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Effect of different sources of copper supplementation on performance, nutrient utilization, blood-biochemicals and plasma mineral status of growing Hariana heifers

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ABSTRACT

Twenty-four, 12–18 months old Hariana heifers were used to determine the effects of organic and inorganic dietary copper (Cu) supplementation on performance, nutrient utilization, blood biochemicals and plasma mineral status. Cu was supplemented (8 mg/kg diet DM) as copper proteinate, copper propionate and copper sulfate ($CuSO_4$). Animals were divided into four treatment groups with 6 animals in each group and were fed basal diet as per NRC (2001) for a period of 120 days. The basal diet contained 8.0 mg of Cu/kg DM. T1 (control) was fed only basal diet with no added copper while in T2: 8 mg/kg DM of copper proteinate; T3: 8 mg/kg DM of copper propionate; T4: 8 mg/kg diet $CuSO_4$ was added respectively.The intake, daily gain, feed:gain ratio, BCS and FCR were not affected by Cu supplementation. The TDN intake and ADF digestibility were significantly higher in both the organic Cu supplemented groups. The intake of Cu was significantly higher in Cu supplemented groups. The concentration of plasma ALT, AST enzymes, total cholesterol and total immunoglobulins were not affected by sources of Cu in diet. The antioxidant activity and plasma Cu concentration were significantly higher in Cu supplemented groups, irrespective of sources. Thus, supplementation of 8 mg/kg DM Cu had no beneficial effect on growth performance and blood biochemicals. In conclusion, chelating agents have no effect on bioavailability of copper. Also, the organic copper can be a preferred form to be supplemented for better digestibility in heifers.

Key words: Copper supplementation, Hariana, Heifers, Mineral status, Nutrient utilization, Performance

Copper (Cu) is an essential element required by cattle and other animal for number of biochemical functions and it functions as a cofactor or component of many essential enzymes such as cytochrome oxidase, lysyl oxidase, ceruloplasmin, and superoxide dismutase (Klasing 1998). Copper deficiency causes reduced growth, anemia, diarrhea as well as impairment of several metabolic enzymes (Suttle 2010). Supplementation of Cu is generally required to overcome the adverse effects caused by the deficiency of this element and to boost the production and reproduction performances. Traditionally, ruminant Cu deficiencies have usually been corrected by supplementation with inorganic mineral supplements (Mondal and Biswas 2007). However, the bioavailabilities of these inorganic forms are lower and hence currently supplementations in the form of chelates, complexes are being popularized in animal diet as alternatives to their inorganic counterparts. Recent advances in mineral research indicate that absorption and utilization of trace elements is higher, if they are supplemented in the organic forms (Mohanta and Garg 2014). Studies in different animal have revealed notable differences in the bioavailability of trace mineral from different sources. Supplementation of Cu and Zn through organic sources as compared to inorganic sources improved gut absorption, and suggested better bioavailability through organic/ chelated sources (Pal *et al.* 2010). Dietary supplementation of copper methionine or Cu-Lysine improved performance in poultry (Singh *et al.* 2015) as compared to $CuSO_4$. Senthil *et al*. (2009) reported that Cu dependent enzymes activity and immune response were highest and respond better against stress in lambs on 14 ppm supplemented Cu from Cu- proteinate source. However, their effect was comparable in goat (Waghmare *et al.* 2014) and cattle (Wang *et al.* 2012). Recent studies have shown that organic copper sources supplementation improve the growth rate and FCR in broiler chickens (Paik 2001) and pigs. However, work is meager particularly assessing the effect of copper supplementation from different sources on growth and nutrient utilization of indigenous cattle. In view of above facts, the present study was designed to

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Table 1. Ingredients and composition (% DM basis) of basal diet fed during the experimental period

Ingredient	Proportion (%)
Maize fodder	35
Wheat straw	15
Barley grain	10
Wheat grain	10
Oat grain	5
Wheat bran	5
Gram chunni	5
Mustard oil cake (expeller extracted)	14
Mineral mixture	2
Nutrient composition	(*% DM basis*)
Dry matter	68.6
Crude protein	13.4
Ether extract	2.9
Total ash	9.9
Neutral detergent fibre	54.7
Acid detergent fibre	34.4
Acid detergent lignin	2.3
Copper (mg/kg DM)	8.0
Zinc (mg/kg DM)	43.18
Iron (mg/kg DM)	199.88
Manganese (mg/kg DM)	30.01

determine the effect of copper supplementation on growth performance, nutrient utilization and blood parameters of growing indigenous Hariana cattle.

MATERIALS AND METHODS

Experimental animal and feeding regimen: A total of 24 female Hariana heifers (aged 12–18 months, 140±1.0 kg body weight) were selected from Instructional Livestock Farm Complex (ILFC) of Veterinary University, India, situated at elevation 191 m above mean sea level, latitude and longitude position being 27° 303 N and 77° 413 E, respectively. The climate of the region is semi-arid.The animal experimental protocol was approved by the Institute Ethical Committee.The animals were divided into four groups of six animals each on the basis of their body weight following randomized block design. The nutrient requirements of heifers were met by feeding basal diet comprising concentrate mixture, wheat straw and maize green fodder. They were fed according to their body weight to meet their nutrient requirements as per NRC (2001) for a period of 120 days. The concentrate mixture was prepared by mixing barley grain, wheat grain, oat grain, wheat bran, gram chunni, mustard oil cake and mineral mixture (BIS Type II, but without copper) in 20, 20, 10, 10, 10, 28 and 2 parts, respectively to meet nutrient requirement except Cu. The basal diet was kept similar in all the four treatment groups except for Cu; T1 (control) no added copper; T2: 8 mg/kg DM commercial feed grade organic sources of Cu chelated with short-chain peptides and amino acids, copper proteinate; T3: 8 mg/kg DM commercial feed grade organic sources of copper chelated with propionic acid, copper propionate; T4: 8 mg/kg DM with commercial feed grade inorganic source of copper, $CuSO_4$. Supplementing 0.065 g, 0.031 g, and 0.033 g of Cu proteinate, Cu propionate, and $CuSO_4$/kg mineral mixture obtained supplemental Cu levels, respectively. Cu proteinate, Cu propionate, and $CuSO_4$ contain 12% Cu, 25% Cu and 24% Cu, respectively. The basal diet fed during experimental period contained 8.0 mg of Cu/kg DM. The composition of the basal diet fed during experimental period is presented in Table 1. The calculated amount of Cu source was first mixed with 250 g of concentrate mixture and was fed individually in a plastic tub.The dose of Cu supplementation was modified as per DM intake every fortnight.The heifers were kept in well-ventilated byre with clean and concrete floor maintained hygienically. They were fed individually respective experimental diets and had *ad lib.* access to fresh and clean drinking water. Deworming was done at the beginning of the experiment.

Observation recorded, sample collection and laboratory analysis: Weighed amount of concentrate mixture and wheat straw was fed at 9.00 AM daily and chaffed maize fodder was offered in the afternoon.The residue, if any, was weighed next morning to calculate the daily feed intake. Representative samples of roughages and concentrate feed were collected daily and dried in hot air oven at 105°C to determine the DM content and calculate the daily DM intake by heifers.The animals were weighed fortnightly on two consecutive days using a digital balance before offering feed and water in the morning. Body weight gain (BWG) and average daily gain (ADG) was obtained by calculations. Body condition score (BCS) was determined using scale given by Anitha *et al.* (2005).

Digestibility trial: A digestibility trial of 7 days duration, involving quantitative collection of feed offered, residues left and feces voided was conducted after 120 days of experimental feeding to assess the nutrient digestibility and balance of nutrients. The dried representative samples of feeds and feces of each animal over the entire collection period were pooled, sampled and ground to pass through 1.0 mm sieve and stored in an air tight polyethylene bags until further analysis.

Analytical methods of feed and faeces: Samples of feeds and faeces were analyzed for proximate constituents according to AOAC (2005) methods. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined according to the procedures described by Van Soest *et al.* (1991). Content of Cu, Fe, Zn and Mn in feedstuffs, refusal left, faeces, and plasma samples were determined by using atomic absorption spectrophotometer (AAS, Model-400, Perkin Elmer, USA).

Blood collection: Blood samples were collected in heparinised vacuutainer tubes (Becton Drive, Franklin Lakes, NJ, USA) on day 0, 30, 60, 90, and 120. Blood samples were centrifuged at 3,000 rpm at 4°C for 20 min and separated plasma was stored at –20°C until further analysis. Cholesterol, ALT, and AST were analyzed by using Span Diagnostic Commercial Kits (Span Diagnostic Ltd, Surat, India). Plasma total immunoglobulin concentration

was measured by using zinc turbidity method (McEvan and Fisher 1970). Ferric reducing antioxidant power (FRAP) assay was used for determination of plasma total antioxidant activity (Benzie and Strain 1999).

Statistical analysis: The data were statistically analyzed using the GLM procedure (SPSS). The data of various parameters were subjected to one-way ANOVA using the dietary source as a variable (Snedecor and Cochran 1994). Duncan's multiple range test was used to detect statistical significance between treatments using a significance level of 0.05.

RESULTS AND DISCUSSION

Performance: The results of growth rate and performance are shown in Table 2. The data indicated that supplementing 8.0 mg Cu/kg DM either in the form of Cu-proteinate, Cu-propionate and Cu sulphate did not affect feed intake, daily gain, feed:gain ratio and BCS in growing Hariana heifers. Similar observations on DMI were also made on supplementation of 7 or 14 ppm Cu in heifers (Mullis *et al.* 2003) and 10, 20 or 30 ppm Cu in Cashmere goats (Zhang *et al.* 2009). As reported in present study, supplementation of Cu as $CuSO_4$ and Cu-methionine did not affect average daily gain and feed:gain ratio in kids (Waghmare *et al.* 2014). No differences in total weight gain or average daily gain were reported in ewes supplemented 5 ppm of Cu-met and 20 ppm of Zn-methionine (Pal *et al.* 2010), in Cu deficient heifers supplemented 8 or 16 ppm Cu-lys (Rabiansky *et al.* 1999), in growing steers fed 20 or 40 ppm Cu (Engle and Spears 2000b).No effect in feed: gain ratio in Simmental steers supplemented with either 10 or 40 ppm Cu (Engle and Spears 2001) or in Simmental and Angus growing heifers supplemented with 7 or 14 ppm Cu in feed (Mullis *et al.* 2003). However, in contrast to present findings, Datta *et al.* (2007) reported improved body weight in kids fed supplemental Cu. The variation in results occurs because there are several factors, such as initial Cu status of the animals, Cu content of basal diet, and concentration of Cu antagonists (Fe, S, and Mo), etc that might affect an animal response to Cu supplementation in ruminants (Solaiman *et al.* 2006, 2007).

As expected, the supplemental Cu did not influence body condition score, since the DM intake did not differ. Results of body condition score were similar to those reported by Uchida *et al.* (2001) and Nocek *et al.* (2006), who did not observe effects of Zn, Mn, Cu or Co supplemented to dairy cows over three lactations on body condition score. Cortinhas *et al.* (2012) also observed no effect of supplementation of organic Zn, Cu and Se neither on body condition score nor on body condition score change during pre- and post-partum.

Digestibility of nutrients and plane of nutrition: The data on nutrient digestibility and plane of nutrition is presented in Table 3. The data reveals that the digestibility of the DM, CP, EE, NDF was not affected except ADF, by dietary supplementation of Cu. The digestibility of ADF was significantly ($P<0.05$) higher in organic Cu supplemented group suggesting a positive role of Cu supplementation in fibre digestion. In agreement with the current experiment, an increase in digestibility of ADF was also found in a study in Muzaffarnagari male lambs supplemented with organic zinc (Garg *et al.* 2008). An increasing trend in CF digestibility with the increase in dose of Cu was observed in Black Bengal kids (Mondal and Biswas 2007). Similar to present findings, Waghmare *et al.* (2014) also reported no effect on the digestibility of DM, OM, CP, EE and NDF on Cu supplementation in kids. Shinde *et al.* (2013) also did not observe any effect of organic Cu supplementation on digestibility of DM, OM, CP, EE, CF and NFE in Chokla rams. Likewise, Mudgal *et al.* (2007) found that supplementation of 10 ppm Cu and 0.3 ppm of Se did not have any effect on nutrient digestibility.

The DMI and digestible CP intake were comparable among the four groups. However, total digestible nutrient (TDN) was significantly ($P<0.05$) higher in group supplemented with organic copper. In accordance with present findings, an increasing trend in TDN intake (g/kg $W^{0.75}$) was found in Cu-proteinate supplemented group compare to $CuSO_4$ (Mondal and Biswas 2007). The intake of DM and CP by heifers of both sources was in accordance with recommended intake (NRC 2001). In contrast with present results, Khan (1978) did not observe any effect on TDN intake when supplemented 20 ppm Cu in the diet of Holstein Friesian calves. Mudgal *et al.* (2007) also did not observe any effect on CP and DCP intakes in the buffalo calves supplemented with 10 ppm of Cu and

Table 2. Effect of different copper sources on performance of growing Hariana heifers

Parameter	Supplemental Cu (8.0 mg/kg DM)				SEM	P value
	Control	Cu-proteinate	Cu-propionate	$CuSO_4$		
Initial body weight	140.17	139.67	139.83	139.67	14.64	0.99
Final body weight	196.83	194.83	197.67	195.67	16.35	0.99
Body weight gain	7.08	6.89	7.23	7.00	0.25	0.81
ADG (kg/d)	0.472	0.459	0.481	0.466	16.85	0.82
DMI (kg/d)	4.35	4.33	4.32	4.20	0.17	0.92
Gain: Feed ratio	0.11	0.11	0.11	0.11	0.00	0.99
BCS	2.57	2.60	2.68	2.63	0.06	0.69
FCR	9.49	9.83	9.74	9.92	0.58	0.96

ADG, Average daily gain; DMI, average daily feed intake; BCS, body condition score; FCR, feed conversion ratio

Table 3. Effect of supplementation of different sources of copper on nutrient intake, digestibility and plane of nutrition of heifers

Parameter	Supplemental Cu (8.0 mg/kg DM)				SEM	P value
	Control	Cu-proteinate	Cu-propionate	$CuSO_4$		
Initial body weight	184.33	183.00	186.00	183.00	16.18	0.999
Final body weight	191.00	189.17	192.50	190.00	16.09	0.999
Total gain	6.67	6.17	6.50	7.00	0.62	0.813
Dry matter intake (kg/d)	5.39	4.96	5.10	5.01	0.47	0.918
CP intake (kg/d)	0.61	0.61	0.63	0.62	0.02	0.680
DCP intake (kg/d)	0.40	0.41	0.43	0.44	0.01	0.148
TDN intake (kg/d)	2.54[a]	2.74[b]	2.82[b]	2.62[a]	0.03	0.000
Digestibility (%)						
DM	63.44	65.81	67.20	65.47	0.93	0.069
CP	65.67	67.04	67.86	66.12	0.80	0.246
EE	74.00	75.03	77.16	75.04	0.75	0.050
NDF	60.80	62.14	58.77	63.25	1.60	0.258
ADF	61.00[a]	64.39[b]	65.05[b]	62.42[a]	0.83	0.005

SEM, Standard error of mean; DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre.

Table 4. Mineral intake and absorption during digestibility experiment in heifers

Mineral	Control	Cu-proteinate	Cu-propionate	$CuSO_4$	SEM	P value
Copper						
Intake (mg/day)	67.84[a]	97.91[b]	98.91[b]	99.25[b]	2.83	0.00
Outgo (mg/day)	61.81[a]	86.13[b]	87.10[b]	87.85[b]	2.48	0.00
Absorption (mg)	6.04[a]	11.78[b]	11.80[b]	11.40[b]	0.42	0.00
Absorption % of intake	8.80[a]	12.03[b]	11.93[b]	11.50[c]	0.24	0.00
Iron						
Intake (mg/day)	1240.83	1241.83	1243.50	1245.50	1.55	0.19
Outgo (mg/day)	904.83	905.16	907.16	905.16	0.89	0.26
Absorption (mg)	336.00	336.66	336.33	340.33	1.78	0.31
Absorption % of intake	27.08	27.11	27.04	27.32	0.11	0.35
Zinc						
Intake (mg/day)	498.83	498.03	500.63	502.23	1.061	0.05
Outgo (mg/day)	398.76	397.77	399.82	401.08	0.97	0.12
Absorption (mg)	100.07	100.26	100.80	101.15	0.95	0.84
Absorption % of intake	20.06	20.13	20.13			

0.3 ppm of Se. The TDN intake was higher for organic sources than inorganic sources. The reason might be that in the organic source the ligand used for complexing affects its stability, ruminal solubility, metabolism and hence increases its bioavailability (Spears *et al.* 2004).

Mineral intake and absorption: The intake of Cu was significantly higher ($P<0.05$) in heifers of Cu-proteinate, Cu-propionate and inorganic $CuSO_4$ supplemented group than non-supplemented group (Control) (Table 4). Supplementation of organic and inorganic forms of Cu influenced the intake and apparent absorption of Cu and it was higher in heifers receiving organic (Cu-proteinate, Cu-propionate) form of Cu than Cu sulphate. Higher absorption from organic sources with similar intake in the present study was in agreement with the findings of Power and Horgan (2000) and it could be believed that the mineral ions from inorganic source in gut are released and possibly recombine with other components in digesta forming insoluble complexes, thus reducing their absorption, while the organic minerals use intestinal transport mechanisms and are more effectively absorbed. Shinde *et al.* (2013) and Pal *et al.* (2010) also observed similar pattern of Cu retention in organic Cu supplemented animals. No ($P>0.05$) significant difference was observed on intake and utilization of Fe, Zn and Mn minerals on supplementing either inorganic or organic source of Cu.

Plasma biochemical response: The effects of supplementation of Cu on hematological profiles are presented in Table 5. The blood biochemicals recorded were within the normal range. Concentration of plasma ALT, AST enzymes were unaffected by the sources of Cu, which indicates that the animals were apparently healthy throughout the experimental duration. The AST and ALT enzymes are the indicators of liver damage. Dietary addition of 30 mg/kg DM Cu has shown no dystrophy in hepatic or other tissues containing ALT, AST enzymes during 90 days feeding period (Mondal and Biswas 2007).

Plasma total Ig was similar among all groups. In

Table 5. Effect of different copper sources on blood metabolites in growing Hariana heifers

Parameter	Supplemental Cu (8.0 mg/kg DM)				SEM	P value
	Control	Cu-proteinate	Cu-propionate	$CuSO_4$		
ALT (IU/l)	25.47	26.14	25.98	25.83	0.27	0.34
AST(IU/l)	67.14	67.78	68.24	66.72	0.52	0.47
Total cholesterol (mg/dl)	197.43	197.73	200.19	199.27	0.88	0.09
Total Ig (mg/ml)	29.73	30.50	29.40	29.23	0.34	0.05
FRAP value (µmol/l)	1048.66[a]	112.51[b]	1117.96[b]	1100.93[b]	7.08	0.00
Plasma mineral						
Calcium (mg/dl)	10.94	10.65	10.82	10.87	0.11	0.30
Phosphorus (mg/dl)	4.57	4.59	4.57	4.58	0.01	0.05
Copper (mg/l)	0.68[a]	0.80[b]	0.78[b]	0.76[b]	0.03	0.05
Iron (mg/l)	1.34	1.34	1.35	1.36	0.01	0.07
Zinc (mg/l)	1.34	1.34	1.35	1.36	0.01	0.91

ALT, Alanine transaminase; AST, aspartate transaminase; FRAP, ferric reducing antioxidant power assay; Ig, immunoglobulins.

accordance with the present results, the total immunoglobulin levels in blood serum (Kinal *et al.* 2007) and colostrum (Kinal *et al.* 2007, Formigoni *et al.* 2011) of newly born calves were higher in the organic mineral supplemented groups. Supplementation of Cu, Mn, and Zn as chelated sources enhanced immune response of early lactation dairy cows compared with cows supplemented with inorganic sources (Nemec *et al.* 2012).

The antioxidant activity (FRAP value) was higher ($P<0.05$) in heifers receiving Cu supplemented diets. Increase in FRAP level indicates the antioxidative activity of copper. Correa *et al.* (2104) also concluded, the antioxidant effect of Cu supplementation at 40 mg/kg, regardless of the source (organic and inorganic), in inducing higher hepatic SOD activity compared with the control. Senthilkumar *et al.* (2009) also reported increased erythrocytic superoxide dismutase activity in Cu supplemented lambs and the effect was more when supplemented with Cu proteinate.

The concentrations of total cholesterol was not affected by source of Cu in diet. Present results were not in agreement with the findings of Cheng *et al.* (2008) who reported reduced plasma total cholesterol concentrations on day 60 by Cu supplementation in lambs and in goats receiving 20 or 40 mg Cu/kg DM (Datta *et al.* 2007). Engle *et al.* (2000a) reported that Cu supplementation (20 or 40 mg Cu/kg DM) reduced serum cholesterol concentration in finishing steers. But in another experiment, serum cholesterol concentration was not affected by treatment or treatment×time interaction (Engle and Spears 2001). It may be speculated that the findings differ due to lower dose of Cu supplementation in present study as it was also found that the addition of higher concentrations of Cu (125 to 250 mg Cu/kg DM) reduced plasma cholesterol (Pesti and Bakalli 1996). However, Cu supplementation did not affect the cholesterol metabolism if the basal diet contained sufficient Cu content. Paik *et al.* (1999) reported that serum cholesterol was not affected by supplementing 125 ppm Cu as Met-Cu chelate when the control diet contained sufficient level of Cu for normal metabolism.

The plasma Cu concentration increased as expected with the Cu supplementation, indicating that dietary Cu levels were reflected in blood Cu concentrations but no differences was found between Cu sources (Table 5). These results were consistent with Zhang *et al.* (2008) and Dezfoulian *et al.* (2012), who reported an increase in plasma Cu of goats and lambs, which received different sources of Cu. There have been similar reports in steers (Engle and Spears 2000b) and calves (Kegley and Spears 1994). Hosienpour *et al.* (2014) also reported increased serum Cu concentration on Cu supplementation. Mondal *et al.* (2007) also observed increased Cu concentration in plasma of Cu supplemented group irrespective of organic or inorganic form. The concentrations of Ca, P, Fe and Zn were not affected ($P>0.05$) by dietary treatments. Similar to present results, supplementation of 10 ppm Cu proteinate did not alter Ca, P, Fe, Zn and Mn concentration in goats (Mondal *et al.* 2007). Wu *et al.* (2015) supplemented Cu at 50,100 and 150 ppm level in mink and reported linear effect of dose of Cu on plasma Cu concentration.

Thus, supplementation of 8 mg/kg DM Cu (as Cu proteinate, Cu propionate and $CuSO_4$) had no beneficial effect on growth performance. The digestibility of the DM, CP, EE, NDF were not affected. Significantly ($P<0.05$) higher values were recorded for TDN intake and ADF digestibility in both the organic copper supplemented groups indicating no effect of chelating agents on bioavailability of copper. The antioxidant activity (FRAP value) and plasma Cu concentration were significantly higher ($P<0.05$) in Cu supplemented groups, irrespective of sources. Hence, the organic form of copper can be supplemented for better digestibility in heifers.

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