Therapeutic Efficacy of Minerals Supplement in Macro-minerals Deficient Buffaloes and its Effect on Haematobiochemical Profile and Production

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ABSTRACT : To record the prevalence of macro-minerals deficiency in buffaloes, a survey was conducted in certain parts of Northern India. The prevalence of soil Ca, P, Mg, Na, P and K deficiency was 21.35%, 23.30%, 28.64%, 3.61% and 6.84%, respectively while that of fodder Ca, P, Mg, Na and K deficiency was 13.88%, 16.55%, 19.72%, 3.54% and 4.86%, respectively. The overall prevalence of serum (buffalo) Ca, P, Mg, Na and K deficiency in certain parts of northern India was 25.48%, 24.66%, 24.36%, 4.42% and 3.28%, respectively. The correlation coefficient of Ca, P, Mg, Na and K in soil, fodder and serum was significant and in most of the cases the values were above 0.6. The highest deficiency of macro-minerals i.e. Ca, P, Mg, Na and K was found in plain regions, followed by Tarai (foot hill of Himalayas) region and finally the hilly region. For therapeutic studies, three types of mineral mixture were prepared according to deficiency obtained and fed to three groups of deficient animals. Observations were recorded on 0, 30, 60 and 75 day. In group A animals normal mineral mixture was provided, where as in group C and D 10% and 25% more of Ca, P, Mg were provided, respectively. There was an increase in body weight, milk yield, haemoglobin concentration, and total erythrocyte count. Alanine aminotransferase, aspartate amino transferase in group D animals. There was a decrease in heart rate, respiratory rate and alkaline phosphatase in group D animal after mineral supplement. Thus showing the efficacy when supplements 3 provided to group D animals. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 9 : 1278-1287)***

Key Words : Macro-mineral Deficiency, Buffalo, Supplement, Haematobiochemical, Production, Therapy, Milk Yield

INTRODUCTION

The population of India has crossed over one billion mark on 2000. With this huge population there is a need to increase the crop production as well as animal food products. The agricultural practice is changing in highly industrialized nations whereby animals are raised in "Soil independent" environment on high quality and multi component feeds (Kirchagnessner, 1978). Low animal productivity may occur as a result of complex climatic social and economic problems but under nutrition is a common factor and marked responses in growth and reproduction have been observed from mineral supplementation.

Soils are reservoirs of nutrients and water for plant growth. Nutrient availability to plants depends on the concentration, content and activity of each nutrient in the soil. The concentrations of mineral elements in plants are dependent on the interaction of a number of factors including soil, plant species, stages of maturity, yield, pasture management and climate. The shedding of the seed is normally responsible for losses of many minerals so that the material remaining e.g. the straw is a poor source (Suttle, 1991). Sometimes, there is a difference of minerals content in soil between hill region and plain land. So the deficiency symptoms of these two areas are quite different.

All animal tissues and all types of food contain inorganic or mineral elements in widely varying amounts and proportion. The mineral elements are solid, crystalline chemical elements and are not decomposed or synthesized by ordinary chemical reaction. Typically, Ca represents above 46%, P about 29% and K, S, Na, Cl and Mg together account for about 25%, while essential trace elements constitute less than 0.3% of the total body weight of animals. The skeleton tissues consist of about 80 to 85% of the total body mineral matter or ash and it is mainly the salts of Ca, P and Mg.

Unlike other nutrients, living organisms cannot synthesize mineral elements. Minerals act as structural components of body organs and tissues, constituents of body fluids and tissues as electrolytes and catalysts in enzyme and hormone systems. The most obvious function of the mineral elements is to provide structural support (skeleton) for the body. The neuromuscular disturbance is observed in animals when the levels of Mg and Ca in blood plasma fall below certain limits. During blood coagulation Ca acts as a major constituent. Phosphorus also participates in a multiplicity of metabolic reaction involving energy transfer.

Availability of minerals also decreases with maturity of fodder (Kumar, 1993). Blood mineral status in cattle depend upon the daily mineral intake through feed, apart from non-nutritional factors such as season, age, weight, pregnancy and lactation state (Khan, 1995).

Under Indian conditions, the metabolic and deficiency diseases are quite common and it is mainly due to non-availability of balanced diet or deficiency of specific

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nutrients in the soil. In addition excess consumption of certain nonspecific elements causes secondary deficiency. Out of different nutritional factors the most important are deficiency or deranged metabolism of Ca, P and Mg. These three minerals play important roles in the development of a number of metabolic and deficiency diseases found in ruminants like cattle, buffalo, sheep and goat.

India has vast and rich livestock resources, which play an important part in contributing to the national economy. A present India stands No. 1 in the total fluid milk production which can be attributed to larger number of animal rather than production per animal, followed by USA. In order to provide 270 gm of daily milk for proper growth as per recommendation of WHO the requirement would be about 90 million tons by 2000 AD (Sharma, 2000).

MATERIALS AND METHODS

The work was conducted in hilly, Tarai and plain region of Uttar Pradesh and Uttaranchal. The eight districts surveyed were Nainital, Almora, Bageshwar, Udham Singh Nagar (U.S. Nagar), Pilibhit, Bareilly, Badaun and Rampur. Soil fodder and blood/sera samples were collected. The soil samples were collected from the field where fodder was cultivated. The samples were taken 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. The samples were grinded and stored in airtight polythene packets for laboratory analysis.

The samples of various fodders, which were being fed as such to the buffaloes, were collected from 8 districts from the owners of animals. A total of 609 fodder samples were collected (Table 1). The collected samples were wheat straw, paddy straw, grass/tree leaves, berseem, maize, sorghum, barley, sugarcane tops, mustard etc. Fodder samples were dried in a hot air oven 100±5°C overnight, grinded and stored in air tight polythene packets for laboratory analysis. A total of 697 blood/sera samples were collected form six districts (Table 1). About 3 ml of blood was drawn from jugular vein in clean vials containing disodium salt of EDTA as an anticoagulant and 10 ml of blood was collected in a sterilized test tube without any anticoagulant for harvesting of serum.

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

Sr. No.	District	No. of soil samples collected	No. of fodder samples collected	No. of blood samples collected	Total
1.	Nainital	73	82	85	240
2.	Almora	67	76	74	217
3.	Bageshwar	65	74	92	231
4.	U.S. Nagar	68	73	89	230
5.	Pilibhit	66	78	92	236
6.	Bareilly	71	75	95	241
7.	Badaun	65	78	83	226
8.	Rampur	72	73	87	232
	Total	547	609	697	1,853

The collected blood samples were examined for total erythrocyte count (TEC), total leucocyte count (TLC) and differential leucocyte count (DLC) as per the procedures given by Jain (1986). Haemoglobin (Hb) concentration was estimated immediately within a day by cyanmethemoglobin method and expressed gm/dl.

Enzymes, serum asparate aminotransferase (AST), serum alanine aminotransferase (ALT) were estimated by the method of Reitman and Frankel's (1957), where as serum alkaline phosphatase (SAP) was estimated by the method of Kind and King's (1954). Values of calcium, magnesium, phosphorus, sodium, potassium in soil, fodder and serum samples were estimated by using atomic absorption spectrophotometer. (AAS_4141 ECIL, India) after digesting the samples.

Soil samples were digested by the method of Franeck (1992) with minor modifications. With 2 g soil samples 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 minutes, the samples were filtered by Whatman filter paper No. 1 by gradually adding triple distilled water making the final volume of filtrate to 50 ml.

The fodder samples were digested by the method of Trolson (1969). One gram of previously grinded and stored samples were taken in digestion tube and 5 ml of concentrated HNO_3 and 1 ml of concentrated H_2SO_4 were added and mixed well. The samples were kept overnight at room temperature followed by digestion on low heat (70-80°C) using heat block (digestion bench), until the volume of samples reduced to about 1 ml. To this 3 ml of double acid mixture (3 part concentrated HNO_3 and 1 part 70% $HCIO_4$) was added and low heat digestion continued until the white fumes appeared from the samples. Digested samples were diluted with 2 ml triple distilled water and filtered through Whatman filter paper No. 1. Repeated washings of digestion tube and filter paper was done by taking 0.5ml triple distilled water. The filtrate was again diluted with triple distilled water to make the final volume to 10 ml.

Serum samples were digested as per procedure described by Kolmer et al. (1951). To 3 ml of serum equal volume of concentrated HNO_3 was mixed in the digestion tube. The samples were kept overnight at room temperature followed by digestion on low heat (70-80°C) using heat block (digestion bench), until the volume of samples reduced to about 1 ml. To this 3 ml of double acid mixture (3 part concentrated HNO_3 and 1 part 70% $HCIO_4$) was added and low heat digestion continued until the digested

samples became watery clear and emitted white fumes. As per need, the addition of 3 ml double acid mixture followed by low heat digestion continued until the digested samples become watery clear and emitted white fumes. Repeated washing with triple distilled water was done to make final volume of 10 ml.

Phosphorus from soil and fodder were estimated by the method of Talapatra et al. (1940) with minor modifications. In this procedure muffle furnace was used for making the samples to ash at about 550°C for 3 h. The acid extract was made by using concentrated HCl. 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. This complex produces a blue coloured compound that is proportional to the phosphorus concentration.

For therapeutic studies and to observe the efficacy of three types of mineral supplements, (Table 2) four group of animals were constituted. To the healthy normal buffalo group A (N=10) normal mineral mixture was given (Supplement-1). To the buffaloes of group C (n=10), the mineral mixture was prepared which contained 8% more of Ca, P and Mg (Supplement-2), whereas buffaloes of group D (n=15) was given that mineral mixture which contained 20% more of Ca, P and Mg (Supplement-3) rest of the ingredients and their composition remained same except common salt (NaCl). About 20 g of mineral mixture was fed to the animals twice daily along with the normal diet being fed to all the groups. Observations were recorded for seventy five days in all the groups.

Statistical analysis was done using *t* test as per Snedecor and Cochran (1967).

Table 2. The composition of three types of mineral and vitamin supplement used in the study

Ingredients	Supplement-1	Supplement-2	Supplements-3
Dicalcium phosphate	25%	27.50%	31.25%
Calcium carbonate	12.5%	13.75%	15.63%
Magnesium sulphate	11%	12.10%	13.75%
Ferrous sulphate	5%	5%	5%
Sodium chloride	45.2%	39.35%	37.07%
Copper sulphate	0.2%	0.2%	0.2%
Manganese oxide	0.3%	0.3%	0.3%
Cobalt chloride	0.1%	0.1%	0.1%
Potassium iodide	0.5%	0.5%	0.5%
Zinc sulphate	1.2%	1.2%	1.2%
Vitamin A*	25×10^6 I.U.	25×10^6 I.U.	25×10^6 I.U.
Vitamin D_3*	15×10^6 I.U.	15×10^6 I.U.	15×10^6 I.U.
Vitamin E*	25 mg	25 mg	25 mg

* Indicates concentration per kg of mineral mixture.

RESULTS

The soil calcium phosphorus and magnesium were estimated (on DM basis) of the eight districts viz. Almora, Nainital, Bageshwar, Pilibhit, U.S. Nagar, Rampur, Bareilly and Badaun (Table 3). The highest concentration of soil Ca, and Mg was found in Almora and the lowest was in Bareilly, where as the maximum soil P was found in Nainital (29.42±1.21 ppm) and the minimum was in Bareilly (14.51±1.68 ppm). It has been observed that highest prevalence of soil Ca deficiency was in Bareilly (26.76%) followed by U.S. Nagar, Pilibhit, Rampur, Badaun, Almora, Bageshwar and finally Nainital (5.48%) (Table 4). The values of soil Na and K were within normal range.

The highest concentration of fodder Ca and P was found in Nainital and the lowest was in Bareilly, whereas the highest concentration of Mg in fodder was in Almora (0.33±0.015%) and the lowest was in Bareilly (Table 5). Amongst fodder samples the highest incidence of calcium deficiency was in Bareilly district (20%), followed by Badaun, Rampur, Pilibhit, U.S. Nagar, Bageshwar, Almora and Nainital (7.32%) in Table 6. From the perusal of Table 6 the highest incidence of fodder P deficiency was in Bareilly (24%) and the lowest was in Almora (7.89%) whereas the maximum incidence of Mg deficiency was in Rampur district (26.03%) and the minimum was in Nainital (8.54%). The fodder Na and K values were optimum and their prevalence of deficiency was negligible.

From the persual of the Table 7, it is evident that the concentration of Ca in the serum of buffaloes was maximum in Nainital (11.64±0.24 mg/dl) followed by Almora, Bageshwar, U.S. Nagar, Pilibhit, Badaun, Rampur and finally Bareilly (8.36±0.08 mg/dl). The highest phosphorus concentration in the serum of buffaloes was in Nainital (5.61±0.22 mg/dl) and the lowest was in Bareilly (3.21±0.09 mg/dl). The maximum concentration of magnesium in the serum of buffaloes was in Almora (2.53±0.09 mg/dl) and the minimum was in Bareilly (1.61±0.04 mg/dl).

In the present study an overall of 25.48% of Ca deficiency was found in the serum of buffalo (Table 8). The highest prevalence of serum Ca deficiency was in Bareilly (33.33%) followed by Rampur, Badaun, U.S. Nagar, Pilibhit, Almora, Bageshwar and finally Nainital (17.02%). An overall 24.66% of phosphorus deficiency was observed in the blood samples of buffaloes. Regarding phosphorus prevalence deficiency the maximum was in Bareilly (32.25%) and the minimum was in Almora (18.96%). The overall prevalence of serum Mg of buffaloes was (24.36%). The maximum prevalence of serum Mg deficiency was in Bareilly (35.19%) and the lowest was in Almora (12.06%).

Table 3. Showing average soil macro-mineral values in a part of northern India

State	Region	District	Ca (ppm)	P (ppm)	Mg (ppm)	Na (meq/100 gm)	K (meq/100 gm)
Uttaranchal	Hilly	Almora	127.31±3.23	27.56±1.18	48.21±1.54	0.144±0.02	0.129±0.02
Uttaranchal	Hilly	Nainital	114.52±1.37	29.42±1.21	47.32±1.43	0.165±0.03	0.158±0.03
Uttaranchal	Hilly	Bageshwar	121.52±3.11	26.78±1.26	47.62±1.34	0.158±0.01	0.032±0.01
Uttar Pradesh	Tarai	Pilibhit	95.45±2.64	20.12±1.13	32.55±1.22	0.218±0.03	0197±0.02
Uttaranchal	Tarai	U.S. Nagar	87.76±2.85	21.44±1.08	34.23±1.13	0.226±0.02	0.214±0.03
Uttar Pradesh	Plain	Rampur	65.44*±3.94	16.23±2.23	28.21*±2.18	0.138±0.02	0.114±0.02
Uttar Pradesh	Plain	Bareilly	61.23*±4.80	14.51*±1.68	27.32*±1.88	0.156±0.03	0.098±0.01
Uttar Pradesh	Plain	Badaun	66.58±2.84	16.73±1.24	29.13±1.94	0.146±0.01	0.121±0.01

Values in the column differ significant at ($p<0.05$).

Table 4. Prevalence of soil macro-mineral deficiency in northern India (%)

State	Region	District	Ca	P	Mg	Na	K
Uttaranchal	Hilly	Almora	08.95	13.16	16.88	4.36	7.43
Uttaranchal	Hilly	Nainital	05.48	09.75	14.02	3.32	3.48
Uttaranchal	Hilly	Bageshwar	07.69	12.16	15.79	3.18	6.56
Uttar Pradesh	Tarai	Pilibhit	22.73	21.21	28.79	1.42	1.36
Uttranchal	Tarai	U.S. Nagar	26.47	24.66	26.83	1.28	1.48
Uttar Pradesh	Plain	Rampur	22.22	27.77	33.33	5.24	7.82
Uttar Pradesh	Plain	Bareilly	26.76	28.17	33.80	3.48	8.54
Uttar Pradesh	Plain	Badaun	21.54	24.62	32.31	4.16	5.12

Table 5. Showing average fodder macro-mineral values (on dm basis) in a part of northern India

State	Region	District	Ca (%)	P (%)	Mg (%)	Na (%)	K (%)
Uttaranchal	Hilly	Almora	0.56±0.041	0.39±0.022	0.33±0.015	0.041±0.02	0.62±0.04
Uttaranchal	Hilly	Nainital	0.58±0.032	0.43±0.026	0.28±0.014	0.036±0.02	0.68±0.06
Uttaranchal	Hilly	Bageshwar	0.54±0.027	0.41±0.025	0.31±0.012	0.038±0.02	0.65±0.06
Uttar Pradesh	Tarai	Pilibhit	0.52±0.021	0.29±0.026	0.24±0.009	0.061±0.003	0.81±0.05
Uttranchal	Tarai	U.S. Nagar	0.47±0.015	0.34±0.011	0.21*±0.13	0.058±0.001	0.78±0.04
Uttar Pradesh	Plain	Rampur	0.48±0.038	0.26±0.024	0.21*±0.012	0.042±0.03	0.75±0.03
Uttar Pradesh	Plain	Bareilly	0.44±0.019	0.23*±0.012	0.18*±0.007	0.046±0.01	0.77±0.04
Uttar Pradesh	Plain	Badaun	0.45±0.026	0.28±0.018	0.23±0.011	0.048±0.01	0.72±0.05

Values in the column differ significant at ($p<0.05$).

Table 6. Prevalence of fodder macro-mineral deficiency in northern India (%)

State	Region	District	Ca	P	Mg	Na	K
Uttaranchal	Hilly	Almora	7.89	7.89	11.84	3.68	6.68
Uttaranchal	Hilly	Nainital	7.32	8.54	8.54	5.12	4.43
Uttaranchal	Hilly	Bageshwar	8.11	9.46	10.81	4.38	5.36
Uttar Pradesh	Tarai	Pilibhit	14.10	16.66	21.79	1.64	1.64
Uttranchal	Tarai	U.S. Nagar	12.33	15.07	20.55	2.36	1.86
Uttar Pradesh	Plain	Rampur	17.81	23.29	26.03	3.45	3.12
Uttar Pradesh	Plain	Bareilly	20.00	24.00	25.33	3.12	2.58
Uttar Pradesh	Plain	Badaun	18.54	23.07	24.62	2.54	3.69

Table 7. Showing average serum macro-mineral values in a part of northern India (mg/dl)

State	Region	District	Ca	P	Mg	Na	K
Uttaranchal	Hilly	Almora	11.48±0.13	4.92±0.17	2.53±0.09	128.39±1.78	4.89±0.39
Uttaranchal	Hilly	Nainital	11.64±0.24	5.61±0.22	2.32±0.11	126.38±1.68	4.86±0.42
Uttaranchal	Hilly	Bageshwar	11.16±0.17	5.08±0.24	2.44±0.14	133.62±1.84	4.78±0.38
Uttar Pradesh	Tarai	Pilibhit	9.13±0.09	3.76*±0.11	2.11± 0.07	153.69±1.96	5.22±0.35
Uttranchal	Tarai	U.S. Nagar	10.29±0.12	4.13±0.13	1.97±0.08	149.56±2.11	5.12±0.45
Uttar Pradesh	Plain	Rampur	8.54±0.12	3.53±0.12	1.86*±0.06	138.46±1.89	4.59±0.53
Uttar Pradesh	Plain	Bareilly	8.36±0.08	3.21*±0.09	1.61*±0.04	142.51±2.19	4.68±0.39
Uttar Pradesh	Plain	Badaun	8.89±0.14	3.46±0.15	1.72±0.12	145.93±2.16	4.76±0.45

* Values in the column differ significant at ($p<0.05$).

Table 8. Prevalence of serum (buffalo) macro-mineral deficiency in northern India (%)

State	Region	District	Ca	P	Mg	Na	K
Uttaranchal	Hilly	Almora	18.96	18.96	12.06	4.54	2.14
Uttaranchal	Hilly	Nainital	17.02	19.14	17.02	5.88	2.65
Uttaranchal	Hilly	Bageshwar	17.43	19.06	15.12	5.18	2.29
Uttar Pradesh	Tarai	Pilibhit	22.07	25.97	20.78	1.96	1.33
Uttranchal	Tarai	U.S. Nagar	24.28	25.71	22.85	2.32	1.46
Uttar Pradesh	Plain	Rampur	28.76	26.03	27.39	4.16	4.16
Uttar Pradesh	Plain	Bareilly	33.33	32.25	35.19	3.77	3.68
Uttar Pradesh	Plain	Badaun	27.45	26.95	33.85	3.24	3.42

The values of serum Na and K were well within the normal range.

Table 9 reveals that in most of the cases of soil-fodder, soil-serum, fodder-serum interrelationship of the minerals Ca, P, Mg, Na and K were significant at 5% and 1% level in the districts of Almora, Nainital, Bageshwar, Pilibhit, U.S. Nagar, Rampur, Bareilly and Badaun.

Therapeutic studies

No significant change was observed in the body temperature of deficiency animal group. There was a significant ($p<0.05$) increase in the respiration rate in the animal of group B, C and D as compared to animals of group A at the 0 day of treatment (Table 10). By the 75th day of treatment the respiration rate of group D returned towards normalcy. Heart rate of group B, C and D increased

Table 9. Correlation coefficients of macro-mineral in soil, fodder and serum of buffalo

	Nainital	Almora	U. S. Nagar	Bageshwar	Pilibhit	Bareilly	Rampur	Badaun
				Calcium				
Soil-F[1]	0.954±0.065**	0.947±0.078**	0.873±0.985**	0.849±0.076**	0.846±0.095**	0.742±0.100**	0.902±0.076**	0.785±0.086**
Soil-S[2]	0.723±0.091**	0.775±0.135**	0.797±0.111**	0.758±0.082**	0.812±0.096**	0.658±0.101*	0.824±0.096**	0.548±0.121
F-S	0.756±0.112**	0.845±0.072**	0.695±0.095*	0.645±0.102*	0.735±0.125**	0.578±0.086	0.823±0.112**	0.845±0.075**
				Phosphorus				
Soil-F	0.795±0.065**	0.900±0.073**	0.703±0.084**	0.753±0.085**	0.813±0.056**	0.568±0.073	0.719±0.093**	0.594±0.089
Soil-S	0.546±0.065**	0.833±0.73**	0.717±0.065**	0.859±0.068**	0.567±0.078	0.665±0.063*	0.782±0.056**	0.725±0.065**
F-S	0.784±0.086**	0.845±0.078**	0.734±0.096**	0.685±0.075*	0.689±0.072*	0.723±0.112**	0.653±0.09*	0.687±0.078*
				Magnesium				
Soil-F	0.873±0.063**	0.937±0.103**	0.734±0.134**	0.692±0.094*	0.786±0.072**	0.693±0.84*	0.764±0.11**	0.768±0.079**
Soil-S	0.734±0.092**	0.812±0.073**	0.685±0.076*	0.738±0.078**	0.646±0.065*	0.531±0.051	0.734±0.074**	0.427±0.113
F-S	0.746±0.085**	0.683±0.132*	0.785±0.096**	0.773±0.883**	0.822±0.064**	0.685±0.083*	0.763±0.121**	0.665±0.097*
				Sodium				
Soil-F	0.768±0.087**	0.748±0.075**	0.849±0.065**	0.681±0.058*	0.654±0.067*	0.728±0.068**	0.283±0.056	0.341±0.065
Soil-S	0.653±0.076*	0.539±0.094	0.714±0.078**	0.643±0.063*	0.426±0.068	0.482±0.075	0.587±0.067	0.551±0.074
F-S	0.763±0.094**	0.746±0.086**	0.962±0.084**	0.782±0.078**	0.856±0.076**	0.683±0.084*	0.738±0.054	0.748±0.053
				Potassium				
Soil-F	0.652±0.053*	0.638±0.058*	0.865±0.078**	0.483±0.067	0.621±0.065*	0.421±0.045	0.346±0.051	0.724±0.065**
Soil-S	0.682±0.078*	0.736±0.065**	0.746±0.069**	0.786±0.065**	0.781±0.068**	0.584±0.058	0.648±0.065*	0.596±0.054
F-S	0.548±0.066	0.632±0.074*	0.843±0.073**	0.714±0.068**	0.876±0.075**	0.845±0.072*	0.764±0.073**	0.734±0.066**

* Indicates significant at 5% level, ** indicates at 1% level. [1] F=fodder, [2] S=Serum.

markedly ($p<0.05$) in respect to control group. By the 75th day of treatment the group D almost reached the normal mark when compared to group A. No changes were observed in the group B animals throughout the duration of treatment (Table 10). In case of group B, C and D a significant ($p<0.05$) decrease of ruminal movement occur in comparison to cattle of group A at the 0 day of treatment (Table 10). By the 75th day of treatment the ruminal movement reached toward normal limit (4.94±0.28/5 min). No significant changes were observed in group B and C animals during the course of treatment.

There was a significant ($p<0.05$) increase in the milk yield in group D. By 60th day in the treated group D (6.85± 0.21 Lt/day) milk yield was markedly increased and returned towards group A (9.72±0.21 Lt/day). By 75th day in the treated group D (7.46±0.25 Lt/day) milk yield almost reached towards healthy control group A. No significant change was observed in group B animals throughout the duration of treatment. In case of group C slow improvement in milk yield was observed.

The body weight of group B, C and D was significantly ($p<0.05$) less than group A at 0 day of treatment. By 75th day of treatment the body weight of group D (478.39± 08.43 kg) as compared to group A (494.34±10.68 kg). The body weight of group C improved slowly.

In case of group B, C and D there was a significant ($p<0.05$) decrease of R.B.C. and Hb level in comparison to buffaloes of group A at 0 day of treatment (Table 11). By 75th day in group D, RBC and Hb values almost reached the normal levels as compared to group A.

A significant ($p<0.05$) decrease was observed in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in buffaloes of group B, C and D as compared to buffaloes of group A at the 0 day of treatment (Table 11). By 60th and 75th day in the treated group D AST and ALT values almost reached the normal levels compared to group A. No significant changes were observed in group B animals throughout the duration of treatment (Table 11).

There was a significant ($p<0.05$) decrease of serum calcium in buffaloes of group B, C and D in comparison to buffaloes of group A at 0 day of treatment (Table 12). By 60th day of treatment the value of group D moved from higher to lower limits of normalcy. No significant change was observed in group B animals throughout the duration of treatment.

In case of P there was a significant ($p<0.05$) decrease in buffaloes of group B, C and D in comparison to buffaloes of group A at 0 day of treatment. By 60th day of treatment there was increase in serum P values of group D (5.21± 0.38 mg/dl).

The serum Mg value was significantly increased in the groups of B, C and D in comparison to buffaloes of group A at 0 day of treatment (Table 12). By 75th day of treatment

Table 10. Effect of various mineral supplements on respiration, heart rate, ruminal movement, milk yield (l/day) and body weight (kg) in deficient

Sr. No.	Respiration rate/minute	Heart rate/minute	Ruminal movement/5 min	Milk yield (L/day)	Body wt. (kg)
Day 0/Group					
A	16.58±1.11	47.34±3.48	5.18±0.36	9.68±0.43	492.25±11.45
B	22.68±1.42*	65.38±3.22*	3.12±0.23*	5.92±0.29*	470.84±09.83*
C	22.72±1.35*	64.73±2.88*	3.42±0.31*	6.11±0.23*	468.92±11.33*
D	21.86±0.65*	63.77±1.73*	3.16±0.13*	6.19±0.18*	469.35±10.22*
Day 30/Group					
A	16.46±1.32	47.16±2.79	5.13±0.24	9.74±0.48	491.56±10.98
B	22.53±1.25*	64.93±2.84*	3.22±0.37*	5.83±0.26*	471.11±09.56*
C	22.46±0.48*	62.52±3.16*	3.65±0.32*	6.48±0.38*	472.41±10.87*
D	21.17±0.44*	58.39±2.18*	3.73±0.17*	6.67±0.22*	473.99±09.11*
Day 60/Group					
A	16.38±1.28	48.38±3.18	5.33±0.19	9.72±0.45	493.74±11.24
B	22.16±1.31*	65.76±3.08*	3.45±0.26*	6.08±0.36*	472.06±10.14*
C	20.11±1.06*	61.14±2.68*	3.89±0.36*	6.54±0.34*	474.44±10.39*
D	20.34±0.63*	56.96±1.83*	4.45±0.24	6.85±0.21	475.36±09.16*
Day 75/Group					
A	16.66±1.36	47.86±2.54	5.64±0.11	9.86±0.49	494.34±10.68
B	22.38±1.24*	65.83±2.81*	3.08±0.18*	5.98±0.28*	471.66±09.18*
C	19.88±0.97*	58.47±2.98*	4.06±0.31*	6.88±0.37*	474.69±10.36*
D	19.73±0.93	52.12±1.31	4.94±0.28	7.46±0.25	478.39±08.43

Values in the column differ significant at ($p<0.05$).

Table 11. Effect of various mineral supplements on Hb, TEC, TLC, AST, ALT and SAP status in deficient buffalo

Sr. No.	Haemoglobin gm%	Total erythrocyte count ($\times 10^6$/µl)	Total leucocyte count (103/µl)	Aspartate amino transferase (R.E. - units/ml)	Alaninine amino transferase (R.E. units/ml)	Alkaline phosphates KA units
Day 0/Group						
A	12.52±0.49	7.12±0.42	7.12±0.63	78.46±3.78	16.84±0.65	04.36±0.32
B	08.84±0.58*	4.56±0.48*	8.76±0.41*	52.36±3.16*	09.36±0.76*	10.29±0.41*
C	09.15±0.42*	4.68±0.39*	8.86±0.36*	51.24±2.74*	09.43±0.73*	10.37±0.34*
D	08.88±0.24*	4.75±0.26*	8.93±0.29*	49.67±2.17*	09.67±0.42*	10.12±0.25*
Day 30/Group						
A	12.67±0.42	7.19±0.39	7.06±0.59	76.54±3.62	17.11±0.68	04.42±0.33
B	08.93±0.40*	4.51±0.41*	8.98±0.48*	53.21±3.24*	09.17±0.62*	09.85±0.27*
C	09.20±0.34*	4.73±0.26*	8.74±0.29*	52.11±2.65*	09.56±0.82*	09.97±0.38*
D	09.12±0.29*	4.94±0.21*	8.67±0.36*	53.59±1.87*	10.54±0.38*	09.23±0.45*
Day 60/Group						
A	12.56±0.46	7.26±0.48	6.84±0.67	77.95±3.74	16.98±0.72	04.29±0.47
B	08.49±0.75*	4.69±0.53*	8.77±0.51*	52.89±3.42*	09.45±0.67*	09.74±0.39*
C	09.42±0.31*	4.87±0.35*	8.68±0.38*	53.16±2.77*	09.74±0.56*	09.56±0.32*
D	09.57±0.16*	5.32±0.36*	8.36±0.29*	58.98±2.16*	11.89±0.38*	08.21±0.49*
Day 75/Group						
A	12.47±0.39	7.04±0.56	6.93±0.52	77.18±3.66	17.26±0.64	04.76±0.54
B	08.56±0.64*	4.38±0.48*	8.94±0.59*	53.13±3.24*	09.78±0.49*	09.12±0.28*
C	09.61±0.46*	4.96±0.29*	8.46±0.34*	53.88±2.63*	10.43±0.44*	09.22±0.43*
D	10.21± 0.12	6.11±0.24	7.94±0.23	66.64±2.39	13.26±0.22	06.44±0.25

* Values in the column differ significant at ($p<0.05$).

Table 12. Effect of various mineral supplements on serum, calcium, phosphorus, magnesium, sodium and potassium status in deficient buffalo (mean±s.e.)

Sr. no.	Calcium (mg/dl)	Phosphorus (mg/dl)	Magnesium (mg/dl)	Sodium (mmol/L)	Potassium (mmol/L)
Day 0/Group					
A	11.59±0.48	6.12±0.35	3.26±0.32	146.87±3.76	5.17±0.46
B	07.65±0.57*	4.34±0.43*	1.84±0.28*	144.75±3.43	4.66±0.35
C	07.42±0.63*	4.41±0.39*	1.78±0.34*	138.46±2.54	4.52±0.29
D	07.28±0.39*	4.29±0.42*	1.89±0.43*	138.12±1.81	4.68±0.38
Day 30/Group					
A	11.47±0.46	6.04±0.46	3.12±0.38	146.42±3.42	5.28±0.53
B	07.69±0.43*	4.13±0.52*	1.86±0.29*	143.31±3.56	4.79±0.48
C	07.58±0.76*	4.64±0.48*	1.94±0.42*	137.12±2.68	4.64±0.41
D	07.87±0.48*	4.86±0.37*	2.16±0.48*	136.18±1.73	4.72±0.44
Day 60/Group					
A	11.65±0.38	6.18±0.52	3.19±0.47	147.54±4.16	5.11±0.53
B	07.48±0.44*	4.26±0.44*	1.92±0.35*	144.22±3.24	4.82±0.36
C	07.69±0.57*	4.79±0.46*	2.04±0.38*	135.15±2.61	4.71±0.29
D	08.55±0.36	5.21±0.38	2.48±0.26	138.98±1.52	4.85±0.46
Day 75/Group					
A	11.41±0.52	5.89±0.38	3.42±0.44	149.21±3.89	5.32±0.37
B	07.68±0.47 *	4.22±0.51*	1.87±0.49*	145.37±3.06	4.75±0.22
C	08.11±0.43*	4.86±0.39*	2.19±0.23*	139.26±3.12	4.78±0.54
D	09.34±0.32	5.46±0.44	2.68±0.25	144.66±1.43	4.92±0.45

* Values in the column differ significant at ($p<0.05$).

the value of group D (2.68±0.25 mg/dl) increased as compared to group A (3.42±0.44 mg/dl). No significant change was observed in group B animals during treatment whereas delayed normalcy was observed in case of group C.

By 75th day of treatment the values of Na and K in case of group D was (144.66±1.43 m.mol/Lt) and (4.92± 0.45 m.mol/Lt) while in-group A the value was 149.21±3.89 m.mol/Lt and 5.32±0.37 m.mol/Lt) (Table 12).

DISCUSSION

From the very early stages minerals have played an important role in life of human beings and its extension to livestock. Before recorded history, common salt was traded to satisfy the salt cravings of grazing animals. Under nutrition is commonly accepted as one of the most important limitation of livestock production in tropical countries. The lack of sufficient energy and protein is often responsible for sub- optimum livestock production (McDowell et al., 1983). The soil Ca, P and Mg value in the three Hilly districts of Uttaranchal was with in the normal limit. Baurah et al. (2000) estimated the different macro- and micro-mineral status of soil in the Hills of Guwahati in Assam, found that Ca, Mg were well within the normal range while inorganic phosphate was deficient. These findings are similar to the present study except that the Uttaranchal have more phosphorus in the soil.

In Tarai region the deficiency of soil Ca, P and Mg was marginal. Tarai area are the most fertile belt but high yielding crops exhaust the mineral concentration of the soil.

In case of plain region the deficiency of soil Ca, P and Mg was maximum. The high prevalence of deficiency in plain region may be due to insecticide spray and excess of pollutants. Similar observations were made by Asthana (1979) and Prasad and Laskar (1985). Rojas et al. (1993) reported that in wet seasons 80% and in dry season 54% of the soil sample were deficient in Ca levels where as 66% and 32% soil sample were deficient in Mg in wet and dry season in south eastern Venezuela.

The fodder Ca, P and Mg deficiency was with in normal limit because the soil mineral quantity was with in the normal level. Similar findings have been reported by Baruah et al. (2000) from the hilly regions of Assam.

Fodder sample of the Tarai regions showed that 15 to 22% fodder samples were deficient in Ca, P and Mg deficiency. The main fodder in this area was Berseem, which has sufficient amount of calcium, Yadav et al. (1998) studied the mineral content of fodder and reported similar finding in Harayana.

The maximum Ca, P and Mg deficiency was found in plain regions. Application of fertilizers that is widely used in these regions, affect the content and availability of minerals to plants (Flemming, 1973). The phosphorus deficiency is widely prevalent throughout the world as P itself is the limiting mineral in the soil (McDowell, 1992; Underwood and Suttle, 1999). The lactating buffaloes showed the highest Ca, P and Mg deficiency in comparison to non-lactating and young one. This is probably due to the fact that lactating cattle require more mineral as Ca, P are the major constituent of milk (Underwood and Suttle, 1999). Mandal et al. (1996) reported similar findings from their study of the mineral status of buffaloes in Mohindergarh district of Haryana. Verma and Paul Gupta (1984) reported that Ca and P deficiency occur in buffaloes when their level fall below 8 and 3.8 mg/dl, respectively. Ramana et al. (2000) reported that Ca, P and Mg deficiency was not observed in non-lactating buffaloes. This may be due to the tendency of owners to leave these animals 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.

The mineral deficiency in hilly, Tarai and plain regions was of same type as the deficient of soil and fodder, because the animals were taking mineral deficient fodder. Das et al. (1992) concluded that mineral status of soil, plant and animal is interrelated.

There is a significant ($p<0.05$ and $p<0.01$) level correlation between soil-fodder, soil-serum and fodder-serum level in Ca, P, Mg, Na and K status in the eight districts viz. Almora, Nainital, Bageshwar, Pilibhit, U.S. Nagar, Rampur, Bareilly and Badaun. These values are similar to the reports of Ramana et al. (2000). Jackson (1997) also reported similar findings.

There was significant rise in the heart rate and respiration rate of the deficient animals. This is probably due to the decrease in Hb concentration. Lack of appetite being the first symptom of Ca and P deficiency (Underwood, 1981) and the animal become anorectic (McDowell, 1992). Thus to compensate the oxygen requirement for the body and the anorectic 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 micro-organisms (Underwood and Suttle, 1999).

There was a significant decrease in the body weight of the deficient animals. Reduction in the feed intake is the major consequence of P deficiency in ruminants (Field et al., 1975; Ternouth and Sevilla, 1990; McDowell, 1992; Underwood and Suttle, 1999). Lee et al. (2001) however reported that carcass length and back fat thickness of finishing pigs were not significantly effected by dietary treatment. In case of deficient buffaloes there was a significant decrease in the milk yield. High yielding cows require more dietary Ca and P (Mc Dowell, 1992). Buchanan and Smith (1978) estimated the Ca and P requirement for a dairy cow weighing 450 kg and producing 4 kg milk daily to be 18.4 g Ca and 17.6 g P. Milk contains maximum amount of Ca hence the milk yield is affected. Sharma and Joshi (2002) reported similar findings in micro filariasis infected cattle.

There was a significant ($p<0.05$) decrease in the Hb concentration and TEC of the deficient animals as compared to healthy control. The loss of appetite is the

characteristic feature of Ca and P deprivation (Little, 1968) followed by anorexia (Call et al., 1978). A decrease in Hb and PCV was noted in cattle and buffaloes, which suffered from P deficiency by Ogawa et al. (1987).

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 phosphatase is one of the major factors in the bone formation. The values of AST and ALT were significantly decreased in the mineral deficient animals. This may probably be due to increase in transmination reaction as a result of Ca and P supplementation (Exton, 1980). There was a significant ($p<0.05$) decrease in the serum Ca, P and Mg in deficient animals and improvement was observed after therapeutic trials. Milking animal receiving roughage with any concentrate in any ratio was unable to meet their Ca requirement (Verma and PaulGupta, 1984). Phosphorus is one of the costliest mineral nutrients in the diet of animal and its deficiency effects the livestock production and health in many parts of India (Joshi et al., 1991)

CONCLUSIONS

It can be concluded that supplement C which was provided to the Group D animals had a significant effect in the body weight, milk yield and haematobiochemical parameters of the macro-mineral deficient animals. It is recommended that this mineral mixture should be provided to the animals of the areas were macro mineral deficiency is prevalent.

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