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Haematobiochemical, vitamin and hormone profile of macro mineral deficient cattle of Northern India

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ABSTRACT

The study was conducted in 2 phases, initially a survey was conducted in certain parts of Northern India to record the prevalence of mineral deficiency in bovine. Soil samples (547), fodder samples (609) and serum samples (638) were collected from the districts of Nainital, Almora, Bageshwar, U.S. Nagar, Pilibhit, Bareilly, Badaun and Rampur. The prevalence of soil Ca, P and Mg deficiency was 21.35%, 23.30% and 28.64%, respectively, while that of fodder Ca, P and Mg deficiency was 13.88%, 16.55% and 19.72%, respectively. The overall prevalence of serum Ca, P and Mg deficiency was 27.15%, 27.61% and 23.13%, respectively. The correlation of coefficient of Ca, P and Mg in soil, fodder and serum was highly significant and in most of the cases the values were above 0.7. The highest deficiency was observed in plain region, followed by Tarai (foothills of Himalayas) region and then hilly region. Regarding haematobiochemical profile significant decrease in Hb, TEC was observed while the values of TLC were slightly higher in deficient buffaloes. The values of serum enzymes, viz. AST, ALT and Cp was lower whereas that of SAP was higher. The values of thyroid hormones (T_3 and T_4) and vitamins (A and E) were significantly lower in deficient animals.

Key words: Cattle, Deficiency, Enzymes, Hormones, Macro-minerals, Vitamins

Blood mineral status in cattle depends upon the daily mineral intake offered through feed apart from non-nutritional factors such as season, age, weight, pregnancy and lactation status (Khan 1995). Minerals act as (i) structural components of body organs and tissues, (ii) constituents of body fluids hormone systems (Underwood 1981).

Under Indian conditions, which is in tropical area (short rainy season 3–4 months, a long dry season 8–9 months) the metabolic and deficiency diseases are quite common and it is mainly due to non-availability of balanced diet or deficiency of specific nutrients in the soil. Out of different nutritional factors, the most important are deficiency or deranged metabolism of Ca, P and Mg. Abdelrahman *et al.* (1998) reported that condition of tropical areas significantly affects the quantity and quality of forages. Thus the deficiency of minerals play a crucial role in the development of various metabolic/deficiency diseases in animals. The present study was planned (i) to map the macro mineral deficient areas of northern India, (ii) to observe the effect, of mineral deficiency on the various haematobiochemical parameters, vitamins and hormones, and (iii) to recommend a suitable mineral mixture of a specific mineral deficient area.

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MATERIALS AND METHODS

To record the prevalence of various minerals deficiency in bovine, a survey was conducted in certain parts of Northern India [average rainfall, 60–70 cms/year, temperatures = 6.9-37.3°C (minimum and maximum) average humidity = 29.5-87.3% (minimum and maximum)]. Eight districts of Uttaranchal and Uttar Pradeshs state were surveyed and soil, fodder and blood samples were collected and analyzed for various enzymes, haematological and mineral values.

The survey was conducted in the Himalayan hill districts, (Nainital, Almora and Bageshwar) tarai (foothills of Himalayas) districts (Udham Singh Nagar and Pilibhit and plain districts (Rampur, Badaun and Bareilly) in Uttaranchal and Uttar Pradesh. Necessary information regarding feeding behaviour of animal, management practices, deworming, vaccination and insecticidal sprays, etc. were collected from farmers. Soil samples (547) were collected (Table 1) with the help of auger up to 15 cm depth. The collected soil samples were dried in hot air oven at 100±5°C overnight. These samples were then ground and stored in airtight polythene packets for laboratory analysis.

The samples of various fodders which were being fed as

Table 1. Total number of collected soil, fodder and blood samples

District	No. of soil samples collected	No. of fodder samples collected	No. of blood/ sera samples collected	Total
Nainital	73	82	78	233
Almora	67	76	77	215
Bageshwar	65	74	76	215
U S Nagar	68	73	82	233
Pilibhit	66	78	84	228
Bareilly	71	75	85	231
Badaun	65	78	75	218
Rampur	72	73	81	226
Total	547	609	638	1,794

such to the cattle were collected from 8 districts from the owner of animals. Fodder samples (609) were collected (Table 1) from grass/tree leaves, paddy straw, wheat straw, berseem, maize, barely, sorghum, sugarcane tops, mustard, oat etc. These were dried in a hot air oven 100±5°C overnight, ground and stored in air tight polythyene packets for laboratory analysis.

While collecting the blood/serum samples, the breed of animal, sex, age, physiological status viz. lactating and nonlactating were noted and 638 blood/serum samples were collected (Table 1). For haematological examination about 3 ml blood was drawn from jugular vein in clean vials containing disodium salt of EDTA as anticoagulant and 10 ml blood was collected in a sterilized test tube without any anticoagulant for harvesting of serum.

Haematological estimation

Differential leucocyte count (DLC), total leucocyte count (TLC) and total erythrocyte count (TEC) were carried out as per Jain (1986). Haemoglobin concentration was estimated by cyanmethemoglobin method and expressed as g/dl. These estimations were done immediately.

Biochemical estimation

Enzymes, serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) and serum alkaline phosphatase (SAP) were analysed by the method of Bergmeyer (1983). Ceruloplasmin (Cp) was estimated as per Wooten *et al.* (1996). Triiodothyronine (T_3) and thyroxine (T_4) hormones were radio immunoassayed (Chopra 1971). Vitamins A and E were estimated by the method of Chawla (2001). Values of calcium, magnesium, sodium and potassium in soil, fodder and serum samples were estimated by using atomic absorption spectrophotometer after digesting the samples.

Digestion of soil samples was done by the method of Franeck (1992) with minor modifications. To 2 g of soil sample 2 ml concentrated HNO_3 was added, mixed well and heated on hot-plate for drying. The samples were allowed to cool down before adding 2 ml concentrated HCl to it. After 15 min, the samples were filtered by Whattman filter paper No. 1 by gradually adding triple distilled water making the final volume of filtrate to 50 ml. The fodder was digested as per Trolson (1969) with slight modifications. Ground and stored sample (18) taken in digestion tube and 5 ml of concentrated HNO_3 and 1 ml concentrated H_2SO_4 were added and mixed well. The samples were kept overnight at room temperature followed by digestion in low heat (70-80°C) using heat block (digestion bench), until the volume of samples was reduced to about 1 ml. To this 3 ml of mixed acid mixture (3 part concentrate HNO_3 and 1 part 70% $HClO_4$) was added and digestion at low temperature continued until the white fumes erupt from the samples. Digested samples were diluted with 2 ml triple distilled water and filtered through whatman filter paper No. 1. Repeated washing of digestion tube and filter paper was done by taking 0.5 ml triple distilled water. The filtrate was again diluted with triple distilled water to make the final volume 10 ml.

Serum samples were digested as per procedure by Kolmer *et al.* (1951) with slight modifications. To 3 ml of serum equal volume of concentrated HNO_3 was mixed in the digestion tube. The samples were digested on mixture (3 part conentrated HNO_3 and 1 part 70% $HClO_4$) was added and digestion on low temperature continued until the digested samples become watery clear and emitted white fumes. Final volume of 10 ml was made by repeated washing with triple distilled water. While digestion of soil, fodder and serum samples, simultaneous digestions of reagent blanks were also undertaken.

Phosphorus from soil and fodder were estimated by the method of Talapatra *et al.* (1940). Samples were ashed by muffle furnace at 550°C for 3 h. Phosphorus was precipitated as ammonium phosphomolybdate by adding nitric acid ammonium molybdate solution. The serum inorganic phosphorus was estimated by the method of Taussky and Shorr (1953). Phosphorus in the form of inorganic phosphate was allowed to react with molybdic acid, producing the phosphomolybdate complex which is a blue-colour compound. The colour is proportional to the phosphorus concentration read on spectrophotometer.

The statistical analysis was made of the results obtained to observe any significant variations by using t-test as per the method described by Snedecor and Cochran (1994).

RESULTS

The soil calcium, phosphorus and magnesium were estimated (on DM basis) in the 8 districts, viz. Nainital, Almora, Bageshwar, Udham Singh Nagar, Pilibhit, Bareilly, Badaun and Rampur.

The highest concentration of Mg and Ca in soil was found in Almora and the lowest was in Bareilly (Table 2). The highest incidence of calcium deficiency was in Bareilly,

Table 2. Average soil, fodder (DM basis) and serum Ca, P and Mg values in Northern India

State	Region	District	Soil (ppm)			Fodder (%)			Serum (mg/dl)		
			Ca	P	Mg	Ca	P	Mg	Ca	P	Mg
Uttaranchal	Hilly	Nainital	114.52	29.42	47.32	0.58	0.43	0.28	11.11	4.93	2.11
			±4.37	±1.21	±1.43	±0.032	±0.026	±0.014	±0.23	±0.21	±0.17
Uttaranchal	Hilly	Almora	127.31	27.56	48.21	0.56	0.39	0.33	10.92	4.72	2.47
			±3.23	±1.18	±1.54	±0.041	±0.022	±0.015	±0.19	±0.16	±0.14
Uttaranchal	Hilly	Bageshwar	121.52	26.78	47.62	0.54	0.41	0.31	10.85	4.68	2.31
			±3.11	±1.26	±1.34	±0.027	±0.025	±0.012	±0.21	±0.19	±0.12
Uttaranchal	Tarai	U S Nagar	87.76	21.44	34.23	0.47	0.34	0.21	8.64	3.94	1.94
			±2.85	±1.08	±1.13	±0.015	±0.01	±0.013	±0.14	±0.13	±0.12
Uttar Pradesh	Tarai	Pilibhit	95.45	20.12	32.55	0.52	0.29	0.24	9.13	3.81	1.77
			±2.64	±1.13	±1.22	±0.021	±0.026	±0.009	±0.17	±0.23	±0.13
Uttar Pradesh	Plain	Bareilly	61.23*	14.51*	27.32*	0.44	0.23*	0.18*	7.85*	3.36*	1.62
			±4.80	±1.68	±1.88	±0.019	±0.012	±0.007	±0.11	0.08	±0.06
Uttar Pradesh	Plain	Badaun	66.58	16.73	29.13	0.45	0.28	0.23	8.13	3.51	1.71
			±2.84	±1.24	±1.94	±0.026	±0.018	±0.011	±0.12	±0.13	±0.07
Uttar Pradesh	Plain	Rampur	065.44*	16.23*	28.21*	0.48	0.26	0.210.	8.24	3.72*	1.68
			±3.94	±2.23	±2.18	0.038	±0.024	±0.12	±0.13	±0.11	±0.09

*Significant at P<0.05.

followed by U S Nagar, Pilibihit, Rampur, Badaun, Almora, Bageshwar and lastly Nainital. The maximum incidence of phosphorus and magnesium deficiency was in Bareilly and the minimum was in Nainital (Table 3).

The highest concentration of fodder Ca and P was found in Nainital and the lowest in Bareilly, whereas the highest concentration of Mg in fodder in Almora and the lowest in Bareilly (Table 2). Amongst fodder samples the highest calcium deficiency was observed in Bareilly district followed by Badaun, Rampur, Pilibhit, U S Nagar, Bageshwar, Almora and Nainital. Highest incidence of fodder P deficiency was in Bareilly and the lowest was in Almora whereas the maximum incidence of Mg deficiency was in Bareilly district and the minimum was in Nainital (Table 3). Concentration of Ca in the serum of cattle was maximum in Nainital followed by Almora, Bageshwar, Pilibhit, U S Nagar, Rampur, Badaun and finally Bareilly (Table 2). Highest phosphorus concentration in the serum of cattle was in Nainital and the lowest in Bareilly. The maximum concentration of magnesium in the serum of cattle was in Almora and the minimum in Bareilly. In the present study an overall 27.15% Ca deficiency was found in the serum of cattle (Table 3). The highest prevalence was in Bareilly followed by Rampur, Badaun, U S Nagar, Pilibhit, Almora, Nainital and finally Bageshwar. An overall 27.61% phosphorus deficiency was observed in the blood samples of cattle. The maximum prevalence was in Bareilly and minimum in Almora. An overall 23.13% Mg deficiency was in Bareilly and the lowest in Almora.

Table 3. Prevalence of soil, fodder and serum Ca, P and Mg deficiency in Northern India

State	Region	District	Soil (%)			Fodder (%)			Serum (%)		
			Ca	P	Mg	Ca	P	Mg	Ca	P	Mg
Uttaranchal	Hilly	Nainital	0.5.48.	09.75	14.02	07.32	8.54	08.54	20.51	24.36	12.81
Uttaranchal	Hilly	Almora	08.95	13.16	16.88	07.89	7.89	11.84	23.37	23.37	10.39
Uttaranchal	Hilly	Bageshwar	07.69	12.16	15.79	08.11	9.46	10.81	19.74	23.68	11.84
Uttaranchal	Tarai	U S Nagar	26.47	24.66	26.83	12.33	15.07	20.55	30.48	30.48	25.61
Uttar Pradesh	Tarai	Pilibhit	22.73	21.21	28.79	14.10	16.66	21.79	27.38	32.14	27.39
Uttar Pradesh	Plain	Bareilly	26.76	28.17	33.80	20.00	24.00	25.33	32.94	34.12	34.12
Uttar Pradesh	Plain	Badaun	21.54	24.62	32.31	18.46	23.07	24.62	30.66	28.00	32.00
Uttar Pradesh	Plain	Rampur	22.22	27.77	33.33	17.81	23.29	26.03	32.10	24.69	30.86

Table 4 revealed that in most of the cases of soil-fodder, soil-serum, fodder-serum inter-relationship was significant at 5% and 1% level in all the districts.

Maximum AST and ALT values were found in Nainital and Almora, respectively, whereas lowest SAP was found in

Table 4. Correlation coefficients of Ca, P and Mg in soil, fodder and serum of cattle

	Nainital	Almora	Bageshwar	U S Nagar	Pilibhit	Bareilly	Badaun	Rampur
Calcium								
Soil-fodder	0.954**	0.947**	0.896**	0.73**	0.846**	0.742**	0.784**	0.902**
	±0.065	±0.078	±0.073	0.098	±0.095	±0.103	±0.084	±0.076
Soil-serum	0.786**	0.725**	0.849**	0.649*	0.861**	0.591	0.831**	0.784**
	±0.123	±0.113	±0.069	±0.124	±0.086	±0.145	±0.065	±0.135
Fodder-serum	0.782**	0.726**	0.758**	0.746**	0.815**	0.687*	0.658*	0.735**
	±0.083	±0.072	±0.071	±0.091	±0.117	±0.011	±0.078	±0.119
Phosphorus								
Soil-fodder	0.795**	0.900*	0.768**	0.703**	0.813**	0.568	0.733*	0.719**
	±0.065	±0.073	±0.073	±0.084	±0.056	±0.073	±0.086	±0.093
Soil-serum	0.734**	0.724**	0.653*	0.734**	0.842**	0.658*	0.694*	0.654*
	±0.073	±0.098	±0.064	±0.088	±0.073	±0.096	±0.131	±0.112
Fodder-serum	0.706**	0.824**	0.749**	0.713**	0.325	0.784**	.852**	0.814**
	±0.100	±0.076	±0.089	±0.089	±0.185	±0.096	±0.092	±0.064
Magnesium								
Soil-fodder	0.873**	0.937**	0.685*	0.734**	0.786**	0.693*	0.785**	0.764**
	±0.063	±1.03	±0.058	±0.134	±0.072	±0.084	±0.089	±0.11
Soil-serum	0.789**	0.784**	0.736**	0.845**	0.824**	0.825**	0.584	0.684*
	±0.072	±0.063	±0.113	±0.053	±0.075	±0.118	±0.072	±0.092
Fodder-serum	0.811**	0.734**	0.832**	0.432	0.785**	0.745**	0.745**	0.796**
	±0.034	±0.065	±0.082	±0.072	±0.063	±0.055	±0.071	±0.065

**Significant at (P<0.01), *significant at (P<0.05).

Almora. From the perusal of Table 5, it was observed that hilly samples have comparatively lower Cp than tarai and plains. Least Cp was found in Bageshwar followed by Bageshwar. Badaun showed the highest Cp.

Regarding the thyroid hormone, lower values of T_3 and T_4 were observed in hilly districts followed by Tarai and

Table 5. Serum enzymes values, viz. AST, ALT, SAP and ceruloplasmin (Cp) in deficient cattle in Northern India

District	AST (RE units/ml)	ALT (RE units/ml)	SAP (RE units/100 ml)	Ceruloplasmin (Cp) (mg/dl)
Nainital	59.30±0.23*	9.00±0.22*	10.00±0.50*	18.00±0.01*
Almora	58.85±0.12*	9.98±0.81*	9.86±0.32*	17.65±0.03*
Bageshwar	48.00±0.59*	9.36±0.32*	11.82±0.86*	16.93±0.50*
U.S. Nagar	40.28±0.46*	9.20±0.23*	11.06±0.12*	19.86±1.10*
Pilibhit	45.05±0.58*	9.03±0.12*	10.98±0.09*	19.98±0.02*
Bareilly	40.20±0.11*	8.45±0.11*	10.70±0.11*	22.25±3.02*
Badaun	41.65±0.50*	8.55±0.20*	10.18±0.05*	23.00±2.00*
Rampur	39.00±0.02*	8.30±0.32*	10.00±0.15*	21.92±3.06*

*Significant at (P<0.05).

Table 6. Haematobiochemical profile of viz. Hb, TEC, TLC, vit. A, vit. E, T_3 and T_4 in deficient cattle in Northern India

District	Hb (g%)	TEC (×10³/µl)	TLC (×10³/µl)	T_3 (mg/dl)	T_4 (µg/dl)	Vit. A (µg/ml)	Vit. E (µg/ml)
Nainital	8.00±0.01*	8.01±0.01	8.88±0.22*	118.61±8.40*	6.83±1.52*	0.73±0.12*	1.60±0.89*
Almora	8.23±0.05*	8.68±0.16	8.23±0.30*	115.25±7.60*	4.69±0.95*	0.71±0.23*	1.05±1.85*
Bageshwar	7.89±0.25*	6.20±0.03	8.50±0.12*	112.05±5.65*	3.66±0.18*	0.70±0.32*	1.36±1.30*
U S Nagar	8.50±0.03*	7.50±0.50	7.60±0.85*	126.39±10.11*	7.00±0.58*	0.84±0.36	3.25±0.80
Pilibhit	8.80±0.01*	7.65±0.23	6.23±0.55*	131.12±9.99*	7.15±0.23*	0.80±0.34	3.03±0.79
Bareilly	7.98±0.26*	5.80±0.02*	9.72±0.16	214.45±30.00	8.42±0.25	0.80±0.50	2.19±1.02
Badaun	7.82±0.12*	5.22±0.10*	9.63±0.11	210.80±25.00	8.35±1.98	0.83±0.12	2.20±1.10
Rampur	8.86±0.26*	5.65±0.19*	8.99±0.02*	213.60±15.00	8.66±1.55	0.79±0.16*	2.00±1.08*

*Significant at (P<0.05).

finally in plains. Maximum T_3 value was found in Bareilly. The highest T_4 value was observed in Rampur. Amongst the vitamins, highest vitamin A and vitamin E concentration was found in U S Nagar (Table 6).

DISCUSSION

In Nainital region 5.48, 9.75 and 14.02% soil samples were deficient in Ca, P, Mg content, respectively, while in Almora deficiency for Ca, P, Mg was 8.95, 13.16, 16.88, respectively. Baruash *et al.* (2000) reported that Ca and Mg were well within the normal range, while inorganic phosphate was deficient in hills of Guwahati in Assam. This finding is similar to present study except that the Uttaranchal have more phosphorus, in the soil.

On estimation of fodder mineral content, Ca deficiency was 7.32% in Nainital and 8.54% in Almora, whereas P deficiency was 8.54% and 7.89% in the hilly district of Nainital and Almora. The low prevalence of the deficiency is due to the fact that soil of this area is also less deficient. Similar findings were reported by Baruah *et al.* (2000) from the hilly regions of Assam. Singh *et al.* (1995) told that utilization of forest based fodder in the mountains of Garhwal and Kumaon with some technological options such as proper management of community land can introduce a new dimension of sustainability in livestock production.

Amongst cattle the highest prevalence of Ca, P and Mg deficiency was in lactating cattle in comparison to non-lactating and young. This is probably due to the fact the lactating cattle require more minerals as Ca, P are the major constituents of milk (Underwood and Suttle 1999). The low prevalence in non-lactating cattle is due to the tendency of owners to leave these animal for grazing, where these can feed various types of fodder. Ramana *et al.* (2000) reported that tree leaves/top feeds are a good source of all minerals. Similar findings were reported by NDRI (1971) from the study of cattle in Assam. However, Talapatra *et al.* (1948) reported that the oxalic acid hampers the absorption of Ca from GI tract. According to Singh *et al.* (1992) the content of oxalates of hilly grasses varied from 0.90 to 2.42%.

The average Ca, P, Mg value in the soil samples of hilly districts are optimum. The average soil, fodder, serum level in 3 districts of hilly region showed that value of Ca, P and Mg was well above the critical level. The prevalence of deficiency is also very less. It is a well documented fact that the soil of Uttaranchal hills are very rich in the mineral content hence due to the food chain, ample amount of mineral reaches in animal body. Agarwal (1988) reported that Nainital soils are less weathered and qualifies to be young soil with high mineral content. Sharma and Joshi (2002) have reported that high parasitic infestation in Tarai region result into mineral deficiency in bovines.

As compared to the hilly region, significantly high deficiency of all the macro mineral was observed in districts of plain region. This is probably due to excessive use of pesticides, which interferes in the absorption of minerals by the fodder. The pesticides also affect the assimilation and absorption of the mineral in the GI tract (Singh *et al.* 1992). There is a significant ($P<0.05$ and $P<0.01$) level correlation between soil-fodder, soil-serum, fodder-serum level in Ca, P and Mg status in all the districts surveyed. These values are similar to the reports of Ramana *et al.* (2000) and Sharma *et al.* (2002).

There was significant rise in the heart rate and respiration rate of the deficient animals. This is probably due the decrease in Hb concentration. Poor appetite being the first symptom of Ca and P deficiency (Underwood 1981). Thus to compensate the oxygen requirement for the body and the conditions, the respiration rate and the heart rate increases. A slight decrease in the ruminal movement was observed in the deficient animals. As the major function of P is to influence the activity of microorganism, hence the decrease in P causes a decrease in the activity of the ruminal microorganisms (Underwood and Suttle 1999).

There was a significant ($P<0.01$) decrease in the Hb concentration and TEC of the deficient animals. A decrease in Hb and PCV was noted in cattle and buffaloes that suffered from P deficiency by Ogawa *et al.* (1987).

AST and ALT significantly decreased in the mineral deficient animals. This may probably be due to increase in transamination reaction as a result of Ca and P supplementation. A rise in the alkaline phosphatase activity was observed in deficient animals. Underwood and Suttle (1999) have reported a rise in serum alkaline phosphatase in a diet, low in phosphorus. This is probably due to increase in serum Ca level, as alkaline phosphate is one of the major factors in the bone formation.

Milch animal receiving roughage with any concentrate in any ratio were unable to meet their Ca requirement (Verma and Paul Gupta 1984). Phosphorus is one of the costliest mineral nutrients in the diet of animal and its deficiency affects the livestock production and health in many parts of India.

T_3 ranged from 112.05–243.60 mg/dl and T_4 ranged 3.00–8.66 µg/dl. These values were in correlation with the findings of Sharma *et al.* (2003). The reduction in both T_3 and T_4 hormones in hilly regions may be due to decreased bio-availability of iodine from different sources of intake.

Lower vitamin A can be correlated with Zn deficiency. Similar findings were reported by Sharma *et al.* (2003) in Haryana State.

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