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Stress appraisal by organic-inorganic zinc and copper supplementation in Beetal kids

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ABSTRACT

To assess the stress tolerance, challenge stressor was induced in 14 Beetal kids of 3 months after random distribution in 2 groups (T_{MM} and T_{OM}). All kids received *ad lib.* concentrate feed supplemented with 2% mineral mixture of similar composition except T_{MM} with inorganic Zn and Cu as sulphate salts and T_{OM} with organic amino acid-mineral complex as Zn-lysine and Cu-lysine. The kids of both groups were challenged with 0.2 ml adjuvant complete freund (ACF) intra-dermally after 90 days of feeding. Hb and PCV declined in T_{MM} group. TLC declined in T_{OM} group. Serum creatinine was higher in T_{MM} group. Serum BUN and albumin elevated after challenge in T_{OM} group. Serum glucose declined after challenge in T_{MM}. At the end of study GPX level was higher in T_{OM} group. It was concluded that organic Zn and Cu had better potential for stress tolerance in goat kids.

Key words: Copper, Inorganic, Kids, Organic, Stress, Zinc

Zinc (Zn) and Copper (Cu) are essential trace minerals having positive influence on animals' productive performances and may be supplemented as inorganic salts or as complex form with organic molecules. The inorganic minerals are lower in cost but with restricted bioavailability due to antagonisms and interactions (Leeson and Summers 2000, Mondal and Biswas 2007). Minerals complexed with organic molecules usually resist many interactions and recommended in animals' diet to counter trace mineral deficiency due to their higher bioavailability (Mondal and Biswas 2007). However, higher cost of organic minerals is major shortcomings for its supplementation (Attaelmannan and Reid 1996). But additional advantages of organic minerals in terms of productivity under stressful conditions (Perucchietti and Litjens 2010) supports its supplementation. It has been indicated that Cu-lysine may be more beneficial than $CuSO_4$ in correcting Cu deficiency (Rabiansky *et al.* 1999). This fact needs to be established in goats under stall-fed conditions where they face many stressors during life cycle. Therefore, present study was undertaken to investigate the effect of immunological challenge stressor, as a model of natural stressors, on haematological and biochemical profiles in Beetal kids fed with mineral mixtures containing organic and inorganic zinc and copper.

MATERIALS AND METHODS

Experimental design: This study was undertaken at Goat Research Farm of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. Study was undertaken after permission of the Institutional Animal Ethics Committee. Fourteen, 90–d old farm born Beetal kids of equal weight and parity, were randomly divided into two groups i.e. T_{MM} and T_{OM} (7 in each). The kids were fed individually. They received either concentrate mixture containing 2% inorganic mineral mixture (T_{MM}) with zinc sulphate and copper sulphate as mineral source or prepared zinc and copper lysine chelate (T_{OM}) in addition to *ad lib.* seasonal green fodder, for 120-d after weaning. The concentrate feed comprised maize 37, soybean meal 30, wheat bran 12, rice bran 18, mineral mixture 2 and salt 1 parts by weight and mixed with 25 g vitamin premix per 100 kg feed. Kids were kept individually in wire meshed iron enclosures of 1.44 m^2 area. Adjustable feeders of 12 × 8 × 5.5 inches and 18 × 12 × 6 inches for concentrate and roughages, respectively, were fixed in each enclosure. All other management practices were similar for both the groups.

Immunological challenge: After 90 days of feeding kids of both groups were immunologically challenged by injecting adjuvant complete freund (ACF) @ 0.2 ml by intra-dermal (I/D) route. The blood samples (2 ml), at monthly intervals from beginning to 120 days, was collected from jugular vein in K_3 EDTA coated vials, as described

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by Jain (1986). Blood was also collected one day before and three days after the challenge with ACF.

Blood and serum profiles: Haemoglobin (Hb) was estimated by Sahli's Method. Packed cell volume (PCV) was analyzed by haematocrit centrifugation method. Total leukocyte count (TLC) was determined by Neubar's counting chamber (Benjamin 1985). Differential leukocyte count (DLC) was estimated Wright-Giemsa stain method (Jain 1986). For serum biochemical profiles, blood was collected in acid free vials without any anticoagulant. Serum was separated and stored in eppendorf's tube in triplicate at –20°C for further use. For serum glucose estimation, blood was collected in sodium fluoride vials. VITROS DT 60 / DT 60 II chemistry system was used for estimation of serum profiles like glucose, total protein, albumin, blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine (CRSC) and iron (Fe). Serum copper (Cu) and zinc (Zn) was analyzed through a commercial firm. The activity of superoxide dismutase (SOD) and glutathione peroxidise (GPX) in erythrocyte lysate was determined as per the methodology of Marklund and Marklund (1974) and Hafeman *et al.* (1974), respectively.

Statistical analysis: The collected data were analyzed by SPSS 16.0 with one way ANOVA using F-test (Snedecor and Cochran 1989) to find out the significant differences between treatments.

RESULTS AND DISCUSSION

Haematological profiles: There was nonsignificant effect of immunological challenge on blood haemoglobin (Hb) in organic mineral group (T_{OM}) and differed significantly ($p<0.05$) in T_{MM} during different phases of challenge (Table 1). This may be due to almost static Hb level throughout study period except on 93rd day where it declined nonsignificantly. In T_{MM} group, Hb level declined significantly ($P<0.05$) between pre and post-challenge period. Static Hb in T_{OM} between pre-challenge (60 days) and at the end of study period as compared to T_{MM} group may be due to better gut absorption and bioavailability of Zn and Cu from organic mineral sources than inorganic one. Amino acids form Zn and Cu complexes with high stability constants, and it has been suggested that such complex formation facilitates Zn and Cu uptake from the intestine (Rucker *et al.* 2008). Hatfield *et al.* (2001) opined that feeding Zn in presence of Cu, increases absorption and retention of Zn. Furthermore, dietary Cu-sulphates of inorganic groups reacts with some of the trace minerals (e.g. Molybdenum) to form an insoluble complex which make Cu biologically less active and less bio-available (Rucker *et al.* 2008). Organic Cu and Zn compounds may be due to unique coordination chemistry, probably permitted the formation of highly soluble, chemically stable products which possibly resisted digestion and interaction with antagonists in the gut (Mondal and Biswas 2007). Since Zn is essential for the function of more than 200 enzymes and Cu improves iron absorption (O'Dell and Sunde 1997), mobilization and utilization, partly because of Cu's role as a redox cofactor in various membrane associated ferrioxidases that oxidize Fe^{+2} and Fe^{+3}, thereby, promoting the transfer of iron to transferring, hence, haemoglobin synthesis. Due to same reason PCV might increased in T_{OM} group and declined significantly ($P<0.01$) in T_{MM} group, at the end of study period (Table 1). Declining trend of Hb and PCV, after immunological challenge, has been observed in both groups which may be due to intense stress in kids of both groups. Sivakumar *et al.* (2010) also reported declining trend of Hb and PCV in heat stressed goats. Naber

Table 1. Haematological profiles of Beetal kids during study period

Parameters	Challenge/ Treatment	Pre-challenge		Post-challenge		P value (F-test)
		Up to 60 days	90th day	93rd day	120th day	
Haemoglobin, g/dl	T_{MM}	9.27^{a}	9.00^{ab}	8.46^{b}	9.01^{ab}	0.013
	T_{OM}	9.51	9.67	9.03	9.63	0.430
PCV,%	T_{MM}	26.00^{bc}	29.57^{a}	28.57^{ab}	25.57^{c}	0.007
	T_{OM}	27.55^{b}	32.00^{a}	30.14^{ab}	27.16^{b}	0.011
TLC, 10^3 /µl	T_{MM}	18.70	22.40	21.70	17.50	0.283
	T_{OM}	20.50^{ab}	25.20^{a}	24.30^{a}	16.50^{b}	0.029
Neutrophil,%	T_{MM}	36.48	33.86	38.43	35.43	0.863
	T_{OM}	34.48	32.86	29.57	38.43	0.374
Lymphocyte,%	T_{MM}	63.33	66.14	60.14	64.29	0.733
	T_{OM}	65.71	66.00	69.86	61.43	0.472
Eosinophil,%	T_{MM}	0.14	0.00	1.43	0.29	0.056
	T_{OM}	0.29	0.00	0.29	0.14	0.682
Neutrophil, 10^3 / µl	T_{MM}	6.84	7.46	8.38	5.99	0.445
	T_{OM}	6.95	8.04	7.14	6.20	0.570
Lymphocyte, 10^3 / µl	T_{MM}	11.80	15.00	13.00	11.40	0.332
	T_{OM}	13.70^{ab}	16.80^{a}	17.00^{a}	10.30^{b}	0.028
Eosinophil, No. / µl	T_{MM}	45.62	0.00	325.14	51.43	0.075
	T_{OM}	52.90	0.00	76.00	0.00	0.470

Means with different superscripts in a row differs significantly.

et al. (1996) observed decreasing serum Zn due to inflammatory stress which is similar to the findings of present study.

Total leukocyte count (TLC) had increasing trend, both in T_{OM} and T_{MM} group, during different periods except at the end of study which had declining trend. This may be due to advancement of age and proper intake of Zn and Cu which helped in proper immunity boost-up (Rucker et al. 2008) in both treatment groups. This built-up immunity with age may be the possible reason for almost constant TLC during challenge phase. Slightly higher TLC in both treatment groups than the reference values (Kramer 2000) may be due to age, breed and environmental differences. Furthermore, none of the animals showed any sign of illness throughout study period irrespective of type of mineral mixture fed. Kramer (2000) hypothesized that endogenous corticosteroid response of excitement and stress produces neutrophilia and lymphopenia. Based on this hypothesis it can be opined that kids of T_{MM} group possibly encountered stress during challenge as they depicted trends of neutrophilia and lymphopenia. It was also opined that in later life of ruminants, lymphocyte acts as dominant leukocyte (Kramer 2000). Nonsignificantly higher trend of lymphocytic count, both per cent and absolute, in T_{OM} than T_{MM} group, during entire study period as well as during pre- and post- challenge periods proved lesser stress in kids fed with organic Cu and Zn. This also proves that organic Zn and Cu probably produced better immunity during challenge period, as reported by Rucker *et al.* (2008), and may be helpful to cope the stressful situation during the lifetime of animals. Though, eosinophilia has been observed in cases related to parasitism, higher eosinophilic count in T_{MM} group immediately after challenge may be due to some reactions related to stress or coccidia proliferation in the gut, as coccidia are opportunistic pathogen inhabiting intestine and get proliferated under the influence of various stressors (Cynthia 2011).

Serum biochemistry: Both serum Zn and Cu elevated (P<0.01) during pre-challenge period (Table 2), irrespective of groups, probably due to sufficient Zn and Cu content in mineral mixture irrespective of sources. After immunological challenge, serum Zn level declined (p<0.01) irrespective of mineral sources and then elevated (P<0.01) to pre-treatment level (Table 2). Declining trend of Zn after challenge may be a body compensatory mechanism to cope stressful situation. Once stress gone, Zn level begun to incline further in both groups. Contrast to Zn, serum Cu level was static till challenge (93 days post-feeding) and then declined. Nonsignificantly higher trend of serum Cu and Zn during study in T_{OM} may be due to its higher

Table 2. Serum biochemical profiles of Beetal kids during study period

Parameters	Challenge/ Treatment	Pre-challenge		Post-challenge		P value (F-test)
		Up to 60 days	90th day	93rd day	120th day	
Zinc, µg/dl	T_{MM}	180.66^{b}	237.80^{a}	91.96^{c}	167.57^{b}	0.000
	T_{OM}	195.16^{b}	236.43^{a}	115.16^{c}	193.09^{b}	0.000
Copper, µg/dl	T_{MM}	79.15^{b}	129.14^{a}	126.61^{a}	61.84^{b}	0.000
	T_{OM}	85.03^{b}	136.30^{a}	133.50^{a}	72.16^{b}	0.000
Iron, µg/dl	T_{MM}	141.24	126.00	148.86	121.71	0.775
	T_{OM}	140.33	122.43	149.29	142.57	0.420
Creatinine, mg/dl	T_{MM}	0.54^{b}	0.40^{b}	0.40^{b}	0.89$^{a}_{x**}$	0.000
	T_{OM}	0.55^{a}	0.34^{b}	0.40^{ab}	0.39$^{ab}_{y**}$	0.016
ALP, U/l	T_{MM}	267.05	253.14	256.71	197.57	0.554
	T_{OM}	252.38	222.29	285.71	329.86	0.404
ALT, U/l	T_{MM}	18.43	10.86	10.14	11.43$_{x**}$	0.313
	T_{OM}	18.19^{a}	10.00^{b}	7.57^{b}	17.29$^{a}_{y**}$	0.001
AST, U/l	T_{MM}	114.52	104.86	83.71	120.00	0.677
	T_{OM}	105.36	90.86	91.00	99.57	0.700
BUN, mg/dl	T_{MM}	22.90	21.29	26.86	20.43	0.112
	T_{OM}	23.19^{b}	22.43^{b}	28.86^{a}	22.57^{b}	0.044
Total protein, g/dl	T_{MM}	6.29$^{ab}_{x**}$	6.73^{a}	6.64^{a}	6.11^{b}	0.048
	T_{OM}	6.76$_{y**}$	7.00	6.89	6.37	0.138
Albumin, g/dl	T_{MM}	2.45$_{x**}$	2.69	2.60	2.44	0.108
	T_{OM}	2.64$^{b}_{y**}$	2.76^{ab}	2.84^{a}	2.57^{b}	0.000
Glucose, mg/dl	T_{MM}	49.57^{b}	62.71^{a}	57.86^{ab}	51.43^{b}	0.024
	T_{OM}	47.52^{b}	63.71^{a}	62.29^{a}	62.14^{a}	0.002
SOD, EU/g Hb	T_{MM}	0.05	0.06	0.06	0.04	0.093
	T_{OM}	0.05	0.06	0.06	0.04	0.154
GPX, EU/g Hb	T_{MM}	0.58^{b}	0.92^{a}	0.15^{c}	0.34$^{c}_{x**}$	0.000
	T_{OM}	0.54^{b}	0.74^{a}	0.15^{d}	0.59$^{b}_{y**}$	0.000

Means with different superscripts (a, b, c, d) in a row and different subscripts (x, y; *P<0.05, **P<0.01) in a column for a particular parameter differs.

absorbability and retention. Similar observations were reported in lambs (Garg *et al.* 2008, Shinde *et al.* 2013) showing higher mineral bioavailability in organic group. Serum Fe level was static during study indicating sufficient Cu for haemopoetic functions, as Cu helps to increase total erythrocyte count in ruminants (Mondal and Biswas 2007). This probably resulted in higher Hb and PCV during study (Table 1) among kids of both groups. Many workers inferred no effect of organic or inorganic mineral supplementation on blood Fe (Garg *et al.* 2008, Jia *et al.* 2009) in lambs.

Normal creatinine for goats ranged between 1.0 to 1.8 mg/dl (Kaneko *et al.* 2008). Among healthy subjects, creatinine molecules are totally eliminated by glomerular filteration. During kidney failure its plasma concentration increases. Values below reference range, in present study, may be due to breed and age where younger animals have lesser creatinine level than adult ones. This also indicated active and efficient filtration of creatinine from kidney under the influence of different trace minerals including Zn and Cu. Higher plasma Zn with lower Cu/Zn ratios are the conditions where lower plasma creatinine level has been observed (Guo and Wang 2013). Furthermore, Zn supplementation ameliorates abnormally high plasma Cu/ Zn ratios and reduces oxidative stress, improves inflammatory status, and maintains immune functions (Guo and Wang 2013). During our study also, lower Cu/Zn ratio immediately after challenge was observed in organic mineral group. This was indicative of lesser oxidative stress and inflammatory responses as well as better stress tolerance by kids of T_{OM} group. Higher Cu/Zn ratio just after challenge probably caused oxidative stress and resulted in significantly higher ($P<0.01$) serum creatinine in T_{MM} over T_{OM}. The serum Alkaline phosphatase (ALP) was within normal range of 93 to 387 U/l in kids of both groups (Kaneko *et al.* 2008). Similar ALP activity after supplementation of inorganic and organic minerals has been reported by many workers (Mandal *et al.* 2008, Shinde *et al.* 2013). ALP is one of the mammalian Zn metallo-enzymes (Rucker *et al.* 2008) and its elevating level, even after challenge, proves better stress tolerance among kids of T_{OM} group. ALP control important vital functions like bone mineralization (Hoffmann and Solter 2008), dephosphorylation of bacterial endotoxin with diminished toxic effect in intestine etc (van Veen *et al.* 2005) which probably resulted in better growth performance of kids of T_{OM} group even after similar concentrate and dry matter intake. In contrast, Jia *et al*. (2009) observed higher ($P<0.05$) ALP in plasma of Cashmere goats fed with $ZnSO_4$ than Zn-Met group. This may be due to different sources of amino acids used to form organic mineral complex or different age, breed and agro-climatic conditions of animal. Serum alanine aminotransferase (ALT) and aspartate aminotransferage (AST) level were within normal physiological range as described by Kaneko *et al*. (2008) and Cynthia (2011), in kids of both groups. This indicated normal liver function and physiology of kids. Similar observations had been reported by many workers (Mandal *et al.* 2008, Mondal and Biswas 2007). Ratio of serum ALT-to-ALP activity is indicative of physiological status of metabolically active glands especially liver. Higher ALT-to-ALP ratio is indicative of hepatic disorders which may be due to some stressful and toxic situation (Hoffmann and Solter 2008). In present study this ratio was higher in T_{MM} group especially after immunological challenge which is indicative of efficient stress coping status of kids in T_{OM} group. Significantly higher ($P<0.05$) serum BUN level after challenge may be due to better protein utilization and albumin status of kids in T_{OM} group even in stressful conditions. Better protein utilization might have resulted in higher growth (Fig. 2) of kids especially during post-challenge phase (Mondal and Biswas 2007). The role of Zn in pituitary hormone function has been identified (Henkin 1976) through growth hormone (GH) mediated action which stimulates cell proliferation and growth. Due to better Zn absorbability, bioavailability, utilization and efficient GH mediation, kids of organic mineral group probably depicted improved growth (Fig. 2). Again, Zn has significant role in protein and carbohydrate metabolism and it may be the reason for comparable total protein, albumin and glucose status in all kids during post-challenge phase in both organic and inorganic groups. Normal physiological ranges of total protein, albumin and glucose (kaneko *et al.* 2008, Cynthia 2011) at the end of study further indicated apparently healthy animals in both groups and signified the role of Zn and Cu supplementation in organic or inorganic form.

Superoxide dismutase (SOD) is a Cu- and Zn- containing enzyme which acts as antioxidant by promoting the dismutation of two O^{-2} molecules to hydrogen peroxide and oxygen and finally prevents the build up of superoxide in cell (Harvey 2008). Glutathione Peroxidase (GPX) also acts as antioxidant by catalyzing the conversion of hydrogen peroxide to water (Harvey 2008). Non-significant differences for SOD between T_{MM} and T_{OM} indicated optimal Zn and Cu level in kids of both groups. Significantly higher ($P<0.01$) level of serum GPX in T_{OM} than T_{MM}, at the end of study (Table 2), may be due to better Zn and Cu utilization from organic mineral source. This also indicated better conversion of hydrogen peroxide to water (Harvey 2008) and prevention of animals from oxidative stress in organic mineral groups. Derouiche *et al.* (2013) also reported increased ($P<0.01$) GPX activity due to copper addition in the diet of rats.

The findings of present study signifies better physiologic role of the organic Zn and Cu for efficient development of haemopoetic activities, balanced serum biochemical levels and stress tolerance of goat kids. Under stall-fed conditions kids encounter many stressors and organic mineral supplementation may be helpful in improving productivity by mitigating different stresses. Finally, it was concluded that organic Zn and Cu had better capability for stress tolerance in goat kids.

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