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Status of Serum Minerals and Biochemical Parameters in Cattle of Organized Farms and Unorganised Farms of Western Uttar Pradesh

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Abstract: Study was conducted in parts of northern India having tropical climate with two prominent type of rearing and management system for cattle. Cattle that are reared and managed by farmers (group A) who rarely supplement mineral mixture in the ration and cattle of organized farms that are managed on good quality ration and supplemented with mineral mixture (group B). Study revealed significant ($p<0.05$) difference in mineral concentration in cattle with respect to copper, zinc, iodine, cobalt, calcium and phosphorus between group A and group B in various districts. Iron was in adequate concentration in both groups. The concentration of serum retinol and α-tocopherol was lower in group A cattle. The concentration of serum ALT and AST in cattle of both group A and B were towards lower side but no significant difference ($p>0.05$) was found between groups. SAP, Cp was significantly ($p<0.05$) different between the groups in cattle of all the four districts. The concentration of both T_3 and T_4 in cattle of group A was lower than healthy cattle. However serum T_4 concentration was better indicator of such deficiencies. Status of serum retinol, α-tocopherol, B_{12}, SAP, Cp, T_3 and T_4 can be used as markers to evaluate status of serum minerals. Mineral supplementation results in better status of minerals in cattle but commercial available mixtures are not appropriate to fulfill the requirement of these cattle.

Key words: Cattle, enzymes, hormone, mineral, serum, vitamin

Introduction

India is agriculture-based country where 25% of its GDP is obtained from this sector. 70% of our population is dependent on agriculture for their livelihood. Though our livestock sector in terms of population and production figures are number one in the world but the average milk production of cattle is quite low. Mineral deficiency limits productivity of livestock in developing tropical countries (McDowell, 1985). Minerals are responsible for various body functions and its deficiency results in impairment of function or induce structural and physiological abnormalities in ways that vary with the minerals, the degree and duration of the dietary deficiencies or toxicity and the age, sex, or species of the animal involved (McDowell, 1992). Minerals exist in cells and tissues of the animal body in a variety of chemical combinations, and in characteristic concentrations, which vary with the mineral and tissue (Underwood and Suttle, 1999). The abnormalities are accompanied by specific biochemical changes and change in vitamin and biochemical parameters (Sharma *et al.*, 2003b). The roles that minerals play in enzymatic reactions range from weak, ionic strength effects to highly specific associations known as metalloenzymes (Underwood, 1971). Most naturally occurring mineral

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deficiencies in herbivores are associated with specific regions and directly related to soil characteristics, fodder fed and subject to amount of mineral supplementation to the livestock. In India, majorities of our livestock are reared by poor farmers for their livelihood and don't have resources to feed their livestock to optimal level and practice of supplementing mineral mixture is very remote possibility. However side-by-side there is organized dairy farms that have many modern amenities and resources to feed their livestock with optimum quantity of feed and practice mineral supplementation. Thus, the status of micro-minerals and related biochemical parameters in cattle of organized farms and that of farmers may be quite different and needs to establish such differences as no such study has been done in India. Keeping these in view, the present study was carried out to evaluate the comparative status of serum minerals, vitamins, hormones and enzymes in cattle of organized farms and of farmers'.

Materials and Methods

Sample Collection

Survey was conducted in western Uttar Pradesh of northern region of India. Four districts were selected on the basis of areas which were not covered for mapping of mineral status of the state viz., Agra, Aligarh, Hathras and Mathura. Necessary information regarding feeding behavior of animals and management practices were collected which revealed that animals were not suffering form malnutrition and incidence of parasitic infection was also low during the survey period (December-March). Survey also indicated that these regions had two basic types of cattle rearing system and accordingly the study was divided into two groups. Group A included cattle of these regions which were reared by farmers who were not supplementing minerals mixture in the ration and consisted mostly of indigenous (Haryana, Tharparkar and Zebu) cattle and only few cross breed cattle. Group B included cattle (crossbred Jersey X Indigenous and Holstein Friesian X Indigenous) reared by organized farms on intensive system of management and supplementing mineral mixture in the ration. A total of 476 blood samples from cattle of group A (n = 254) and group B (n = 222) was collected. About 10 mL blood was collected from jugular vein using 20 mL disposable syringe in a sterilized test tube without any anticoagulant and kept at room temperature without disturbing it. After, 2-4 h the clots were broken with the help of pasture pipette and serum was collected using micropipette in micro centrifuge tubes and properly labeled and brought in ice pack and stored at -4°C in refrigerator. In addition fodder (n = 204) samples were collected from the four districts form both organized farms and farmers and soil (n = 172) samples were also collected from field were these fodder were grown.

Minerals

Serum sample was digested as per procedure described by Kolmer *et al.* (1951). Three milliliter of serum with equal volume of concentrated Nitric acid (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 bench, until the volume of samples was reduced to about 1 mL. To this 3 mL of double acid mixture (concentrated HNO_3 and 70% perchloric acid in 3:1 ratio) 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 was repeated couple of times. Further heating was continued to reduce the volume to approximately 0.5 mL. Final volume of filtrate was made up to 10 mL with triple distilled deionized water after luke warming the solution. The fodder samples were digested by the method of Trolson (1969) using concentrated HNO_3 and concentrated sulphuric acid in 5:1 ratio. Digested sample was diluted with 2 mL triple distilled deionized water and

filtered through Whatman filter paper No.1. and the final volume of the filtrate was made up to 10 mL. Similarly digestion of soil samples was done by the method of Franeck (1992). Analysis of soil, fodder and serum copper, cobalt, zinc, iron, calcium and magnesium was done using atomic absorption spectrophotometer (Model No. AAS 4141, ECIL, Hyderabad) using air/acetylene as reducing flame and respective lamps. Inorganic iodine was determined by the method of Aumont and Tressol (1967). Serum inorganic phosphorus was estimated by the method of Tausky and Shorr (1953). Phosphorus in soil and fodder were estimated by the method of Talapatra *et al.* (1940) by making acid (hydrochloric acid) extract of ashed material.

Vitamins

Serum vitamin A (retinol) and vitamin E (α-tocopherol) of cattle was estimated by the procedure of Chawla and Kaur (2001) using HPLC method at the National Dairy Research Institute, Karnal. HPLC was carried out on Water equipment (Milford M.A., USA) using Millennium Software. Stock solution of retinol (20 μg mL^{-1}) and α-tocopherol (200 μg mL^{-1}) was prepared in 100% ethanol. Requisite aliquots of individual stock solution were taken in brown colored volumetric flasks and dried under nitrogen in water bath at room temperature. The dried standards were reconstituted in mobile phase prior to injection in HPLC. A working standard 2.0, 20.0 μg mL^{-1} of retinol and α-tocopherol was prepared at the time of estimation. Three extractions were done for maximal vitamin extract using petroleum ether from 0.2 mL deproteinised (with an equal volume of 95% ethanol containing 3% ascorbic acid) serum. The combined ether extract was dried at 40°C under nitrogen and redissolved in mobile phase and filtered through 0.22 micro filters for HPLC use. Twenty microliter of standard was injected in HPLC column for chromatographic separation. The system consisted of a model 510 pump, rheodye injector with 20 μL loop, model 486 tunable absorbance detector using multiwave length detection and C-18 μ Bondapak (USA) silica column (3.9X300 mm). Solvent system used for the separation of vitamins consisted of acetonitrile, Tetrahydrafuran (THF) and HPLC water in ration of 47:42:11. The programs for HPLC analysis of retinol (325 nm) and α-tocopherol (290 nm) on a single run had charge time of 0.00 and 2.50 min, respectively and retention time of 2.17 and 3.25 min, respectively. Serum vitamin B_{12} concentration was measured by radioimmunoassay (Lau *et al*., 1965).

Hormones

Serum thyroid hormones (T_3 and T_4) of adult cattle were estimated by Radioimmunoassay (RIA) technique using gamma scintillation counter (I^{125} elaborated), Cobra 7, Germany by the method of Chopra (1972) at nuclear research laboratory of Indian Veterinary Research Institute, Izatnagar.

Enzymes

Enzymes activities in adult cattle of aspartate amino transferase (AST), alanine amino transferase (ALT) was estimated by the method of Reitman and Frankel's (1957) and Serum Alkaline Phosphatase (SAP) were estimated by the King *et al.* (1951) method using test kit obtained from Span Diagnostic Ltd., India and enzyme Ceruloplasmin (Cp) was determined with p-phenylenediamine by the method of Wooten *et al.* (1996).

Statistical Analysis

The data from each district were analyzed separately, and analysis of variance was applied to data for a nested design with unequal subclass replications and multiple t-test (Snedecor and Cochran, 1987). Significance was noted at $p<0.05$, unless otherwise stated.

Results and Discussion

Cattle of farmers suffered from poor health, poor reproductive performance with irregular estrus, poor conception, repeat breeding and alternate-year calving, retained placentas, weak calves and high calf mortality, anaemia, stomatitis and poor appetite, achromotrichia, diarrhoea, decreased milk production in lactating cattle, hoof deformities and lameness, dry hair and skin, severely underweight cattle. Similar symptoms have been reported by Radostits *et al.* (2000) and Sharma *et al.* (2003a, b). In contrast cattle of organized farms were better in health and production and supplemented commercially available mineral mixture in ration on regular basis.

Soil and Fodder Minerals

The mean (±SE) concentration (ppm) of soil calcium, phosphorus and magnesium in all the four districts of Uttar Pradesh were 61.23±4.80, 14.23±1.86 and 31.42±2.04, respectively. The mean (±SE) concentration (ppm) of soil copper, zinc, iron and cobalt in these four district of Uttar Pradesh was 1.18±0.11, 1.01±0.04, 43.09±1.92 and 0.32±0.02, respectively. The average soil pH and range in the district of Agra, Aligarh, Hathras and Mathura was 8.3 (7.2-9.3), 8.4 (7.3-10.2), 7.8 (7.2-8.1) and 8.1 (7.3-10.9), respectively. The overall concentration on dry matter basis (%) of fodder calcium, phosphorus and magnesium in all the four districts of Uttar Pradesh were 0.41±0.026, 0.25±0.031 and 0.24±0.011, respectively. The mean (±SE) concentration (ppm) of fodder copper, zinc, iron and cobalt in these four districts was 10.75±0.52, 25.67±1.52, 282.74±13.41 and 0.18±0.05, respectively Results indicated deficiency of zinc, copper, calcium, cobalt, phosphorus and magnesium in both soil and fodder. While iron in sufficient concentration in both soil and fodder. The present findings may be attributed to alkalinity in the soil and application of fertilizers and pesticides and intensive farming (obtaining three crops in a year) except in Hathras were farmers were utilizing organic farming and minimal application of fertilizers and pesticides.

Serum Minerals

The analysis of Table 1 showed significant ($p<0.05$) difference in mineral concentration in cattle with respect to copper, zinc, iodine, cobalt, calcium and phosphorus between group A and group B in various districts. However non-significant ($p>0.05$) differences in concentration serum iron and magnesium were observed between groups. Cattle of both groups in Hathras district showed little difference with respect to serum mineral status. The overall prevalence of deficiency of serum copper, zinc, cobalt, iodine, calcium, phosphorus and magnesium in cattle of group A were 48.81, 55.91, 35.83, 40.15, 29.52, 34.25 and 22.45%, respectively. Cattle of group A were deficient in serum zinc, copper, iodine, cobalt, calcium, and phosphorus and marginally deficient in magnesium. Present findings may be attributed to deficient mineral status in alkaline soil and fodder of these regions (Kumar, 2003) and application of fertilizers and pesticides in soil and fodder for better production (Horvath and Reid, 1980; Dey *et al.*, 1997). Cattle of both groups of district Hathras showed non significant difference which may be attributed to soil pH, lack of industrialization in the district and farmers utilizing organic farming and avoiding fertilizers and pesticides to minimum levels. Serum iron concentration was adequate in cattle of both the groups. Deficiency of copper is also attributed to high iron level in soil, fodder and cattle of these areas of India. This finding is supported by findings of Campbell *et al.* (1974), who suggested that high level of iron over extended periods of time have an influence on copper availability. Humphries *et al.* (1983) and Sharma *et al.* (2002; 2003b) have also reported similar findings. However, the prevalence of deficiency of serum mineral in group B was less compared to group A cattle. The prevalence of deficiency of serum copper, zinc, cobalt, iodine, calcium,

Table 1: Concentration of serum minerals in cattle of group A and group B

	Copper (ppm)		Cobalt (ppm)	
District	Group A	Group B	Group A	Group B
Agra	0.572±0.124 n = 84	0.842±0.089^{b} n = 52	0.036±0.004 n = 84	0.041±0.002 n = 52
Aligarh	0.302±0.041^{a} n = 40	0.515±0.023^{ab} n = 48	0.029±0.001 n = 40	0.053±0.011^{b} n = 48
Hathras	0.749±0.097 n = 56	0.891±0.102 n = 40	0.037±0.004 n = 56	0.044±0.003 n = 40
Mathura	0.454±0.065^{a} n = 74	0.759±0.067^{b} n = 82	0.023±0.002 n = 74	0.054±0.003^{b} n = 82
	Iodine (μg dL^{-1})		Calcium (mg dL^{-1})	
District	Group A	Group B	Group A	Group B
Agra	5.46±0.06 n = 84	7.46±0.14^{a} n = 52	6.21±0.14 n = 84	7.72±0.26^{b} n = 52
Aligarh	7.73±0.09 n = 40	10.58±0.12 n = 48	6.65±0.48 n = 40	7.86±0.34 n = 48
Hathras	10.27±0.04 n = 56	11.41±0.09 n = 40	7.84±0.29 n = 56	8.12±0.52 n = 40
Mathura	8.01±0.03 n = 74	13.51±0.18^{b} n = 82	5.82±0.22^{a} n = 74	8.01±0.37^{b} n = 82

Table 1: Continue

	Zinc (ppm)		Iron (ppm)	
District	Group A	Group B	Group A	Group B
Agra	0.791±0.085^{a} n = 84	1.392±0.042^{b} n = 52	1.671±0.308 n = 84	1.584±0.203 n = 52
Aligarh	0.685±0.045^{a} n = 40	1.215±0.191^{b} n = 48	1.722±0.412 n = 40	1.612±0.164 n = 48
Hathras	1.073±0.051 n = 56	1.332±0.085 n = 40	1.461±0.313 n = 56	1.708±0.212 n = 40
Mathura	0.769±0.071^{a} n = 74	1.384±0.039^{b} n = 82	1.689±0.251 n = 74	1.481±0.143 n = 82
	Phosphorus (mg dL^{-1})		Magnesium (mg dL^{-1})	
District	Group A	Group B	Group A	Group B
Agra	3.82±0.21^{a} n = 84	5.71±0.43^{b} n = 52	1.68±0.09 n = 84	1.89±0.26 n = 52
Aligarh	3.14±0.38^{a} n = 40	4.84±0.34^{b} n = 48	1.75±0.12 n = 40	1.91±0.34 n = 48
Hathras	4.78±0.29 n = 56	5.12±0.30 n = 40	1.74±0.33 n = 56	1.81±0.15 n = 40
Mathura	3.11±0.15^{a} n = 74	5.64±0.57^{b} n = 82	1.52±0.14 n = 74	1.69±0.39 n = 82

'n' indicates number of samples analyzed, Values with superscript 'a' differ significantly ($p<0.05$) between districts, Values with superscript 'b' differ significantly ($p<0.05$) between groups

Table 2: Concentration of serum vitamin and hormone in cattle of group A and group B

District	Retinol (μg mL^{-1})	α-tocopherol (μg mL^{-1})	Vit. B_{12} (ng mL^{-1})	T_3 (ng mL^{-1})	T_4 (μg dL^{-1})
Group A					
Agra n = 84	0.213^{a}±0.02	1.51^{a}±0.06	0.25±0.009	0.84±0.051	2.15±0.024
Aligarh n = 40	0.296^{a}±0.11	1.63^{a}±0.13	0.21±0.007	0.87±0.104	2.95±0.008
Hathras n =56	0.565±0.09	2.31±0.09	0.23±0.004	1.02±0.08	3.23±0.013
Mathura n = 74	0.254^{a}±0.04	1.59^{a}±0.12	0.19±0.003	0.93±0.049	2.08±0.037
Group B					
Agra n = 52	0.539^{b}±0.14	3.52^{b}±0.05	0.27±0.001	1.13±0.109	5.17^{b}±0.09
Aligarh n = 48	0.513^{b}±0.08	2.84^{b}±0.12	0.38±0.004	1.080.091	4.98^{b}±0.013
Hathras n = 40	0.601±0.12	2.44±0.10	0.30±0.004	1.31±0.053	5.04^{b}±0.012
Mathura n = 82	0.544^{b}±0.07	3.14^{b}±0.07	0.51^{b}±0.002	1.24±0.039	4.69^{b}±0.008

'n' indicates number of samples analyzed, Values with superscript 'a' differ significantly ($p<0.05$) between districts, Values with superscript 'b' differ significantly ($p<0.05$) between groups

phosphorus and magnesium deficiency in cattle of group B were 20.27, 23.42, 15.75, 19.37, 16.22, 22.07 and 15.77%, respectively. Significant difference between groups with respect to concentration of serum copper, cobalt, zinc, iodine, calcium and phosphorus may be attributed to supplementation of commercially available mineral mixture and balanced ration feed to cattle of organized farms.

Table 3: Concentration of serum enzymes in cattle of group A and group B

Districts	ALT (RE Units mL^{-1})	AST (RE Units mL^{1})	SAP (RE Units mL^{1}.)	Cp (IU L^{-1})
Group A				
Agra n = 84	13.98±1.68	41.32±6.18	1.41^{a}±0.58	21.47±0.51
Aligarh n = 40	15.39±2.11	48.58±5.44	1.29^{a}±0.87	10.68^{a}±0.66
Hathras n = 56	14.51±2.08	53.32±5.44	2.82±0.28	28.33±1.23
Mathura n = 74	11.44±2.06	39.76±4.93	1.25^{a}±0.34	15.21^{a}±0.32
Group B				
Agra n = 52	14.23±1.34	42.53±5.91	3.87^{b}±0.63	37.84^{b}±1.07
Aligarh n = 48	15.89±3.15	52.27±4.37	3.12^{b}±0.97	32.46^{b}±1.42
Hathras n = 40	16.09±2.91	54.64±3.88	4.41^{b}±0.41	42.53^{b}±0.91
Mathura n = 82	14.69±2.44	47.54±4.13	3.76^{b}±0.38	37.12^{b}±0.84

'n' indicates number of samples analyzed, Values with superscript 'a' differ significantly ($p<0.05$) between districts, Values with superscript 'b' differ significantly ($p<0.05$) between groups

However, the concentration of these minerals in group B cattle was also towards lower side. This may be attributed to improper composition of available commercial mineral mixtures. These commercial mineral mixtures are broad based and not based on area-wise requirements. Concentration of magnesium and iron did not vary much between groups as these were in normal concentration in cattle as well as soil and fodder of this region.

Serum Vitamins

Concentration of serum retinol, α-tocopherol and vitamin B_{12} in cattle of group A and group B were compared between groups and between districts and significance observed at $p<0.05$ (Table 2). Concentration of serum retinol in cattle of group A was lower as compared to reference value of healthy cattle (0.53 mg L^{-1}) reported by Linderberg *et al.* (1999). Lower levels of retinol in cattle of group A may be attributed to lower serum zinc and copper concentration. Zinc deficiency interferes with hepatic synthesis of retinol binding protein, resulting in impaired mobilization of retinol from liver (Flodin, 1979). These findings were in agreement with the findings of Facho (1995) and Carvens and Vaden (1994). The analysis showed significant ($p<0.05$) difference in retinol and α-tocopherol concentration in cattle between group A and group B in all the three districts except Hathras. While serum retinol in cattle of group B was towards normalcy and is attributed to better status of serum minerals. Similarly concentration of serum α-tocopherol in cattle of group A was significantly ($p<0.05$) lower in all the three districts except Hathras compared to the cattle of group B. The concentration in cattle of group A was much lower as compared to 2.25-3.41 μg mL^{-1} in healthy cattle reported by Hidiroglou *et al.* (1994). While serum α-tocopherol was towards normalcy in cattle of group B. Such findings are attributed to zinc deficiency as it has a profound effect on the intestinal absorption and body status of lipid soluble vitamins. Similar reports of lower α-tocopherol status in rat due to zinc deficiency has been reported by Kim Eul *et al.* (1998). Lower concentration of micro-minerals may influence the concentration serum vitamins that may also contribute to lower fertility associated with micro-mineral deficiency. Concentration of serum vitamin B_{12} was lower in all the districts in cattle of group A and group B (except Mathura) cattle. However, significant ($p<0.05$) difference between groups was observed only in Mathura district which may be attributed to higher cobalt concentration in cattle of Mathura district. Lower concentration of vitamin B_{12} in cattle of group A and B is attributed to lower concentration of serum cobalt in cattle of both groups. Thus status of serum retinol, α-tocopherol and B_{12} in cattle may be used as marker to access the mineral status of animals.

Serum Enzymes

Mineral deficiency affects various metabolic pathways due to altered enzymatic functions (Underwood, 1977). The concentration of serum ALT and AST in cattle of both group A and B were towards lower side but no significant difference was found between groups (Table 3). This may be due to the decrease in transamination reaction as a result of calcium and phosphorus deficiency. Status of serum ALT and AST were not much affected by the concentration serum minerals. SAP and Cp activity was significantly ($p<0.05$) different between groups in cattle of all the four districts (Table 3). SAP and Cp was lower in cattle of group A. Decrease in SAP enzyme with concurrent deficiency of micro-minerals, especially zinc has been reported by Parisi and Vallee (1969), Snaith and Leuvy (1968). Cp is copper dependent enzyme (Radostits *et al*., 2000) and thus decreases with decrease in level of copper in body. Thus SAP and Cp can give indication about micro-mineral deficiencies and can be used as a marker for such deficiencies. Decrease concentration of Cp due to copper deficiency cause disturbance of iron metabolism resulting in its sequestration (Mills *et al.*, 1976). Cattle of group B showed higher concentration of SAP and Cp attributed to higher concentration of serum minerals. SAP and Cp are good markers for zinc and copper deficiency while serum ALT and AST are not suitable measure of such deficiency.

Serum Hormones

Concentration of serum thyroxine (T_4) and triidothyronine (T_3) in cattle of group A and group B were compared between groups and between districts and significance observed at $p<0.05$ (Table 2) The concentration of both T_3 and T_4 in cattle of group A was lower than healthy cattle (Kaneko *et al*., 1997). Higher concentrations of these hormones in group B may be attributed to higher serum iodine, zinc and copper. However serum T_4 concentration was better indicator of such deficiencies. Significant difference between groups were observed with respect to concentration of T_4 in cattle of all the four districts. Serum T_3 was non-significantly ($p>0.05$) different between groups in cattle of all the districts. Present findings corroborate with the findings of Sharma *et al.* (2003b) who have reported correlation between serum zinc and Thyroxine (T_4) status of the animal and showed decrease in level of T_3 and T_4 on decreasing serum zinc level. Copper deficiency impairs secretion of tyrosine hydroxylase and dopamine beta enzyme system, which are both copper containing in hypothalamic neurons. This causes inhibition of synthesis of thyroid releasing factors. The copper containing peroxidase enzyme of the thyroid gland impairs thyroid hormone secretion (Singh *et al.*, 2002). Thus status of thyroid hormones can give indication of the micro-mineral deficiencies especially that of iodine, copper and zinc.

Thus the present study signifies the importance of comparative study of these parameters with respect to cattle reared and managed under different systems as the concentration of these parameters varies. Status of serum vitamins (retinol, α-tocopherol and B_{12}), enzymes (SAP, Cp) and hormones (T_3 and T_4) are suitable markers to evaluate status of serum minerals as these varies with change in status of minerals in body. Practice of mineral supplementation results in better status of minerals in cattle but commercial available broad-based mineral mixtures are not appropriate to fulfill the requirement of these cattle for optimal production. Thus there is need to develop area-specific mineral mixture as per the concentration of these minerals in soil, fodder and cattle.

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