Journal of Trace Elements in Medicine and Biology 50 (2018) 415–423

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Nutrition

Selenium and copper interaction at supra-nutritional level affecting blood parameters including immune response against *P. multocida* antigen in Murrah buffalo (*Bubalus bubalis*) calves

Vishal Mudgal[a,*], Anil Kumar Garg[a], Ram Sharan Dass[a], Mayank Rawat[b]

[a] *Mineral and Vitamin Nutrition Laboratory, Centre for Advanced Faculty Training in Animal Nutrition, ICAR-Indian Veterinary Research Institute, Izatnagar, 243 122, UP, India*

[b] *Biological Standardization Division, ICAR-Indian Veterinary Research Institute, Izatnagar, 243 122, UP, India*

ARTICLE INFO

Keywords:
Antioxidant profile
Buffalo calves
Haematology
Immunity
Supra-nutritional selenium and copper

ABSTRACT

Minerals play important role in the diet of an animal. Bio-availability of minerals largely gets affected by absolute as well as the relative amount of each mineral present in the diet of an animal. Copper and selenium are two such an essential elements affect utilization of each other in the gastrointestinal tract. The present study elucidates the utilization of copper and selenium at supra-nutritional levels (higher than nutritional requirements). Male Murrah buffalo (*Bubalus bubalis*) calves (n = 10, 8–9 months, 111.7 ± 12.55 kg body weight) were divided equally into two groups and fed either a standard (Control) diet or the same diet supplemented with 0.3 ppm selenium (Se) and 10 ppm copper (Cu) (Treatment). Supplementation was made using liquid solutions of two inorganic mineral sources after mixing in the concentrate mixture and study lasts for a period of 80 days. Blood samples were collected just before starting supplementation (designated as 0 day of study) and at day 40 and 80 after starting supplementation. Blood samples were subjected to haematological parameters, plasma minerals and various oxidative stress-related parameters were determined with the cell-mediated and humoral immune response against antigen *P. multocida* (P_{52} strain). Supra-nutritional Se with Cu had higher blood monocytes ($P < 0.05$) and plasma selenium ($P < 0.01$) levels, while other hematological parameters and plasma minerals (except zinc, which was lower ($P = 0.025$) at day 80 in the treatment group) remained unaffected. Among markers for oxidative stress in blood, levels of lipid peroxidation were lesser ($P < 0.01$), at day 80 and overall mean values of the enzyme glutathione peroxidase and catalase were higher ($P < 0.05$) in the supra-nutritional group against control values. The overall mean activity of other oxidative stress markers including reduced glutathione, ceruloplasmin as well as the concentration of α tocopherol, retinol, and β carotene remained unaffected due to supra-nutritional Se and Cu. Although cell-mediated immune response remained comparable ($P > 0.05$) between groups, higher ($P < 0.05$) overall mean antibody titer values, as well as the values at day 80, was reported in supra-nutritional Se + Cu group. The study concluded that supra-nutritional Se with Cu in the ration of growing Murrah buffalo calves was helpful to reduce the oxidative stress and to enhance the humoral immune response. Simultaneously, higher plasma Se level and number of monocytes in blood highlighted the additional role of selenium and copper in a ration of growing buffalo calves as compared to its normal recommended dose.

1. Introduction

Buffaloes play important role in the Indian economy due to its major contribution in total milk production [1]. Lack of balanced feeding is a major hurdle for improvement of their production and reproduction performances. Disproportionate use of trace elements not only interferes with normal physiology or immune regulation but also causes many diseases [2,3]. Selenium and copper are two essential trace elements act as cofactors in numerous biochemical reactions. Selenium plays an important role in the regulation of several metabolic processes of the body and is an integral part of different selenoproteins [4,5]. However, the physiological role of selenium is chiefly concentrated on the activity of glutathione peroxidase (GSHPx). It has been suggested that selenium might enhance immunity, growth, reproductive

* Corresponding author. Present address: Mineral Nutrition Laboratory, Division of Animal Nutrition and Feed Technology, ICAR – Central Institute for Research on Buffaloes, Hisar, 125 001, Haryana, India.
E-mail address: vishal.mudgal@icar.gov.in (V. Mudgal).

https://doi.org/10.1016/j.jtemb.2018.08.008
Received 13 April 2018; Received in revised form 29 August 2018; Accepted 31 August 2018

performance, and the ability to resist disease [6,7]. Copper is essential for erythropoiesis, the transport and utilization of iron in the biosynthesis of hemoglobin, normal growth and osteogenesis [8]. It also has an anticancer effect [9] with participating role in the pigmentation of hair. It is also required as a specific cofactor in the antioxidant enzyme Cu/Zn superoxide dismutase [10] and is incorporated into ceruloplasmin [11] and cytochrome C oxidase [12].

Now a day's these trace elements are especially in focus when supplied at concentrations higher than suggested nutritional requirements (supra-nutritional level). Se has anticancer effects [13] and Cu reported to have enhanced humoral immunity at supra-nutritional levels [14].

Additionally, metabolic interactions between selenium and copper have been demonstrated in several species [15–19]. Research has focused on ruminants because they are at risk for acquiring selenosis, but the results of studies of any beneficial effect of copper supplementation in the prevention of selenium toxicity have been inconclusive [20–23]. Therefore, an optimal balance between selenium and copper in feed and organisms is essential for normal performance and health. Several hypotheses were proposed to explain the above observations [24] however; knowledge on the influence of enhanced levels of selenium and copper on buffalo health is still fragmentary. The aim of this work was to investigate the effect of supra-nutritional dietary doses of selenium with copper on selected blood parameters including antibody response against *P. multocida* in buffalo calves.

2. Materials and methods

2.1. Animals: selection and grouping

Before starting the experiment, ethical approval was obtained from the Institute level Animal Ethical Committee (IAEC) for performing different activities associated with animal handling. Buffalo calves were procured from the Institute's Animal Farm section. Calves were dewormed a month prior to the start of experimental feeding and tested for antibody titer against *P. multocida* (P_{52} strain) antigen. Ten healthy male Murrah buffalo (*Bubalus bubalis*) calves (8–9 months, 111.7 ± 12.55 kg body weight, antibody titer 2662–2789) were selected and divided equally into two groups of five animals each on the basis of their body weights and antibody titer.

2.2. Housing and management

Murrah buffalo calves were housed in a well-ventilated, clean, and concrete-floored shed and fed individually. Strict management and hygiene practices were adopted throughout the experimental period. Clean drinking water was provided *ad-libitum* twice a day at about 9 A.M. and 3 P.M.

2.3. Feeds and feeding

Calves were offered concentrate mixture (50% wheat bran, 27% soybean meal, 20% ground maize grain, 2% mineral mixture, and 1% common salt) and *ad-libitum* wheat straw to meet their nutrient requirements for body weight gain of 500 g/day [25]. The amounts of the concentrate mixture offered were revised fortnightly as per change in the body weights of calves. Calves were additionally provided with about two kilograms of available green fodder (maize/oats/berseem) daily. Feeding schedule was similar in both the groups, except for additional selenium and copper (0.3 mg/kg and 10 mg/kg of dry matter intake, respectively) supplementation in the concentrate mixture of treatment group's animals. Control animals also fed the same concentrate mixture, but without any supplementation. Aqueous solutions of sodium selenite and cupric sulphate were the inorganic source of selenium and copper, which were daily, mixed in measured amounts with the weighed amount of the concentrate mixture of each animal as per their dry matter intake. The quantity of mineral solution was revised every week, according to weekly dry matter intake of the individual animal. Experimental feeding was done for a period of 80 days.

2.4. Feed analysis

Feeds and fodder samples were analysed for different chemical constituents after drying at 60 °C and grinding to pass the 1 mm screen in a Wiley mill using standard procedures [26,27]. Calcium content in feed samples was analysed by the Talapatra method [28] and phosphorus was determined by the method of Association of Official Analytical Chemists [26]. Trace elements like Cu, Zn, Mn, and Fe were estimated in the feed using atomic absorption spectrophotometer [AAS, model 4141, Electronic Corporation of India Limited (ECIL), Hyderabad, India] with dry ashing. Wet digestion was carried out in a 100-ml Kjeldahl flask after soaking feed samples overnight in 10–20 ml double acid mixture (Nitric and Perchloric acid, 4:1) for selenium estimation and vapour generation assembly was additionally used along with AAS for the same. Representative blanks were also taken into consideration following the same acid and procedure of operation done with samples. Certified reference standards procured from Sigma Aldrich Limited worked as a base for making a standard curve to compare with test samples.

2.5. Body weight recording with blood collection and serum/plasma separation

Blood samples from buffalo calves were collected initially (0 day) before starting supplementation and subsequently on day 40 and 80 after starting supplementation through jugular venipuncture, including recording of body weights. All aseptic precautions were taken in the morning (before watering and feeding) and blood was collected in clean and dry test tubes and kept in slanting position for 45 min, followed by centrifugation at 700 × *g* for 15 min to separate out serum. For plasma, blood was collected in a heparin-coated clean tube and was separated at 700 × *g* for 15 min. Whole blood was also collected for haematological studies using EDTA at 1 mg/ml of blood. The serum/plasma samples were stored in 2 ml plastic vials at −20 °C till further analysis.

2.6. Hematological parameters

Whole blood samples were used for the estimation of hemoglobin using a diagnostic kit (Glaxo), manufactured by Sigma Diagnostic Pvt. Limited, Baroda, India. Other Hematological observations like Packed cell volume (PCV), total leukocyte count (TLC) as well as differential leukocyte count (DLC) was determined as per the standard protocol [29].

2.7. Plasma minerals

A suitable amount of plasma samples were taken in a 70-ml capacity glass digestion tube, soaked overnight in 10 ml double acid mixture (Nitric and Perchloric acid, 4:1), and digested. Representative blanks were also taken into consideration following the same procedure of operation done with samples. These digested plasma samples were analysed for different trace minerals like zinc, copper, manganese, iron, and selenium using same procedures and AAS mentioned above, while plasma Ca and inorganic P levels were estimated directly in plasma utilizing diagnostic kits (Glaxo, India) manufactured by Sigma Diagnostic Pvt. Limited, Baroda, India. For estimation of selenium vapour generation assembly was used along with AAS.

2.8. Plasma antioxidant vitamins

β-carotene, retinol, and α-tocopherol were estimated [30] in plasma with the help of high-pressure liquid chromatography (HPLC, make