Iranian Journal of Applied Animal Science

Administration of Different Levels of Arginine and Lysine Coupled with Copper for Change the Copper Concentration of Milk in the Lactating Zandi's Ewes

Research Article

O. Pordel[1], H. Khazali[1*], H. Rokni[2] and A. Hosseini[1]

[1] Department of Animal Physiology, Faculty of Life Science and Biotechnology, Shahid Beheshti University, Tehran, Iran
[2] Department of Education, Institute of Applied Scientific Education of Jihad-e-Agriculture, Tehran, Iran

Received on: 7 Aug 2017
Revised on: 6 Oct 2017
Accepted on: 31 Oct 2017
Online Published on: Jun 2018

*Correspondence E-mail: h_khazali@sbu.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

Copper (Cu) plays an essential role in the human and animal body system. One of the most important sources of Cu is milk. The purpose of this study was to determine the effect of different doses of injection of inorganic Cu and organic Cu as Cu-arginine and Cu-lysine on Cu concentrations of milk in the lactating Zandi's ewes. This experimental research involved 54 lactating Zandi's ewes collected from the department of State Organization Agriculture of Tehran province, Iran. They were allocated into three main groups included: group 1) which received inorganic form of Cu as copper chloride, groups 2 and 3) which received organic Cu as Cu-arginine or Cu-lysine (25, 50 and 100 mg kg^{-1} of body weight (BW)) in the chelate form, respectively. The milk samples were taken 30 min before and 6 hours after intravenous injection of Cu preparations. The Cu concentration in milk was measured using the flame atomic absorption spectrometric method. There was no significant difference among the groups in milk's Cu concentration before administration of either inorganic or organic Cu. Data analysis showed that the administration of inorganic Cu caused a significant increase (P<0.01) of Cu concentration in the milk in a dose dependent manner. Furthermore, the administration of Cu-arginine or Cu-lysine in chelate form caused a significant increase (P<0.01) in milk Cu concentration compared to the inorganic Cu. In conclusion, administration of different forms of Cu influenced the Cu concentration in the milk. The organic forms of Cu led to a significant increase in milk Cu concentration in a dose dependent manner compared to the inorganic form of Cu.

KEY WORDS arginine, copper, ewe milk, lactation stage, lysine.

INTRODUCTION

Copper (Cu) is one of the essential trace element which is bound to different proteins and enzymes in the animals and human that are involved in a broad range of physiological processes (Nemec *et al.* 2012). The decrement or increment in body Cu concentration might cause many disorders; therefore, preventing the trace mineral deficiencies has long been recognized as a factor of utmost importance in the maintenance of the production, reproduction and health of animals (Nemec *et al.* 2012). One of the most important sources for Cu is milk (Pechova *et al.* 2009). Recently, several attempts have been made in order to provide a sufficient status of this microelement in both animals and humans (Pechova *et al.* 2009). Traditionally, the range of supplements that were used for elevating the Cu concentration, contained mostly the inorganic zinc like sulfate salts (Pechova *et al.* 2009). The organic Cu forms including metal amino acid chelates, metal complexes, metal methionine hydroxy analog chelates, metal proteins, and metal propionates have been developed to increase the intestinal absorption and the mineral bioavailability (Nemec *et al.* 2012). Hatfield *et al.* (2001) demonstrated that providing mature ewes with complex organic minerals resulted in higher concentrations of Cu in the liver than the inorganic source. Another study suggests that ewes supplemented with the dietary Cu in the form of Cu-methionine hada higher Cu concentration in different tissues than ewes which received Cu-sulfate (Pal *et al.* 2010).

The Cu cell uptake might be through different mechanisms; for instance, it could be through its specific channel or co-transporting system (Lönnerdal, 2007). The Cu concentration in human milk is about 20-25% of that of serum (Lönnerdal, 2007). In human, the major fraction of Cu in serum is tightly bound to ceruloplasmin, whereas a minor fraction is loosely associated with the serum albumin, amino acids, and low-molecular-weight chelators (Lönnerdal, 2007); the Cu transportation into milk is assumed to be via an active process (Freestone *et al.* 2014). The mammary gland has been found to have three Cu-specific transporters which are; copper transporter 1 (Ctr1), ATP7A, and ATP7B (Kelleher and Lonnerdal, 2006; Michalczyk *et al.* 2000).

Regarding these transporters, Ctr1 has been found to be of the essence for the cellular Cu import as Ctr1 knockout mice die at an early embryonic stage (Lee *et al.* 2000; Lee *et al.* 2001).

This study aimed to determine the effect of different doses of injection of inorganic Cu and organic Cu as Cu-arginine and Cu-lysine on concentrations of Cu in milk, in the lactating Zandi's ewes.

MATERIALS AND METHODS

Animal and experimental design

In this experiment, 54 lactating Zandi ewes weighting 45 ± 5 kg were used. All ewes were in the third or fourth months of the first lactation and were collected from Department of State Organization Agriculture of Tehran. This study was approved and conducted in accordance with the regulations of the Animal Ethics Committee of Animal Science and Veterinary Faculty of Tehran University, Iran. Gross energy and chemical composition of feed stuffs consisted of dry mater, crude protein, crude fiber, ether extract, total ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium and phosphorous were analyzed in animal science research institute of Karaj. Diets were formulated based on AFRC (1995) (Table 1).

The ewes were assigned into three main groups and in each group, there are the levels of treatments doses as follows (n=6); Groups which received copper in the inorganic form as copper chloride ($CuCl_2$; Merck, Germany), groups which received either organic Cu as Cu-arginine (Cu-R) or Cu-lysine (Cu-K) in different doses (25, 50 and 100 mg kg^{-1} of body weight (BW)) (Felix *et al.* 2012). A single dose of the Cu preparations were administered individually by the intravenous (Jugular veins) injection in all treatment groups at 8:00-9:00 a.m. The basal diet was contained 5 mg kg^{-1} Cu.

Table 1 Experimental rations and nutrients

Ingredients (g/kg)	
Wheat straw (g/day)	260
Alfalfa (hay) (g/day)	50
Corn (grain) (g/day)	220
Corn gluten meal (g/day)	85
Bone meal (g/day)	0.47
Salt (g/day)	1.22
Vitamin and mineral supplement	3.50
Nutrients	
Metabolizable energy (MJ/kg)	9.73
Crude protein (%)	13.72
Calcium (%)	0.24
Phosphorous (%)	0.24
Sodium (%)	0.21
Magnesium (%)	0.11
Dry mater intake (g/day)	620
Metabolizable energy intake (MJ/day)	6.03
Metabolizable protein intake (g/day)	55.37

Sample collection and analysis

Milk samples were collected 30 min before and 6 hours after the administration of Cu preparations (Leone and Mercer, 1999). The samples were collected in clean polypropylene tubes and kept under refrigeration until the time of their processing.

The milk Cu concentrations were determined using the flame atomic absorption spectrometric (F-AAS) method and the Analytical CTA-2000 (Chemtech, UK) device (Cu wavelength=324.8 nm). The milk was mineralized by the wet process with hydrogen dioxide and nitric acid addition (2 mL milk+1 mL H_2O_2+2 mL HNO_3) using the microwave digestion technique in the MLS-1200 (Milestone, Italy) microwave oven.

At the end, the Cu concentration in milk was expressed as ppm using the calibration curve (Moreno-Rojas *et al.* 1993).

Statistical analysis

This experiment was designed under randomized double-blinded control study. The statistical analysis was performed using SPSS software (SPSS, 2011). The Kolmogorov-Smirnov test was used to demonstrate the normal distribution of the data. Comparisons between initial group means were by one-way analysis of variance (ANOVA) or '*t*' test. Analysis of results following treatment was by repeated measures, two-way ANOVA (mixed model) and the Tukey's test to compare means. The results are displayed as a mean value with the standard error of the mean (mean±SEM). A level for $P < 0.05$ was considered to be significant. The drawing of charts were done using the Microsoft excel software.

RESULTS AND DISCUSSION

The influence of inorganic Cu administration on the milk Cu concentration

The Cu concentrations in milk are displayed in Figure 1 and Table 2. At the onset of the experiment, there were no differences in the Cu concentration among the groups, where as, after the administration of the inorganic Cu, the Cu concentrations of Cu in milk were significantly increased ($P<0.01$) after doses 25, 50 and 100 mg kg^{-1} it was 0.327 ± 0.016 ppm, 0.482 ± 0.013 ppm, and 0.71 ± 0.011 ppm, respectively, in comparison to before administration of Cu.

The influence of Cu-R administration on milk Cu concentration

The concentrations of Cu in milk before and after administration of Cu-R are exhibited in Figure 2 and Table 2. Despite the fact that at the nascent stages of the experiment there were, no differences among the groups in the concentration of Cu in milk (the differences are shown in Table 1), statistical analysis showed that average concentrations of Cu in milk of different groups were affected after the administration of Cu-R.

Significant increase ($P<0.001$) has been found among the groups which received the organic Cu-R with doses of 25, 50 and 100 mg kg^{-1} where the Cu concentration was 0.75 ± 0.013 ppm, 0.821 ± 0.032 ppm, and 1.033 ± 0.013 ppm, respectively in comparison with before Cu administration groups. Moreover, significant increment was observed in the milk Cu concentration in groups, which received Cu-R at dose 100 mg kg^{-1} in comparison with that received 100 mg kg^{-1} inorganic Cu (the differences are shown in Table 2).

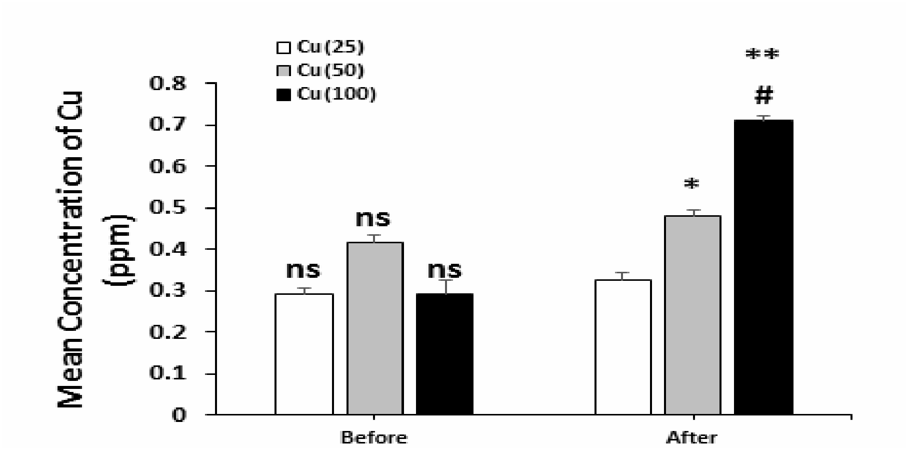

Figure 1 Mean Cu (ppm) concentration in milk before and after administration of inorganic Cu (25, 50 and 100 mg kg^{-1}) in treatment groups
Data expressed as mean ± SE (n=6)
* ($P<0.01$), ** ($P<0.001$) *vs*. Cu (25), # ($P<0.001$) *vs*. Cu (50) in correspondence after groups
NS: non-significant for either corresponding before or after administration

Table 2 Mean milk Cu concentration (ppm) in Zandi lactating ewes before and after administration of organic or inorganic Cu preparations (Mean±SE) (n=6)

Treatments*	Mean milk Cu concentration (ppm)		
	Before	After	P-value
Cu (25)	0.291±0.014[b]	0.327±0.016[a]	0.01
Cu (50)	0.415±0.019[b]	0.482±0.013[a]	0.01
Cu (100)	0.291±0.034[b]	0.710±0.011[a]	0.01
Cu-R (25)	0.301±0.019[b]	0.750±0.013[a]	0.001
Cu-R (50)	0.408±0.028[b]	0.821±0.032[a]	0.001
Cu-R (100)	0.326±0.039[b]	1.033±0.013[a]	0.001
Cu-K (25)	0.378±0.037[b]	0.781±0.037[a]	0.001
Cu-K (50)	0.366±0.012[b]	1.080±0.035[a]	0.001
Cu-K (100)	0.399±0.055[b]	1.252±0.029[a]	0.001

SE: standard error.
The means within the same row with at least one common letter, do not have significant difference ($P>0.05$).

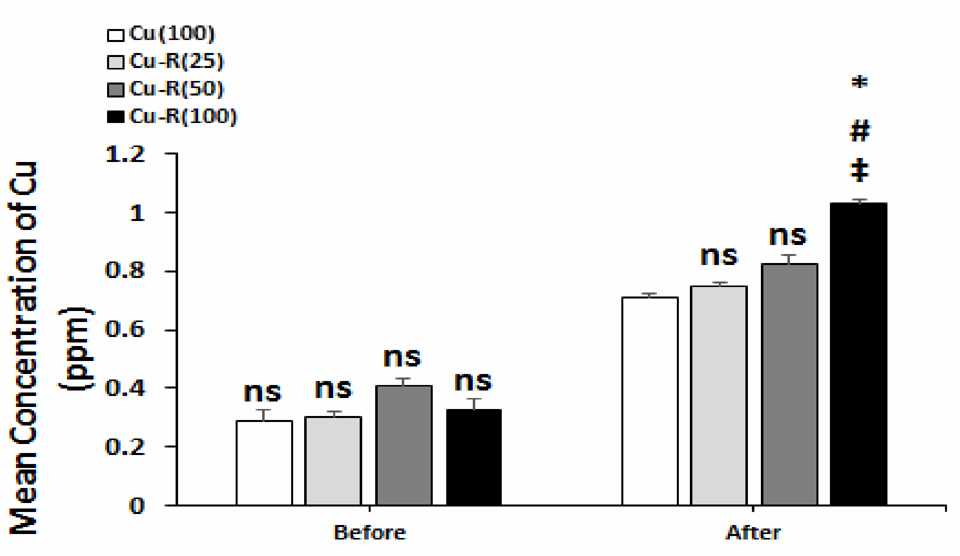

Figure 2 Mean Cu (ppm) concentration in milk before and after administration of organic Cu as Cu-arginine (Cu-R) in treatment groups (Cu-R (25), Cu-R (50) and Cu-R (100))
Data expressed as mean ± SEM (n=6)
* (P<0.001) *vs.* Cu (100), # (P<0.001) *vs.* Cu-R (25), ‡ (P<0.001) *vs.* Cu (100)-R (50) in correspondence after groups
NS: non-significant for either corresponding before or after administration

The influence of Cu-K administration on the milk Cu concentration

The concentrations of Cu in milk before and after the administration of Cu-Kare shown in Figure 3 and Table 2. At the start of the experiment there were, no differences among the groups in the Cu concentration in milk (Table 2), the average Cu concentrations of milk in all groups were affected after administration of Cu-K with different level of doses. A significant increase (P<0.001) has been found among the groups for the average concentrations of Cu in milk for doses of 25 to100 mg kg^{-1} Cu-K where the milk Cu concentration was 0.781 ± 0.037 ppm, 1.08 ± 0.035 ppm, and 1.252 ± 0.029 ppm, respectively, in comparison with before administration. Also, asignificant increase (P<0.001) was observed in the milk Cu concentration in groups which received Cu-K with different doses (50 and 100 mg kg^{-1}) where the milk Cu concentration was 1.08 ± 0.035 ppm, and 1.252 ± 0.029 ppm, respectively, in comparison with that received inorganic Cu (100 mg kg^{-1}) it was 0.71 ± 0.011 ppm. The ewes which were supplemented with either Cu-R or Cu-Khad higher milk Cu concentrations compared with the corresponding control groups. Also, the supplementation with the organic form of Cu showed a significant increase when compared with the inorganic Cu.

In other words, the supplementation of Cu with organic complex significantly increased the excretion of the Cu ion into milk (6 hours after administration) as compared to the inorganic Cu.

Studies focusing on the nexus between the milk Cu concentration and the level of supplementation are scarce and the results revealed very different conclusions. Fantuz *et al.* (2013) did not detect an increase of Cu concentration in milk or in plasma of donkey which has dietary trace element supplementation of 36 mg Cu for three months. The study of Wang *et al.* (2012) indicated that the supplementation of the methionine hydroxy Cu during 120 days' challenges tended to result in the increment of in the milk yield and might have helped the cows to cope with the stress. In the literature, supplementation of the organic forms of Cu to cattle ration under a variety of stresses result in improved Cu status (Nockels *et al.* 1993) and higher milk production (Olkowski *et al.* 1990).

On the other hand, Hackbart *et al.* (2010) observed that replacing a portion of inorganic supplemental trace minerals with an equivalent amount of these organic trace minerals (Zinc (Zn), Manganese (Mn), Cu and Cobalt (Co)) increasmilk production in mid-lactation. These findings were in agreement with our finding where our results revealed that organic Cu significantly elevate the milk Cu concentration when compared with the inorganic Cu which could be attributed to the higher bioavailability of the organic source of Cu.

Previous study indicated that supplementation of the organic form of the essential trace elements provide a higher bioavailability than the inorganic form (Sobhanirad *et al.* 2010).

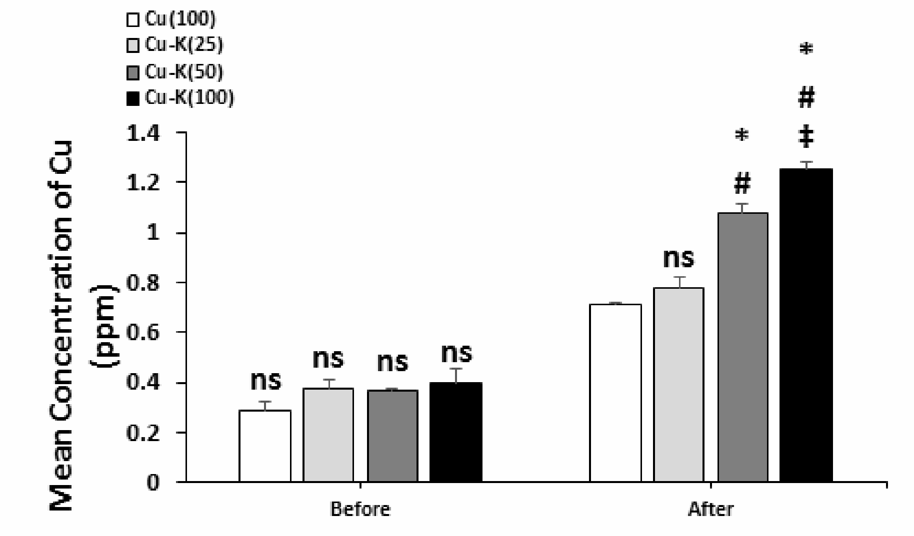

Figure 3 Mean copper (Cu) (ppm) concentration in milk before and after administration of organic Cu as Cu-lysine (Cu-K) in treatment groups (Cu-K (25), Cu-K (50) and Cu-K (100))
Data expressed as mean ±SEM (n=6)
* (P<0.001) *vs.* Cu (100), # (P<0.001) *vs.* Cu-K (25), ‡ (P<0.001) *vs.* Cu-K (50) in correspondence after groups
NS: non-significant for either corresponding before or after administration

In the current study, the supplementation with the organic Cu significantly elevated the level of milk Cu concentration when compared with the inorganic Cu that could be related to the higher availability of the organic source of Cu.

The Cu bound to the organic compounds is more available for the absorption than Cu from the inorganic sources (Sobhanirad *et al*. 2010).

Cu is essential for the milk production (Kirchgessner and Weigand, 1982) and milk is one of the mediums for the endogenous excretion of the excess minerals (Miller, 1975). When the dairy animals are fed with the metal amino acid chelates, the uptake by the tissues (like the mammary glands) in greater quantities, influence the milk production and increase the level of the minerals in milk (Ashmead, 2012).

CONCLUSION

Based upon the current results, we conclude that administration of different forms of Cu influences the concentration of Cu in milk. The organic form forms of the Cu causes a significant increase in milk Cu concentration in a dose dependent manner in comparison with inorganic Cu. Finally, in this preliminary report, it can be said that organic Cu present in milk in greater quantities. This may be due to the presence of amino acids co-transporter in milk epithelial tissue. Further research is warranted to proffer a more vivid picture in this issue.

ACKNOWLEDGEMENT

The authors declare that they have no conflict of interest in this work. The authors would like to thank the Department of State Organization Agriculture of Tehran for providing the animals for the execution of this work. This study was financially supported by Shahid Beheshti University, Tehran, Iran.

REFERENCES

AFRC. (1995). Energy and Protein Requirements of Ruminants. CAB International, Wallingford, UK.

Ashmead H.D. (2012). Amino Acid Chelation in Human and Animal Nutrition. CRC Press, New York.

Fantuz F., Ferraro S., Todini L., Mariani P., Piloni R. and Salimei E. (2013). Essential trace elements in milk and blood serum of lactating donkeys as affected by lactation stage and dietary supplementation with trace elements. *Animal*. **7,** 1893-1899.

Felix T.L., Weiss W.P., Fluharty F.L. and Loerch S.C. (2012). Effects of copper supplementation on feedlot performance, carcass characteristics, and rumen sulfur metabolism of growing cattle fed diets containing 60% dried distillers grains. *J. Anim. Sci.* **90,** 2710-2716.

Freestone D., Cater M.A., Ackland M.L., Paterson D., Howard D.L., Jonge M.D. and Michalczyk A. (2014). Copper and lactational hormones influence the CTR1 copper transporter in PMC42-LA mammary epithelial cell culture models. *J. Nutr. Biochem*. **25,** 377-87.

Hackbart K.S., Ferreira R.M., Dietsche A.A., Socha M.T., Shaver

R.D., Wiltbank M.C. and Fricke P.M. (2010). Effect of dietary organic zinc, manganese, copper, and cobalt supplementation on milk production, follicular growth, embryo quality, and tissue mineral concentrations in dairy cows. *J. Anim. Sci.* **88,** 3856-3870.

Hatfield P.G., Swenson C.K., Kott R.W., Ansotegui R.P., Roth N.J. and Robinson B.L. (2001). Zinc and copper status in ewes supplemented with sulfate- and amino acid-complexed forms of zinc and copper. *J. Anim. Sci*. **79,** 261-266.

Kelleher S.L. and Lonnerdal B. (2006). Mammary gland copper transport is stimulated by prolactin through alterations in CTR1 and ATP7A localization. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. **291,** 1181-1191.

Kirchgessner M. and Weigand M. (1982). Optimal zinc requirements of lactating dairy cows based on various dose-response relationships. *Arch. Tierenahr*. **32,** 569-578.

Lee J., Prohaska J.R., Dagenais S.L., Glover T.W. and Thiele D.J. (2000). Isolation of a murine copper transporter gene, tissue specific expression and functional complementation of a yeast copper transport mutant. *Gene*. **254,** 87-96.

Lee J., Prohaska J.R. and Thiele D.J. (2001). Essential role for mammalian copper transporter CTR1 in copper homeostasis and embryonic development. *Proc. Natl. Acad. Sci. USA*. **98,** 6842-6847.

Leone A. and Mercer F.B.J. (1999). Copper Transport and Its Disorders: Molecular and Cellular Aspects. Kluwer Academic, Plenum Publishers, New York.

Lönnerdal B. (2007). Trace element transport in the mammary gland. *Ann. Rev. Nutr*. **27,** 165-177.

Michalczyk A.A., Reiger J., Allen K.J., Mercer J.F. and Ackland M.L. (2000). Defective localization of the Wilson disease protein (ATP7B) in the mammary gland of the toxic milk mouse and the effects of copper supplementation. *Biochem. J.* **352,** 565-571.

Miller W.J. (1975). New concepts and developments in metabolism and homeostasis of inorganic elements in dairy cattle: A review. *J. Dairy. Sci*. **58,** 1549-1560.

Moreno-Rojas R., Amaro-Lopez M.A. and Zurera-Cosano G. (1993). Micronutrients in natural cow, ewe and goat milk. *Int. J. Food Sci. Nutr*. **44,** 37-46.

Nemec L.M., Richards J.D., Atwell C.A., Diaz D.E., Zanton G.I. and Gressley T.F. (2012). Immune responses in lactating Holstein cows supplemented with Cu, Mn, and Zn as sulfates or methionine hydroxy analogue chelates. *J. Dairy. Sci.* **95,** 4568-4577.

Nockels C.F., DeBonis J. and Torrent J. (1993). Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. *J. Anim. Sci*. **71,** 2539-2545.

Olkowski A.A., Gooneratne S.R. and Christensen D.A. (1990). Effects of diets of high sulphur content and varied concentrations of copper, molybdenum and thiamine on *in vitro* phagocytic and candidacidal activity of neutrophils in sheep. *Res. Vet. Sci.* **48,** 82-86.

Pal D.T., Gowda N.K., Prasad C.S., Amarnath R., Bharadwaj U., Suresh Babu G. and Sampath K.T. (2010). Effect of copper- and zinc-methionine supplementation on bioavailability, mineral status and tissue concentrations of copper and zinc in ewes. *J. Trace Elem. Med. Biol*. **24,** 89-94.

Pechova A., Misurova L., Pavlata L. and Dvorak R. (2009). The influence of supplementation of different forms of zinc in goats on the zinc concentration in blood plasma and milk. *Biol. Trace Elem. Res*. **132,** 112-121.

Sobhanirad S., Carlson D. and Bahari Kashani R. (2010). Effect of zinc methionine or zinc sulfate supplementation on milk production and composition of milk in lactating dairy cows. *Biol. Trace Elem. Res*. **136,** 48-54.

SPSS Inc. (2011). Statistical Package for Social Sciences Study. SPSS for Windows, Version 20. Chicago SPSS Inc.

Wang F., Li S.L., Xin J., Wang Y.J., Cao Z.J., Guo F.C. and Wang Y.M. (2012). Effects of methionine hydroxy copper supplementation on lactation performance, nutrient digestibility, and blood biochemical parameters in lactating cows. *J. Dairy. Sci*. **95,** 5813-5820.