Studies on Vitamin Mineral Interactions in Relation to Passive Transfer of Immunoglobulins in Buffalo Calves

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ABSTRACT : Status of blood minerals and their absorption by neonate calves as influenced by fat soluble vitamins supplementation in their respective mothers, mineral supplementation in calves themselves has been evaluated. The objective was to know the impact of antioxidant vitamin supplementation to advance pregnant buffaloes, on enhanced acquired immunity during first few hours after birth, in relation to weight gain in buffalo calves. Advance pregnant buffaloes (n = 30) consisting of average body weight of 550±15 kg and of 4-6 parity were fed on 25 kg green (green Jawar-*Sorghum bicolor*), 2-3 kg wheat straw and 3-4 kg concentrate mixture individually per day. Intramuscular injections of vitamin triplex A D_3 E consisting of -2,500,000 IU of vit A -Palmitate; 2,500,000 IU of vitamin D_3 and 1,000 IU of vit E (dl-alpha tocopherol acetate) were given per dose, a month prior to parturition, twice at 15 days interval to 15 dams. Rest of the 15 pregnant buffaloes served as negative controls. Secretion of immune proteins, immunoglobulin (Ig) enhanced by 80% in colostrum. The blood serum levels of Zn, Cu, Ca, Mg were measured from birth to 90 days in calves. A significant ($p<0.05$) difference between the blood serum Zn levels of calves born to vitamin supplemented and non-supplemented dams was measured and a positive correlation between blood serum Zn levels and injections of vitamins was identified. Association of Zn and Cu with passive immunity status has been identified in these calves. A significant positive correlation between Zn and Cu was also identified which showed a change under the impact of vitamin supplementation in buffaloes. The study signifies the role of micronutrients supplementation in dams prior to parturition, in calf immunity development. The study indicates significant mineral - vitamins interactions during this process. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 6 : 825-830)***

Key Words : Fat Soluble Vitamin, Advance Pregnancy, Minerals, Buffalo Calves, Passive Immunity

INTRODUCTION

Transfer of immunoglobulin (Ig) from colostrum to neonates is of utmost significance in view of calf development. Passively acquired immunity during this period of initial few hours after birth is well associated with the antimicrobial protection of calves through readily available colostral immune bodies (Lemke et al., 2004). Also, the neonatal imprinting is done by exposing the calves to maternal Ig which, in turn, guides the development of nascent immune system in calves during the first 2-3 weeks (Lemke and Lange, 2002). Absorbed levels of blood serum Ig within 24 h after birth has been well associated with growth performance of buffalo calves (Sikka et al., 1997). It indicates that big scope of growth improvement lies with raising the serum Ig status of calves. Secretion of Ig in colostrum is known to be governed by several cell level biochemical changes in mammary tissues (Wender, 1984). Development and maintenance of cell membranes against oxidative damages is the basic need for propagation of various vital processes of immunity (Nockel, 1996) and growth (Ivan and Grieve, 1974). Supplementation of fat soluble vitamins as antioxidants, in pregnant buffaloes prior to parturition, raised the levels of secreted Ig in colostrum up to 80% (Sikka et al., 2002). Absorption of Ig in calves born to such dams was 30% more in terms of acquired passive immunity in this study. Serum levels of the micronutrients as Zn, Cu, Ca and Mg are well associated with enzymatic processes having antioxidant role to protect the tissues (Goswami et al., 2005). Blood serum levels of these micronutrients which also propagate the immunity in calves are likely to depend on pre- parturition feeding of dams (Panigrahi et al., 2005). Impact of antioxidant vitamin supplementation of dams on various mineral level interactions in their respective calves was taken up with respect to passive immunity improvement of buffalo calves in present study.

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MATERIAL AND METHODS

Advance pregnant buffaloes (n = 30; 550±15 kg body wt) of 4 to 6 parity were randomly selected from the institute herd. Each animal was fed *Sorghum bicolor* grass (25 kg), straw (2-3 kg) and concentrate mixture (3-4 kg) per day. Buffaloes were allocated to two groups, injected (Ttdm) and un-injected (Utdm) control, each consisting of fifteen buffaloes. Animals of injected group were administered antioxidant vitamins, AD_3E Triplex (i/m), twice at 15 days interval, a month prior to their expected calving. Triplex consisted of vitamin A (Palmitate, 2,500,000 IU), vitamin D_3 (2,500,000 IU) and dl-alpha tocopherol acetate, vitamin E (1,000 IU) per dose. Calves (n =15) born in each group were further divided into three

Table 1. Blood serum mineral levels (Mean±SE) in calves consequent to micronutrients supplementation and fat soluble vitamin injections to buffaloes

Mineral	Treatment	Age (Days (d))						
		At birth	1	15	30	45	60	90
Zn	Control	2.1±0.2	3.4±0.9*	3.9±0.9	2.3±0.3	1.3±0.2	4.9±1.2	1±0.2
(μg/ml)		(2.6±0.2)	(3.6±0.2)	(2.8±.8)	(1.5±0.5)	(2.3±0.6)	(4.5±0.7)	(1±0.2)
	Mineral	2.1±0.3	2±0.9*	2.8±0.6	1.2±0.5	0.6±0.5	1.4±0.5	3±1.0
		(2.3±0.5)	(1.1±0.2)	(1.9±1)	(4.8±1.2)	(2.1±0.5)	(1.1±0.3)	(2.7±0.)
	Mineral+	2.4±1.2	3.6±0.5*	3.2±0.8	1.8±1.4	2±1.3	0.9±0.7	7.4±1.9
	Vitamins	(3±0.8)	(5±0.7)	(1.5±0.6)	(2.6±0.8)	(1.4±0.6)	(2.1±0.9)	(4.2±1.2)
	Mean	2.5±0.2	3.9±0.3	2.4±0.5	1.6±0.2	2.5±0.5	1.7±0.2	3.2±0.5
		(2.6±0.3)	(3.2±1.9)	(2±0.6)	(2.9±1.7)	(1.9±0.4)	(2.5±1.7)	(2.6±1.5)
Cu	Control	1.2±0.1	1±0.2	0.8±0.2	1.3±0.3	1.5±0.3 [a]	1.1±0.4 [b]	0.9±0.4
(μg/ml)		(1.4±0.2)	(1.7±0.3)	(1±0.2)	(1.1±0.7)	(1.2±0.2)	(0.7±0.3)	(0.9±0.3)
	Mineral	0,8±0.3	0.6±0.2	0.6±0.3	1.2±0.6	1.2±0.6 [a]	1.5±0.6 [b]	1.3±0.7
		(1.1±0.2)	(1.3±0.2)	(0.8±0.3)	(1.0±0.5)	(0.7±0.3)	(0.8±0.2)	(0.8±0.4)
	Mineral+	0.8±0.4	1±0.4	1.1±0.5	1.2±0.3	1.1±0.3 [a]	0.8±0.2 [b]	0.8±0.5
	Vitamins	(1±0.5)	(1.1±0.3)	(1.1±0.4)	(1.2±0.2)	(1.1±0.2)	(0.8±0.3)	(0.4±0.3)
	Mean	1.18±0.1	1.3±0.1	1.2±0.1	1.2±.04	1.02±0.1	0.96±0.1	0.85±0.3
		(1.2±0.2)	(1.4±0.3)	(1±0.2)	(1.1±0.1)	(1±0.3)	(0.8±0.1)	(0.7±0.3)
Mg	Control	19±0.3 [c]	27±0.9 [cc]	15±0.9*	25±1.3	22±0.9 [cc]	26±0.8 [c]	26±2.1*
(μg/ml)		(23±0.6)	(54±5) [cc]	(20±1.2)	(24±3.5)	(28±1.8)	(27±1.8)	(19±1.5)
	Mineral	18±0.9 c	16±0.7 [cc]	18±1.8 *	28±0.8	17±0.7 [cc]	17±2.8 [c]	24±1.2*
		(18±1.1)	(18±1.2)	(32±1.8)	(23±1.0)	(11±0.8)	(22±1.1)	(23±1.9)
	Mineral+	16±0.8 c	18±0.5	24±1.5 *	15±0.6	23±1.6 [cc]	17±1.3 [c]	17±1.8 *
	Vitamins	(12±0.9)	(22±1.4)	(30±2.2)	(18±2.5)	(19±1.9)	(14±1.8)	(23±2.2)
	Mean	20.4±1.2	26.1±1.8	21.9±1.1	20.9±0.8	20.5±0.8	19.6±1.1	19±0.8
		(17.7±6)	(31.3±20)	(27.3±7)	(21.6±3)	(19.3±8.5)	(21±7)	(21.6±2)
Ca	Control	110±0.7	106±2.7	107±3.7	137±2.5 [a]	119±2.2	114±3.1 [a]	78+5
(μg/ml)		(119±0.5)	(100±2.3)	(90±2.3)	(80±2.4)	(100±2.6)	(164±3.0)	(57±1.2)
	Mineral	115±1.1	110±2.7	123±5.9	153±3 [a]	110±5.1	116±3.7 [a]	105±1.4
		(113±3)	(108±2.3)	(120±0.8)	(120±2.9)	(83±2.6)	(119±5.8)	(81±2.2)
	Mineral+	112±1	128±1.8	111±2.5	93±2.3 [a]	118±1	101±3.1 [a]	143±5.5
	Vitamins	(87±4.8)	(95±1.2)	(85±2.9)	(118±7.3)	(67±2.6)	(102±4.1)	(91±0.8)
	Mean	136.5±21	119.7± 9	114.5± 6	118±6.8	123±5.1	115±11	91±8.8
		(106±1.4)	(101±6.4)	(98±19)	(106±23)	(83±16)	(128±32)	(76±17)

Figures in parenthesis refer to the levels in un-injected buffalo's calves.
* Significant ($p<0.05$) difference between the injected vs. un-injected buffaloes and due to the calf treatments as well.
[a] Significant ($p<0.05$) difference due to injected vs. un-injected buffaloes.
[b] Significant ($p<0.05$) difference due to interaction of both calf treatments and dam's treatment.
[cc] Significant difference due to calf treatment at ($p<0.01$).
[c] Significant difference due to calf treatment at ($p<0.05$).

subgroups. Control (Co), mineral supplemented (Ms) and combination of mineral-vitamin supplemented (Vms) groups having 5 calves in each of them. Treatments given to calves of different sub-groups are as below.

Control (Co)

Calves were fed on colostrum/milk with some concentrate mixture for a month. Feeding of green (*Sorghum bicolor*), and wheat straw was initiated at the age of six weeks.

Mineral supplemented (Ms)

Calves underwent the oral supplementation of mineral mixture (5 gram/day), from age of day 1 to 60. One dose of mixture generally consisted of Ca (1,305 mg), Mg (66 mg), Cu (2.3 mg), Mn (1.45 mg), Zn (6.25 mg) and Fe (16.5 mg) along with sodium chloride, cobalt chloride, potassium iodide and sodium fluoride (as per the requirement).

Vitamin-mineral (Vms)

Calves were supplemented with fat soluble vitamins orally, from age of day one to 30 in addition to mineral supplementation. Vitamin dose consisted of vit E- acetate (60 mg), vitamin A (100,000 IU) and vitamin D (10,000 IU) for 25 kg body weight calf.

Estimations

Blood mineral levels were estimated in calves from birth to three months age at the interval of 15 days (Table1). Effect of vitamin - mineral interaction in calves and it's effect on acquired passive immunity was studied.

Mineral content was estimated in calf serum by Atomic absorption spectrophotometer (Perkin Elmer 2380). Blood collection was done prior to colostrum feeding and day 1, 15, 45, 60, 75 and 90 after birth. Serum (5 ml) was digested with mixture (10 ml) of nitric and perchloric acid (4:1) as per the method of AOAC (1984). Digested samples were made-up to 50 ml volume in flask and serum Ca, Mg, Zn and Cu were estimated against suitable standards. Serum Ig levels were estimated as per the reference method of zinc sulfate turbidimetry (Pfeiffer, 1977). Records of body weight and health were regularly updated for these calves. Statistical analysis was done using SAS Package (Little et al., 1991). General linear model was applied to identify the level of significance in variation of serum constituent during the study days. Repeated Measured Analysis of Variance was used to identify the level of significance in data due to the treatments of vitamin injections to dams; the treatments given to calves and their interactions, influencing the serum constituent levels over days of study. The correlations between each of the different serum minerals and Ig levels were determined on different days after birth of calves, by regression analysis. Coefficients of correlations were tested for significance.

RESULTS AND DISCUSSION

Blood mineral levels were estimated in neonatal calves from birth to age of 90 days. Effect of supplementation of mineral mixture to calves and fat soluble vitamins injections in their dams before parturition, on buffalo calf serum mineral levels was studied.

Zinc

The overall mean blood serum zinc level in calves was estimated as 2.58 μg/ml in thirty buffalo calves. Zinc level varied significantly during first fifteen days with respect to age and treatment given to the calves as well, especially, during first fifteen days after birth. Levels increased within 24 hours after colostrum feeding (Table 1). Serum zinc level reduced significantly ($p<0.01$) in calves supplemented with mineral mixture. Fat soluble vitamins supplementation in addition to mineral mixture in calves prevented the suppression of zinc level in serum. Serum zinc levels increased by 28.6 to 39% from 15 to 30 days age in calves of injected buffaloes. Zinc level in such calves were significantly higher ($p<0.05$) than mineral supplemented calves. The calves born to un-injected dams showed higher ($p<0.01$) level of suppression in zinc absorption due to mineral supplementation in calves. It indicates a positive interaction between injection of fat soluble vitamins in buffaloes and zinc absorption in their respective calves. It corroborates with earlier reports (Sikka et al., 2002, 2004) showing buffaloes secreting significantly ($p<0.01$) higher zinc in colostrum when injected with fat soluble vitamins, prior to the calving. It suggests higher uptake of zinc by udder tissue from higher serum zinc levels in vitamin injected buffaloes when compared with un-injected ones. An effect of negative feed back in zinc absorption emerged due to oral feeding of mineral mixture in calves. It was at much lower level in calves born to vitamin injected dams. Mineral supplementation in calves reduced the serum zinc levels significantly ($p<0.01$).

Copper

Overall mean serum copper levels were estimated as 1.05 μg/ml in thirty buffalo calves. Vitamin injection in buffaloes before parturition increased the copper uptake by mammary tissue showing more than 40% increase in copper levels of first day colostrum as reported earlier (Sikka et al., 2004). In contrast to the earlier reports (Mee et al., 1995), the serum copper levels reduced significantly ($p<0.05$) in minerals supplemented calves, especially, during first fifteen days after birth (Table 1). Vitamin supplementation in addition to minerals to these calves improved the copper absorption. Also, the absorption of copper was higher in calves born to vitamin injected dams after two weeks of age. A significant effect of vitamin injection to dams on absorption of copper in their respective calves was noted at the age of 45 days ($p<0.05$) and at 2 months ($p<0.01$). It corresponds with earlier reports of higher uptake of copper by mammary tissues and it's secretion in colostrum by vitamin injected dams in comparison to the un-injected ones (Sikka et al., 2004) in buffaloes.

Calcium

The overall mean serum calcium level was estimated as 112.9 μg/ml in calves. Vitamin injections in dams, prior to the parturition, increased the calcium absorption by the respective calves (Table 1). Higher colostrum calcium levels were estimated in injected dams resulted by higher uptake of calcium by udder tissue (Chatterjee et al., 2003; Sikka et al., 2004) which indicated high calcium level in blood serum of injected dams after injections. Antioxidant property of some of the fat soluble vitamins might have controlled the calcium depletion from tissue in injected buffaloes during early lactation. Oral supplementation of mineral mixture in calves increased the absorbed calcium levels in calves. A significant reduction ($p<0.05$) in absorbed levels of calcium was noted when vitamins were supplemented along-with the minerals in these calves. No significant effect of vitamin injections in dams on calcium

Table 2. Regression coefficients among the blood minerals of buffalo calves

Micronutrients in calves		Regression coefficients (r) between blood mineral levels in calves of injected vs. un-injected dams									
		Ig		Ca		Mg		Cu		Zn	
		UT	T	UT	T	UT	T	UT	T	UT	T
Zn	C	0.16	0.53	0.74*	0.57	0.15	0.32	0.079*	-0.54	1	1
	M	0.48	-0.01	0.07	-0.37	0.13	-0.03	0.02	-0.36	1	1
	VM	0.45	-0.51	0.06	0.88*	-0.04	-0.23	-0.24	-0.10	1	1
Cu	C	-0.12	-0.24	-0.37	0.27	0.74*	0.197	1	1		
	M	-0.61	-0.32	0.28	0.02	0.02	0.33	1	1		
	VM	0.42	0.05	-0.19	-0.2	-0.06	0.10	1	1		
Mg	C	0.06	-0.07	0.09	0.23	1	1				
	M	-0.31	-0.23	0.59	0.69*	1	1				
	VM	0.20	0.02	-0.02	0.01	1	1				
Ca	C	0.31	0.55	1	1						
	M	-0.14	-0.04	1	1						
	VM	-0.13	-0.24	1	1						
Ig	C	1	1								
	M	1	1								
	VM	1	1								

* Significant ($p<0.05$) r values.

absorption in calves was noted. However the negative interraction of mineral and vitamin supplementation in calves was reduced significantly ($p<0.05$) in calves born to vitamin injected dams.

Magnesium

The overall mean level of serum magnesium was estimated as 21.35 μg/ml in thirty buffalo calves. Serum magnesium levels were higher in calves born to un-injected buffaloes significantly ($p<0.05$) during the days under study. The calves supplemented with interactive feeding of mineral and vitamins showed reduced level of serum magnesium, though a definite trend was lacking. An indication of suppressed absorption of magnesium in calves born to vitamin injected buffaloes was noted.

Interrelations among serum minerals in calves

Correlation coefficients (r) between the minerals and Ig levels were estimated to study the interrelation between the two with respect to micro-nutrient supplementation in calves (Table 2). High zinc and low copper absorption is observed in calves, born to vitamin A-D-E supplemented buffaloes contrary to the positive ($p<0.05$) correlation between these two minerals levels in un-injected dam's calves. These calves, when supplemented with mineral mixture, showed a decrease in zinc and copper absorption levels indicating a negative feedback effect of mineral mixture on absorption of zinc and copper. An increase in zinc and decrease in copper absorption in calves born to injected dams is identified as an antagonistic effect of vitamin injections in dams on absorption of two minerals in calves. A negative correlation between zinc and copper has also been observed (r = -5.4*, -0.36, -0.10), respectively, in control, mineral and vitamin mineral supplemented calves. Level of zinc decreased and copper levels increased with advanced age. Negative correlation between zinc supplementation and copper content in liver has been elucidated earlier (Ivan and Grieve, 1974).

A significant positive correlation (r = 0.735, $p<0.05$) between zinc and calcium is identified in un -supplemented calves. Mineral supplementation reduced the correlation coefficient to insignificant level in contrast with calves supplemented with both vitamins and minerals(r = 0.88, $p<0.05$). Mineral supplementation to calves suppressed the absorption of zinc but enhanced the absorption of calcium levels due to which correlation between zinc and calcium were insignificant in mineral fed calves (Table 2). However, the combined supplementation of vitamins and minerals showed a positive additive effect on zinc absorption and an improvement in absorption levels of zinc and calcium (r = 0.88, $p<0.05$).

Change in serum mineral levels with respect to passive immunity

Acquisition of passive immunity by calves has been found to be a function of absorption capacity of calves (Sikka et al., 1996) and secretion of immune bodies in colstrum by mammary tissue in respective dams (Sikka et al., 2002). Role of zinc (Fletcher et al., 1988) and copper (Lukaswycz and Prohaska, 1990) has been associated with the proliferation level of immune bodies in establishing humoral immunity; their uptake by mammary tissue of dams and secretion in colostrum, for their transfer to calves subsequently. Change in serum levels of macro (Ca and Mg) and micro (Cu and Zn) elements was correlated with the absorbed level of immunoglobulins, in view of suggesting a role of these elements in acquisition of passive immunity in calves during first few hours after birth (Table

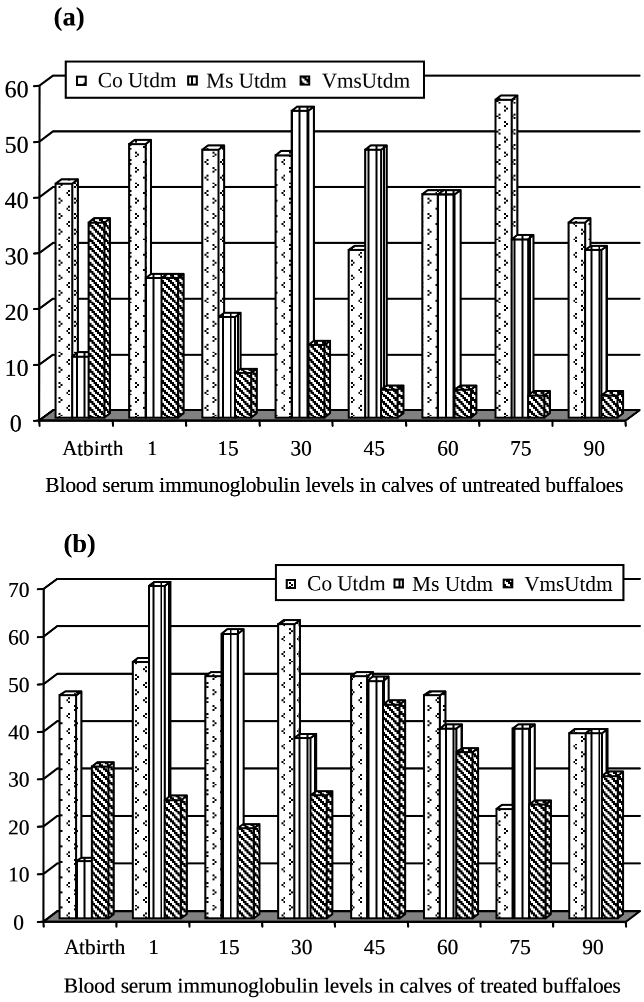

Figure 1. Blood serum immunoglobulin levels in calves.

2). Injection of vitamins in dams showed a higher correlation between absorption of Ig and zinc absorption in calves. More than 60% enhancement has been reported earlier (Sikka et al., 2002, 2004) in zinc levels and significantly higher level of Ig in first day colostrum of injected dams due to more secretion of these constituents from mammary tissue of vitamin injected dams. Probably due to an antioxidant effect of fat soluble vitamins, an increase in colostral transfer of zinc and Ig to calves is possible. Oral feeding of mineral mixture to calves, decreased serum zinc levels due to an expected negative feed back effect of mineral mixture on absorbed zinc levels, reducing the positive relationship between zinc and Ig as shown in Table 2. Less absorption of zinc in comparison to an increased Ig absorption from colostrum consisting of higher zinc and Ig shows a negative correlation between zinc and immunoglobulin levels in calf serum (Figure 1). It corroborates with the earlier studies showing immune system as a process of continuous cell proliferation which produces the immunoglobulins as a zinc dependent (Goswami et al., 2005). It shows that higher Ig in serum of calves, born to injected dams is due to the higher passive transfer of colostral Ig from these dams (Figure 1), however, zinc absorption in calves is affected by high zinc levels in colostrum. Oral supplementation of minerals and vitamins simultaneously, lowered the Ig absorption in calves inspite of its higher transfer from injected dams. Common supplementation of vitamin and minerals averted the negative feed back effect of mineral supplementation on absorption of zinc in calves although, the Ig acquisition from its passive transfer was suppressed in these calves.

Present observations suggest that mineral supplementation in calves decreased the absorption of zinc and copper in calves but increased the absorption of calcium and Ig. Dual supplementation of mineral and vitamins, increased the absorption of zinc and copper but decreased Ig and calcium levels in serum, suggesting that vitamin supplementation in calves did not show any benefit, however, their injections in adult buffaloes, prior to calving, enhanced the level of humoral immunity, improved Ig levels in colostrums, their transfer to calves and subsequent absorption. Vitamin supplementation to calves did not stimulate Ig absorption. A positive interaction between zinc and calcium was identified in suckling calves of un-injected dams. Association of suppressed levels of zinc in serum of calves with higher acquisition of Ig in this study is due to the impact of mineral feeding to calves which affected the zinc absorption in calves. However, fat soluble vitamins injections to pregnant buffaloes during advance pregnancy actually improves the passive immunity in calves and may be useful in reducing the calf mortality rates.

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