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The Effect of Oral Levothyroxine Sodium on Serum Zn, Fe, Ca and Mg Levels During Acute Copper Sulfate Toxication in Rabbits

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Abstract: This study was carried out to determine the effect of oral levothyroxine sodium (T_4) application on some serum mineral (Zn, Fe, Ca and Mg) concentrations during $CuSO_4$ toxication (1%). Forty male New Zealand rabbits (6 months-old, weighing 2.16±0.08 kg) were allocated to 4 groups. All groups received 1% $CuSO_4$ (*$5H_2O$) (Copper (II) sulfate pentahydrate M102787) in drinking water. Except for control group (group A), T_4 was orally applied to 3 experimental groups at doses of 1.67 (group B), 3.33 (group C) and 6.67 μg kg^{-1} per bw per day (group D) for 2 days. Serum Cu measurements were performed by Atomic Absorption Spectrometer equipped with Flame system. Serum Zn levels in groups C and D were higher than in group A, while serum Ca levels in groups C and D were found to be lower than in group A ($p<0.05$), on the last day. There were significant differences for all mineral levels with respect to time ($p<0.001$). There was also a significant interaction between the dose of T_4 applied (groups A-D) and time ($p<0.01$), except for Fe levels ($p>0.05$). However, there were significant differences only in Zn and Ca levels in multiple comparisons ($p<0.05$). A significant correlation was also found between T_4 and Zn (r: -0.537, $p<0.001$) as well as T_4 and Ca (r:-0.593, $p<0.001$) levels. In addition, administration of T_4 along with 1% $CuSO_4$ affected serum Zn, Ca and Mg levels significantly ($p<0.05$). At the result, T_4 administration at different doses with 1% $CuSO_4$ caused an increase in serum Zn but a decrease in serum Ca levels. However, the administration of this combination showed no significant effect on serum Fe and Mg levels.

Key words: Thyroxine, $CuSO_4$ toxication, serum, Cu, Zn, Fe, Ca, Mg

INTRODUCTION

Stressful conditions like diseases could induce fluctuations in serum Cu levels (Karademir, 2007b, c; Orr *et al*., 1990; Rahelie *et al*., 2006). Soil and pastures with low Cu level can cause Cu deficiencies in grazing animals (Dargatz *et al*., 1999; Gambling *et al*., 2008; Kurt *et al*., 2001. Similarly, different time periods of the year may affect serum Cu levels (Cimtay and Olculu, 2000; Erdogan *et al*., 2003). Cu is a component of the ceruloplasmin and Cu/Zn-SOD in the body and insufficient serum Cu level may cause inadequate activation of these structures (Bulbul *et al*., 2008). Therefore, oral Cu supplementation may be required (Aziz *et al*., 2008; Oztabak and Ozpinar, 2005; Sahin *et al*., 2001; Uzlu *et al*., 2007). However, over-dose of Cu may be applied involuntarily by animal owners or technicians. Additionally, Cu toxicity was reported to occur in some territories (Maclachlan and Jhonston, 1982). Thyroidal Hormones (TH), particularly thyroxin (T_4), could have a great effect on the energy metabolism (Alcalde *et al*., 1999; Braunlich, 1984; Canavan *et al*., 1994; Dickson, 1996; Nakhoul *et al*., 2000). Active transport mechanism, which is an energy dependent process is important for intestinal absorption of Cu and some other nutrients (Lonnerdal, 2008; McDowell, 1992). Thyroxin is used in hypothyroidism or in cases of thyroidal gland extirpation (Uzlu *et al*., 2007; Van Hoek and Daminet, 2009; Dickson, 1996; Page *et al*., 2009).

Zn, Fe, Ca and Mg are important minerals for animal organism (McDowell, 1992; Baxter, 1986). Minerals are involved in many metabolic functions (Karademir, 2006; Kurt *et al*., 2001; O'Brien *et al*., 2000) as well as in the formation of many enzymes (Aziz *et al*., 2008). Interactions among minerals were reported to occur in the diet and/or within the body (Karademir, 2006; Kurt *et al*., 2001; Witkowska *et al*., 1991). It was also reported that administration of T_4 could increase serum Cu levels in a dose dependent manner (Karademir, 2009). Consumption of high Cu levels during T_4 therapy may occur in some

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cases. However, there is no detailed information about serum mineral status during T_4 application along with high level $CuSO_4$. Therefore, the purpose of this study was to elucidate the effect of T_4 application and acute $CuSO_4$ toxication on serum Zn, Fe, Ca and Mg levels.

MATERIALS AND METHODS

Animals and procedures: Fourty male clinically healthy New Zealand Rabbits (6 months old, 2.16±0.08 kg) were used in this study. The animals were divided into 4 equal experimental groups. 1% Copper (II) sulfate pentahydrate ($CuSO_4*5H_2O$, Merck 102787) in drinking water, which is toxic dose (Chapman *et al*., 1962), were given *ad-libitum* to all groups including control. Group A was kept as control and received tap water only. Levothyroxine sodium (T_4) (Levotiron tablet®) at doses of 1.67, 3.33 and 6.67 μg kg per bw per day in tap water was orally applied to group B-D, respectively. T_4 applied at ten o'clock daily at a single dose for 2 days (first and second days after the blood collections). The applications of T_4 and Cu were given simultaneously.

The animals were fed with a commercial animal food. The food and water were given *ad libitum* before and during the experiment. Cu, Zn, Fe, Ca and Mg contents of food and water was determined by flame system Atomic Absorption Spectrometer (FAAS) (Thermo Elemental S4, Thermo Electron Corporation, Cambridge, UK) (Karademir, 2007a, b). Levels of Cu, Zn, Fe, Ca and Mg in feed were found to be 12.56, 98.46, 475.83, 33715,4 and 2533.61 mg kg^{-1} in Dry Matter (DM) and 0.013, 0.074, 0.058, 103.66 and 31.79 mg L^{-1} in tap water, respectively.

The commercial food was purchased from Bayramoglu Yem ve Un San. Tic. A.S. ISO 9001:2000, ISO 22000:2005 and the composition is given in Table 1.

Blood collections and laboratory analysis: Two milliliters of the blood were collected via cardiac puncture under the light ether anesthesia on first, second and last days. Serum was separated by centrifugation at 3500 rpm for 15 min. Serum mineral status was also determined by FAAS (Karademir, 2007a, b). Standard solutions for Cu, Zn, Ca and Mg were supplied from Fluka Chemie GmbH, Switzerland (Fluka 61147, 96457, 21049 and 63046, respectively) and for Fe from Merck KgaA, Germany (119781).

Accuracy control of FAAS was performed using previously known standard solutions for all mineral measurements. These standard solutions were aspirated for 6 times per 10 samples during analyses and mineral levels were measured. Coefficients of Variations (CV) for this parameter was calculated from this obtained findings. CV were found to be Cu: 4.16, Zn: 3.78, Fe: 2.36, Ca: 2.18 and Mg 1.73% (Karademir, 2007a, b). All lab-ware used were made of PTFE material.

Table 1: Ingredients of diet given to rabbits

Diet composition	Units
Dry matter (%)	88
Crude protein (%)	17
Crude cellulose (%)	12
Crude ash (%)	10
Acid insoluble ash (%)	1
Calcium (%)	1.5
Phosphorus (%)	0.75
NaCl (%)	0.6
Vitamin A (IU kg^{-1})	5000
Vitamin D3 (IU kg^{-1})	600
Vitamin E (mg kg^{-1})	25
Metabolic energy (kcal kg^{-1})	2600

Raw-materials for this composition: Barly, corn, corn chaff, corn glutein, wheat, rye, craff, cottonseed meal, sunflower meal, dicalcium phosphate, vitamin, mineral

Statistical analysis: Statistical analyses were performed using SPSS statistical software version 10.0.1 (SPSS, 1999). Data were presented as means±SEM.

One-Way ANOVA was used for comparison of days in each T_4 groups (A-D) (Ergun and Saktas, 2009). Differences of mineral levels throughout the experimental period as well as interaction between time and groups were analyzed by repeated measurement ANOVA (RM ANOVA). One-Way ANOVA were also used to compare the serum mineral levels between groups in days (for first, second, third, fourth and last days). Duncan test was employed for multiple comparisons. Pearson correlation test was used to determine the relationship between the dose of T_4 and individual average values of serum mineral levels on second and last days. Pearson correlation test was also employed for the analyses of relationship between serum Zn, Fe, Ca and Mg levels. Linear regression analysis was used to observe the effect of T_4 on serum Zn, Fe, Ca and Mg levels.

RESULTS AND DISCUSSION

Oral levothyroxine sodium at various doses with toxic dose copper sulfate was applied to the rabbits. The applications were continued for 2 days. One-Way ANOVA test results for differences among days (groups for days of first-last):

For Zn: There was no difference among days in groups A and B ($p>0.05$). However, differences were found between first day and other days in groups C and D ($p<0.05$ and $p<0.01$) (Fig. 1).

For Fe: There was no difference among the experimental days in all groups (A-B) ($p>0.05$) (Fig. 2).

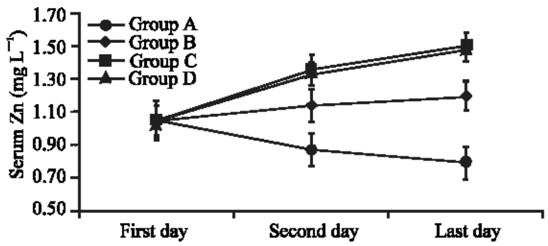

Fig. 1: Serum Zn levels of groups in relation to time

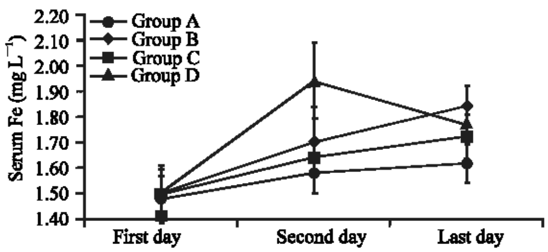

Fig. 2: Serum Fe levels of groups in relation to time

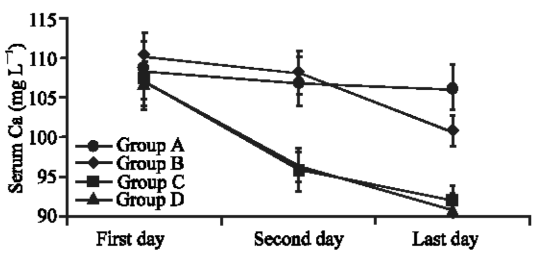

Fig. 3: Serum Ca levels of groups in relation to time

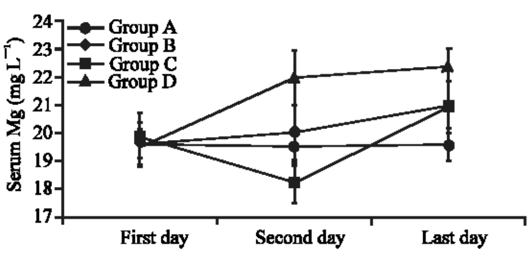

Fig. 4: Serum Mg levels of groups in relation to time

For Ca: There was no difference among days of group A ($p>0.05$). There were differences between last day and other days ($p<0.05$) in group B. There were also significant differences between first day and other days in groups C ($p<0.01$) and D ($p<0.001$) (Fig. 3).

For Mg: There was no difference among days in groups A and B ($p>0.05$). There was a significant difference between last and second day in group C ($p<0.05$), but no difference was found between first and other days in group C ($p>0.05$). There was a significant difference between first day and other days in group D ($p<0.05$) (Fig. 4).

Repeated measurements ANOVA (RM ANOVA) test results for differences among groups (A-D) within time

For Zn: There were significant differences within time ($p<0.001$). There were also significant interactions between time and mineral levels of groups ($p<0.001$). Post Hoc test results showed that there were significant differences between group A and other groups ($p<0.05$) (Fig. 1).

For Fe: There were significant differences within time ($p<0.001$), but no interaction was found between time and mineral levels of groups ($p>0.05$). There was also no difference among groups according to Post Hoc test ($p>0.05$) (Fig. 2).

For Ca: There were significant differences within time ($p<0.001$) and interactions were observed between time and mineral levels of groups ($p<0.01$). According to Post Hoc test, there were differences between groups A-B and C-D ($p<0.05$) (Fig. 3).

For Mg: There were significant differences within time ($p<0.001$) and interactions were observed between time and mineral levels of groups ($p<0.001$). However, there was no difference among groups according to Post Hoc test ($p>0.05$) (Fig. 4).

One-Way ANOVA test results for differences among groups within particular days (first-last days): No significant differences were found in mineral levels of groups on the first days. However, significant differences were observed in mineral levels of groups on other days for minerals (Zn, Fe, Ca and Mg). These findings were given as follows:

For Zn: There was a significant difference between group A and other groups on second day ($p<0.01$). There were significant differences between group A and other groups as well as B and other groups on the last day ($p<0.001$) (Fig. 1).

For Fe: No differences were found in Fe levels for all days (Fig. 2).

For Ca: On the second day, Ca level of group A was similar to group B. In addition, group C was similar to group D. There was no difference between each other ($p>0.05$). However, there was a significant difference between group A and groups C, D as well as among group B and groups C, D ($p<0.01$). In contrast to second day, a difference was found between group A and B on the last day ($p<0.001$) (Fig. 3).

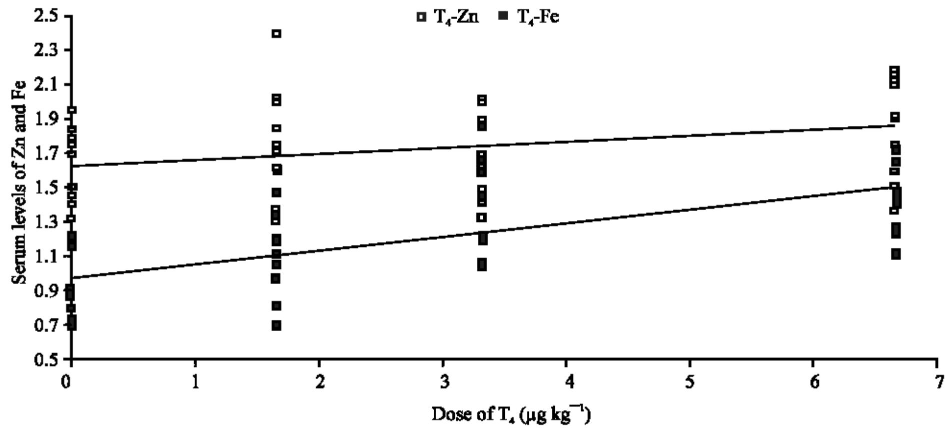

Fig. 5: Illustration of correlation test results between T_4-Zn and T_4-Fe according to average values of second and last days' data

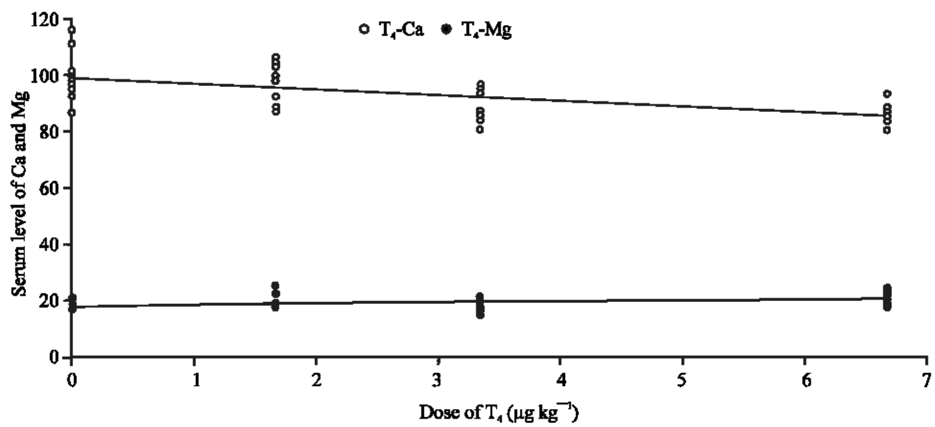

Fig. 6: Illustration of correlation test results between T_4-Ca and T_4-Mg according to average values of second and last days' data

Table 2: The effects of T_4 on serum Zn, Fe, Ca and Mg levels during 1% $CuSO_4$ toxication

Regression equation	r^2 (%)	Significance
Zn = 0.968 + 0.0809 Aplied T_4	32.8	p<0.001
Fe = 1,63 + 0.0325 Aplied T_4	8.5	p>0.05
Ca = 106 − 1.12 Aplied T_4	35.2	p<0.001
Mg = 19.4 + 0.360 Aplied T_4	13.4	p<0.05

Unite of minerals: mg L^{-1}, unite of T_4: µg kg^{-1} per BW per day

For Mg: On the second day, Mg level in group D was significantly different from those of other groups except group B (p<0.05). However, on the last day, there was a significant difference between group A and D (p<0.05) but no difference was observed between group A and B as well as group A and C (p>0.05) (Fig. 4).

The correlation between the dose of T_4 and average mineral levels in the second and last days was also evaluated by Pearson correlation test. It was found that there were significant correlation between the dose of T_4-Zn (r: 0.537, p<0.001) and T_4-Ca (r: -0.593, p<0.001) but not between the dose of T_4-Fe (r: 0.291, p>0.05) and T_4-Mg (r: 0.336, p>0.05) levels (Fig. 5 and 6).

Regression analyses were employed to test the effect of T_4 on serum Zn, Fe, Ca and Mg levels during $CuSO_4$ toxication. T_4 affected the serum Zn, Ca and Mg levels, significantly (p<0.05) but not serum Fe level (p>0.05). The results of regression analyses are given in Table 2.

Average values of second and last days of Zn, Fe, Ca and Mg levels correlations test results were shown in Table 3. Only a negative correlation was detected between Zn and Ca levels.

Chemical and biological interactions among the minerals were reported to occur in many conditions (Karademir, 2006; Kurt *et al.*, 2001). These interactions were reported to be affected by intestinal absorption site, transport site in the intestinal mucosa, other membrane barriers, functional metal binding proteins, chemical forms, electrical structure of minerals (O'Dell, 1989).

Table 3: Correlation values between Zn, Fe, Ca and Mg levels of average values of second and last days (r)

Chemical components	Zn	Fe	Ca
Fe	0.114	-	-
Ca	-0.644*	-0.118	-
Mg	0.167	0.012	-0.113

*p<0.01

In the previous study, we showed that serum Cu levels were affected by oral T_4 applications along with 0.1% $CuSO_4$. During for 3 days, serum Cu levels were increased with the increased doses of T_4 (Karademir, 2009). The purpose of the present study was to investigate the effect of various doses of T_4 applications along with acute Cu toxication (1% $CuSO_4$) on some serum mineral levels (Zn, Fe, Ca and Mg). Findings of this study showed that some fluctuations occurred on serum Zn, Fe, Ca and Mg levels in a dose-dependent manner in response to T_4 treatment with toxic dose of $CuSO_4$ (1%). Possible mechanisms for these fluctuations may be associated with increased Cu levels in the body (Kurt *et al*., 2001; Witkowska *et al*., 1991) or the effect of T_4 on intestinal absorption of these minerals via altering the active transport system (Lonnerdal, 2008; McDowell, 1992) as well as altered mineral excretion via urinary system (Braunlich, 1984; Van Hoek and Daminet, 2009).

Group A (only 1% $CuSO_4$ applied group without T_4) showed slight decreases in levels of serum Zn and Ca ($p>0.05$), slight increase in Fe level ($p>0.05$) and a stable course in the level of Mg ($p>0.05$). However, T_4 treatments affected the serum Zn, Ca and Mg levels during 1-4 days ($p<0.05$) (Fig. 1-4).

Xin *et al*. (1993) measured serum Cu levels during, before and after 8 weeks of lactation with or without Cu supplementation. In the study, Zn levels were reported to be decreased with oral Cu supplementations.

The decrease was more prominent when Cu level was increased. Multiple type of interaction among Cu, Zn and Fe were reported for intestinal absorption or metabolism. There is a positive interaction between Cu and Fe, but a negative interaction could occur between Cu and Zn for absorption (O'Dell, 1989). Findings of the study were seen in group A (Cu supplementation without T_4) with the slight decrease of Zn and slight increase of Fe levels. Nevertheless, 3.33 μg kg^{-1} and higher dose of T_4 applications significantly increased the levels of serum Zn ($p<0.05$). Serum Fe showed a slight increase in all groups ($p>0.05$). In addition, while serum Ca levels were decreased with all T_4 doses ($p<0.05$), serum Mg levels were increased with 3.33 μg kg^{-1} and higher dose of T_4 applications ($p<0.05$). The possible cause of this mineral fluctuations could be related to intestinal absorption (Lonnerdal, 2008; Nakhoul *et al*., 2000), urinary excretion (Braunlinch, 1984; Canavan *et al*., 1994) or interactions of minerals (Gambling *et al*., 2008; Dargatz *et al*., 1999; Karademir, 2007b, c; 2009; Maclachlan and Jhonston, 1982).

Positive correlation was observed between T_4 and serum Zn levels (Fig. 5). On the other hand, opposite result could be expected due to the negative effect of increased Cu level on serum Zn level (Witkowska *et al*., 1991; Xin *et al*., 1993). This condition may be due to the effect of T_4 on absorption, distribution or excretion of body Zn level (Alcade *et al*., 1999; Braunlich, 1984; Lonnerdal, 2008).

$CuSO_4$ toxication caused a non-significant slight decrease in serum Ca level of group A ($p>0.05$). The decrease in serum Ca level was found to be statistically significant with the T_4 application ($p<0.05$) (Fig. 3). Negative interaction between Cu and Ca is known (McDowell, 1992). The results of this investigation showed that the effect of negative interaction increased with the T_4 applications.

The cause of this condition may be expected from metabolic effect of T_4 on the organism (Dickson, 1996). Negative interaction between Zn and Ca levels are also known (Baxter, 1986) and the increased Zn levels may also contribute to the decreased Ca level seen in this study. The correlation results between T_4-Zn, T_4-Ca and Zn-Ca supported our opinion on the fluctuation of Zn and Ca under the effect of T_4 and $CuSO_4$ toxication (Fig. 5, 6 and Table 3). The effect of T_4 with $CuSO_4$ toxication on serum Zn and Ca levels were found highly significant ($p<0.001$) (Table 2).

Serum Mg is a macro mineral like Ca, whereas serum Fe is a micro mineral like Zn and Cu. Mg has a close relation to Ca in the living organism. Serum Fe has also a close relation with Zn and Cu (Karademir, 2009; O'Dell, 1989). T_4 applications with $CuSO_4$ toxication affected serum Mg and Fe less compared to serum Ca and Zn levels (Table 2, 3 and Fig. 4-6). The animal organisms have compensatory mechanisms for the fluctuations of some metabolic cases. The urinary system is one of them (Dickson, 1996). In addition, T_4 applications may affect this compensatory system (Alcalde *et al*., 1999; Braunlich, 1984; Van Hoek and Daminet, 2009).

Differences for all minerals (Zn, Fe, Ca and Mg) among groups (A-D) were observed in relation to time during T_4 and $CuSO_4$ applications ($p<0.001$). Most prominent differences were observed in serum Zn and Ca levels. The serum levels of Zn were increased, while serum

Ca levels decreased in time with T_4 applications during $CuSO_4$ toxication. In group A, Zn level was lower, while Zn levels were increased with T_4 treatment in other groups. The differences between group A and groups B-C were significant ($p<0.05$) (Fig. 1).

Decreased serum Zn during Cu applications has been known (Witkowska *et al*., 1991; Xin *et al*., 1993). We observed in the present study that T_4 application increased the serum Zn levels in a dose dependent manner. Similarly, a negative interaction between Cu and Ca levels are known (Karademir, 2006; Kurt *et al*., 2001; McDowell, 1992).

Nevertheless, T_4 applications effectively decreased the level of serum Ca compared to control group. Interactions between groups and time were significant within time ($p<0.01$) and according to Post Hoc analyses there were significant differences between A-B groups and C-D groups ($p<0.05$).

These results showed that high level T_4 is more effective than low dose on decreasing serum Ca level. T_4 with $CuSO_4$ increased serum Fe level within time ($p<0.001$), but no interaction was found between time and groups ($p>0.05$). Similarly, there was no difference between groups according to multiple comparisons ($p>0.05$).

These results suggest that there is no effect of T_4 along with $CuSO_4$ toxication on serum Fe levels. The elevation in serum Fe level may be due to positive interaction with Cu (O'Dell, 1989).

There was a significant difference in serum Mg levels within time period of the treatment ($p<0.001$). There was an interaction between time and groups ($p<0.001$). However, in multiple comparisons no significant differences between groups were found ($p>0.05$). The cause of this situation may be due to the wider standard deviations in groups.

The interaction between Serum Ca and Mg was reported in a previous study (O'Dell, 1989). However, levels of Mg showed no significant fluctuations ($p>0.05$). T_4 application may be a causative event for unaffected Mg level.

The results of One-way ANOVA for the analyses of differences between groups within particular days (first-last days) also supported the results obtained from RM ANOVA.

As the results of this study, oral T_4 administration increased serum Zn levels, but decreased serum Ca levels during $CuSO_4$ toxication. However, serum Fe and Mg levels were not significantly affected by these combinations.

T_4 therapy is widely used in the treatment of hypo-thyroidal functional disorders. Furthermore, there are many locations in the world which is Cu deficient or contains toxic level of Cu. Cases of Cu deficiencies may need Cu therapy. Consequently, simultaneous intake of T_4 and high amount of Cu could occur either voluntarily or involuntarily.

CONCLUSION

The elevation in serum Zn levels or Ca deficiency could occur during T_4 therapy along with $CuSO_4$ toxication. This combination can be utilized for the treatment of short time Zn deficiency or Ca toxication.

REFERENCES

Alcalde, A.I., M. Sarasa, D. Raldua, J. Aramayona, R. Morales, J. Biber, H. Murer, M. Levi and V. Sorribas, 1999. Role of thyroid hormone in regulation of renal phosphate transport in young and aged rats. Endocrinology, 140: 1544-1551. PMID: 100 98486.

Aziz, B., T. Bulbul S. Kucukersan, M. Sireli and A. Eryavuz, 2008. Effects of dietary supplementation of organic and inorganic Zn, Cu and Mn on oxidant/antioxidant balance in laying hens. Kafkas Univ. Vet. Fak. Derg., 14 (1): 19-24. http://vetdergi.kafkas.edu.tr/extdocs/2008_1/19_24.pdf.

Baxter, J.T., 1986. Deficiencies of Mineral Nutrient. Current Veterinary Therapy 2. In: Howard, J.L. (Ed.). Food Animal Practice. W.B. Saunders. Com. Philadellphia, pp: 278-292. ISBN: 0-7216-1526-0.

Braunlich, H., 1984. Postnatal development of kidney function in rats receiving thyroid hormones. Exp. Clin. Endocrinol., 83: 243-250. PMID: 6540694.

Bulbul, A., T. Bulbul, S. Kucukersan, M. Sireli and A. Eryavuz, 2008. Effects of dietary supplementation of organic and inorganic Zn, Cu and Mn on Oxidant/Antioxidant balance in laying hens. Kafkas Univ. Vet. Fak. Derg., 14 (1): 19-24. http://vetdergi.kafkas.edu.tr/extdocs/2008_1/19_24.pdf.

Canavan, J.P., J. Holt, J. Easton, K. Smith and D.F. Goldspink, 1994. Thyroid-induced changes in the growth of the liver, kidney and diaphragm of neonatal rats. J. Cell. Physiol., 161: 49-54. PMID: 7929607.

Chapman, J.R.H.L., S.L. Nelson, R.W. Kidder, W.L. Sippel and C.W. Kidder, 1962. Toxicity of cupric sulfate for beef cattle. J. Anim. Sci., 21: 960-962. http://jas.fass.org/cgi/reprint/21/4/960.pdf.

Cimtay, I. and A. Olculu, 2000. Investigation on blood plasma and hair copper levels in Clinically healty cattle in Elazig and it's vicinity. Turk. J. Vet. Anim. Sci., 24: 267-273. http://journals.tubitak.gov.tr/veterinary/issues/vet-00-24-3/vet-24-3-12-9901-17.pdf.

Dargatz, D.A., F.B. Garry, G.B. Clark and P.F. Ross, 1999. Serum copper concentrations in beef cows and heifers. J. Am. Vet. Med. Assoc., 215: 1828-1832. PMID: 10613217.

Dickson, W.M., 1996. Endocrin Glands. Endocrinology, Reproduction and Lactation. 11th Edn. In: Swenson, M.J. and W.O. Reece (Eds.). Dukes' Physiplogy of Domestic Animals, Cornell University Press, London, pp: 629-664. ISBN: 0-8014-2804-1.

Erdogan, S., Z. Erdogan and N. Sahin, 2003. Mevsimsel olarak merada yetistirilen koyunlarda serum bakir, cinko ve seruloplazmin duzeyleri ile yun bakir ve çinko degerlerinin arastirilmasi. Ankara Univ. Vet. Fak. Derg., 50: 7-11. http://www.ankara.edu.tr/kutuphane/Veteriner_Web_HTML/veteriner2003_1.htm.

Ergun, G. and S. Saktas, 2009. ANOVA modellerinde kareler toplami yontemlerinin karsilastirilmasi. Kafkas Univ. Vet. Fak. Derg., 15 (3): 481-484. http://vetdergi.kafkas.edu.tr/extdocs/ 2009_3/481_484.pdf.

Gambling, L., H.S. Andersen and H.J. McArdle, 2008. Iron and copper and their interactions during development. Biochem. Soc. Trans., 36: 1258-1261. PMID: 19021536.

Karademir, B., 2006. Molybdenum and enzymatic functions for animals. Kafkas Univ. Vet. Fak. Derg., 12 (2): 217-224. http://vetdergi.kafkas.edu.tr/extdocs/12_2/217_224.pdf.

Karademir, B., 2007a. Comparisons of some sample preparation methods for blood-serum copper and zinc at atomic absorption spectrometer. Kafkas Univ. Vet. Fak. Derg., 13 (1): 61-66. http://vetdergi.kafkas.edu.tr/extdocs/13_1/61_66.pdf.

Karademir, B., 2007b. Effect of stress induced by vaccination on blood plasma copper, zinc, potassium and magnesium. Kafkas Univ. Vet. Fak. Derg., 13 (1): 49-54. http://vetdergi.kafkas.edu.tr/extdocs/13_1/49_54.pdf.

Karademir, B., 2007c. Serum copper and zinc levels of akkaraman and tuj sheep according to age and sex under the winter condition. Kafkas Univ. Vet. Fak. Derg., 13 (1): 55-59. http://vetdergi.kafkas.edu.tr/extdocs/13_1/55_59.pdf.

Karademir, B., 2009. The effects of oral Thyroxine aplication on serum Cu concentration in Rabbits. Kafkas Univ. Vet. Fak. Derg. in progress. http://vetdergi.kafkas.edu.tr.

Kurt, D., O. Denli, Z. Kanay, C. Guzel and K. Ceylen, 2001. An investigation of the Cu, Zn and Se levels of blood serum and the Cu and Zn levels of wool of Akkaraman ewes in the Diyarbakir region. Turk. J. Vet. Anim. Sci., 25: 431-436. http://journals.tubitak.gov.tr/veterinary/issues/vet-01-25-4/vet-25-4-3-9906-7.pdf.

Lonnerdal, B., 2008. Intestinal regulation of copper homeostasis: A developmental perspective. Am. J. Clin. Nutr., 88 (3): 846S-850S. PMID: 18779306.

Maclachlan, G.K. and W.S. Jhonston, 1982. Copper poisoning in sheep from north roneldsay maintained on diet of terrestrial herbage. Vet. Rec., 111 (13): 299-301. PMID: 7147642.

McDowell, L.R., 1992. Minerals in animal and human nutrition. Academic Press Inc., London. ISBN: 0-12-483369-1.

Nakhoul, F., C.B. Thompson and A.A. McDonough, 2000. Developmental change in Na,K-ATPase alpha1 and beta1 expression in normal and hypothyroid rat renal cortex. Am. J. Nephrol., 20: 225-231. PMID: 10878407.

O'Brien, K.O., N. Zavaleta, L.E. Caulfield, J. Wen and S.A. Abrams, 2000. Prenatal iron supplements impair zinc absorption in pregnant Peruvian women. J. Nutr., 130: 2251-2255. PMID: 10958820.

O'Dell, B.L., 1989. Mineral interactions relevant to nutrient requirements. J. Nutr., 119 (12): 1832-1838. PMID: 269 3644.

Orr, C.L., D.P. Hutcheson, R.B. Grainger, J.M. Cummins and R.E. Mock, 1990. Serum copper, zinc, calcium and phosphorus concentrations of calves stressed by bovine respiratory disease and infectious bovine rhinotracheitis. J. Anim. Sci., 68: 2893-2900. PMID: 22 11419.

Oztabak, K. and A. Ozpinar, 2005. Effect of feding newborn lambs with colostrum and sterilised cow's milk on serum copper and zinc contentration. Istanbul Univ. Vet. Fak. Derg., 31 (1): 75-81. http://www.istanbul.edu.tr/fakulteler/veteriner/vetfakdergi/yayinlar/2005-1/makale-8.pdf.

Page, R.B., R.J. Monaghan, J.A. Walker and S.R. Voss, 2009. A model of transcriptional and morphological changes during thyroid hormone-induced metamorphosis of the axolotl. Gen. Comp. Endocrinol., 162 (2): 219-232. PMID: 19275901.

Rahelie, D., M. Kujundzie, Z. Romie, K. Brkie and M. Petrovecki, 2006. Serum concentration of zinc, copper, manganese and magnesium in patients with liver cirrhosis. Coll. Antropol., 30 (3): 523-528. PMID: 17058518.

SPSS, 1999. SPSS reference manual (Release 10.0.1) for Windows. SPSS Inc, USA. http://www.spss.com/downloads/Papers.cfm?ProductID=00077&Name=SPSS%20Statistical%20Services%20for%20Microsoft%20SQL%20Server%202005.

Sahin, T., I. Cimtay, G. Aksoy and A. Olculu, 2001. Effects of copper sulfate administration on body weight gain some blood parameters in lambs. Turk. J. Vet. Anim. Sci., 25: 933-938. http://journals.tubitak.gov.tr/veterinary/issues/vet-01-25-6/vet-25-6-18-0008-23.pdf.

Uzlu, E., M. Citil, O. Atakisi, K. Yapar and H.M. Erdogan, 2007. Tavsanlarda farkli nonsteroidal antiinflamatorik ilaçlarin serbest triiyodotronin (fT_3), serbest troksin (fT_4) ve Troid Stimulan Hormon (TSH) konsantrasyonlarina etkileri. Kafkas Univ. Vet. Fak. Derg., 13 (1): 185-189. http://vetdergi.kafkas.edu.tr/extdocs/2007_2/185_189.pdf.

Van Hoek, I. and S. Daminet, 2009. Interactions between thyroid and kidney function in pathological conditions of these organ systems: A review. Gen. Comp. Endocrinol., 160 (3): 205-215. PMID: 19133263.

Witkowska, J., D. Czerwińska, A. Kiepurski and W. Roszkowski, 1991. Harmful elements versus iron, zinc and copper: Their interactions in animals and humans. I. Mercury, tin, nickel, selenium, fluorine and aluminum. Rocz. Panstw. Zakl. Hig., 42 (1): 15-23. PMID: 1788508.

Xin, Z., D.F. Watermen, R.W. Hemken and R.J. Harmon, 1993. Copper status and requirement during the dry period and early lactation in multiparous Holstein cows. J. Dairy Sci., 76: 2711-2716. PMID: 8227673.