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IMPACT OF PERIPARTUM NUTRITIONAL SUPPLEMENTATION ON PLASMA MINERALS PROFILE AND POSTPARTUM FERTILITY IN BUFFALOES

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Abstract: Eighty five advanced pregnant (~8 months) pluriparous buffaloes selected at farmers' doorstep in three tribal villages of Gujarat were randomly divided into control (n=45) and nutrients treatment (n=40) groups to study their mineral profile and postpartum fertility. The buffaloes of treatment group, in addition to farmers feeding schedule (control), received daily 1.5 kg compounded concentrate mixture (22 % CP) and 50 g of chelated ASMM for 2 months each pre- and post-partum. Further, 15 buffaloes each of control and treatment group were injected intramuscularly with 5 ml of micro-minerals (each ml containing Se, Zn, Cu and Mn @ 5, 40, 15 and 10 mg, respectively), twice 2 months before and on the day of calving, keeping rest of the animals (control, n=30 and treatment, n=25) as control. Blood sampling done on days -60, -30, -15, 0, 15, 30, 45 and 60 peripartum revealed that the plasma calcium levels in treatment group were significantly higher ($p<0.05$) than control group at most intervals including the overall mean, and dropped significantly on the day of calving. The mean plasma inorganic phosphorus levels fluctuated non-significantly during pre- and postpartum periods, and reduced significantly $p<0.05$) on the day of calving. The plasma magnesium values under treatment group were apparently lower at all intervals than the control group and did not vary between periods. The plasma zinc levels especially in micro-minerals injected subgroups were significantly higher ($p<0.05$) at most intervals, suggesting sustained release of zinc from the site of injection. The plasma iron and copper levels were lower significantly ($p<0.05$) in both the groups at calving as compared to pre- and postpartum profile. The levels in micro-minerals injected subgroups were apparently higher than in non-injected subgroups. The plasma manganese levels were consistently higher in treatment as compared to control group at all the peripartum intervals, but varied significantly ($p<0.05$) only on the day of calving and day 45 postpartum. Further, the subgroups injected with micro-minerals showed consistently higher plasma manganese levels over non-injected subgroups. It was concluded that nutrients supplementation in terms of high protein concentrate, ASMM and injection of sustained release micro-minerals (Se, Zn, Cu and Mn) during transition period was beneficial in escalating blood minerals profile that improved the postpartum reproductive performance significantly in respect of first postpartum estrus, service period and conception rate in buffaloes under field.

Keywords: Buffalo, Transition period, Nutritional management, Minerals profile, Fertility.

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INTRODUCTION

Sustainability of a dairy production system is fully dependent on efficient reproductive performance of cows and buffaloes, which in turn is influenced by peripartum nutritional and health status. Factors such as limited energy intake, lower body reserves and peripartum diseases can delay the uterine involution and thereby the ovarian recrudescence. Mineral status of animals is a direct reflection of their presence, absence, deficiency or excess in soil, fodder and ration. There are hardly few studies (Patel *et al.* 2009; Khan *et al.* 2015) addressing transitional period through nutritional interventions in terms of energy, protein and minerals in buffaloes under Indian context, which has been proved highly beneficial in reducing puerperal disorders and improve postpartum fertility in cattle (Sihag and Yadav 2007; Theodore 2015). Hence, this study was aimed to assess the effect of incorporating protein and minerals in the ration of transitional buffalo-cows including micro-minerals injections on their plasma minerals profile and postpartum fertility.

MATERIALS AND METHODS

The advanced pregnant (~8 months) pluriparous buffaloes (n=85) of 2-4 parity were selected in 3 tribal villages of Mahisagar district in Gujarat. The experiment was initiated at about two month prepartum by dividing the selected animals randomly into control and treatment groups in each village. All the registered animals were dewormed using Fenbendazole plus Ivermectin bolus 3.0 g (Fendikind plus, Vet Mankind) orally twice 2 months before and again on the day of calving, and were maintained hygienically at farmers' doorstep. They were fed green fodder (8-10 kg), paddy/maize straw (5-7 kg), home-made or compounded concentrate – Panchamrut dan (1.5-2.0 kg) and mineral mixture (30-35 g), as per farmers feeding practices in the region. The buffaloes of treatment group, in addition to farmers feeding schedule/control, received daily extra 1.5 kg compounded concentrate mixture (22 % CP) and 50 g of area specific chelated mineral mixture-ASMM (both developed by AAU, Anand) from 2 months prepartum to 2 months postpartum. The composition (on DM basis) of chelated mineral mixture & concentrate used was as under.

Chelated Mineral Mixture (%)		***Compounded Concentrate (%)***	
Calcium:	20.00	Maize	11.00
Phosphorus:	12.00	Molasses	10.00
Sulphur:	02.00	Soyabean	17.50
Zinc:	2.25	De-oiled rice bran	56.50
Manganese:	0.12	Min. Mixture	2.00

Cobalt:	0.014	Salt	1.00
Copper:	0.20	Urea	1.00
Iodine:	0.030	Bypass fat	1.00

Further, the animals of both control and treatment groups were randomly subdivided into two subgroups for parentral injection of sustained release micro-minerals. The buffaloes of subgroup-I (n=15 each) were additionally injected with 5 ml of micro-minerals (Stimvet$^{(R)}$, Wellcon Animal Health Pvt Ltd; each ml containing Se, Zn, Cu, Mn 5, 40, 15 and 10 mg, respectively), twice 2 months before and on the day of calving, while those of subgroup-II (control, n=30; treatment, n=25) served as Stimvet$^{(R)}$ controls. The animals were regularly monitored for periparturient events. The period for uterine involution (by weekly rectal palpation) and first estrus postpartum were recorded. The buffaloes exhibiting estrus beyond 55-60 days postpartum were only inseminated. Pregnancy was confirmed per rectum by 45 days after last AI.

Blood samples were collected from the representative animals of both the groups on the days -60, -30, -15, 0, 15, 30, 45, and 60 peripartum by jugular vein puncture in heparinized vacutainers. The blood samples were immediately centrifuged and plasma samples separated out were stored at -20°C with a drop of merthiolate (0.1%) until analyzed. The levels of plasma calcium, phosphorus and magnesium were estimated by using standard procedures and assay kits of Coral Clinical System, Goa on biochemistry analyzer (Nova 2021, Analytical Technologies Pvt Ltd). The micro-minerals, zinc, iron, copper, cobalt and manganese were estimated in blood plasma samples after 1:2 dilution with Milli-Q water without wet digestion on Atomic Absorption Spectrophotometer (Model AAS 4141, ECI Ltd). The data generated on different plasma minerals profiles were analysed statistically using ANOVA and ‘t’ test to compare differences between periods in treated and control groups and between micro-minerals injected and non-injected subgroups on different days peripartum and correlated with postpartum fertility traits.

RESULTS AND DISCUSSION

The mean plasma concentrations of macro (Ca, P, Mg) and micro (Zn, Fe, Cu, Co, Mn) minerals recorded during different fortnightly intervals peripartum in the buffaloes under treatment and control groups and subgroups are presented in Table 1 and 2**.**

Effect on Plasma Macro-Minerals Profile

The mean plasma calcium levels in the buffaloes under both treatment and control groups and subgroups fluctuated non-significantly during prepartum period, and dropped suddenly and significantly ($p<0.05$) on the day of calving. These values then gradually increased in the subsequent postpartum days with occasional significant difference in some group and subgroup (Table 1). Further, the mean plasma calcium levels in the treatment group were significantly higher ($p<0.05$) as compared to control group at most intervals including the overall pooled mean (8.37±0.06 vs. 7.69±0.07 mg/dl). This may be attributed to peripartum nutrient supplementation in the form of chelated mineral mixture and high protein concentrate in the ration. These findings corroborated with the observations of Hadiya *et al.* (2009). Theodore (2015) also found sudden drop in calcium level at calving and thereafter increase on various days postpartum in crossbred cows supplemented with bypass fat and minerals peripartum, and similar were the observations of Khan *et al.* (2015) and Patel *et al.* (2015) in buffaloes. In the present study, the calcium levels were found to be lower yet within the normal physiological range (8-11 mg/dl).

The mean plasma inorganic phosphorus levels fluctuated non-significantly during peripartum periods, but reduced significantly ($p<0.05$)) on the day of calving. The overall mean values in the treatment and control group did not vary at any of the intervals. Moderate reduction in the levels of phosphorus at and after calving might be due to the necessity of this element for the colostrum synthesis and enhanced carbohydrate metabolism. The plasma inorganic phosphorus values in the treatment subgroups remained almost constant at all the days, except on day 15 postpartum (Table 1). The present trend and mean values concurred with Shah *et al.* (2003) and Singh *et al.* (2005). However, others documented much lower values than the present one (Kumar *et al.* 2000).

The mean plasma magnesium values under treatment group were apparently lower at all intervals when compared with the control group, without much variation between fortnightly intervals peripartum, but differed significantly only in the overall pooled means (2.43±0.05 vs. 2.59±0.04 mg/dl; Table 1). The trend and mean magnesium concentrations found in the present study were in agreement with the report of Piccione *et al.* (2012). On the contrary, higher values have been documented by Hadiya *et al.* (2009), Yokus *et al*. (2010) and Patel *et al.* (2015).

Effect on Trace Minerals Profile

The zinc levels especially in Stimvet injected subgroups were significantly higher ($p<0.05$) than non-injected subgroups at most intervals peripartum (Table 2), suggesting sustained release of zinc from the injection site. The present findings with respect to values and trend are in accordance with Khan *et al.* (2015). The plasma zinc concentration in treatment group was significantly ($p<0.05$) lower as compared to control group on days 45 and 60 postpartum and thereby the overall pooled values (0.99±0.018 vs. 1.11±0.027 ppm), may be because of its association with folliculogenesis and steroidogenesis, as it did not vary during prepartum and early postpartum periods. These findings were in accordance with the observations of Singh *et al.* (2004), Patel *et al.* (2006) and Hadiya *et al.* (2009). The present values are however lower as compared to those reported by Parikh (2009).

The mean plasma iron levels were significantly lower in both treatment and control groups at calving as compared to pre- and postpartum profile, which can be a normal physiological phenomenon due to voluminous loss of blood/haemolysis at parturition. The mean plasma iron concentration showed an increasing trend from day 60 to 15 prepartum and decreased on the day of calving and then gradually increased in treatment group (Table 2). This trend of iron concurred with Akhtar *et al.* (2009), who also reported decreasing tendency of iron levels towards parturition. The overall pooled plasma concentrations of iron in treatment and control groups were almost similar. The overall pooled mean values were in agreement with the reports of Patel *et al.* (2006) and Ammu *et al.* (2013). However, relatively higher values have been documented by Hadiya *et al.* (2009) in cattle. Iron is abundantly present in all the feed and fodder; hence a deficiency in adult ruminant seems improbable.

The mean plasma copper levels followed the trend of iron with significantly lower values on the day of calving in most subgroups. The overall pooled mean plasma copper in treatment group was significantly lower ($p<0.05$) as compared to control group (1.10±0.016 vs. 1.17±0.024 ppm). The levels in micro-minerals injected subgroups in both nutrients treated and control groups were also apparently and/or significantly higher than in non-injected subgroups (Table 2). These findings of copper over different peripartum periods closely corroborated with the report of Khan *et al.* (2015) in summer calving buffaloes. Similar trend has also been documented by Akhtar *et al.* (2009) and Parikh (2009) in buffaloes and Hadiya *et al.* (2009) in crossbred cows.

The effect of period or days of pre- and postpartum phase was observed to be non-significant on mean plasma cobalt levels, as the values were consistent in all groups. Moreover, the mean values on day of calving and overall pooled only were significantly higher in micro-

minerals injected than non-injected control subgroup (Table 2). The overall pooled values of cobalt in treatment and control groups were identical (0.03±0.000 ppm), showing no any beneficial effect of nutritional and mineral supplementation. In contrast, some workers reported relatively higher plasma cobalt values (Singh *et al.,* 2004 and Patel *et al.,* 2006).

The mean plasma manganese concentrations (ppm) were consistently higher in the nutrient treatment group as compared to control group at all the peripartum intervals studied, but varied significantly ($p<0.05$) only on the day of calving (0.05±0.003 vs. 0.03±0.002) and on day 45 postpartum (0.05±0.003 vs. 0.04±0.002). Further, the micro-minerals injected subgroups of both treatment and control groups showed consistently higher plasma manganese levels over non-injected subgroups, but the differences were significant only on the day of calving and in overall means under nutrient treated group, and on days 30-60 postpartum in control group. These observations clearly indicated the beneficial effect of injected manganese. The deficiency of manganese suppresses fertility in both the male and the female. It is responsible for silent estrus and anoestrus or irregular estrus (Brown and Casillas, 1986), decreased conception rate and abortions in females. Manganese is important in cholesterol synthesis (Keen and Zidenberg-Cheer, 1990), which in turn is necessary for the synthesis of steroids like progesterone and estrogen. Decreased concentration of these steroids in circulation following manganese deficiency may lead to reproductive abnormalities. The present findings are to some extent in accordance with the observations of Khan *et al.* (2015) during peripartum period in buffaloes, but their values were almost ten times higher than the present ones.

Postpartum Fertility

As compared to control group, the mean time for uterine involution (29.39±0.50 vs 32.12±0.82 days, $p<0.05$), first postpartum estrus (67.65±1.67 vs 79.43±3.06 days, $p<0.01$) and service period (90.89±4.41 vs 105.09±4.76 days, $p<0.05$) were significantly shorter with higher overall conception rate (55.00 and 40.00 %) in treatment group. Further, the buffaloes injected with Stimvet had apparently or significantly beneficial effect on these traits in both treatment and control groups. Similar beneficial results with nutrients and/or vitamin-mineral supplementation peripartum in dairy cows and buffaloes have also been documented by some workers (Patel *et al.* 2009; Abdulkareem *et al.* 2012; Dhami *et al.* 2015; Khan *et al.* 2015). The role of micro-nutrients, energy and protein in animal reproduction is well established. Early onset of postpartum estrus with satisfactory conception rates have been attributed to combined effect of mineral and vitamin supplementation because of their positive effect on

steroid synthesis and release, follicular growth and symptoms of ovulatory estrus (Srivastava 2008).

Table 1: Mean plasma macro-minerals levels during peripartum period in buffaloes under nutrients supplemented and control groups and subgroups

Days peri-partum	Treatment group			Control group		
	Subgroup-I (n=9)	Subgroup-II (n=9)	Pooled (n=18)	Subgroup-I (n=7)	Subgroup-II (n=7)	Pooled (n=14)
Plasma calcium levels (mg/dl)						
-60	8.03±0.28	7.89±0.23^{ab}	7.96±0.18^{b}	7.93±0.31	7.72±0.34^{ab}	7.82±0.22^{b}
-30	8.74±0.37	8.71±0.23^{b}	8.72^{p}±0.21^{b}	7.92±0.23	7.89±0.32^{ab}	7.90^{q}±0.19^{b}
-15	8.60±0.20	8.47±0.23^{b}	8.54^{p}±0.15^{b}	7.83±0.21	7.70±0.24^{ab}	7.77^{q}±0.15^{b}
0	7.93±0.20	7.73±0.20^{a}	7.83^{p}±0.14^{a}	7.10±0.20	7.03±0.27^{a}	7.06^{q}±0.16^{a}
15	8.44±0.27	8.12±0.20^{ab}	8.28±0.17^{ab}	7.82±0.48	7.67±0.29^{ab}	7.74±0.27^{b}
30	8.21±0.21	8.51±0.29^{b}	8.36^{p}±0.18^{b}	7.68±0.27	7.81±0.17^{ab}	7.74^{q}±0.15^{b}
45	8.30±0.29	8.40±0.22^{ab}	8.35^{p}±0.18^{b}	7.70±0.12	7.78±0.30^{ab}	7.74^{q}±0.16^{b}
60	8.46±0.27	8.43±0.23^{ab}	8.44^{p}±0.17^{b}	7.58±0.19	7.94±0.12^{b}	7.76^{q}±0.12^{b}
Overall	8.40±0.09	8.34±0.08	8.37^{p}±0.06	7.70±0.10	7.69±0.10	7.69^{q}±0.07
Plasma inorganic phosphorus levels (mg/dl)						
-60	5.33±0.13^{b}	5.16±0.16^{b}	5.25±0.10^{b}	5.27±0.09^{bc}	5.17±0.24^{b}	5.22±0.12^{bc}
-30	5.23±0.14^{b}	5.25±0.17^{b}	5.24±0.11^{b}	5.32±0.18^{c}	5.16±0.19^{b}	5.24±0.13^{c}
-15	5.12±0.17^{b}	5.03±0.13^{ab}	5.08±0.10^{bc}	5.30±0.13^{bc}	4.87±0.27^{ab}	5.09±0.15^{bc}
0	4.41±0.11^{a}	4.67±0.12^{a}	4.54±0.08^{a}	4.64±0.11^{a}	4.36±0.19^{a}	4.50±0.11^{a}
15	4.54^{x}±0.13^{a}	5.15^{y}±0.09^{b}	4.84±0.11^{b}	4.87±0.16^{ab}	4.85±0.05^{ab}	4.86±0.08^{b}
30	5.00±0.22^{b}	5.01±0.16^{ab}	5.01±0.13^{bc}	5.09±0.10^{bc}	4.99±0.20^{b}	5.04±0.11^{bc}
45	5.02±0.15^{b}	5.14±0.13^{b}	5.08±0.10^{bc}	5.26±0.14^{bc}	4.88±0.14^{ab}	5.07±0.11^{bc}
60	5.12±0.18^{b}	5.17±0.08^{b}	5.15±0.09^{bc}	5.31±0.16^{bc}	5.04±0.11^{b}	5.17±0.10^{bc}
Overall	4.97±0.06	5.07±0.05	5.02±0.04	5.13^{x}±0.06	4.92^{y}±0.07	5.02±0.05
Plasma magnesium levels (mg/dl)						
-60	2.47±0.28	2.63±0.16	2.55±0.16	2.70±0.16	2.63±0.22	2.66±0.13
-30	2.45±0.24	2.49±0.13	2.47±0.13	2.44±0.11	2.67±0.10	2.56±0.08
-15	2.53±025	2.37±0.12	2.45±0.14	2.54±0.15	2.69±0.19	2.61±0.12

0	2.34±0.24	2.35±0.12	2.35±0.13	2.42±0.18	2.51±0.14	2.46±0.11
15	2.49±0.29	2.32±0.12	2.41±0.15	2.43±0.12	2.68±0.16	2.56±0.10
30	2.42±0.26	2.26±0.17	2.34±0.15	2.47±0.14	2.67±0.16	2.57±0.11
45	2.41±0.24	2.63±0.10	2.52±0.13	2.60±0.13	2.68±0.12	2.64±0.09
60	2.30±0.30	2.47±0.11	2.38±0.15	2.60±0.13	2.59±0.13	2.59±0.09
Overall	2.41±0.09	2.44±0.05	2.43^{p}±0.05	2.54±0.05	2.64±0.05	2.59^{q}±0.04

Subgroup-I = Stimvet 5 ml i/m; Subgroup-II = Stimvet control; Day 0 = Day of calving.
Means bearing uncommon superscripts within the column differ significantly between periods (abc) and those within the row differ significantly between sub-groups (xy) or between pooled values (pq) (P<0.05).

Table 2: Mean plasma Trace minerals profile during peripartum period in buffaloes under nutrients supplemented and control groups and subgroups

Days peri-partum	Treatment group			Control group		
	Subgroup-I (n=9)	Subgroup-II (n=9)	Pooled (n=18)	Subgroup-I (n=7)	Subgroup-II (n=7)	Pooled (n=14)
Plasma zinc levels (ppm)						
-60	0.83 ± 0.104^{a}	0.94±0.040	0.89 ± 0.056^{a}	$1.11^{x}\pm0.062^{a}$	0.93^{y}±0.046	1.02±0.045
-30	1.09 ± 0.112^{abc}	0.93±0.036	1.01 ± 0.060^{ab}	$1.35^{x}\pm0.073^{abc}$	0.97^{y}±0.036	1.16±0.066
-15	$1.22^{x}\pm0.109^{c}$	0.93^{y}±0.037	1.07 ± 0.066^{b}	$1.29^{x}\pm0.085^{abc}$	0.87^{y}±0.029	1.08±0.074
0	$1.04^{x}\pm0.055^{abc}$	0.87^{y}±0.057	0.96 ± 0.044^{ab}	$1.17^{x}\pm0.061^{ab}$	0.83^{y}±0.038	0.99±0.059
15	$1.22^{x}\pm0.069^{c}$	0.91^{y}±0.032	1.07 ± 0.053^{b}	$1.42^{x}\pm0.082^{bc}$	0.82^{y}±0.046	1.12±0.095
30	1.12 ± 0.063^{bc}	0.96±0.047	1.03 ± 0.043^{ab}	$1.44^{x}\pm0.102^{c}$	0.94^{y}±0.037	1.19±0.086
45	1.03 ± 0.071^{abc}	0.90±0.033	$0.96^{p}\pm0.041^{ab}$	$1.51^{x}\pm0.113^{c}$	0.93^{y}±0.090	1.22^{q}±0.106
60	0.92 ± 0.077^{ab}	0.94±0.041	$0.93^{p}\pm0.042^{ab}$	$1.31^{x}\pm0.074^{abc}$	0.92^{y}±0.059	1.12^{q}±0.071
Overall	1.06^{x}±0.032	0.92^{y}±0.014	0.99^{p}±0.018	1.33^{x}±0.032	0.90^{y}±0.018	1.11^{q}±0.027
Plasma iron levels (ppm)						
-60	2.46 ± 0.143^{ab}	2.58 ± 0.105^{b}	2.52 ± 0.087^{bcd}	2.73 ± 0.187^{c}	2.62 ± 0.173^{b}	2.67 ± 0.123^{c}
-30	2.69 ± 0.215^{b}	2.55 ± 0.096^{b}	2.62 ± 0.115^{cd}	2.69 ± 0.177^{c}	2.65 ± 0.187^{b}	2.67 ± 0.124^{c}
-15	2.74 ± 0.178^{b}	2.62 ± 0.107^{b}	2.68 ± 0.102^{d}	2.56 ± 0.098^{bc}	2.53 ± 0.162^{ab}	2.55 ± 0.091^{bc}
0	1.99 ± 0.113^{a}	2.14 ± 0.114^{ab}	2.06 ± 0.080^{a}	1.99 ± 0.141^{a}	2.14 ± 0.107^{a}	2.07 ± 0.088^{a}
15	2.38 ± 0.158^{ab}	2.27 ± 0.180^{ab}	2.32 ± 0.117^{abc}	2.21 ± 0.072^{ab}	2.20 ± 0.141^{ab}	2.21 ± 0.076^{a}
30	2.29 ± 0.126^{ab}	2.44 ± 0.159^{ab}	2.37 ± 0.100^{abcd}	2.28 ± 0.123^{ab}	2.38 ± 0.146^{ab}	2.33 ± 0.093^{ab}
45	2.41 ± 0.143^{ab}	2.36 ± 0.168^{ab}	2.39 ± 0.107^{abcd}	2.17 ± 0.074^{ab}	2.39 ± 0.087^{ab}	2.28 ± 0.063^{ab}
60	2.49 ± 0.132^{b}	2.05 ± 0.232^{a}	2.27 ± 0.140^{ab}	2.21 ± 0.082^{ab}	2.45 ± 0.088^{ab}	2.33 ± 0.067^{ab}
Overall	2.43±0.058	2.37±0.056	2.40±0.040	2.36±0.054	2.42±0.052	2.39±0.037
Plasma copper levels (ppm)						

-60	1.09±0.073^{ab}	1.03±0.036	1.06±0.040^{ab}	1.16^{x}±0.052^{a}	0.89^{y}±0.072^{a}	1.03±0.056^{a}
-30	1.26±0.085^{b}	1.09±0.019	1.18±0.046^{b}	1.42^{x}±0.080^{b}	0.98^{y}±0.065^{abc}	1.19±0.078^{ab}
-15	1.17±0.063^{ab}	1.05±0.045	1.11±0.040^{ab}	1.44^{x}±0.049^{b}	0.92^{y}±0.050^{ab}	1.18±0.080^{ab}
0	1.01±0.076^{a}	0.97±0.032	0.99±0.040^{a}	1.14^{x}±0.025^{a}	0.91^{y}±0.044^{a}	1.03±0.040^{a}
15	1.21±0.082^{ab}	1.08±0.054	1.14±0.050^{b}	1.42^{x}±0.073^{b}	1.01^{y}±0.037^{abc}	1.22±0.070^{ab}
30	1.19±0.064^{ab}	1.08±0.049	1.14±0.041^{b}	1.46^{x}±0.103^{b}	1.06^{y}±0.029^{bc}	1.26±0.076^{b}
45	1.23^{x}±0.059^{ab}	0.98^{y}±0.064	1.10±0.052^{ab}	1.33^{x}±0.074^{ab}	1.08^{y}±0.035^{c}	1.21±0.053^{ab}
60	1.17±0.073^{ab}	1.06±0.046	1.11^{p}±0.044^{ab}	1.42^{x}±0.076^{b}	1.13^{y}±0.020^{c}	1.27^{q}±0.055^{b}
Overall	1.16^{x}±0.026	1.04^{y}±0.016	1.10^{p}±0.016	1.35^{x}±0.028	0.99^{y}±0.019	1.17^{q}±0.024
Plasma cobalt levels (ppm)						
Overall	0.03±0.001	0.03±0.000	0.03±0.000	0.03^{x}±0.000	0.03^{y}±0.000	0.03±0.000
Plasma manganese levels (ppm)						
Overall	0.05^{x}±0.001	0.04^{y}±0.001	0.05±0.001	0.05^{x}±0.007	0.03^{y}±0.001	0.04±0.004

Subgroup-I = Stimvet 5 ml i/m; Subgroup-II = Stimvet control; Day 0 = Day of calving.
Means bearing uncommon superscripts within the column differ significantly between periods (abc) and those within the row differ between sub-groups (xy) or pooled values (pq) ($P<0.05$).
The period effect was in-significant in all groups for cobalt & manganese, hence data are not shown.

Conclusion

It was concluded that nutrient supplementation in terms of high protein concentrate, ASMM and injection of sustained release micro-minerals (Se, Zn, Cu and Mn) during transition period was beneficial in escalating blood minerals profile that independently as well as collectively improved the postpartum reproductive performance significantly in respect of uterine involution, first postpartum estrus, service period and conception rate in buffaloes under field conditions.

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