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Copper status and enzyme, hormone, vitamin and immune function in heifers

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Abstract

A survey was conducted in 10 districts of Northern India to record the copper deficiency in soil, fodder and serum samples. Significant deficiency of copper was observed in soil, fodder and serum samples of heifers. The copper deficient animals were listless, showed depigmentation of skin, stiff gait and were anaemic and diahorric.

The purpose of the study was to evaluate the magnitude of copper deficiency in Northern India and to examine the various haematobiochemicals, enzymes, vitamin and immune function which are affected by the deficiency of copper, so as to identify the parameters which can be of diagnostic importance in copper deficiency.

Forty hypocuperemic heifers were selected from these areas and were randomly divided into two groups A and B. The heifers in group A were provided with mineral mixture containing copper sulphate and in group B without copper sulphate. Significant improvement ($P < 0.01$) was observed in the haemoglobin (Hb), total leukocyte count (TLC) and total erythrocyte count (TEC) level at the 30th day of treatment in the animals of group A. Significant ($P < 0.01$) improvement in serum. Ceruloplasmin (Cp) level was observed within 30 days of treatment, while significant ($P < 0.01$) improvement in monoamine oxidase and liver cytochrome oxidase was observed at the 60th day of treatment in group A animals.

Regarding hormones significant improvement was observed in T_3 and T_4, in the animals of group A within 60 days of treatment. The values of vitamin A and E showed significant ($P < 0.01$) improvement within 30th days of treatment.

The phagocytic activity of neutrophils against *Candida albicans* significantly ($P < 0.01$) improved in group A within 60 days of treatment. Similarly significant improvement in superoxide dismutase activity in RBC was observed at the 30th day, and WBC and whole blood at 60th day in group A animals.

Significant improvement in liver Cu level was observed at the 30th day of treatment, while in group B the liver Cu was significantly ($P < 0.01$) depleted at the 60th day of experimentation. Additional Cu supplementation improved growth performance significantly in group A.

Keywords: Copper deficiency; Haematology; Monoamine oxidase; Liver cytrochrome oxidase; Ceruloplasmin; Thyroid hormones; Vitamins A and E; Superoxide dismutase; *Candida albicans*; Neutrophil; Mineral mixture; Treatment

1. Introduction

As far back in 1928, Hart et al. reported the essentiality of copper for the growth and haemoglobin formation in the rats. There are extensive copper deficient areas throughout the world which has adverse effects on the crops and livestock (Underwood and Suttle,

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1999). Copper play various important biological roles in the body of the animals through several copper dependent enzymes. Some of important copper dependant enzymes are cytochrome oxidase (E.C.1.9.3.1) widely involved in many oxidative reactions, monoamine oxidase (E.C.1.4.3.4) associated with mat uration of collagen and elasitn; and ceruloplasmin (E.C.1.16.3.1) involved in mobilization and utilization of iron stored in the liver.

High difficulty arises in selecting suitable indicator for assessment of the copper status in the body of the animals (Xin et al., 1991). Liver Cu level has been suggested to be a satisfactory indicator of the Cu status of the animal but there are certain limitations like problem of sampling, processing of the samples and assay procedure (Suttle and Angvs, 1976). Plasma Cu concentration can be misleading due to homeostatic mechanism (Davis and Mertz, 1987).

There is a close relationship between the dietary Cu level, activity of enzymes and cellular and immune functions. Copper deficiency in human subject is reflected in the low Cu content in leucocytes in comparison of the healthy persons (Thomas et al., 1988). Super oxide dismutase (SOD) is an important enzyme functionally associated with Cu in different tissues. The determination of Cu in SOD is more reliable than the Cu of whole blood (Mccord and Fridovich, 1969).

The status of Cu level in the body also influences the level of vitamins like A and E, and thyroid hormones (T_3 and T_4); and dietary supplementation of microminerals has shown significant elevation in these constituents in animals kept under non-technical management in some rural parts of India (Sharma et al., 2003).

The status of Cu and its interrelationship with SOD activity, immune function, various enzymes, hormones and vitamins in the livestock of India is not well known. Therefore, the present study was conducted to compare the sensitivity of these parameters for establishing the interrelationship with Cu and to evaluate the immune response and neutrophil function in relation to copper deficiency.

2. Materials and methods

A survey was conducted in 10 districts of Northern India viz., Nainital, Almora, Bageshwar, Pithoragarh, Pilibhit, Udham Singh Nagar, Badaun, Bareilly, Rampur and Shahjhanpur to record the mineral status in soil, fodder and serum of heifers (Table 10).

2.1. Sample collection

A total 986 samples of soil and 1012 samples of fodder grown in the same fields were collected. For harvesting serum blood samples without the use of anticoagulant were collected from 842 heifers and another heparinized blood samples from 369 heifers of same areas were collected for haematological estimations.

2.2. Mineral estimation

Minerals viz. calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), zinc (Zn), copper (Cu), iron (Fe) and cobalt (Co) were estimated in soil, fodder and serum samples after digestion. The samples of soil, fodder and serum were digested by the method of Franeck (1992), Trolson (1969), Kolmer et al. (1951) respectively. Mineral content in soil, fodder and serum samples was estimated by atomic absorption spectrophotometer (AAS) (ECIL 4141, Hyderabad, India). Phosphorus was determined in soil and fodder by the method of Talapatra et al. (1940) with minor modifications. Serum inorganic phosphorus was estimated by the method of Taussky and Shorr (1953). For mineral estimation in liver, samples were obtained by biopsy under local anesthesia and digested by wet ashing procedure for mineral estimation through AAS. All the collected samples of soil, fodder and serum were analysed individually.

2.3. Therapeutic study

2.3.1. Animals and diets

In a cooperative farm located in a highly copper deficient area, forty cross-bred (Sahiwal × Holstein Friesian) hypo cuperemic heifers of an average 200 kg initial body weight and 1.5 years of average age were selected for the study. The heifers were randomly divided into two comparable groups A and B. The basal diet was composed of 2 kg commercial concentrate mixture of medium quality, 10 kg green berseem (*Trifolium alexandrinum*) fodder and wheat straw ad libitum. The ration was composed to meet the requirement of growing cross-bred heifers as far as possible during the 180 days feeding trial. Perspective mineral mixtures A and B were fed in concentrate mixture @ 50 g per head per day in groups A and B, respectively. The basal diet was supplemented with mineral mixtures A and B (Table 1) with or without copper supplement respectively. The animals of both the groups A and B were homogenous to non-nutritional factors. The chemical composition of the basal feeds is given in Table 2 on dry matter basis. The body weights were estimated with the help of measurements applied to the following formula.

$$\text{Body weight(lbs)} = \frac{L(\text{in.}) \times G^2(\text{in.})}{300},$$

where L is the body length and G is the body girth at heart.