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Growth rate, metabolic parameters and carcass quality in turkeys fed diets with different inclusion levels and sources of supplemental copper

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ABSTRACT. The aim of this experiment was to determine whether the inclusion levels of supplemental copper (Cu) in turkey diets can be decreased without compromising important metabolic functions, growth parameters and carcass quality, and whether the above goals can be achieved with the involvement of Cu nanoparticles. The experiment was carried out on 648 one-day-old Hybrid Converter turkeys divided into 6 groups, with 6 replicates per group (18 birds per replicate). The experiment had a two-factorial design, with Cu sulphate (Cu-SUL) and Cu nanoparticles (Cu-NP) as 2 dietary sources of Cu, and 3 dietary inclusion levels of Cu (2, 10 and 20 mg/kg). It was demonstrated that the replacement of Cu-SUL with Cu-NP and a decrease in a dose of supplemental Cu from 20 to 10 mg/kg or even 2 mg/kg of the diet did not affect the growth parameters or the carcass quality of turkeys. The few effects exerted by the substitution of Cu-SUL with Cu-NP included an increase in haemoglobin levels and an improvement in the antioxidant status of fresh breast meat. However, fresh meat was characterised by optimal redox parameters when the dietary dose of Cu was decreased to 10 mg/kg. The results of the present study cannot be generalised, but they significantly expand the knowledge about Cu-NP as an efficient source of Cu for turkeys.

Introduction

In a modern agricultural practice, poultry diets are routinely supplemented with micronutrients, including copper (Cu), because the Cu content of basic feed components does not fully meet the nutrient requirements of fast-growing birds. According to the latest recommendations, the diets of broiler turkeys should contain up to 30 mg Cu/kg (Hybrid Turkeys, 2016), which is significantly more than the previously recommended Cu dose of 8 mg/kg (NRC, 1994) as well as the rates recommended by the European Commission's Scientific Committee for Animal Nutrition (European Commission, 2003). According to European Commission (2003), the dietary inclusion levels of available Cu from organic sources can be lower (up to 20 mg Cu/kg of feed), and the total Cu content in animal diets should not exceed 35 mg/kg. The European Food Safety Authority (EFSA FEEDAP Panel, 2016) has recently published the newly proposed maximum content (NPMC) of Cu in complete feeds for target animals. The NPMC for poultry, including turkeys for fattening, was set at 25 mg/kg.

According to some postulates, the recommended doses of supplemental Cu should be decreased because Cu excreted with animal faeces increases the Cu load in the environment. Research has demonstrated that the excretion of Cu and other minerals increased linearly with an increase in their dietary intake (Bao et al., 2007). In laying hens, the Cu content per kg of excreta dry matter (DM) increased from 25.3 to 397 mg/kg when the basal diet was supplemented with $CuSO_4$ (Cu-SUL) at 0 and 240 mg/kg (Skřivan et al., 2006). In many experiments it was demonstrated that organic trace elements, including amino acid chelates, increase nutrient bioavailability (Leeson and Caston, 2008; Jegede et al., 2011) and decrease faecal nutrient losses (Mikulski et al., 2009). Amino acid chelates are absorbed from the intestines at a significantly higher rate than soluble inorganic metal salts, but their supplementation is often difficult and not economically viable (Andersen, 2004). Copper nanoparticles (Cu-NP) are a new and alternative source of dietary Cu with potentially high availability (Gonzales-Eguia et al., 2009; Ognik et al., 2016; 2018; Kozłowski et al., 2018; Jankowski et al., 2019). The metabolic rate and development of broiler embryos were improved when Cu-NP were injected *in ovo* or included in hen diets (Pineda et al., 2013). Diets supplemented with more available nanoparticles (44.0 vs 34.2% for $CuSO_4$) improved growth performance in piglets (Gonzales-Eguia et al., 2009), but similar experiments are rarely conducted on growing poultry. In our preliminary study on young turkeys, a decrease in the dose of supplemental Cu, both Cu-SUL and Cu-NP, from 20 to 2 mg/kg of the diet did not compromise growth parameters. Copper in a dose of 20 mg/kg induced oxidation reactions and was less effective in conferring antioxidant protection than Cu in a dose of 2 mg/kg. Dietary supplementation with Cu-NP also exerted a more beneficial influence on the carbohydrate metabolism and antioxidant status of young turkeys than supplementation with conventional Cu-SUL (Kozłowski et al., 2018). According to Sawosz et al. (2018), Cu inclusion levels in poultry diets can be decreased and environmental Cu loads can be minimised by replacing Cu-SUL with Cu-NP without compromising growth parameters.

The aim of this study was to determine whether the inclusion levels of supplemental Cu in turkey diets can be decreased without compromising important metabolic functions, growth parameters and carcass quality, and whether the above goals can be achieved with the involvement of Cu-NP.

Material and methods

Birds, management and diet

The experiment and the slaughter protocol were approved by the local Ethical Committee for Experiments on Animals in Olsztyn (permission No. 30/2015; 2015.04.29). In total, 648 one-day-old Hybrid Converter female turkeys were placed in 36 pens and kept according to the breeder's recommendations that were adjusted for the birds' age. Turkeys were divided into 6 groups, with 6 replicates per group (18 birds per replicate). The experiment had a two-factorial design, with copper sulphate (Cu-SUL) and Cu nanoparticles (Cu-NP) as 2 dietary sources of Cu, and 3 dietary inclusion levels of Cu (2, 10 and 20 mg/kg). Turkeys had free access to water and feed that was prepared locally by the 'Agrocentrum' Feed Mill Ltd. Crumbled (1–28 days of age) and pelleted (next feeding stage) experimental diets (Table 1) were supplemented

Table 1. The composition and nutrient content of experimental diets, % as-fed basis

Indices	Feeding period, days		
	1–42	43–70	71–98
Ingredients			
wheat	43.11	46.20	61.66
soybean meal	38.97	30.46	15.95
faba bean	10.00	10.00	10.00
rapeseeds	-	5.00	6.00
soybean oil	2.80	3.86	3.54
sodium sulphate	0.15	0.15	0.15
salt	0.20	0.16	0.17
limestone	1.60	1.57	0.85
monocalcium phosphate	1.75	1.32	0.67
L-methionine, 99%	0.37	0.26	0.20
DL-lysine HCl, 75%	0.44	0.40	0.37
L-threonine, 99%	0.12	0.12	0.05
mineral-vitamin premix[1]	0.50	0.50	0.40
Calculated nutrient density			
crude protein	26.50	23.00	18.50
crude fibre	3.40	3.98	3.57
crude fat	4.23	7.16	7.37
AME[2], kcal/kg	2750	2950	3100
arginine	1.76	1.52	1.18
lysine	1.74	1.50	1.17
methionine	0.71	0.57	0.45
Met + Cys	1.13	0.95	0.78
threonine	1.05	0.93	0.68
tryptophan	0.32	0.29	0.22
Ca	1.15	1.05	0.65
P	0.55	0.45	0.30
Na	0.15	0.13	0.13

[1] per kg of diet: IU: vit. A 24999.75, vit. D 35000, vit. E 100; mg: tocopherol 91, vit. K 4, vit. B_1 5, vit. B_2 15, vit. B_6 6, vit. B_{12} 0.04, niacin 100, pantothenic acid 30, folic acid 4, choline chloride 700, calcium D-pantothenate 32.665, biotin 0.35, total Se 0.3, total Fe 60, total Mn 100, total Zn 100, J 1.5; g: Ca 1.0435; [2] AME – Apparent Metabolizable Energy

with vitamin-mineral premixes containing different amounts and sources of Cu. The Cu-SUL diet contained conventional copper sulphate, and the Cu-NP diet contained Cu nanoparticles (25 nm in size) powder with 99.8% purity (purchased from Sky Spring Nanomaterials Inc., Houston, TX, USA). The tested Cu supplements were added to the vitamin-mineral premix using a carbohydrate carrier (glucose).

Growth trial and sample collection

Body weights (BW), body weight gains (BWG) and feed intake were recorded and calculated on a pen basis. Daily feed intake (DFI) per bird was calculated based on total feed consumption per pen for the entire experimental period and on selected days of the experiment. The feed conversion ratio (FCR; kg of feed/kg of BWG) was calculated per pen based on BWG and feed intake. Mortality rates, including the cause of death, were recorded daily, and the BW of dead birds were used to adjust the average BWG, average DFI and FCR.

After 14 weeks of feeding, seven turkeys from each group (with an average BW of the group) were slaughtered at 98 day of age at the Department's slaughterhouse, 8 h after feed withdrawal. Before slaughter, blood 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. Subsamples of the *pectoralis major* muscle were used to determine meat colour upon deboning (24 h post mortem). The remaining portion of breast meat was vacuum-packaged, frozen at −20 °C, and stored for further analysis.

Laboratory analyses

The Cu contents in feed and tissue (blood, liver and meat) samples were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Inc., Palo Alto, CA, USA). The contents of zinc (Zn), calcium (Ca), phosphorus (P) and magnesium (Mg) were determined by flame atomic absorption spectrometry (FAAS, Varian Inc., Palo Alto, CA, USA). Haemoglobin (Hb) and haematocrit (Ht) levels were measured using an automatic haematology analyser (Abacus Junior Vet, Diatron, Budapest, Hungary). The concentrations of glucose (GLU), triacylglycerols (TAG), total cholesterol (TC), uric acid (UA), urea (UREA) and total protein (TP), and the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were measured in the blood plasma of turkeys using an automatic biochemical analyser (Plasma Diagnostic Instruments Horiba, Kyoto, Japan).

The yields of whole carcasses, breast muscles and thigh muscles were determined relative to live BW. The colour of breast muscles was determined 24 h post mortem by the optical reflection method in the CIELAB system, where the values of L^* (lightness, lower values indicate a darker colour), a^* (redness, higher positive values indicate a higher contribution of redness) and b^* (yellowness, higher positive values indicate a higher contribution of yellowness) were measured with the MiniScan XE Plus portable spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA, USA). The average of two readouts from the cross-section of each right breast muscle free from colour defects, bruising and haemorrhages was recorded. The redox status of turkey breast meat was assessed using the methods described by Ognik and Wertelecki (2012), and the following indicators were determined: antioxidant indicators: superoxide dismutase (SOD) and catalase (CAT) activity, total glutathione (GSH + GSSG) content and oxidant indicator: malondialdehyde (MDA) content.

Statistical analysis

A single pen (n = 6) was regarded as a replicate experimental unit in a statistical analysis of performance parameters. Individual birds were regarded as experimental units in analyses of the biochemical and antioxidant parameters of tissues. The biochemical and antioxidant parameters of the blood plasma were analysed in 36 birds representing 6 replications from each of the 6 experimental treatments. Two-way ANOVA was performed to determine the effects of different inclusion levels (2, 10 and 20 mg/kg) and sources of supplemental Cu (Cu-SUL or Cu-NP), and to determine the interactions between both factors (inclusion level × source; IL × S). In significant IL × S interactions, the significance of differences between the mean values of the analysed parameters in groups was estimated by Tukey's multiple-range test. Treatment effects were considered to be significant at $P < 0.05$. The results were processed in the Statistica PL ver. 13.1 (StatSoft Corp., Kraków, Poland) application.

Results

The Cu content in all experimental diets approximated the values assumed in the experimental design (Table 2). The observed minor differences between groups could have resulted from different

Table 2. The content of Cu and selected minerals in turkey diets

Indices	Feeding period, days		
	1–42	43–70	71–98
Cu content of experimental diets[1], mg/kg			
$Cu\text{-}SUL_{20}$	31.2	29.1	30.7
$Cu\text{-}NP_{20}$	28.4	27.2	26.9
$Cu\text{-}SUL_{10}$	21.1	17.9	18.8
$Cu\text{-}NP_{10}$	20.4	18.3	17.6
$Cu\text{-}SUL_{2}$	14.9	12.6	12.9
$Cu\text{-}NP_{2}$	13.7	13.4	12.5
Content of selected minerals in the diet, mg/kg			
Ca	12.8	11.5	7.10
P	8.70	7.90	5.20
Zn	172	140	148
Fe	258	229	221

[1] diets supplemented with 2, 10 and 20 mg of copper sulphate ($Cu\text{-}SUL_2$, $Cu\text{-}SUL_{10}$, $Cu\text{-}SUL_{20}$) or copper nanoparticles ($Cu\text{-}NP_2$, $Cu\text{-}NP_{10}$, $Cu\text{-}NP_{20}$)

Cu concentrations in the 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 indicated that the major feed ingredients supplied approximately 11 mg/kg Cu in total. The basal diet supplemented with different doses of Cu supplied identical amounts of Ca, P, Zn and Fe to the experimental diets, and the concentrations of these nutrients decreased as the birds grew older.

The applied dietary treatments had no influence on Ca and Mg concentrations in the blood plasma (Table 3). Regardless of the Cu source, plasma P concentration was higher in the treatment with the lowest Cu supplementation (2 mg/kg) than in both treatments with higher supplementation levels (10 and 20 mg/kg). An interaction between the inclusion level and the source of Cu was observed in plasma Zn concentrations ($P < 0.001$) which decreased in birds whose diets were supplemented with 20 and 10 mg of Cu-NP/kg (vs Cu-SUL), whereas an opposite effect was noted in birds whose diets were supplemented with 2 mg of Cu-NP/kg. The above interaction was also observed in plasma Cu concentrations which were comparable in all four groups receiving 10 and 2 mg/kg of supplemental Cu, whereas the highest and the lowest Cu plasma concentrations were noted in groups $Cu\text{-}NP_{20}$ and $Cu\text{-}SUL_{20}$, respectively ($P < 0.05$).

The values of Ht and the most of the analysed blood biochemical indicators, including uric acid levels and the activity of ALT, AST, GGT and ALP, were similar in all groups of birds whose diets were supplemented with different amounts of Cu-SUL and Cu-NP (Table 4). Two-way ANOVA revealed that Cu-NP supplementation significantly increased blood Hb concentrations relative to the Cu-SUL treatment. Regardless of the source of supplemental Cu, the lowest Cu level of 2 mg/kg significantly increased plasma TC concentrations relative to the two higher inclusion levels of Cu.

Table 3. The content of selected minerals in the blood plasma of turkeys

Indices	Minerals				
	Cu, µmol/l	Zn, µmol/l	Ca, mmol/l	P, mmol/l	Mg, mmol/l
Group[1]					
$Cu\text{-}SUL_{20}$	2.81^{b}	39.8^{a}	2.44	1.98	0.83
$Cu\text{-}NP_{20}$	4.62^{a}	36.3^{b}	2.38	2.28	0.81
$Cu\text{-}SUL_{10}$	3.93^{ab}	38.9^{a}	2.13	2.16	0.85
$Cu\text{-}NP_{10}$	3.96^{ab}	36.5^{b}	2.33	2.28	0.94
$Cu\text{-}SUL_{2}$	3.70^{ab}	35.8^{b}	2.19	2.62	0.93
$Cu\text{-}NP_{2}$	3.36^{ab}	39.0^{a}	2.08	2.70	0.86
Cu source					
Cu-SUL	3.48	38.1	2.25	2.26	0.87
Cu-NP	3.98	37.3	2.26	2.42	0.87
Cu inclusion level, mg/kg					
20	3.72	38.0	2.41	2.13^{b}	0.82
10	3.94	37.7	2.23	2.22^{b}	0.89
2	3.53	37.4	2.14	2.66^{a}	0.90
SEM	0.179	0.380	0.054	0.067	0.019
P-values					
Cu source (S)	0.147	0.186	0.916	0.163	0.990
Cu inclusion level (IL)	0.610	0.748	0.128	0.001	0.160
IL × S interaction	0.028	<0.001	0.446	0.705	0.248

[1] diets supplemented with 20, 10 and 2 mg of copper sulphate ($Cu\text{-}SUL_{20}$, $Cu\text{-}SUL_{10}$, $Cu\text{-}SUL_2$) or copper nanoparticles ($Cu\text{-}NP_{20}$, $Cu\text{-}NP_{10}$, $Cu\text{-}NP_2$); SEM – standard error of the mean (standard deviation for all birds divided by the square root of the number of birds, n = 36); [a,b] – two-way ANOVA was applied, means within the same column with different superscript letters are significantly different across groups ($P \leq 0.05$) in Tukey's test (calculated only if the IL × S interaction was significant)

After 16 weeks of the feeding trial during which turkey diets were supplemented with different amounts and sources of Cu, none of the experimental factors affected BW (Table 5) or the carcass quality of turkeys (Table 6). Neither the source nor the inclusion level of supplemental Cu in the experimental diets affected mortality rates, BW, feed intake or the FCR in any of the three analysed rearing periods. The FCR values across treatments revealed marginally significant interactions between the source and inclusion level of Cu (P = 0.052) only during the entire experiment (days 1–98) because lower Cu-NP doses induced a greater decrease in FCR values than the corresponding doses of Cu-SUL. The dressing

Table 4. Haematological and biochemical parameters in the blood of turkeys[1]

Indices	Ht, l/l	Hb, g/l	GLU, mmol/l	TP, g/l	UREA, mmol/l	TAG, mmol/l	TC, mmol/l	UA, µmol/l	ALT, U/l	AST, U/l	GGT, U/l	ALP, U/l
Group[2]												
$Cu\text{-}SUL_{20}$	38.1	9.64	18.6	31.4	0.28	0.46	2.64	227	6.18	274	3.19	1422
$Cu\text{-}NP_{20}$	40.4	10.9	17.6	28.7	0.30	0.55	2.47	174	5.34	217	3.44	1399
$Cu\text{-}SUL_{10}$	38.5	10.3	16.6	31.2	0.34	0.49	2.40	184	6.63	208	2.66	1419
$Cu\text{-}NP_{10}$	38.0	10.5	17.5	33.5	0.38	0.54	2.39	219	5.33	237	4.09	1464
$Cu\text{-}SUL_{2}$	37.8	10.1	18.2	34.2	0.42	0.51	2.84	216	4.29	307	2.89	1500
$Cu\text{-}NP_{2}$	38.5	10.6	18.7	30.9	0.32	0.69	2.77	218	5.93	245	2.84	1466
Cu source												
Cu-SUL	38.2	10.0[b]	17.8	32.3	0.35	0.48	2.62	209	5.70	263	2.91	1447
Cu-NP	39.0	10.6[a]	17.9	31.0	0.33	0.59	2.55	204	5.23	233	3.45	1443
Cu inclusion level, mg/kg												
20	39.3	10.3	18.1	30.1	0.29	0.50	2.56[b]	200	5.76	246	3.31	1410
10	38.3	10.4	17.1	32.4	0.36	0.52	2.40[b]	202	5.98	223	3.38	1441
2	38.2	10.3	18.4	32.5	0.37	0.60	2.80[a]	217	5.11	276	2.86	1483
SEM	0.309	0.135	0.299	0.659	0.022	0.030	0.051	10.42	0.267	10.63	0.253	15.00
P-values												
Cu source (S)	0.181	0.023	0.861	0.343	0.733	0.076	0.409	0.802	0.748	0.142	0.300	0.883
Cu inclusion level (IL)	0.265	0.898	0.149	0.225	0.288	0.375	0.004	0.764	0.367	0.105	0.678	0.151
IL × S interaction	0.182	0.228	0.389	0.157	0.382	0.660	0.770	0.246	0.054	0.125	0.473	0.515

[1] data represent the mean values of 6 birds per treatment; [2] diets supplemented with 20, 10 and 2 mg of copper sulphate ($Cu\text{-}SUL_{20}$, $Cu\text{-}SUL_{10}$, $Cu\text{-}SUL_{2}$) or copper nanoparticles ($Cu\text{-}NP_{20}$, $Cu\text{-}NP_{10}$, $Cu\text{-}NP_{2}$); SEM – standard error of the mean (standard deviation for all birds divided by the square root of the number of birds, n = 36); Ht – haematocrit; Hb – haemoglobin; GLU – glucose; TP – total protein; ALB – albumin; TAG – triacylglycerols; TC – total cholesterol; UA – uric acid; ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGT – gamma-glutamyl transferase; ALP – alkaline phosphatase; [a,b] – two-way ANOVA was applied; – means within the same column with different superscript letters are significantly different across groups ($P \leq 0.05$) in Tukey's test (calculated only if the IL × S interaction was significant)

Table 5. Growth performance of turkeys

Indices	Body weight, kg				Daily feed intake, g per bird				FCR, kg/kg				Mort., %
	MC_1	MC_{42}	MC_{70}	MC_{98}	1–42	41–70	71–98	1–98	1–42	41–70	71–98	1–98	
Group[1]													
$Cu\text{-}SUL_{20}$	0.07	2.74	5.93	9.55	96.0	242	348	244	1.52	2.09	3.08	2.18	2.78
$Cu\text{-}NP_{20}$	0.07	2.79	5.97	9.55	98.5	246	353	257	1.52	2.17	3.20	2.21	1.85
$Cu\text{-}SUL_{10}$	0.07	2.69	5.97	9.51	97.4	249	354	245	1.56	2.11	3.20	2.24	0.00
$Cu\text{-}NP_{10}$	0.07	2.73	5.91	9.46	97.6	246	352	252	1.54	2.10	3.18	2.19	0.93
$Cu\text{-}SUL_{2}$	0.07	2.75	5.89	9.43	98.9	243	365	258	1.55	2.16	3.36	2.25	0.93
$Cu\text{-}NP_{2}$	0.07	2.76	5.97	9.56	99.2	243	351	251	1.55	2.09	3.13	2.17	0.93
Cu source													
Cu-SUL	0.07	2.73	5.93	9.49	97.4	245	356	249	1.54	2.12	3.22	2.22	1.24
Cu-NP	0.07	2.76	5.95	9.53	98.4	245	352	253	1.54	2.12	3.17	2.19	1.24
Cu inclusion level, mg/kg													
20	0.07	2.77	5.95	9.55	97.3	244	351	250	1.52	2.13	3.14	2.19	2.32
10	0.07	2.71	5.94	9.49	97.5	247	353	249	1.55	2.10	3.19	2.22	0.46
2	0.07	2.76	5.93	9.50	99.1	243	358	255	1.55	2.13	3.25	2.21	0.93
SEM	<0.001	0.014	0.023	0.043	0.429	2.165	2.866	2.140	0.006	0.018	0.037	0.009	0.449
P-values													
Cu source (S)	0.599	0.228	0.662	0.733	0.252	0.953	0.491	0.369	0.477	0.980	0.528	0.074	0.866
Cu inclusion level (IL)	0.133	0.246	0.944	0.828	0.184	0.729	0.603	0.511	0.149	0.779	0.509	0.529	0.272
IL × S interaction	0.533	0.783	0.487	0.741	0.458	0.774	0.396	0.173	0.609	0.238	0.177	0.052	0.781

[1] diets supplemented with 20, 10 and 2 mg of copper sulphate ($Cu\text{-}SUL_{20}$, $Cu\text{-}SUL_{10}$, $Cu\text{-}SUL_{2}$) or copper nanoparticles ($Cu\text{-}NP_{20}$, $Cu\text{-}NP_{10}$, $Cu\text{-}NP_{2}$); SEM – standard error of the mean (standard deviation for all birds divided by the square root of the number of birds, n = 36)

Table 6. The results of turkey carcass analysis, relative weights of selected organs (body weight = 100%) and breast meat colour

Indices	Dressing percentage	Breast muscle	Thigh muscle	Drumstick muscle	Abdominal fat	Breast meat colour		
						L	*a*	*b*
Group[1]								
$Cu\text{-}SUL_{20}$	79.2	21.1	10.9	8.19	1.46	54.1	3.86	12.7
$Cu\text{-}NP_{20}$	78.3	20.3	10.8	8.06	1.36	53.5	4.65	13.0
$Cu\text{-}SUL_{10}$	78.9	20.0	10.9	8.17	1.41	53.0	4.60	12.4
$Cu\text{-}NP_{10}$	79.2	20.6	10.7	7.65	1.53	54.5	4.40	13.0
$Cu\text{-}SUL_{2}$	78.8	21.5	10.7	8.05	1.41	54.4	4.12	13.1
$Cu\text{-}NP_{2}$	79.0	20.5	11.0	8.14	1.48	54.2	3.71	12.6
Cu source								
Cu-SUL	78.9	20.9	10.8	8.13	1.43	53.9	4.19	12.7
Cu-NP	78.8	20.5	10.8	7.95	1.46	54.0	4.25	12.9
Cu inclusion level, mg/kg								
20	78.8	20.7	10.8	8.12	1.41	53.7	4.28	12.9
10	79.1	20.3	10.8	7.91	1.47	53.8	4.49	12.7
2	78.9	21.0	10.9	8.09	1.45	54.3	3.91	12.9
SEM	0.194	0.181	0.074	0.091	0.054	0.329	0.181	0.145
P-values								
Cu source (S)	0.807	0.271	0.946	0.325	0.794	0.779	0.870	0.574
Cu inclusion level (IL)	0.852	0.265	0.974	0.598	0.905	0.772	0.426	0.897
IL × S interaction	0.388	0.130	0.382	0.406	0.717	0.433	0.371	0.292

[1] diets supplemented with 20, 10 and 2 mg of copper sulphate ($Cu\text{-}SUL_{20}$, $Cu\text{-}SUL_{10}$, $Cu\text{-}SUL_{2}$) or copper nanoparticles ($Cu\text{-}NP_{20}$, $Cu\text{-}NP_{10}$, $Cu\text{-}NP_{2}$); SEM – standard error of the mean (standard deviation for all birds divided by the square root of the number of birds, n = 36)

Table 7. Redox status of fresh and frozen breast meat

Indices	Fresh meat				Frozen meat			
	SOD, U/g	CAT, U/g	GSH+GSSG, µmol/kg	MDA, µmol/kg	SOD, U/g	CAT, U/g	GSH+GSSG, µmol/kg	MDA, µmol/kg
Group[1]								
$Cu\text{-}SUL_{20}$	2.74	3.48	1.00	2.63^{a}	4.53	10.9	0.92	2.98
$Cu\text{-}NP_{20}$	3.70	4.11	0.96	1.92^{b}	4.56	11.6	0.83	2.84
$Cu\text{-}SUL_{10}$	3.28	3.99	1.09	1.87^{b}	3.78	12.5	0.86	2.45
$Cu\text{-}NP_{10}$	3.56	5.82	1.02	1.86^{b}	4.40	12.2	0.92	2.79
$Cu\text{-}SUL_{2}$	3.27	4.21	1.15	2.01^{b}	4.70	11.2	1.00	1.93
$Cu\text{-}NP_{2}$	3.55	5.30	1.10	2.62^{a}	5.17	12.0	0.98	2.70
Cu source								
Cu-SUL	3.10^{b}	4.21^{b}	1.08^{a}	2.17	4.34	11.5	0.93	2.45
Cu-NP	3.61^{a}	5.30^{a}	1.03^{b}	2.13	4.71	11.9	0.91	2.78
Cu inclusion level, mg/kg								
20	3.22	3.80^{b}	0.98^{c}	2.28^{a}	4.55^{ab}	11.3^{b}	0.88^{b}	2.91^{a}
10	3.42	4.91^{a}	1.06^{b}	1.86^{b}	4.09^{b}	12.3^{a}	0.89^{b}	2.62^{ab}
2	3.41	4.75^{a}	1.13^{a}	2.32^{a}	4.93^{a}	11.6^{ab}	0.99^{a}	2.31^{b}
SEM	0.118	0.170	0.014	0.071	0.123	0.169	0.017	0.097
P-values								
Cu source (S)	0.033	<0.001	0.025	0.722	0.107	0.200	0.582	0.069
Cu inclusion level (IL)	0.732	0.002	<0.001	0.002	0.016	0.024	0.013	0.027
IL × S interaction	0.391	0.177	0.802	<0.001	0.554	0.302	0.216	0.115

[1] diets supplemented with 20, 10 and 2 mg of copper sulphate ($Cu\text{-}SUL_{20}$, $Cu\text{-}SUL_{10}$, $Cu\text{-}SUL_{2}$) or copper nanoparticles ($Cu\text{-}NP_{20}$, $Cu\text{-}NP_{10}$, $Cu\text{-}NP_{2}$); SEM – standard error of the mean (standard deviation for all birds divided by the square root of the number of birds, n = 36); [a,b] – two-way ANOVA was applied, means within the same column with different superscript letters are significantly different across groups ($P \leq 0.05$) in Tukey's test (calculated only if the IL × S interaction was significant)

percentage, relative weights of major muscle groups (breast, thigh, drumstick) and breast meat colour were similar in all experimental subgroups.

The experimental factors differentiated most redox indicators in fresh breast meat and induced less pronounced differences in frozen breast meat (Table 7). The substitution of Cu-SUL with Cu-NP in turkey diets increased SOD and CAT activities, and decreased the total glutathione content of fresh meat ($P < 0.001$, $P = 0.033$ and $P = 0.025$, respectively). An analysis of MDA content revealed a significant interaction between the source and inclusion level of Cu, where the highest and lowest dose of Cu-NP led to a decrease and an increase in MDA levels, respectively. A decrease in the dietary Cu dose from 20 mg/kg to 10 and 2 mg/kg did not affect SOD activity, but increased CAT activity ($P = 0.002$) in fresh breast meat. In fresh breast meat, total glutathione content increased ($P < 0.001$) subject to the applied dose of supplemental Cu, whereas MDA levels decreased in the group receiving a moderate dose of Cu and were not affected by the source of supplemental Cu. The analysed redox parameters did not differ in frozen meat. The frozen meat of turkeys whose diets were supplemented with the lowest Cu dose was characterised by higher total glutathione content ($P < 0.001$) relative to the remaining groups) and significantly lower MDA levels ($P < 0.027$) relative to the group receiving the highest dose of supplemental Cu.

Discussion

In the present experiment, supplemental Cu doses of 2, 10 and 20 mg/kg increased the total Cu content of turkey diets to approximately 14, 21 and 30 mg/kg, respectively. The above values were lower, similar and higher, respectively, than the inclusion rate of 25 mg/kg recommended for poultry diets in the EU (EFSA FEEDAP Panel, 2016). The difference between the total Cu content in diets and supplemental Cu doses indicates that the major feed ingredients supplied approximately 11 mg/kg Cu in total.

In the current study, dietary supplementation with Cu-NP increased Cu concentration in the blood plasma, but only in turkeys whose diets were supplemented with the highest Cu dose. At the same time, plasma Zn levels decreased in response to higher plasma Cu levels and increased in response to lower plasma Cu levels (interaction between Cu source and Cu level). The Cu dose had no effect on Ca and Mg levels in the blood, and the only change observed was in blood P levels which increased in response to lower dietary Cu supplementation. The correlation between Cu-NP supplementation and intestinal absorption of Zn as also confirmed in the study on chickens (Ognik et al., 2016). The cited authors observed that intestinal absorption of Zn was improved in line with a decrease in the dietary dose of Cu nanoparticles. Copper and Zn concentrations in cells are regulated by metallothioneins (MTs), which are low-molecular-weight proteins abundant in cysteine residues. A single MT molecule is capable of binding seven divalent Zn ions and up to 12 monovalent Cu ions. Due to similarities in the coordination chemistry of Zn and Cu, Cu is able to compete for Zn binding sites and push Zn away from the molecule (Gaetke and Chow, 2003).

The results of previous studies investigating the effect of dietary Cu levels on microelement and macroelement concentrations in the blood are inconclusive. Chickens fed diets supplemented with 75 mg Cu-SUL/kg were characterised by increased plasma concentrations of Cu and Zn at 42 days of age, relative to control group birds whose diets were not supplemented with Cu (Samanta et al., 2011). In another experiment, dietary supplementation with Cu at 50 mg/kg increased Cu concentration, decreased Zn concentration and had no significant effect on Fe concentration in the blood plasma of 8-week-old chickens. In 18-week-old birds, dietary Cu had no influence on the plasma concentrations of Zn, Fe or Mn (Adegbenjo et al., 2014). Bao et al. (2007) reported similar concentrations of trace minerals in the blood plasma of chickens fed diets supplemented with 4 and 40 mg Cu/kg (Bioplex-Cu). In our experiment, supplemental Cu doses of 2, 10 and 20 mg/kg did not differentiate Cu, Zn or Mg levels in the blood plasma.

Copper plays an important role in Fe metabolism, Hb synthesis and erythrocyte production (Tapiero et al., 2003; Mroczek-Sosnowska et al., 2013; Ognik et al., 2018) because ceruloplasmin transports around 95% of Cu in the blood stream and also participates in iron metabolism. Therefore, red blood cell indicators (RBC, Hb) increased in the few experiments where chicken diets were supplemented with Cu nanoparticles (Mroczek-Sosnowska et al., 2013; Miroshnikov et al., 2015; Ognik et al., 2018). In the present experiment, the substitution of Cu-SUL with Cu-NP increased Hb values. In broiler chickens, plasma Hb levels increased when diets were supplemented with 75–150 mg Cu/kg relative to the control group, whereas a decrease in plasma Hb levels was noted in response to the

highest Cu dose of 250 mg/kg (Samanta et al., 2011). In an experiment performed on growing turkeys (Makarski et al., 2014), a dietary Cu-SUL dose of 50 mg/kg decreased Hb levels relative to the control group where the diet was not supplemented with Cu. In the present study, diets supplemented with up to 30 mg Cu/kg did not induce changes in Hb levels in growing turkeys. In a study by Ognik et al. (2018), haematological parameters were influenced by the inclusion levels of Cu nanoparticles in chicken diets. In treatments where Cu levels were below the values recommended by the National Research Council (NRC, 1994), the supplementation of chicken diets with Cu nanoparticles increased Hb and Ht levels as well as RBC counts. Haemoglobin, Ht and RBC values decreased in chickens when the total Cu content in diets increased by 13% or more in excess of the recommended levels (NRC, 1994) due to supplementation with Cu nanoparticles. Ghasemipoor and Zolghadri (2014) also reported a decrease in the Hb levels of chickens whose diets were supplemented with nano-CuO at 16 mg/kg BW for 35 days compared with birds fed control unsupplemented diets.

In our experiment on young turkeys, the dietary supplementation with Cu-NP, in particular at the inclusion level of 20 mg/kg, decreased plasma glucose concentrations in turkeys (Kozłowski et al., 2018). Mroczek-Sosnowska et al. (2016) also observed a decrease in plasma glucose levels of chickens administered Cu-NP *in ovo* during embryogenesis. However, in the present study, the above effects were not confirmed when Cu-SUL was replaced with Cu-NP and when the dose of supplemental Cu was decreased. No differences were found in most blood biochemical parameters of turkeys fed diets with graded Cu levels.

A model study revealed that Cu is able to down-regulate lipid metabolism, especially cholesterol biosynthesis (Huster and Lutsenko, 2007). The above could partly explain the results of the present study where total plasma cholesterol concentration was the highest in turkeys fed diets with the lowest dose of supplemental Cu. Similar findings were reported by Kaya et al. (2006) who observed symptoms of hypertriglyceridemia, hypercholesterolemia and anaemia in chickens fed diets deficient in Cu (3.5 mg Cu/kg in feed ingredients) relative to birds fed diets containing 8 mg Cu/kg.

According to many studies, Cu can promote growth in chickens at dietary doses of 100 to 450 mg/kg (Pekel and Alp, 2011; Samanta et al., 2011). However, Sawosz et al. (2018) observed that the standard inclusion level of Cu (7.5 mg/kg of the diet) recommended by the NRC (1994) can significantly improve chicken performance if 25% of the $CuSO_4$ dose is replaced with Cu nanoparticles. Mroczek-Sosnowska et al. (2016) confirmed the beneficial effect of Cu nanoparticles injected *in ovo* on the performance of growing chickens. However, in the present study, the replacement of Cu-SUL with Cu-NP and the decrease in the dose of supplemental Cu from 20 mg/kg to 10 and 2 mg/kg did not affect the growth performance or the carcass quality of turkeys. In one of the few experiments performed on growing turkeys, the supplementation of bird diets with 50 mg Cu-SUL/kg did not induce changes in growth performance (Makarski et al., 2014). In the present experiment, the growth performance of birds and slaughter yield were similar in the compared treatments, regardless of the dose of supplemental Cu. The above could indicate that the Cu content of basic feed components (approx. 11 mg/kg) fulfilled the nutrient requirements of turkeys. This observation is also supported by similar slaughter yield and carcass quality traits in the analysed turkeys.

It is generally accepted that the oxidative stability of meat can be improved with diet modification (Estévez, 2015). For this reason, poultry diets are supplemented with antioxidants, such as vitamin E and selenium, to protect meat against oxidative damage. Research has demonstrated that Cu may act as an antioxidant or a pro-oxidant in living birds, depending on the dose and other feeding parameters (Ajuwon and Idowu, 2010; Xu et al., 2012). High Cu concentrations decreased glutathione levels and 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase activity, and limited cholesterol synthesis (Bakalli et al., 1995). Broilers whose diets were supplemented with 250 mg Cu-SUL/kg for 6 weeks were characterised by increased lipid peroxidation in the liver and plasma, lower CAT activity and lower levels of SOD and GSH (Ajuwon et al., 2011). However, the elimination of iron and Cu from the diet decreased oxidation values in cooked broiler leg meat in the thiobarbituric acid reactive substances assay (Ruiz et al., 2000). Bozkaya et al. (2001) observed intensified lipid peroxidation, an increase in MDA levels, a decrease in Cu-dependent SOD activity, and an increase in CAT activity in chickens fed Cu-deficient diets. In the current study, the replacement of Cu-SUL with Cu-NP increased SOD and CAT activity and decreased the total glutathione content of fresh breast meat. In frozen

meat, the above parameters were similar regardless of the source of supplemental Cu. In fresh meat, the optimal ratio of total glutathione (average values) to MDA (lowest value relative to the remaining treatments) was noted when turkey diets were supplemented with a moderate dose of Cu. In frozen meat, glutathione content was the highest and MDA content was the lowest when turkey diets were supplemented with the lowest Cu dose. A decrease in the glutathione content of meat could indicate that Cu nanoparticles lowered the antioxidant potential of meat, which stimulated SOD and CAT activity. However, the observed effect was moderate and short-lived, it did not increase MDA levels and was not confirmed in frozen meat. A decrease in the dose of supplemental Cu from 20 mg/kg to 10 and 2 mg/kg did not compromise the antioxidant potential of meat. The optimal redox parameters were noted in the fresh meat of turkeys receiving a moderate Cu dose and in the frozen meat of turkeys receiving the lowest Cu dose.

Conclusions

It can be concluded that the replacement of Cu sulphate (Cu-SUL) with Cu nanoparticles (Cu-NP) and a decrease in the dose of supplemental Cu from 20 to 10 mg/kg or even 2 mg/kg of the diet did not affect the growth parameters or the carcass quality of turkeys.

The few effects exerted by the substitution of Cu-SUL with Cu-NP included an increase in haemoglobin levels and an improvement in the antioxidant status of fresh breast meat. However, fresh meat was characterised by optimal redox parameters when the dietary dose of Cu was decreased to 10 mg/kg. The results of the present study cannot be generalised, but they significantly expand our knowledge about Cu-NP as an efficient source of Cu for turkeys.

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