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Original article

Metabolic parameters in young turkeys fed diets with different inclusion levels of copper nanoparticles

K. Kozłowski[1], J. Jankowski[1], K. Otowski[1], Z. Zduńczyk[2], K. Ognik[3]

[1] Department of Poultry Science, Faculty of Animal Bioengineering,
University of Warmia and Mazury in Olsztyn,
Oczapowskiego 5, 10-719 Olsztyn, Poland
[2] Institute of Animal Reproduction and Food Research
of the Polish Academy of Sciences, Tuwima 10, 10-748 Olsztyn, Poland
[3] Department of Biochemistry and Toxicology,
Faculty of Biology, Animal Sciences and Bioeconomy,
University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

Abstract

The aim of this study was to verify the hypothesis postulating that the supplementation of turkey diets with Cu nanoparticles can lower dietary inclusion levels of Cu without compromising the growth rate and antioxidant status of turkeys. The experiment was carried out on 648 one-day-old Hybrid Converter turkeys divided into 6 groups with 6 replicates per group, in a two-factorial design with 3 dietary inclusion levels of Cu (20, 10 and 2 mg/kg) and 2 dietary sources of Cu - copper sulfate (Cu-SUL) and Cu nanoparticles (Cu-NP). At 42 days of age, blood samples were collected from 2 birds per replicate (12 birds per group), after slaughter livers were collected for analyses. Blood and liver samples were assayed for: Cu, Zn, Ca, P, Mg, GLU, TP, ALB, UREA, TAG, TC, UA, ALT, AST, ALT, GGT, ALP, SOD, GPx, CAT, VIT C, FRAP, GSH+GSSG, LOOH, MDA.

The results of this experiment demonstrate that a decrease in the dietary inclusion levels of Cu from 10 mg/kg to 2 mg/kg does not compromise the growth performance of turkeys, but weakens antioxidant defense mechanisms. A Cu dose of 20 mg/kg induces oxidation reactions and has a much more inhibitory effect on the antioxidant defense system than dietary Cu content of 2 mg/kg. In turkeys, dietary supplementation with Cu-NP has a more beneficial effect on carbohydrate metabolism and antioxidant status compared with Cu-SUL. The results of analyses examining the antioxidant and metabolic status of young turkeys indicate that 10 mg/kg is the optimal dietary inclusion level of Cu.

Key words: nano-copper, turkey, blood, liver, redox status

Correspondence to: K. Ognik, e-mail: kasiaognik@poczta.fm

Introduction

Copper (Cu) is an essential micronutrient for healthy growth, development and functioning of living organisms. This element is present in the active sites of many enzymes that participate in key metabolic processes (Festa and Thiele 2011) such as mitochondrial respiration, protection against free radicals, neurotransmitter synthesis, production of collagen and elastin, melanin synthesis and iron metabolism (Gupta and Lutsenko 2009). Copper participates in immune responses and inhibits inflammatory processes by influencing the metabolism of arachidonic acid and prostaglandin synthesis. The element mediates thiol oxidation to disulfides, stabilizes the permeability of cell and intracellular membranes and binds to histamine to minimize its adverse effects during inflammatory processes (Angelova et al. 2011).

Inside cells, Cu is bound mostly to thiol-containing intracellular proteins with high affinity for Cu ions. According to the literature, 60-70% of Cu in blood plasma is bound to ceruloplasmin, 10-30% to transcuprein, and 15-20% to albumin (Lutsenko et al. 2007). Free Cu ions which are not bound to ceruloplasmin promote free radical damage to proteins, lipids and nucleic acids (Brewer 2007). By directly bonding with free thiol groups, Cu can promote the oxidation and cross-linking of proteins, which can lead to enzyme inactivation and damage to cell structural proteins (Dusek and Jankovic 2012; Ognik et al. 2017). Poultry diets, including turkey diets, are supplemented with copper sulfate. According to the recommendations of the National Research Council (NRC 1994), the inclusion levels of Cu in diets fed to growing turkeys should reach 6-8 mg Cu/kg. The inclusion levels recommended by breeding companies are higher at 20 mg/kg. In line with EU regulations, the Cu content of poultry diets should not exceed 25 mg/kg (EFSA 2016). Copper concentration in animal diets has to be monitored due to growing levels of environmental contamination with this element. These risks spur the search for alternative chemical forms of Cu, mostly chelates with amino acids which are highly bioavailable and which decrease the amount of Cu released to the environment (Karimi et al. 2011). Poultry diets can also be supplemented with Cu nanoparticles which are highly physically active and chemically neutral. The biological activity of nanoparticles can be attributed to their large surface area which enables Cu atoms to directly reach target cells (Pineda et al. 2013). If Cu nanoparticles are capable of exerting similar effects to macroparticles, their inclusion levels in poultry diets can be reduced on account of higher biological reactivity, which will decrease the amount of Cu released to the environment.

The aim of this study was to verify the hypothesis postulating that the supplementation of turkey diets with Cu nanoparticles can lower dietary inclusion levels of Cu without compromising the growth rate and antioxidant status of turkeys.

Materials and Methods

Experimental design and diet composition

A total of 648 one-day-old Hybrid Converter female turkeys purchased from the Grelavi Hatchery in Kętrzyn were randomly placed in 36 pens with a surface area of 3.7 m^2, with 18 birds per pen. The pens were bedded with wood shavings. Stocking density was 4.86 birds /m^2 until week 6, and 3.2 birds/m^2 from week 6 until the end of the experiment. Turkeys had free access to feed and water, and management conditions were consistent with the recommendations of the breeder company and adjusted to the birds' age. Turkeys were divided into 6 groups with 6 replicates per group, in a two-factorial design with 3 dietary inclusion levels of Cu (20, 10 and 2 mg/kg) and 2 dietary sources of Cu – copper sulfate and Cu nanoparticles (Cu-SUL and Cu-NP). Copper nanoparticles (25 nm in size) in the form of 99.8% purity powder, purchased from the Sky Spring Nanomaterials Inc. (USA), were added to a vitamin-mineral premix using a carbohydrate carrier.

Basal diets whose composition is given in Table 1 were supplemented with different doses of Cu. Diets were prepared in the "Agrocentrum" Feed Mill Ltd., in two stages: (1) basal diets without the vitamin-mineral premix, (2) diets for experimental groups were supplemented with vitamin-mineral premixes containing different levels and sources of Cu, thoroughly mixed, pelleted and crumbled. At 42 days of age, blood samples were collected from 2 birds per replicate (12 birds per group) representing average BW; after slaughter, livers were collected for biochemical analyses.

Laboratory Analysis

Blood for analysis was collected from the wing vein into test tubes with an anticoagulant (heparin). Blood samples were centrifuged at 3,000 g for 10 min, and plasma was collected for further analysis. The concentrations of glucose (GLU), triacylglycerols (TAG), total cholesterol (TC), uric acid (UA), total protein (TP), albumin (ALB), creatinine (CREAT), and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate and gamma-glutamyl transferase (GGT) were measured using an automatic biochemical analyzer (Plasma Diagnostic Instruments Horiba, Kyoto, Japan).

In erythrocytes, superoxide dismutase (SOD) activity was measured with the Ransod kit (Randox), glutathione peroxidase (GPx) activity was measured with the Ransel kit (Randox), and catalase (CAT) activity was measured according to Aebi (1984). Total antioxidant potential (FRAP) was determined in blood plasma by the method described by Ognik and Wertelecki (2012). The concentrations of total glutathione (GSH + GSSG), malondialdehyde (MDA) and lipid hydroperoxides (LOOH), and the activities of SOD and CAT were determined in blood plasma and liver by the methods proposed by Ognik and Wertelecki (2012). Copper content in samples of diets and blood plasma was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). The content of zinc (Zn), calcium (Ca), phosphorus (P) and magnesium (Mg) in samples of diets, blood plasma and liver were determined by flame atomic absorption spectrometry (FAAS).

Statistical Analysis

Two-way ANOVA was performed to determine the effects of Cu inclusion level (20, 10 and 2 mg/kg) and form (Cu-SUL or Cu-NP) and the interaction between both factors (level x form). The significance of differences between mean values of the analyzed parameters in groups was estimated by Duncan's multiple range test. Data were processed in the STATISTICA PL 12.0 application.

Results

The effect of different dietary inclusion levels of Cu on metabolic parameters in turkeys

The Cu content of experimental diets was close to the expected value (Table 1). Minor differences could have resulted from the inaccuracy of the analytical technique. The difference between the total Cu content of diets and supplemental Cu doses shows that major feed ingredients provided approximately 11 mg/kg Cu in total. After 6 weeks of feeding diets supplemented with various amounts of Cu, none of the experimental factors affected the body weights of turkeys, diet intake or feed conversion ratio (FCR) (Table 2). In comparison with subgroups where diets were supplemented with the lowest and medium Cu doses, turkeys fed diets with the highest addition of Cu were characterized by higher Cu level) and lower Zn levels in blood plasma (Table 3). The plasma levels of Ca and P were higher in turkeys fed diets with the lowest inclusion level of Cu than in birds receiving diets with the medium and highest inclusion level of Cu. The blood plasma level of Mg increased in response to increasing supplemental Cu doses. A significant interaction between the inclusion level and form of Cu was also noted for Mg - higher Cu doses in the form of Cu-NP increased plasma Mg concentrations. The lowest dietary inclusion level of Cu reduced average plasma TP levels due to a high decrease in TP levels in the subgroup where diets were supplemented with the lowest dose of Cu-NP (L × F interaction) (Table 4). The highest dietary inclusion level of Cu increased plasma TAG concentrations in comparison with the remaining treatments, whereas other blood biochemical parameters were not affected by dietary supplementation with Cu. The addition of the highest Cu dose to turkey diets increased the activities of SOD and CAT, and MDA concentrations in blood plasma, relative to subgroups with the lowest and medium Cu doses (Table 5). Different dietary inclusion levels of Cu exerted varied effects on FRAP values and GSH+GSSG concentrations in the blood plasma of turkeys. Higher FRAP values and lower GSH+GSSG concentrations were observed in turkeys fed diets with the lowest and highest addition of Cu, compared with the subgroup receiving diets with the medium Cu dose. Turkeys fed diets with the lowest inclusion level of Cu were characterized by lower plasma vitamin C concentrations than birds in the remaining treatments. The highest supplemental Cu dose decreased GSH+GSSG concentrations in the liver, relative to subgroups with the lowest and medium Cu doses. The values of the remaining redox status parameters in the liver were not influenced by the dietary inclusion levels of Cu (Table 6).

The effect of different dietary forms of Cu on metabolic parameters in turkeys

Dietary Cu sources (Cu-SUL vs. Cu-NP) had no effect on the growth performance of turkeys (Table 2) or the plasma concentrations of Cu, Zn, Ca, P and Mg (Table 3). Cu-NP lowered average GLU and ALB levels in the blood plasma of turkeys, which resulted from a high decrease in the above parameters in the subgroup where diets were supplemented with the lowest dose of Cu-NP (L x F interaction) (Table 4). Cu-NP, in comparison with Cu-SUL, increased GSH+GSSG concentrations, and decreased MDA levels and CAT activity in the blood plasma of turkeys (Table 5). The replacement of Cu-SUL with Cu-NP in turkey diets had no influence on the redox status parameters in the liver (Table 6).

Discussion

In the present experiment, supplemental Cu doses of 2, 10 and 20 mg/kg increased the total Cu content of

Table 1. Diet composition and nutrient content.

Composition of basal diet		Total Cu content of experimental diets	
Ingredients	%	Diet[2]	mg/kg
Wheat	43.11	20 – SUL	31.2
Soybean meal	38.96	20 – NP	28.4
Faba bean	10.00	10 – SUL	21.1
Soybean oil	2.80	10 – NP	20.4
Sodium sulfate	0.15	2 – SUL	14.9
Salt	0.20	2 – NP	13.7
Limestone	1.60		
Monocalcium phosphate	1.75		
Methionine	0.37		
Lysine	0.44		
Threonine	0.12		
Vitamin-mineral premix[1]	0.50		
Analyzed nutrients			
Crude protein %	25.88		
Ca %	1.28		
P %	0.87		
Zn mg/kg	172		
Fe mg/kg	258		

[1] Per kg of diet: Vit. A – 24999.75 I.U, Vit. D – 35000 I.U, Vit. E – 100 I.U, Tocopherol – 91 mg, Vit. K – 4 mg, Vit. B_1 – 5 mg, Vit. B_2 – 15 mg, Vit. B_6 – 6 mg, Vit. B_{12} – 0.04 mg, niacin – 100 mg, pantothenic acid – 30 mg, folic acid – 4 mg, choline chloride – 700 mg, calcium d-pantothenate – 30.0 mg, biotin – 0.35 mg, total Se – 0.3 mg, total Fe – 60 mg, total Mn – 100 mg, total Zn – 100 mg, J – 1.5 mg, Ca – 1.0435 g

[2] Diets supplemented with 2, 10 and 20 mg of Cu in the form of sulfate (Cu-SUL_2, Cu-SUL_{10}, Cu-SUL_{20}) or 2, 10 and 20 mg of Cu in the form of nanoparticles (Cu-NP_2, Cu-NP_{10}, Cu-NP_{20})

Table 2. Growth performance of turkeys.

	Body weight, kg		Daily feed intake, g	FCR kg/kg
	day 1	day 42		
Dietary supplementation with Cu mg/kg[1]				
20 Cu – SUL	0.065	2.741	96.0	1.523
20 Cu – NP	0.065	2.792	98.5	1.520
10 Cu – SUL	0.065	2.685	97.4	1.564
10 Cu – NP	0.065	2.733	97.6	1.537
2 Cu – SUL	0.065	2.752	98.9	1.546
2 Cu – NP	0.065	2.759	99.2	1.549
Cu inclusion levels, mg/kg				
20	0.065	2.767	97.3	1.522
10	0.065	2.709	97.5	1.550
2	0.065	2.755	99.1	1.548
Form of Cu				
Cu – SUL	0.065	2.726	97.4	1.544
Cu – NP	0.065	2.761	98.4	1.535
SEM	<0.001	0.014	0.429	0.006
P value				
Inclusion level (L)	0.133	0.246	0.184	0.149
Form (F)	0.599	0.228	0.252	0.477
L × F interaction	0.533	0.783	0.458	0.609

[1] Diets supplemented with 2, 10 and 20 mg of Cu in the form of sulfate (Cu-SUL_2, Cu-SUL_{10}, Cu-SUL_{20}) or 2, 10 and 20 mg of Cu in the form of nanoparticles (Cu-NP_2, Cu-NP_{10}, Cu-NP_{20})

Table 3. Concentrations of selected macroelements and microelements in the blood plasma of 42-day-old turkeys.

	Cu μmol l^{-1}	Zn μmol l^{-1}	Ca mmol l^{-1}	P mmol l^{-1}	Mg mmol l^{-1}
Dietary supplementation with Cu, mg/kg[1]					
20 Cu – SUL	3.32	41.1	3.00	1.61^{b}	0.32^{ab}
20 Cu – NP	3.67	42.3	3.26	1.82^{b}	0.34^{a}
10 Cu – SUL	2.09	45.8	3.25	1.67^{b}	0.29^{b}
10 Cu – NP	2.06	43.0	3.45	1.54^{b}	0.31^{ab}
2 Cu – SUL	1.80	44.3	4.34	1.74^{b}	0.29^{b}
2 Cu – NP	2.50	45.3	4.53	2.23^{a}	0.26^{c}
Cu inclusion levels, mg/kg					
20	3.50^{a}	41.7^{b}	3.13^{b}	1.72^{b}	0.33^{a}
10	2.07^{b}	44.4^{a}	3.35^{b}	1.61^{b}	0.30^{b}
2	2.15^{b}	44.8^{a}	4.43^{a}	1.99^{a}	0.27^{c}
Form of Cu					
Cu – SUL	2.41	43.7	3.53	1.67	0.30
Cu – NP	2.74	43.5	3.75	1.87	0.30
SEM	0.137	0.478	0.194	0.058	0.006
P value					
Inclusion level (L)	<0.001	0.011	0.014	0.012	<0.001
Form (F)	0.092	0.805	0.562	0.066	0.771
L × F interaction	0.328	0.126	0.995	0.049	0.039

[1] Diets supplemented with 2, 10 and 20 mg of Cu in the form of sulfate (Cu-SUL_2, Cu-SUL_{10}, Cu-SUL_{20}) or 2, 10 and 20 mg of Cu in the form of nanoparticles (Cu-NP_2, Cu-NP_{10}, Cu-NP_{20})

Table 4. Biochemical blood plasma parameters in 42-day-old turkeys.

	GLU mmol l^{-1}	TP g l^{-1}	ALB μmol l^{-1}	UREA mmol l^{-1}	TAG mmol l^{-1}	TC mmol l^{-1}	UA μmol l^{-1}	ALT U l^{-1}	AST U l^{-1}	GGT U l^{-1}	ALP U l^{-1}
Dietary supplementation[1]											
20 Cu – SUL	13.9^{bc}	34.4^{a}	184^{a}	0.48	0.34	2.50	275	9.83	532	3.74	593^{ab}
20 Cu – NP	14.3^{ab}	35.2^{a}	188^{a}	0.42	0.36	2.43	337	9.69	543	3.99	658^{a}
10 Cu – SUL	14.9^{a}	34.3^{a}	193^{a}	0.43	0.33	2.75	310	8.45	534	2.88	664^{a}
10 Cu – NP	14.5^{ab}	33.7^{a}	188^{a}	0.46	0.28	2.66	270	10.2	554	3.33	679^{a}
2 Cu – SUL	15.1^{a}	34.6^{a}	195^{a}	0.39	0.31	2.77	298	9.45	517	2.15	682^{a}
2 Cu – NP	13.0^{c}	28.8^{b}	169^{b}	0.52	0.27	2.49	275	9.39	532	3.61	534^{b}
Cu inclusion levels, mg/kg											
20	14.1	34.8	186	0.45	0.35^{a}	2.46	306	9.76	537	3.86	626
10	14.7	34.0	190	0.44	0.31^{b}	2.70	289	9.33	544	3.10	672
2	14.0	31.7	182	0.46	0.29^{b}	2.63	286	9.42	524	2.88	608
Form of Cu											
Cu – SUL	14.7^{a}	34.4	190^{a}	0.43	0.33	2.67	294	9.24	528	2.92	646
Cu – NP	14.0^{b}	32.6	182^{b}	0.46	0.30	2.53	294	9.76	543	3.64	624
SEM	0.16	0.57	2.15	0.02	0.009	0.07	10.14	0.193	19.58	0.282	16.49
P value											
Inclusion level (L)	0.105	0.055	0.211	0.962	0.013	0.466	0.708	0.620	0.926	0.340	0.225
Form (F)	0.014	0.078	0.026	0.442	0.100	0.354	0.987	0.170	0.715	0.209	0.466
L × F interaction	0.002	0.031	0.008	0.158	0.144	0.853	0.100	0.074	0.995	0.647	0.018

[1] Diets supplemented with 2, 10 and 20 mg of Cu in the form of sulfate (Cu-SUL_2, Cu-SUL_{10}, Cu-SUL_{20}) or 2, 10 and 20 mg of Cu in the form of nanoparticles (Cu-NP_2, Cu-NP_{10}, Cu-NP_{20})

Table 5. Blood redox status parameters in 42-day-old turkeys.

	VIT C µmol l^{-1}	CAT U g Hb	SOD U g Hb	GPx U g Hb	GSH+GSSG µmol l^{-1}	FRAP µmol l^{-1}	MDA µmol l^{-1}	LOOH µmol l^{-1}
Dietary supplementation[1]								
20 Cu – SUL	63.3	1639	2462^{a}	44.5	0.17	364	1.38	36.7
20 Cu – NP	59.4	1338	1868^{b}	39.6	0.23	368	1.02	30.6
10 Cu – SUL	54.3	1010	1432^{b}	50.3	0.50	281	1.35	30.7
10 Cu – NP	59.5	299	1823^{b}	40.9	0.63	296	0.92	35.7
2 Cu – SUL	50.0	484	1345^{b}	43.4	0.18	383	1.17	37.3
2 Cu – NP	43.4	383	1551^{b}	40.3	0.35	4240	0.65	37.4
Cu inclusion levels mg/kg								
20	61.4^{a}	1488^{a}	2165^{a}	42.1	0.20^{b}	366^{a}	1.20^{a}	33.7
10	56.9^{a}	655^{b}	1628^{b}	45.6	0.57^{a}	288^{b}	1.13^{b}	33.2
2	46.7^{b}	434^{b}	1448^{b}	41.8	0.27^{b}	404^{a}	0.91^{b}	37.4
Form of Cu								
Cu – SUL	55.9	1044^{a}	1746	46.1	0.28^{b}	343	1.30^{a}	34.9
Cu – NP	54.1	673^{b}	1747	40.3	0.41^{a}	363	0.86^{b}	34.6
SEM	1.51	109.2	96.0	1.52	0.22	14.9	0.05	1.15
P value								
Inclusion level (L)	<0.001	<0.001	0.003	0.524	<0.001	0.005	0.022	0.280
Form (F)	0.476	0.035	0.996	0.061	0.007	0.472	<0.001	0.888
L × F interaction	0.132	0.338	0.048	0.683	0.587	0.854	0.755	0.147

[1] Diets supplemented with 2, 10 and 20 mg of Cu in the form of sulfate (Cu-SUL_2, Cu-SUL_{10}, Cu-SUL_{20}) or 2, 10 and 20 mg of Cu in the form of nanoparticles (Cu-NP_2, Cu-NP_{10}, Cu-NP_{20})

Table 6. Liver redox status parameters in 42-day-old turkeys.

	VIT C µmol kg^{-1}	CAT U g protein	SOD U g protein	GSH+GSSG µmol kg^{-1}	LOOH µmol kg^{-1}	MDA µmol kg^{-1}
Dietary supplementation[1]						
20 Cu – SUL	60.7	67.9	4.64	0.40	2.15	3.39
20 Cu – NP	58.6	69.2	4.33	0.42	2.38	3.68
10 Cu – SUL	61.1	67.0	3.97	0.48	2.30	3.26
10 Cu – NP	60.6	70.1	3.80	0.45	2.52	3.39
2 Cu – SUL	63.4	69.3	4.02	0.46	2.49	4.10
2 Cu – NP	66.0	66.3	3.90	0.47	2.11	3.87
Cu inclusion levels, mg/kg						
20	59.7	68.6	4.49	0.408^{b}	2.26	3.54
10	60.9	68.5	3.89	0.464^{a}	2.41	3.33
2	64.7	67.8	3.96	0.466^{a}	2.30	3.98
Form of Cu						
Cu – SUL	61.8	68.1	4.21	0.445	2.31	3.58
Cu – NP	61.7	68.5	4.01	0.447	2.34	3.65
SEM	1.493	1.770	0.139	0.010	0.107	0.205
P value						
Inclusion level (L)	0.376	0.980	0.170	0.029	0.854	0.433
Form (F)	0.994	0.907	0.480	0.916	0.915	0.876
L × F interaction	0.817	0.790	0.961	0.654	0.420	0.880

[1] Diets supplemented with 2, 10 and 20 mg of Cu in the form of sulfate (Cu-SUL_2, Cu-SUL_{10}, Cu-SUL_{20}) or 2, 10 and 20 mg of Cu in the form of nanoparticles (Cu-NP_2, Cu-NP_{10}, Cu-NP_{20})

turkey diets to approximately 14, 21 and 30 mg/kg, respectively. The noted Cu concentration was below, similar to and higher than the inclusion rate of 25 mg/kg recommended for poultry diets in the EU (EFSA, 2016).

According to the literature, supplemental Cu can promote growth in chickens at very high doses reaching 100–450 mg/kg (Pekel and Alp 2011; Samanta et al. 2011). In our study, the supplementation of turkey diets with Cu-SUL or Cu-NP at 2, 10 and 20 mg Cu/kg for 42 days did not influence the growth performance of female turkeys. Similar results were reported by Makarski et al. (2014), in whose study, diets supplemented with 15 mg/kg or 65 mg Cu /kg had no effect on the performance parameters of turkeys. In this experiment, the highest dietary inclusion level (20 mg/kg) of Cu-SUL and Cu-NP increased Cu levels and decreased Zn levels in the blood plasma of turkeys. In a previous study of chickens, a higher dietary dose of Cu nanoparticles inhibited Zn absorption in the intestines (Ognik et al. 2016). In the work of Adegbenjo et al. (2014), dietary supplementation with Cu at 50 mg/kg also increased Cu concentration and lowered Zn concentration in the blood plasma of 8-week-old chickens. Contrary results were reported in an experiment where chicken diets were supplemented with 75 mg Cu/kg. The plasma concentrations of both Cu and Zn increased in the experimental birds relative to control chickens whose diets were not supplemented with Cu (Samanta et al. 2011). Copper and Zn cations are metabolic antagonists (Bjorklund 2013), therefore, higher dietary inclusion levels of Cu can decrease blood Zn concentrations. In the present experiment, plasma P and Ca levels decreased in turkeys whose diets were supplemented with the highest and medium Cu doses. These results could suggest that in young turkeys, an increase in Cu concentration can decrease the availability of nutrients bound to phytic acid, in particular P. The above observation has been confirmed by studies where high dietary inclusion levels of Cu (250 mg/kg or higher) decreased apparent P retention (Banks et al. 2004).

The observed decrease in plasma Ca levels in response to increased dietary Cu content is consistent with the results of a previous experiment on chickens where dietary supplementation with Cu-NP decreased Ca absorption in the intestines (Ognik et al. 2016). Unlike other nutrients, Ca can be absorbed via different mechanisms, including TRPV6 and calbindin-D_{9K} Ca transport proteins, active transport of Ca^{2+}-ATPase, passive diffusion of Na^{+}/Ca^{2+} ions, and direct paracellular transport from the digestive tract to bodily fluids (Hoenderop 2005). The above mechanisms of Ca absorption could be compromised under exposure to higher dietary levels of Cu. In our study, an increase in plasma Mg levels was also noted in turkeys fed diets with the highest and medium Cu doses. Phosphorus and Ca are antagonists of Mg, therefore, a decrease in P and Ca levels could increase Mg concentration.

Surprisingly, plasma TAG levels were higher in turkeys fed diets supplemented with 20 mg Cu/kg. In an experiment by Kaya et al. (2006), diets deficient in Cu (3.5 mg Cu/kg in feed ingredients) were more likely to induce hypertriglyceridemia, hypercholesterolemia and anemia than diets containing 8 mg Cu/kg. In a study where chicken diets were supplemented with 50, 100 and 150 mg Cu/kg, plasma TC, LDL and TAG concentrations decreased with a rise in Cu inclusion levels (Jegede et al. 2011). Dietary Cu did not influence the blood lipid profile in turkeys fed diets supplemented with 50 mg Cu/kg (Makarski et al. 2014) or chickens fed diets supplemented with 75 mg Cu/kg (Samanta et al. 2011). In our experiment, the increase in plasma TAG concentration could have resulted from a higher Cu:Zn ratio in blood plasma. Hypercholesterolemia induced by Zn deficiency due to higher dietary intake of Cu was reported in a study of rats (Muhammad et al. 2012). In our experiment, plasma TP concentration increased with a rise in the dietary inclusion levels of Cu. Almonsuor (2006) supplemented quail diets with Cu-SUL in doses of 100 to1000 mg/kg and observed an increase in plasma TP concentration in birds fed diets with the addition of 750 and 1000 mg Cu/kg.

In a previous study of turkeys (Mikulski et al. 2009), an increase in dietary inclusion levels of Cu from 11 to around 30 mg/kg did not influence SOD activity in blood. In the present experiment, the increase in the dietary dose of Cu from 2 to 20 mg/kg increased FRAP and MDA values and SOD and CAT activity in blood, and decreased GSH+GSSH levels in blood and liver. Our results could suggest that unlike diets with low inclusion levels of Cu, the highest Cu dose contributes to oxidative stress. Intensified lipid peroxidation enhances the production of new radical forms, which could lead to the depletion of endogenous antioxidants. The above suggestion is supported by the noted decrease in GSH+GSSG levels. The described process has negative effects because GSH+GSSG participate in free radical reactions and the elimination of many xenobiotics. In studies of chickens whose diets were supplemented with very high doses of Cu (100 mg/kg or higher), oxidation processes were intensified in tissues (Min et al. 2009, Cao et al. 2016). Min et al. (2009) found that an increase in hydroxy radical activity was accompanied by a rise in MDA concentration in brain tissue and an increase in SOD and GPx activity in response to Cu doses of 100 and 200 mg/kg, whereas Cu doses of 400–800 mg Cu/kg decreased the activity

of the analyzed enzymes. A decrease in GSH levels and an increase in MDA concentration in blood plasma were also reported in chickens administered 50 μg Cu-SUL in ovo relative to control group chickens (Oguz et al. 2014).

In our experiment, dietary supplementation with Cu-NP, in particular at the inclusion level of 20 mg/kg, decreased plasma glucose and albumin concentrations in turkeys. A decrease in glucose levels with a simultaneous increase in albumin concentration were also reported in chickens fed diets without and with very high inclusion levels of Cu-SUL (100-400 mg/kg) (Kumar et al. 2013). Mroczek-Sosnowska et al. (2016) also observed a decrease in plasma glucose levels of chickens administered Cu-NP in ovo during embryogenesis.

Nanoparticles are characterized by small size, large surface area and high reactivity, which enables them to bind cell membrane proteins and cell surface receptors during transamination of metals in cells. Proteins with high affinity for nanoparticles are adsorbed on the surface of nanoparticles to create hard corona complexes, whereas low affinity proteins create soft corona complexes (Monopoli et al. 2011). Hard corona proteins interact directly with the surface of nanoparticles, whereas soft corona proteins interact with hard corona proteins via weak protein-protein interactions (Walkey et al. 2012). In the group of hard corona proteins with affinity for nanoparticles, albumins interact most readily with the surface of this nanometal. The interactions between albumins and the nanometal or other proteins can decrease plasma levels of free albumin. Our findings and the results reported by other authors (Brewer 2007, Ajuwon et al. 2011) indicate that excessive dietary inclusion levels of Cu, in particular Cu-SUL, can intensify oxidation processes in poultry. In our study, the supplementation of turkey diets with Cu-NP in doses of 2, 10 and 20 mg Cu/kg had a more beneficial effect on the antioxidant status of birds than the corresponding doses of Cu-SUL. These observations were confirmed by lower MDA levels, lower CAT activity and higher GSH+GSSH concentrations in the blood of turkeys receiving Cu-NP than in turkeys administered Cu-SUL.

Conclusions

The results of this experiment demonstrated that a decrease in the dietary inclusion levels of Cu from 10 mg/kg to 2 mg/kg does not compromise the growth performance of turkeys, but weakens antioxidant defense mechanisms. A Cu dose of 20 mg/kg induces oxidation reactions and has a much more inhibitory effect on the antioxidant defense system than dietary Cu content of 2 mg/kg. In turkeys, dietary supplementation with Cu-NP has a more beneficial effect on carbohydrate metabolism and antioxidant status compared with Cu-SUL. The results of analyses examining the antioxidant and metabolic status of young turkeys indicate that 10 mg/kg is the optimal dietary inclusion level of Cu.

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