Studies on Serum Micro-mineral, Hormone and Vitamin Profile and Its Effect on Production and Therapeutic Management of Buffaloes in Haryana State of India

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ABSTRACT : A survey was conducted in certain parts of Haryana to record the prevalence of micro mineral deficiency in buffaloes. The prevalence of soil Cu, Co, Zn and Fe deficiency was 55.26%, 6.9%, 59.12% and 7.89% respectively. While that of fodder Cu, Co, Zn and Fe was 60.64%, 6.7%, 61.22% and 11.37% respectively. The overall prevalence of serum Cu, Co, Zn and Fe deficiency in Haryana was 59.2%, 19.1%, 59.2% and 19.9% respectively. The correlation co-efficient of Cu, Co, Zn and Fe in soil, fodder and serum was significant in most of the cases the values were above 0.8. Blood examination revealed significant decrease in haemoglobin and TEC level. However, no variation in level of TLC were observed in mineral deficient buffaloes. In micro mineral deficiency, thyroid hormone (T_3 and T_4) levels were decreased in buffaloes. Marginally lower concentration of vitamin A and E were observed in mineral deficient buffaloes in Haryana. The highest deficiency of micro minerals was 61.76% in copper at Ambala followed by 65.86% in zinc at Rhotak. For therapeutic studies a mineral mixture was prepared according to defiency obtained and fed to three groups of animals. Observation was recorded on 0, 15, 30, 45 and 60 days. Group A consist of normal healthy animals and group B mineral deficient animal untreated and group C mineral deficient animal, treated with prepared mineral mixture. 25 gram of mineral mixture was fed daily along with normal ration. There was increase in body weight, milk yield, haemoglobin concentration and total erythrocyte level in group C animals when compared to group B animals. The milk yield in group C animals increased to 6.970±0.41 after 60th day of supplement in comparison to 0 day where it was 5.910±0.37, similarly the body wt. of group C animals increased from 129.42±01.13 (at 0 day) to 159.31±03.61 at 60th day of treatment. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 4 : 519-528)***

Key Words : India, Haryana, Buffaloes, Mineral Deficiency, Thyroid Hormone, Vitamin A and E, Production

INTRODUCTION

Livestock rearing is an integral part of Indian rural life. The livestock sector has a crucial role in national economy particularly rural economy. Livestock sector contribute 26% of 31% of GDP (Dairy India 1997) India leads first in bovine population with39 million buffaloes in 1997. At present India is the top milk producer with 78 million tonnes. The country annual milk production can increase rapidly if milk animals are fed properly with better health care. Under nutrition is a major cause and marked response in growth and reproduction have been obtained from mineral supplements. Minerals are soild crystaline substances which cannot synthesised in the body. Its acts as a structural components of body organs and tissues, and consituents of body fluid, electrolytes and a catalyst in bio chemical function (Under wood, 1981). As these minerals are present in trace amount in nature their deficiency is frequently encountered. Micro mineral deficiency leads to anaemia due to decrease production of erythrocytes. Dairy animals are more prone to nutritional anemia which is mainly due to lack of iron, copper and cobalt resulting in insufficient haemoglobin formation. Under Indian condition which is tropical the mineral deficiency diseases are quite common and is mainly due to non availability of balanced diet or deficiency of minerals in soil and fodder. Abdul Rahman et al. (1998) reported that conditions of tropical areas significantly affect the quality and quantity of forages. Heavy rainfall, leaching of soils, making them deficient in plant minerals (Pfonder, 1971). The mineral deficiency can be corrected by supplementation which can save huge economic losses.

The objective of the present study was to identify the mineral deficient areas of Haryana and observe the efficacy of mineral supplements by various haematological parameters, milk yield, body weight and to recommend a suitable mineral mixture of a specific mineral deficient area.

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Table 1. Total number of samples of soil, fodder and serum / blood collceted from various districts of Haryana state

Districts	Soil	Fodder	Serum/blood
Karnal	51	68	45
Ambala	34	39	44
Kurukshetra	39	42	33
Sonepat	37	32	54
Yamunanagar	36	35	36
Rhotak	29	34	34
Hissar	49	46	83
Nabha[ab]	29	47	73
Total	304	343	402

[ab] Regional station of CIRB Hissar in Punjab State.

MATERIALS AND METHODS

To record the prevalence of various micro mineral deficiency in buffaloes, a survey was conducted in certain parts of Haryana. The work was carried out in 2 phases.

In first phase 7 districts of Haryana namely Karnal, Kurukshetra, Rhotak, Sonepat, Yamuna Nagar, Hissar and Ambala, one district of Punjab and organised farm CIRB regional station at Nabha, were surveyed and soil, fodder and serum/blood samples were collected and analysed for haemotological, vitamins and mineral concentrations. The information regarding feeding, behaviour of animals, management, dewormings, vaccinations and inseticide sprays etc. were collected from farmers.

A total of 304 soil sample were collected (Table 1). The sample were taken with 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 grinded and stored in air tight polythene packet for lab analysis.

The samples of various fodders which were being fed as such to the buffalo were collected from 7 districts of Haryana and 1 district of Punjab. A total of 343 fodder samples were collected (Table 1). The collected samples included natural grasses, wheat straw, berseem, maize, sorghum, mustard, sugarcane tops and oats etc. These were dired in a hot air oven 100±5°C overnight, grinded and stored in air tight polythene packet for laboratory analysis.

While collecting the blood/serum samples the information of breed, age, sex, physiological status, viz, lactating, non lactating were noted. A total of 402 blood/serum samples were collected (Table 1). About 3 ml of blood was drawn from jugular vein in clean vials containing disodium salt of EDTA as an anticoulant and 10 ml blood was collected in sterilised test tube with out any anticoagulant for havesting the serum.

The total Leucocyte Count (TLC) and Total Erythrocyte Count (TEC) were carried out as per the procedures given by Jain (1986). Haemoglobin concentration was estimated by cyanmethaemoglobin method and expressed in gm/dl. These estimations were carried out immediately after blood collection.

Digestion of soil samples was done by the method of Franeck (1992). Two gms of soil sample +2 ml of conc. HNO_3 was added mixed well and heated and hot plate for drying. The samples were allowed to cool down before adding 2 ml concentrated HCl to it. After 15 minute the samples were filtered by what man filter paper No. 1 by gradually adding triple distilled water making the final volume to 50 ml.

The fodder was digested by the method of Troelson (1969). One gram of grinded and stored samples were taken in digestion tube and 5 ml of conc. HNO_3 and 1 ml conc. 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) untill the volume of samples was reduced to 1 ml. To this 3 ml of double acid mixture 3 part conc. (HNO_3 and 1 part 70% $HCLO_4$) was added and low heat digestion continued until the white fumes comes from the samples. Digested samples were diluted with 2 ml triple distilled water and filtered through what man filter paper No. 1. Repeated washing of digestion tube and filter paper was done by taking 0.5 ml triple distilled water. The filterate was again diluted with triple distilled water to make the final volume 10 ml.

Serum sample were digested per procedure by Kolmer et al. (1951). Three ml of serum and equal volume of conc. HNO_3 was mixed in the digestion tube. The samples were digested by low heat (70-80°C) until the volume reduced to about 1 ml. To this 3 ml of double acid mixture (3 part concentrated HNO_3 and 1 part of 70%. $HCLO_4$) was added and low heat digestion continued until the digested samples become watery clear and emitted white fumes. Final volume at 10 ml was made by repeated washing with triple distilled water. While digestion of soil, fodder and serum samples, simultaneous digestion of reagent blanks were also under taken.

The serum thyroid hormone status Tri-iodothyronin (T_3) and Thyroxin (T_4) were estimated by RIA with the method of Chopra et al. (1972) using gamma scientillation counter at Nuclear Research Lab, IVRI. The serum vit. A and E level was determined by HPLC at Dairy Cattle Nutrition Division National Dairy Research Institute, Karnal by method of Chawla and Kaur (2001).

THERAPEUTIC MANAGEMENT

In the second phase for therapeutic studies and to observe the efficacy of prepared mineral mixture (Table 8) trial was conducted in 3 group of animals at a farmer's dairy. Group A consist (n=10) normal healthy buffaloes. Group B (n=8) consists animal deficient untreated whereas group C consists (n=8) mineral deficient animal treated with prepared mineral

Table 2. Average value of soil and fodder minerals in various districts of Haryana State (Mean±SE)

districts		Total no. of sample	Cu (ppm)	Co (ppm)	Zn (ppm)	Fe (ppm)
Karnal	S	51	1.12±0.37*	5.12±0.21	0.56±0.01*	2.12±1.08
	F	68	7.64±0.03*	0.04±0.02*	26.62±0.34*	83.2±1.03
Sonepat	S	37	0.71±0.23*	2.54±0.18*	0.65±0.08*	3.77±0.99
	F	32	11.61±0.01	0.12±0.01	22.19±0.39*	63.79±2.03
Yamunanagar	S	36	1.28±0.85	1.78±0.47*	0.75±0.08*	2.40±0.21
	F	35	6.64±0.01*	0.15±0.03	15.02±0.38*	138.0±1.95
Rhohtak	S	29	0.32±0.64*	3.36±0.13	0.70±0.02*	2.96±1.76
	F	34	13.73±0.02*	0.14±0.16	22.57±0.28*	90.78±2.32
Ambala	S	34	1.28±0.80	3.84±0.22	0.78±0.61*	2.81±0.34
	F	39	8.68±0.02*	0.07±0.11	21.05±0.35*	134.5±0.04
Kurukshetra	S	39	1.01±0.64*	3.29±0.88	0.94±0.68*	4.85±0.52
	F	42	5.81±0.01*	0.06±0.15*	16.13±0.70*	113.0±1.72
Hissar	S	49	2.06±0.49	3.36±0.18	0.62±0.13*	1.74±0.61*
	F	46	6.66±0.40*	0.05±0.02*	14.50±0.29*	164.4±1.12
Nabha[ab]	S	29	1.00±0.63*	4.24±0.13	0.69±0.19*	6.69±0.72
	F	47	13.45±0.09*	0.12±0.01	22.94±0.88*	178.5±1.79

S=Soil, F=Fodder

[ab] Regional station of CIRB Hissar in Punjab.

* Significant at ($p<0.05$).

Table 3. Prevalence of soil and fodder mineral deficiency in various districts of Haryana state

districts		Total no. of sample	Cu (ppm)	Co (ppm)	Zn (ppm)	Fe (ppm)
Karnal	S	51	27 (52.94)	3 (5.8)	32 (62.74)	5 (9.8)
	F	68	42 (61.96)	9 (13.23)	43 (63.20)	11 (16.17)
Sonepat	S	37	19 (57.35)	2 (5.40)	23 (62.16)	3 (8.10)
	F	32	20 (62.5)	1 (3.12)	21 (65.62)	2 (6.25)
Yamunanagar	S	36	19 (52.78)	4 (11.12)	22 (61.12)	1 (2.78)
	F	35	23 (65.7)	1 (2.85)	21 (60.00)	2 (5.71)
Rhohtak	S	29	17 (58.62)	2 (6.8)	19 (65.86)	4 (13.79)
	F	34	21 (61.76)	2 (5.8)	25 (73.52)	2 (5.84)
Hissar	S	49	30 (61.23)	3 (6.12)	30 (61.22)	4 (8.16)
	F	46	28 (60.86)	6 (13.04)	29 (63.0)	9 (19.56)
Kurukshetra	S	39	22 (56.41)	2 (5.12)	24 (61.53)	1 (2.56)
	F	42	26 (61.9)	2 (4.76)	26 (61.9)	7 (16.67)
Ambala	S	34	21 (61.76)	3 (8.82)	19 (55.89)	5 (14.70)
	F	39	25 (64.10)	1 (2.56)	24 (61.53)	3 (7.69)
Nabha[ab]	S	29	13 (44.82)	2 (6.8)	11 (37.93)	1 (2.56)
	F	47	23 (48.93)	1 (2.12)	19 (40.42)	3 (6.38)

S=Soil, F=Fodder

[ab] Regional station of CIRB Hissar in Punjab.

Figures in parenthesis indicate percentage

mixture. Twenty five gram mineral mixture was given along with normal diet. All these animals were homogenous to non nutritional factors. The prepared mineral mixture contained 10% more of zinc sulphate, copper sulphate and cobalt chloride as compared to normal feed additives (Table 8). Observation were recorded at 0, 15, 30, 45 and 60 day regarding serum micro mineral profile, thyroid hormones and vit. A and E, TEC, TLC, Hb and effect on milk yield. For body weight in calves 6-12 month of age were divided into 3 group group A-consist (n=6) at normal healthy calves, group B calf deficient (untreated) in mineral (n=6) and group C mineral deficient calf treated with prepared mineral mixture (n=6) daily. The statistical analysis were made by the results, obtained to observed any significant variation using 't' test as per the method described by Snedecor and Cochran (1969).

RESULTS

The soil, copper, cobalt, zinc and iron were estimated (on DM basis) of 7 districts of Haryana viz. Karnal, Kurukshetra, Ambala, Rhotak, Yamuna Nagar, Sonepat and Hissar and one district of Punjab as CIRB regional station at Nabha. The highest prevalence of soil copper deficiency was 61.76% in

Table 4. Average serum minerals in buffaloes in various districts of Haryana state (Mean±SE)

districts	Cu (ppm)	Co (ppm)	Zn (ppm)	Fe (ppm)
Karnal	0.12±0.71*	0.40±0.12	2.03±0.71*	255.47±0.46
Ambala	0.32±0.12*	0.40±0.23	3.00±0.16*	212.10±0.28
Kurukshetra	0.59±0.11	0.22±0.38*	2.81±0.41*	313.12±0.09
Sonepat	0.45±0.16	0.36±0.17*	2.92±0.37*	292.99±0.61
Rhohtak	0.14±0.32*	1.10±0.41	3.44±1.68*	268.11±0.12
Yamunanagar	0.36±0.54*	0.60±0.57	2.05±0.28*	336.70±0.46
Hissar	0.30±0.19*	1.12±0.39	3.86±1.01*	284.01±0.81
Nabha[ab]	0.41±0.12	0.46±0.17	2.55±0.72*	363.12±0.76

L-Lactating animal where the author shows the data of Lacting animals.

Significant at (P<0.05).

[ab] Regional station of CIRB Hissar in Punjab State.

Table 5. Overall prevalence of serum mineral deficiency in buffaloes in various district of Haryana State

Districts	Total No. of sample	Cu	Co	Zn	Fe
Karnal	45	28(62.3)	9(20.0)	30(66.7)	10(22.3)
Ambala	44	26(59.09)	9(20.4)	29(65.90)	10(22.7)
Kurukshetra	33	20(60.71)	6(18.9)	20(60.7)	6(18.19)
Sonepat	54	37(68.5)	12(22.3)	35(64.81)	12(22.3)
YamunaNagar	56	22(61.12)	7(19.4)	22(61.2)	8(22.3)
Rhotak	34	19(64.70)	7(20.5)	22(64.7)	8(23.5)
Hissar	83	51(61.45)	15(18.0)	53(63.8)	15(18.1)
Nabha[ab]	73	35(47.91)	12(16.4)	27(36.98)	11(15.06)
	(402)	238(59.20)	77(19.1)	238(59.20)	80(19.90)

[ab] Regional station of CIRB Hissar in Punjab State.

Figures in parenthesis indicate percentage

Ambala district followed by 61.23% at Hissar. The the highest concentration of copper was observed at Hissar (2.06±0.49 ppm) followed by 1.28±0.85 at Yamuna Nagar and 1.28±0.80 in Ambala district (Table 2).

The overall incidence of copper deficiency was 55.26% in Haryana in soils. The highest cobalt deficiency was 11.12% at Yamuna Nagar followed by 8.82% at Ambala. The highest concentration of cobalt was observed in Karnal (5.12±0.21) followed by 4.24±0.13 ppm at CIRB regional station at Nabha. Significant lower (p<0.05) concentration was observed in Sonepat (2.54±0.18) and YamunaNagar (1.78±0.47) respectively (Table 2).

The highest zinc deficiency was observed in Rhotak (65.86%) followed by Karnal district (62.74%) (Table 3).

The highest zinc concentration in soil was observed in Kurukshetra district 0.94±0.68 ppm followed by 0.78±0.61 in Ambala district. Significant low zinc level was observed in all the districts. The highest incidence of iron deficiency in soil was observed at Ambala (14.70%) followed by Rhotak (13.79%). However, significantly lower (p<0.05) concentration of iron in soil samples were observed in Hissar district.

In fodder highest copper concentration was observed in Rhotak 13.73±0.02 followed by 11.61±0.01 ppm in Sonepat district. The copper concentration was significantly low in all the districts except Sonepat (Table 2). The highest copper deficiency in fodder was 65.7% in Yamuna Nagar followed by 64.01% in Ambala district. The overall deficiency of copper in Haryana state was 60.64% in fodder (Table 3).

The highest cobalt concentration was found in Yamuna Nagar (0.15±0.039) followed by Rhotak (0.14±0.16 ppm).

The value in Karnal, Krukshetra and Ambala was significantly low (p<0.05) (Table 4). The highest cobalt deficiency was observed in Karnal district (13.23%) followed by Hissar (13.04%). The overall deficiency of cobalt in Haryana was 6.7%. The highest prevalence of zinc deficiency was observed in Rhotak (73.52%) followed by Sonepat (65.52%). The overall deficiency of zinc in Haryana was 61.22% in fodder (Table 3). The highest zinc concentration was observed as 26.62±0.34 in Karnal district followed by 22.57±0.28 at Rhotak. Among zinc concentration in fodder all the districts were significant low at 5% level (Table 2).

The overall incidence of 11.37% iron deficiency was observed in Haryana state. The highest incidence of 19.56% iron deficiency was observed in Hissar district. However the iron concentration of 164.4±1.12 ppm at Hissar was observed of Hissar (Table 2).

Among serum minerals in buffaloes the overall deficiency of copper in Haryana was 59.20%. The highest prevalence of copper deficiency was observed in Sonepat district (68.5%) followed by Rhotak district 64.70% (Table 5). In Karnal district the copper deficiency in buffaloes was 62.3%. Hissar district showed 61.45% deficiency. The copper concentration was lowest in all the district viz. Kurkshetra (0.59±0.11 ppm) followed by Sonepat district (Table 4). The copper concentration of Karnal, Ambala, Yamuna Nagar and Rhotak was 0.12±0.71, 0.32±0.12, 0.36±0.54 and 0.14±0.32 ppm respectively in lactating buffaloes which is significantly lower (p<0.05) (Table 4). The CIRB regional station at Nabha showed 47.91% copper deficiency (Table 5).

The highest cobalt deficiency of 22.3% was recorded at Sonepat followed by Rhotak district 20.5%. The overall deficiency of cobalt in Haryana was 19.1% (Table 5). The highest level of cobalt concentration was 1.12±0.39 ppm in Hissar district followed by 0.60±0.57 ppm in Yamuna Nagar buffaloes. The cobalt concentration was significantly low (p<0.05) at Kurukshetra and Sonepat (0.22±0.38 and 0.36±0.17 ppm). Significantly low cobalt concentration was observed in Kurukshetra 0.22±0.38 ppm (Table 4).

Among zinc deficiency in serum in buffaloes the overall deficiency in Haryana was 59.20%. The highest incidence of zinc deficiency was observed in Karnal district (66.7%)

Table 6. Average haematological, hormonal and vitamin values in buffaloes in various districts of Haryana state (Mean±SE)

Districts	Hb (g/dl)	TLC ($\times10^6$/µl)	TEC ($\times10^6$/µl)	T_3 (ng/ml)	T_4 (ng/ml)	Vit A (ng/ml)	Vit E (ng/ml)
Karnal	7.96±0.16	6641±0.14	4.49±0.24	1.54±0.03	22.64±1.42	1.85±0.11	1.98±0.03
YamunaNagar	8.80±0.39	6456±0.26	4.06±0.42	1.39±0.07	19.10±1.28	0.86±1.42	2.01±1.12
Kurukshetra	7.82±0.68	7891±0.52	5.21±0.48	1.08±0.76	22.23±2.31	1.23±1.18	3.18±0.17
Sonepat	7.42±0.32	6785±0.19	5.15±0.56	1.51±0.19	22.86±1.19	1.64±0.09	3.10±0.13
Ambala	7.46±0.19	6520±0.51	5.30±0.22	0.82±0.31	18.17±1.09	1.26±0.42	2.03±0.34
Hissar	8.20±0.27	7600±0.67	5.80±0.29	0.99±1.12	15.16±1.52	1.28±0.06	4.97±0.21
Rhotak	8.52±0.43	6792±0.21	5.57±0.53	0.86±0.18	16.04±1.77	1.61±0.03	1.99±0.79
Nabha[ab]	9.14±0.26	8482±0.16	6.60±0.48	1.76±1.36	24.60±1.07	1.51±0.07	4.34±0.16

* Significant at ($p<0.05$), [ab] Regional station of CIRB Hissar in Punjab State.
T_3=Tri-iodothyronin, T_4=Thyroxin,

followed by Ambala (65.90%). Among zinc concentration in serum significantly low ($p<0.05$) level was observed 3.00±0.16 at Ambala, 2.92±0.37 at Sonepat, 2.81±0.41 at Kurukshetra and 2.05±0.28 ppm at Yamuna Nagar (Table 4). The overall deficiency of iron was 19.90% in Haryana. The highest incidence 23.5% in Rhotak followed by 22.3% in Karnal, Sonepat (22.3%) and Yamuna Nagar (22.3%) respectively (Table 5). The soil-fodder, soil-serum, fodder-serum interrelationship was significant at 5% and 1% in most of the districts (Table 7).

Significantly low ($p<0.05$) Hb level (7.46±0.19 g/dl) was observed at Ambala followed by 7.82±0.68 at Kurukshetra in mineral deficient buffaloes (Table 6). Significant decrease in erythrocyte level in Yamuna Nagar ($4.06\pm0.42\times10^6$ µl) followed by $4.49\pm0.24\times10^6$ /µl at Karnal was observed. Non significant decrease ($p<0.05$) in TLC level was observed at Yamuna Nagar 6,456±0.26 followed by 6,520±0.51 at Ambala (Table 6).

The level of T_3 hormone was significantly decreased ($p<0.05$) in Ambala (0.82±0.31), followed by Rhotak (0.86±0.18), Hissar (0.99±1.12), Kurukshetra (1.08±0.76) and Yamuna Nagar (1.39±0.07 ng/ml) respectively where as non significant decrease was observed in Sonepat (1.57±0.19), Karnal (1.54±0.03) and Nabha (1.76±1.36 ng/ml) respectively (Table 6). T_4 Concentration was significantly low in Hissar (15.16±1.52), Rhotak (16.04±1.77), Ambala (18.17±1.09), Yamuna Nagar (19.10±1.28 ng/ml). Whereas non significant decrease level was observed in Kurukshetra (22.23±2.31), Karnal (22.64±1.42), Sonepat (22.86±1.19) and finally at Nabha (24.60±1.07 ng/ml) respectively (Table 6).

Among vitamin A non significant ($p<0.05$) low values were observed in Sonepat (1.64±0.09), Rhotak (1.61±0.03), Nabha (1.51±0.07), Hissar (1.28±0.06), Ambala (1.26±0.42) and Kurukshetra (1.23±1.18 ng/ml). Significant low ($p<0.05$) value was observed in Yamuna Nagar (0.86±1.42 ng/ml) (Table 6).

In case of vitamin E non significant low value ($p<0.05$) was observed in Nabha (4.34±0.16), Kurukshetra (3.18±0.17), Sonepat (3.10±0.13 ng/ml). Whereas significantly low ($p<0.05$) concentration was observed in Karnal (1.98±0.03), Rhotak (1.99±0.79), Yamuna Nagar (2.01±1.12) and at Ambala (2.03±0.34 ng/ml) (Table 6).

Table 8. Composition of mineral supplement prepared* for the present study

Ingredients	Percentage
Dicalcium phosphate	48.3
Sodium chloride	43.83
Calcium carbonate	13.91
Magnesium sulphate	4
Ferrous sulphate	0.041
Zinc sulphate	0.026
Copper sulphate	0.008
Cobalt chloride	0.006
Manganease oxide	0.009
Potassium iodide	0.001

* The amount of these compounds were 10% more as compared to normal feed additives.

THERAPEUTIC MANAGEMENT

There was significant increase in milk yield in group C when compared to deficient untreated group 'B'. By 60th day the treated group C shows 6.97±0.41 lit/day. No significant increase was observed in mineral deficient untreated group 'B' animals throughout the duration of treatment (Table 9).

The body weight of group B and C animal was significant low (127.76±8.16 kg) and (129.42±1.13 kg) respectively when compared to normal healthy animal of group 'A'. By 30th day of feeding mineral supplement the value of group 'C' animal started increasing at a faster rate and on 60th day treatment it was 159.31±3.61 kg in comparison to group B and A (Table 9). There was significant decrease in Hb level in buffaloes group B and C (8.11±0.24 and 9.15±0.37 g/dl) on 0 day. By 60th day treatment in group C, Hb level reached to normal level 10.50±0.32 gm/dl as compared to control group 12.41±0.56 gm/dl (Table 9). No significant change was observed in group 'C' in case of TLC. However TEC level reached to normal level 6.10±0.72 as compared to group normal healthy animal ($6.76\pm0.21\times10^6$ µl) (Table 9).

There was significant increase in serum Cu level in group

Table 7. Correlation co-efficients of Cu, Co, Zn and Fe in soil, fodder and serum buffaloes in Haryana State

	Karnal	Sonepat	Rhotak	Yamuna Nagar	Ambala	Kurukshetra	Hissar	Nabha
Copper								
Soil-fodder	0.910±0.056**	0.576±0.087	0.070±0.423	0.348±0.095	0.715±0.10**	0.249±0.076	0.933±0.09**	0.685±0.01*
Soil-serum buffaloes	0.018±0.072	0.737±0.153**	0.654±0.121*	0.635±0.091*	0.428±0.101	0.293±0.076	0.872±0.18**	0.804±0.07**
Fodder-serum buffaloes	0.855±0.112**	0.438±0.007	0.654±0.095*	0.394±0.112	0.623±0.071	0.623±0.121*	0.073±0.19	0.760±0.21**
Cobalt								
Soil-fodder	0.957±0.061**	0.073±0.073	0.106±0.084	0.944±0.065**	0.580±0.071	0.448±0.093	0.377±0.06	0.045±0.98
Soil-serum buffaloes	0.423±0.056	0.423±0.037	0.384±0.056	0.938±0.087**	0.595±0.063	0.422±0.051	0.947±0.08**	0.109±0.81
Fodder-serum buffaloes	0.201±0.086	0.984±0.087**	0.609±0.096*	0.782±0.072**	0.800±0.112**	0.413±0.08	0.506±0.36	0.631±0.02*
Zinc								
Soil-fodder	0.661±0.036*	0.345±0.102	0.537±0.121	0.512±0.072	0.226±0.084	0.868±0.121**	0.587±0.21	0.794±0.08**
Soil-serum buffaloes	0.892±0.092**	0.518±0.37	0.747±0.031**	0.395±0.65	0.625±0.051*	0.865±0.072**	0.842±0.81**	0.526±0.16
Fodder-serum buffaloes	0.753±0.08**	0.840±0.123**	0.201±0.93	0.794±0.071	0.596±0.081	0.760±0.121**	0.274±0.01	0.200±0.21
Iron								
Soil-fodder	0.301±0.08	0.67±0.21*	0.176±0.72	0.087±0.01	0.087±0.01	0.577±0.08	0.139±0.04	0.485±0.05
Soil-serum buffaloes	0.680±0.32*	0.533±0.03	0.742±0.04**	0.750±0.46**	0.750±0.46**	0.566±0.26	0.604±0.26*	0.439±0.32
Fodder-serum buffaloes	0.478±0.09	0.478±0.76	0.718±0.03**	0.344±0.86	0.344±0.86	0.516±0.13	0.135±0.01	0.122±0.86

* Significant at ($p<0.05$).
** Significant at ($p<0.01$).

C (0.21±0.11 ppm) on 15th day treatment (Table 10). By 60th day of treatment the level of copper reached to almost normal level 0.53±0.63 ppm when compared to normal healthy animal 0.61±0.72 ppm. Regarding serum cobalt there was significant decrease in buffaloes of group B and C (0.22±0.75 and 0.21±0.72 ppm) in comparison to group A (0.41±0.53 ppm) on 0 day treatment. By 30th day there was significant increase in serum cobalt level in group C (0.29±0.67 ppm). By 60th day of treatment the value of group C increased to almost normal range 0.39±0.32 ppm (Table 10).

Regarding serum zinc level in group B a significant decrease (2.03±0.32 and 2.04±0.86 ppm) was observed on 0 day treatment. By 15th day there was marked improvement in serum zinc in group C (3.12±0.32 ppm). By 60th day of treatment the serum Zn level was 7.80±0.43 ppm in group C when compared to group A (10.01±0.22 ppm). Significant increase serum iron level was observed in group C after feeding mineral supplement (from 209±6.46 ppm on 0 day to 221±1.86 ppm by 60th day treatment) (Table 10).

The mean serum T_3 concentration were increased from 0.85±0.14 ng/ml on 0 day to 0.89±0.14 on 15th day, 0.96±0.39 on 30 day and reaches to normal level 1.49±0.49 ng/ml on 60th day in treated group C in comparison to untreated group B. Similarly T_4 level also starts increase from 14.19±1.60 ng/ml on 0 day to 14.21±1.14 on 15th day, 16.23±1.80 ng/ml on 30th day and reaches nearer to normal

Table 9. Effect of mineral supplement on TEC, TLC, Hb and body weight in calves and milk yield in buffaloes (Mean±SE)

Groups	Days	Hb (g/dl)	TEC ($\times10^6$/μl)	TLC ($\times10^6$/μl)	B.Wt. (kg)	Milk yield (kg)
A NHA (n=10)	0	11.16±0.31[a]	6.32±0.46[a]	6,456±0.72[a]	156.81±10.82[a]	7.102±0.34[a]
B ADM (UT) (n=8)		8.11±0.24[b]	4.10±0.52[b]	6,061±0.43[a]	127.76±8.10[b]	5.013±0.19[b]
C ADM (T) (n=8)		9.15±0.37[b]	4.63±0.21[b]	6,664±0.24[a]	129.42±1.1[b]	5.910±0.3[b]
A NHA (n=10)	15	12.49±0.71[a]	6.41±0.19[a]	6,427±0.16[a]	158.71±9.21[a]	7.302±0.21[a]
B ADM (UT) (n=8)		8.01±0.29[b]	4.70±0.16[b]	6,021±0.21[a]	129.62±8.34[b]	5.152±0.21[b]
C ADM (T) (n=8)		9.59±0.26[b]	5.08±0.22[c]	6,710±0.86[a]	132.72±4.31[b]	6.210±0.19[b]
A NHA (n=10)	30	12.41±0.32[a]	6.85±0.24[a]	6,123±0.59[a]	161.06±2.41[a]	7.550±0.13[a]
B ADM (UT) (n=8)		8.31±0.39[b]	4.88±0.42[b]	5,998±0.62[a]	131.01±7.52[b]	5.210±0.17[b]
C ADM (T) (n=8)		9.88±0.16[c]	5.97±0.48[c]	6,040±0.71[a]	145.12±5.15[b]	6.710±0.28[c]
A NHA (n=10)	45	12.48±0.34[a]	6.79±0.56[a]	6,186±0.19[a]	165.21±4.11[a]	7.732±32[a]
B ADM (UT) (n=8)		8.29±0.21[b]	4.92±0.39[b]	5,962±0.14[a]	133.81±5.17[b]	5.220±24[b]
C ADM (T) (n=8)		10.01±0.61[c]	6.01±0.31[c]	5,932±0.51[a]	148.71±4.98[c]	6.710±31[c]
A NHA (n=10)	60	12.41±0.56[a]	6.76±0.21[a]	6,211±0.56[a]	171.42±3.95[a]	7.817±37[a]
B ADM (UT) (n=8)		8.20±0.21[b]	4.92±0.18[b]	5,715±0.48[a]	135.09±5.26[b]	5.290±0.19[b]
C ADM (T) (n=8)		10.50±0.32[c]	6.10±0.72[c]	5,898±0.21[a]	159.31±3.61[c]	6.970±0.41[c]

NHA=Normal healthy animal
ADM (UT)=Animal deficient of minerals (untreated)
ADM (T)=Animal deficient of minerals (treated)
Values with super script b differ significantly at 1% level with values of superscript a.
Values with superscript c differ significantly at 5% level as compared to 0 day of treatment.

Table 10. Effect of mineral supplement on serum Cu, Co, Zn and Fe status in deficient buffalo (Mean±SE)

Groups	Days	Cu (ppm)	Co (ppm)	Zn (ppm)	Fe (ppm)
A NHA (n=10)	0	0.50±0.26[a]	0.49±0.53[a]	10.01±0.43[a]	215±07.32[a]
B ADM (UT) (n=8)		0.12±0.32[b]	0.22±0.75[b]	2.03±0.32[b]	192±01.36[a]
C ADM (T) (n=8)		0.15±0.27[b]	0.21±0.72[b]	2.04±0.86[b]	209±06.46[a]
A NHA (n=10)	15	0.51±0.87[a]	0.51±0.73[a]	9.79±0.44[a]	218±04.72[a]
B ADM (UT) (n=8)		0.11±0.39[b]	0.21±0.46[b]	2.09±0.14[b]	197±01.73[a]
C ADM (T) (n=8)		0.21±0.11[b]	0.25±0.31[b]	3.12±0.32[b]	211±03.46[a]
A NHA (n=10)	30	0.53±0.23[a]	0.49±0.76[a]	10.12±0.76[a]	208±01.35[a]
B ADM (UT) (n=8)		0.10±0.46[b]	0.20±0.54[b]	2.20±0.31[b]	199±02.32[a]
C ADM (T) (n=8)		0.25±0.81[c]	0.29±0.67[c]	5.09±0.36[c]	219±08.74[a]
A NHA (n=10)	45	0.57±0.21[a]	0.45±0.43[a]	9.81±0.41[a]	219±01.85[a]
B ADM (UT) (n=8)		0.32±0.96[b]	0.23±0.74[b]	2.19±0.24[b]	207±06.31[a]
C ADM (T) (n=8)		0.31±0.34[c]	0.32±0.55[c]	6.90±1.02[c]	220±1.24[a]
A NHA (n=10)	60	0.61±0.72[a]	0.46±0.27[a]	10.01±0.22[a]	217±0.43[a]
B ADM (UT) (n=8)		0.32±0.85[b]	0.21±0.17[b]	2.18±0.54[b]	219±1.76[a]
C ADM (T) (n=8)		0.40±0.63[c]	0.39±0.32[c]	7.80±0.43[c]	221±1.86[a]

NHA=Normal healthy animal
ADM (UT)=Animal deficient of minerals (untreated)
ADM (T)=Animal deficient of minerals (treated)
Values with super script b differ significantly at 1% level with values of superscript a.
Values with superscript c differ significantly at 5% level as compared to 0 day of treatment.

level 20.14±1.02 ng/ml respectively on 60th day in treated group C when compared to untreated group B. Similarly, the vitamin A and E level were also increased in treated group from 0.80±0.31 and 2.07±0.22 ng/ml on 0 day to 1.28±0.12 and 2.16±0.18 ng/ml in treated group `C' when compared to untreated group `B' (Table 11).

DISCUSSION

Trace mineral imbalances and deficiencies have been increasingly implicated in heatlh problem of dairy animals. Scientific community has began to recognise that trace mineral can substantially influence the production potential of dairy animals. From ancient times common salt was fed to

Table 11. Effect of mineral supplement on serum T_3, T_4 and Vit. A, E status in deficient buffalo (Mean±SE)

Groups	Days	T_3 (ng/ml)	T_4 (ng/ml)	Vit A (ng/ml)	Vit E (ng/ml)
A NHA (n=10)	0	1.55±0.08[a]	24.28±1.62[a]	1.92±0.21[a]	2.34±0.07[a]
B ADM (UT) (n=8)		0.83±0.42[b]	14.14±1.23[b]	0.82v0.09[b]	2.02±0.14[a]
C ADM (T) (n=8)		0.85±0.14[b]	14.19±1.60[b]	0.80±0.31[b]	2.07±0.22[a]
A NHA (n=10)	15	1.58±0.31[a]	24.33±0.98[a]	1.95±0.06[a]	2.35±0.81[a]
B ADM (UT) (n=8)		0.81±0.28[b]	14.15±1.32[b]	0.80±0.21[b]	1.97±0.33[b]
C ADM (T) (n=8)		0.89±0.14[b]	14.21±1.32[b]	0.83±0.14[b]	2.09±0.10[b]
A NHA (n=10)	30	1.60±0.23[a]	24.30±1.86[a]	1.96±0.13[a]	2.34±0.07[a]
B ADM (UT) (n=8)		0.80±0.66[b]	14.10±1.09[b]	0.81±0.08[b]	2.05±0.11[b]
C ADM (T) (n=8)		0.96±0.39[c]	16.23±1.80[b]	0.91±0.03[b]	2.10±0.28[b]
A NHA (n=10)	45	1.21±0.61[a]	24.41±0.71[a]	1.99±0.01[a]	2.56±0.26[a]
B ADM (UT) (n=8)		0.84±0.20[b]	14.16±1.41[b]	0.79±0.18[b]	2.05±0.16[b]
C ADM (T) (n=8)		1.21±0.61[c]	18.07±1.20[b]	1.09±0.29[b]	2.11±0.07[b]
A NHA (n=10)	60	1.70±0.28[a]	24.62±1.86[a]	2.06±0.05[a]	2.71±0.06[a]
B ADM (UT) (n=8)		0.83±0.12[b]	14.19±1.61[b]	0.76±0.09[b]	2.01±0.22[a]
C ADM (T) (n=8)		1.49±0.49[c]	20.14±1.02[b]	1.28±0.12[c]	2.16±0.18[a]

NHA=Normal healthy animal
ADM (UT)=Animal deficient of minerals (untreated)
ADM (T)=Animal deficient of minerals (treated)
Values with super script b differ significantly at 1% level with values of superscript a.
Values with superscript c differ significantly at 5% level as compared to 0 day of treatment.

satisfy the salt carvings of grazing animals under domestic conditions.

In the present study, it was observed that soil copper level was 1.12±0.37 ppm in Karnal with overall deficiency of 52.94%. Copper deficiency is wide spread in Haryana soils. Randhawa and Kanwar (1964) and Yadav et al. (1998) described 0.32-1.26 mg/kg^{-1} with mean value of 0.74 mg/kg^{-1} in Rewari district of Haryana. In Hissar deficiency of 61.23% in soils with copper content of 2.06±0.4 ppm. Gupta et al (1995), Yadav & Khirumar (1999) reported similar findings . The overall prevalence shows 6% cobalt deficiency. The overall cobalt concentration was 3.44±0.12 ppm. Similar reports have been given by Sarkar et al. (1980) in Bengal area. In case of zinc deficiency in soil, the overall deficiency was 59.21%. Kadyan (1985) also reported similar wide spread zinc deficiency. Safaya et al. (1974). Iron content of soil reflected low to marginal level deficiency in Haryana with the overall 7.5% deficiency. Singh et al. (1980) indicated 1.6-7.00 ppm in Rhotak, 3.4-13.4 ppm in Sonepat and 2.28 ppm in Mohindergarh district. The present study corroborates with these findings. Singh et al. (1980) mentioned total dietary intake of iron in buffalo was sufficient to meet the prescribed level and there seems to be no deficiency of iron in soils.

On estimation of fodder, copper was found to be highly deficient follwed by zinc. The overall copper deficiency in Haryana was 92%. The copper content of fodder varies from 5.81±0.01 in Kurukshetra to 13.73±0.02 ppm in Rhotak district. Yadav et al. (1998) found that 7.30±3.90 ppm in green fodder in Rewari district of Haryana. Singh et al. (1979) reported copper content of fodder was 13.14 mg/kg in Rhotak and 13.67 mg/kg in Sonepat and 11.15 mg/kg in Mohindergarh.

The present study revealed lower value of zinc than the values reported by Lal et al. (1996) in Hissar i.e. 13.6 mg/kg in fodder. Zinc is deficient in fodder in Karnal 26.62±0.34 ppm. Yadav (1998) reported dry roughage were deficient in trace minerals. Low content of zinc in soil is attributed to low content of zinc in fodders, Gupta et al. (1995). The iron content of fodder show adquacy to higher levels. Yadav et al. (1998), Mandal et al. (1996) and Singh et al. (1980) reported iron content of fodder ranged from 45.5-770 ppm in roughages. The iron content of fodder is sufficient to meet the requirement 100 ppm NRC (1971). Study of the prevalence of serum minerals deficiency in buffalo shows an overall deficiency of copper of 63.15% in Karnal with mean value of 0.16±0.23 ppm in lactating animals. The serum copper level 0.45±0.16 ppm at Sonepat and 0.14±0.32 ppm at Rhotak. Yadav et al. (1999), Mandal (1996) reported 0.21-2.34 mg/kg in Haryana. Kadyan et al. (1985) reported 0.21-2.34 mg/kg in Haryana Yadav and Khirmar (1999) revealed copper content 0.5 µg/ml at Jind district in Haryana. The serum zinc concentration in lactating-buffaloes was 2.03±0.71 ppm in Karnal followed by Ambala districts 3.00±0.16 ppm. This is lower than values of 1.34-3.85 mg with the average of 2.73 mg/kg. Yadav and Khirmar (2000). The average zinc concentration in serum lactating buffaloes is similar to the findings of Lal et al. (1994) and Yadav and Khirmar (2000).

The prevalence of iron deficiency in buffalo shows overall 19.91%. The iron concentration varies from 212.10±0.28 in Ambala to 363.12±0.76 ppm in Nabha. However Singh et al. (1980) reported 282.75 in Rhotak, 211.98 in Sonepat and 222.07 ppm in Mohindergarh. The findings of present study corroborate with the values of Singh et al. (1980) and Arneja et al. (1977).

There was significant decrease in Hb level. Singh et al. (2001) and Krauss et al. (1997) reported similar values. Singh et al. (2001) reported significant decrease in Hb, PCV, TEC with no changes in DLC and TLC in thio urea induced goitrous goats. The present study reveals no variation in monocytes, esinophills, and basophills. Boyne and Arthur (1986) also reported similar findings.

The low level of T_3 indicate the metabolic status of animals. Singh (2001), Shukla et al. (1994) and Dixit et al. (1984) also reported significant reduction in serum zinc and copper and in metabolic regulation of thyroxine hormone. The reduction in T_3, T_4 level also observed by Singh et al. (2001), Tsuneyoshi et al. (1995), the T_4 concentration was 20.56±2.18 ng/ml similar to the study of Dixit et al. (1984). Kohl et al. (1987) reported 11.16±2.04 in summer and 11.36±1.35 mg/ml in winters in buffaloes bulls of Haryana. Marginally low vitamin A and E level was also reported by in cows (1.21 mg/µl) by Chawla et al. (2001). The vit. A levels of the present study are similar to values of Weiss et al. (1997). Micheal et al. (1994), Raja raman et al. (1997) also reported similar findings.

There was a significant decrease in milk yield of the deficiency buffaloes. After the therapeutic management an increase in the milk yield was observed in-group C. from 15th day onwards, while no improvement was seen in-group B i.e. untreated group. Sharma et al. (2002) also reported similar findings.

The level of hemoglobin also raised significantly in deficient animal after supplementation (Underwood, 1999). The value of serum copper shows marked increase in the group C (treated group) and it reached upto normalcy by 60th day of treatment (0.15±0.27 ppm on day zero to 0.53±0.63 ppm on 60th day treatment). The results of the present study also corroborates with the findings of Yadav et al. (1998).

There was a marked improvement in the serum zinc level in group C by the 60th day therapy. The improvement was prominent in group C in comparison to group A (i.e.) 7.80±0.43 and 10.01±0.22 ppm respectively. Similar findings have also been reported by Sharma et al. (2002) in their work at Uttaranchal and U.P.

On the basis of this therapeutic trial of mineral supplements, it is noticed that by providing mineral supplements animal can gain body weight as well as milk production will also increase.

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