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Different Sources and Levels of Copper Supplementation on Performance and Nutrient Utilization of Castrated Black Bengal (*Capra hircus*) Kids Diet

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ABSTRACT : Twenty eight 3-4 month old castrated Black Bengal kids (*Capra hircus*) were used to determine the effects of source and level of dietary copper (Cu) concentration on their performance and nutrient utilization. Cu was supplemented (0, 10, 20 and 30 mg/kg diet DM) as copper sulfate ($CuSO_4$, $5H_2O$) or copper proteinate (Cu-P). Kids were fed a basal diet containing maize (19.5%), soybean (17.0%), deoiled rice bran (56.5%), molasses (4.0%), di-calcium phosphate and salt (1.0% each) and mineral and vitamin mixture (0.5% each) supplements at 3.5% of body weight to meet NRC (1981) requirements for protein, energy, macro minerals and micro minerals, excluding Cu. The basal diet contained 5.7 mg Cu/kg, 122.5 mg Fe/kg, 110 mg Zn/kg, 0.26 mg Mo/kg and 0.32% S. $CuSO_4$ or Cu-P was added to the basal diet at the rate of 10, 20 and 30 mg/kg. Kids were housed in a well ventilated shed with facilities for individual feeding in aluminum plated metabolic cages. Blood samples were collected from the jugular vein on d 0, 30, 60 and 90 to determine hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and serum enzymes (alkaline phosphatase, alanine transferase and aspertate transferase). A metabolism trial of 6 days duration was conducted after 90 days of experimental feeding. Statistical analysis revealed that source and level of Cu supplementation improved live weight gain ($p<0.04$) and average daily gain ($p<0.01$). No significant contribution of source and level of Cu to alter serum serum enzymes was evident. Goats fed Cu-P tended to have higher Hb, PCV and TEC than with $CuSO_4$ supplementation. Cu-P increased digestibility of ether extract (EE, $p<0.02$) and crude fiber ($p<0.05$) and showed an increasing trend ($p<0.09$) for digested crude protein (CP) and crude fiber (CF). Supplemental dose of Cu linearly improved ($p<0.02$) digestibilities of dry matter (DM), organic matter (OM), EE and nitrogen free extract (NFE). Though the absorption of nitrogen (N) was not affected ($p>0.10$) by both source and dose of Cu, N retention was affected ($p<0.04$) and there was a significant Source×Dose interaction ($p<0.05$). Final body weight (BW) was not influenced ($p>0.10$) by the source of Cu but increasing dose of Cu increased ($p<0.04$) the BW of kids. TDN intake (g/kg $W^{0.75}$) was higher ($p<0.05$) with the increased dose of Cu and there was a significant Source×Dose interaction. It was concluded that supplementation of Cu from different sources and varying dose level in a concentrate based diet may improve performance, nutrient utilization and plane of nutrition in castrated Black Bengal kids. The effects on performance and nutrient utilization are more pronounced with Cu-P than $CuSO_4$ supplementation. Higher dose of Cu showed better result than lower dose. (**Key Words :** Copper Proteinate, Copper Sulfate, Performance, Nutrient Utilization, Kid)

INTRODUCTION

Copper (Cu) is an essential element required by cattle and other animal for number of biochemical functions (Davis and Mertz, 1987). In the past, ruminant Cu deficiencies have usually been corrected by supplementation with inorganic mineral supplements. During the last several years, free choice of Cu supplementation to grazing cattle has increased and chelated minerals have become popular for use in free choice mineral supplements. Trace minerals complexed with organic molecule have been implemented to be more bioavailable than inorganic trace minerals (Brown and Zeringue, 1994). However, a shortcoming of supplemental organic Cu compounds is their highest cost (Attaelmannan and Reid, 1996). Some researchers (Nockels et al., 1993; Rabiansky et al., 1999) have indicated that Cu-lysine may be more beneficial than $CuSO_4$ in correcting Cu deficiency in animals. The physiological advantage afforded by organic Cu compounds may be due to the unique coordination chemistry of Cu, which permits the formation of highly soluble, chemically stable products that resists digestion and interaction with antagonist in the guts.

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Table 1. Chemical composition of basal diets on dry matter basis

Chemical composition (DM basis)	
Nutrient content	
Dry matter (%)	90.75
Organic matter (%)	88.29
Crude protein (%)	19.95
Ether extract (%)	2.34
Crude fiber (%)	11.71
Nitrogen free extract (%)	53.92
Mineral content	
Total ash (%)	11.71
Ca (%)	1.60
P (%)	1.10
Cu (ppm)	5.7
Zn (ppm)	110
Mo (ppm)	0.26
S (%)	0.32

Manspeaker et al. (1987) reported that chelated minerals were absorbed more readily than their inorganic counterpart.

The overall growth promoting effect of Cu is well established and is amply reviewed by different workers (Underwood, 1981; McDowell, 1992). To overcome the detrimental influences on animal performance mineral supplementation are essential to the livestock. A number of reports reveal that supplementation of various minerals improve the performance of animals (Heindi and Kirchgessner, 1993; Lall et al., 2000). Organic Cu sources, including Cu-proteinate (Cu-P) and copper lysine have been utilized to overcome effects of antagonistic minerals. Improvements in the digestibility of proteins (Braude, 1965; Castell and Bowland, 1968) and retention of nitrogen (Braude, 1965) have been reported in young pigs fed diet containing added Cu. Studies by Dove and Haydon (1992) and Dove (1995) have indicated that addition of 250 ppm Cu improved digestibility and utilization of the fat of weaned pigs, but results have limited and inconclusive for ruminant studies. Therefore, the main objective of this study was to investigate the effect of source and level of supplemental Cu on performance and nutrient utilization of castrated growing Black Bengal kids fed high concentrate diets.

MATERIALS AND METHODS

Animals and experimental design

The study was carried out in the Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Kolkata from February 2004 and May 2004 representing summer season of the year. Twenty eight 3-4 month old castrated Black Bengal kids (BW: 6.91±0.46) were procured from nearby villages to University goat farm and used as the experimental units. Prior to the beginning of the study the kids were fed on pasture for 30 days adaptation period with gradual introduction of mash feed and curtailing the grazing time. During the last 15 day of the adaptation period 100 mg/kg Mo (as $(NH_4)_6Mo_7O_{24}$, $4H_2O$; Merck Ltd., Mumbai, India, MW: 1,235.86, minimum assay 99%) was supplemented with the feed to reduce body Cu reserves. After 30 day of adaptation period, the kids were distributed into one control and six treatment groups (n = 4 per group) in such a way that the average age and mean BW were similar ($p>0.05$) among the groups.

Supplementation of the diet with graded dose of Cu

The kids were fed with the unsupplemented basal diet for a period of 30 days. Basal diet was formulated with ground maize (19.5%), deoiled rice bran (56.5%), soybean (17%), molasses (4%), dicalcium phosphate and salt (1% each), minerals and vitamins mixture (0.5% each) to meet or excess nutrient requirement (NRC, 1981) except Cu. After this preliminary feeding, the kids were supplemented with 0, 10, 20 or 30 mg elemental Cu/kg dietary dry matter (DM) as either copper sulfate pentahydrate ($CuSO_4$, $5H_2O$; Merck Limited, Mumbai, India, MW: 249.68; minimum assay 99%) or copper proteinate (Bioplex copper, supplied by the Alltech Inc. Nicholasville, USA; elemental Cu 15%) for a period of 90 days. To prepare the graded dose of copper from copper sulfate 1.965, 3.929 and 5.894 g of $CuSO_4$ was mixed with premix (required major and trace minerals mixed with rice bran) and subsequently prepared 50 kg diet. Similarly 3.333, 6.667 and 9.999 g of copper proteinate was mixed with premix to prepare 50 kg diet. Control group of animals received concentrate mixture without copper supplementation. Analysis of copper content of control diet was 5.7 mg/kg DM. Cu content of $CuSO_4$ supplemented diet was 14.2, 25.6 and 34.8 mg/kg DM respectively and in copper proteinate supplemented diet it was 15.9, 25.1 and 36.1 respectively. The chemical analyses of concentrate mixture are presented in Table 1. The kids were individually fed the treatment diets at 3.5% of body weight in two times daily (8.00 and 14.30 h). The kids were offered *ad lib* fresh and clean drinking water during the day. Feed intake was adjusted every 14 days during the 90 days feeding trial.

Record keeping

Body weight was recorded as initial at the beginning of experiment and further measurement at 30, 60, and 90 d for two consecutive days in the morning before offering any feed to the animal. Body weight gain (BWG) and average daily gain (ADG) was obtained by calculations.

Blood collection and analysis

Jugular blood (10 ml approx.) samples were collected from each kid in both non-heparinized and heparinized vacutainer tube at 0, 30, 60 and 90 d of the experiment. The

Table 2. Performance of castrated Black Bengal kids supplemented with Cu-sulfate and Cu-proteinate during 90 days feeding trial of copper supplementation (n = 4 in each treatments)

Attributes	Source and dose of supplemental copper mg/kg diet DM							SE	Source	Dose		Month	
	Control	Copper sulfate			Copper proteinate					Linear	Quadratic	Linear	Quadratic
	0	10	20	30	10	20	30						
Body weight													
Day 0	7.71	7.68	7.17	8.05	8.09	8.35	8.09	0.458	0.173	0.600	0.867	0.000	0.000
Day 30	8.86	8.97	8.63	9.76	9.54	10.10	9.75	0.324	0.089	0.156	0.998		
Day 60	10.05	10.23	9.84	10.79	10.52	11.37	11.52	0.324	0.038	0.082	0.784		
Day 90	11.02	11.56	11.17	12.14	11.58	12.06	12.47	0.324	0.293	0.036	0.842		
Body weight gain													
Day 0-30	1.16	1.29	1.46	1.71	1.45	1.75	1.66	0.019	0.006	0.000	0.280	0.000	0.257
Day 31-60	1.18	1.26	1.21	1.03	0.98	1.27	1.77	0.016	0.000	0.165	0.326		
Day 61-90[1]	0.97	1.33	1.33	1.35	1.06	0.68	0.95	0.018	0.000	0.553	0.731		
Over all[2]	3.31	3.88	4.00	4.09	3.48	3.70	4.38	0.048	0.003	0.000	0.930		
Average daily gain													
Day 0-30	38.56	43.00	48.56	57.11	48.22	58.33	55.22	0.633	0.006	0.000	0.280	0.000	0.257
Day 31-60	39.44	42.00	40.44	34.44	32.56	42.33	59.00	0.517	0.000	0.165	0.326		
Day 61-90[1]	32.44	44.44	44.22	44.89	35.33	22.78	31.67	0.603	0.000	0.553	0.731		
Over all[2]	36.82	43.15	44.41	45.48	38.70	41.15	48.63	0.053	0.003	0.000	0.930		

[1] Salt×Dose interaction ($p<0.05$). [2] Salt×Dose interaction ($p<0.01$).

uncoagulated blood was used for hematological assessment viz. hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), and total leukocyte count (TLC). The non-heparinized blood was allowed to clot and the separated serum samples were decanted into autoclaved plastic vial and stored in Eppendorf tubes at -20°C till analyses. Hb from the collected blood was estimated by cyanmethaemoglobin method (Cannan, 1958). PCV, TEC, TLC was determined as per standard method of Schalm et al. (1975). Serum samples were analyzed for alkaline phosphatase, aspertate transferase and alanine transferase in an automatic blood analyzer (Microlab 200 from EMerck India Ltd., Mumbai, India) using commercial kit (from Emerck, India Ltd., Mumbai, India).

Metabolism trial

A metabolism trial of 6 days duration, involving quantitative collection of feeds, feces and urine was conducted after 90 days of experimental feeding in order to assess the digestibility, balance of nutrients and plane of nutrition. The kids were individually placed in stainless steel metabolic cages equipped with wire mesh screens and drain pans that allow separate collection of feces and urine. Kids were acclimatized in the metabolic cages for 5 days before commencing the collection. Feces voided and urine excreted during a 24 h period was quantified. The dried representative samples of feeds (100 g) and feces (100 g) of each animal over the entire collection period were bulked, sampled and ground to pass through 1.5 mm sieve and stored in an air tight polyethylene bags until further analyses. Representative aliquots of urine (50 ml) from individual animals were immediately preserved for nitrogen determination.

Analytical methods of feed and faeces

Samples of feeds and feces were analyzed for proximate constituents and urine for nitrogen according to AOAC (1995) methods. P content of feeds was determined as per Talapatra et al. (1940). Sulfur content of feed was estimated as per the method of Ward and Johnston (1962). For the analyses of Cu, Zn, Fe and Mo content of the concentrate mixture was dry ashed at 400°C for 4 h in a muffle furnace, treated with concentrated HNO_3, solubilized in dilute HCl (1:1) and filtered through the Whatman® grade 42 filter paper (AOAC, 1995) and the final volume made upto 50 ml. subsequent minerals analysis was done in an flame atomic absorption spectrophotometer (A analyst 100, Perkin Elmer Inc., USA). Lanthanum chloride was mixed with samples aliquot while estimating Ca and Mg to minimize the interference from other trace minerals. Concentration of P in feeds was determined by colorimetric procedure (AOAC, 1995).

Statistical analysis

Data were analyzed by the general linear model of the SPSS (1997) with individual kids as the experimental units. The data obtained after metabolism trial (after 90 days) were analyzed separately to determine the effects of the sources and doses of supplemental Cu. For this the 'source' and 'dose' were used as the fixed factors in the model. Polynomial contrasts were applied to determine the linear and quadratic effects of different dose levels (0 vs. 10, 20 and 30 mg) of supplemental Cu. Salt×Dose interaction was also calculated. A probability of $p<0.05$ was considered to be statistically significant and that of $p<0.10$ was described as a trend.

Table 3. Serum enzymes of Black Bengal kids supplemented with Cu-sulfate and Cu-proteinate at the beginning (day 0) and at monthly interval (day 30, 60 and 90) of copper supplementation (n = 4 in each treatments)

Attributes	Source and dose of supplemental copper mg/kg diet DM							SE
	Control	Copper sulfate			Copper proteinate			
	0	10	20	30	10	20	30	
Alkaline phosphatase (U/L)								
Day 0	285.7	283.7	283.7	284.3	284.3	285.3	286.0	21.228
Day 30	291.0	295.3	298.7	299.3	286.7	287.7	286.0	26.878
Day 60	289.3	297.7	300.0	302.3	291.0	290.7	292.7	24.802
Day 90	292.0	299.7	299.7	340.3	297.7	298.0	305.3	28.065
Alanineamino transferase (U/L)								
Day 0	18.3	19.3	18.7	18.7	18.7	19.7	19.0	6.396
Day 30	19.3	20.0	20.0	20.0	20.0	21.0	20.7	4.471
Day 60	22.7	24.0	24.0	22.7	23.0	24.0	22.0	8.294
Day 90	25.3	25.0	26.0	25.3	24.7	23.3	24.0	7.537
Aspertate aminnotranferase (U/L)								
Day 0	161.3	161.3	158.3	159.0	161.3	160.0	160.0	12.802
Day 30	160.5	164.3	162.7	166.0	166.0	165.0	166.0	14.626
Day 60	167.3	166.3	168.7	167.3	168.0	167.7	168.0	11.811
Day 90	169.0	168.3	169.7	169.3	168.3	171.0	169.0	16.838

Table 4. Haematological profile of Black Bengal kids supplemented with Cu-sulfate and Cu-proteinate at the beginning (day 0) and at monthly interval (day 30, 60 and 90) of copper supplementation (n = 4 in each treatments)

Attributes	Source and dose of supplemental copper mg/kg diet DM							SE	Source	Dose	
	Control	Copper sulfate			Copper proteinate						
	0	10	20	30	10	20	30			Linear	Quadratic
Haemoglobin (g/dl)											
Day 0	10.22	10.22	10.17	10.19	10.19	10.13	10.20	0.023	0.657	0.524	0.549
Day 30	10.31	10.28	10.28	10.29	10.27	10.28	10.30	0.020	1.000	0.801	0.464
Day 60	10.36	10.73	10.88	11.04	10.74	10.89	11.18	0.030	0.398	0.000	0.257
Day 90	10.38	10.83	10.81	10.61	10.87	10.97	11.23	0.013	0.000	0.001	0.024
Packed cell volume (%)											
Day 0	27.33	27.67	27.67	27.33	27.67	27.33	27.67	0.151	1.000	0.816	0.577
Day 30	27.67	28.33	29.33	30.33	28.67	29.33	30.33	0.126	0.689	0.823	0.736
Day 60	28.33	30.00	29.33	31.00	29.67	30.67	32.00	0.208	0.159	0.000	1.000
Day 90	27.67	31.00	29.00	29.33	31.00	30.33	31.00	0.184	0.025	0.012	0.006
Total erythrocyte count ($\times 10^6$ cumm)											
Day 0	12.35	12.35	12.29	12.31	12.31	12.24	12.32	0.031	0.659	0.529	0.536
Day 30	12.46	12.43	12.42	12.43	12.41	12.42	12.45	0.022	0.983	0.780	0.460
Day 60	12.52	12.96	13.14	13.34	12.98	13.15	13.52	0.022	0.398	0.000	0.273
Day 90	12.54	13.08	13.06	12.82	13.13	13.26	13.56	0.022	0.000	0.001	0.023
Total leukocyte count											
Day 0	7,300.0	7,300.0	7,200.0	7,233.3	7,266.7	7,333.3	7,266.7	44.671	0.652	0.696	1.000
Day 30	7,300.0	7,333.3	7,400.0	7,266.7	7,300.0	7,300.0	7,200.0	54.502	0.580	0.745	0.533
Day 60	7,400.0	7,333.3	7,350.0	7,386.7	7,383.3	7,366.7	7,350.0	48.588	0.925	0.834	0.784
Day 90	7,266.7	7,500.0	7,600.0	7,526.7	7,460.0	7,666.7	7,643.3	39.056	0.580	0.008	0.106

RESULTS

Performance

Body wt. changes of Black Bengal kids with supplementation of $CuSO_4$ and Cu-P at monthly interval were presented in Table 2. Body wt was higher in Cu-P supplemented kids after 30 d ($p<0.09$) and 60 d ($p<0.04$) of supplementation. Effect of source on BW was not found after 90 d of supplementation of Cu salts. Increases in dose Cu from both sources linearly improved BW from 60 d ($p<0.09$) to the end ($p<0.04$) of feeding trial. Quadratic response of dose was not found. Body wt. gain (BWG) and average daily gain (ADG) were varied ($p<0.01$) throughout the feeding trial with the variation of source of Cu salt. Overall BWG and ADG were higher ($p<0.01$) in Cu-P supplemented kids compared to $CuSO_4$ supplementation except during first 30 days of trial. Numerically highest rate of gain was found in 20 mg Cu-P/kg supplemented kids during first 30 days, in 30 mg Cu-P/kg during 30-60 days. in 30 mg $CuSO_4$/kg during 61-90 d feeding trial. Rate of

gain in BW due to Cu-P supplementation was more just after the starting of experiment but gradually reduces in course of time, which was found in present experiment. Over all ADG was highest (48.63 g/d) in 30 mg Cu-P/kg supplemented kids. Effect of month on BW was best linear quadratic ($p<0.01$). But on BWG and ADG was only linear ($p<0.01$). Salt×Dose interaction was noted for BWG and ADG during 61-90 day ($p<0.05$) and over all gain ($p<0.01$).

Serum enzymes

Concentration of serum alkaline phosphatase, aspertate transaminase (AST) and aspertate alanine transaminase (ALT) in Black Bengal kids supplemented with two sources of Cu were presented in Table 3. No significant effect of treatment or interactions was found for these three serum enzymes. For this reason, only means of each serum enzymes at 30 days interval were given in the table. Concentrations of these serum enzymes signified that the animals were apparently healthy throughout the experimental period. It can be concluded from this table that supplementation of Cu upto 30 mg/kg couldn't able to produce any dystrophy in hepatic or other tissues containing these enzymes during the 90 days feeding period. Thus no clinical symptom of Cu toxicity was developed.

Haematology

Effects of supplementation of Cu on haematological profiles were presented in Table 4. Whole blood haemoglobin (Hb; g/dl) was similar across the treatment upto 30 days of supplementation of Cu from two sources with different dose. Linear effect ($p<0.01$) of dose was noted from d 60 onwards till end of experiment. Effect ($p<0.02$) of salt on blood Hb concentration was noted at the final day of blood collection, where Cu-P showed better result compare to $CuSO_4$. Same type of response was noted for packed cell volume (PCV; %), total erythrocyte count (TEC; $\times 10^6$/cumm). Salt×dose interaction was noted at d 90 for Hb, PCV and TEC. Total leukocyte count (TLC) throughout the experimental period were similar ($p<0.10$) in all the treatment groups.

Digestibility of nutrients

Effects of two sources of supplemental Cu with different dose level on nutrient digestibility were presented in Table 5. Supplemental Cupper sources had no effect ($p>0.10$) on DM, OM, NFE digestibility whereas Cu-P improved digestibility of EE ($p<0.02$) and CF ($p<0.05$) compare to $CuSO_4$. The amount digested CP (g/d) tended to be higher ($p<0.09$) in Cu-P supplemented kids. Increase in

Table 5. Nutrient intake (g/d), digested (g/d) and apparent digestibility (%) of castrated Black Bengal kids supplemented with Cu-sulfate and Cu-proteinate at the end of 90 day copper supplementation (n = 4 in each treatments)

Attribute	Source and dose of supplemental copper mg/kg diet DM							SE	Significance of treatments effects		
	Control	Copper sulfate			Copper proteinate				Source	Dose	
	0	10	20	30	10	20	30			Linear	Quadratic
Dry matter											
Intake[1]	385.70	404.60	390.95	424.90	405.30	422.10	436.45	7.233	0.592	0.007	0.012
Digested	224.59	238.63	230.58	250.82	237.26	248.28	259.56	3.176	0.378	0.167	0.238
Digestibility[1]	58.23	58.98	58.98	59.03	58.54	58.82	59.47	0.132	0.651	0.001	0.482
Organic matter											
Intake	339.11	355.72	343.72	373.57	356.34	371.11	383.73	6.471	0.316	0.016	0.008
Digested	222.69	237.52	230.02	250.41	236.00	247.68	262.09	4.965	0.076	0.031	0.087
Digestibility[1]	65.67	66.77	66.92	67.03	66.23	66.74	68.30	0.153	0.159	0.000	0.993
Crude protein											
Intake	76.95	80.72	77.99	84.77	80.86	84.21	87.07	1.261	0.478	0.012	0.427
Digested	41.64	44.06	42.55	46.32	43.97	46.02	47.70	1.021	0.081	0.096	0.251
Digestibility	54.12	54.58	54.55	54.64	54.38	54.65	54.78	0.235	0.562	0.156	0.651
Ether extract											
Intake[1]	9.03	9.47	9.15	9.94	9.48	9.88	10.21	0.352	0.476	0.015	0.291
Digested	5.61	5.96	5.75	6.25	5.92	6.18	6.45	0.201	0.026	0.059	0.212
Digestibility	62.16	62.95	62.84	62.82	62.38	62.52	63.11	0.096	0.018	0.019	0.352
Crude fiber											
Intake	45.17	47.38	45.78	49.76	47.46	49.43	51.11	0.731	0.421	0.039	0.325
Digested	25.58	27.11	25.87	28.45	27.03	28.47	29.24	0.211	0.086	0.072	0.126
Digestibility	56.63	57.23	56.50	57.18	56.95	57.60	57.22	0.015	0.046	0.081	0.436
Nitrogen free extract											
Intake	207.93	218.12	210.76	229.06	218.50	227.55	235.29	3.862	0.212	0.021	0.159
Digested	150.17	160.30	155.88	169.48	159.04	167.02	178.82	2.011	0.189	0.052	0.503
Digestibility	72.22	73.49	73.96	73.99	72.79	73.40	76.00	0.215	0.176	0.008	0.592

[1] Salt×Dose interaction ($p<0.05$).

Table 6. Nitrogen balance and plane of nutrition of castrated Black Bengal kids supplemented with Cu-sulfate and Cu-proteinate at the end of 90 day copper supplementation (n = 4 in each treatments)

Attribute	Source and dose of supplemental copper mg/kg diet DM							SE	Significance of treatments effects		
	Control	Copper sulfate			Copper proteinate				Source	Dose	
	0	10	20	30	10	20	30			Linear	Quadratic
Nitrogen balance											
Intake (g/d)	12.31	12.91	12.48	13.56	12.94	13.47	13.93	0.191	0.478	0.012	0.427
Output (g/d)											
Faecal	5.65	5.87	5.67	6.15	5.90	6.11	6.30	0.112	0.212	0.162	0.492
Urinary	4.41	4.46	4.44	4.58	4.46	4.49	4.62	0.091	0.206	0.421	0.328
Absorbed (g/d)	6.66	7.05	6.81	7.41	7.04	7.36	7.63	0.158	0.081	0.096	0.251
Retained (g/d)	2.25	2.59	2.37	2.83	2.58	2.87	3.01	0.041	0.051	0.036	0.128
Absorption (%)	54.12	54.58	54.55	54.64	54.38	54.65	54.78	0.086	0.562	0.156	0.651
Retention (%)[1]	18.30	20.05	18.97	20.87	19.91	21.33	21.62	0.072	0.036	0.012	0.126
Plane of nutrition											
Final weight (kg)	11.02	11.56	11.17	12.14	11.58	12.06	12.47	0.324	0.293	0.036	0.842
DMI (g/kg $W^{0.75}$)	63.77	64.54	63.99	65.33	64.56	65.22	65.77	1.021	0.213	0.293	0.496
DCPI (g/kg $W^{0.75}$)	6.89	7.03	6.96	7.12	7.00	7.11	7.19	0.361	0.239	0.166	0.418
TDN (g/kg $W^{0.75}$)[1]	38.03	39.06	38.83	39.72	38.77	39.47	40.73	0.421	0.088	0.041	0.412

[1] Salt×Dose interaction ($p<0.05$).

dose of Cupper irrespective of sources increased intake of DM ($p<0.01$), OM, CP and EE ($p<0.02$), CF ($p<0.04$) and NFE ($p<0.03$), which is due to the increase in body weight. Digestibility of DM, OM, EE and NFE increased ($p<0.02$) when the dose of Cu increased to 30 mg Cu/kg diet. Higher EE digestibility was noted in 30 mg Cu-P/kg diet kids. CP digestibility were similar ($p>0.10$) among the treatments. There was an increasing trend ($p<0.09$) in CF digestibility with the increase in dose of Cu. All the response of dose of Cu in digestibility of nutrient was linear. Salt×Dose interaction ($p<0.05$) was noted for DM intake (DMI), DM and OM digestibility, EE intake. Quadratic response ($p<0.02$) of dose was noted for DMI and OM intake.

Nitrogen balance

Nitrogen (N) balance of kids supplemented with $CuSO_4$ and Cu-P were presented in Table 6. N retention (%) increased ($p<0.04$) with the supplementation of Cu-P compare to $CuSO_4$. Increasing trend of absorbed (g/d; $p<0.09$) and retained (g/d; $p<0.06$) amount of N was found in Cu-P supplementation than $CuSO_4$. Linear dose response was noted for N intake and retention ($p<0.02$) and retained N ($p<0.04$). Retention (%) and retained (g/d) on N increased with the increase in dose of supplemental Cu. Though absorption (%) of N was not affected by both source and dose of supplemental Cu but retention (%) and retained (g/d) of N was affected by both source and dose, which indicate that supplemental source and dose of Cu reduces the rate of excretion of N in kids and thus helps in N metabolism and increased the BW gain. Salt×Dose interaction ($p<0.05$) was found for N retention (%).

Plane of nutrition

Final BW of the experimental kids was not influenced ($p<0.10$) by source of Cu, but BW was increased linearly ($p<0.04$) with the increasing dose of Cu irrespective of source. Highest BW at the end of 90 d supplemental period was recorded in 30 mg Cu-P supplemented kids. No salt×dose interaction ($p>0.10$) was found for final BW of kids. No effect of Cu source, dose and their interaction was found for DMI (g/kg $W^{0.75}$) and digestible CP intake (DCPI; g/kg $W^{0.75}$). Increasing trend ($p<0.09$) in TDN intake (g/kg $W^{0.75}$) was found in Cu-P supplementation compare $CuSO_4$, whereas TDN intake increased linearly ($p<0.05$) with increase in dose of supplemental Cu in the diet. Salt×Dose interaction ($p<0.05$) was also found for TDN intake.

DISCUSSION

Trace minerals complexed with organic molecules have been implied to be more bioavailable than inorganic trace minerals (Brown and Zeringue, 1994; Lim and Paik, 2006). Some researchers (Nockels et al., 1993; Rabiansky et al., 1999) have indicated that Cu-lysine may be a more available source of Cu than $CuSO_4$ in cattle. The physiological advantage afforded by organic Cu compounds may 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). However, in the present study, only two Cu sources ($CuSO_4$ and Cu-P) effect were detected. In this experiment supplementation of Cu increased BW of kids and the performances from Cu-P supplementation was better than $CuSO_4$ supplementation. In agreement with the present findings, Cu supplementation to finishing cattle had increased performance (Engle and Spears, 1999; Engle and Spears, 2000b). In another study,

finishing steers supplemented with 10 or 40 mg Cu kg^{-1} dry matter (DM) had higher ADG than control (Engle et al., 2000a). In contrast Ward et al. (1993), reported that addition of 5 mg Cu kg^{-1} DM to a growing diet (containing 6.5 mg Cu kg^{-1} DM) didn't affect BWG of Angus steers during 96 days study. However in the later experiment in growing Angus steers supplementation of 5 mg Cu kg^{-1} DM to a corn silage diet containing 5.2 mg Cu kg^{-1} DM increased intake but didn't significantly affect gain (Ward and Spears, 1997). But it has been reported that growth performance wasn't affected by Cu supplementation when fed over the entire finishing period in finishing steers (Engle and Spears, 2000a, Engle et al., 2000b). A 28% improvement in ADG was found, when Nubian does were supplemented with Cu at level of 100-150 mg/day for 23 weeks (Solaiman et al., 2001). At lower levels of 10 and 30 mg Cu/kg diet, no effect of Cu supplementation on feed intake and ADG of meat goats has been reported (Luginbuhl et al., 2000). It is evident that conflicting performance results exist in ruminant consuming high concentrate diets supplemented with Cu. The reasons for the discrepancy between results are not clear. There are many factors that could potentially affect an animal response to Cu supplementation, such as the Cu concentration of the basal diet, the duration and concentration of Cu supplementation, forms of Cu supplementation (organic or inorganic) the absence and presence of dietary Cu antagonists (S, Mo, Zn, Fe), initial Cu status of the animal, environmental and health factors and breed differences in Cu metabolism. Cu also has been shown to stimulate growth hormone secretion from bovine pituitary explants *in vitro*, (LaBella et al., 1973). Therefore, it is possible that Cu enhances protein synthesis by stimulating hormones and growth factors in young kids.

Concentration of serum AKP, ALT, AST enzymes were unaffected by the increase in dose of Cu from both the sources of Cu, which signifies that the animals were apparently healthy throughout the experimental period. Levels of plasma AST, ALT, γ-glutamyltransferase were positively correlated with increased Cu intake and indicative of hepatic damage starting with 300 mg Cu /head/day (Haenlein, 2004). So it can be concluded that supplementation of Cu upto 30 mg/kg could not able to produce any dystrophy in hepatic or other tissues containing these enzymes during 90 days feeding period. Thus no clinical symptom of Cu toxicity was developed.

Whole blood Hb was increased with the supplementation of Cu and improvement was better in Cu-P supplementation compare to $CuSO_4$. Gardner et al. (1976) and Gengelbatch et al. (1994) reported that Mo inhibits Cu mediated hemopoitic functions in ruminants. In the present experiment concentration of Mo was low (0.26 ppm), so it can be opined that Cu increased TEC count by mediating hemopoitic function in ruminants and subsequently PCV was also increased. The present findings inconsistence with the findings of Eckert et al. (1999) who found that supplementation of Cu at 30 mg/kg diet tended to reduce Hb concentration. They explained that sheep were more prone to haemolytic anaemia which is one of the adverse condition caused by Cu toxicity, would infact lower PCV, TEC and Hb values (Frenger et al., 1992). Thomson and Todd (1974) reported that serum AST activity begin to rise during the prehaemolytic period. Lamand (1981) reported that the toxicity limit in Nubian goats is approximately 30 mg/kg DM. However, Kessler (1991) cited a study of Hussein (1985) with Swedish goats, which were fed 15 mg Cu/kg live weight for seven weeks showing no sign of toxicity. A new study by Solaiman et al. (2001) confirms that goats can not only tolerate high doses of Cu may even required higher Cu level. Growing Nubian does were given upto 150 mg Cu/day for 23 weeks without exhibiting sign of Cu toxicity. But in the present experiment higher level of Cu was 30 mg/kg DM and this dose was not found to be toxic upto 90 days of feeding trial in castrated Black Bengal goats.

There are many mineral interactions in the feed ration influencing net absorption (Haenlein, 1992). Mineral ions compete for anionic legends to form insoluble precipitates. Mineral ions compete for transport proteins. Competing mineral ions block enzyme reactions. Vitamins affect absorption chelation between amino acids, and influence mineral absorption (Haenlein, 1992). Different chemical forms of Cu have been shown to be more bioavailable than $CuSO_4$. Ward et al. (1996) have shown Cu-P to be more available than iso-amounts of $CuSO_4$ in heifers fed diets high in Mo and S. tribasic Cu chloride was more bioavailable than $CuSO_4$ (Spears et al., 1997). Whereas, antagonist (S- 0.32%, Mo- 0.26 mg/kg) was not too high in the diet to affect Cu absorption. Effects of Cu supplement in different doses were to improve DM, CF, NFE and OM digestibilities in the present investigation. Similar results have been reported earlier in pigs (Dove, 1995; Luo and Dove, 1996) and in Black Bengal kids (Mondal et al., 2004). In one of the previous study Lopez-Guisa and Satler (1992) reported an increase in CF digestibility when Holstein cows were supplemented with Cu compare to cows consuming low Cu diet. Improvement in fat digestibility was also observed due to addition of Cu. Ho and Elliot (1974) suggested that Cu enhanced the specific activity of the fatty acyl desaturase systems thus altering composition of depot fat. Adams and Jensen (1985) reported that the apparent digestibility of fat was decreased as protein level increased in diet. In the present investigation lower CP level (19.95%) could have contributed higher digestibility of dietary indigenous fat by the same way found in pigs. The

improved fat digestibility resulting from Cu addition could lead to the increased absorption of fatty acid and fat soluble vitamins and affects other aspect of nutrient metabolism in the body and therefore stimulate growth of Black Bengal kids. Improvement of fat metabolizability due to Cu addition may be partially due to increase in lipase and phospholipase activity in the small intestine. Activities of the pancreatic enzymes were not affected by Cu possible due to low Cu level in pancreas (<1.5 ppm, not presented in table). Cu functions bio-chemically as a component of several Cu depended enzymes and as a cofactor for numerous other enzymes (Zapsalis and Beck, 1985; Soreansen, 1987). It is possible that high dietary Cu concentration enhance growth of kids by stimulating activities of the enzymes involved in nutrient utilization. The higher BW can also be confirmed with the high intake of TDN (g/kg $W^{0.75}$) found in this experiment. The intake of DM and CP by kids of both sources and levels were inaccordance with recommended intake (NRC, 1981; Kearl, 1982).

Addition of Cu improved N retention (%) without altering N absorption (%) can be well compared with the previous work in pigs (Braude, 1965; Castell and Bowland, 1968; Dove, 1995) and in kids (Mondal et al., 2005). Zhou et al. (1994) demonstrated that Cu given in either intravenous or oral intake increased serum mitogenic activity (mitogenic peptides, an indicator of blood growth factor activity) in weanling pigs and numerically increased pituitary growth hormone mRNA concentration. Cu also had been shown to stimulate growth hormone secretion from bovine pituitary explants *in vitro* (LaBella et al., 1973). Therefore it is possible that Cu enhance protein retention and protein synthesis by stimulating hormone and growth factors in Black Bengal kids.

The data from this feeding trial indicate that supplementation of Cu plays an important role in the performance, digestion of dry matter, fat and nitrogen utilization in castrated Black Bengal kids. Effect of organic Cu (Cu-P) for the improvement of performance and digestibilities are better than the supplemental inorganic Cu source ($CuSO_4$). Goats can tolerate upto 30 mg supplemental Cu/kg diet without showing any toxic symptoms or haemolytic anemia as evident from serum enzyme and haematology. However, further long term trials are warranted to consolidate the present findings.

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