RESEARCH ARTICLE

Influence of Bypass Fat and Mineral Supplementation during Transitional Period on Plasma Trace Minerals Profile and Postpartum Fertility in Jafarabadi Buffaloes

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Abstract

This investigation was carried out on 40 advanced pregnant Jafarabadi buffaloes of 2–4 parity equally divided into two groups, viz., control and treatment groups. The control animals were maintained on standard routine farm feeding, and the animals of the treatment group received additional oral supplements of 50 g chelated mineral mixture and 150 g bypass fat daily along with concentrates for 6 weeks prepartum and 8 weeks postpartum. The bypass fat was given @ 15 g/liter of milk produced limiting up to 200 g/head/day from 15–60 days postpartum. Ten animals 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 ecbolic Exapar (n = 5) 2 boli/day for 4 consecutive days postpartum. Blood samples were obtained from all animals by jugular vein puncture on the day –45, –30, –7 and 0 (day of calving), 7, 15, 30, 45, 60 peripartum for estimation of plasma trace minerals, *viz.,* zinc, iron, copper, cobalt, and manganese. The plasma zinc and copper concentrations were found to be higher on day 30 prepartum and again on day 7 postpartum in Stimvet injected groups.Moreover, the overall pooled mean concentration of zinc was significantly higher in nutrients supplemented than the control group. The plasma concentrations of iron, cobalt, and manganese did not reveal any specific trend or significant variations between groups or between periods within any of the groups. Further, the peripartum oral nutrients supplementation significantly shortened the periods of placental expulsion time and uterine involution, with early onset of postpartum first estrus, apparently shorter service period and enhanced pregnancy rate in comparison to control group. The influence of Stimvet and Exapar alone or in combination, however, did not show a significant beneficial effect on these traits, except shortened placental expulsion time and higher plasma zinc and cobalt status. Hence, the Jafarabadi buffalo keepers may be advised to provide additional oral nutrients supplementation in the form of bypass fat and chelated minerals over routine feeding to their animals during a transitional period for improved postpartum fertility.

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Introduction

Minerals have a direct or indirect relationship with the productive and reproductive health of animals. Deficiencies and imbalance of minerals during the periparturient period are associated with disorders like retention of fetal membranes, dystocia, abortion, weak calf syndrome, and vulval discharge (Gupta *et al*., 2005). It is established facts that, apart from energy status, macro and micro-nutrients play an important role in animal reproduction as they form components of metallo-enzymes and enzyme co-factors (Kalasariya *et al.,* 2016; Vala *et al.,* 2018). Some of these minerals are the components of hormones and thus directly regulate the 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). Deficiency of minerals during advance pregnancy especially calcium, zinc, and selenium upsets the uterine and placental functions. Lower concentrations of circulatory minerals result in impaired reproductive function leading to the cessation of cyclic activity (Martson *et al*., 1972). Infertility due to nutritional deficiency is usually characterized by a failure of estrus or cessation of the estrous cycle in pubertal and postpartum animals. Hence this study was aimed to evaluate the role of peripartum supplementation of chelated mineral

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mixture and bypass fat on plasma trace minerals profile and postpartum fertility in Jafarabadi buffaloes.

Materials and methods

The study was conducted from September 2015 to June 2017 on 40 advanced pregnant Jafarabadi buffaloes of 2–4 parity selected from Cattle Breeding Farm, JAU, Junagadh. The prior approval from the Institutional Animal Ethics Committee was obtained for the use of farm animals in this study. All the 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 randomly 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 @ 50 g chelated mineral mixture (developed by AAU, Anand, Composition: Calcium 20%, Phosphorus 12%, Sulfur 02%, Zinc 2.25 %, Manganese 0.12%, Cobalt 0.014%, Copper 0.20% and Iodine 0.030%) and 150 g bypass fat (Sunergy, Polchem, Malaysia) along with concentrates for 6 weeks prepartum and 8 weeks postpartum, and then bypass fat was however given @ 15 g/lit of milk limiting to 200 g/day/head from 15-60 days postpartum/lactation. Both the main groups were 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, Wellcon Animal Health Pvt Ltd, Mumbai) around day 45 prepartum and on day of calving, keeping 10 animals each as Stimvet controls. They were further subgrouped and one of them (n = 5) received ecbolic boli Exapar (Ayurvet Ltd., Delhi) 2 boli/day for 4 consecutive days postpartum. The puerperal events, uterine involution and first/fertile estrus postpartum were recorded for each animal.

Blood samples (7 mL) were obtained from all animals by jugular vein puncture in heparinized vacutainers on days -45, -30, -7, 0, 7, 15, 30, 45 and 60 (day 0 is the day of parturition). The plasma was separated immediately after the collection of blood by centrifugation of samples at 3000 rpm for 10 minutes. The plasma was stored at -80°C with a drop of merthiolate until analyzed. The plasma samples (0.5 mL each) were wet digested using mixture (4.5 mL) of perchloric-nitric- and sulphuric acid in the ratio of 1:4:1 on a hot plate in volumetric flasks till white transparent residues. The residues were then diluted with triple glass distilled water, and volume was made up to 25 mL. The plasma zinc, iron, copper, cobalt, and manganese were determined on ICP-OES (Optical Emission Spectrometer; Model Optima 7000 DV; Perkin-Elmer, USA) machine against standard curves. The data on a mineral profile were analyzed statistically using online SPSS software version 20.0 for ANOVA, DMRT, and 't' test for group and period effects (Snedecor and Cochran, 1994).

Results and discussion

The mean plasma levels of trace minerals, *viz.,* Zn, Fe, Cu, Co, Mn found during peripartum periods from -45 days till +60 days in Jafarabadi buffaloes of control and treatment groups as well as Simvet, Exapar, S + E and non-treated subgroups (irrespective of main control or treatment groups) are presented in Tables 1 and 2. The variations in mean values for subgroups treated with Stimvet, Exapar, S+E and none under main control and oral nutrient treatment (bypass fat + ASMM) were found to be insignificant between subgroups and between periods within the subgroup or showed a consistent trend; hence their pooled values are only presented in two tables here.

Plasma Zinc and Iron

The peripartum mean plasma zinc and iron levels were high in Treatment group as compared to Control group almost throughout the study period with significant (p <0.01) difference in overall pooled means (zinc 1.184 ± 0.005 vs. 1.093 ± 0.006 ppm; iron 2.571 ± 0.014 vs. 2.375 ± 0.010 ppm, (Table 1), although the differences between periods were not significant for any of these elements. The higher zinc in Treatment group can be attributed to oral supplementation of an extra chelated mineral mixture containing zinc in this group as compared to the control group. These findings are in accordance with the observations made by Khan *et al.* (2015) and Kalasariya *et al.* (2016).

Further, the plasma zinc and iron concentrations fluctuated inconsistently between Stimvet, Exapar, Stimvet+Exapar, and non-treated control subgroups irrespective of major Control and Treatment groups. Yet the values of zinc were higher in Stimvet treated subgroups, particularly around day 30 prepartum and day 7 postpartum, *i.e.*, in periods subsequent to parenteral injections of micro-minerals (Table 1). This could be due to the cumulative effect of supplementation of these minerals through both oral ASMM and Stimvet injection. Moreover, the non-significant differences fond between groups and between periods may be attributed to oral ASMM already being fed to all the animals on the farm as a routine. Similar observations were reported by Theodore *et al.* (2016) in crossbred cows and Kalasariya *et al.* (2016) in buffaloes using oral mineral supplements with or without parenteral injections of micro-minerals.

Plasma Copper, Cobalt and Manganese

The plasma levels of copper and cobalt were consistently higher (p <0.05) in oral nutrients supplemented/treatment group over Control group at all the intervals throughout the study with highly significant (p <0.01) difference in overall pooled means (copper 1.217±0.08 vs. 1.028±0.005 ppm; cobalt 0.543 ± 0.011 vs 0.447 ± 0.011 ppm (Table 2). The present findings on mean plasma copper and cobalt levels and a fluctuating trend observed in the peripartum period in buffaloes of both the groups compared well with the

Table 1: Effect of nutrient supplementation (treatment) over control as well as Stimvet and Exapar alone or in combination on peripartum plasma zinc and iron concentrations in buffaloes

	Overall nutrient groups		*Treatment subgroups*			
Peri-partum period	*Control (N = 20)*	*Treatment (n = 20)*	*Stimvet (N = 10)*	*Exapar (N = 10)*	*S+E (N = 10)*	*None (N = 10)*
			Plasma zinc concentration (ppm)			
–45 day	1.085 ± 0.019	1.123 ± 0.009	1.125 ± 0.031	1.110 ± 0.028	1.108 ± 0.021	1.113 ± 0.022
–30 day	1.091 ± 0.018	1.177 ± 0.019	1.180 ± 0.047	1.117 ± 0.019	1.117 ± 0.019	1.162 ± 0.030
–7 day	1.102 ± 0.017	1.168 ± 0.010	1.142 ± 0.024	1.129 ± 0.022	1.127 ± 0.021	1.142 ± 0.024
0 day	1.085 ± 0.010	1.208 ± 0.023	1.143 ± 0.022	1.128 ± 0.024	1.131 ± 0.020	1.184 ± 0.052
7 day	1.099 ± 0.017	1.178 ± 0.013	1.172 ± 0.033	1.125 ± 0.022	1.120 ± 0.020	1.137 ± 0.023
15 day	1.070 ± 0.005	*1.198 ± 0.012	1.137 ± 0.026	1.130 ± 0.025	1.116 ± 0.019	1.154 ± 0.027
30 day	1.090 ± 0.017	1.169 ± 0.010	1.138 ± 0.028	1.117 ± 0.023	1.116 ± 0.018	1.148 ± 0.024
45 day	1.098 ± 0.018	1.194 ± 0.020	1.175 ± 0.046	1.149 ± 0.026	1.110 ± 0.019	1.149 ± 0.025
60 day	1.120 ± 0.025	1.178 ± 0.011	1.141 ± 0.029	1.151 ± 0.030	1.151 ± 0.028	1.153 ± 0.030
Overall	1.093 ± 0.006	**1.184 ± 0.005	1.151 ± 0.011	1.130 ± 0.008	1.124 ± 0.007	1.150 ± 0.010
			Plasma iron concentration (ppm)			
–45 day	2.376 ± 0.025	2.379 ± 0.029	2.386 ± 0.063	2.363 ± 0.061	2.366 ± 0.044	2.415 ± 0.035
–30 day	2.369 ± 0.025	2.450 ± 0.032	2.489 ± 0.046	2.438 ± 0.076	2.479 ± 0.060	2.482 ± 0.045
–7 day	2.368 ± 0.023	2.528 ± 0.043	2.477 ± 0.064	2.446 ± 0.040	2.460 ± 0.058	2.408 ± 0.059
0 day	2.379 ± 0.032	2.611 ± 0.044	2.457 ± 0.063	2.550 ± 0.062	2.533 ± 0.057	2.440 ± 0.079
7 day	2.363 ± 0.024	2.573 ± 0.038	2.496 ± 0.061	2.493 ± 0.058	2.449 ± 0.053	2.435 ± 0.057
15 day	2.352 ± 0.027	2.563 ± 0.030	2.439 ± 0.063	2.502 ± 0.046	2.463 ± 0.059	2.424 ± 0.044
30 day	2.342 ± 0.026	2.550 ± 0.059	2.362 ± 0.073	2.419 ± 0.082	2.491 ± 0.048	2.420 ± 0.072
45 day	2.385 ± 0.024	2.556 ± 0.042	2.496 ± 0.055	2.532 ± 0.048	2.422 ± 0.039	2.432 ± 0.074
60 day	2.439 ± 0.057	2.609 ± 0.058	2.678 ± 0.098	2.473 ± 0.070	2.571 ± 0.094	2.375 ± 0.043
Overall	2.375 ± 0.010	**2.57 ± 0.014	2.487 ± 0.023	2.487 ± 0.020	2.481 ± 0.019	2.437 ± 0.019

N, Number of animals; *p <0.05, ** p <0.01 between control and treatment group

findings of Akhtar *et al*. (2009) and Kalasariya *et al.* (2016). In contrast to the findings of the present study, some workers found relatively lower plasma copper values (Khan *et al.,* 2015). In the present study, significantly higher copper and cobalt values observed in Treatment group compared to Control group from day 30 prepartum to day 60 postpartum may be due to the effect of supplemented mineral mixture and injection Stimvet used in this group. This observation corroborated well with findings of Kalasariya *et al.* (2016), who also used similar products in transitional buffaloes of tribal farmers. The mean plasma manganese levels appeared more or less constant throughout the study in all groups, with the overall means of 0.049 ± 0.001 and 0.055 ± 0.001 ppm in control and nutrient supplemented groups, respectively, which was however in contradiction to the findings of Kalasariya *et al.* (2016), who found significantly higher manganese profile in Stimvet injected and treatment group than the control group of transitional buffaloes of tribal belt.

However, the influence of Stimvet and Exapar alone or in combination when analyzed irrespective of oral nutrients supplementation did not reveal any significant or consistent trend on these elements, perhaps due to optimized requirements of nutrients through oral supplement itself, thus masking the additional supply through parenteral route (Tables 2), but it had a clear beneficial effect in nonoral supplemented control group. Hidiroglou (1979) stated that various minerals (Cu, Co, Se, Mn, Zn, I) can influence the reproductive performance of ruminants. Reproductive failure may be induced by deficiency of single or combined trace elements and by their imbalances. Reproductive failure in the female is a manifestation of zinc deficiency. Kalasariya *et al.* (2016) in their experiment on the high plane of nutrition with Stimvet injection in transitional buffaloes of tribal areas found significantly higher plasma zinc and manganese levels (p <0.05) at most intervals compared to control groups. The plasma iron and copper levels were significantly (p <0.05) lower in both the groups at calving as compared to pre- and postpartum profile and apparently, higher in treated than the control group. The plasma manganese levels varied significantly (p <0.05) only on the day of calving and day 45 postpartum between groups. Theodore *et al.* (2016) found

Table 2: Effect of nutrient supplementation (treatment) over control as well as Stimvet and Exapar alone or in combination on peripartum plasma copper, cobalt and manganese levels in buffaloes

	Overall nutrient groups			*Treatment subgroups*		
Peripartum Period	*Control (N = 20)*	*Treatment (N = 20)*	*Stimvet (N = 10)*	*Exapar (N = 10)*	*S+E (N = 10)*	*None (N = 10)*
			Plasma copper concentration (ppm)			
–45 Day	1.023 ± 0.013	1.103 ± 0.021	1.100 ± 0.040	1.108 ± 0.033	1.117 ± 0.044	1.112 ± 0.038
–30 Day	1.031 ± 0.014	*1.195 ± 0.018	1.147 ± 0.047	1.124 ± 0.030	1.135 ± 0.050	1.126 ± 0.033
–7 Day	1.033 ± 0.013	*1.198 ± 0.025	1.118 ± 0.044	1.109 ± 0.043	1.093 ± 0.031	1.140 ± 0.040
0 Day	1.027 ± 0.011	*1.268 ± 0.028	1.127 ± 0.051	1.175 ± 0.050	1.151 ± 0.057	1.136 ± 0.043
7 Day	1.038 ± 0.022	*1.200 ± 0.023	1.148 ± 0.049	1.097 ± 0.040	1.127 ± 0.037	1.105 ± 0.039
15 Day	1.007 ± 0.010	*1.248 ± 0.020	1.119 ± 0.041	1.136 ± 0.036	1.136 ± 0.055	1.119 ± 0.049
30 Day	1.024 ± 0.015	*1.205 ± 0.027	1.109 ± 0.049	1.092 ± 0.038	1.136 ± 0.050	1.121 ± 0.034
45 Day	1.032 ± 0.020	*1.219 ± 0.023	1.146 ± 0.047	1.154 ± 0.047	1.085 ± 0.046	1.117 ± 0.031
60 Day	1.040 ± 0.019	*1.178 ± 0.020	1.109 ± 0.033	1.090 ± 0.036	1.122 ± 0.040	1.113 ± 0.034
Overall	1.028 ± 0.005	**1.217 ± 0.08	1.125 ± 0.014	1.121 ± 0.013	1.124 ± 0.015	1.121 ± 0.012
			Plasma cobalt concentration (ppm)			
–45 Day	0.494 ± 0.045	0.530 ± 0.038	0.556 ± 0.062	0.517 ± 0.058	0.499 ± 0.053	0.477 ± 0.065
-30 Day	0.425 ± 0.021	*0.540 ± 0.032	0.546 ± 0.048	0.470 ± 0.047	0.432 ± 0.029	0.490 ± 0.338
–7 Day	0.469 ± 0.043	*0.572 ± 0.024	0.578 ± 0.050	0.450 ± 0.051	0.530 ± 0.045	0.525 ± 0.058
0 Day	0.436 ± 0.027	*0.503 ± 0.027	0.478 ± 0.042	0.448 ± 0.034	0.466 ± 0.044	0.495 ± 0.041
7 Day	0.427 ± 0.029	*0.525 ± 0.027	0.502 ± 0.042	0.505 ± 0.050	0.446 ± 0.039	0.451 ± 0.038
15 Day	0.497 ± 0.034	*0.559 ± 0.034	0.505 ± 0.043	0.555 ± 0.058	0.555 ± 0.058	0.497 ± 0.035
30 Day	0.450 ± 0.023	*0.573 ± 0.037	0.582 ± 0.039	0.553 ± 0.050	0.442 ± 0.042	0.468 ± 0.064
45 Day	0.413 ± 0.041	*0.487 ± 0.029	0.456 ± 0.043	0.483 ± 0.061	0.399 ± 0.033	0.463 ± 0.063
60 Day	0.407 ± 0.029	**0.596 ± 0.032	0.502 ± 0.053	0.450 ± 0.051	0.495 ± 0.051	0.559 ± 0.056
Overall	0.447 ± 0.011	**0.543 ± 0.011	0.523 ± 0.016	0.491 ± 0.017	0.474 ± 0.015	0.492 ± 0.017
			Plasma manganese concentration (ppm)			
Overall	0.049 ± 0.001	0.055 ± 0.001	0.053 ± 0.001	0.052 ± 0.001	0.049 ± 0.001	0.054 ± 0.001

N = Number of animals; *p <0.05, ** p <0.01 between control and treatment group.

highest mean copper values on day 3 prepartum and day of calving, while iron showed a consistently increasing trend from day 3 prepartum to postpartum period in bypass fat and mineral supplemented transitional crossbred cows.

Further, the zinc values fluctuated between the pre- and postpartum days with the lowest on the day of calving, while the mean cobalt values showed no distinct changes in the periods studied. Moreover, no significant differences were observed for the microminerals profile between treatment and control groups. The present findings to some extent are in accordance with these reports.

Puerperal Event and Postpartum Fertility

The peripartum feeding of bypass fat and chelated minerals in buffaloes significantly reduced placental expulsion time (3.93 ± 0.24 *vs* 7.18 ± 0.72 hrs; p <0.01) and period of uterine involution (32.75 ± 0.57 *vs* 37.00 ± 0.56 days; p <0.05) over control. The mean service period observed was also significantly (p <0.05) reduced (107.10 ± 4.43 *vs* 133.65 ± 6.04 days) in nutrients supplemented group. However, no significant effect of Stimvet injection and Exapar boli either alone or in combination was observed over control group on these traits. These findings corroborated well with the reports of Mavi *et al.* (2006) for vitamin E and Se supplemented buffaloes. Similarly, Khan *et al.* (2015), Patel *et al.* (2015), Mane *et al.* (2016), Kalasariya *et al.* (2016) and Theodore *et al.* (2016) found a beneficial effect of peripartum nutritional supplementation on uterine involution and postpartum fertility in cattle and buffaloes. The present observations clearly indicated that there is a positive effect of peripartum nutrient supplementation in buffaloes so far as uterine involution and fertility are concerned. Dhakal (1999) recorded expulsion of the placenta in 100 percent animals with a conception rate of 72 vs. 40 % in buffaloes and 55 vs. 25 % in cows following the use of Exapar. Similarly, a significant positive effect on the expulsion of the placenta and uterine involution was reported in dairy animals following the use of Exapar by Thakur *et al.* (2013). 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.

Conclusion

In general, the plasma zinc and copper concentrations were found to be higher on day 30 prepartum and again on day 7 postpartum in Stimvet injected and pooled groups. Moreover, the overall pooled mean concentrations were significantly ($p < 0.01$) higher in Treated than Control group. This could be due to the cumulative effect of supplementation of zinc and copper through both oral ASMM and Stimvet injection. However, the iron, cobalt and manganese levels did not show such variations, even though manganese is also there in Stimvet injection. The present finding of insignificant variations in trace minerals profile could be due to optimum supplementation of these nutrients in the ration of experimental animals that probably masked the additional supplements used, though it significantly improved uterine health and postpartum fertility.

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