

# Impact of copper (Cu) at the dose of 50 mg on haematological and biochemical blood parameters in turkeys, and level of Cu accumulation in the selected tissues as a source of information on product safety for consumers

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## Abstract

**Introduction.** The current state-of the art points to a positive impact of copper (Cu) supplements on the general health status in poultry. Copper induces beneficial changes in the haematological and biochemical blood parameters. It also displays immunostimulating properties and helps maintain a proper microbiological balance in the digestive tract.

**Objective.** The objective of this study was to investigate the impact of Cu at the dose of 50 mg/kg BW, administered in organic and inorganic form, on the haematological and biochemical blood parameters and level of Cu bioaccumulation in the liver and pectoral muscle.

**Materials and method.** The study was carried out on 45 BUT-9 turkeys which had been reared for 16 weeks. They were divided into 3 experimental groups: I – the control group; II – fed with CuSO<sub>4</sub> at the dose of 50 mg Cu·dm<sup>-3</sup> H<sub>2</sub>O; III – received a Cu chelate with lysine at the same dose.

**Results.** The administration of Cu at the dose exceeding the nutritional recommendations did not induce beneficial changes in the examined birds. This indicates that it is not necessary to administer Cu doses higher than the recommended levels. The extent of Cu accumulation in the pectoral muscle increased by 40% compared to the control group, whereas in the liver it was higher by 30–35% than in the birds without Cu administration. The level of Cu in tissues does not pose a risk to consumers.

**Conclusions.** The supplementation of Cu at the dose of 50 mg has a negative impact on the level of the analyzed parameters. The results of the presented study indicate that the administered Cu dose exceeds birds' demand for this element.

## Key words

copper, turkeys, feeding, mineral elements, food safety.

## INTRODUCTION

Previous studies [1] with turkeys administered Cu at the doses of 10 and 20 mg Cu dm<sup>-3</sup> H<sub>2</sub>O confirmed the health-promoting properties of copper indicated earlier by many authors. Trace elements, such as Cu, are essential for the normal growth in poultry and are involved in numerous processes in the digestive tract and the whole body [2, 3].

The role of Cu in the body results mainly from its presence in the structure of many enzymes that take part in oxidative and reductive processes, such as cytochrome oxidase, lysyl oxidase, superoxide dismutase, ceruloplasmin, and metallothioneins [4].

For many years, copper has been used as a nutritional supplement in poultry production owing to its microbiological properties, potential to increase body weight [5, 6, 7, 8, 9], and positive impact on meat quality [5]. However, attention should

be paid to differing availability of chemical forms of copper used in poultry feeding. A study by AOYAGI AND BAKER [10] indicates that a chelate of copper with lysine is better absorbed than its inorganic forms (by 126%). This finding is further confirmed by studies conducted by GUO et al. [11]. Supplementation of organic Cu allows for better utilization of this element by animals which translates into a reduction of Cu content in faeces, and thereby reduces environmental contamination [12]. Research by PAIK et al. [13] indicates that a Cu-amino acid complex, as opposed to copper sulfate, is better absorbed and increases Cu accumulation in the pectoral muscle, simultaneously reducing its accumulation in the liver.

According to the National Research Council (NRC) [14], the content of Cu in feedstuffs for turkey growers should range from 8–6 mg/kg of feed, depending on the age of birds. Studies by WANGA et al. [5], carried out with turkey broilers on the utilization of trace elements administered as premixes with Zn, Cu and Fe sulfates at doses higher than the recommended values, indicate a lack of significant differences in body weight gains in broilers fed with doses

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exceeding the NRC recommended levels by 20–100% [5, 14]. The lack of significant changes in body weight gains in broilers has also been reported in a study on Cu, Fe, Zn and Mn supplementation; however, it showed an improvement in meat quality [15].

**Objective.** The objective of this study was to determine the impact of copper chelate with lysine and copper sulfate at the dose of 50 mg Cu dm<sup>-3</sup> H<sub>2</sub>O on the haematological and biochemical blood parameters in turkeys, and on the level of Cu accumulation in the liver and pectoral muscle in turkeys. Measurements of Cu concentration in the selected tissues aim at identifying the safety of meat from Cu-supplemented turkeys for consumers.

## MATERIALS AND METHOD

The study was carried out with 45 BUT-9 turkey, reared for 16 weeks. The turkeys were randomly divided into 3 experimental groups, each with 15 birds. The first group constituted the control. Group II was administered CuSO<sub>4</sub> in water at the dose of 50 mg Cu·dm<sup>-3</sup> H<sub>2</sub>O. In group III, the birds received a chelate of Cu with lysine at the dose of 50 mg Cu·dm<sup>-3</sup> H<sub>2</sub>O. The experimental preparations were administered in drinking water in order to eliminate antagonistic effects of feed mixture components on the availability of this element [16].

The birds were kept in cages on straw litter. The same hygienic and zootechnical conditions were provided for all birds according to the requirements for production of slaughter turkeys. The birds had a constant (*ad libitum*) access to feed and water. They were kept on straw litter and fed with conventional complete feed mixtures, adjusted to age and available on the Polish market. The quality of drinking water was consistent with the sanitary regulations specified in the Regulation of the Ministry of Health of 4 May 4 1990 (Official Journal of Laws, No 3, item 250/1990) for drinking water and water for farming purposes.

The biological experiment was based on the 5-stage feeding programme with loose feed mixtures the nutritive value of which was balanced depending on the age and physiological requirements of turkeys. Table 1 presents the composition of feed mixture declared by the manufacturer.

Feed mixtures were determined for contents of selected minerals, i.e. Cu, Zn, Mn, Fe, K, Mg, Ca, and P. Respective results are provided in Table 2.

Before slaughter at the age of 16 weeks, blood was sampled from the brachial vein of the birds for haematological and biochemical analyses. The haematological parameters assayed included: red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb) and haematocrit (Ht), determined by clinical methods. In addition, an automatic blood analyzer, Hitachi 704, was used to measure the following biochemical parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (CHOL), low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides (TG), uric acid (UA), glucose (GLU), and mineral compounds in blood plasma (Ca, Mg, K, Cu, Zn and P). Contents of Cu and Zn were determined with the AAS method.

Dissections were performed after slaughter to collect samples of liver and pectoral muscle. The samples were then

**Table 1.** Nutritive value of complete feed mixtures for turkeys

Feeding period	0–3 week	4–6 week	7–9 week	10–12 week	13–16 week
Metabolizable energy (MJ)	12.08	11.94	12.10	12.50	12.80
Total protein (%)	28.00	27.00	26.00	24.00	21.00
Fat [%]	4.11	4.00	4.70	4.60	4.30
Crude fibre (%)	3.57	4.00	3.50	4.00	4.00
Lysine (%)	1.80	1.70	1.58	1.45	1.25
Methionine (%)	0.68	0.62	0.60	0.57	0.53
Methionine + cystine (%)	1.15	1.10	0.98	0.92	0.87
Tryptophan (%)	0.31	0.32	0.26	0.24	0.22
Calcium (%)	1.35	1.30	1.30	1.20	1.20
Available phosphorus (%)	0.63	0.70	0.72	0.65	0.65
Sodium (%)	0.16	0.15	0.16	0.15	0.15
Vitamin A [IU/kg]	14000	14000	13500	13500	12500
Vitamin D <sub>3</sub> [IU/kg]	3500	3640	3500	3500	3250
Vitamin E [IU/kg]	65	65	50	50	45

**Table 2.** Content of mineral elements in complete feed mixtures for turkeys

Feed mixtures (feeding period)	Minerals							
	Cu	Zn	Mn	Fe	K	Mg	Ca	P
	(mg · kg <sup>-1</sup> )							
0–3 week	16.00	16.50	1.150	24.00	4.835	3.095	12.88	6.940
4–6 week	15.00	19.00	1.250	18.50	4.595	3.610	14.13	7.250
7–9 week	20.00	19.50	1.130	23.00	3.980	3.235	14.65	7.100
10–12 week	22.00	17.00	1.300	17.50	3.940	3.205	7.86	8.060
13–16 week	15.00	18.50	1.250	18.50	2.935	2.645	12.10	6.970

determined for the content of Cu with an atomic absorption spectrophotometer (AAS) on a UNICAM 939 apparatus.

The numerical data were analyzed statistically with one-way analysis of variance (ANOVA) at p=0.05 using STATISTICA 5.0 PL 97 software.

## RESULTS

Table 3 presents final body weights of turkeys at 16 weeks of age. The body weight of turkeys from the control group was 8,410.7 g. The birds administered CuSO<sub>4</sub> reached the lowest body weight, i.e. 8,090.0g, whereas the body weight of the turkeys from group III amounted to 8,340.0 g.

**Table 3.** Final body weights of turkey hens after 16 weeks rearing

Index	Group I	Group II	Group III
	Control	Cu SO <sub>4</sub>	Chelate Cu - Lys
	50 mg Cu · dm <sup>-3</sup> H <sub>2</sub> O		
Body weight (g)	8,410.77 ±782.35	8,090.00 ±777.08	8,340.00 ±796.21

Haematological analyses of blood are a basic source of information on the health status of the body. Table 4 depicts the levels of haematological blood parameters in 16-week-old turkeys. RBC and WBC decreased in groups II and III. In the group supplemented with organic Cu, these values

**Table 4.** Levels of haematological blood indices in 16-week-old turkeys

Index	Group I Control		Group II CuSO <sub>4</sub> (50 mg Cu/l H <sub>2</sub> O)		Group III Chelate Cu-Lys (50 mg Cu/l H <sub>2</sub> O)	
	$\bar{x}$	$\pm SD$	$\bar{x}$	$\pm SD$	$\bar{x}$	$\pm SD$
RBC [10 <sup>12</sup> /l]	1.83 <sup>a</sup>	0.40	1.43 <sup>b</sup>	0.37	1.39 <sup>b</sup>	0.18
WBC [10 <sup>9</sup> /l]	76.23 <sup>a</sup>	3.12	68.96 <sup>b</sup>	4.37	63.60 <sup>b</sup>	26.26
Hb [g/dl]	11.63 <sup>a</sup>	2.43	9.17 <sup>b</sup>	2.33	8.84 <sup>b</sup>	1.12
Ht [%]	28.14 <sup>a</sup>	6.12	22.03 <sup>b</sup>	5.79	21.52 <sup>b</sup>	2.68

were lower than in the birds administered CuSO<sub>4</sub>. Similar observations were reported for haemoglobin concentration. In the birds from group III, the level of Hb was lower by 24% than in the control group. The haematocrit index was 28.14 in the control group, 22.03 in the group administered CuSO<sub>4</sub>, and 21.52 in the group fed with Cu-Lys.

Table 5 presents levels of selected biochemical parameters in blood plasma in 16-week-old turkeys. The activity of blood plasma enzymes was measured in order to determine the toxicity of administered Cu doses. A decrease in AST was accompanied by an increase in ALT activity. In group II, the difference in AST activity was almost 60% in comparison with the control group. In the birds administered Cu, the increase was reported in ALT activity, particularly in group III (7.33 U/l).

**Table 5.** Levels of biochemical indices in blood serum of turkeys

Index	Group I Control		Group II CuSO <sub>4</sub> 50 mg Cu · dm <sup>-3</sup> H <sub>2</sub> O		Group III Chelate Cu-Lys 50 mg Cu · dm <sup>-3</sup> H <sub>2</sub> O	
	$\bar{x}$	$\pm SD$	$\bar{x}$	$\pm SD$	$\bar{x}$	$\pm SD$
UA [mg/dl]	4.34	1.31	4.26	2.03	4.05	1.54
GLU [mg/dl]	301.00 <sup>a</sup>	11.11	299.60 <sup>b</sup>	25.53	269.00 <sup>ab</sup>	50.85
CHOL [mg/dl]	140.00	22.76	152.00	9.97	153.0	25.97
LDL [mg/dl]	30.19 <sup>b</sup>	16.15	42.30 <sup>ab</sup>	17.92	45.43 <sup>a</sup>	18.64
HDL [mg/dl]	89.48 <sup>a</sup>	8.52	82.82 <sup>ab</sup>	22.15	79.12 <sup>b</sup>	15.85
TG [mg/dl]	115.89 <sup>b</sup>	27.44	131.28 <sup>ab</sup>	27.54	141.95 <sup>a</sup>	42.96
ALT [U/l]	4.90	3.25	5.11	3.06	7.33	3.35
AST [U/l]	360.83 <sup>a</sup>	190.09	152.33 <sup>b</sup>	61.25	258.75 <sup>ab</sup>	163.85

**Table 6.** Content of mineral elements in blood serum of turkeys with copper supplementation

Index [mg/dl]	Group I Control		Group II CuSO <sub>4</sub> (50 mg Cu/l H <sub>2</sub> O)		Group III Chelate Cu-Lys (50 mg Cu/l H <sub>2</sub> O)	
	$\bar{x}$	$\pm SD$	$\bar{x}$	$\pm SD$	$\bar{x}$	$\pm SD$
Ca	14.94 <sup>a</sup>	1.35	13.79 <sup>ab</sup>	0.55	11.97 <sup>b</sup>	1.74
Mg	3.11 <sup>b</sup>	0.20	3.19 <sup>b</sup>	0.26	4.38 <sup>a</sup>	1.44
K	139.44 <sup>a</sup>	5.06	125.88 <sup>ba</sup>	15.85	121.91 <sup>b</sup>	12.14
Cu	0.210 <sup>b</sup>	0.06	0.291 <sup>b</sup>	0.029	1.33 <sup>a</sup>	0.150
Zn	2.200	0.20	2.220	0.21	2.35	0.25
P	9.19	1.17	8.31	0.90	8.53	0.79

The supplementation with Cu resulted in a statistically significant increase in TG in blood plasma or serum of the turkeys (Tab. 5). These changes were especially tangible in

group III. The level of glucose decreased to a considerable degree in the birds that received Cu as the amino acid chelate.

The administration of copper supplements modified the mineral composition of blood plasma or serum in turkeys. The results of analyses are summarized in Table 6. The level of copper increased over six times in the group that received the chelate Cu-lysine. Interestingly, the levels of K and Ca were low in groups II and III.

Chemical analysis of pectoral muscle and liver was carried out in order to determine the degree of copper accumulation in the birds. Results of this analysis are presented in Table 7. The highest level of Cu was detected in the group administered organic Cu (liver: 7.15 mg/dl, pectoral muscle: 1.92 mg/dl). The concentration of copper in the pectoral muscle and the liver of the birds administered CuSO<sub>4</sub> also increased in comparison with the control group.

**Table 7.** Content of mineral elements in the liver and pectoral muscles of turkeys

Element [mg/dl]	Group I Control		Group II CuSO <sub>4</sub> (50 mg Cu/l H <sub>2</sub> O)		Group III Chelate Cu-Lys (50 mg Cu/l H <sub>2</sub> O)	
	$\bar{x}$	$\pm SD$	$\bar{x}$	$\pm SD$	$\bar{x}$	$\pm SD$
Liver	5.31 <sup>b</sup>	0.37	6.92 <sup>a</sup>	2.05	7.15 <sup>a</sup>	0.83
Pectoral muscles	1.27 <sup>b</sup>	0.25	1.83 <sup>a</sup>	0.58	1.92 <sup>a</sup>	0.31

## DISCUSSION

The content of mineral compounds, i.e. Ca, P, K, Mg, Fe and Mn, determined in the feed mixture did not differ significantly from the nutritional standards specified by FARUGA [17] and SMULIKOWSKA [18]. Based on the results achieved, it was concluded that the feed mixtures used in the study contained less Zn than the recommended level [17], which might result from excessive losses of this element during dry mineralization of feed at 550°C. The concentration of Cu in the tested feedstuffs ranged from 15 – 22 mg · kg<sup>-1</sup> and was within the standard values [19].

Interestingly, the differences in the activity of aminotransferases do not explicitly explain the lesions in the liver of the tested birds. Our previous studies involving the supplementation of Cu at doses of 10 and 20 mg · dm<sup>3</sup> H<sub>2</sub>O also demonstrated significant variations in aminotransferase activity [1]. Other authors have reported a significantly lower level of AST and ALT with the supplementation of organic and inorganic copper at the dose of 30 mg/kg [20].

The changes in TG blood level detected in the Cu-supplemented birds are a negative phenomenon since they are related to lipogenesis in the liver and fat tissue and to pathologies in carbohydrate metabolism. The other lipid indices were also deteriorated. These results, however, are inconsistent with findings published by Sevcikov et al. [21] who supplemented copper at 35 and 175 mg/kg in the inorganic form, and as an amino acid chelate. In this experiment, they focused on the analysis of lipid indices in poultry meat. The dose of 175 mg Cu/kg in the form of a chelate with glycine resulted in cholesterol content decrease by 24.9%, compared to the control group [21]. In turn, an increase in total cholesterol and triglycerides in birds administered a Cu-lysine chelate was reported by Dmoch et al. [20].

The supplementation with copper preparations slightly modified the mineral composition of plasma or serum. These results are consistent with previous studies [22]. The administration of Cu ions caused an increase in potassium concentration; similar results were reported by Dmoch et al. [20].

The degree of Cu accumulation in the liver and pectoral muscle increased in the CU-supplemented groups. However, in one of the studies supplementation with Cu at 35 mg/kg as CuSO<sub>4</sub> did not cause significant accumulation of copper in tissues. Once the dose was increased five times, the content of Cu in the liver did not change significantly, whereas with other form of copper administered – it was higher by app. 7%. The concentration of Cu in the pectoral muscle upon administration of 175 mg Cu/kg was significantly increased regardless of the form which the supplement was administered in [21]. The tendency of these changes is comparable with our results.

The content of Cu in the liver and pectoral muscle is particularly important for consumers. Cu is essential for erythropoiesis and proper effectuation of numerous metabolic processes. An adult person's demand for copper ranges from 1.5–4 mg/day. This increases in pregnant women, children and elderly people due to poorer absorption [23, 24]. Cu deficiency may contribute to the development of cardiovascular diseases. Furthermore, it also increases the risk of heart failure and thrombosis [4]. Compared to literature data [4], the results presented in Table 7 do not indicate hazards to consumers. Meat from turkeys administered Cu may be a source of this element in the human diet. Consumption of such meat may be an alternative to Cu supplements, the use of which is recommended by Hordyjewska et. al. to maintain a proper level of Cu in an everyday diet [4].

The results of the presented study confirm the findings of the majority of studies and indicate a lack of positive effects reflected in the haematological and biochemical blood parameters in turkeys. In addition, they demonstrate a lack of expected improvement in health with supplementation of Cu at doses higher than the levels recommended by, for instance, NRC, INRA or GfE for growing turkey broilers [25, 26, 27, 28].

## CONCLUSIONS

The results of the presented study confirm a lack of indications for the administration of higher Cu doses than the levels recommended for turkey growers (e.g. NRC). The dose of 50 mg Cu·dm<sup>-3</sup> H<sub>2</sub>O did not produce beneficial changes in the haematological and biochemical blood parameters. This dose resulted in a higher accumulation of Cu in the tissues. Meat from turkeys administered Cu may provide a source of this element in the everyday diet of humans and thus prevent the diseases associated with Cu deficiency.

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