±0.22	5.51±0.15	5.91±0.18
0 Day	4.82 ± 0.14^{a}	5.33 ± 0.18^{abc}	5.08 ± 0.12^{a}	5.07±0.20	5.10±0.30	4.79±0.21	5.36±0.24
7 Day	5.19 ± 0.12^{ab}	5.43 ± 0.15^{abc}	5.31 ± 0.10^{ab}	5.28±0.26	5.16±0.16	5.18±0.19	5.61±0.12
15 Day	5.16 ± 0.18^{ab}	5.36 ± 0.17^{abc}	5.26 ± 0.13^{ab}	5.23±0.26	5.27±0.29	5.20±0.29	5.32±0.19
30 Day	5.37 ± 0.14^{bc}	5.20 ± 0.15^{b}	5.29 ± 0.10^{ab}	5.20±0.18	5.22±0.23	5.32±0.25	5.41±0.17
45 Day	5.26 ± 0.16^{abc}	5.11 ± 0.14^{a}	5.19 ± 0.11^{ab}	5.27±0.20	4.80±0.22	5.21±0.23	5.47±0.18
60 Day	5.28 ± 0.14^{abc}	5.46 ± 0.17^{abc}	5.37 ± 0.11^{abc}	5.50±0.23	5.27±0.26	5.28±0.22	5.41±0.19
Overall	**5.29±0.05**	***5.50±0.06**	**5.39±0.04**	**5.36±0.08**	**5.33±0.09**	**5.27±0.08**	**5.62±0.07**

N= Number of animals; *P<0.05 between control and supplemented groups.
Means bearing uncommon superscripts within the column differ significantly between periods (p<0.05).

combination subgroups (Table 2), probably due to competitive bioavailability of various minerals from these products.

The mean plasma magnesium concentrations were found to be almost consistent during entire peripartum period studied in both Treatment and Control groups with apparently higher values in Treatment group than the Control group at most of the intervals (Table 3). These observations are in agreement with the findings of Hadiya *et al.* (2010) and Patel (2014). However, Theodore *et al.* (2016) found comparatively lower magnesium concentration in nutrients treatment than control group of cows. Very similar trend was also found in Stimvet and oral Exapar alone or in combination groups, being little higher in Stimvet injected group (Table 3). There was a non-significant decline in plasma level of magnesium at calving in all groups.

Table 3: Plasma magnesium concentrations (mg/dl) in Jafarabadi buffaloes given peripartum nutrients supplementation and treatment with Stimvet and Exapar alone or in combination

Peri-partum Period	Overall nutrient groups			Treatment subgroups			
	Control (N=20)	Supplemented (N=20)	Pooled (N=40)	Stimvet (N=10)	Exapar (N=10)	S+E (N=10)	None (N=10)
-45 Day	2.64±0.12	2.84±0.10	2.74±0.08	2.73±0.10	2.77±0.18	2.52±0.19	2.94±0.14
-30 Day	2.51±0.07	2.87±0.12	2.69±0.07	2.79±0.13	2.52±0.14	2.70±0.19	2.74±0.13
-7 Day	2.52±0.10	3.00±0.10	2.76±0.08	2.73±0.13	2.70±0.16	2.86±0.20	2.74±0.17
0 Day	2.45±0.08	2.80±0.09	2.62±0.06	2.75±0.13	2.62±0.12	2.59±0.14	2.53±0.14
7 Day	2.58±0.10	2.87±0.09	2.72±0.07	2.82±0.13	2.62±0.13	2.73±0.17	2.73±0.12
15 Day	2.65±0.10	3.00±0.10	2.82±0.07	2.74±0.14	2.81±0.06	2.94±0.22	2.80±0.15
30 Day	2.59±0.09	2.82±0.09	2.70±0.07	2.75±0.09	2.70±0.01	2.67±0.20	2.69±0.12
45 Day	2.47±0.11	2.72±0.09	2.60±0.07	2.73±0.12	2.51±0.14	2.48±0.18	2.66±0.13
60 Day	2.53±0.09	2.91±0.11	2.72±0.08	2.83±0.13	2.69±0.10	2.71±0.24	2.65±0.13
Overall	**2.55±0.03**	***2.87±0.03**	**2.71±0.02**	**2.76±.0.04**	**2.66±0.04**	**2.69±0.06**	**2.71±0.04**

N= Number of animals; *P<0.05 between control and supplemented groups.
Means within the column do not differ significantly (p>0.05).

Conclusion

The results of peripartum supplementation of chelated ASMM and bypass fat to Jaffarabadi buffaloes revealed that this supplement was beneficial for significant increase in plasma calcium and inorganic phosphorus levels over control group, with drop in the levels on the day of calving, and this rise in minerals was associated with early uterine involution and shorter periods of first estrus postpartum and conception, however, plasma magnesium levels did not reveal significant variation between groups.

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Conflict of Interest:

All authors declare no conflict of interest.

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