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REDOX AND IMMUNOLOGICAL STATUS OF TURKEYS FED DIETS WITH DIFFERENT LEVELS AND SOURCES OF COPPER*

Jan Jankowski[1], Krzysztof Kozłowski[1], Katarzyna Ognik[2], Zenon Zduńczyk[3], Kamil Otowski[1], Ewa Sawosz[4], Jerzy Juśkiewicz[3]♦

[1]Department of Poultry Science, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-719 Olsztyn, Poland
[2]Department of Biochemistry and Toxicology, Faculty of Biology, Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland
[3]Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10, 10-748 Olsztyn, Poland
[4]Faculty of Animal Science, Department of Animal Nutrition and Biotechnology, Warsaw University of Life Sciences, Ciszewskiego 8, 02-786 Warszawa, Poland
♦Corresponding author: j.juskiewicz@pan.olsztyn.pl

Abstract

This study, performed on turkeys aged 1 to 98 days, aimed to investigate whether different dietary inclusion levels (20, 10, 2 mg kg^{-1}) of copper nanoparticles (Cu-NP) as a substitute for copper sulphate (Cu-SUL) affect redox and immunological status of turkeys' tissues. No significant differences in the final body weights of turkeys were found across the dietary treatments. A comparison of the physiological effects of Cu-NP and Cu-SUL revealed equivocal metabolic responses including decreased superoxide dismutase (SOD) activity in the liver, increased SOD and catalase activities in breast muscles, decreased total glutathione concentrations in breast muscles, and decreased plasma IgY concentrations. An analysis of the antioxidant and immune status parameters in the blood, liver and breast meat of turkeys indicates that 10 mg/kg is the optimal inclusion level of additional Cu. Both two-fold higher and five-fold lower Cu supplementation levels have a negative influence on selected parameters of the antioxidant and immune status of birds. Lower supplementation levels of Cu-NP (2 and 10 mg/kg) exert similar physiological effects to Cu-SUL, whereas higher addition of Cu-NP (20 mg/kg) may negatively affect selected redox parameters and stimulate the synthesis of the proinflammatory cytokine IL-6. The results of the present study indicate that further research is needed to establish the actual dietary requirements for Cu in turkeys and the efficacy of nanoparticles as a new additional Cu source in turkey nutrition.

Key words: turkey, nano-copper, redox status, immunity, breast meat

Copper (Cu) participates in most of enzymatic reactions and cellular metabolism. In addition, Cu is essential for a wide range of health and performance-related

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functions in all animal species (Klasing, 1998). An adequate amount of dietary Cu is required for the proper functioning of the circulatory system in animals (Kim et al., 1992), which is an important consideration in view of the sudden death syndrome and other cardiovascular diseases in fast-growing birds. Therefore, Cu is one of the ingredients of typical mineral premixes routinely used in poultry nutrition, and the recommended amount of supplemental Cu has been increased from 8 mg kg^{-1} to 20 mg kg^{-1} (NRC, 1994; Hybrid Turkeys, 2013, respectively). However, the amount of Cu used in the poultry diets can reach up to 25 mg kg^{-1}. The European Food Safety Authority (EFSA) published the newly proposed maximum content of Cu in complete diets for targeted animals to reduce the amount of Cu released into the environment (EFSA, 2016). This is an important consideration since a large proportion of inorganic Cu salts such as sulphate remains in poultry excreta and contaminates the environment (Maheshwari, 2013). On the other hand, organic forms of Cu, which are better utilised by poultry (Nollet et al., 2008; Mikulski et al., 2009), are not in wide use.

Recent research has shown that nano-minerals, including Cu nanoparticles, could be used as feed additive to improve growth rate, digestion and absorption in poultry and livestocks (Bunglavan et al., 2014; Ognik et al., 2016; Hill and Li, 2017; El Sabry et al., 2018). Due to the special characteristics of nanoparticles such as shape, small size, large surface area, which enhance activity of this form of minerals, some new properties of nanoparticles could be expected (Albanese et al., 2012; Majewski et al., 2017; Tomaszewska et al., 2017; El Sabry et al., 2018). This may be a key feature of Cu nanoparticles in view of the need to reduce the amount of Cu in animal diets and to minimize the amount of Cu released into the environment. From the consumer's point of view, a reduction in Cu accumulation in edible carcass parts as well as the health benefits and high quality of meat are of utmost importance. Therefore, the aim of this study was to investigate whether the dietary inclusion of Cu-NP as a substitute for Cu-SUL affects the growth performance, redox and immune status of turkeys.

Material and methods

Birds and dietary treatments

The experiment was approved by the local Ethics Committee for Experiments on Animals in Olsztyn (permission No. 30/2015; 2015.04.29). A total of 648 one-day-old Hybrid Converter female turkey poults were placed in 36 pens, and were raised in accordance with the recommendations of a breeder management guide. Turkey poults were divided into 6 groups with 6 replicates per group (each of 18 poults), in a two-factorial design with 3 dietary inclusion levels of Cu (20, 10 and 2 mg/kg) and 2 dietary forms that contained either Cu – conventional copper sulphate (Cu-SUL) or Cu nanoparticles (Cu-NP) (25 nm in size) in the form of 99.8% purity powder (purchased from the Sky Spring Nanomaterials Inc., Houston, TX), which were added to a vitamin-mineral premix using a carbohydrate carrier. All birds had free access to water and feed prepared in the local "Agrocentrum" Feed Mill Ltd. The composition of pelleted and crumbled experimental diets is shown in Table 1.

Table 1. Composition and the calculated nutritional value of experimental turkey basal diets (g kg^{-1} as-fed basis)

Item	Experimental period (days of age)		
	1–42	43–70	71–98
Ingredients (g kg^{-1})			
wheat	431.1	462.0	616.6
soybean	389.7	304.6	159.5
faba bean	100.0	100.0	100.0
rapeseed	-	50.0	60.0
soybean oil	28.0	38.6	35.4
sodium sulphate	1.5	1.5	1.5
salt	2.0	1.6	1.7
limestone	16.0	15.7	8.5
monocalcium phosphate	17.5	13.2	6.7
methionine	3.7	2.6	2.0
lysine	4.4	4.0	3.7
threonine	1.2	1.2	0.5
vitamin-mineral premix*	5.0	5.0	4.0
Nutritional value**			
AME (kcal kg^{-1})	2750	2950	3100
protein	265.0	230.0	185.0
fibre	34.0	39.8	35.7
fat	42.3	71.6	73.7
arginine	17.6	15.2	11.8
lysine	17.4	15.0	11.7
methionine	7.1	5.7	4.5
methionine and cysteine	11.3	9.5	7.8
threonine	10.5	9.3	6.8
tryptophan	3.2	2.9	2.2
calcium	11.5	10.5	6.5
non-phytin phosphorus	5.5	4.5	3.0
natrium	1.5	1.3	1.3

*Per kg of diet: vitamin A – 24999.75 IU, vitamin D – 35000 IU, vitamin E – 100 IU, tocopherol – 91 mg, vitamin K – 4 mg, vitamin B_1 – 5 mg, vitamin B_2 – 15 mg, vitamin B_6 – 6 mg, vitamin B_{12} – 0.04 mg, niacin – 100 mg, pantothenic acid – 30 mg, folic acid – 4 mg, choline chloride – 700 mg, calcium d-pantothenate – 32.665 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

**The content of nutrients and non-nutrients was calculated according to the Polish Feedstuff Analysis Tables (Smulikowska and Rutkowski, 2005).

The turkey poults were weighed at the beginning of the experiment and then after 6, 10 and 14 weeks of experimental feeding. In none of the periods were there any differences between the experimental treatments. At the termination of the study, one bird was selected from each pen for the collection of physiological tests. The body

weight of the selected turkeys, 6 per each nutritional treatment, was in the range of +/–10% of the experimental mean, 9.51 kg.

Sampling and analyses

Blood samples (5 ml) were collected from the wing vein into test tubes with an anticoagulant (heparin), which were centrifuged at 380 g for 10 min at 4°C, and plasma was collected for further analysis. The levels of haemoglobin (Hb) and haematocrit (Ht) in blood were measured using an automatic haematology analyser (Abacuss Junior Vet, Diatron, Hungary). In erythrocytes, superoxide dismutase (SOD) activity was measured using the Ransod kit (Randox), glutathione peroxidase (GPx) activity was measured by the Ransel kit (Randox). Catalase (CAT) activity, the level of total antioxidant potential (FRAP), concentrations of total glutathione (GSH + GSSG) and malondialdehyde (MDA) in blood plasma, as well as redox parameters in liver and breast muscle samples were measured as previously described (Ognik and Wertelecki, 2012). The immune system response of turkey poults was determined based on immunoglobulins (IgA, IgM, IgY) level in the plasma (using the ELISA kits from Elabscience Biotechnology Co., Ltd.), interleukin 6 (IL-6, using USCN Life Science Inc. kits), ceruloplasmin (Cp) and lysozyme. Blood plasma lysozyme activity was determined with the sandwich ELISA kit, and ceruloplasmin (Cp) was determined by the *p*-phenylenediamine colorimetric method according to Sunderman and Nomoto (1970). The Cu content of samples of diets and tissues was determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

Statistical analysis

Two-way ANOVA was performed to determine the effects of different inclusion levels (20, 10 and 2 mg/kg) and forms of additional Cu (Cu-SUL or Cu-NP), and the interaction between both factors (addition × form; A×F). In the case of a significant A×F interaction, the significance of differences between mean values of the analysed parameters in groups was estimated by Duncan's multiple range test. Data were checked for normality before statistical analyses were performed. Treatment effects were considered to be significant at $P<0.05$. The results were processed in the STATISTICA PL 12.0 application.

Results

The Cu content of all experimental diets was close to the value assumed in the experimental design and slightly decreased as the birds grew older (Table 2). The minor differences observed between groups could have resulted from different Cu concentrations in feed ingredients used for formulating diets in successive stages of the feeding trial. The difference between the total Cu content of diets and supplemental Cu doses indicates that major feed ingredients provided approximately 11 mg kg^{-1} Cu in total.

Table 2. The total content of Cu in turkey experimental diets (mg kg^{-1})

Experimental diets*	Experimental period (days)		
	1–42	43–70	71–102
Cu-SUL_{20}	31.2	29.1	30.7
Cu-NP_{20}	28.4	27.2	26.9
Cu-SUL_{10}	21.1	17.9	18.8
Cu-NP_{10}	20.4	18.3	17.6
Cu-SUL_{2}	14.9	12.6	12.9
Cu-NP_{2}	13.7	13.4	12.5

*Diet supplemented with 20, 10 and 2 mg of additional Cu in the form of sulphate (Cu-SUL_{20}, Cu-SUL_{10}, Cu-SUL_{2}) or nanoparticles (Cu-NP_{20}, Cu-NP_{10}, Cu-NP_{2}). The total Cu content originated from the basal diets and supplemental copper.

Table 3. Cu level and redox status parameters of blood/plasma of birds*

	Cu (umol L^{-1})	SOD (U g^{-1})	CAT (U g^{-1})	GPx (U g^{-1})	GSH+GSSG (μmol L^{-1})	FRAP (μmol L^{-1})	MDA (μmol L^{-1})
Group							
Cu-SUL_{20}	2.81 b	1784	574 a	56.0 c	0.114	499 a	0.56
Cu-NP_{20}	4.62 a	1516	339 b	50.1 c	0.120	551 a	0.79
Cu-SUL_{10}	3.93 ab	1097	399 b	51.2 c	0.107	568 a	0.65
Cu-NP_{10}	3.96 ab	1666	381 b	54.5 c	0.102	506 a	0.70
Cu-SUL_{2}	3.70 ab	2337	220 c	70.4 b	0.161	420 b	0.95
Cu-NP_{2}	3.36 ab	2082	237 c	79.3 a	0.112	339 c	0.94
SEM	0.179	105.4	20.502	4.344	0.008	14.75	0.032
Cu addition (A)							
20 mg kg^{-1}	3.72	1650 b	456	53.0	0.117	525	0.68 b
10 mg kg^{-1}	3.94	1382 b	390	52.8	0.105	537	0.67 b
2 mg kg^{-1}	3.53	2209 a	229	74.9	0.136	379	0.94 a
P values	0.610	0.003	0.001	0.009	0.227	0.001	0.001
Cu form (F)							
Cu-SUL	3.48	1739	398	59.2	0.127	496	0.72
Cu-NP	3.98	1755	319	61.3	0.111	465	0.81
P values	0.147	0.933	0.002	0.040	0.285	0.126	0.110
A×F interaction	0.028	0.123	0.001	0.001	0.287	0.015	0.164

*SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GSH+GSSG, total glutathione; FRAP, ferric reducing ability of plasma; MDA, malondialdehyde

a, b, c – mean values within a column with unlike letters were shown to be significantly different ($P<0.05$); differences among the groups Cu-SUL_{20}, Cu-SUL_{10}, Cu-SUL_{220}, Cu-NP_{20}, Cu-NP_{10}, and Cu-NP_{2} were indicated with superscripts only in the case of a statistically significant interaction A×F ($P<0.05$).

After 14 weeks of feeding the average body weight of turkeys from individual treatments was within a narrow range of 9.43–9.56 kg ($P>0.05$; data not shown in tables). For diets supplemented with various amounts and forms of Cu, none of the experimental factors affected the body weights. A level × form interaction was observed for plasma Cu concentrations, which were comparable in all four groups

treated with 10 and 2 mg kg^{-1} of additional Cu whereas the highest and the lowest Cu plasma concentrations were noted in groups Cu-NP_{20} and Cu-SUL_{20}, respectively ($P<0.05$) (Table 3). An interaction was noted for blood CAT and GPx activity which was highest in the Cu-SUL_{20} treatment. The lowest blood CAT activity was observed in turkeys fed diets supplemented with Cu at 2 mg kg^{-1}, irrespective of Cu form ($P<0.05$ vs. all other groups). A level × form interaction was also noted for GPx blood activity: a dietary Cu dose of 2 mg kg^{-1} led to an increase in GPx activity, compared with the remaining two doses, and the highest blood GPx activity was observed in the Cu-NP_2 group ($P<0.05$ vs. all other groups). The opposite trend was noted in FRAP values (cf. the interaction), which were lowest in the Cu-NP_2 group ($P<0.05$ vs. all other groups) and higher in groups Cu-SUL_{20}, Cu-NP_{20}, Cu-SUL_{10}, and Cu-NP_{10} ($P<0.05$ vs. Cu-SUL_2 and Cu-NP_2). Regardless of Cu form, the lowest inclusion level of Cu (2 mg kg^{-1}) contributed to an increase in blood SOD activity and MDA concentration ($P<0.05$ vs. 10 and 20 mg kg^{-1}).

Table 4. Hepatic Cu concentration and redox status parameters* in the birds' liver

	Cu (mg kg^{-1})	SOD (U g^{-1})	CAT (U g^{-1})	GSH+GSSG (μmol kg^{-1})	MDA (μmol kg^{-1})
Group					
Cu-SUL_{20}	7.80 bc	10.7	50.4	0.91	2.99
Cu-NP_{20}	9.84 a	8.70	44.7	0.98	2.95
Cu-SUL_{10}	9.16 ab	8.09	74.8	1.04	2.20
Cu-NP_{10}	8.03 abc	6.83	68.1	1.02	2.75
Cu-SUL_2	6.20 cd	8.31	69.7	1.16	2.96
Cu-NP_2	5.79 d	7.77	76.1	1.04	2.77
SEM	0.326	0.239	2.119	0.023	0.107
Cu addition (A)					
20 mg kg^{-1}	8.82	9.68 a	47.6 b	0.94 b	2.97
10 mg kg^{-1}	8.60	7.46 b	71.5 a	1.03 ab	2.48
2 mg kg^{-1}	6.00	8.04 b	72.9 a	1.10 a	2.87
P values	0.001	0.001	0.001	0.015	0.143
Cu form (F)					
Cu-SUL	7.72	9.03 a	65.0	1.03	2.72
Cu-NP	7.89	7.77 b	63.0	1.01	2.82
P values	0.753	0.001	0.427	0.622	0.616
A × F interaction	0.045	0.273	0.071	0.200	0.321

*SOD, superoxide dismutase; CAT, catalase; GSH+GSSG, total glutathione; MDA, malondialdehyde;

a, b, c, d – mean values within a column with unlike letters were shown to be significantly different ($P<0.05$); differences among the groups Cu-SUL_{20}, Cu-SUL_{10}, Cu-SUL_{220}, Cu-NP_{20}, Cu-NP_{10}, and Cu-NP_2 were indicated with superscripts only in the case of a statistically significant interaction A×F ($P<0.05$).

The highest and the lowest Cu concentrations in the liver were noted in turkeys fed diets with 20 and 2 mg kg^{-1} of additional Cu in the form of nanoparticles, respectively (Table 4). Higher hepatic Cu accumulation was observed in response to dietary Cu supplementation in the form of nanoparticles at 20 mg kg^{-1} (cf. level × form interaction). Irrespective of Cu form, the 20 mg kg^{-1} treatment was associated with the lowest CAT activity and the highest SOD activity in the liver ($P<0.05$ vs. 10 and 2 mg kg^{-1} treatments). Additionally, hepatic SOD activity was lower in turkeys fed diets containing Cu-NP ($P<0.05$ vs. Cu-SUL treatments). The highest concentration of total glutathione (GSH+GSSG) in the liver was noted upon dietary Cu supplementation at 2 mg kg^{-1} ($P<0.05$ vs. the 20 mg kg^{-1} treatment).

Table 5. Cu concentration and redox status parameters* in the breast meat of turkeys

	Cu (mg kg^{-1})	SOD (U g^{-1})	CAT (U g^{-1})	GSH+GSSG (µmol kg^{-1})	MDA (µmol kg^{-1})
Group					
$Cu\text{-}SUL_{20}$	4.25	2.74	3.48	1.00	2.63 a
$Cu\text{-}NP_{20}$	4.41	3.70	4.11	0.96	1.92 b
$Cu\text{-}SUL_{10}$	3.43	3.28	3.99	1.09	1.87 b
$Cu\text{-}NP_{10}$	2.94	3.56	5.82	1.02	1.86 b
$Cu\text{-}SUL_{2}$	2.65	3.27	4.21	1.15	2.01
$Cu\text{-}NP_{2}$	2.48	3.55	5.30	1.10	2.62 a
SEM	0.198	0.118	0.170	0.014	0.071
Cu addition (A)					
20 mg kg^{-1}	4.33 a	3.22	3.80 b	0.98 c	2.28
10 mg kg^{-1}	3.18 b	3.42	4.91 a	1.06 b	1.86
2 mg kg^{-1}	2.57 b	3.41	4.75 a	1.13 a	2.32
P values	0.001	0.732	0.002	<0.001	0.002
Cu form (F)					
Cu-SUL	3.44	3.10 b	4.21 b	1.08 a	2.17
Cu-NP	3.28	3.61 a	5.30 a	1.03 b	2.13
P values	0.638	0.033	0.001	0.025	0.722
A × F interaction	0.751	0.391	0.177	0.802	0.001

*SOD, superoxide dismutase; CAT, catalase; GSH+GSSG, total glutathione; MDA, malondialdehyde;

a, b – mean values within a column with unlike letters were shown to be significantly different ($P<0.05$); differences among the groups $Cu\text{-}SUL_{20}$, $Cu\text{-}SUL_{10}$, $Cu\text{-}SUL_{220}$, $Cu\text{-}NP_{20}$, $Cu\text{-}NP_{10}$, and $Cu\text{-}NP_{2}$ were indicated with superscripts only in the case of a statistically significant interaction A×F ($P<0.05$).

Two-way ANOVA revealed that regardless of Cu form, the highest Cu supplementation level caused a decrease in CAT activity and total glutathione concentrations in the breast muscles of turkeys ($P<0.05$ vs. the other treatments) (Table 5). The highest GSH+GSSG concentrations in breast muscles were noted in the 2 mg kg^{-1} treatment ($P<0.05$ vs. 10 and 20 mg kg^{-1} treatments). An analysis of dietary

Cu forms indicated that Cu-NP increased CAT and SOD activities, and decreased total glutathione concentrations in breast muscles, as compared with Cu-SUL. Surprisingly, the level × form interaction revealed the highest MDA concentrations in breast muscles in groups Cu-SUL_{20} and Cu-NP_2 ($P<0.05$ vs. all other groups).

Table 6. Levels of lysozyme, ceruloplasmin (Cp), immunoglobulins (IgA, IgY and IgM), and interleukine-6 (IL-6) in the blood plasma of turkeys

	Lysozyme (μg mL^{-1})	Cp (mg L^{-1})	IgY (ng mL^{-1})	IgM (ng mL^{-1})	IgA (ng nmL^{-1})	IL-6 (pg mL^{-1})
Group						
Cu-SUL_{20}	2.77	0.80	797	339	24.5	6.06 b
Cu-NP_{20}	2.05	0.88	893	330	22.8	8.62 a
Cu-SUL_{10}	2.85	1.13	753	369	20.6	5.21 b
Cu-NP_{10}	2.31	0.76	877	288	20.7	3.21 c
Cu-SUL_2	2.64	0.57	741	390	29.0	3.80 c
Cu-NP_2	2.41	0.69	723	357	25.6	5.71 b
SEM	0.093	0.050	15.36	11.331	0.860	0.296
Cu addition (A)						
20 mg kg^{-1}	2.41	0.84 a	845 a	334	23.6 ab	7.34
10 mg kg^{-1}	2.58	0.94 a	815 a	328	20.6 b	4.21
2 mg kg^{-1}	2.52	0.63 b	732 b	373	27.3 a	4.76
P values	0.738	0.025	0.002	0.200	0.005	<0.001
Cu form (F)						
Cu-SUL	2.75 a	0.83	763b	366	27.7	5.02
Cu-NP	2.26 b	0.78	831a	325	23.0	5.85
P values	0.008	0.532	0.011	0.067	0.285	0.013
A×F interaction	0.524	0.066	0.063	0.401	0.655	<0.001

a, b, c – mean values within a column with unlike letters were shown to be significantly different ($P<0.05$); differences among the groups Cu-SUL_{20}, Cu-SUL_{10}, Cu-SUL_{220}, Cu-NP_{20}, Cu-NP_{10}, and Cu-NP_2 were indicated with superscripts only in the case of a statistically significant interaction A×F ($P<0.05$).

Irrespective of Cu form, the 2 mg kg^{-1} treatment caused a significant decrease in Cp and IgY concentrations in the blood plasma of turkeys in comparison with the remaining two Cu doses (Table 6). In all treatments, nutritional levels of IgM were similar, however the 2 mg kg^{-1} treatment contributed to a significant increase in plasma IgA concentrations vs. the Cu dose of 10 mg kg^{-1} ($P<0.05$). Dietary supplementation with Cu-NP increased IgY concentrations and decreased lysozyme concentrations in the blood plasma of turkeys in comparison with the Cu-SUL treatment. As demonstrated by the addition × form interaction, turkeys from the Cu-NP_{20} group had elevated plasma IL-6 concentrations as compared with all other groups.

Discussion

As summarized in a recent review article (Hill and Li, 2017), many reports have shown that nanoparticles may be effective animal growth promoters. But, a faster growth rate of broiler chickens was also reported in response to very high (100–450 mg kg^{-1}) dietary levels of conventional source of Cu (Samanta et al., 2011). At moderate addition of Cu to turkey diets, i.e. 15 and 65 mg kg^{-1}, no differences in the growth performance parameters of birds were noted (Makarski et al., 2014). In the present study, supplemental Cu doses of 2, 10 and 20 mg kg^{-1} increased the total dietary Cu content to approximately 14, 21 and 30 mg kg^{-1}, respectively. It should be stressed that the above values obtained in the present experimental schema were below, similar to and higher than the inclusion rate of 25 mg kg^{-1} recommended for poultry diets in the EU (EFSA, 2016). But, neither the differences in dietary Cu supplementation levels nor the total Cu content of feed affected the final body weights of turkeys.

In the present study, blood plasma Cu concentration increased in response to the highest dietary addition of Cu-NP, relative to the identical amount of Cu-SUL. It can be assumed that such nanoparticles treatment led to enhanced Cu absorption in the gastrointestinal tract. Many *in vitro* experiments demonstrated that nanoparticles with reduced size and greater area exhibit a high rate of absorption in the stomach and intestines (Hussain et al., 2001; Tomaszewska et al., 2017).

Intensified oxidation processes in poultry tissues, manifested by a decrease in SOD and CAT activity, were noted in studies investigating the effect of high dietary Cu doses, above 250 mg kg^{-1} (Ajuwon et al., 2011). It is well known that superoxide dismutase is involved in the first line of antioxidant defence against toxic reactive oxygen species (McCord, 1983), and diminished activities of antioxidant enzymes could negatively affect cellular resistance against the oxidant-induced damage of cell genome (Amstad et al., 1994). In an earlier experiment, a decrease in dietary Cu content from 30 to approximately 11 mg kg^{-1} did not influence SOD activity in the blood of turkeys (Mikulski et al., 2009). In the current study, a decrease in dietary Cu content from 20 and 10 to 2 mg kg^{-1} increased SOD and GPx activities in birds' blood. No differences were found in the levels of important antioxidant, namely plasma glutathione. However, irrespective of the Cu source elevated plasma MDA levels followed dietary treatment with 2 mg of Cu kg^{-1}. Low-grade Cu-NP contributed to a decrease in FRAP values. Our results could suggest that the highest and medium Cu doses exerted antioxidant effects, relative to diets with the lowest Cu content. Such observations were also made by other authors who found that high cytoplasmic Cu concentrations may enhance GPx activity and more effectively protect cells against peroxide-induced damage (Freedman and Wolterbeek, 1989). The above findings indicate that in comparison with the addition of 2 mg Cu kg^{-1}, dietary supplementation at 10 and 20 mg kg^{-1} did not exert pro-oxidant effects which were noted in response to higher pharmacological concentrations of dietary Cu (Ajuwon et al., 2011).

Previous studies have shown that hepatic Cu concentration is influenced by variations in dietary Cu levels (Bao et al., 2007). In the present experiment, reduced

dietary Cu content (from 20–10 to 2 mg kg^{-1}) led to a decrease in Cu concentration and an increase in total glutathione levels in the liver. It has been reported that Cu reduces the concentration of hepatic glutathione through the stimulation of 3-hydroxyl-3-methylglutaryl coenzyme reductase (Kim et al., 1992). In our experiment, despite the noted decrease in glutathione levels, MDA concentration did not increase in the liver. An increase in MDA concentration, indicative of intensified oxidation processes in the liver, was observed in broiler chickens fed diets supplemented with 250 mg Cu per one kg (Ajuwon et al., 2011). In another experiment, a low dose of supplemental Cu (50 mg kg^{-1}) only numerically decreased MDA concentration in the liver. In our study, an increase in dietary Cu content from 2 to 10 mg kg^{-1} had no effect on the analysed indicators of the liver redox status. The highest dose of supplemental Cu did not increase MDA concentration, but it increased SOD activity, decreased CAT activity and further reduced GSH+GSSG and vitamin C levels with a simultaneous decrease in MDA concentration in the liver. Our findings could suggest that dietary supplementation with 20 mg Cu per kg exerted both antioxidant and pro-oxidant effects in turkeys. The replacement of Cu-SUL with Cu-NP resulted in decreased CAT activity but it did not affect other indicators of the liver redox status.

Other authors (Xiang et al., 2009) found no differences in MDA concentration in the muscles of broiler chickens fed diets without and with different inclusion levels of Cu, from 50 to 350 mg kg^{-1}. In the present experiment, a decrease in MDA concentration was noted if Cu addition was reduced from 20 to 10 mg kg^{-1} or if Cu-SUL was replaced with Cu-NP. However, the lowest dose of Cu-NP increased MDA levels in breast meat. A decrease in dietary Cu addition from 20 to 10 mg kg^{-1} lowered Cu levels and increased CAT activity and total glutathione concentrations in breast muscles. The lowest Cu addition admittedly increased total glutathione levels, however, it did not improve other indicators of the antioxidant status of breast meat, compared with the medium dose of Cu. The replacement of Cu-SUL with Cu-NP resulted in similar concentrations of Cu, an increase in SOD and CAT activities, and a decrease in total glutathione levels in meat.

In brief, the antioxidant and immune status of the blood, liver and breast muscles in turkeys indicates that 10 mg kg^{-1} is the optimal inclusion level of additional dietary Cu. Both two-fold higher and five-fold lower Cu addition negatively influenced selected parameters of the antioxidant and immune status of birds. The replacement of Cu-SUL with Cu-NP induced both desirable and undesirable changes in selected parameters of the analysed tissues, but the dose of 10 mg kg^{-1} should be considered as safest and most effective. Nevertheless, the efficacy of metal nanoparticles in turkey nutrition requires further research, and this issue will be addressed in our future studies.

Trace elements, including Cu, have a considerable effect on the immune system (Percival, 1998), and a Cu deficit may lead to a decrease in Cp levels (Wang et al., 2011). In the present experiment, Cp concentration decreased in response to an increase in dietary Cu content from 20 and 10 to 2 mg kg^{-1}. At the same time, the level of the most important immunoglobulin, Y, decreased. This is consistent with the results of another experiment (Wang et al., 2011) where the blood levels of IgM,

IgG and IgA depended on the addition of Cu to broiler chicken diets. In our experiment, decreased Cp levels were noted in turkeys fed diets with the lowest Cu doses, whereas IL-6 levels increased in birds fed diets supplemented with 20 mg Cu kg^{-1}, in comparison with the remaining treatments. It has been suggested that serum Cp levels increase as part of the stress response of hepatocytes and the release of inflammatory mediators (Malavolta et al., 2015), and that interleukin-6 is the key cytokine responsible for the activation of B cells and stimulation of the synthesis of acute phase proteins, including Cp (Dinant and Dijkmans, 1999).

Conclusions

In conclusion, the turkeys' redox and immune status of blood, liver and breast muscles, characterized in this study, indicates that 10 mg kg^{-1} is the optimal dietary inclusion level of additional Cu. Both two-fold higher and five-fold lower Cu addition to a diet had a negative influence on selected antioxidant and immune parameters of birds. The results also indicate that the replacement of Cu-SUL with Cu-NP induced both desirable and undesirable changes in selected parameters of blood (increase in IgY and IL-6 levels), liver (decreased SOD activity) and breast muscles (increased CAT and SOD activities, decreased GSH+GSSG levels). Lower addition levels of Cu-NP (2 and 10 mg kg^{-1}) exerted similar physiological effects to Cu-SUL, whereas 20 mg kg^{-1} of Cu-NP negatively affected the redox status and stimulated the synthesis of the proinflammatory cytokine IL-6. It can be concluded that the efficacy of Cu nanoparticles in turkey nutrition remains debatable. Further research, including an economic analysis, is needed to investigate the advantages and disadvantages of this feed additive.

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