



Pharmacological levels of copper and selenium augmenting good cholesterol in serum of healthy male buffalo (*Bubalus bubalis*) calves

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ABSTRACT

Dietary pharmacological/supra-nutritional level of copper (Cu) and selenium (Se) was investigated in the ration of dairy buffalo calves. Male Murrah buffalo (20) calves (8–9 months, 112.1±7.69 kg body weight) were divided into 4 equal groups and fed basal diet (control, Cu =10.4 ppm and Se = 0.23 ppm) or basal diet supplemented with 10 ppm Cu (T1), 0.3 ppm Se (T2) or combination of 10 ppm Cu with 0.3 ppm Se (T3) for a period of 120 days. During experimental period blood samples were collected on days 0, 40, 80, and 120 and subjected for analysis of serum total cholesterol and high-density lipoprotein (HDL) for assessment of the pharmacological effect of trace minerals Cu and Se either alone or in combination. Supplementation of Cu alone (T1) or in combination with Se (T3) had reduced total cholesterol level from 80th day onward, whereas HDL remained high in all the supplemented groups at 120th day of observation as compared to control. It may be concluded that pharmacological level of Cu is helpful in reducing serum cholesterol levels, but Cu and Se was helpful for the augmentation of HDL levels of serum in buffaloes.

Keywords: Buffalo calves, Cholesterol, Copper, High-density lipoprotein, Selenium

Copper and selenium are essential trace elements (Patterson *et al.* 1957, Davis and Mertz 1987), required for a number of biochemical functions in living organisms. Both these elements had scientific records of interaction between them, especially in ruminants. McChowell and Gawthorpe (1985), Deol *et al.* (1994) and Van Ryssen *et al.* (1998) reported that supplementing high concentration of copper resulted in increased liver selenium. It occurs due to indirect interaction, resultant damage caused in the liver by high levels of copper. In some studies, high level of copper (35 ppm) was not found unsafe by European Union (EC No 1334/2003/EC) and was justified in view of the interference of Cu with other micronutrients, mainly molybdenum and sulfur (S), but also iron (Fe) and zinc (Zn) (Kendall *et al.* 2001). Previous studies in cattle in NW Spain have found significant positive correlations between Cu and Se in the liver (López-Alonso *et al.* 2004) or in the kidney (Blanco-Penedo *et al.* 2006). In another study, the negative effect of Cu supplementation on muscular Se status in calves emphasizes the need for new research on Cu–Se interaction for a better understanding of the risk of Se deficiency (García-Vaquero *et al.* 2011). Cu interactions with other metals (mainly Mn, Zn, and Fe) have been recently related with the pathogenesis of a great number of neurological

diseases affecting both animals and humans, like for example Alzheimer's disease (Maynard *et al.* 2005) and prion related diseases like spongiform encephalopathy in cattle (Tsenkova *et al.* 2004, Deloncle *et al.* 2006).

Cu and Se had peculiar roles to play in the metabolism of cholesterol and high-density lipoprotein (HDL) to affect cardiac health (Alarcon-Corredor *et al.* 2004, Rayman *et al.* 2011, DiNicolantonio *et al.* 2018). Pharmacological/Supra-nutritional levels of Cu (Mudgal *et al.* 2019) or Se (Hall *et al.* 2011) either alone or in combination (Mudgal *et al.* 2018) had a positive influence on various blood parameters including antioxidant parameters as well as the immune performance of dairy or beef ruminants.

However, the investigation on the effect of pharmacological levels of these elements on blood cholesterol and HDL levels of dairy buffaloes are scanty and thus in the present study two levels of both of these elements in buffalo calves were investigated with assessment of an interaction effect in male Murrah buffalo calves.

MATERIALS AND METHODS

Animals (selection and grouping): After getting the Institute Animal Ethical Committee approval for animal experimentation, 20 healthy male Murrah buffalo (*Bubalus bubalis*) calves (8–9 months, 112.1±7.69 kg body weight) were selected for this study and dewormed 1 month prior

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to start of experimental feeding. These calves were divided into four groups of five animals each on the basis of their body weight following randomized block design (RBD).

Housing and management: Murrah buffalo calves were housed in a well-ventilated, clean, and concrete-floored shed and fed individually. Strict management and hygienic practices were adopted throughout the experimental period. Clean drinking water was provided *ad lib.* twice a day at about 09:00 and 15:00.

Feeds and feeding: Concentrate mixture including 50% wheat bran, 27% soybean meal, 20% crushed maize grain, 2% mineral mixture, and 1% common salt was used in the feeding of buffalo calves with *ad lib.* wheat straw. Feeding was done to meet nutrient requirements of calves for a body weight gain of 500 g/d (Pathak and Verma 1993). The amount of concentrate mixture offered was regularly revised fortnightly as per changed body weights of calves. Calves were additionally provided with about two and a half kilograms of available green fodder (maize/oats/berseem), twice a week to meet vitamin A requirements. The feeding schedule was similar for all buffalo calves, except for mineral supplementation, which was not made in group control, 10 ppm copper (Cu) in group T1, 0.3 ppm selenium (Se) in group T2, 10 ppm Cu + 0.3 ppm Se in group T3. Supplementation of Cu and Se was done using aqueous solutions of cupric sulfate and sodium selenite respectively, which were daily mixed in a measured amount in the weighed amount of concentrate mixture of each animal as per their dry matter intake. The quantity of mineral solution was revised every week according to total dry matter intake of individual animals. Experimental feeding was done for a period of 120 days.

Chemical analysis: Total mixed ration (TMR, calculated on the basis of proportion of concentrate mixture and wheat straw consumed during entire experimental period) was analyzed for different chemical constituents after drying at 60°C and grinding to pass 1 mm screen in a Wiley mill using standard procedures of AOAC (2000): DM after drying at 100°C for 24 h; crude protein (CP) by analysis of nitrogen (N × 6.25) by Kjeldahl method after acid digestion; ether extract (EE) after extraction with petroleum ether by the Soxhlet method; total ash by igniting at 550°C for 3 h in a muffle furnace. Neutral detergent fiber (aNDF) was analyzed by a method of Van Soest *et al.* (1991), where the feed sample was refluxed for 1 h with ND solution without sodium sulfite and with the use of a heat-stable alpha-amylase. Acid detergent fiber (ADF) was analyzed sequentially on the same sample by a method of AOAC (2000). aNDF and ADF were expressed with residual ash. Hemicellulose was determined by subtracting the value of ADF from aNDF. For estimation of calcium (Ca) and phosphorus (P), a hydrochloric acid (HCl) extract was prepared after dissolving the ash of the feed sample in 0.1N HCl. Ca content in feed samples was analyzed by the method of Talapatra *et al.* (1940) and P was determined by the method of AOAC (2000). For analysis of Cu and Se, a suitable amount of feed sample was taken in a 100 mL

Kjeldahl flask, soaked overnight in about 20 mL mixture of nitric and perchloric acid (4:1) and digested. The digested sample was used for the estimation of Cu using atomic absorption spectrophotometer [model 4141, Electronic Corporation of India Limited (ECIL), Hyderabad, India], along with vapour generation assembly for estimation of Se.

Blood collection, serum separation, and analysis: Blood samples from buffalo calves were collected initially (0 day) and subsequently at 40 day's interval through jugular venipuncture, observing all aseptic precautions in the morning (before watering and feeding), into 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. The serum samples were stored in 2 mL of plastic vials at -20°C until further analysis. Serum samples were analyzed for total cholesterol and HDL utilizing diagnostic kits (Glaxo, India).

Statistical analysis: Data generated in the experiment were analyzed statistically using analysis of variance (ANOVA, Snedecor and Cochran 1989) and Duncan's multiple range tests to compare the means (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Chemical composition of feeds: The chemical composition of the total mixed ration (calculated on the basis of the proportion of concentrate mixture and wheat straw consumed during the entire experimental period) were comparable to the levels recommended for growing buffalo calves for 500 g daily gain (Pathak and Verma 1993). The contents of crude protein (CP) was 10.60%, ether extract (EE) 2.03%, total carbohydrates (TCHO) 79.63%, neutral detergent fiber (NDF) 62.92%, acid detergent fiber (ADF) 34.55% and hemicelluloses 28.36% in the total mixed ration. The Cu (10.40 ppm) and Se (0.23 ppm) contents in the diet were also comparable to the levels recommended for cattle (NRC 2001) with 0.86% calcium and 0.44% total phosphorus in the total mixed ration for buffalo calves.

Intake and growth: Intake of digestible crude protein (5.97–6.08 g/kgW^{0.75}) and total digestible nutrients (48.42 – 50.03 g/kgW^{0.75}) also remained comparable among four groups with values of dry matter intake (82.06 – 85.18 g/kgW^{0.75}). The average daily body weight gain of four groups also remained comparable (P>0.05) with their values of 577, 607, 611 and 626 g in control, T1, T2, and T3 groups, respectively.

Serum cholesterol: The overall mean (of 40, 80 and 120 d) values of total cholesterol as well as at different points of time after starting supplementation have been depicted in Table 1 with their standard errors.

The overall mean of total cholesterol values was 106.0, 101.9, 108.9 and 101.4 mg/dl (Table 1). It was observed that on day 80 (P<0.05) and 120 (P<0.01), the values of total cholesterol were significantly reduced in the groups supplemented with Cu either alone (T1) or in combination with Se (T3), indicating that supplementation of Cu had

Table 1. Effect of copper (Cu) or/and selenium (Se) supplementation on serum cholesterol (mg/dL) levels

Group	Day 0	Day 40	Day 80*	Day 120**	Overall Mean
Control	84.0±1.86	83.0±7.67	108.9 ^b ±3.43	126.0 ^c ±2.21	106.0±3.86
Cu	84.5±2.13	100.7±7.44	96.4 ^a ±3.12	108.7 ^a ±2.51	101.9±3.02
Se	86.0±2.45	98.8±7.61	105.7 ^{ab} ±2.62	122.1 ^{bc} ±2.17	108.9±3.59
Cu + Se	86.7±0.96	93.0±6.41	97.2 ^a ±3.03	114.0 ^{ab} ±2.29	101.4±2.92

Overall Mean is the mean of different periodical observations except at day 0; Means bearing different superscripts in a row differ significantly at *(P<0.05) and **(P<0.01).

reducing effect on total cholesterol level in the serum. However, there was no influence on the total cholesterol values due to supplementation of Se alone (T2) at day 80.

In contrast to the present findings, Engle and Spears (2001) found no effect on total cholesterol levels due to Cu supplementation (10 or 40 ppm) in Simmental steers. Likewise, Correa *et al.* (2012) also did not report any effect of 10 and 40 ppm Cu supplementation on plasma cholesterol levels in Nellore beef cattle. It appears that differences in the breed had influenced Cu metabolism as in a study, Mullis *et al.* (2003) observed a different kind of response for Cu supplementation in Simmental heifers to that of Angus heifers. Simmental steers showed no difference in cholesterol level in blood due to Cu supplementation, whereas reducing effect was observed in Angus heifers. However, several reports on the effect of Cu supplementation on the cholesterol level in blood are consistent with our findings. Engle *et al.* (2000a) in Angus steers found reduced serum cholesterol concentration at 84 and 112 days due to supplemental Cu (@ 10 ppm). Similarly, Engle *et al.* (2000b, 2000c) and Engle and Spears (2000) in Angus steers reported decreased serum cholesterol levels due to supplementation of 20 ppm Cu. Likewise, Fagari-Nobijari *et al.* (2013) reported reduced serum cholesterol by supplementation of 30 ppm Cu over control (4.7 ppm) diet in young Holstein bulls.

In respect of the effect of Se supplementation on blood cholesterol levels, our findings were also supported by earlier workers. In one of the experiments, Arthur *et al.* (1988) did not find any effect on the concentration of plasma total cholesterol due to supplementation of 0.1 ppm Se in steers. Similarly, Singh *et al.* (2002) also reported no effect on cholesterol values when supplemented Se, even at a high level of 8.54 ppm in the diet of buffalo calves. Likewise, Netto *et al.* (2014) also did not report any influence on serum cholesterol levels when supplemented selenium (2

ppm) in Brangus bulls.

When we consider interaction effect of Cu and Se, in contrast to our findings Netto *et al.* (2014) reported no effect on serum cholesterol level when supplemented Cu (40 ppm) with selenium (2 ppm) in the diet of Brangus bulls, whereas the level of cholesterol was reduced in longissimus dorsi muscle. The level of Se used by Netto *et al.* (2014) was very high and maybe a reason to not influence the serum cholesterol level.

Serum high-density lipoprotein: Overall mean values of HDL were found to be high (P<0.05) in the mineral supplemented groups, as compared to control as these were 66.49, 77.04, 75.06 and 72.48 mg/dl in group T1 to T4, respectively (Table 2). It was further observed that the values of HDL were significantly higher (P<0.05) on 120th day in all the mineral supplemented groups as compared to control group. It indicated that supplementation of both Cu and Se either alone or in combination resulted in raised HDL level in buffalo calves. However, Engle *et al.* (2000b) did not find any significant effect on HDL values due to Cu supplementation (@ 20 or 40 ppm) in steers. The reason for the difference in result might be a difference in supplemental levels of Cu, as they used high levels of copper (20/40 ppm) as compared to 10 ppm of Cu used in the present experiment.

In human studies supplementation of Cu (Alarcon-Corredor *et al.* 2004) remained associated with augmenting the levels of HDL with simultaneous reduction of total cholesterol and thus was supportive in reducing the incidences of cardiovascular disease as well.

Ratio of total cholesterol with HDL: The overall mean (of 40, 80 and 120 day) value of ratio of total cholesterol to HDL were 1.74, 1.37, 1.47 and 1.43 in groups T1 to T4, respectively (Table 3). The ratio remained significantly high (P<0.01) in different treatment groups as compared to the control one. Likewise was the position at day 40 of the

Table 2. Effect of copper (Cu) or/and selenium (Se) supplementation on serum HDL (mg/dL) levels

Group	Day 0	Day 40	Day 80*	Day 120**	Overall Mean
Control	54.6±1.60	49.0±4.31	77.2±2.28	73.3 ^a ±1.89	66.4±3.01
Cu	54.8±2.13	62.3±5.74	85.7±4.45	83.2 ^b ±2.83	77.0±3.59
Se	54.1±1.68	59.2±2.18	81.8±1.98	84.2 ^b ±3.06	75.1±3.20
Cu + Se	54.4±2.75	56.8±4.61	79.5±2.01	81.1 ^b ±4.00	72.5±3.18

Overall Mean is the mean of different periodical observations except at day 0; Means bearing different superscripts in a row differ significantly at *(P<0.05).

Table 3. Effect of copper (Cu) or/and selenium (Se) supplementation on Total Cholesterol: HDL levels

Group	Day 0	Day 40	Day 80*	Day 120**	Overall Mean
Control	1.55±0.06	2.09 ^b ±0.22	1.41 ^b ±0.05	1.72±0.04	1.74 ^b ±0.07
Cu	1.55±0.07	1.66 ^a ±0.15	1.14 ^a ±0.09	1.31±0.06	1.37 ^a ±0.08
Se	1.59±0.04	1.67 ^a ±0.11	1.29 ^{ab} ±0.03	1.45±0.05	1.47 ^a ±0.04
Cu + Se	1.61±0.10	1.66 ^a ±0.12	1.22 ^a ±0.03	1.41±0.04	1.43 ^a ±0.03

Overall Mean is the mean of different periodical observations except at day 0; Means bearing different superscripts in a row differ significantly at *(P<0.05) and **(P<0.01).

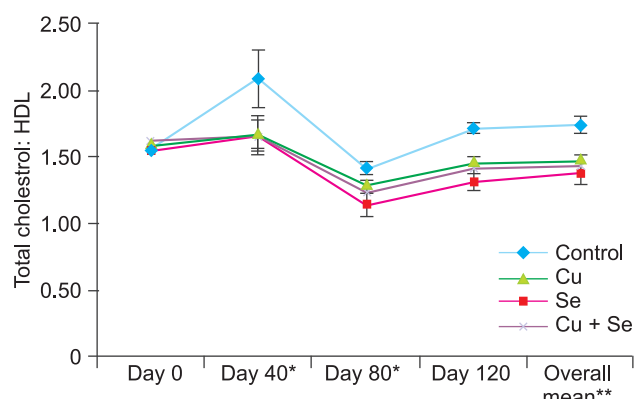


Fig. 1. Effect of copper (Cu) or/and selenium (Se) supplementation on Total Cholesterol: HDL levels.

Overall Mean is the mean of different periodical observations except at day 0. Day 40, *(P<0.05) with the difference between control × Cu, Control × Se and Control × Cu + Se, Day 80: *(P<0.05) with the difference between control × Cu and Control × Cu + Se; **(P<0.01) with the difference between control × Cu, Control × Se and Control Cu + Se.

study but at low level of significance (P<0.05). The response was slight variable at day 80, when the effect (P<0.05) was apparent only in groups supplemented with Cu either alone or with Se (Fig. 1) but significance was missing from the group supplemented with Se alone.

Rayman *et al.* (2011) also reported beneficial association of supplementing Se on ratio of total cholesterol and HDL in human subjects.

Low levels of cholesterol and high level of HDL were considered beneficial for heart health (DiNicolantonio *et al.* 2018) and in this respect supplementation of these minerals (Cu and or Se) at present supplementation level interprets their positive influence on animal's health.

On the basis of the present observations, it may be concluded that supplementation of Cu either alone or in combination with Se was helpful in reducing total cholesterol levels, whereas supplementation of Cu or/and Se was helpful for augmentation in the serum HDL levels of buffaloes.

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