



Effect of different levels and sources of copper supplementation on blood biochemical constituents and copper status in lambs

P SENTHILKUMAR¹, D NAGALAKSHMI² and Y RAMANA REDDY³

Sri Venkateswara Veterinary University, Rajendranagar, Hyderabad, Andhra Pradesh 500 030 India

Received: 7 June 2011; Accepted: 23 October 2011

Key words: Biochemical, Copper, Haematological, Lambs

The NRC (1985) gave the Cu requirement for sheep as 7–11 mg/kg DM, while Underwood and Suttle (1999) estimated Cu requirements that varied between 4.3–28.4 mg/kg DM. Similarly, Senthilkumar *et al.* (2009) reported higher requirements of Cu for immune response in lambs. The variations in copper requirements at various stress condition, relationships between dietary copper source and level, related enzyme activities, growth performance, nutrient digestibility and immune function recently have attracted considerable interest among scientists (Arthington 2005, Solaiman *et al.* 2007). But the information regarding the influence of copper supplementation at different dietary concentrations and from organic and inorganic source on blood parameter in sheep is limited, in spite of their economic importance. Hence, in this study an attempt was made to determine the possible effect of copper supplementation at different levels, from two different sources, on hematological parameters, blood biochemical constituents and Cu status in lambs.

Animal and feeding management: Male Nellore Brown lambs (30), 3–5 months old; weighing 15.5±0.06 kg, housed indoors were randomly allotted to 5 dietary groups in a completely randomized design. A basal diet (BD) was prepared from the locally available feed ingredients (sorghum straw 40, maize 45, molasses 6, groundnut cake 5, limestone powder 1.4, di-calcium phosphate 1.3, urea 1, trace mineral and vitamin mixture 0.06 and salt 1%) met the nutrient requirements (NRC 1985) of lambs except for Cu. The feed grade CuSO₄·5H₂O (minimum assay 21.1%) was the inorganic source and copper proteinate (minimum assay 10%) was the organic source of Cu used in the present study and were added in the experimental diets to supply Cu at 7 and 14 mg/kg from these sources. The Cu content in the BD was

7.38 mg/kg DM of diet. The animals of group-T1 (basal) were offered BD (no Cu supplementation), group T2 and T3 were offered BD supplemented with 7 mg/kg Cu from CuSO₄·5H₂O and Cu-proteinate, respectively and group T4 and T5 were offered BD supplemented with 14 mg/kg Cu from inorganic and organic source, respectively for a period of 180 days.

Sample collection: Blood samples were collected from jugular vein into heparinized and non heparinized vacutainer tubes from each lamb at 0-, 90- and 180-days of feeding for haematological and biochemical constituents, respectively. Collected blood in non heparinized tubes was then centrifuged at 1,100 × g for 10 min to obtain serum and stored at –20°C.

Analytical procedures: Serum Cu concentration was estimated by AAS (Arenza *et al.* 1977). After 180 days of feeding, 4 representative animals from each dietary treatment were slaughtered and about 100g of liver from central lobe was collected for analysis of Cu content (AOAC 1998). The blood haemoglobin (Hb) (Cannan 1958), total erythrocyte count (TEC) (Coles 1986) and total leukocyte count (TLC) (Schalm *et al.* 1986) were estimated after each collection immediately. Serum samples were analyzed for cholesterol (Wybenga and Pileggi 1970), glucose (Cooper and McDaniel 1970), urea nitrogen (Rahmatulla and Boyde 1980), total protein (Reinhold 1953), albumin (Gustafsson 1976) and globulin concentration was calculated by subtracting the albumin from total protein.

Statistical analysis: Data was analyzed by the GLM procedure of SPSS (1997) for a completely randomized design with level and source of Cu as the variables. The differences among means were determined using preplanned single degree of freedom contrasts. The single degree of freedom contrasts statements were as follows: (1) control vs Cu, (2) 7 mg/kg vs 14 mg/kg, (3) 7 mg/kg Cu from CuSO₄ vs 7 mg/kg Cu from Cu-proteinate, (4) 14 Cu from CuSO₄ vs 14 mg/kg Cu-proteinate. The means were separated by Duncan's multiple range tests at 5% level (Duncan 1955).

Present address: ¹Veterinary Assistant Surgeon (drpskumar 2003@yahoo.com), Veterinary dispensary, Chettikulam, Perambalur, Tamil Nadu 621 104.

^{2,3}Associate Professor (²dnlakshmi@rediffmail.com, ³ramanayr19@yahoo.co.in), Department of Animal Nutrition, College of Veterinary Science.

Hematological profile: Supplementation of Cu had significant effect on blood hemoglobin level whereas the TEC and TLC were not affected by supplemental Cu (Table 1). On days 90 ($P<0.05$) and 180 ($P<0.01$) of feeding trial, lambs supplemented with Cu had significantly higher Hb level than control. Lambs supplemented with 14 mg/kg Cu had higher ($P<0.05$) Hb level than 7 mg/kg Cu supplemented lambs on day 90. The 14 mg/kg Cu supplementation from Cu-proteinate significantly ($P<0.01$) decreased Hb level whereas at 7 mg/kg supplementation, the Cu-proteinate significantly ($P<0.01$) improved the Hb concentration. The Hb concentration in lambs fed diets varying in Cu concentration throughout the feeding trial was within the normal range of 9–14 (g/dl) (Kaneko 1980). The increased Hb level in this study might be due to increased absorption of Fe from gut at supplementation levels of 14mg/kg and the difference in effect from various sources of Cu supplementation was due to variation in its bioavailability (Eckert *et al.* 1999) and also due to the age, breed and Cu status of the animals. The physiological advantage afforded by organic Cu compounds might be due to the unique coordination chemistry of Cu, which permits the formation of highly soluble, chemically stable products that resist interaction with antagonists in the gut (Brown and Zeringue 1994). The ceruloplasmin is required for Fe metabolism, transport of Cu and regulation of certain amines and its concentration was higher in Cu supplemented lambs (Senthilkumar *et al.* 2009), correlated with increased Hb level in the present study. In contrast, no effect on Hb was observed in kids fed either 100 or 200 mg Cu/d (Solaiman *et al.* 2007) or 20 or 40 mg Cu/kg DM (Dorton *et al.* 2003). Eckert *et al.* (1999) reported supplementation of Cu at 30 mg/kg tended to decrease Hb concentration due to production of haemolytic anaemia as a result of high levels of Cu. But in the present study no such depression on Hb concentration was observed, indicating no toxic effect of feeding 14 mg/kg supplemented Cu from either organic or inorganic source even when fed up to 180 days. Datta *et al.* (2007) observed no effect on TEC and TLC throughout the experimental period of 84 days in kids fed 20 or 40 ppm supplemental Cu from CuSO_4 or Cu-proteinate corroborating with the present findings.

Biochemical profile: Influence of supplemental Cu at different concentration and from different sources was not significant with regard to serum glucose, but the cholesterol and total protein levels in serum were altered in this study (Table 1). Similarly, the blood glucose levels were not affected in kids on Cu supplementation at 10 (Mondal *et al.* 2004), 20 or 40 mg/kg diet (from CuSO_4 or Cu-proteinate) (Datta *et al.* 2007). Lambs supplemented with 14 mg/kg Cu from Cu-proteinate had lower cholesterol level compared to CuSO_4 (Table 1). Within each supplemental level, the cholesterol concentration was lower in those fed Cu-proteinate in comparison to CuSO_4 . The cholesterol concentration increased in BD fed lambs from 0 to 90 and

further to 180 days. Whereas in Cu supplemented groups, the serum cholesterol level gradually reduced from 0 to 180 days. The hypercholesterolemia observed on low Cu diets might be due to increase in the activity of 3-hydroxy-3-methyl glutaryl CoA (HMG-CoA) reductase, the rate limiting enzyme in cholesterol synthesis (Kim *et al.* 1992). High concentration of liver Cu regulates cholesterol biosynthesis indirectly by decreasing the reduced form of glutathione (GSH) and increases the oxidized form of glutathione (GSSH) (Kim *et al.* 1992). Higher cellular GSSH concentration has been shown to reduce the activity of HMG CoA reductase (Gilbert 1990), thus reducing the carbon flux through the mevalonate pathway thereby decreasing cholesterol synthesis. In the present study, the higher serum and liver Cu concentration in Cu supplemented lambs was correlated with the reduced serum cholesterol. Such significant increase in hepatic Cu accumulation with consistent decrease in serum total cholesterol was observed by Mondal *et al.* (2007) and Datta *et al.* (2007) in kids and by Engle *et al.* (2000) in steers fed 20 or 40 mg supplemental Cu/kg DM.

Copper supplementation did not influence the serum protein concentration up to 90 days of feeding and on day 180, lambs supplemented with Cu had significantly ($P<0.01$) higher serum protein compared to BD but no dose or source effect was observed (Table 1). At 90 days of feeding the Cu supplemented lambs had higher ($P<0.01$) albumin and lower ($P<0.01$) globulin levels compared to BD fed lambs which might be due to increased ceruloplasmin activity in response to inflammation associated to antigen (20% chicken RBC), administered to estimate immune response on day 90 of feeding trial (Senthilkumar *et al.* 2009). In contrast, Cu supplementation did not have any significant effect on the serum protein, albumin, globulin and albumin- globulin ratio in kids (Mondal *et al.* 2004, Datta *et al.* 2007).

Copper status: The average Cu intake was 6.07, 11.67, 11.55, 17.49 and 17.06mg/d in lambs fed BD, BD supplemented with 7 and 14 mg/kg from inorganic and organic source, respectively resulting in higher Cu concentration in serum and liver in Cu supplemented lambs in dose dependent manner (Table 2). Lambs supplemented with 14 mg/kg Cu had higher ($P<0.01$) serum Cu compared to 7 mg/kg supplementation on 90 and 180d of feeding. On 90d of feeding trial, no source effect was observed at 7 mg/kg Cu supplementation, but at 14 mg/kg Cu supplementation, the CuSO_4 group showed increased serum Cu concentration compared with Cu-proteinate. Dorton *et al.* (2003) reported higher ($P<0.01$) serum Cu in steers with Cu supplementation and steers supplemented with 20 mg Cu/kg DM had higher ($P<0.01$) serum and liver Cu concentration than 10 mg Cu/kg DM. Steers supplemented with 20 mg Cu/kg DM from organic Cu had higher ($P<0.03$) plasma and liver Cu concentration than from CuSO_4 .

Table 1. Effect of dietary copper concentration and source on haemato-biochemical parameters in lambs

Attribute	Cu supplementation					SEM	P-value			
	Basal diet (T ₁)	7 mg/kg		14 mg/kg			Basal vs Cu	7mg/kg vs 14mg/kg	T ₂ vs T ₃	T ₄ vs T ₅
		Inorganic (T ₂)	Organic (T ₃)	Inorganic (T ₄)	Organic (T ₅)					
<i>Haemoglobin (g/dl)</i>										
0d	9.77	9.91	9.95	9.98	9.82	0.05	0.22	0.70	0.77	0.30
90d‡	10.20 ^d	10.39 ^{cd}	10.69 ^b	11.06 ^a	10.51 ^b	0.07	0.01	0.05	0.001	0.001
180d‡	10.83 ^b	11.33 ^b	12.86 ^a	12.80 ^a	10.93 ^b	0.22	0.03	0.66	0.001	0.001
<i>Total erythrocyte count (× 10⁶/mm³)</i>										
0d	9.19	9.31	9.15	9.19	9.31	0.08	0.90	0.88	0.56	0.66
90d	9.21	9.26	9.24	9.21	9.26	0.57	0.32	0.33	0.27	0.90
180d	9.21	9.47	9.46	9.47	9.48	0.05	0.02	0.96	0.94	0.95
<i>Total leucocyte count (× 10³/mm³)</i>										
0d	9.93	9.93	9.86	9.94	9.90	0.02	0.62	0.55	0.30	0.52
90d	9.73	9.58	9.69	9.94	10.0	0.12	0.79	0.08	0.76	0.86
180d	9.74	9.56	9.79	10.4	9.98	0.12	0.71	0.32	0.75	0.52
<i>Glucose (mg/dl)</i>										
0d	51.2	51.4	51.3	51.4	51.6	0.08	0.51	0.32	0.63	0.08
90d	51.4	51.5	51.5	51.6	51.4	0.07	0.30	0.11	0.78	0.12
180d	51.3	51.4	51.6	51.3	51.6	0.09	0.24	0.13	0.55	0.00
<i>Cholesterol (mg/dl)</i>										
0d	65.1	65.1	65.3	65.8	65.7	0.14	0.42	0.10	0.66	0.80
90d	67.1 ^d	62.1 ^c	61.3 ^c	58.3 ^b	56.9 ^a	0.07	0.001	0.001	0.03	0.001
180d†	70.3 ^e	60.5 ^d	58.7 ^c	57.0 ^b	52.7 ^a	1.09	0.001	0.001	0.001	0.001
<i>Urea nitrogen (mg/dl)</i>										
0d	14.4	14.2	14.3	14.4	14.3	0.10	0.74	0.29	0.34	0.61
90d	13.9 ^b	14.4 ^a	14.2 ^{ab}	14.3 ^a	13.9 ^b	0.13	0.07	0.20	0.40	0.02
180	14.06	14.34	14.19	14.27	14.36	0.106	0.07	0.61	0.33	0.60
<i>Total Protein (g/dl)</i>										
0d	6.17	6.15	6.10	6.11	6.13	0.02	0.25	0.91	0.7	0.62
90d	6.19	6.19	6.20	6.19	6.19	0.00	1.00	1.00	0.52	1.00
180d	6.51 ^b	6.58 ^a	6.60 ^a	6.60 ^a	6.61 ^a	0.01	0.001	0.27	0.23	0.44
<i>Albumin (g/dl)</i>										
0d	2.60	2.61	2.62	2.60	2.61	0.01	0.73	0.37	0.95	0.92
90d	2.56 ^b	2.65 ^{ab}	2.65 ^{ab}	2.69 ^a	2.69 ^a	0.02	0.002	0.00	0.97	0.94
180d	2.61 ^b	2.66 ^a	2.69 ^a	2.69 ^a	2.69 ^a	0.01	0.00	0.23	0.07	0.86
<i>Globulin (g/dl)</i>										
0d	3.57	3.54	3.48	3.50	3.53	0.20	0.23	0.86	0.43	0.70
90d	3.63 ^a	3.54 ^b	3.54 ^b	3.51 ^b	3.50 ^b	0.02	0.002	0.001	0.85	0.91
180d	3.91	3.92	3.91	3.91	3.92	0.01	0.77	0.94	0.70	0.70
<i>Albumin : Globulin</i>										
0d	0.73	0.74	0.75	0.74	0.74	0.01	0.46	0.47	0.78	1.00
90d	0.71 ^b	0.75 ^a	0.75 ^a	0.77 ^a	0.77 ^a	0.01	0.002	0.001	0.93	0.87
180d	0.67	0.68	0.69	0.69	0.69	0.00	0.03	0.51	0.18	0.73

abcd, means with different superscripts in a row differ significantly (P<0.05); ‡Level × source (P<0.01).

Liver and serum Cu concentration in BD fed lambs in the present study remained higher than the critical levels (< 20 mg of Cu/kg DM in liver and < 0.6 mg/L of Cu in serum, respectively) (McDowell et al. 1985). Also, Cu accumulation in liver was higher when Cu was supplemented as CuSO₄ compared to Cu-proteinate. In accordance with present findings, Mondal *et al.* (2004) observed increased liver Cu concentration in kids fed diets supplemented with Cu as

CuSO₄ compared to Cu-proteinate.

This study indicated that lambs fed basal diet containing 7.38 mg Cu/kg DM, were physiologically normal. The supplementation of Cu (7 or 14 mg/kg DM) to the basal diet improved the Hb and Cu status of the lambs and had no adverse effects on other haematological parameters and biochemical constituents. The serum cholesterol level reduced in 14 mg/kg Cu supplemented lambs and the effect

Table 2. Effect of dietary copper concentration and source on serum and liver copper concentration in lambs

Attribute		Cu supplementation				SEM	P-value			
Basal diet (T ₁)	7 mg/kg		14 mg/kg		Basal vs Cu		7mg/kg vs 14mg/kg	T ₂ vs T ₃	T ₄ vs T ₅	
	Inorganic (T ₂)	Organic (T ₃)	Inorganic (T ₄)	Organic (T ₅)						
<i>Serum copper (mg/dl)</i>										
0 days	0.15 ^{ab}	0.14 ^b	0.15 ^a	0.15 ^a	0.15 ^a	0.001	0.93	0.16	0.02	0.75
90 days	0.14 ^c	0.16 ^b	0.16 ^b	0.18 ^a	0.16 ^b	0.002	0.001	0.004	0.52	0.001
180 days	0.16 ^c	0.22 ^b	0.27 ^b	0.31 ^{ab}	0.37 ^a	0.02	0.002	0.01	0.24	0.10
<i>Liver copper (mg/kg DM)</i>										
180 days	190.1 ^d	250.3 ^b	215.4 ^c	291.4 ^a	250.9 ^b	7.958	0.001	0.002	0.001	0.001

abc, means with different superscripts in a row differ significantly (P<0.05).

was more when supplementation was in the form of Cu proteinate.

SUMMARY

An experiment was conducted on 3- to 5- month-old 30 male Nellore lambs (15.45 kg body weight) to assess the effect of the copper (Cu) supplementation in diet from inorganic (CuSO₄) and organic (Cu proteinate) sources on haematological, blood biochemical constituents and copper status. The total erythrocyte and leukocyte counts, serum glucose, globulin and urea nitrogen were not affected by level and source of Cu supplemented. The haemoglobin (Hb) content was higher at 7 than 14 mg Cu/kg supplementation. The serum cholesterol reduced with Cu supplementation and was dose and source related, lower in lambs fed 14 than 7 mg Cu/kg and reduction was higher in Cu-proteinate than CuSO₄. Serum total protein and albumin content was higher in Cu supplemented lambs at 180 days. Cu supplemented lambs had greater liver and serum Cu concentration. The Cu accumulation in liver was greater at 14 mg/kg supplementation from CuSO₄. These results indicated that Cu supplementation enhanced the Hb synthesis and Cu status of the lambs, decreased serum cholesterol and had no effect on other haematological parameters and biochemical constituents.

REFERENCES

- AOAC. 1998. *Official Methods of Analysis*. 16th edn. Association of Official Analytical Chemists, Gaithersburg, M D.
- Arenza J S, Hathi S D, Singh B and Verma P N. 1977. Status of some microminerals in neonatal buffalo calves and their mothers. *Indian Journal of Dairy Science* **30**: 255.
- Arthington J D. 2005. Effects of copper oxide bolus administration or high-level copper supplementation on forage utilization and copper status in beef cattle. *Journal of Animal Science* **83**: 2894–2900.
- Brown T F and Zeringue L K. 1994. Laboratory evaluations of solubility and structural integrity of complexed and chelated trace mineral supplements. *Journal of Dairy Science* **77**: 181–89.
- Cannan R K. 1958. Clinical chemistry 4: 246–251. C F. *Text book of Clinical Practical Biochemistry*. Vol. I, 5th edn, pp 479–80. (Ed.) Varley, H. 1991. CBS publisher and Distributors.
- Coles E H. 1986. *Veterinary Clinical Pathology*. 4th edn, p. 40. W B Saunders Company, a division of Harcourt Brace and Company, Philadelphia.
- Cooper G R and Mc Daniel. 1970. *Standard Methods of Clinical Chemistry*. p. 159. (Ed.) Seligson D. Academic Press, New York.
- Datta C, Mondal M K and Biswas P. 2007. Influence of dietary inorganic and organic form of copper salt on performance, plasma lipids and nutrient utilization of Black Bengal (*Capra hircus*) kids. *Animal Feed Science and Technology* **135**: 191–209.
- Dorton K C, Engle T E, Hamar D W, Siciliano P D and Yemm R S. 2003. Effects of copper source and concentration on copper status and immune function in growing and finishing steers. *Animal Feed Science and Technology* **110**: 31–44.
- Duncan D B. 1955. Multiple range and multiple F tests. *Biometrics* **11**: 1–42.
- Eckert G E, Gren L W, Carstens G E, Ramsey W S. 1999. Utilization of copper in copper proteinate, copper lysine and cupric sulfate or copper proteinate. *Journal of Animal Science* **77**: 224–49.
- Engle T E, Spears J W, Armstrong T A, Write C L and Odle J. 2000. Effects of dietary copper source and concentration on carcass characteristics and lipid on cholesterol metabolism in growing and finishing steers. *Journal of Animal Science* **78**: 1053–59.
- Gilbert H F. 1990. Molecular and Cellular aspects of thiol-disulfide exchange. *Advance Enzymology* **63**: 169–72.
- Gustafsson E J. 1976. Improved specificity of serum albumin determination and estimation of acute phase of reactants by use of the bromocresol green. *Quinchemistry* **22**: 616–22.
- Kaneko J J. 1980. *Clinical Biochemistry of Domestic Animals*. 3rd edn. Academic Press. Inc. Orlando, Florida 32887.
- Kim S, Chao P Y and Allen G D. 1992. Inhibition of elevated hepatic glutathione abolishes copper deficiency cholesterolemia. *FASEB* **6**: 2467–71.
- Mc Dowell L R. 1985. Nutrition of grazing ruminants in warm climates. p. 216. (Ed) Smith A J. University of Edinburg Press, Edinburg, Scotland.
- Mondal M K, Roy B and Biswas P. 2004. Effect of supplementation of copper on nutrient utilization by Black Bengal kids. *Indian Journal of Animal Nutrition* **21**: 261–64.
- Mondal M K, Biswas P, Roy B and Mazumdar D. 2007. Effect of

- copper sources and levels on serum lipid profiles in Black Bengal (*Capra hircus*) kids. *Small Ruminant Research* **67**: 28–35.
- Nockels C F, Debonis J and Torrent J. 1993. Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. *Journal of Animal Science* **73**: 2539–45.
- NRC 1985. *Nutrition Requirements of Sheep*. 6th edn. National Academy Press. Washington, DC.
- Rahmatullah M and Boyde T R C. 1980. Improvement in the determination of urea using diacetylmonoxime with or without deproteinization. *Clinical Chemistry. Acta* **107**: 3–9.
- Reinhold J G. 1953. *Standard Methods of Clinical Chemistry*. p. 88. (Ed.) Rynner, M. Academic Press, New York.
- Schalm, O W, Jain, N C and Carrol E J. 1986. *Veterinary Haematology*. 4th edn, P.15. Lea and Febiger, Philadelphia.
- Senthilkumar P, Nagalakshmi D, Ramana Reddy Y and Sudhakar K. 2009. Effect of different level and source of copper supplementation on immune response and copper dependent enzyme activity in lambs. *Tropical Animal Health and Production* **41**: 645–53.
- Solaiman S G, Craig Jr T J, Reddy G and Shoemaker C E. 2007. Effect of high levels of Cu supplement on growth performance, rumen fermentation and immune response in goat kids. *Small Ruminant Research* **69**: 115–23.
- SPSS 1997. Statistical Package for Social Sciences, Base Applications Guide 7.5. SPSS, Chicago, USA
- Underwood E J and Suttle N F. 1999. *The Mineral Nutrition of Livestock*. 3rd edn. CAB International Publishing, U.K.
- Wybenga D R and Pileggi V J. 1970. *Clinical Chemistry* **16**, 980.