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Research Article

EFFICACY OF AREA SPECIFIC MINERAL MIXTURE SUPPLEMENTATION ON HEMATOLOGICAL AND MINERAL PROFILE IN DAIRY ANIMALS OF PUNJAB

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ABSTRACT

The study was conducted to assess the mineral status of dairy animals of Kandi belt of Punjab. Blood samples were collected from 200 dairy animals (76 cattle and 124 buffaloes) randomly selected from 9 villages of 4 blocks of the 2 districts (Hoshiarpur and Nawanshahar). Fodder (25) and soil (27) samples were also collected simultaneously from all the dairy units. These animals were grouped on the basis of age as group I (1-4 years), group II (5-7 years of age) and group III (>7 years). Plasma analysis revealed significant subclinical deficiency of calcium (64.47%), inorganic phosphorus (69.73%), iodine (84.21%) and copper (53.94%) in cattle and of Ca (58.06 %), magnesium (51.61%) and iodine (74.19%) in buffaloes. High mean plasma Molybdenum concentration was detected in cattle (47.36%) as well as buffaloes (66.93%). Overall prevalence of anemia in cattle was higher as compared to that of buffaloes. Soil mineral analysis revealed significant deficiency of inorganic phosphorus (55.55%) and copper (51.85%). Fodder mineral profile indicated significant deficiency of copper (56%) and manganese (48%). During therapeutic trial, daily feeding of area specific mineral mixture (ASMM) @ 50 g/animal/day for 90 days resulted in significant improvement in mean haemoglobin and packed cell volume values in supplemented group as compared to the un-supplemented group of cattle. Significant improvement was recorded in mineral profile of cattle and buffaloes in comparison to the control group. After 30 days of cessation of supplementation of ASMM significant decrease in mean haemoglobin and packed cell volume values of cattle and in mineral profile of both cattle and buffaloes was observed. Hence, it is concluded that area specific mineral mixture supplementation should be continued for longer duration for more significant effects.

Key words: Area specific mineral mixture, Buffaloes, Cattle, Fodder, Plasma, Soil.

Kandi area, the sub-mountainous zone, is located in north-eastern parts of Gurdaspur, Hoshiarpur, Nawanshahar, Ropar and Patiala districts and comprises of about 9 percent of the total geographical area of Punjab. This zone is economically weaker region of the state. There is an acute shortage of forages in Kandi area and animals are under-fed in terms of macronutrients and micronutrients (Hundal *et al.,* 2009). Livestock depends on forages for their mineral requirements, which are deficient in several minerals as they grow on deficient soils (Sharma *et al.,* 2005). Due to their increased requirement for lactation, dairy cows are more prone to mineral deficiencies (Mc Dowell *et al.,* 1983). The deficiency of certain minerals like calcium (Ca), inorganic phosphorus (Pi), copper (Cu), and zinc (Zn) in dairy cattle has been reported under field conditions and the supplementation of area specific mineral mixture (ASMM) in improving productive and reproductive efficiency is gaining importance (Gowda *et al.,* 2008).

Keeping above things into consideration, the present study was conceptualized to assess the effect of area specific mineral mixture supplementation on plasma mineral profile and hematological parameters of dairy animals of sub-mountainous zone (Kandi area) of Punjab in relation to the mineral status of soil and fodder of the area.

MATERIALS AND METHODS

Experimental design: 100 dairy animals (32 cattle and 68 buffaloes) were randomly selected from the surveyed group of 200 dairy animals (76 cattle and 124 buffaloes) for the therapeutic trial and fed area specific mineral mixture @ 50g/animal/day for 3 months since the animals of Kandi area were deficit of micro and macro minerals as per the previous surveys conducted by other researchers (Singh, 2002 and Hundal *et al*., 2009) in this region. 32 animals (15 cattle and 17 buffaloes) were kept as control. Fodder (25) and soil (27) samples were also collected simultaneously from all the dairy units. These animals were grouped on the basis of age as group I (1-4 years), group II (5-7 years of age) and group III (>7 years).

Tests and procedures: About 20 ml of blood sample was collected from each selected animal in heparinised mineral free glass vials. The samples were centrifuged @ 3000 rpm for 10-15 minutes immediately after collection and the separated plasma samples were stored at -10° C temperature in deep freezer for subsequent mineral analysis. Also 2 ml of blood was collected in sterile plastic vials, containing disodium ethylene di-amine tetra acetic acid (Na_2EDTA) as anticoagulant, for estimation of hematological parameters. Samples were collected on days 0 and 90 after feeding and one month after the cessation of mineral supplementation i.e. on day 120. Fodder and soil samples were collected following standard procedures and were packaged in plastic bags of suitable size for further processing.

Hematology: Hemoglobin (Hb) was estimated by Drabkin's / cyanomethaemoglobin method and packed cell volume (PCV) by microhematocrit method. Hb and PCV were sufficient to know the anaemic state of the animals and less time consuming.

Mineral analysis: For Ca and Mg estimation, samples were diluted with 0.1 per cent lanthanum chloride and

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Table 1
Plasma mineral status, Hb and PCV in cattle and buffalo of different age groups (Mean±S.E.)

Parameter	Group I (1-4 years)		Group II (5-7years)		Group III (>7years)		Total	
	Cattle (N=24)	Buffalo (N=23)	Cattle (N=42)	Buffalo (N=73)	Cattle (N=10)	Buffalo (N=28)	Cattle (N= 76)	Buffalo (N= 124)
Hb (g/dl)	10.20±0.59	12.81±0.58	9.08±0.36	10.67±0.36	12.17±0.89	11.50±0.44	9.83±0.32	11.25±0.27
PCV (%)	28.88±1.82	36.65±1.65	25.14±1.09	31.55±1.10	35.30±2.63	34.36±1.37	27.64±0.97	33.1±0.80
Ca (mmol/l)	2.07 ± 0.26	2.45 ± 0.27	2.02 ± 0.14	2.61 ± 0.18	2.08 ± 0.31	2.36 ± 0.33	2.00 ± 0.12	2.52±0.14
Mg (mmol/l)	1.03 ± 0.13[b]	0.81±0.13[b]	1.10±0.09[c]	0.69±0.05[a]	0.72±0.10[a]	0.64±0.08[a]	1.03±0.07	0.70±0.04
Pi (mmol/l)	1.25 ± 0.08	1.54 ± 0.12	1.31 ± 0.06	1.59 ± 0.06	1.28 ±0.11	1.88 ± 0.07	1.27 ± 0.04	1.65±0.05
Fe (µmol/l)	89.12±8.53[b]	46.42±5.60	48.86± 4.48[a]	50.27 ± 3.48	51.31±7.25[c]	37.52±4.23	53.75±3.42	47.54±2.64
Cu (µmol/l)	10.35±1.16[a]	14.13±1.34	11.52±0.75[ab]	11.28±0.73	12.78±2.51[b]	10.44±0.87	11.48± 0.63	11.56±0.55
Zn (µmol/l)	17.83± 3.21	27.41±4.06	25.04 ± 2.50	28.91 ± 2.00	28.59±7.71	25.12±3.71	28.67± 2.20	27.67±1.62
Mn (µmol/l)	0.79 ± 0.08	0.78 ± 0.09	0.91 ± 0.08	0.75 ± 0.05	0.99±0.16	0.67 ± 0.08	0.88 ± 0.05	0.74 ± 0.04
Mo (ppm)	0.26 ± 0.04[a]	0.58 ± 0.10	0.47 ± 0.06[b]	0.63 ± 0.05	0.43±0.07[b]	0.66 ± 0.07	0.65 ± 0.06	0.63 ± 0.04
I (ppm)	1.52 ± 0.06	1.73 ± 0.07	1.6 ± 0.04	1.68 ± 0.04	1.75 ± 0.08	1.61 ± 0.06	1.61 ± 0.03	1.68 ± 0.03

analyzed in Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer Analyst 700, USA). For micro mineral estimation (Cu, Zn, Fe, Mn and Mo), plasma samples were first digested and then diluted with double glass distilled water and analyzed in AAS. Iodine concentration in plasma was measured by Digital Ion-analyzer equipped with an iodide specific electrode (Orion Research Model 4 star ISE, 96-53-BNWP).

Fodder samples: The fodder samples were allowed to air dry for few days. These samples were then dried in hot air oven at 65°C overnight followed by acid digestion and dilution with double glass distilled water and analyzed in AAS for micro mineral analysis (Cu, Zn, Mn and Fe). For Ca and Mg estimation, samples were diluted with 0.1 per cent lanthanum chloride and analyzed in Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 700, USA). Plant phosphorus levels were measured by method given by AOAC (2000).

Soil samples: The soil samples were air-dried and treated with 20 ml of AB-DTPA (Ammonium Bicarbonate-Diethylene triaminepentaaceticacid) solution for estimating various mineral contents (Ca, Mg, Cu, Zn, Fe and Mn) as described for fodder samples. Plant available phosphorus was estimated by method given by Olsen (1953).

Statistical analysis: Mean, standard error of mean and range of various parameters were estimated and test of significance (2-way split analysis of variance, chi-square test and Fischer's exact test) between different groups were performed using SPSS for Windows (version 16.0; Microsoft).

RESULTS AND DISCUSSION

Haematology: The overall mean values of Hb and PCV in cattle were 9.83 ± 0.32 g/dl and 27.64 ± 0.97 per cent, respectively (Table 1). According to Feldman *et al*. (2000), Hb and PCV values in cows vary from 8.0 to 15.0 g/dl and 24 to 46 per cent, respectively, with respective mean values of 11g/dl and 35 per cent. Hence, it could be inferred that cows were having values for Hb and PCV in lower normal range. Animals were anaemic with respect to the mean normal values of Hb (11 g/dl) and PCV (35%). These findings were comparable to those of Ozukum (2011) who reported mean Hb and PCV values of 9.89 ± 0.14 g/dl and 25.44 ± 0.34 per cent, respectively, in cattle of Bathinda district of Punjab. Wide variations in the hematological parameters exist due to many physiological factors, such as health status of the animal, environmental temperature, stress, age, muscular activity, quality of the diet, time of sampling, stage of hydration, emotional state, parasitic burden, and breed differences (Feldman *et al.,* 2000).

Anaemia is characterized by decrease in hemoglobin (Hb), haematocrit (PCV) or total erythrocyte count (TEC) levels. The overall prevalence of anaemia in cattle was 26.31 percent on Hb basis. Age-wise the incidence of anaemia in cattle was highest (30.95 %) in group II (5-7 years of age) followed by 25 per cent and 10 per cent in group I (1-4 years) and group III (>7 years), respectively. The overall prevalence of anaemia in cattle based on PCV values was 34.21 per cent. On the basis of PCV values, incidence of anaemia in cattle was highest (42.85 %) in group II (5-7 years), followed by 29.17 per cent and 10 per cent in group I (1-4 years) and group III (>7 years), respectively (Table 2).

The overall mean values of Hb and PCV in buffaloes were 11.25 ± 0.27 g/dl and 33.13 ± 0.80 per cent, respectively (Table 1) which were within the normal range. These results were comparable with the respective mean values of 10.67g/dl and 31.88 per cent, as reported in adult buffaloes by Singh (2002). On the basis of Hb and PCV values, the overall prevalence of anemia in buffaloes was 10.48 and 21.77 per cent, respectively. The highest percentage of anemia was observed in group II (5-7 years

Table 2
Percentage prevalence of anemia on the basis of Hb and PCV and plasma minerals status in cattle on the basis of age

Parameter	Status	Group I (1-4 years) (N=24)	Group II (5-7 years) (N=42)	Group III (>7years) (N=10)	Total (N= 76)	Overall Percent
Hb (g/dl)	Normal (>8)	18	29	9	56	73.68
	Marginal (6-7.9)	5	10	1	16	21.05
	Low (<6)	1	3	0	4	5.26
	Deficiency (%)	25	30.95	10		26.31
PCV (%)	Normal (>24)	17	24	9	50	65.79
	Marginal (18-23.9)	6	15	1	22	28.94
	Low (<18)	1	3	0	4	5.26
	Deficiency (%)	29.17	42.85	10		34.21
Ca(mmol/l)	Normal (>2)	8	13	6	27	35.52
	Marginal (1.6-1.99)	7	13	1	21	27.63
	Low (<1.6)	9	16	3	28	36.84
	Deficiency (%)	66.66	69.04	40		64.47
Mg (mmol/l)	Normal (>0.6)	15	32	6	53	69.73
	Marginal (0.4 – 0.6)	5	5	1	11	14.47
	Low (<0.4)	4	5	3	12	15.79
	Deficiency (%)	37.5	23.8	40		30.26
Pi (mmol/l)	Normal (>1.45)	6	14	3	23	30.26
	Marginal(1.29-1.45)	3	15	3	21	27.63
	Low (<1.29)	15	13	4	32	42.1
	Deficiency (%)	75	66.66	70		69.73
Fe (µmol/l)	Normal (>17.9)	24	36	9	68	89.47
	Low (<17.9)	0	6	1	8	10.52
	Deficiency (%)	0	14.28	10		10.52
Cu (µmol/l)	Normal (>9.6)	9	20	6	35	46.05
	Marginal (7.9 - 9.59)	7	8	0	15	19.73
	Low (<7.9)	8	14	4	26	34.21
	Deficiency (%)	62.5	52.38	40		53.94
Zn (µmol/l)	Normal (>12.2)	12	27	7	46	60.52
	Marginal(9.8-12.1)	2	7	1	10	13.15
	Low (<9.8)	10	8	2	20	26.31
	Deficiency (%)	50	35.71	30		39.47
Mn (µmol/l)	Normal (>0.37)	21	37	9	67	88.15
	Low (<0.37)	3	5	1	9	11.84
	Deficiency (%)	12.5	11.9	10		11.84
Mo (ppm)	Normal (<0.3)	15	20	5	40	52.63
	Toxic (>0.3)	9	22	5	36	47.36
	Toxicity (%)	37.5	52.38	50		47.36
I (ppm)	Normal (>2)	2	6	4	12	15.79
	Low (<2)	22	36	6	64	84.21
	Deficiency (%)	91.66	85.71	60		84.21

of age) in terms of both Hb and PCV (Table 3).

Similar to the present findings, Singh (2013), recorded overall 21.19 and 33.90 per cent prevalence of anemia in cattle on Hb and PCV basis, respectively, in Central zone of Punjab which was higher than that of buffaloes (13.11 and 13.11 per cent on Hb and PCV basis, respectively).

Mineral profile: Plasma mineral status in cattle and buffaloes of different age groups is represented in Table 1. In the present study, the overall Ca deficiency in cattle was found to be 64.47 per cent. Previous studies conducted in Punjab (Randhawa, 1999; Singh, 2002 and Chhabra, 2006) also revealed a higher level of hypocalcaemia in cows (90.0, 42.8 and 54.7 per cent, respectively). The overall prevalence of hypocalcaemia in buffaloes in the present study was recorded to be 58.06 per cent. On the contrary, Randhawa *et al.* (2008) observed hypocalcaemia in 21.8 per cent of animals which was lower than the present findings.

Overall prevalence of hypomagnesaemia in cattle and buffaloes was found to be 30.26 and 51.61 per cent, respectively. Lactational drainage could be the reason for the fall in plasma Mg levels in cows in the present study.

In cattle, overall prevalence of hypophosphataemia observed was 69.73 per cent, whereas Singh *et al*. (2005) and Siddique (2011) had recorded a prevalence of hypophosphataemia in 27.38 and 29.80 per cent of cows, respectively. Both these findings were lower than that of the present study. These differences in the level of Pi in various studies could be the result of differences in dietary levels and composition, breed, season and other factors like sample preparation, degree of hemolysis, temperature, duration of sample collection and plasma preparation time. Overall prevalence of hypophosphataemia recorded for buffaloes was 31.45 per cent, which was in agreement with Singh *et al*. (2008) and Randhawa *et al*. (2009) who reported similar prevalence of subclinical hypophosphataemia (26.70 and 21.79 per cent, respectively) in buffaloes surveyed in Sub-mountainous region and central districts of Punjab.

In the present study, the overall prevalence rates of Cu, Fe, Zn, Mn and I deficiencies in cattle recorded were 53.94, 10.52, 39.47, 11.84 and 84.21 percent, respectively. However, Singh *et al*. (2003) indicated lower incidence of hypocupraemia (25.60%) in cows of Sub-mountainous regions of Punjab. These variations could be a result of difference in dietary copper levels, Cu: Mo ratio, method of sampling, geographical areas under study and analytical techniques involved. In buffaloes, the overall prevalence rates of Cu, Fe, Zn, Mn and I deficiencies recorded were 36.29, 8.06, 18.54, 27.41 and 74.19 percent,

Table 3
Percentage prevalence of anemia (on the basis of Hb and PCV) and plasma minerals status in different age groups of buffaloes

Parameter	Status	Group I (1-4 years) (N=23)	Group II (5-7 years) (N=73)	Group III (>7 years) (N=28)	Total (N=124)	Overall Percent
Hb (g/dl)	Normal (>8)	21	65	25	111	89.51
	Marginal (6-7.9)	0	2	1	3	2.42
	Low (<6)	2	6	2	10	8.06
	Deficiency (%)	8.69	10.95	10.71		10.48
PCV (%)	Normal (>24)	19	56	22	97	78.22
	Marginal (18-23.9)	1	13	0	14	11.29
	Low (<18)	3	4	6	13	10.48
	Deficiency (%)	17.39	23.28	21.42		21.77
Ca (mmol/l)	Normal (>2)	10	33	9	52	41.93
	Marginal (1.6-1.99)	9	20	8	37	29.83
	Low (<1.6)	4	20	11	35	28.22
	Deficiency (%)	56.52	54.8	67.85		58.06
Mg (mmol/l)	Normal (>0.6)	12	35	13	60	48.38
	Marginal (0.4 – 0.6)	5	18	6	29	23.38
	Low (<0.4)	6	20	9	35	28.22
	Deficiency (%)	47.82	52.05	53.57		51.61
Pi (mmol/l)	Normal (>1.45)	14	46	25	85	68.54
	Marginal(1.29-1.45)	3	13	1	17	13.7
	Low (<1.29)	6	14	2	22	17.74
	Deficiency (%)	39.13	36.98	10.71		31.45
Fe (µmol/l)	Normal (>17.9)	21	68	25	114	91.93
	Low (<17.9)	2	5	3	10	8.06
	Deficiency (%)	8.69	6.84	10.71		8.06
Cu (µmol/l)	Normal (>9.6)	17	46	16	79	63.7
	Marginal (7.9 - 9.59)	2	7	5	14	11.29
	Low (<7.9)	4	20	7	31	25
	Deficiency (%)	26.08	36.98	42.85		36.29
Zn (µmol/l)	Normal (>12.2)	19	63	19	101	81.45
	Marginal (9.8-12.1)	0	1	0	1	0.8
	Low (<9.8)	4	9	9	22	17.74
	Deficiency (%)	17.39	13.7	32.14		18.54
Mn (µmol/l)	Normal (>0.37)	18	53	19	90	72.58
	Low (<0.37)	5	20	9	34	27.41
	Deficiency (%)	21.73	27.39	32.14		27.41
Mo (ppm)	Normal (<0.3)	9	26	6	41	33.06
	Toxic (>0.3)	14	47	22	83	66.93
	Toxicity (%)	60.86	64.38	78.57		66.93
I (ppm)	Normal (>2)	7	20	5	32	25.8
	Low (<2)	16	53	23	92	74.19
	Deficiency (%)	69.56	72.6	82.14		74.19

respectively. Lower prevalence rate was recorded by other research workers viz. Singh *et al*. (2004), Randhawa *et al*. (2009) and Mircha (2009) detected 7.37, 10.13 and 25.30 per cent hypocupraemia, respectively, in dairy animals from Central, Sub-mountainous and Moga districts of Punjab. The differences observed in various studies could be due to the fact that many factors affect an animal's response to trace mineral supplementation such as the duration and concentration of trace mineral supplementation, physiological status of an animal (i.e., pregnant vs. non-pregnant), the absence or presence of dietary antagonists, environmental factors, and the influence of stress on trace mineral metabolism.

Table 4
Comparative changes in Hb and PCV values in cattle (Mean±S.E)

Parameter	Group	Day 0	Day 90	Day 120	Sig.
Hb (g/dl)	Treatment (n=32)	9.13± 0.13	9.47 ± 0.12	9.15 ± 0.12	T* Txt* t*
	Control (n=15)	8.06± 0.22	7.90 ± 0.21	7.81 ± 0.19	
PCV (%)	Treatment (n=32)	27.00± 0.40	28.19 ± 0.34	27.09 ± 0.38	T* Txt t
	Control (n=15)	23.47± 0.62	23.20 ± 0.66	23.00 ± 0.59	

Where Sig. = Significance for, T = Treatment, Txt = time-treatment interaction and t = time, * = Significance below 0.05 ($p \leq 0.05$)

Table 5
Comparative changes in Hb and PCV values in buffaloes (Mean ± S.E)

Parameter	Group	Day 0	Day 90	Day 120	Sig.
Hb (g/dl)	Treatment (n=68)	9.99± 0.31	10.68 ± 0.30	10.23 ± 0.29	T Txt t
	Control (n=17)	8.88 ± 0.32	8.53 ± 0.32	8.69 ± 0.37	
PCV (%)	Treatment (n=68)	28.94 ± 0.96	31.63 ± 0.93	30.32 ± 0.87	T Txt t
	Control (n=17)	26.29 ± 1.04	25.24 ± 1.00	25.71 ± 1.11	

Where Sig. = Significance for, T = Treatment, Txt = time-treatment interaction and t = time, * = Significance below 0.05 ($p \leq 0.05$)

Table 6
Comparative changes in plasma macro minerals in cattle (Mean ± S.E)

Parameter	Group	Day 0	Day 90	Day 120	Sig.
Ca (mmol/l)	Treatment (n=32)	1.74± 0.12	1.79± 0.12	1.75± 0.12	T Txt* t*
	Control (n=15)	1.50± 0.15	1.45± 0.15	1.43± 0.14	
Mg (mmol/l)	Treatment (n=32)	0.70± 0.07	0.82± 0.07	0.77± 0.07	T Txt t
	Control (n=15)	0.62± 0.09	0.59± 0.09	0.56± 0.09	
Pi (mmol/l)	Treatment (n=32)	1.31± 0.06	1.38± 0.06	1.35± 0.06	T* Txt* t
	Control (n=15)	1.13± 0.09	1.11± 0.09	1.07± 0.09	

Where Sig. = Significance for, T = Treatment, Txt = time-treatment interaction and t = time, * = Significance below 0.05 ($p \leq 0.05$)

In the present study, the overall mean plasma Mo level recorded in cattle was 0.65 ± 0.06 ppm (Table 1) which indicates Mo toxicity (level >0.3 ppm). Randhawa (1999) reported considerably higher levels of Mo ranging from 1.60 to 1.89 ppm among cross bred cattle from rural herds of Punjab. Similarly, Sandhu *et al.* (1992) had observed

Table 7
Comparative changes in blood micro minerals in Cattle (Mean ± S.E)

Parameter	Group	Day 0	Day 90	Day 120	Sig.
Cu (mmol/l)	Treatment (n=32)	10.94± 0.64	11.28± 0.69	11.13± 0.68	T Txt t
	Control (n=15)	9.28± 0.92	9.23± 0.91	9.20± 0.91	
Zn (µmol/l)	Treatment (n=32)	28.52± 2.85	29.40± 2.73	27.69± 2.59	T Txt t
	Control (n=15)	25.27± 4.58	25.19± 4.58	24.83± 4.35	
Fe (µmol/l)	Treatment (n=32)	55.13± 5.09	57.27± 5.15	55.38± 5.08	T* Txt* t
	Control (n=15)	38.80± 5.59	38.07± 5.48	37.32± 5.37	
Mn (µmol/l)	Treatment (n=32)	0.80± 0.09	0.88± 0.09	0.84± 0.09	T Txt* t
	Control (n=15)	0.75± 0.08	0.72± 0.08	0.69± 0.08	
Mo (ppm)	Treatment (n=32)	0.69± 0.09	0.72± 0.09	0.73± 0.09	T Txt* T
	Control (n=15)	0.40± 0.09	0.38± 0.09	0.36± 0.09	
I (ppm)	Treatment (n=32)	1.58± 0.05	1.66± 0.04	1.63± 0.04	T Txt t
	Control (n=15)	1.45± 0.07	1.48± 0.08	1.46± 0.07	

Where Sig. = Significance for, T = Treatment, Txt = time-treatment interaction and t = time, * = Significance below 0.05 ($p \leq 0.05$)

significantly higher levels of plasma Mo in animals. However, significantly lower values of 0.123 ± 0.01 ppm were reported by Ward (1978). Baruah *et al*. (1998) had detected mean level of 0.08 ppm in Jersey heifers of Assam. These variations in the observations might be due to differences in nature of diet, status of Cu-Mo-S interactions in soil-plant-animal system, geographical area of study and differences in rates of absorption and excretion from the body (Underwood and Suttle, 1999). Age-wise analysis showed significant changes in mean plasma Mo levels in cattle (Table 1); highest values being recorded in group II and lowest in group I animals. Similar trend was recorded

Table 8
Comparative changes in blood macro minerals in buffaloes (Mean ± S.E)

Parameter	Group	Day 0	Day 90	Day 120	Sig.
Ca (mmol/l)	Treatment (n=68)	2.17±0.14	2.38±0.14	2.30±0.13	T Txt* t
	Control (n=17)	1.82±0.24	1.73±0.21	1.67±0.19	
Mg (mmol/l)	Treatment (n=68)	0.63±0.04	0.78±0.05	0.73±0.05	T Txt t*
	Control (n=17)	0.56±0.09	0.50±0.08	0.62±0.10	
Pi (mmol/l)	Treatment (n=68)	1.60±0.07	1.77±0.09	1.64±0.08	T Txt t
	Control (n=17)	1.51±0.07	1.44±0.08	1.43±0.07	

Where Sig. = Significance for, T = Treatment, Txt = time-treatment interaction and t = time, * = Significance below 0.05 ($p \leq 0.05$)

Table 9
Comparative changes in plasma micro minerals in buffaloes (Mean ±S.E)

Parameter	Group	Day 0	Day 90	Day 120	Sig.
Cu (µmol/l)	Treatment (n=68)	11.27±0.65	12.14±0.59	11.61±0.55	T Txt T
	Control (n=17)	10.96±0.43	10.84±0.42	11.05±0.41	
Zn (µmol/l)	Treatment (n=68)	25.96±2.23	27.58±2.09	26.62±2.04	T Txt T
	Control (n=17)	22.42±3.76	21.61±3.61	21.65±3.64	
Fe (µmol/l)	Treatment (n=68)	46.60±3.30	51.66±3.32	49.84±3.34	T Txt* t*
	Control (n=17)	39.18±6.77	39.54±6.59	38.71±6.61	
Mn (µmol/l)	Treatment (n=68)	0.69±0.06	0.82±0.06	0.77±0.06	T Txt t*
	Control (n=17)	0.55±0.09	0.59±0.09	0.56±0.09	
Mo (ppm)	Treatment (n=68)	0.59±0.05	0.53±0.05	0.60±0.05	T Txt t
	Control (n=17)	0.40±0.08	0.46±0.08	0.48±0.83	
I (ppm)	Treatment (n=68)	1.64±0.04	1.71±0.04	1.69±0.04	T Txt* t
	Control (n=17)	1.61±0.08	1.55±0.07	1.56±0.07	

Table 10
Mean soil mineral levels in the districts of Hoshiarpur and Nawanshahar

Minerals	Critical Limit (ppm)	Hoshiarpur (N=14)	Nawanshahar (N=13)	Over all (N=27)
Ca	71	157.95±10.38	180.76± 19.26	168.93± 10.74
Mg	15	75.74±15.22 ^{b}	53.35± 5.79 ^{a}	64.96± 8.51
P	10	9.09±0.51 ^{a}	12.53± 1.34 ^{b}	10.75± 0.76
Fe	30	58.01±3.55 ^{b}	54.69± 8.17 ^{a}	56.41± 4.27
Zn	1.5	2.37± 0.23	1.85± 0.18	2.12± 0.15
Cu	2	2.12± 0.15	1.80± 0.10	1.96± 0.10
Mn	10	16.33± 1.20	14.33± 1.07	15.37± 0.82

Figures with different superscripts in a row differ significantly at ($p<0.05$)

Table 11
Percentage prevalence of mineral deficiencies in soil samples

Minerals	Critical Limit (ppm)	Hoshiarpur (N=14)	Nawanshahar (N=13)	Over all (N=27)
Ca	71	0	0	0
Mg	15	0	0	0
P	10	11(78.57%)	4 (30.76%)	15 (55.55%)
Fe	30	0	3 (23.07%)	3 (23.07%)
Zn	1.5	2 (14.28%)	4 (30.76%)	6 (22.22%)
Cu	2	5 (35.71%)	9 (69.23%)	14 (51.85%)
Mn	10	0	2 (15.38%)	2 (15.38%)

Table 12
Mean fodder mineral levels in the districts of Hoshiarpur and Nawanshahar

Minerals	Critical Limit	Hoshiarpur (N=9)	Nawanshahar (N=16)	Over all (N=25)
Ca (%)	0.3	0.37 ± 0.07	0.38 ± 0.03	0.37 ± 0.03
Mg (%)	0.2	0.46 ± 0.06	0.41± 0.04	0.43 ± 0.04
P (%)	0.25	0.43 ± 0.05	0.39 ± 0.04	0.41 ± 0.03
Fe (ppm)	30	105.10±28.21	156.52±18.49	138.0±16.05
Zn (ppm)	30	35.52 ± 3.49	30.92± 1.63	32.58 ± 1.65
Cu (ppm)	10	12.39 ± 1.05	7.07 ± 0.91	8.98 ± 0.86
Mn (ppm)	30	30.00 ± 3.46	38.82 ± 4.12	35.64 ± 2.99

Values in a row do not differ significantly at ($p < 0.05$)

Table 13
Percentage prevalence of mineral deficiencies in fodder samples

Minerals	Critical Limit	Hoshiarpur (N=9)	Nawanshahar (N=16)	Over all (N=25)
Ca (%)	0.3	4 (44.44%)	5 (31.25%)	9 (36%)
Mg (%)	0.2	1(11.11%)	1 (6.25%)	2 (8%)
P (%)	0.25	1(11.11%)	3 (18.75%)	4 (16%)
Fe (ppm)	30	4 (44.44%)	2 (12.5%)	6 (24%)
Zn (ppm)	30	3 (33.33%)	5 (31.25%)	8 (32%)
Cu (ppm)	10	2 (22.22%)	12 (75%)	14 (56%)
Mn (ppm)	30	5 (55.55%)	7 (43.75%)	12 (48%)

for Mo toxicity (Table 2). However, Siddique (2011) observed age related rise in Mo levels. Contrary to this, Randhawa (1999) recorded age related fall in plasma Mo levels in crossbred cows of Punjab. Considering 0.3 ppm as critical level, 47.36 per cent of cattle had toxic levels of Mo which was in agreement with the findings of Randhawa (1999) and Singh (2002) who recorded 52.70 per cent prevalence of molybdenosis from other districts of Punjab. The overall prevalence of Mo toxicity in buffaloes was found to be 66.93 percent (Table 3) which was comparable to the findings of Randhawa (1999) and Singh, M (2002) who also reported higher prevalence of Mo toxicity as 40.0 per cent and 24.05 per cent, respectively in dairy animals of Punjab.

Effect of supplementation of area specific mineral mixture on blood parameters: The results indicated non-significant increase in mean Hb and PCV levels in cattle after supplementation of area specific mineral mixture. Non-significant decrease in levels on 120th day might be due to cessation of mineral mixture supplementation after 3 months of regular feeding. Effect of supplementation on hematological parameters of cattle and buffaloes is shown in tables 4 and 5. Effect of mineral mixture supplementation on mineral profile of cattle and buffaloes is shown in tables 6, 7 and tables 8, 9, respectively.

Soil and Fodder Analysis: Soils of Kandi area in Punjab surveyed in the present study are deficient in P and Cu and the fodder samples are deficient in Cu and Mn. (Tables 10 to 13).

It is concluded that area specific mineral mixture should be fed to the animals for longer duration of time in order to have better and significant effects.

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