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Effect of inorganic and organic zinc and copper supplementation on performance, nutrient utilization, rumen fermentation and blood biochemistry of kids

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ABSTRACT

Aim of the present study was to assess the effect of inorganic and organic zinc and copper supplementation on intake, nutrient utilization, performance, feed efficiency and hemato, blood biochemicals of kids. Barbari male kids (60) were randomly divided into 4 equal groups. Control group of kids were supplemented inorganic Zn and Cu (iZn-iCu), one group had organic Zn and Cu (OrZn-OrCu), while other groups had either inorganic Zn and organic Cu (iZn-OrCu), or organic Zn and inorganic Cu (OrZn-iCu). Organic minerals were provided at 0.75 of their inorganic level. Growth, feed intake and feed conversion efficiency was similar in either form or combinations of Cu and Zn supplementation. The kids during 150 days of experimental feeding had live weight gain 8.6 to 8.8 kg and average daily gain 57.0 to 58.8 g. Kids consumed feed dry matter 618 to 710 g/d, which accounted 2.96 to 3.47% of live weight, with feed conversion ratio (kg feed/ kg gain) of 10.4 to 10.6 kg. Intake and digestibility of OM, CP and NDF were also similar among 4 groups. All kids had positive and similar N balance, which ranged from 8.1 to 9.9 g/d. Growing Barbari kids under present feeding protocol consumed 567 g feed and 14.3g nitrogen to have 59 g daily gain. Rumen fermentation characteristics studied at 4 h post feeding revealed that total volatile fatty acid contents were similar among 4 groups, whereas rumen fluid pH was lower and NH_3-N was higher in OrCu and iZn fed, and TCA- ppt-protien was lower in OrCu and OrZn fed kids. Supplementation of organic Zn and Cu improved blood hemoglobin. Red and white blood corpuscles (WBC) counts were similar amongst 4 groups, but WBC decreased linearly with progress in feeding period. The lymphocytes, granulocytes, eosinophils, monocytes, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (MCHC) were similar. Kids fed OrCu and OrZn had lower protein, globulin and blood urea nitrogen content. Plasma Zn and Cu contents did not change, while plasma Fe content reduced in OrZn and Cu supplemented kids. It is concluded that kids may be fed diets with OrZn and Cu at 25% lower level than the required inorganic Zn and Cu during active growth phase without compromising performance. The combination of OrCu and iZn tended to promote feed DM intake per unit BW, reduced rumen fluid pH, and increased NH_3-N level.

Key words: Feed intake, Feed efficiency, Goat, Growth, Organic minerals, Performance

The supplementation of Zn and Cu in animal feeding produced beneficial effects in terms of growth, milk production and reproduction (Hureta *et al.* 2002, Ashmead and Samford 2004, Stahlhut *et al.* 2006, Mandal *et al.* 2007, Griffiths *et al.* 2007). Trace mineral complexes with organic molecules are more bio-available than inorganic trace minerals (Brown and Zeringue 1994). The physiological advantage of organic trace minerals is offered by the unique chemistry of organic metal complex, which permits highly soluble, chemically stable product that resists interactions with antagonists in the gut (Brown and Zeringue 1994, Spears 1996). The animal feeds naturally contain trace minerals primarily as organic complexes. The metal complex can be absorbed as such or can be modified to a chemical form of the mineral that can be absorbed (Spears 1996). Bio-availability of organic minerals in animal system is higher in comparison to inorganic counter parts and the organic Cu supplementation is reported to reduce fecal excretion (Mondal *et al.* 2007). The physiological advantage afforded by organic Cu to be due to the formation of highly soluble and chemically stable products (Brown and Zeringue 1994) that did not affect bio-availability of Zn (Eckert *et al.*1999). Organic Cu complexes are absorbed via a mechanism that differs from the one controlling absorption of inorganic Cu, and does not interfere with Zn and Fe absorption (Du *et al.*1996). The Zn is an essential element for ruminants, its deficiency may reduce appetite causing low growth rate and

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wool growth (Reis and Sahlu 1994, White *et al.* 1994, Sandoval *et al.*1997, Jia *et al.* 2008). An interrelationship exits between Cu and Zn utilisation, Cu deficient rations causing alleviated Zn levels and Zn deficient diets increase Cu levels to their indicator tissues (Haenlein and Anke 2011), thus creating mineral imbalance by affecting utilisation. It is reported that organic Cu and Zn supplementation increase nutrient utilisation over inorganic forms (Datta *et al.* 2007, Grag *et al.* 2008, Mondal *et al.* 2007) and increase their level in serum (Mandal *et al.*2007). Environmental pollution concern resulted in restricted use of trace elements in animal feeding. Feeding of mineral elements at higher levels may cause soil and water pollution through animal excreta (Mandal *et al.*2007). The concentration and bio-availability of minerals in diet determines the level of mineral excretion in faeces and urine by the animals (Spears 1989, Mendoza *et al.*1993, Datta *et al.* 2007, Garg *et al.* 2008).

Organic sources of trace minerals protect from antagonism and has 31 to 40% greater bio-availability than inorganic sources (Hansen *et al.* 2008). Based on apparent absorption to tissue and blood concentration, it is suggested that organic trace minerals are considerably better absorbed than inorganics (Spears 1996).The organic source of copper (Cu-proteinate) stimulated more growth in kids than equivalent supplement of $CuSO_4$ (Datta *et al.* 2007), organic zinc (zinc-methionine) improved gain and feed efficiency in heifers (Spears 1989) and lambs (Garg *et al.* 2008). The copper-glycinate complex showed higher bio-availability but complexed with lysine, methionine or other amino acids did not have consistent advantage over $CuSO_4$ supplementation (Suttle 2010, Cheng *et al.* 2010). Supplementation of equivalent amount of $CuSO_4$ or copper- proteinate in kids did not improve performance (Mondal *et al.* 2007), and dairy cows supplemented zinc, copper and selenium as carboamino complex did not produce beneficial effects over in organic sources (Cortinhas *et al.* 2010). Since higher bio-availability of organic trace minerals has been confirmed by several researchers, we hypothesized that organic trace minerals supplementation at lower level than the required inorganic trace minerals may fulfill animal's requirement. Hence, we supplemented organic trace minerals at 25% lower levels than required in present experiment. Therefore, present experiment aim was to assess the effect of required inorganic and low level of organic zinc and copper supplementation on performance, intake, nutrient utilization, rumen fermentation, feed efficiency and hemato- and blood biochemical attributes of kids.

MATERIALS AND METHODS

Animals, housing and feeding: Sixty, weaner (100.3± 2 d old and 9.2± 1.35 kg BW) male Barbari kids were randomly divided into 4 equal groups and fed for 150 days, while penned in well-ventilated enclosures for the experiment. Animals were supplemented Zn and Cu, which was mixed with concentrate mixture and fed at 20 g/ kg BW to provide adequate essential nutrient required (Table 1) to support 50 to 100 g daily gain (ICAR 1998). Each kid offered 500 g green fodder (barseem, *Trifolium alexandrinum*) to meet vitamin requirements, while *ad lib.* dry roughage (gram straw; *Cicer arietinum*) was available to animals in an excess to allow refusal of 10%. The animals of control group were fed inorganic Zn and Cu (iZn-iCu), while other kid groups were fed either organic Zn and Cu (OrZn-OrCu), inorganic Zn and organic Cu (iZn-OrCu), or organic Zn and inorganic Cu

Table 1. Chemical composition of diet

	Composition of concentrate fed to animal groups*				Roughage	
	iZn-iCu	iZn-OrCu	OrZn-iCu	OrZn-OrCu		
Ingredient composition						
Barley (g/kg)	740	740	740	740		
Groundnut oil cake (g/kg)	120	120	120	120		
Wheat bran (g/kg)	120	120	120	120		
Common salt (g/kg)	20	20	20	20		
$CuSO_4.5H_2O$ (g/100kg)	7.86	-	7.86	-		
$ZnSO_4.7H_2O$ (g/100kg)	43.9	43.9	-	-		
Organic-Zn product (g/100kg)	-	-	50.0	50.0		
Organic-Cu product (g/100kg)	-	10.0	-	10.0		
Chemical composition (g/kg DM)					Dry#	Green'
Dry matter	920.7	947.2	963.6	946.4	943.9	216.6
Organic matter	960.4	968.0	971.4	963.2	925.9	950.9
Crude protein	192.6	192.5	192.2	192.5	95.0	147.5
Neutral detergent fibre	476.8	455.4	463.9	469.6	576.6	438.4

*Dietary treatments groups: iZn-iCu, Inorganic Zn and Cu; iZn-OrCu, Inorganic Zn and Organic Cu; OrZn-iCu, Organic Zn and Inorganic Cu; OrZn-OrCu, organic Cu and Zn.#Gram straw (*Cicer arietinum*); 'green, barseem (*Trifolium alexandrinum*) and cow pea (*Vigna sinensis*).

(OrZn-iCu). The mineral supplements were mixed with concentrate; organic minerals were added at 0.75 of the required inorganic Zn and Cu to support optimum kid growth. The concentrate mixture fed to animals contained groundnut oil cake meal 0.12, wheat bran 0.12, barley 0.74 and common salt 0.02. Animals received fresh feed once a day at 9:30 h, after discarding the residue of previous days. Feed records were maintained throughout experiment. Feed samples were collected at weekly intervals for dry matter (DM) determination and 3 or 4-week samples were pooled for chemical analyses. Free choice water was available. Animals were dewormed at the beginning of the experiment against roundworms, lungworms and fluke using levamisole hydrochloride and oxyclozamide respectively, 7 mg and 15 mg/ kg live weight.

Growth and metabolism experiment: A feeding-cum-growth trial lasted 150 days in a randomized design during which kids BW were recorded for 2 consecutive days every 15 days and these values were used to determine BW gain. Pattern of growth was calculated based on 15 days periods BW change. A metabolism trial was conducted near at 130^{th} d on 6 randomly selected kids from each treatment, for 10 day (i.e. 3 day adaptation followed by 7 day of sampling) during which daily feed intake, output of faeces and urine were recorded and collected. Sample of feed, orts, faeces and urine were collected every morning. Faeces and urine were collected into acidified containers containing 100 ml H_2SO_4 (100 ml concentrated H_2SO_4 diluted to 1,000 ml with distilled water) using a total collection method. Samples of feed, faeces and ort were dried to a constant weight in a hot air oven at 70°C for chemical analysis. Dried samples of the 7 days collection were pooled, ground to pass a 1mm screen and preserved for chemical analyses.

Blood collection and biochemical determinations: Blood was collected through jugular vein puncture method at 0, 75 and 150 days of experimental feeding. Blood was collected in EDTA impregnated tubes for hematology study, plasma was separated and frozen stored at –20°C to estimate biochemical attributes. Haematological observations were determined in whole blood using blood analyzer dedicated for goat hematology. Plasma protein, albumin, urea nitrogen and total cholesterol contents were estimated using commercial kit. Trace minerals (Zn, Cu and Fe) in plasma were estimated by AAS (atomic absorbance spectrophotometer).

Rumen fermentation: Rumen fluid samples were collected at 4 h post feeding at 90 d of feeding from all animals to estimate change in pH, ammonia nitrogen (NH_3-N), total volatile fatty acids (TVFA) and trichloroacetic acid perceptible protein (TCA-ppt protein). The samples were collected directly from rumen using stomach tube, a 50 ml rumen fluid was collected and pH measured within 5 min of aspiration. The sample was strained by 4 layers of muslin cloth, a portion of 10 ml was centrifuged at 150g for 15 min to eliminate feed particles from the sample and supernatant was used to determine fermentation metabolites to which 2 drops of saturated $HgCl_2$ was added to arrest fermentation immediately. These samples were stored frozen at –20°C until analyzed.

Chemical analysis: The DM of feed, orts and feces was analyzed by drying at 100 °C for 24 h or to constant weight. The AOAC (2000) analytical procedures were used for the OM determination by ashing at 550°C for 4 h and N estimation by a Kjeldahl technique. Neutral detergent fibre (NDF) was determined following the procedure of Van Soest *et al.* (1991) without sodium sulfite or α-amylase. The NDF is expressed with residual ash. The NH_3-N in rumen fluid was estimated following the procedure of Chany and Marbach (1962), TVFA by Barnett and Reid (1957) procedure and protein by Lowry *et al.* (1951) in TCA-ppt.

Statistical analysis: Results of feed intake, feed efficiencies, nutrient utilization and rumen fermentation were subjected to analysis of variance for statistical significance test using general linear mathematical model as:$Y_{ijk} = m + T_i + e_{ij}$, where: Y_{ijk}, observation mean; m, general mean; T_i, effect of i^{th} treatment (i = 1, 4), e_{ij}, random error. The growth and blood data were analysed using repeated measures procedure of general linear model (GLM) of SPSS Base 15.0 (2006). The diet, blood collection period, and their interactions were analysed using mathematical model as: $Y_{ijkl} = \mu + T_i + P_j + (T \times P)_{ij} + e_{ijk}$, where Y_{ijkl}, observation mean; μ, general mean; T_i, effect of ith treatment (i = 1, 4); P_j, observation at collection period (j = 1, 3); $(T \times P)_{ij}$, interaction between ith dietary treatment and observation of jth period, e_{ijk}, random error. The model also tested the significance in terms of linear and quadratic effects of feeding on blood attributes and significant means were separated considering least significant difference.

RESULTS AND DISCUSSION

The initial, final and total BW gain, average daily gain (ADG), feed intake and feed efficiency were similar among kids of 4 groups fed organic or inorganic Zn and Cu supplemented diets (Table 2). Kids under 4 dietary treatments had similar growth pattern (Fig. 1). Feed intake ranged from 421 to 792 g with mean intake 567 g/d to have ADG ranging between 27 to 94 g with average 59 g (Fig. 2), but feed intake between 580 to 680 g/d seems optimum to support an ADG 70 to 90 g/day. Total daily DM intake was not different among four kid groups, while supplementation of organic trace minerals had increasing trend (P= 0.09) of DM in terms of g/kg $W^{0.75}$ or per cent of BW. The kids supplemented i Zn and OrCu had more pronounced trend of increased DMI (g/ kg$W^{0.75}$ or kg 100/kg BW). Intake and digestibility of OM, CP and NDF were also similar among 4 kid groups. Total and urinary N excretion was higher in kids supplemented iCu and OrZn. All animal groups had positive and similar N balance (Table 3). Nitrogen intake have polynomial effect

Table 2. Growth performance, feed intake and feed efficiency of kids supplemented organic or inorganic Zn and Cu

	Dietary treatments groups*				SEM
	iZn-iCu	iZn-OrCu	OrZn-iCu	OrZn-OrCu	
Growth performance					
Initial BW (kg)	9.1	9.7	9.1	9.0	0.221
Final BW (kg)	17.9	18.5	17.9	17.5	1.521
Gain (kg)	8.8	8.8	8.8	8.6	1.009
Average daily gain (g/day)	58.8	58.4	58.6	57.0	15.21
Feed DM intake (g/day)					
Roughage intake					
Dry	207.6	191.7	189.7	185.9	20.324
Green	85.1	84.6	85.6	82.5	9.824
Total	292.7	276.3	275.3	268.4	14.391
Concentrate	244.8	244.8	260.1	244.8	8.994
Total feed intake	537.5	521.1	535.4	513.2	4.729
Feed efficiency					
Per cent	10.9	11.2	19.4	11.1	0.322
kg feed/ kg gain	9.1	8.9	9.1	9.0	0.289

*Dietary treatments groups: iZn-iCu, Inorganic Zn and Cu; iZn-OrCu, Inorganic Zn and Organic Cu; OrZn-iCu, Organic Zn and Inorganic Cu; OrZn-OrCu, organic Cu and Zn.

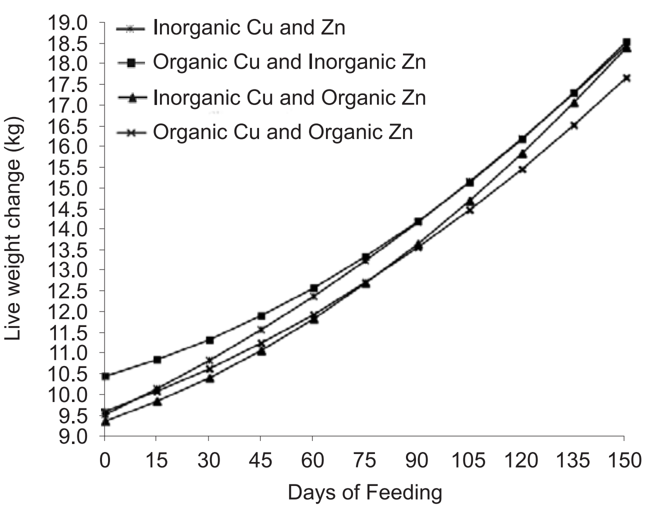

Fig. 1. Live weight change of kids fed organic or inorganic sources of zinc and copper

Live weight (kg, ×) = $0.0293X^2 + 0.54019x + 8.8121$, $R^2 = 0.991$,

Live weight (kg, ■) = $0.046X^2 + 0.2559x + 10.005$, $R^2 = 0.985$,

Live weight (kg, ▲) = $0.0469X^2 + 0.3405x + 8.827$, $R^2 = 0.991$,

Live weight (kg, ×) = $0.0371X^2 + 0.3611x + 9.0677$, $R^2 = 0.984$.

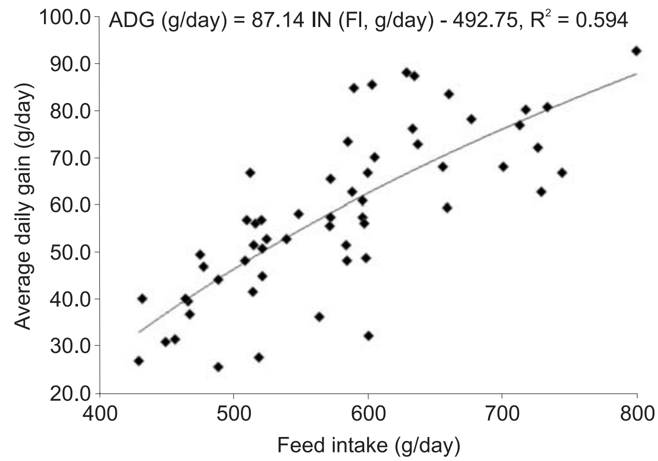

Fig. 2. Relationship of daily feed intake with average daily gain (ADG) in kids.

on average daily gain in present study and N intake ranged from 10.3 to 20.0 g/ day with mean 14.3g, to have mean daily gain of 59g, however an intake of 14.5 to 17 g/day could be considered desirable to have ADG between 70 to 90g/ day (Fig. 3). Rumen fluid pH, NH_3-N and TCA-ppt-protein were significantly different ($P<0.05$), while TVFA content was similar among 4 kid groups. Rumen fluid pH was lower ($P<0.001$), while NH_3-N was higher ($P<0.002$) in kids supplemented OrCu and iZn, whereas rumen fluid pH and NH_3-N levels were not different among other 3 kid groups. The TCA-ppt protein was lower in OrCu and OrZn supplemented kids (Table 4). Haemoglobin (Hb) was lower ($P=0.031$) in kids fed inorganic trace minerals, and Hb had linear and quadratic increase ($P<0.001$) with progress of experimental feeding, interaction between treatment and blood sampling period were also significant. The haemtocrit (Hc) was similar amongst kids of 4 groups but increased linearly ($P<0.02$) with progress of feeding periods. Red blood cell (RBC) and white blood cell (WBC) counts were similar

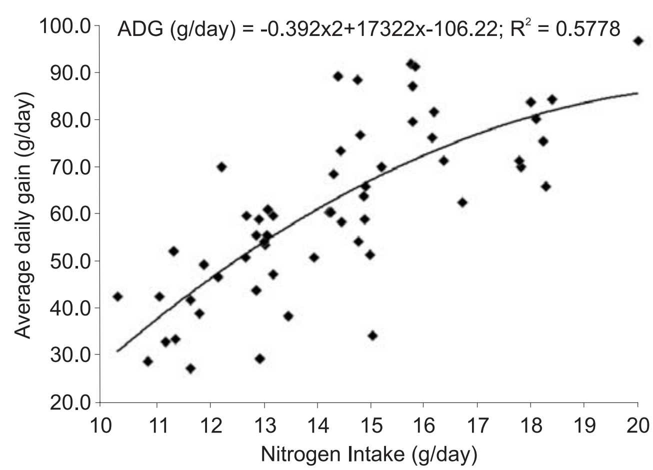

Fig. 3. Relationship of nitrogen intake with average daily gain (ADG) in kids.

Table 3. Nutrient intake and utilization in kids supplemented organic or inorganic Zn and Cu

	Dietary treatments groups*				SEM	*P- value*
	iZn-iCu	iZn-OrCu	OrZn-iCu	OrZn-OrCu		
Body weight (BW, kg)	20.5	20.5	20.3	19.7	0.302	0.812
Dry matter intake						
Green (g/d)	65.3	79.4	75.5	71.6	02.460	0.221
Straw (g/d)	188.07	276.7	192.6	194.8	14.019	0.063
Concentrate (g/d)	348.6	353.9	382.5	352.1	6.572	0.242
Total (g/d)	601.9	710.0	650.6	618.5	17.572	0.132
g $kg^{-1}W^{0.75}$	62.8	73.7	67.8	66.2	1.563	0.084
kg100 kg^{-1}BW	2.96	3.47	3.19	3.14	0.073	0.090
Dry matter digestibility (%)	67.87	67.66	65.08	70.62	01.060	0.399
Organic matter						
Intake (g/d)	564.4	666.3	614.1	580.5	16.496	0.127
Digestibility (%)	70.21	69.90	67.60	72.62	0.994	0.384
Crude protein						
Intake (g/d)	95.2	16.9	103.7	97.6	2.165	0.196
Digestibility (%)	78.38	78.76	78.45	82.05	1.049	0.551
Natural detergent fibre						
Intake (g/d)	301.6	353.6	319.9	307.3	9.277	0.193
Digestibility (%)	58.04	57.09	54.38	61.45	1.417	0.384
N- utilization						
N-intake (g/d)	15.2	17.1	16.6	15.6	0.346	0.196
N-voided (g/d)						
Faeces	3.3	3.5	3.7	2.8	0.203	0.453
Urine	3.3b	3.0b	5.1a	4.5ab	0.296	0.026
Total	6.6b	6.6b	8.8a	7.3ab	0.325	0.040
N-balance (g/d)	8.7	9.9	8.1	8.3	0.361	0.321
ME of diet (MJ/kg DM)	16.44	14.59	14.87	16.59	0.514	0.409
N of diet (g/kg DM)	25.3a	24.2b	25.6a	25.3a	0.208	0.049
ME intake (MJ/day)	9.87	9.83	9.56	10.21	0.139	0.448

*Dietary treatments groups: iZn-iCu, Inorganic Zn and Cu; iZn-OrCu, inorganic Zn and organic Cu; OrZn-iCu, Organic Zn and Inorganic Cu; OrZn-OrCu, organic Cu and Zn.

Table 4. Rumen fermentation characteristics of kids supplemented organic or inorganic Zn and Cu

	Dietary treatments groups*				SEM	*P- value*
	iZn-iCu	iZn-OrCu	OrZn-iCu	OrZn-OrCu		
Rumen fluid pH	6.51a	5.93b	6.41a	6.41a	0.041	<0.001
NH_3-N (mg/dl)	29.76b	40.99a	20.92b	28.41b	1.966	0.002
TVFA (mmol/dl)	10.44	8.75	9.09	10.33	0.460	0.468
TCA-ppt-protein (mg/dl)	123.47a	122.97a	136.30a	81.83b	4.504	<0.001

* Dietary treatments groups: iZn-iCu, Inorganic Zn and Cu; iZn-OrCu, inorganic Zn and organic Cu; OrZn-iCu, organic Zn and inorganic Cu; OrZn-OrCu, organic Cu and Zn.

amongst 4 groups, but WBC decreased linearly ($P<0.001$) with progress in feeding period (Table 5). The lymphocytes ranged from 58.5 to 65% were not different amongst the kid groups. The granulocytes were higher ($P=0.043$) in OrCu and OrZn supplemented kids. Eosinophils and monocytes were not different amongst 4 animal groups. Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration (MCHC) were also similar among the 4 groups, while (MCHC) had linear ($P=0.030$) and quadratic ($P=0.02$) increase with progress in feeding period. Plasma protein and globulin (g/dl), and blood urea nitrogen (BUN, mg/dl) contents were significantly different amongst kid groups (Table 6), while albumin and total cholesterol were similar. Kids supplemented OrCu and OrZn had lower protein ($P<0.001$) and globulin ($P=0.004$) content, while other 3 groups had similar levels. The protein content was not different between 0 and 150 d of experiment while albumin, albumin: globulin ratio, BUN and cholesterol contents reduced and globulin increased at 150 d of feeding. Plasma Zn and Cu contents were similar among 4 kid groups,

Table 5. Hematology of kids supplemented organic or inorganic Zn and Cu

	Dietary treatments groups*				Mean	Significance (P-value)**			
						Treat	Period		T×P
	iZn-iCu	iZn-OrCu	OrZn-iCu	OrZn-OrCu			Lin	Qud	
Hemoglobin (Hb, g/dl)									
0 day	8.5	8.6	8.8	8.8	8.7b	0.031	<0.001	<0.001	0.054
75 day	8.6	8.8	8.5	8.5	8.6b				
150 day	8.5	9.6	9.9	10.0	9.5a				
Mean	8.5b	9.0a	9.0a	9.0a	8.92				
Haematocrit (Hc,%)									
0 day	23.1	23.3	24.2	24.0	23.6	0.532	0.02	0.209	0.946
75 day	23.2	22.5	22.8	25.2	23.5				
150 day	24.5	25.2	24.6	25.5	25.0				
Mean	23.6	23.7	23.9	24.9	24.0				
Red blood corpuscles (RBC, 10^6/dl)									
0 day	17.6	18.8	17.4	18.9	18.2	0.344	0.168	0.346	0.027
75 day	17.7	19.9	16.8	17.6	18.0				
150 day	17.4	18.5	21.2	18.5	18.8				
Mean	17.6	19.0	18.5	18.3	18.3				
White blood corpuscles (WBC, 10^3/μl)									
0 day	18.3	23.8	18.6	23.5	21.1a	0.367	<0.001	0.450	0.059
75 day	19.7	22.2	16.8	18.6	19.4a				
150 day	16.4	15.2	17.9	16.3	16.4b				
Mean	18.1	20.4	17.8	19.5	18.9				
Lymphocytes (%)									
0 day	61.4	63.0	64.5	42.6	58.4	0.074	0.264	0.494	0.016
75 day	59.3	63.5	64.5	63.4	62.6				
150 day	55.2	67.7	65.4	80.7	66.7				
Mean	58.58	65.0	64.9	63.9	63.0				
Granulocytes (%)									
0 day	29.8	30.4	29.5	51.6	34.6	0.043	0.471	0.996	0.056
75 day	29.3	28.2	31.3	32.3	30.2				
150 day	28.7	24.3	27.9	19.4	25.3				
Mean	29.3	27.2	29.5	32.8	29.6				
Eosinophils (%)									
0 day	9.1	6.5	11.5	4.9	7.8	0.531	0.561	0.816	0.037
75 day	11.3	10.1	5.4	12.4	10.2				
150 day	17.9	18.6	0	0	18.2				
Mean	12.5	11.2	7.8	9.0	10.6				
Monocytes (%)									
0 day	1.3	1.8	0.6	1.0	1.2	0.742	0.443	0.597	0.389
75 day	1.7	1.3	1.9	2.1	1.8				
150 day	7.1	1.6	0	0	5.2				
Mean	3.2	1.5	1.3	1.7	1.4				
Mean corpuscular volume (MCV, fl)									
0 day	13.2	13.0	13.8	12.9	13.2	0.520	0.523	0.975	0.634
75 day	13.7	11.9	13.7	14.8	13.5				
150 day	14.3	13.4	12.1	13.9	13.4				
Mean	13.7	12.8	13.1	13.9	13.4				
Mean corpuscular haemoglobin (MCH, pg)									
0 day	4.9	4.8	5.0	4.7	4.9	0.875	0.646	0.589	.996
75 day	5.1	5.0	5.1	5.0	5.0				
150 day	5.0	5.1	4.8	5.5	5.1				
Mean	5.0	5.0	5.0	5.1	5.0				
Mean corpuscular haemoglobin concentration (MCHC, g/dl)									
0 day	36.8	36.7	36.6	36.6	36.7	0.703	0.030	0.029	0.867
75 day	37.1	40.4	37.4	35.7	37.6				
150 day	35.3	38.3	40.0	39.8	38.3				
Mean	36.4	38.4	38.0	37.4	37.54				

* Dietary treatments groups: iZn-iCu, Inorganic Zn and Cu; iZn-OrCu, Inorganic Zn and Organic Cu; OrZn-iCu, Organic Zn and Inorganic Cu; OrZn-OrCu, organic Cu and Zn.

** Treat, effect of treatment; T*P, treatment and period interaction; Lin, linear effect and Quad, quadratic effect of feeding period.

Table 6. Blood bio-chemicals of kids supplemented organic or inorganic Zn and Cu

	Dietary treatments groups*				Mean	Significance (P-value)**		
	iZn-iCu	iZn-OrCu	OrZn-iCu	OrZn-OrCu		Treat	Period	T×P
Plasma protein (g/dl)								
0 day	8.11	6.89	6.68	7.12	7.16	<0.001	0.629	<0.001
150 d	7.29	8.07	7.70	6.09	7.28			
Mean	7.65a	7.5a	7.23a	6.60b	7.23			
Albumin (g/dl)								
0 day	3.63	3.76	3.72	3.49	3.65	0.453	<0.001	0.435
150 day	2.73	2.75	2.60	2.70	2.70			
Mean	3.13	3.24	3.12	3.10	3.15			
Globulin (g/dl)								
0 day	4.47	3.13	2.95	3.63	3.52	0.004	<0.001	<0.001
150 day	4.57	5.32	5.10	3.39	4.59			
Mean	4.53a	4.26a	4.11a	3.15b	4.08			
A:G Ratio								
0 day	0.97	1.27	1.30	2.07	1.44	0.475	0.018	0.776
150 day	0.64	0.53	0.56	0.80	0.63			
Mean	0.78	0.89	0.90	1.43	1.017			
Blood urea-N (mg/dl)								
0 day	24.38	22.35	24.69	19.56	22.51	<0.001	0.001	<0.001
150 day	25.02	21.31	19.02	19.70	21.24			
Mean	24.74a	21.81b	21.64b	19.63c	21.84			
Cholesterol (mg/dl)								
0 day	124.31	124.06	112.74	113.28	118.59	0.605	0.010	0.054
150 day	110.92	108.02	113.58	113.28	111.45			
Mean	117.61	116.04	113.16	113.28	115.02			

*Dietary treatments groups: iZn-iCu, Inorganic Zn and Cu; iZn-OrCu, Inorganic Zn and Organic Cu; OrZn-iCu, Organic Zn and Inorganic Cu; OrZn-OrCu, organic Cu and Zn; **Treat, effect of treatment; T*P, treatment and period interaction.

while plasma Fe content reduced ($P<0.001$) in organic elements supplemented kids. The plasma Fe content reduced ($P=0.005$) at 150 day of sampling than occurred at initiation of the experiment (Table 7).

Present experiment demonstrated that supplementation of organic copper and zinc, and also combinations of inorganic and organic Cu and Zn at 0.75 of required inorganic Zn and Cu level in kids diet did not alter growth, intake, most of rumen fermentation characteristics and blood variables. These are in agreement with the previous findings with kids (Mondal *et al.* 2007), lambs (Cheng *et al.* 2008), sheep (Luo and Dove 1996), calf (Mulhlenbein *et al.*2001) and steers (Wanger *et al.* 2008, Spears *et al.*1991a), who also not demonstrated advantage of feeding trace minerals complex with amino acids. Contrary to present findings, Datta *et al*. (2007) and Garg *et al*. (2008) reported improved nutrient utilization, daily gain and feed efficiency, respectively, in kids fed organic Cu and lambs fed organic Zn. While, Spears (1989) reported 8.1% faster growth and 7.3% higher feed efficiency in heifers and Johnson *et al.* (1988) reported 10.7% faster gain in calves supplemented zinc-methionine in comparison to zinc oxide supplemented diets. Spears *et al.* (1991b) also observed higher gain and feed efficiency in calves supplemented organic zinc. Supplementation of copper and zinc in either form or in combinations did not alter growth, feed intake and feed efficiency. The copper and zinc supplemented in inorganic form were copper sulphate and zinc sulphate, while organic counterparts were organic minerals having amino acid ligation. It was presumed that lower levels of organic zinc and copper could meet the requirement of kids against required levels of inorganic Cu and Zn in diet. It appears that supplementation of organic zinc and copper did not have consistent performance improvements in ruminants. The studies reported improvement on feeding organic trace minerals, when these were fed above the control diets or animals were under experiment after a transit. Spears *et al.* (1991b) and Johnson *et al*. (1988) carried out experiments on calves after transit, while Datta *et al.* (2007) and Garg *et al.* (2008) fed higher levels than control and reported advantages. The present experiment supplemented organic zinc and copper at 0.75 level of required inorganic copper and zinc, hence improvement did not exhibit. However, animals receiving 25% less organic copper and organic zinc maintained performance and plasma zinc and copper levels similar to control lambs. The steers supplemented copper sulphate or copper-proteinate had similar copper status (Witteriberg *et al.*1990). Since copper and zinc are components of several

Table 7. Plasma minerals of kids supplemented organic or inorganic Zn and Cu

	Dietary treatments groups*				Mean	Significance (P-value)**		
	iZn-iCu	iZn-OrCu	OrZn-iCu	OrZn-OrCu		Treat	Period	T×P
Plasma Zn (µg/ml)								
0 day	3.11	3.32	3.80	3.44	3.31	0.493	0.170	0.839
150 day	2.9	2.92	3.10	3.42	3.13			
Mean	3.00	3.12	3.40	3.43	3.22			
Plasma Cu (µg/ml)								
0 day	1.30	1.36	1.08	0.75	1.19	0.139	0.711	0.073
150 day	1.09	1.00	1.14	1.07	1.07			
Mean	1.20	1.18	1.13	0.95	1.12			
Plasma Fe (µg/ml)								
0 day	22.76	13.82	22.14	24.75	19.99	<0.001	0.005	0.029
150 day	23.82	9.92	12.13	10.50	13.95			
Mean	23.27a	11.87b	13.38b	15.82b	16.42			

*Dietary treatments groups: iZn-iCu, Inorganic Zn and Cu; iZn-chCu, Inorganic Zn and Organic Cu; chZn-iCu, Organic Zn and Inorganic Cu; chZn-chCu, organic Cu and Zn; **Treat, effect of treatment; T*P, treatment and period interaction.

enzymes or enzyme systems and co-factor for several enzymes in biological system, the growth promotion of both zinc and copper is through stimulation of enzymes responsible for nutrient utilization and of metabolic activities. Therefore, animals fed 25% less copper and zinc as organic complex as its inorganic level did not affect performance, intake and maintained similar levels of plasma copper and zinc.

In present study, daily feed DM intake did not improve but feed DM intake per unit live weight showed a trend of improvement (P=0.090) by the organic trace minerals supplementation. Marked improvement was with animals supplemented inorganic Zn and organic Cu. The digestibility of DM, OM, CP, NDF, N-balance and ME intake did not change, which were in agreement of previous researchers (Garg *et al.* 2008, Puchala *et al.*1999, Mondal *et al.* 2007, Spears *et al.*1991b), who reported improved intake and no improvement of digestibility and N-balance upon organic trace minerals supplementation. The digestibility of nutrient only improved, when dose of the trace minerals varied, therefore, feed DM intake and digestibility may get altered with higher levels of copper and zinc but form of supplementation did change (Cheng *et al.* 2010).

Present findings of rumen fermentation characteristics are within the reported range of normal variations of native growing goat kids (Dutta *et al.* 2009, Chaudhary, 2011, Bhatta *et al.* 2007, Kushwaha 2011) offered diets with varying roughage to concentrate ration or crude protein and energy levels. The mean rumen fluid pH in the present study remained within the critical rumen pH range of 6.0 to 7.0 reported for optimum nutrient utilisation and microbial growth (Stewart 1977, Terry *et al.* 1969). The mean values of rumen ammonia-nitrogen obtained in this study are mostly within the range of 50–250 mg NH_3-N/l of rumen liquor recommended as being adequate for microbial growth with higher levels more preferable for continuous supply of high levels of rumen ammonia-nitrogen for more efficient microbial activities (Preston and Leng 1987). Dietary copper is known to eliminate or reduce protozoa from rumen (Mendoza *et al.*1993, Engle and Spears 2000) and dietary zinc inhibits activity of cellulolytic enzymes produced by fibre digesting ruminal microbes (Arelovich *et al.* 2000, Eryavuz and Dehority 2009), thus affecting rumen fermentation and nutrient utilization. Possibly organic copper and zinc supplementation improved ruminal concentration and availability of zinc and copper, which reduced protozoa and other fibre degrading microbes activity which resulted in lowered TCA-ppt protein levels. The reduced ruminal fluid pH and increased ammonia nitrogen in kids supplemented with organic copper and inorganic zinc may be due to reduced bio-availability of zinc and higher bio-availability of copper creating a mineral imbalance in rumen, as higher copper is known to reduce bioavailability of zinc (Suttle 2010, McDonald *et al.* 2008).

Reduced plasma protein, albumin and blood urea nitrogen in organic Zn and Cu supplemented kids of present study corroborated favorably with the findings of Mondal *et al.* (2007) and Cheng *et al.* (2010), who reported significantly altered protein synthetic activities of liver upon supplementation of organic copper and zinc. Reduced plasma protein and blood urea nitrogen levels in organic zinc and copper supplemented kids is possibly due to higher bio-availability of zinc and cooper, which reduced protein synthetic and increased lipolytic activities in liver (Cheng *et al.* 2010) causing reduced levels of plasma proteins with prolonged feeding. Except haemoglobin, feeding of organic or inorganic zinc and copper did not change hematology of lambs, while hemoglobin, PCV increased and WBC reduced linearly with progress in feeding period. This showed that organic copper and zinc improved hemoglobin synthesis because both zinc and copper are known to accelerate hemoglobin synthesis via several iron transformation and incorporation pathways (Suttle 2010, McDonald *et al.* 2008). Non infectious disease conditions and stressed conditions increases WBC counts (Solaiman *et al.* 2007), reduced WBC indicated that with progressed feeding animal status of copper and zinc improved resulting better health status because supplementation of copper and zinc known to improve health status and reduce stress (Spears *et al.*1991b, Johnson *et al.*1988). Serum levels of copper, zinc and iron of present study are in accordance with the normal range reported for goats and kids (Ahmed *et al.* 2001, Haenlein and Anke 2011). Plasma zinc, copper and iron reduced with progress in feeding experiment and organic minerals reduced plasma levels of iron. Dietary zinc and copper known to reduce bio-availability of iron in ruminants and both have antagonism to iron (Suttle 2010). Garg *et al*. (2008) also reported lower levels of serum iron in lambs fed organic zinc.

It is concluded that **s**upplementation of organic zinc and copper at 0.75 level of inorganic requirement did not change intake, performance, nutrient utilization, hematology and blood biochemistry of kids. The combination of inorganic Zn and organic Cu supplementation tended to improve feed DM intake (per unit BW), reduced rumen fluid pH, increased rumen NH_3-N in kids. The growing Barbari kids under present feeding protocol consumed 567 g feed and 14.3g nitrogen to have 59 g daily gain. Supplementation of organic Zn and Cu improved blood hemoglobin and reduced cholesterol showed health promoting effects. Therefore, kids may be fed diets with organic Zn and Cu at 25% lower level than the required inorganics during active growth phase without compromising performance.

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