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Original article

Effect of various levels of dietary copper on liver transaminases, total immunoglobulin and antioxidant status in hariana heifers

V.P. Gupta[a], V. Kumar[b,*], D. Roy[b], M. Kumar[b]

[a]*M.V.Sc student, Department of Animal Nutrition, College of Veterinary Sciences & Animal Husbandry, DUVASU, Mathura -281001(UP), India.*

[b]*Assistant Professor, Department of Animal Nutrition, College of Veterinary Sciences & Animal Husbandry, DUVASU, Mathura -281001(UP), India.*

*Corresponding author; Assistant Professor, Department of Animal Nutrition, College of Veterinary Sciences & Animal Husbandry, DUVASU, Mathura -281001(UP), India.

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ABSTRACT

This study was conducted to evaluate the effect of different levels of copper containing mineral supplements on liver transaminase, total immunoglobulin and antioxidant status in heifers (Hariana; Bos indicus). Eighteen heifers were randomly allotted to 3 dietary treatments (average body weight 145.0±1.0 kg, age 14-18 months) having 6 animals in each and fed for a period of 60 days. Control group G1 was fed basal ration (9.8 mg Cu/kg) comprising of concentrate mixture, berseem and wheat straw without mineral supplementation, G2 (50.1 mg Cu/kg) and G3 (90.4 mg Cu/kg) were supplemented with mineral supplements containing 2.1 and 4.2% CuSO4, respectively. During 60 days feeding trial, blood samples were collected to analyze for liver enzymes (ALT and AST), total immunoglobulin and total antioxidant activity (FRAP assay). Supplementation of Cu containing mineral mixture above (50 or 90 mg Cu/kg DM) physiological requirement did not adversely affected (P>0.05) liver transaminase activity, immune status and antioxidant status. It may be concluded that a Cu level (50 ppm) higher than recommended (10 ppm) may be supplemented in the diet of growing animals without compromising health, improving tissue quality and productivity.

1. Introduction

Copper (Cu) is an essential micronutrient required for various biological functions through several enzymes such as superoxide dismutase and ceruloplasmin (Halliwell and Gutteridge, 1999; Engle, 2011). A primary deficiency of Cu can occur in bovines when their diet is deficient in Cu or due to antagonistic effect of excess Mo, S, and Fe concentration in diet (Spears, 2003). Copper deficiency produced inconsistent immune function responses in calves (Galyean et al., 1999; Ward and Spears, 1999). Heifers fed diets marginal in Cu (6–7 mg/kg diet) had a greater percentage (60% vs. 36%) of infected quarters at calving than heifers supplemented with 20 mg Cu/kg diet (Harmon, 1998). In cattle, Cu is included at concentrations of 10 to 100 mg/kg of diet to reduce mortality in calves and for growth promotion in feedlot cattle (NRC, 2000). Dietary Cu above physiological concentrations has been shown to alter lipid metabolism in calves (Jenkins and Kramer, 1989), beef cattle (Engle, 2011) and goats (Solaiman et al., 2006). It also has been reported that dietary supplements leading to Cu accumulation in the liver at concentration above normal (125 mg/kg of wet weight) induce negative effects on animal performance in finishing steers (Engle and Spears, 2000). A hepatic concentration of Cu is significantly correlated to hepatic enzyme (aspartate transaminase and g-glutamyltransferase) activities (Lopez-Alonso et al., 2006). The dietary Cu supplementation of more than recommended levels (10 mg/kg, NRC 2001) for better health response and carcass quality have shown inconsistent performance in cattle (Engle and Spears, 2000). Effect of high supplementation need to be studied on critical blood biochemical parameters. Based on this background present study was conducted to evaluate the effect of different dietary concentration of Cu on liver transaminase activity, total immunoglobulin and antioxidant status in Hariana heifers.

2. Materials and methods

2.1. Experimental design and feeding

For this experiment eighteen Hariana heifers were randomly assigned to three treatment groups (n= 6) (145.0±1.0 kg body weight, age 14-18 months). Heifers were fed on treatment diets for 60 days. De-worming of all the heifers was done before the start of the experiment. The nutrient requirements of animals were met by feeding concentrate mixture, berseem and wheat straw. Heifers either received a basal diet devoid of supplemental mineral mixture (G1) or were supplemented with mineral mixture (G2) and mineral mixture (G3). Concentrate mixture of G1 group was consisted of barley grain (56 parts), wheat grain (11.7 parts) and mustard cake (32.3 parts). The concentrate mixture of G2 and G3 was consisted of barley grain (55 parts), wheat grain (10 parts), mustard cake (33 parts) and mineral mixture (2 parts). Experimental mineral mixture supplemented in G2 and G3 treatment groups were formulated on the basis of Cu concentration in the plasma samples of dairy animals which was observed to be below critical levels. Therefore, two times more copper sulphate was incorporated in G3 mineral mixture than G2 mineral mixture to see the effect of two level of copper sulphate on certain parameters. Mineral mixtures were prepared by using dicalcium phosphate, calcium carbonate, salt, iron sulphate, copper sulphate, zinc sulphate, cobalt chloride, manganese chloride, magnesium sulphate and potassium iodide and their percent incorporation is presented as footnote in Table 1.

2.2. Sampling and observations

During the feeding trial, feed intake was recorded daily and body weight at fortnightly before feeding and watering in the morning. Feed conversion ratio was calculated as kg feed per kg body weight gain. Blood samples were collected at fortnightly interval in heparinised vacutainer tube posing minimum stress to animals. Plasma was separated by centrifuging blood at 3000 rpm at 4ºC for 20 minute. Separated plasma was stored at -20 ºC till further analysed.

2.3. Samples analysis

Samples of the feed offered and ort left were collected daily in polyethylene sachets and pooled at fortnightly intervals. DM, CP, EE, CF and total ash was determined as per AOAC (1995). NDF and ADF were analyzed according to the methods described by Van Soest et al. (1991). Mineral content except phosphorus in feeds, ort left and faeces were estimated by Atomic Absorption Spectrophotometer (AAS 400; Perkin Elmer, USA). Phosphorus content of feeds and residue was analysed by AOAC (1995). Alanine amino transferases (ALT), aspartate amino transferase (AST) were analyzed by auto-analyzer (Mindray) using commercial kits (Span Diagnostics Ltd, Surat, India). Plasma total immunoglobulins were estimated by zinc turbidity method (McEwan and Fisher, 1970). Total antioxidant activity was measured by FRAP assay of Benzie and Strain (1999). FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing easily reduced oxidant system present in stoichimetric excess.

2.4. Statistical analysis

Data on feed intake, body weight and plasma parameters were analyzed using the MIXED model with repeated measures using statistical software SPSS version 19 (SPSS Inc., Chicago, IL, USA). The statistical model was used to estimate sampling period effect, treatment group and their interaction:

$Yijk = \mu + Ti + Dj + (T\times D)ij + eijk$

Where, Yijk = dependent variable

μ = overall mean of the population

Ti = mean effect of the treatment group.

Dj = mean effect of day of sampling (j = 0, 15, 30, 45 and 60th days) with day as a repeated factor

$(T\times D)ij$ = effect of the interaction between treatment and period of sampling

eijk = unexplained residual element assumed to be independent and normally distributed.

The pair-wise comparison of homogenous subset was done by using 'Tukey's honestly significant difference (HSD) test

3. Results and discussion

Ingredient and nutrient composition of basal diets supplemented with or without mineral mixture is presented in Table 1. Concentrate mixture, berseem and wheat straw were fed to heifers in proportion of 40: 35: 25 respectively. The nutrient composition of feed ingredients was within range reported in earlier studies (Amrutkar et al., 2012; Vaswani et al., 2012; Tiwary et al., 2010).

3.1. Nutrient intake

The basal diets provided to three groups were provided sufficient nutrients as per NRC (2001) requirement. DCP and TDN intakes of heifers range from 434.4-486.9 g/day and 3.06-3.24 kg/day, respectively. Similarly, basal diet mineral content was marginally above requirement in Ca, P, Zn, Mn and excess of Fe; however, it was marginally deficient in Cu. NRC (2001) requirement of Cu is 10 mg/kg of DM and in present study the level of Cu was 9.8 mg/kg of DM in non supplemented group (G1), 50.11 and 90.43 mg/kg in mineral supplemented groups G2 and G3, respectively. Total DM intake was 4.88, 4.98 and 4.99 kg/day and as % of body weight it was 3.14, 3.16 and 3.15 kg in group G1, G2 and G3, respectively. Similarly, feed conversion ratio was 10.37, 9.31 and 7.52 (kg feed/kg gain) and daily weight gain was 464.6, 552.8 and 613.6 g in group G1, G2 and G3, respectively (Table 2). Total DM intake, daily gain and feed conversion ratio (FCR) were similar ($P>0.05$) among three groups. The results of present finding on DM, daily gain and FCR were in agreement to earlier studies on heifers supplemented with minerals (Saxena et al., 2010; Sharma et al., 2011).

Table 1
Ingredient and nutrient composition of experimental diets fed to heifers.

Attribute	Treatments1		
	G1	G2	G3
	Ingredients (%)		
Wheat straw	25.0	25.0	25.0
Berseem	35.0	35.0	35.0
Barley grain	22.4	22.0	22.0
Wheat grain	4.68	4.0	4.0
Mustard oil cake	12.92	13.2	13.2
Mineral mixture	-	0.802	0.803
Composition (%)4			
Dry Matter	63.47	63.42	63.43
Organic Matter	90.56	90.44	90.41
Ether Extract	3.00	2.77	2.63
Crude Protein	14.44	14.61	14.61
Total Ash	9.44	9.56	9.59
Nitrogen Free Extract	56.13	56.04	56.13
NDF	46.26	46.11	46.24
ADF	25.71	25.67	25.65
Hemicellulose	20.55	20.45	20.59
ADL	11.23	11.28	11.30
Cellulose	14.48	14.38	14.35
Ca	0.56	0.73	0.72
P	0.44	0.49	0.49
Na	0.04	0.15	0.15
Cu (mg kg-1)	9.84	50.11	90.43
Zn (mg kg-1)	42.34	60.46	58.94
Fe (mg kg-1)	223.97	264.37	263.45
Mn (mg kg-1)	49.65	59.96	60.12

[1]Treatment: G1= Basal ration; G2 = Basal ration with 0.8% mineral mixute2; G3 = Basal ration with 0.8% mineral mixture3
[2] Contained CaHPO4 (40%); CaCO3 (30%); NaCl (22%); FeSO4 (2.5%); CuSO4 (2.1%); ZnSO4 (0.75%); CoCl2 (0.05%); MnCl2 (0.6%); MgSO4 (2.0%); KI (0.01%).
[3]Contained CuSO4 (4.2%); CaHPO4 (37.9%) beside all other salts same as in G2.
[4]Calculted values.

3.2. Liver transaminase activity

Plasma AST and ALT concentrations are the indicators of hepatic function (Forsyth et al., 1999). Mean levels of AST were 101.98, 95.38 and 94.32 U/L in G1, G2 and G3, respectively. Similarly, ALT levels were 27.14, 29.23 and 27.10 U/L in G1, G2 and G3, respectively (Table 3). The mean plasma liver transaminase activity (AST and ALT) were similar (P>0.05) in three groups and there was no effect (P>0.05) of supplementation over period of time (Figure 1-2). This indicates that there was no liver accumulation of Cu at toxic level as ALT activity is an excellent predictive indicators of hepatic copper accumulation in cattle (Minervino et al., 2008). Levels of livers transaminase were within range reported by authors in Zebu cattle (Mahima et al., 2013, Bhan et al., 2012; Sharma et al., 2011; Mondal et al., 2009).

Table 2
Average body weight gain, dry matter consumption and feed conversion ratio in heifers.

Attribute	Treatments1			SEM	P-value
	G1	G2	G3		
Body weight (kg)					
Initial	145.79	146.08	145.38	7.20	0.99
Final	176.67	179.25	182.20	7.86	0.91
Total gain	27.87	33.17	36.82	2.13	0.23
Average daily gain (g/day)	464.58	552.78	613.61	35.51	0.23
Dry matter intake					
kg day-1	4.88	4.98	4.99	0.83	0.18
% body weight	3.14	3.16	3.15	0.49	0.70
g/kg W0.75	110.19	111.37	110.94	0.75	0.28
Feed conversion ratio	10.37	9.31	7.52	0.73	0.29

1Trt: G1= Basal ration (9.8 mg Cu/kg DM); G2 (50.1 mg Cu/kg DM) and G3 = Basal ration (90.4 mg Cu/kg DM) with 0.8% mineral mixture (see Table 1), SEM: standard error of mean.

3.3. Total immunoglobulin (TIg)

Total plasma immunoglobulin level was in 25.40, 26.52 and 26.66 (mg/ml) in three respective groups (Table 3). Plasma TIg level were similar (P>0.05) between the three groups. The average mean of TIg during first fortnightly increased and remains static in mineral supplemented group (G2 and G3), whereas it showed a declining trend (P=0.06) in non supplemented group (G1) (Figure 3). This decline in TIg may be due to oxidative stress (Chatterjee et al., 2003). Cu-containing enzymes, ceruloplasmin, binds up to 95% of circulating Cu, regulates iron availability, takes part in oxidation-reduction reactions, and may regulate immune function (Healy and Tipton, 2007). The levels of TIg were within ranges reported for Zebu cattle (Chatterjee et al., 2003; Mutoni et al., 2012).

Table 3
Average plasma values of certain welfare parameters of heifers supplemented with different levels of copper containing mineral supplement.

Attribute	Treatments			SEM	P-value		
	G1	G2	G3		Trt1	Prd2	Trt × Prd
AST (IU/L)	101.98	95.38	94.32	1.88	0.08	0.24	0.54
ALT (IU/L)	27.14	29.23	27.10	0.61	0.97	0.07	0.27
TIg(mg/ml)	25.40	26.52	26.66	0.37	0.13	0.06	0.66
FRAP (µ mol/l)	1044.9	1038.2	1155.5	34.1	0.19	0.91	0.96

[1]Trt: G1= Basal ration (9.8 mg Cu/kg DM); G2 (50.1 mg Cu/kg DM) and G3 = Basal ration (90.4 mg Cu/kg DM) with 0.8% mineral mixture (see Table 1).
[2]Period of sampling: 0, 15, 30, 45 and 60 day of feeding trail.

3.4 Plasma total antioxidant activity (FRAP Assay)

Plasma total antioxidant activity (FRAP value) during the 60 days mineral supplementation averaged 1044.9, 1038.2, 1155.5 µ mol/l in group G1, G2 and G3, respectively. Overall plasma FRAP value in present study was similar (P>0.05) among three groups. A declining trend in total antioxidant activity was visible in non supplemented group (G1) over the period (Figure 4) indicating an improvement in antioxidant activity in mineral supplemented groups. FRAP value were within ranges reported in Zebu cattle (Chatterjee et al., 2003; Agarwal and Chandra, 2013)

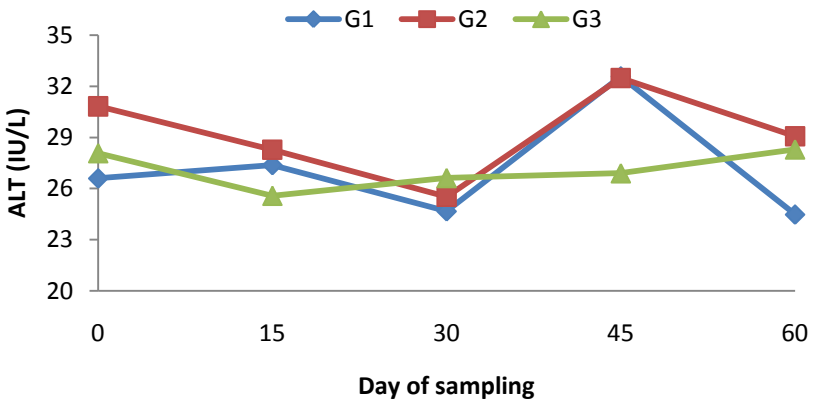

Fig. 1. Effect of different levels of copper containing mineral supplement on aspartate aminotransferase activity (IU/L) of heifers.

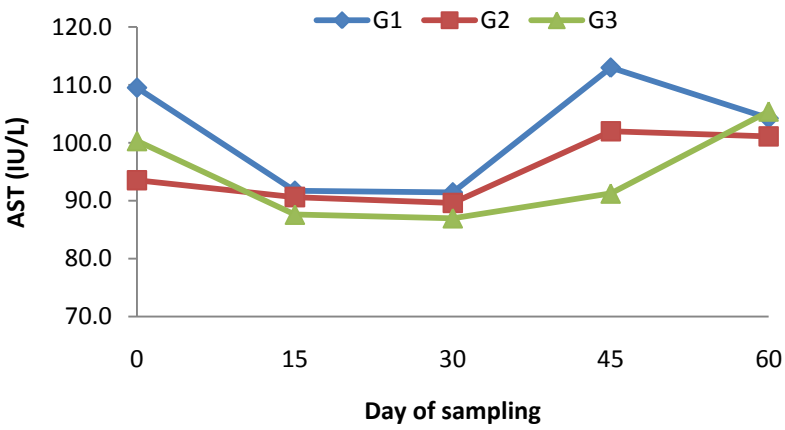

Fig. 2. Effect of different levels of copper containing mineral supplement on alanine amino transaminase activity (IU/L) of heifers

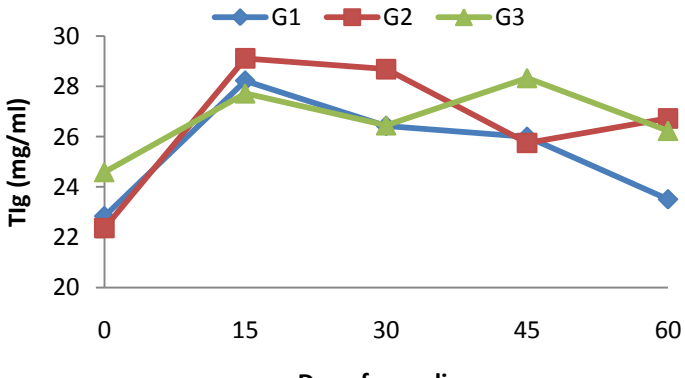

Fig. 3. Effect of different levels of copper containing mineral

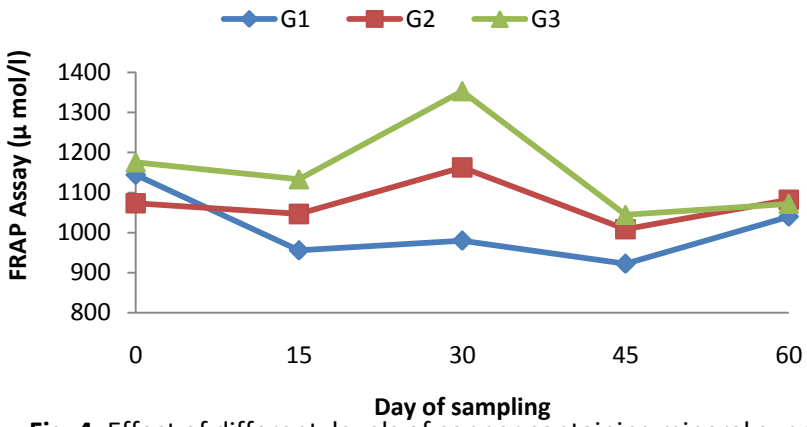

Fig. 4. Effect of different levels of copper containing mineral supplement on ferric reducing antioxidant power assay (µ mol/l) in plasma of heifers

4. Discussion

Supplementation of Cu containing mineral mixture above (50 or 90 mg Cu/kg DM) physiological requirement did not adversely affected liver transaminase activity, immune status and antioxidant status. Thus a Cu level (50 ppm) higher than recommended (10 ppm) may be supplemented in the diet of growing animals for improving health and productivity.

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