EFFECT OF ORGANIC MINERAL SUPPLEMENTATION ON BLOOD BIOCHEMISTRY, PLASMA ANTIOXIDANT ENZYME ACTIVITIES AND THEIR CORRELATION TO SPERM FUNCTIONAL CHARACTERISTICS IN OSMANABADI BUCKS

A. ARANGASAMY[1*], SHARANYA J NAIDU[2], K. HEMALATHA[3], B. RENU SHARMA[4], S. SELVARAJU[5], M. VENKATA KRISHNAIAH[6], G.P. RANI[7], B. K. BINSILA[8], J.P. RAVINDRA[9] AND B. RAGHAVENDRA[10]

Reproductive Physiology Laboratory,Animal Physiology Division, ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, Karnataka -560030, INDIA

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ABSTRACT

This study was carried out to investigate the effect of organic copper (Cu) and zinc (Zn) supplementation on blood biochemical parameters and antioxidant enzyme properties in growing Osmanabadi goats at the onset of puberty and semen quality. Forty bucks (five months) were allocated randomly into ten groups, all were fed with basal roughage and a concentrate mixture (50 : 50) supplemented (for a period of 8 months) either with different doses of organic Zn : 20 mg (T1), 40 mg (T2), 60 mg (T3), organic Cu : 12.5 mg (T4), 25 mg (T5), 37.5 mg (T6) and combination of Cu + Zn: 12.5 mg + 20 mg (T7), 25 mg + 40 mg (T8), 37.5 mg + 60 mg (T9), respectively, per kg dry matter (DM) or fed without additional mineral supplementation (Control). Blood samples collected on days 120 and 240 of treatment for analysis oftotal protein; albumin; cholesterol; high density lipoprotein (HDL) and glucose as well as enzyme activities like Catalase (CAT), Superoxide dismutase (SOD), Glutathione reductase (GR) and Glutathione peroxidase (GPx) were analyzed and correlated with spermatozoa functional characteristics. Significantly increased ($P<0.05$) enzymatic activities at days 120 and 240 were observed with positive correlations to the spermatozoa functional characteristics and enzyme activities. Varying level ofalbumin, glucose, HDL and protein in Cu treated groups of the experiment. Cu supplementation affects protein, glucose, HDL and albumin levels in goats; and supplementation of both organic Zn and Cu significantly increased the antioxidant defense enzyme activities in goats with positive correlation to sperm functional attributes.

Key words: Zn, Cu, Goat, Blood plasma, Antioxidant enzymes, Sperm functional attributes

INTRODUCTION

Organic Zinc and Copper supplementation influences the semen characteristics, fertilityand also protect the spermatozoa from oxidative damages in goats (Arangasamy *et al*. 2018a; Arangasamy *et al*. 2018b; Hemalatha *et al*. 2018; Narasimhaiah *et al*. 2018; Rahman *et al*. 2014) .In addition, the organic forms of trace minerals have significant effects on maintaining optimum reproductive function because of their role in spermatogenesis, sperm production and fertility (Rowe *et al*. 2014; Dance *et al*. 2016). Protein, mineral, energy diets are supplemented at prepubertal age for advancing the onset of puberty, enhanced sperm quality and fertility in farm animals (Dance *et al.* 2016; Geary *et al.* 2016). Supplementation of trace minerals leads to changes in sperm membrane to protect sperm cells from cryo damages or to improve the quality of the sperm production ((Renard *et al.* 1996; Sánchez-Partida *et al.* 1999).

Recently, it was reported that in goats, organic mineral supplementation lowered the lipid peroxidation in spermatozoa and seminal plasma along with a stronger antioxidant immune system(Arangasamy *et al.* 2018b; Narasimhaiah *et al.* 2018). Analyzing the blood biochemical components and plasma antioxidant enzyme activities in mineral supplemented bucks will aid in exploring the possible correlation between the enzyme activities and sperm functional characteristics and may also serve as good indicators of optimal physiological functions.

[1]Principal Scientist (arangasamyars@gmail.com), [2,6]Ph.D Scholar, [3-4]Junior Research Fellows, [5]ICAR-National Fellow, [7]Post-Doc, [8]Scientist, [9]Principal Scientist & Head (Rtd), 1-9 Animal Physiology Division, [10]Director, ICAR NIANP.

MATERIALS AND METHODS

Experimental animals, feeding and management

Forty bucks (n = 40) of five months old, were allocated randomly to ten groups of four bucks each. All the goats were fed with the basal roughage and a concentrate mixture (50 : 50) as per the ICAR recommendation (Ranjhan, 1998) supplemented (for a period of 8 months) either with different doses of organic Zn : 20 mg (T1), 40 mg (T2), 60 mg (T3), organic Cu : 12.5 mg (T4), 25 mg (T5), 37.5 mg (T6) and combination of Cu + Zn: 12.5 mg + 20 mg (T7), 25 mg + 40 mg (T8), 37.5 mg + 60 mg (T9), respectively, per kg dry matter (DM) or fed without additional mineral supplementation (Control). The complete feeding pattern and composition of diet ingredients were followed as previously reported(Narasimhaiah*et al.* 2018).

BIOCHEMICAL PARAMETERS STUDIED IN BLOOD PLASMA

Biochemical parameters such asblood glucose, albumin, HDL and cholesterol were estimated using Autospan diagnostic kits, India, following the supplier's procedure using microplate photometer (Thermo Scientific multiskan FC microplate photometer, Finland). Total protein was estimated using BCA (Bicinchoninic Acid) kit as per the supplier's procedure.A working solution was prepared by adding 50 parts of BCA and 1 part of copper solution. Standard and protein samples (25 il each) were added followed by transfer of 200 ìl of working solution into a microplate well and incubated for 30 minutes (37ºC). The absorbance was measured in a microplate photometer (Thermo Scientific multiskan FC microplate photometer, Finland) at 562nm. The absorbances of samples were subtracted from the blank and a standard curve was plotted from 2 mg to 15.625 mg to determine protein concentrations.

ESTIMATION OF ENZYME ACTIVITIES IN BLOOD PLASMA

Antioxidant enzymes such ascatalase, superoxide dismutase, glutathione reductase and glutathione peroxidase were estimated in the study. The CAT (Aebi 1984), SOD (Marklund and Marklund 1974), GR (Carlberg and Mannervik 1975), and GPx (Paglia and Valentine 1967) activity assays were carried out as per the given standard protocol. Assays were monitored for 3 minutes at 30 sec interval in UV spectrophotometer (Biochrom Libra S32 UV/Vis Spectrophotometer, UK).

STATISTICAL ANALYSIS

Statistic analysis of the data was done using Statistical Package for the Social Sciences (SPSS) version 20, One-way Analysis of variance (ANOVA) was done while LSD was used for mean separation of antioxidant enzymes and biochemical parameters in blood plasma for the comparison of treatment groups with that of control. Anitoxidant enzymes were correlated with sperm functional attributes using Pearson correlation coefficient. Data was expressed as mean±SEM, and the values were considered to be significant at Pd"0.05.

RESULTS AND DISCUSSION

Effects of feeding organic trace mineral (Zn and Cu) at the onset of puberty, semen characteristics, cooling and equilibration, cryopreservation and *in vitro* fertility in indigenous Osmonabadi goat have been studied earlier(Arangasamy *et al.* 2018a and 2018b; Hemalatha*et al.* 2018; Narasimhaiah *et al.* 2018). The present investigation was the continuation of our earlier report(Arangasamy *et al.* 2018a and 2018b; Hemalatha *et al.* 2018; Narasimhaiah *et al.* 2018). The thrust of the continuous investigation is to assess the effects of supplemented mineral (Cu and Zn alone or in combinations) on blood plasma total protein, albumin, glucose, cholesterol, HDL, CAT, SOD, GPx and GR.

Alterations in the serum hematological and biochemical components are observed in goat upon supplementation of trace mineral. Biochemical parameters in blood plasma from mineral supplemented groups weredescribed in Table 1 the effect of mineral supplement on blood biochemical parameters. The treatment had significant (P<0.05) effect on albumin, cholesterol, HDL, glucose and total protein. Albumin level decreased significantly in T3, T6, T7, T8, and T9 groups of 120 days treatment and in T5, T6, T7, T8, and T9 groups of 240 days treatment compare to control. Cholesterol increased significantly in T6 of 120 days treatment as compared to control. Significantly lower level of cholesterol was observed in T2 and T5 groups of 240 days treatment as compare to control. HDL increased significantly in T4, T5, T6, T7, T8 and T9 groups of 120 days of treatment and no significant difference was observed in 240 days of treatment as compared to control. Although glucose showed no significant difference at 120 days of mineral treatment, significantly decreased levels were observed in T7, T8, and T9 groups at 240 days of treatment as compare to control. Protein was lower in T2 of 120 days treatment and in T9 group of 240 days treatment compare to control.

In the present study, glucose and cholesterol levels (except T6 group) were not influenced by the supplemented trace minerals in goats at 120 days. There was a significant (*P*<0.05)reduction in the levels of cholesterol (medium dose @ T2 and T5 group) and glucose (combination group: T7, T8, and T9 group) at 240 days in selected treatment groups. Studies have shown that, human patients supplemented with zinc had reduced level of serum cholesterol(Milbury and Richer 2007; Nakashima and Dyck 2009).The studies in steer indicated that supplementation of Zn did not influence the serum glucose and cholesterol(Malcolm-Callis *et al.* 2000; Whitman *et al.* 2007). The mechanism involved in reducing the level of glucose and cholesterol in Zn and Cu treated goats could be their involvement in glucose transport and energy conversion mechanism(May and Contoreggi 1982). Copper treated groups also showed a reduced level of cholesterol and the probable mechanism could be an involvement of Cu in lipid metabolism(Samanta *et al.* 2011). There was significant increase in HDL levels in Cu treated groups of 120 days of treatment and no effect was observed in 240 days treatment. Increase in HDL level in the Cu supplemented groups was in contrast to the findings in rats (Carr and Lei, 1989). The observedsignificantly lowered (P<0.05) level of albumin in certain groups Cu treated goats compared to control group is similar to the earlier report

conducted in male mink(Xuezhuang Wu *et al*. 2015). In contrast, the supplementation of zinc components had no effect on serum protein and albumin in buffalo calves(Mandal and Dass 2010). The variations in the observed biochemical effect due to supplementation of zinc and copper in some of the species might be due to dose dependent effect or could be of strong association between the type of mineral supplemented or incompatibility in the action of mineral in either alone or in combination forms.

Cu and Zn relatively have shown high antioxidative property in the present study through enhanced level of SOD, CAT, GPx and GR activities and positive correlation with various sperm functional parameters. No supporting report was available to correlate enzyme activities with mineral supplementation. Zn and Cu reduced oxidative stress by increasing the enzyme defense system and had positive correlation of CAT, SOD, GPx, GR activities with various sperm functional parameters. These observations are similar to the previous work conducted in goat through supplementation of Cu and Zn(Narasimhaiah *et al.* 2018). Narasimhaiah and co-workers observed a correlation between goat seminal plasma enzymes activities and sperm functional characteristics. In another study, (Patricio *et al.* 2016) noticed a positive correlation between sperm functional characteristics of spermatozoa and seminal plasma enzymes in human. It is necessary to maintain a balance between CAT and SOD activities for proper serum homeostasis as studied for sperm motility and relationship among CAT and SOD(Hsieh *et al.* 2006). It was found that, the activities of SOD diminished, and the level of damage to cell membranes increased in copper deficient in the individual(Harris, 2001). The SOD maturation and activation are the vastly regulated processes and are controlled through post-translational modifications. SOD1 action in normal scenario is initiated by the incorporation of copper and zinc ions and then the continuation of disulfide oxidation that leads to the formation of enzymatically active homodimers (Vonk *et al*. 2010, 2012). Feeding of trace minerals in our study contributed to better blood homeostasis and protected against antioxidant stimulating molecules in the body. The SOD activities were non-significantly higher in all the 120 days of mineral supplemented groups than control and T3, T8 and T9 shows (P < 0.05) higher activity. The SOD activities were higher (P<0.05) in T3, T4, T5, T6, T7, T8, and T9 at 240 days of mineral supplemented groups as compared to control (Table 1). Activities of this enzyme at 240 days of treatment had (P<0.05) positive correlation with volume (r=.40, p =.012) and fast progressive motility (r =.40, p=.011).The reproductive performance of small ruminants is having major links with the enzyme activities and the supplemented Cu and Zn (Ramirez-Lozano and Lozano 2009). In the previous studies, a positive correlation between the spermaotozoa mass motility character and expression level of WBC *SOD1* was observed in the mineral treated bucks.

The catalase enzyme happen to be an essential antioxidant enzyme in somatic as well as male reproductive tract function(Collins *et al*. 2004). The CAT activities were (P <0.05) higher in T7 and T8 groups and (P <0.05) lower in T9 group at 120 days of mineral supplementation as compared to control. At 240 days of treatment, CAT activities were higher in T1, T2, T4 and T7 groups as compared to control (Table 1). Activities of this enzyme at 240 days of treatment had (P < 0.05) positive correlation with mass (r =.35, p =.027), concentration (r =.34, p =.034) and type A spermatozoa (r =.48, p =.002). The GR activities were higher in all the 120 days mineral supplemented groups (except in T5, T7, and T9) compared to control and significantly (P <0.05) higher in T2, T3, and T4. The GR activities were non-significantly higher in all the 240 days mineral supplemented groups (except in T3) compared to control (Table 1). Activities of this enzyme at 240 days of treatment had (P <0.05) positive correlation with mass (r = .35, p =.025), volume (r = .47, p =.002), type A spermatozoa (r = .40, p =.011). The GPx activities were (P <0.05) higher in T2 and T7 at 120 days of mineral supplementation and in T1, T2, T4, and T7 at 240 days as compared to control (Table 1). Activities of this enzyme at 240 days of treatment had (P <0.05) positive correlation with live and dead (r = .41, p =.009), individual progressive motility (r = .33, p =.035), and VAP (r = .34, p =.034).

The CAT, GPx4 and GR expression levels were correlated and associated with sperm functional parameters and male fertility (Macanovic *et al.* 2015). Some earlier studies have shown the impact of organic minerals in the sperm functional characters, enzyme activities and infertility (Milbury and Richer 2007; Arangasamy *et al.* 2018a and 2018b;Narasimhaiah *et al.* 2018). GSH-Px and catalase activities in seminal plasma showed a (P<0.05) positive correlation with spermatozoa motility and morphology characteristics in men(Giannattasio *et al*. 2002; Khosrowbeygi *et al.* 2004). Shamsi *et al.*(2010) also reported that serum Catalase, SOD and GSH levels were positively correlated to spermatozoa count and motility in men. Similarly in our study, CAT had (P <0.05) positive correlation with mass motility, concentration and type A spermatozoa. SOD had (P <0.05) positive correlation with the volume and fast progressive motility. GR had (P <0.05) positive correlation with mass motility, volume and type A spermatozoa. GPx had positive correlation with live and dead, VAP and individual progressive motility. Antioxidant enzymes like SOD, CAT, GR and GPx are shown to have positive correlation with sperm motility characteristics. This indicates an augmented defense mechanism to

overcome the reactive oxygen formation in the extracellular fluid during the mineral supplementation period.

In conclusion, Cu supplementation affects protein, glucose, HDL and albumin levels in goats supplementation; and supplementation of both organic Zn and Cu in the growing goats lead to an improvement in the plasma antioxidant defense mechanism through increased SOD, CAT, GPx and GR activities with positive correlation to spermatozoa functional attributes.

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STATEMENT OF ANIMAL RIGHTS

Animal experimentation was conducted with the approval obtained from the institutional IAEC committee ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru.

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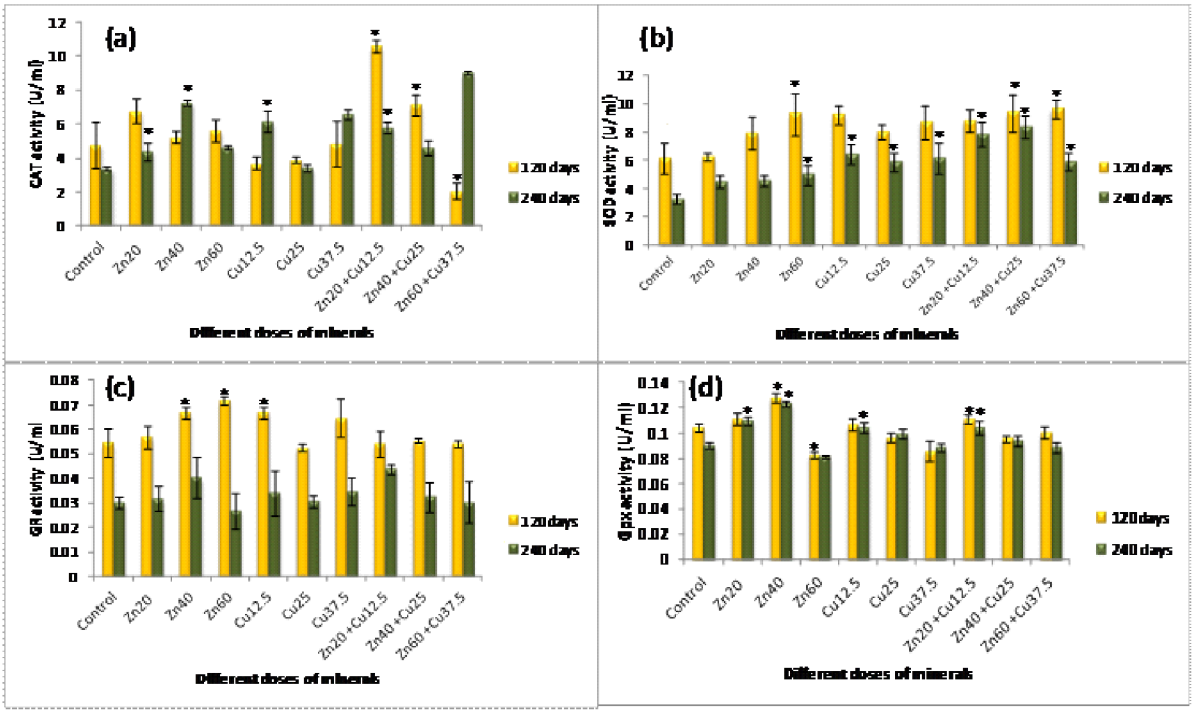

Figure 1. Effect of different doses of organic mineral supplementation on (a) CAT enzyme activity (b) SOD enzyme activity (C)GR enzyme activity (d)GPxenzyme activity in blood plasma of bucks. * indicates significant difference. *p*>0.05. CAT, catalase; SOD, superoxide dismutase; GR, glutathione reductase; GPx, glutathione reductase

Table 1 : Biochemical parameters and Enzyme activities in control and treatment groups (T1–T9) of 120 and 240 days treatment

Biochemical parameters	Days	Control	T1 (Zn-20)	T2 (Zn-40)	T3 (Zn-60)	T4 (Cu-12.5)	T5- (Cu-25)	T6 (Cu-37.5)	T7 (Zn-20+Cu 12.5)	T8 (Zn-40+Cu25)	T9 (Zn-60+Cu37.5)
Biochemical Parameters											
Albumin (g/dL)	120	4.1±0.2	3.6±0.4	3.6±0.1	3.1±0.1*	3.8±0.4	3.7±0.0	3.4±0.2*	3.5±0.6*	2.9±0.2*	2.7±0.0*
	240	3.8±0.1	3.6±0.2	3.4±0.3	3.6±0.0	3.4±0.1	2.8±0.1*	2.8±0.2*	2.8±0.2*	2.7±0.0*	2.8±0.1*
Cholesterol (mg/dL)	120	67.3±1.0	69.2±3.9	66.2±1.0	62.9±5.0	60.4±3.4	72.2±4.1	77.1±4.0*	72.2±2.0	73.6±0.7	73.8±1.1
	240	69.5±2.3	64.5±1.4	60.2±1.0*	69.1±2.3	65.8±8.2	63.3±3.1*	68.1±1.1	67.3±0.5	67.2±0.5	65.2±1.0
HDL (mg/dL)	120	17.4±2.0	22.6±3.9	23.7±6.2	23.5±2.7	40.3±5.0*	32.2±4.4*	41.7±6.9*	31.4±4.1*	41.9±3.7*	33.8±3.0*
	240	36.7±6.1	32.3±4.4	44.7±1.7	45.8±5.7	44.1±3.2	37.1±3.8	40.6±4.4	39.7±5.0	47.6±4.9	36.8±2.9
Glucose (mg/dL)	120	99.4±6.1	97.4±2.6	95.4±2.2	91.5±1.9	87.7±4.9	96.5±7.8	98.3±2.8	104.3±4.9	104.5±2.3	97.6±6.1
	240	97.8±6.0	90.5±2.8	84.1±10.4	105.7±4.5	99.0±9.3	89.7±4.9	82.1±2.6	80.7±1.8*	81.5±0.3*	79.7±5.2*
Protein(g/L)	120	87.1±2.7	92.5±8.0	68.7±4.9*	78.2±4.2	76.7±8.6	88.8±8.7	84.3±2.3	72.1±3.6	72.5±3.5	80.8±6.8
	240	97.1±10.5	107.2±9.4	94.8±5.9	100.7±5.8	99.6±4.6	92.5±3.0	88.9±9.8	100.2±10.6	101.7±4.1	68.1±3.7*
Enzyme activities											
SOD (U/ml)	120	6.1±1.1	6.3±0.2	7.9±1.1	9.3±1.5*	9.2±0.7	8.0±0.6	8.7±1.2	8.8±0.8	9.3±1.3*	9.6±0.7*
	240	3.3±0.4	4.5±0.4	4.6±0.4	5.0±0.7*	6.4±0.7*	5.8±0.6*	6.1±1.1*	7.8±0.9*	8.3±0.8*	5.9±0.6*
CAT (U/ml)	120	4.8±1.4	6.8±0.7	5.2±0.4	5.6±0.6	3.7±0.4	3.9±0.2	4.8±1.3	10.6±0.4*	7.1±0.6*	2.1±0.5*
	240	3.4±0.1	4.4±0.5*	7.3±0.2*	4.6±0.1	6.1±0.6*	3.4±0.2	6.6±0.3	5.8±0.3*	4.6±0.4	9.0±0.1
GR (U/ml)	120	0.05±0.0	0.06±0.0	0.07±0.0*	0.07±0.0*	0.07±0.0*	0.05±0.0	0.06±0.0	0.05±0.0	0.06±0.0	0.05±0.0
	240	0.03±0.0	0.03±0.0	0.04±0.0	0.03±0.0	0.03±0.0	0.03±0.0	0.03±0.0	0.04±0.0	0.03±0.0	0.03±0.0
Gpx (U/ml)	120	0.10±0.0	0.11±0.0	0.12±0.0*	0.08±0.0*	0.10±0.0	0.10±0.0	0.08±0.0	0.11±0.0*	0.10±0.0	0.10±0.0
	240	0.09±0.0	0.11±0.0	0.12±0.0*	0.08±0.0	0.10±0.0*	0.10±0.0	0.09±0.0	0.10±0.0*	0.09±0.0	0.09±0.0

* indicates significant difference in a same row (P <0.05) as compared to control