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Effects of High Levels of Dietary Copper Sulfate and Copper Proteinate on Growth Performance, Retention for Copper and Zinc of Rats

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Abstract: This study was conducted to determine the effects of high levels of inorganic Copper Sulfate ($CuSO_4$) and organic Copper Proteinate (CuP) supplementation to the rat diet on grwth performance, Copper (Cu) and Zinc (Zn) contents of liver, heart, kidney, spleen and bone in addition Cu contents of faeces and serum Cu levels. A total of 66 Wistar weanling male rats were used in the trial. The following treatments were applied in experiment: control (no supplemental Cu), 15 mg Cu kg^{-1} diet from $CuSO_4$, 15 mg Cu kg^{-1} diet from CuP. Feed intake was decreased by feeding the diet containing $CuSO_4$ and CuP however, Cu supplementation had a significant effect positively on body weight gains and feed conversion ratio ($p<0.05$). Cu contents of liver, heart, kidney and spleen were increased by feeding the diet containing $CuSO_4$ and CuP at the rate of 15 g Cu kg^{-1} of feed as compared to the control at 28 day ($p<0.05$). Serum Cu levels were highest in the rat fed CuP and there were significant differences between CuP and control ($p<0.05$). In addition to bone Cu and Zn and feaces Cu concentrations of the rats fed organic and inorganic Cu sources were similar ($p>0.05$). Zn contents of liver were increased by feeding the diet containing $CuSO_4$ and CuP while Zn contents of kidney were decreased ($p<0.05$). Zn contents of heart and spleen were decreased by CuP ($p<0.05$). The results from the study were generally in favour of the group with $CuSO_4$ addition however, it can be stated that organic copper source was absorbed beter, taking into account the high serum Cu concentration in the group with CuP addition.

Key words: Rat, copper proteinate, copper sulfate, retention for copper, zinc, Turkey

INTRODUCTION

In recent years, a considerable interest has grown in the use of trace element complexes or chelates of organic origin in rations instead of inorganic salts (sulfate, oxide, carbonate, chloride). This is due to the results obtained from studies demonstrating that rations with organic trace element additives improve the development, reproduction and health of different animal species. For some chelated elements are easily absorbed and are thus effective in target tissues owing to their electrical neutrality in some pH conditions. This was based on their high bioavailability (Puchala *et al*., 1999; Uchida *et al*., 2001; Acda and Chae, 2002). This is also important in terms of environmental pollution. At the present time, various commercial organic trace element additives are available in complex, chelate or proteinate form. Many studies that use different animal species are available on the comparison of organic trace elements with different inorganic sources or their different doses in rats (Du *et al*., 1996; Aydemir and Ozcan, 2003) on the other hand in calves (Kincaid *et al*., 1986) in pigs (Coffey *et al*., 1994; Zhou *et al*., 1994; Apgar *et al*., 1995; Apgar and Kornegay, 1996) and in steers (Engle *et al*., 2000). Some of the research results report that organic trace elements have higher bioavailability than inorganic additives while others record no difference between them in terms of bioavailability. It is reported that in animal nutrition, in general, inorganic copper sulfate has higher bioavailability than carbonate and oxide forms (Ledoux *et al*., 1987; Clark *et al*., 1993) and among organic copper sources, amino acid or organic acid complexes of copper are used intensively (Wapnir, 1998). The purpose of this study is to demonstrate the bioavailability of copper added at high levels to rat diet in the form of copper proteinate for an organic complex as compared to the addition of inorganic copper sulfate at an equivalent level. The study was conducted in order to determine Cu and Zn contents of liver, heart, kidney, spleen and bone. In addition Cu contents of faeces and serum Cu levels and growth performance.

MATERIALS AND METHODS

A total of 66, 30 days olds male Wistar rats (average weight 90 g) were individually weighed and distributed

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Table 1: The composition of the basal diets (as fed basis%)

Ingredient	Values
Albumin (min. CP 80%)	28.00
Maize starch	30.47
Sucrose	25.00
Fibre (sawdust)	5.00
Cotton oil	5.00
Vitamin mixture[a]	0.50
Choline (Choline 70)[b]	0.12
Mineral mixture[c]	5.91
Analyzed composition (%)	
Dry matter	93.50
Crude protein	23.32
Ether extract	5.39
Crude fiber	1.99
Crude ash	6.65
Total copper (mg kg^{-1})	0.07
Total zinc (mg kg^{-1})	24.48

[a]Supplied mg or IU kg^{-1} of diet: 48390 IU g^{-1} Vit-A, 9680 IU g^{-1} Vit-D3, 97000 IU kg^{-1} Vit-E, 9700 mg kg^{-1} Vit-K, 9700 mg kg^{-1} thiamin, 32000 mg g^{-1} riboflavin, 13000 mg kg^{-1} pyridoxine, 71 mg kg^{-1} Vit-B12, 226000 mg kg^{-1} niacine, 80000 mg kg^{-1} pantothenic acid, 3200 mg kg^{-1} folic acid, 645 mg kg^{-1} biotin. [b]Purchased from Purina, containing: 700000 mg kg^{-1} choline. [c]Supplied mg or g kg^{-1} of diet: 6 g Ca ($CaCO_3$), 4 g P (K_2HPO_4), 4 g K (K_2HPO_4), 2 g Na (NaCl), 2 g Cl (NaCl), 0.65 g Mg ($MgSO_4.H_2O$); 5 mg F (NaF), 20 mg Zn ($ZnCl_2$), 65 mg Mn ($MnSO_4.H_2O$), 3.2 mg Co ($CoCl_2.6H_2O$), 0.8 mg Mo ($(NH_4)_6Mo_7O_{24}.4H_2O$), 0.6 mg I (KI), 0.2 mg Se (Na_2SeO_3), 60 mg Fe ($FeC_6H_5O_7.5H_2O$), 3 mg Cr ($CrCl_3.6H_2O$)

into 3 groups of 22 rats each. Rats were individually housed in stainless stell cages in a room maintained at 22±1°C with lighting regulated to provide 16:8 h light/dark cysles. The three groups were fed *ad libitum* on semi-purified diets containing 0 mg Cu (no supplemental Cu, control), 15 mg Cu (inorganic copper source, $CuSO_4.5H_2O$, Uparc Chemical) or 15 mg Cu kg^{-1} (organic copper source, Bioplex Copper, Alltech, Nicholasville, KY, USA) for 28 day. The experimental diets used are shown in Table 1. Feed and distilled water were consumed *ad libitum*. The basal diet was ground through a 1 mm screen in preparation for chemical analysis. Dry matter content was determined by oven-drying at 105°C for 16 h. Crude ash content was determined by muffle at 550°C for 16 h. Ash obtained was digested by 10 mL of a 1:1 HCl solution and into 250 mL glass bottle with warm distilled water. The solution was then read directly in the flame atomic absorption spectrophotometry (Model: Perkin-Elmer, 2380) for the analysis of total Cu and Zn contents of diet. The Kjeldahl method was used for the analysis of total nitrogen content of diets and crude protein was expressed as nitrogen×6.25. Ether extract content was obtained by the Soxhlet extraction using anhydrous diethyl ether. The crude fibre content was determined using 12.5% H_2SO_4 and 12.5% NaOH solutions (Naumann and Bassler, 1993).

Sixty four rats were sampled at 28 days of age, weighed and killed by exposure to diethyl ether. Blood samples from heart of all rats from each treatment were collected at the end of the trial into tubes (Gel Clot Activator isotherm, Lot: 070364) before death, immediatelly. Tubes were kept at room temperature for 30 min to obtain serum for determination of Cu concentrations and stored in Eppendorf tubes at -20°C until analysis. Cu in serum samples were using commercial kits (Copper Liquid, Randox Chemical, U.K.). Livers, spleens, kidneys and hearts of rats were removed after blood collection, weighed and stored at -20°C. The whole organs were dried at 100°C for 24 h, weighed and digested using a wet-ashing procedure by 12 mL of a 4:1 mixture of 65% HNO_3 and 70% $HClO_4$. Ash was filtreted into 100 mL glass bottle with warm distilled water. The solution was analyzed for Cu and Zn via flame atomic absorption spectrophotometry (Model: Perkin-Elmer AAnalyst 400). Bone samples (femur+tibia+fibula) from each rat were cleaned of adhering soft tissue. Faeces were collected from individual rats daily at 23-28 days by total collection of faeces from each cage. In all, 64 fresh faeces samples were collected in plastic trays, weighed and stored in an air tight plastic bag in a freezer until faeces samples were required for analysis when they were homogenized and analyzed for Cu concentrations. Bone and faeces sampels dried at 100°C for 24 h, weigted and ashedat 550°C. Ash obtained was digested by 10 mL of a 1:1 HCl solution and into 250 mL glass bottle with read warm distilled water. The solution was then directly in the flame atomic absorption spectrophotometry (Model: Perkin-Elmer 2380).

Data were subjected to ANOVA using General Linear Models (SPSS, 2002). The models included control, $CuSO_4$ and CuP. Means were separated using Duncan's multiple range tests with a 5% level of probability was used. The results of statistical analysis were shown as mean values and Standard Error (SE) in the tables.

RESULTS AND DISCUSSION

The feed intakes were 17.30, 14.11 and 13.40 g day^{-1} for rats consuming control, $CuSO_4$ and Cu proteinate (CuP) with 15 mg Cu kg^{-1} feed supplementation (Table 2). The feed intakes were highest ($p<0.05$) in control group but this was similar in treatment groups. This were not consistent with the previous studies that organic mineral complexes increased feed intake than inorganic mineral sources in pigs (Coffey *et al*., 1994; Zhou *et al*., 1994) and rats (Du *et al*., 1996). There have not been consistent reports on the effects of organic or inorganic Cu complexes on the performance or Cu retention of rats. In this experiment results are in agreement with the finding of some researchers, in contrast the others. On the other hand, body weight gains of rats supplemented with $CuSO_4$ were significantly higher than control groups and

Table 2: Effects of dietary $CuSO_4$ and CuP on the body weight gain, feed intake and feed conversion ratio

Parameters	Control (n = 20)	$CuSO_4$ (n = 22)	CuP (n = 22)
Initial body weight (g)	86.69±2.23[a]	86.79±1.38[a]	87.98±1.38[a]
Body weight gain (g day^{-1})	2.99±0.17[b]	3.39±0.09[a]	3.15±0.08[ab]
Feed intake (g day^{-1})	17.30±0.44[a]	14.11±0.24[b]	13.40±0.32[b]
Feed conversion ratio (feed intake/weight gain)	6.05±0.34[a]	4.23±0.13[b]	4.30±0.14[b]

[a,b]Different letters in the same row are statistically different ($p<0.05$), ±Standard error

however, this was similar for group supplemented with CuP (Table 2). This finding was in agreement with the studies demonstrating that daily body weight gain increased with the addition of inorganic and organic copper to the diet without any difference between the sources of copper (Stansbury *et al*., 1990; Van Heugten and Coffey, 1992; Aoyagi and Baker, 1993; Coffey *et al*., 1994; Zhou *et al*., 1994) however, not in agreement with the study indicating that animals feeding on organic sources seem to have a tendency to exhibit higher daily body weight gains as compared to animals feeding on inorganic sources (Apgar and Kornegay, 1996). The differences between the results of these studies may be attributed to differences in diet composition.

Feed conversion ratio for rats were similar in treatment groups and significantly ($p<0.05$) lower than in control groups. Although, the treatment groups consumed a lower quantity of feed they exhibited a higher rate of body weight gain indicating better feed efficiency. Moreover, though there were no significant differences between copper sources in terms of feed conversion ratio, the group with CuP addition tended to have quantitatively higher rates as compared to the group with $CuSO_4$ addition. This finding was in agreement with studies reporting there are no differences between copper sources in terms of feed conversion ratio and an improvement in this parameter as compared to control group (Coffey *et al*., 1994; Zhou *et al*., 1994; Apgar and Kornegay, 1996).

Copper concentration in liver, kidney and heart of the rats in the groups with $CuSO_4$ and CuP addition were significantly ($p<0.05$) higher than those in the control group however was similar for different copper sources (Table 3). In general there was no difference between treatment groups however, quantitatively higher values were obtained in the group with $CuSO_4$ addition as compared to the group with CuP addition. This finding was in agreement with the results reported for Cu concentration in liver (Zhou *et al*., 1994; Du *et al*., 1996; Eckert *et al*., 1999; Engle *et al*., 2000) and kidney (Apgar *et al*., 1995) of rats fed on diets with a high level of copper addition. Researchers think that this is related to the fact that increasing Cu content of the diet would result in a decrease in Cu concentration in the liver since the

Table 3: Effects of dietary $CuSO_4$ and CuP on the Cu and Zn contents in organs, bone, faeces and serum

Parameters	Control (n = 17)	$CuSO_4$ (n = 22)	CuP (n = 22)
Cu content (μg g^{-1} of dry tissue)			
Liver	11.37±1.360[b]	28.99±0.460[a]	28.43±1.690[a]
Kidney	32.62±1.710[b]	145.25±7.720[a]	141.34±7.300[a]
Heart	30.37±1.580[b]	300.57±5.430[a]	275.68±4.850[a]
Spleen	59.97±6.920[c]	693.32±45.86[a]	512.99±49.06[b]
Bone (μg g^{-1} dry weight)	8.89±0.770[b]	138.92±4.900[a]	123.65±9.320[a]
Faeces (μg g^{-1} dry weight)	33.26±3.270[a]	20.91±0.610[b]	20.42±1.500[b]
Serum (μmol L^{-1})	15.78±5.470[b]	23.80±4.470[ab]	34.29±5.500[a]
Zn content (μg g^{-1} of dry tissue)			
Liver	60.89±7.420[b]	105.58±2.050[a]	97.86±5.150[a]
Kidney	121.27±1.910[a]	84.77±4.910[b]	77.15±4.170[b]
Heart	96.68±12.28[a]	83.30±7.490[ab]	63.95±9.900[a]
Spleen	178.27±24.41[a]	176.19±18.68[a]	93.37±9.350[b]
Bone (μg g^{-1} dry weight)	152.25±10.35[b]	321.70±6.920[a]	288.07±21.25[a]

decrease in the liver with increasing copper consumption is a mechanism to prevent copper toxicity in the organism. In the study, the fact that Cu concentration in the liver was not too high despite the high copper content of the diet may probably be attributed to this.

The highest Cu concentration in the spleen of the rats was observed in the group with $CuSO_4$ addition and the Cu concentration in the spleen of the group with CuP addition was higher than that in the control group ($p<0.05$). This finding was not in agreement with the results reporting that rats consuming organic copper complexes accumulated more copper in their spleens (Du *et al*., 1996) and Cu-aminoacid complex had higher bioavailability than $CuSO_4$ (Acda and Chae, 2002). The significant differences ($p<0.05$, Table 3) of spleen Cu content among the treatment groups suggested that spleen Cu content was a sensitive indicator of Cu status and utilization of Cu for rats.

In the study, addition of high levels of copper to the diet did not adversely effect Zn concentration in the liver in the treatment groups. Zinc concentration in the liver of the rats was significantly ($p<0.05$) higher in the treatment groups however, there were no differences between sources of copper (Table 3). This finding was in agreement with the results reporting that addition of high levels of inorganic copper to the diet increased Zn concentration in the liver in rats (Aydemir and Ozcan, 2003) however was not in agreement with the results reporting that organic copper complexes caused higher accumulation of Zn in the liver (Du *et al*., 1996). The kidney Zn content of control group was significantly higher than $CuSO_4$ and CuP groups and however this found no significant differences ($p>0.05$) between Cu sources. Regarding zinc concentration in the heart, there were differences between the test groups ($p<0.05$) however, control group had quantitatively higher values

than the group with $CuSO_4$ addition and the group with $CuSO_4$ addition higher than the group with CuP addition. On the other hand, there were no difference in Zn concentration in the spleen of the rats in the control group and the group with $CuSO_4$ addition ($p>0.05$). Moreover, this parameter had significantly low values in the group with CuP addition (Table 3). The values obtained in the experiments for Zn concentration in kidney and heart were in agreement with the results reported by Du *et al*. (1996) however, the values for Zn concentration in the spleen were not. Zn concentration in kidney, heart and spleen of the rats were lower at high levels of copper addition as compared to the Cu accumulation in the same organs. This is expected since zinc is one of the antagonists of copper. Moreover, because the Zn content of the diet is adequate (24.48 mg kg^{-1} Zn) for the rats fed on the basal diet without copper addition, Zn concentration in kidney, heart and spleen in the control group were found to be significantly higher than that in the treatment groups. This situation may be attributed to higher Zn accumulation in the organs depending on the copper deficit in the basal diet.

Serum and liver Cu concentration in rats are parameters that adequately reflect copper bioavailability and there is positive correlation ($r = 0.67$) between them (Lee *et al*., 1988). In the experiments, the highest serum Cu concentration was found in the group with CuP addition ($p<0.05$, Table 3). This finding is in agreement with the results demonstrating that copper absorption rate is higher for and organic copper than for inorganic forms (Ward *et al*., 1996; Acda and Chae, 2002) but was not in agreement with the results reporting that serum Cu concentration is not affected by copper sources (Apgar *et al*., 1995). However, it was found in the study that despite the high levels observed in serum Cu concentration in the group with CuP addition, copper accumulation in the organs was not in parallel. This situation may be attributed to the conclusion that although organic copper sources have high absorption rates in the intestines they are probably metabolized in different ways in the tissues.

Cu and Zn concentrations in bones of rats were high in the treatment groups ($p<0.05$) but were no difference between copper sources (Table 3). Addition of high levels of copper to the diet increased the Cu concentration in the bones however, did not adversely affect Zn concentration. This situation may be attributed to the elimination of the antagonistic effect of addition of high level of copper to the diet when the Zn level in the diet is adequate (Du *et al*., 1996; Wapnir, 1998).

Cu concentration in the faeces of rats was significantly high in the control group however, there was no difference between copper sources (Table 3). The high Cu concentration in the faeces of the rats in the control group may be resulting from endogenic losses of copper depending probably on the copper deficiency in the basal diet (Wapnir, 1998). The low levels of Cu concentration in the faeces in the groups with $CuSO_4$ and CuP addition indicate that both sources of copper have similar levels of bioavailability and although statistically insignificant, the group with CuP addition had better absorption. This finding was in agreement with the studies reporting that copper source had no effect on bioavailability of copper (Stansbury *et al*., 1990; Aoyagi and Baker, 1993).

CONCLUSION

In the study, when high level of copper in $CuSO_4$ and CuP forms was added in the diet of rats, the daily feed intake decreased and similarly, daily body weight gain and feed efficiency rates were improved. For both sources of copper, Cu concentrations in liver, heart and kidney increased and these increases were at comparable levels. Regarding Cu concentration in the spleen, the significant differences between the treatment groups demonstrated that this parameter was a good indicator to reflect bioavailability of copper. The results from the study were generally in favour of the group with $CuSO_4$ addition however, it can be stated that organic copper source was absorbed better, taking into account the high serum Cu concentration in the group with CuP addition.

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