

Effect of dose and source of copper supplementation on antioxidant status, humoral immune response and carcass characteristics of lambs

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ABSTRACT

An experiment was conducted on 30 male Nellore lambs with average body weight (15.45 ± 0.06 kg) to determine the level of the copper (Cu) supplementation in diet from inorganic and organic sources for higher immunity, carcass production and antioxidant activity by allotting them randomly to 5 groups in completely randomized design. The dietary treatments were, viz. basal diet (BD, no supplemental Cu), BD supplemented with 7 ppm Cu as copper sulphate (CuSO_4) and Cu proteinate, and BD supplemented with 14 ppm Cu from CuSO_4 and Cu proteinate. The lambs were fed the respective diet at 3.5% of body weight to meet the ICAR requirements except Cu throughout the feeding trial of 180 days. The BD (DM basis) contained 7.38 ppm Cu. The oxidative enzymes i.e. lipid peroxidase (LPx), RBC catalase, RBC superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity were estimated at 90 days of feeding trial and the lambs were sensitized with *Brucella abortus* antigen at 90th day to estimate humoral immune response on 0, 7, 14, 21, 28 and 35 days of post sensitization. At the end of 180 days feeding, 4 lambs from each treatment were slaughtered to study the carcass characteristics. The LPx activity was lower in Cu supplemented lambs and the RBC SOD and catalase activities were higher in Cu-proteinate supplemented lambs at both levels. The antibody titres were higher on 14 ppm Cu supplemented lambs compared to BD lambs. No effect of Cu supplementation was observed on carcass characteristics. This result indicated that supplementation of 14 ppm Cu to a basal diet containing 7.38 ppm Cu increased the oxidative enzyme activities and humoral immune response while Cu supplementation did not influence the carcass traits.

Key words: Carcass traits, Cu-proteinate, Cu sulphate, Immunity, Lambs, Oxidative enzymes

Copper plays important biological roles in animals through several copper dependent enzymes such as superoxide dismutase (Turnlund *et al.* 2004) playing an important role in combating oxidative stress. Copper deficiency has also been associated with reduced immune function and increased susceptibility to diseases (Stable and Spears 1990). Kincaid *et al.* (1986) reported that Cu proteinate was more available than copper sulphate in growing calves, and greater ceruloplasmin activity was observed in ewes fed copper from copper proteinate than copper sulphate (Eckert *et al.* 1999). Sheep is an important source of animal protein after poultry in our country. There is need to maximize the profits in sheep rearing by minimizing the losses from diseases by improving the disease

resistant capacity and antioxidant defense mechanisms of the lambs. Thus, the study was undertaken to understand the effect of level and source of copper supplementation in lambs on antioxidant status, humoral immune response and carcass traits in lambs.

MATERIALS AND METHODS

Animals, feed and management: Nellore Brown lambs (30; 15.5 ± 0.06 kg) were dewormed and randomly allotted into 5 groups of 6 each in a completely randomized design. A basal diet (BD) was prepared from the locally available feed ingredients that were mostly low in Cu to meet nutrient requirements (ICAR 1998) of lambs except Cu. The ingredient composition and chemical composition in the BD is presented in Table 1. The feed grade $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was the inorganic source and copper proteinate was the organic source of Cu used in the present study. The appropriate amounts of Cu supplements were added to the basal diet to supply Cu at 7 and 14 ppm from these sources. The animals of group-1 (basal) were offered BD (no Cu supplementation), group 2 and 3 were offered BD supplemented with 7 ppm

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Table 1. Ingredient and chemical composition (% DM basis) of basal diet

Ingredient	Composition (%)
Sorghum straw	40
Maize	45
Groundnut cake	5
Molasses	6
Urea	1
Limestone powder	1.4
Dicalcium phosphate	1.3
Salt	1
Trace mineral and vitamin mixture*	0.06
<i>Chemical composition</i>	
Dry matter	98.50
Crude protein	13.72
Neutral detergent fibre	55.20
Acid detergent fibre	24.75
Calcium	0.99
Phosphorus	0.37
Copper (ppm)	7.38
Molybdenum (ppm)	0.62
Sulphur (ppm)	0.15

*Trace mineral premix provided (mg/kg diet): iron, 30; manganese, 40; zinc, 30; cobalt, 0.2; molybdenum, 0.5; iodine, 0.25; selenium, 0.2. Vitamin A and E were provided to supply 940 IU and 210 IU per kg diet, respectively.

Cu from $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and Cu-protein, respectively and group 4 and 5 were offered BD supplemented with 14 ppm Cu from the above inorganic and organic source, respectively. Lambs were fed the respective diets at 3.5% of their body weight (BW) for 180 days of experimental feeding. Animals were housed indoor with individual feeding and watering facility, fed 3-times a day and fresh drinking water was made available at all times, dewormed at regular intervals and standard vaccination schedule was followed throughout the study.

Enzyme assays: The oxidative enzymes, viz. RBC catalase, lipid peroxidase, glutathione peroxidase and RBC superoxide dismutase activities were estimated in haemolysate. The blood was collected after 90 days of experiment in a clean sterilized vial containing ethylene diamine tetraacetate @ 1 mg/ml and centrifuged at 1 500 rpm for 10 min and the red blood cells (RBC) were washed with phosphate buffer saline (PBS) for 3-times and then 1 ml of RBC was added to 4 ml of distilled water to prepare 5% erythrocyte lysate.

The lipid peroxidase (LPx) activity was estimated as per Placer *et al.* (1966) and expressed as nmol MDA/mg protein. The glutathione peroxidase (GSH-Px) activity was determined by kinetic method (Paglia and Valantine 1967) and the enzyme activity was reported as IU/mg protein. The RBC superoxide dismutase (SOD) and catalase activities were determined as per McCord and Fridovich (1969) and

Bergmeyer (1983), and the activities were expressed as $\mu\text{mole/min/g Hb}$ and units/mg Hb , respectively. The Hb in haemolysate was estimated colorimetrically by using Drabkin's solution according to the procedure described by Cannan (1958).

Humoral immune response: The effect of the dietary treatments on the humoral immunity of lambs was assessed using heat killed *Brucella abortus* S₉₉ (IVRI, India). At 90d of experimental feeding, all lambs were sensitized with heat killed *Brucella abortus* S₉₉, administered intramuscularly as per the BW and a booster dose of antigen was given after 15 days. Prior to administration of antigen, lambs were screened for brucellosis with the help of Rose Bengal plate test (RBPT) according to Alton *et al.* (1975). Serum was collected from sensitized lambs on 0, 7, 14, 21, 28 and 35th day of post sensitization (PS) to assess humoral immune response by indirect ELISA (Perlman and Engvall 1971). The dose of autoclaved *B. abortus* S₉₉ was determined by Lowry's method (Lowry *et al.* 1951). The dose of the above antigen was determined by checkerboard analysis. Accordingly, the protein concentration of antigen was fixed at 40 $\mu\text{g/ml}$ and serum samples were diluted to 1: 400. The optical density and hence the seroreactivity was measured at 492 nm in ELISA reader.

Carcass traits: Four representative lambs from each group were slaughtered at the end of 180 days of experimental feeding. The preweighed lambs were fasted overnight with a free access to water and were slaughtered the next day by halal method after noting the overnight fasted weight. After skinning and evisceration, the weight of hot carcass was recorded. Weight recorded after deducting gut fill from preslaughter weight was noted as empty body weight. Carcass was divided into 5 major primal cuts namely leg, loin, rack, breast and foreshank, and neck and shoulder. Each cut weight was recorded and expressed as percentage of carcass weight. The weight of hot carcass was expressed as percentage of preslaughter weight as well as empty body weight to arrive at dressing percentage. The weight of liver, heart, kidney, lungs with trachea and gastrointestinal tract (GIT) (full and empty) were expressed as percentages of preslaughter weight. The weight of edibles included the weight of carcass, liver, heart, kidneys, testes, dressed head and feet and was expressed as percent of preslaughter weight. The weight of inedible offals (blood, empty GIT, skin, spleen and lungs with trachea) was recorded. The cut wise separated bone, meat and fat of each animal were weighed and expressed as percentage of respective cuts. Loin eye area (cm^2) was determined by tracing the cut section of *Longissimus dorsi* (LD) muscle between the 10th and 11th rib on butter paper and measuring with the help of graph paper partitioned to 1 mm.

Statistical analysis: The data were subjected to the test of significance with each lamb as a replicate in a completely randomized design with Cu level and source as the 2 factors

of variation (Snedecor and Cochran 1980). The means were compared using Duncan's multiple range test (Duncan's 1955).

RESULTS AND DISCUSSION

Oxidative enzymes: Copper supplementation influenced the oxidative enzyme activities in lambs (Table 2). Administration of *B. abortus* antigen at 90d of feeding might have caused stress to the lambs as evident from the lipid peroxidase activity. Lipid peroxidation is the best marker of oxidative stress (Saygili *et al.* 2003), its concentration increasing during stress condition. The length of propagation of lipid peroxidation depends upon chain breaking antioxidants such as the enzyme RBC catalase and SOD and GSH-Px (Diniz *et al.* 2003). The superoxides formed during lipid peroxidation are scavenged by SOD by forming hydrogen peroxides. The peroxides formed by the SOD are detoxified by GSH-Px and catalase (Mates and Sanchez 1999). The LPx activity was lower ($P<0.01$) and GSH-Px activity was higher ($P<0.01$) in Cu supplemented lambs compared to BD fed lambs. This indicated that Cu supplemented lambs reacted better against stress induction than unsupplemented lambs in the present investigation. The GSH Px and RBC SOD activities were dose dependent, the activities increased as the level of Cu in diet increased. Similar results were observed in dairy steers in which, RBC SOD activity increased in steers fed diet supplemented with 20ppm Cu compared to copper deficient steers (Xin *et al.* 1991). The SOD activity had a increasing trend in steers

supplemented with 0, 10 and 20ppm of Cu, but the differences were not statistically significant in studies of Dorton *et al.* (2003). Higher LPx activity was observed in Cu-overloaded sheep (drenched with 10 ml $\text{CuSO}_4/\text{kg BW}$ for 5 day a week) (Sansinanea *et al.* 1997). Similarly higher LPx and lower RBC SOD activities were observed in rats fed 60ppm Cu supplemented rats than 10ppm Cu or unsupplemented rats (Galhardi *et al.* 2004). But such a trend was not observed in the present study even at higher (14ppm) level of Cu supplementation indicating that 14ppm of Cu supplementation did not cause imbalance of oxidant antioxidant system. The source of Cu supplementation had influence on RBC SOD and catalase activities, being higher in lambs fed organic source of Cu. The RBC catalase activity was higher ($P<0.01$) at Cu supplementation of 14ppm from either source and of 7ppm in the form of Cu proteinate.

Humoral immune response: The antibody titres against *B. abortus* as assessed by ELISA is given in Table 3. No difference was observed on antibody response on 0day due to variation in dietary Cu. Cu supplementation influenced the antibody titres on 7, 14, 21, 28 and 35 d post sensitization. The titres were higher on 14ppm Cu supplemented lambs compared to BD fed lambs on all days except 35d post sensitization. In comparison to BD fed lambs, the lambs on 7ppm Cu supplementation in form of Cu proteinate had higher ($P<0.01$) titres. Comparison among the sources at 14ppm Cu supplementation, titres were higher when supplemented as Cu proteinate than Cu sulphate on 14th and 28th day PS. Dorton *et al.* (2003) reported higher ($P<0.04$)

Table 2. Oxidative enzyme activities in lambs fed diets varying in copper concentration

Diet	Lipid peroxidase (nmole MDA/mg protein)	RBC catalase (U/mole/min/g Hb)	RBC superoxide dismutase (Units/mg Hb)	Glutathione peroxidase (IU/mg protein)
BD ¹	33.42 ^a	8.71 ^d	0.23 ^c	3.59 ^c
7-I	18.83 ^b	9.31 ^{cd}	0.34 ^d	8.24 ^b
7-O	20.76 ^b	11.91 ^b	0.40 ^c	7.46 ^b
14-I	22.04 ^b	11.52 ^{bc}	0.52 ^b	9.37 ^{ab}
14-O	19.47 ^b	15.92 ^a	0.61 ^a	11.02 ^a
P	0.001	0.001	0.005	0.001
BD vs Cu ¹	0.001	0.014	0.001	0.001
Main factors				
Cu supplementation (ppm)				
0	33.42 ^a	8.71 ^b	0.23 ^c	3.59 ^c
7	19.80 ^b	10.61 ^b	0.37 ^b	7.85 ^b
14	20.75 ^b	13.72 ^a	0.57 ^a	10.20 ^a
P	0.001	0.001	0.001	0.001
Source				
Inorganic	20.44	10.42 ^b	0.43 ^b	8.81
Organic	20.11	13.92 ^a	0.51 ^a	9.24
P	0.872	0.003	0.001	0.661
SEM	1.336	0.578	0.026	0.558

^{abc}Means with different superscripts in a sub-column differ significantly: $P<0.05$; $P<0.01$.

¹BDVsCu- Basal diet vs Cu supplementation- P value; 7-I and 14-I – 7 and 14ppm Cu supplementation from CuSO_4 ; 7-O and 14-O- 7 and 14ppm Cu supplementation from Cu-proteinate.

Table 3. Humoral immune response against *B. abortus* in lambs fed diets varying in copper concentration

Diet Days PS ¹	ELISA (A ₄₉₂)					
	0	7	14	21	28	35
BD ²	0.096	0.176 ^c	0.284 ^d	0.468 ^c	1.01 ^c	0.734 ^b
7-I	0.083	0.206 ^{bc}	0.304 ^{cd}	0.560 ^b	1.102 ^{bc}	0.792 ^{ab}
7-O	0.095	0.239 ^a	0.350 ^{ab}	0.648 ^a	1.205 ^a	0.849 ^a
14-I	0.093	0.232 ^{ab}	0.333 ^{bc}	0.642 ^a	1.114 ^b	0.777 ^{ab}
14-O	0.101	0.243 ^a	0.390 ^a	0.652 ^a	1.281 ^a	0.827 ^a
P	0.268	0.001	0.001	0.001	0.001	0.072
BD ² vs Cu	0.633	0.001	0.006	0.001	0.001	0.024
Main factors						
<i>Cu supplementation (ppm)</i>						
0	0.096	0.176 ^b	0.284 ^b	0.468 ^b	1.013 ^b	0.734
7	0.089	0.222 ^a	0.327 ^a	0.604 ^a	1.154 ^a	0.821
14	0.096	0.237 ^a	0.362 ^a	0.650 ^a	1.197 ^a	0.802
P	0.338	0.001	0.004	0.001	0.003	0.068
<i>Source</i>						
Inorganic	0.088	0.219	0.319	0.601	1.108	0.785
Organic	0.098	0.241	0.370	0.650	1.243	0.838
P	0.103	0.048	0.005	0.043	0.001	0.079
SEM	0.003	0.006	0.009	0.015	0.002	0.002

^{abc}Means with different superscripts in a sub-column differ significantly: P<0.05; P<0.01.

¹Post sensitization; ²BD vs Cu- Basal diet Vs Cu supplementation- P value; 7-I and 14-I – 7 and 14ppm Cu supplementation from CuSO₄; 7-O and 14-O- 7 and 14ppm Cu supplementation from Cu-proteinate.

Table 4. Slaughter and carcass characteristics of lambs fed diets varying in copper concentration

	PSW	EBW	CW	DW %SW	LEA	Legs	Lion	Rack	B&FS	N&S	Edible %SW	NE %SW	Meat	Bone	Fat
BD ¹	26.60	25.67	13.65	51.13	25.25	33.69 ^a	11.94 ^a	12.22	14.44	27.36	63.94	22.89 ^{ab}	58.33 ^{ab}	27.22	11.85
7-I	26.83	25.88	12.37	46.22	25.31	30.52 ^b	10.75 ^{bc}	13.81	14.40	28.04	60.08	20.78 ^b	54.09 ^b	20.39	12.55
7-O	25.85	25.08	12.89	49.85	25.81	33.17 ^a	11.60 ^{ab}	12.34	14.38	27.77	62.45	20.18 ^b	59.79 ^a	29.34	10.85
14-I	25.63	24.85	12.94	50.80	22.65	34.00 ^a	9.69 ^c	13.87	13.02	22.88	63.08	21.36 ^b	58.08 ^{ab}	32.52	13.17
14-O	25.38	24.55	12.85	50.92	26.93	33.60 ^a	11.30 ^{ab}	12.01	15.54	26.85	63.79	25.18 ^a	62.16 ^a	28.69	8.88
P	0.914	0.937	0.812	0.053	0.746	0.019	0.003	0.206	0.466	0.101	0.465	0.037	0.010	0.055	0.519
BD vs Cu ¹	0.698	0.734	0.374	0.388	0.981	0.476	0.090	0.461	0.935	0.657	0.490	0.584	0.936	0.906	0.851
Main factors															
<i>Cu supplementation (ppm)</i>															
0	26.60	25.67	13.65	51.13	25.25	33.69 ^a	11.94 ^a	12.22	14.44	27.36	63.94	22.89	58.33	27.22	11.85
7	26.34	25.48	12.63	48.04	25.56	31.84 ^b	11.17 ^{ab}	13.07	14.39	27.91	61.27	20.48	56.94	24.86	11.70
14	25.50	24.70	12.89	50.86	24.79	33.80 ^a	10.49 ^b	12.94	14.28	24.87	63.43	23.27	60.12	30.60	11.03
P	0.709	0.742	0.504	0.067	0.935	0.049	0.043	0.655	0.989	0.124	0.284	0.079	0.200	0.174	0.913
<i>Source</i>															
Inorganic	26.23	25.37	12.65	48.51	23.98	32.26	10.22	13.84	13.71	25.46	61.58	21.07	56.08	26.45	12.86
Organic	25.61	24.81	12.87	50.38	26.37	33.38	11.45	12.17	14.96	27.31	63.12	22.68	60.98	29.01	9.87
P	0.690	0.713	0.789	0.315	0.437	0.349	0.030	0.041	0.331	0.399	0.495	0.380	0.024	0.556	0.245
SEM	0.670	0.650	0.391	0.762	1.190	0.472	0.260	0.412	0.510	0.846	0.890	0.712	0.941	1.661	1.004

^{abc}Means with different superscripts in a sub-column differ significantly: P<0.05; P<0.01.

¹BD vs Cu- Basal diet Vs Cu supplementation- P value; 7-I and 14-I – 7 and 14ppm Cu supplementation from CuSO₄; 7-O and 14-O- 7 and 14ppm Cu supplementation from Cu-proteinate.

antibody titer specific for ovalbumin (OVA) in steers supplemented with 20 mg Cu/kg DM than steers supplemented with 10 mg Cu/kg DM and steers supplemented with 20ppm Cu from organic source had

greater (P<0.03) antibody titre concentration than from CuSO₄. Supplementing 10ppm Cu to a semipurified diet (1.5ppm Cu) to calves increased the antibodies against *Pasteurella hemolytica* on d21 post sensitization compared

to unsupplemented lambs (Stable *et al.* 1993). The source of Cu in diet had significant influence on humoral immune response in the present study, being higher when supplemented from organic source corroborating with the findings of Dorton *et al.* (2003) in steers. While Salyer *et al.* (2004) found higher titres against OVA in heifers fed 10ppm dietary Cu as CuSO₄ in comparison to Cu polysaccharide.

Carcass characteristics: The carcass characteristics, yield of wholesale cuts, proportion of edible and non-edible and proportion of meat, bone and fat ratio is presented in Table 4. No effect of Cu supplementation was observed on most of the carcass characteristics. The dressing% among various groups varied from 46.22 to 51.13%. No dose (7 or 14 ppm Cu) or source effect (CuSO₄ or Cu-proteinate) was observed for any of the carcass traits, except weight of legs and loin. The percentage of lion was lower ($P<0.01$) in lambs fed CuSO₄ compared to those fed Cu proteinate and BD fed lambs and loin percentage of lambs fed Cu proteinate was similar to BD fed lambs. The weight of legs was comparable among various groups, except lower leg proportion in lambs fed 7ppm Cu as CuSO₄. The lower weight of leg and loin in lambs on 7ppm Cu supplementation from inorganic source resulted in lower ($P<0.01$) meat weight, compared to other groups. Copper supplementation at 10 or 40ppm to finishing diet containing 9.8ppm Cu had no effect on dressing percentage and weight of hot carcass of steers (Engle and Spears 2001). Similarly supplementation of 20ppm Cu from CuSO₄ or Cu proteinate had no effect on dressing percentage, but the hot carcass weight reduced ($P<0.05$) compared to unsupplemented lambs (Engle *et al.* 2000). Ward and Spears (1997) reported that long-term Cu supplementation to diets marginal in Cu increased *Longissimus muscle* area in steers. While, the loin eye area was unaffected due to Cu supplementation in the present study. Similarly, the *Longissimus muscle* area was not affected by the Cu supplementation (up to 40ppm as CuSO₄ or Cu proteinate) in steers in studies of Engle and Spears (2001).

The present study indicated that supplementation of 14ppm Cu to a basal diet with 7.38ppm Cu increased the activities of antioxidant enzymes and humoral immune response in lambs, preferably in the form to Cu proteinate compared to CuSO₄. The Cu supplementation (7 or 14ppm) from either source had no influence on carcass traits in lambs.

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