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Effects of copper glycine chelate on liver and faecal mineral concentrations, and blood parameters in broilers

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The aim of the study was to determine the influence of Cu-glycine chelate on the chemical composition of the liver and blood parameters of broiler chickens. A total of 250 one-day-old Ross 308 male chicks were allotted into 5 groups with 5 replicates of 10 birds each. Rearing of birds lasted 42 days. In the experiment Cu was added to the premix in the form of $CuSO_4$ (16 mg, 8 mg Cu), and in the form of Cu glycine chelate (16 mg, 8 mg, 4 mg Cu). The parameters in the chickens' blood remained within the range of physiological norms when lower levels of the analyzed elements were added. Adding lower levels of Cu (8 or 4 mg·kg^{-1}) in comparison with the recommended doses (16 mg·kg^{-1}) for broilers, in the form of highly assimilable organic sources, did not reduce the content of minerals Cu, Fe, and Zn in the chickens' liver, but reduced the faecal Fe, Cu and Zn concentrations compared to $CuSO_4$.

Key words: broiler chickens, copper, organic minerals, mineral excretion, blood indices

Introduction

The issue of optimising mineral nutrition for contemporary meat crossbreds needs continuous updating. This is a result of the rapidly progressing genetic improvement of chickens and simultaneous attempts at reducing environmental pollution by increasing doses of mineral components. The goals can be achieved thanks to more accurately balanced diets and improved accessibility of the components.

The requirement of minerals in contemporary meat crossbreds can be satisfied by including these components in feeding stuffs or using feed additives. As regards mineral components, among other additives, a phytase enzyme (PHY) is used to increase the availability of phosphorus (P) and other mineral components such as zinc (Zn), copper (Cu) and iron (Fe) (Świątkiewicz et al. 2001, Shelton et al. 2006). In addition to its mineral availability properties, PHY has been shown to decrease P excretion and reduce P-linked pollution in intensive poultry production (Waldroup et al. 2000).

The majority of previous studies revealed that organic compounds containing certain minerals in their structure are better utilized by animals. Such compounds include chelates, which are composed of ion metals bound with amino acids, mainly methionine and lysine (Banks et al. 2004, Predieri et al. 2005, Wang et al. 2007, Vieira 2008). It is believed that elements derived from mineral and organic compounds can be absorbed in an unchanged form by the intestinal mucous membrane through the amino acid transport system and are thus better assimilated by the body (Predieri et al. 2005, Wang et al. 2007, Vieira 2008). Recently, new additives have been produced in which methionine or lysine is replaced by glycine-an amino acid with a lower molecular mass. Chemically, these compounds are combinations of one to three glycine molecules with microelements such as Cu or Fe. Such a form seems to guarantee greater stability and better chemical and physical homogeneity. Glycine chelates can be even better assimilated and guarantee proper functioning of metabolic processes in the body, assessed, for example, on the basis of biochemical and haematological blood indices, than chelates with higher molecular mass (Männer et al. 2006, Feng 2007, 2009, Ettle et al. 2008). Feng et al. (2009), studying the effect of an Fe-glycine chelate additive, found an increased concentration of Fe in the hearts, livers, kidneys and spleens. In their previous studies Feng et al. (2007) demonstrated that along with increasing the amount of Fe supplied as Fe-glycine chelate, the level of haemoglobin, the haematocrit and blood Fe increased. In addition, Männer et al. (2006) determined that the stability and availability of intestinal chelates based on the smallest amino acid, i.e. glycine, is 25% higher than in older generation chelates based on lysine or methionine.

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Copper is an element with a very broad action spectrum, and thus it is involved in numerous metabolic transformations in the body and as a result it can significantly affect the health status of the animals (Reeves and DeMars 2004, Arredondo and Nunez 2005). This contributes to increasing the mitogenic activity in plasma and excretion of the growth hormone or peptides (Nys 2001). A supply of 100 – 250 mg·kg^{-1} stimulates the growth of chicks (Nys 2001); however, such high doses increase its emission to the environment, which is not allowed by European Union regulations. Copper additive in feed has a beneficial effect on weight gain, the conversion of feed and modification of the bacterial microflora in the alimentary tract (Ruiz et al. 2000, Nys 2001, Makarski 2002). One possible mechanism by which Cu may benefit birds is shifting the gastrointestinal microbiota, thereby reducing the susceptibility of birds to disease, reducing intestinal lymphocyte recruitment and infiltration and thus increasing nutrient absorption (Arias and Koutsos 2006). Some researchers have demonstrated that supplementing broiler diets with high dietary Cu regulates intestinal microbiota through its bactericidal or bacteriostatic functions (Xia et al. 2004, Arias and Koutsos 2006). However, Aydin et al. (2010) reported that supplementing 250 ppm of Cu from Cu proteinate had no significant effect on the number of pathogenic bacteria in the ileum or on the carcass.

However, due to the low level of absorption and retention of Cu in animals, it is extremely difficult to determine its assimilability from different products added to feeding stuffs. It is believed that the best method to determine the bio-availability of Cu is to directly measure its retention in birds' livers (Luo et al. 2005).

The study touches upon a very current issue, both on a national and international scale, regarding improvement in the availability of minerals in the diet of chicks and reduced emission of minerals. The results of previous studies (Kwiecień et al. 2015) indicate that the use of organic Fe did not decrease the production performance of chickens and their blood parameters, which could suggest that minerals administered in the form of glycine chelate are better assimilable in comparison to those supplied in an inorganic form even if their amount is lower than recommended. Therefore, a decision was made to verify whether the use of Cu in the form of glycine chelate administered in various amounts will not have a negative effect on the bodies of broiler chickens. The aim was to determine the influence of Cu in the form of glycine chelate (Cu-Gly) on the liver and faecal mineral concentrations, biochemical and haematological blood parameters in Ross 308 chickens.

Material and methods

Animals and experimental design

All procedures used during the study were approved by the Local Ethics Committee for Animal Testing at the University of Life Sciences in Lublin, Poland (Resolution No. 37/2011 of 17 May 2011).

A total of 250 one-day-old Ross 308 male chicks were allotted into 5 groups with 5 replicates of 10 birds each. The birds were weighed individually at the beginning of the experiment. They were wing-banded and distributed randomly into 5 treatments of 250 chicks. They were kept under similar conditions of management throughout the experimental period. Artificial lighting was used to provide chicks with 24 h light during the whole experimental period. The initial brooding temperature was 33 °C in the first week of age which was gradually reduced by 2 °C per week to 23 °C. Then it remained constant. Feed and water were provided ad libitum throughout the experimental period which lasted until the chicks were 42 days of age.

Feed mixtures were prepared on the basis of corn and wheat meals and soybean meal (Table 1). The birds were fed with a starter mixture (S) from the 1st to the 21st day of rearing, grower mixture (G) from the 22nd to the 35th day and finisher mixture (F) from the 36th to the 42nd day of rearing. The G and F mixtures were administered in the form of pellets, while the S mixture was in the form of a crumble. Following the instructions, basic S, G and F mixtures were prepared and, next, diversified with an addition of Cu in the form of sulphates and chelates. 1 kg of basic diet not supplemented with Cu contained S-6.10 mg, G-6.21 mg, and F-5.95 mg Cu. The premix was formulated to contain the required amount of trace elements either in inorganic ($CuSO_4$ $5H_2O$, 25% Cu) or organic combination (Cu-Gly, 16% Cu and 37% glycine).

The copper requirement of Ross 308 broilers is 16mg kg^{-1} feed (Aviagen 2013). According to these recommendations, the content of Cu should be identical at each rearing stage, which was taken into account during the experiment. In the studies Cu was introduced into the mixtures at 2 levels of $CuSO_4$: at 100% of the requirement or at 50% and at 3 levels of Cu-Gly: at 100% of the requirement or at 50% or 25%.

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Table 1. Composition and nutritional value of basal diets

Ingredients (%)	Mixture		
	starter (1-21 days)	grower (22-35 days)	finisher (36-42 days)
Maize	24.44	40.00	40.00
Wheat	42.99	27.84	28.84
Soybean meal (46% protein)	25.00	24.97	22.87
Soybean oil	2.50	3.69	3.98
Monocalcium phosphate	0.90	0.90	0.81
Limestone	1.40	1.13	1.09
Sodium bicarbonate	0.08	0.08	0.08
NaCl	0.29	0.25	0.26
Vitamin–mineral premix[a]	0.50[b]	0.50[c]	0.50[d]
Fat-protein concentrate[e]	1.00	-	1.00
DL-methionine 99%	0.30	0.23	0.23
L-lysine HCl	0.42	0.28	0.27
L-threonine 99%	0.18	0.13	0.07
One kg feed mixtures contains			
Metabolizable energy (ME, MJ kg^{-1})	12.7	13.1	13.2
Available P, %	0.42	0.41	0.39
Total Ca/available P	2.12	1.90	1.92
Crude protein, %	20.2	18.2	18.1
Crude fibre, %	3.06	2.99	2.99
Crude fat, %	4.66	6.08	6.43
Lysine, %	1.29	1.13	1.09
Methionine+cysteine, %	0.93	0.83	0.81
Total Ca, %	0.88	0.78	0.75
Total P, %	0.66	0.65	0.63
Cu, mg			
16mg CuSO$_4$	22.10	22.21	21.95
8mg CuSO$_4$	13.98	14.02	13.89
16mg Cu-Gly	21.98	22.15	22.02
8mg Cu-Gly	14.12	14.21	14.01
4mg Cu-Gly	10.10	10.21	9.98
Fe, mg	40.31	39.82	38.61
Zn, mg	99.71	98.50	98.52

[a] the premix was added Cu as CuSO4 in an amount of 16 or 8 mg kg^{-1}, and Cu-Gly in an amount of 16, 8 or 4 mg kg^{-1}
[b] composition of the premix per kg of starter diet: Mn 100 mg, J 1 mg, Fe 40 mg, Zn 100 mg, Se 0.15 mg, vit. A 15 000 UI, vit. D3 5 000 UI, vit. E 75 mg, vit. K3 4 mg, vit. B1 3 mg, vit. B2 8 mg, vit. B6 5 mg, vit. B12 0.016 mg, biotin 0.2 mg, folic acid 2 mg, nicotic acid 60 mg, pantothenic acid 18 mg, choline 1 800 mg
[c] composition of the premix per kg of grower diet: Mn 100 mg, J 1 mg, Fe 40 mg, Zn 100 mg, Se 0.15 mg, vit. A 12 000 UI, vit. D3 5 000 UI, vit. E 50 mg, vit. K3 3 mg, vit. B1 2 mg, vit. B2 6 mg, vit. B6 4 mg, vit. B12 0.016 µg, biotin 0.2 mg, folic acid 1.75 mg, nicotic acid 60 mg, pantothenic acid 18 mg, choline 1 600 mg
[d] composition of the premix per kg of finisher diet: Mn 100 mg, J 1 mg, Fe 40 mg, Zn 100 mg, Se 0.15 mg, vit. A 12 000 UI, vit. D3 5 000 UI, vit. E 50 mg, vit. K3 2 mg, vit. B1 2 mg, vit. B2 5 mg, vit. B6 3 mg, vit. B12 0.011 µg, biotin 0.05 mg, folic acid 1.5 mg, nicotic acid 35 mg, pantothenic acid 18 mg, choline 1 600 mg
[e] 1kg of fat-protein concentrate contain: 2% crude fat, 39% crude protein, 10,8 MJ ME
Cu-Gly = copper glycine chelate

Three levels of chelate were used for the following reasons: firstly, the chicks' requirement of minerals as per the technical instructions of the breeder of Ross chicks (100% of the requirement) was covered; secondly the bioavailability of minerals declared by the manufacturer of chelates, i.e. ca. 50% (50% of the requirement) was taken into account; and thirdly, scientific research information indicating that the levels of mineral components in poultry mixtures often exceed the requirement (25% of the requirement) was considered.

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In the experiment Cu was added to mixtures S, G and F in an amount of 16 or 8 mg kg^{-1} in the form of $CuSO_4$ (at 100% recommended levels for Ross broiler chicks or 50%) - diet 16 mg and 8 mg $CuSO_4$; or in the form of Cu-Gly in an amount of 16, 8 or 4 mg kg^{-1} (at 100%, 50% and 25% of the recommended levels for Ross broiler chicks) - diets 16 mg Cu-Gly, 8 mg Cu-Gly, 4 mg Cu-Gly.

The basal diets were formulated using NRC (1994) guidelines. According to these standards and industry-specific nutrition recommendations for Ross 308 broiler chicks (Aviagen 2013), the requirement of Cu is covered regardless of the amount supplied by typical feed components. The control group was a group receiving additional Cu in the form of $CuSO_4$ $5H_2O$, corresponding to 100% of the requirement. With regard to good assimilability, as a standard, the sources of microelements used in the production of feed mixtures include well soluble inorganic forms. Therefore, the source of Cu in the experiment was $CuSO_4$ $5H_2O$. The experiment involved the use of *GLYSTAR FORTE* chelate (2:1 glycine-metal ratio) made by ARKOP Sp. z.o.o.

Experimental measurements

All feathers were carefully removed from the faeces by hand. Whole-stool samples were collected on the last, i.e. 42nd, day of the experiment, from 10 birds from each group. Then the faeces were mixed within respective groups (collective sample), placed in sterile plastic containers and frozen at a temp. of – 20 °C for future chemical analysis. Prior to the analyses the material was thawed at room temperature. Afterwards, the samples were dried at a temp. of 60 °C over 24 h and ground in an electrical grinding mill. A 50 g representative sample of the material prepared as described above was collected and placed in a sterile plastic container at room temperature. After faecal collection, birds were returned to their original pens.

The birds were slaughtered after 10 h of starvation during which they had unlimited access to water (EC 2009). All the animals were clinically healthy. On the last day of rearing, the birds were weighed and 10 from each group with body weight closest to the average weight in the group were selected for slaughter. After slaughter, a simplified dissection analysis (Ziołecki and Doruchowski 1989) was performed during which the livers were weighed, packed into labelled plastic bags and frozen (at a temperature of – 25 °C) until the time of analysis. Blood samples (10 samples per treatment) for analysis were taken from the wing vein (*vena cutanea ulnaris*) in the morning of the slaughter day. Blood for haematology tests was sampled into 2 ml Vacutest tubes containing K_3EDTA anticoagulant. The material for biochemical tests was blood sampled into 6 ml Vacutest tubes containing lithium heparin. During transport blood was stored at a temperature of 2 to 8 °C.

Chemical analysis

Liver

The livers were used to determine the content of dry matter, crude protein, crude fat and crude ash with the use of AOAC (2000). Samples of liver were dried at 100 °C for 24 h and ashed for 10 h at 550 °C. The ashed samples were dissolved in a nitric acid-perchloric acid mixture (1:1) and diluted with deionised water for mineral analysis. The contents of Fe, Cu, Zn and Ca were measured using flame atomic absorption spectrophotometry (Unicam 939/959AA-6300, Shimadzu Corp., Tokyo, Japan).

Feed and faeces

The contents of dry matter, crude ash, crude protein, crude fat and crude fibre in feed samples were determined with standard AOAC (2000). The thawed faeces were thoroughly mixed, weighed, and dried for 24 h (65 °C). The Cu, Fe, Zn and Ca content in feed and Cu, Fe and Zn in faecal samples was determined using the AAS flame technique in a Unicam 939 (AA Spectrometer Unicam, Shimadzu Corp., Tokyo, Japan) apparatus, after ashing at 550 °C, according to the methods of AOAC (2000). Total P content in the feed was determined colorimetrically (PN-76/R-64781, 1976) with a Helios Alpha UV-VIS apparatus (Spectronic Unicam, Leeds, United Kingdom).

The amino acid composition in feed was determined by ion-exchange chromatography using an INGOS AAA 400 amino acid analyser (Ingos Ltd., Czech Republic) with post-column ninhydrin derivatization and spectrophotometric detection according to the standard manufacturer's procedure and MCMiAŻ/PB-03 test procedure.

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The samples were hydrolyzed in an aqueous solution (6N HCl+0.5% phenol at a temperature of 110 °C for 24 h). Sulphur amino acids (cysteine and methionine) were determined in a separate analysis as oxidised derivatives (cysteic acid and methionine sulfone) resulting from oxidation with performic acid and then released from proteins during acid hydrolysis. Assimilable lysine was determined based on the difference between total lysine and the so-called residual lysine which did not react with DNFB (dinitrofluorobenzene). Following this reaction, the tested samples were again subjected to acid hydrolysis (Žilic et al. 2006).

Blood

Complete blood analysis was performed within three hours of collection using an ABACUS Junior Vet haematology analyser (Diatron, Vienna, Austria). The complete blood count determined the haematocrit value (Ht), concentration of haemoglobin (Hb), red blood cell (RBC) and white blood cell count (WBC).

Plasma for the analysis of biochemical indicators was obtained by centrifugation of complete blood at 3000 rpm over 15 minutes in a laboratory centrifuge (MPW-350R, MPW Medical Instruments, Warsaw, Poland) at a temperature of 4 °C. The plasma was analysed within four hours of collection; zinc, calcium, copper, iron, total protein, glucose levels, uric acid, triacylglycerols (TG), total cholesterol and high-density lipoprotein (HDL) were determined along with the activity of the following enzymes: alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). The above-mentioned components of blood plasma were analysed by colorimetric methods, described in the manual, using BioMaxima reagent sets (Lublin, Poland) in a Metrolab 2300GL random-access biochemical analyzer (Metrolab SA, Buenos Aires, Argentina). Low-density cholesterol fraction (LDL) was calculated from the formula designed by Friedewald et al. (1972):

$$LDL\ (mmol\ l^{-1}) = cholesterol - HDL - (TG/2.2)$$

Statistical analysis

The results of laboratory and manufacturing tests were statistically analyzed, using the Statistica software ver. 10 (Statsoft Inc., Tulsa, USA). The elements calculated were measures of location (arithmetic mean) and absolute measures (SEM), and single-factor analysis of variance. Significant statistical differences were set at the level of $p<0.05$ and $p<0.01$. The significance of the differences between mean values in particular diets was assessed using Duncan's multiple range test.

Results

Introducing organic Cu into broiler mixes at levels lower than those recommended did not deteriorate the effectiveness of chicken fattening and mortality of chicks compared to inorganic forms where microelements were sourced from $CuSO_4$ (Table 2).

An addition of Cu in the form of $CuSO_4$ satisfying up to 50% of the requirement $p<0.01$ increased the content of crude protein in the chickens' livers, compared to the remaining groups (Table 2). No influence of Cu added to the mixtures on the content of other basic components was demonstrated.

The lowest share of Cu (4.79 mg·kg^{-1}) was observed in the livers of the chickens obtaining an addition of Cu in the form of $CuSO_4$ corresponding to 50% of the requirement, while the lowest content of Zn and Ca was recorded in the group of birds fed with $CuSO_4$ corresponding to 100 and 50% of the requirement.

The addition of Cu in the form of Cu-Gly had a significant effect on reducing the amount of Fe, Cu and Zn excreted with chicken droppings (Table 2); on average it reduced the amount of Fe excreted with droppings by ca. 8% compared to diets with the addition of $CuSO_4$.

Also, it decreased the amount of excreted Cu by ca. 5% compared to the recommended dose of 16 mg $CuSO_4$. An increased excretion of Zn with droppings was observed after the introduction of $CuSO_4$ compared to Cu-Gly.

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Table 2. Effects of copper glycine chelate on final body weight, mortality, liver weights and chemical composition of liver and on mineral contents in the faeces of chickens (mean value)

Cu source	CuSO$_4$		Cu-Gly				
Cu addition (mg·kg^{-1})	16	8	16	8	4	SEM	p value
Final BW (g)	2430	2324	2485	2479	2359	18.21	0.244
Share of liver in the body weight (%)	1.72	1.87	1.73	1.64	1.76	0.259	0.238
Mortality (%)	2.00	2.00	0.00	0.00	2.00	6.380	0.234
Chemical composition of liver (%)							
Dry matter	23.2	22.9	22.6	22.9	22.8	0.130	0.683
Crude ash	1.18	1.25	1.19	1.21	1.21	0.011	0.317
Crude protein	17.4^B	18.3^A	16.9^B	17.3^B	16.9^B	0.124	<0.001
Crude fat	4.83	4.06	4.21	4.10	4.41	0.079	0.645
Mineral composition of liver (mg·kg^{-1})							
Cu	6.00^A	4.79^B	5.59^A	5.50^A	5.62^A	0.078	0.001
Fe	84.4	84.1	92.8	90.6	90.0	1.201	0.092
Zn	34.1^B	31.4^B	56.1^A	53.5^A	54.6^A	1.458	<0.001
Ca	20.5^C	18.2^C	25.6^B	30.2^A	25.7^B	0.001	<0.001
Mineral contents in faecal (mg·kg^{-1})							
Fe	132.7^a	131.6^a	122.3^b	120.9^b	121.8^b	2.585	0.022
Cu	24.6^a	24.2^{ab}	23.4^b	23.4^b	23.6^b	0.251	0.030
Zn	177.1^A	181.3^A	159.3^B	158.9^B	159.4^B	4.949	0.006

$^{A, B}$ - means with different superscripts in lines differ at $p<0.01$
$^{a, b}$ - means with different superscripts in lines differ at $p<0.05$
BW = body weight
Cu-Gly = copper glycine chelate
EEI = European Efficiency Index
SEM = standard error of the means

The addition of Cu-Gly resulted in an increased content of total protein and Cu and a lower content of glucose, uric acid, Fe and Ca in the chickens' blood, with reference to Cu in an inorganic form (Table 3). Introducing an addition of Cu, covering up to 100% of the requirement, in the form of Cu-Gly, significantly reduced the share of Zn in blood compared to the remaining experimental groups. The highest concentration of Fe (22.4 µmol·l^{-1}) in blood was noted in the group of chickens administered 8 mg·kg^{-1} of Cu in the form of CuSO$_4$, while the lowest content of this element (20.1 µmol·l^{-1}) was observed in chickens receiving Cu-Gly at the level of 8 mg·kg^{-1} (Table 3).

The concentration of total cholesterol, TG and LDL fraction, irrespective of the amount of Cu in the mixtures, was significantly lower in the groups of chickens receiving organic copper additive (Table 3). HDL fraction was also found to have increased with the addition of Cu-Gly at the level of 100%, 50% and 25% of the requirement ($p<0.01$) compared to diets obtaining a mixture with Cu added in an inorganic form.

The activity of enzymes in the plasma of broiler chickens using organic and inorganic forms of Cu is presented in Table 3. An addition of Cu in the form of CuSO$_4$, corresponding to up to 50% of the requirement for this element, resulted in reducing the activity of AST in comparison to the other groups ($p<0.01$). Moreover, a significantly higher activity of ALT was observed in groups administered Cu-Gly chelate compared with its activity in the plasma of chickens receiving feed with inorganic Cu additive. The highest LDH activity was recorded in the plasma of chickens fed Cu additive in the form of Cu-Gly amounting to 25%.

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Table 3. Effects of copper glycine chelate on some biochemical parameters in chicken blood (mean value)

Cu source	CuSO$_4$		Cu-Gly				
Cu addition (mg·kg^{-1})	16	8	16	8	4	SEM	p value
Glucose (mmol·l^{-1})	9.84^A	9.94^A	8.82^B	8.98^B	8.86^B	0.098	<0.001
Total protein (g·l^{-1})	28.6^B	27.9^B	30.3^A	30.5^A	31.1^A	0.244	<0.001
Uric acid (mmol·l^{-1})	0.35^A	0.37^A	0.24^B	0.26^B	0.27^B	0.010	<0.001
Total cholesterol (mmol·l^{-1})	3.37^A	3.33^A	2.70^B	2.73^B	2.65^B	0.068	<0.001
HDL-cholesterol (mmol·l^{-1})	2.22^B	2.25^B	2.65^A	2.66^A	2.70^A	0.046	<0.001
LDL-cholesterol (mmol·l^{-1})	0.79^a	0.79^a	0.60^b	0.61^b	0.59^b	0.027	0.024
TG (mmol·l^{-1})	0.18^A	0,18^A	0.14^B	0.15^B	0.15^B	0.004	<0.001
ALP (U·l^{-1})	998	1099	1056	1040	1163	39.80	0.760
ALT (U·l^{-1})	11.3^B	11.1^B	12.6^A	12.5^A	12.6^A	0.168	0.001
AST (U·l^{-1})	261^A	236^B	279^A	277^A	273^A	3.423	<0.001
LDH (U·l^{-1})	1216^B	1196^B	1208^B	1215^B	1360^A	12.92	<0.001
Cu (µmol·l^{-1})	1.33^B	1.46^B	2.04^A	2.27^A	2.04^A	0.057	<0.001
Fe (µmol·l^{-1})	21.9^A	22.1^A	20.4^B	20.1^B	20.5^B	0.535	<0.001
Zn (µmol·l^{-1})	28.5^b	29.1^a	26.4^b	29.1^a	28.4^a	0.301	0.017
Ca (mmol·l^{-1})	1.99^A	2.01^A	1.73^B	1.72^B	1.70^B	0.026	<0.001

$^{A, B}$ - means with different superscripts in lines differ at $p<0.01$
$^{a, b}$ - means with different superscripts in lines differ at $p<0.05$
Cu-Gly = copper glycine chelate
SEM = standard error of the means
TG = triacylglycerols
ALP = alkaline phosphatase
ALT = alanine transaminase
AST = aspartate transaminase
LDH = lactate dehydrogenase

The highest WBC count was revealed with the use of feed with an addition of Cu-Gly covering 25% of the requirement. On the other hand, an addition of Cu in the form of CuSO$_4$, covering 50% of the requirement, significantly reduced the WBC count compared to chickens receiving feed with an addition of 8 mg and 4 mg Cu in the form Cu-Gly (Table 4). The highest RBC count was observed with the addition of 16 mg CuSO$_4$ which was significantly higher than the value of this index in the remaining groups. Lower values of Ht were noted with the use of the inorganic form added at the level satisfying 100% and 50% of the Cu requirement. An addition of Cu in the form of Cu-Gly, irrespective of the level applied, resulted in a significant increase in the Ht value.

Table 4. Effects of copper glycine chelate on some haematological indices of chicken blood (mean value)

Cu source	CuSO$_4$		Cu-Gly				
Cu addition (mg·kg^{-1})	16	8	16	8	4	SEM	p value
WBC (10^9·l^{-1})	28.8^c	29.2^{ab}	29.9^{abc}	30.8^{abc}	30.9^a	0.291	0.027
RBC (10^{12}·l^{-1})	1.91^A	1.75^B	1.74^B	1.71^B	1.77^B	0.018	0.001
Hb (mmol·l^{-1})	8.13	8.00	8.28	8.54	8.56	0.075	0.180
Ht (l·l^{-1})	0.25^B	0.25^B	0.26^A	0.25^B	0.27^A	0.002	0.003

$^{A, B}$ - means with different superscripts in lines differ at $p<0.01$
$^{a, b, c}$ - means with different superscripts in lines differ at $p<0.05$
Cu-Gly = copper glycine chelate
SEM = standard error of the means
WBC = white blood cell
RBC = red blood cell
Hb = haemoglobin
Ht = haematocrit

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Discussion

Chemical composition of liver and faeces

The earlier results of own studies (Kwiecień et al. 2014, Winiarska and Kwiecień 2015) form evidence of the beneficial effect of Gly-Cu additive in feed mixtures on the growth and development of broiler chickens. Throughout the rearing period the replacement of $CuSO_4$ with Cu-Gly did not significantly alter the weight of chickens. The addition of Gly-Cu chelate in the chicken diets did not change the feed to gain ratio, dressing percentage and content of breast and thigh meat in the carcass.

The levels of Cu applied in the experiment did not lead to significant changes in the content of the basic components in the birds' livers. Only with an addition of Cu in organic form, was a significant reduction in the content of crude protein noted in the birds' livers. Similarly, in studies published by Makarski et al. (2006) a significant decrease (of 11.8%) in total protein was recorded in the livers of birds obtaining an addition of Cu-Lys chelate amounting to 10 mg·kg^{-1}.

The source of copper significantly affects Cu concentration in the liver of broiler chickens (Wang et al. 2007). In the author's own studies, the lowest concentration of Cu in the chickens' livers was noted with the addition of Cu, limited to 50%, in the form of $CuSO_4$. Makarski (2002) observed an increase in the content of Cu in the liver of turkeys with the addition of Cu in the form of a chelate amounting to 30 mg·dm^{-3}. Also, later studies by Makarski et al. (2009b) suggest that adding 50 mg·dm^{-3} of Cu chelate to water resulted in a statistically significant increase in Cu concentration and a slightly higher concentration of Ca, Zn and Fe in the birds' livers. Güçlü et al. (2008) recorded an increased level of Cu in the liver when the diet was supplemented with Cu protein amounting to 300 and 450 mg·kg^{-1}. Conversely, Pierce et al. (2005) observed a higher content of Cu in the birds' livers when 250 ppm of Cu was used in the form of a sulphate. On the other hand, Bao et al. (2007) did not observe any significant differences in Cu concentration in the livers of chickens administered low levels of Cu in an organic form (2 mg kg^{-1}), medium (4 mg kg^{-1}), high (8 mg kg^{-1}), and in an inorganic form. It is believed that better assimilability of metals from organic forms than from inorganic forms in birds depends on how easily these compounds are converted into adequate organic combinations which are biologically active.

The use of Cu-Gly in the mixture, regardless of its level, significantly decreased the excretion of Fe, Zn and Cu with droppings into the environment compared to the recommended dose of $CuSO_4$. The results of literature-based studies indicate that decreasing Cu in the diet to the level of nutritional requirements is an effective method of reducing the excretion of Cu with droppings. The results of studies involving chickens carried out by Nollet et al. (2005) indicate that the replacement of the mineral form of added copper with a bioplex at the same level (Cu - 12.5 ppm) made it possible to reduce the excretion of Cu in birds. Using 12 ppm Cu in the form of sulphate in the control mixture and 2.5 ppm Cu bioplex in the experimental mixture, it was found that the excretion of Cu with droppings was lower by 55% with reference to sulphates (Nollet et al. 2007). Nollet et al. (2008) supplementing Cu at 5 levels in the form of two- and three-peptide bioplex found that the smallest loss of Cu was recorded at the lowest doses (2.5 ppm and 5 ppm). Dozier et al. (2003) administered from 4 to 12 mg Cu, both in inorganic form (sulphates), organic form (amino acid chelate) and as a mineral-organic combination. The results suggest that the supply of Cu decreased from 12 to 4 mg reduced the excretion of this element into the environment by 35%. It seems that improved assimilability of metals from organic than inorganic forms is determined by the ease of conversion of such compounds by animals into adequate biologically active organic combinations.

Biochemical indicators of blood

In the experiment the effect of using organic forms of Cu on the level of glucose, total protein and uric acid was observed (Ganong 2005). Changes in the level of glucose in blood reflect the changes of sugar in the body. The use of Cu-Gly resulted in lowering the level of glucose in blood, which suggests that glucose metabolism can be regulated with the help of Cu chelate compounds. The author's own studies confirmed that Cu has "insulin-like" properties because it lowers the level of glucose in the plasma, thus stimulating the bio-synthesis of protein. Makarski (2002) in his studies involving turkeys observed a decrease in the level of glucose in the birds' blood after adding lysine chelate to drinking water at the amount of 10, 20 and 30 mg Cu·l^{-1}. On the other hand, the content of total protein with partial and/or total replacement of $CuSO_4$ with its organic equivalent was significantly higher. The increase in the content of protein may suggest accelerated bio-synthesis of issue protein or decelerated catabolic processes of proteins. However, with no data available in the literature, interpreting the obtained results is quite problematic.

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Additionally, the obtained results suggest that increased doses of Gly-Cu in feed result in reducing the level of uric acid in blood. The observed differences were confirmed by statistics ($p < 0.01$). The reduction in the level of uric acid, suggesting a decrease in the catabolism of amino acids in the process of deamination in the case of increased use of amino acids from the so-called pool of free amino acids in blood, can testify to the influence of Cu on the increased utilisation of amino acids in protein synthesis.

After the 42nd day of rearing blood samples were taken in order to determine the content of Cu, Fe, Zn and Ca. The concentration of Cu was significantly higher in groups of chickens receiving Cu in an organic form in comparison with groups fed mixtures with $CuSO_4$. This may suggest better assimilability of copper from organic forms. The use of Cu (10 mg·kg^{-1}) in an organic form in studies by Dobrzański et al. (2008) resulted in a significant increase in the concentration of Cu in hens' blood. An increase in the concentration of Fe and Cu and a lower concentration of glucose in blood were recorded in the studies by Makarski et al. (2002) with an addition of Cu in the form of Cu-lysine chelate and Cu-methionine. Aksu et al. (2010) noted a drop in the level of Cu between chickens fed 2/3 of the recommended dose (26 mg·kg^{-1} Zn, 5 mg·kg^{-1} Cu, 40 mg·kg^{-1} Mn) and those with full requirement satisfied (40 mg·kg^{-1} Zn, 8 mg·kg^{-1} Cu, 60 mg·kg^{-1} Mn) for elements in the form of organic compounds, which could be caused by an interaction between Zn and Cu. Different results were obtained by Dmoch and Polonis (2007) who did not observe any influence of the form of Cu on its content while using Cu-lysine chelate; however, they noted a slight decrease in the content of Ca, P, Fe and Zn in the chickens' plasma. Similarly, Mondal et al. (2007) noted that the source of Cu in the diet did not affect the balance of the main minerals or their concentration in the plasma.

Following partial or complete replacement of inorganic Cu in the feed with its organic equivalent, the level of Ca in blood was significantly reduced. It seems that the influence of Cu on the concentration of Ca in blood may occur in the liver through the inhibition of 25-hydroxylase, which catalyses production of 25-hydroxycholecalciferol responsible for absorbing Ca in the upper section of the small intestine, by active transportation. $1.25\text{-}(OH)_2D_3$ models the synthesis of protein responsible for binding Ca^{2+} ions in the cells of intestinal mucous membrane. It should be supposed that by the 42nd day the chickens had not accumulated enough Cu to stimulate the synthesis of $1.25\text{-}(OH)_2D_3$. Additionally, a drop in the concentration of Ca could be related to the dynamics of the development of the skeletal system in maturing birds, which requires a high intake of Ca from blood, and Cu ions favour the process of bone mineralization. On the other hand, a lower level of Fe in plasma observed under the influence of adding Cu-Gly may suggest that this element is better utilized in the process of heme synthesis.

Changes in the content of total cholesterol, its lipoprotein molecules of high and low density and TG, may suggest lipid transformations occurring in the body (Ganong 2005). Cholesterol released from endogenous and exogenous lipoproteins is esterified to HDL, and its molecule participates in the reverse transportation to the liver. HDL concentration should account for more than 40% of total cholesterol so its reduction is unfavourable (Winnicka 2008).

The available literature provides little information about the effect of the use of Cu chelates on the content of cholesterol in the blood of broilers. It may be supposed that the organic compound of Cu affects lipid transformations in the animals' organisms. Previously published studies by Winiarska-Mieczan and Kwiecień (2015) indicated also that in the meat of chicken receiving Cu in the form of chelate the content of cholesterol was significantly ($p < 0.05$) lower compared to chicken receiving Cu in an organic form.

An addition of Cu in the form of Cu-Gly introduced into feeding stuffs, irrespective of the level applied, significantly contributed to a decrease in the level of total cholesterol, TG and LDL, as well as an increase in HDL, compared to the values of these parameters obtained with the use of their equivalents in the form of $CuSO_4$. The observed reduction in the level of TG and total cholesterol may suggest a positive effect of the experimental agents applied. TG provide a cell with a source of energy which is released during their disintegration and during the oxidation of fatty acids. A reduced level of TG in the blood plasma of the birds is a positive phenomenon as it suggests a weakened process of lipogenesis in the liver and fat tissue, which seems to confirm "insulin-like" properties of copper. The content of the analyzed parameters may be conditioned by genetic factors, as well as by environmental agents, especially dietary ones, which may account for differences between the results of the author's own studies and those conducted by other authors. Ševčikova et al. (2003) observed the level of cholesterol being reduced by 24.9% with an addition of Cu in the form of glycine chelate, compared to the control group which was not administered an addition of Cu. Similarly, Aksu et al. (2010) noted a drop in the level of total cholesterol and the LDL fraction in the plasma of chickens fed with mixtures supplemented with Zn, Cu and Mn in an organic form corresponding to 2/3 of the requirement (26 mg of Zn, 5 mg of Cu, 40 mg of Mn) or with recommended doses (40 mg of Zn, 8 mg of Cu, 60 mg of Mn), along with an increased amount of HDL. Mondal et al. (2007) recorded a reduction in cholesterol in chickens' blood when 200 mg·kg^{-1} of Cu in the form of protein was added to the mixture. Similar observations were made by Makarski and Zadura (2006) studying the influence of different doses of Cu

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on the level of lipid fractions in plasma. The lowest amount of cholesterol in chickens' plasma was demonstrated when 50 mg·dm^{-3} of Cu and lysine chelate were added to water, along with a limited level of TG. Also, Chowdhury et al. (2004) noted that an increased level of Cu and methionine chelate (100 mg and 150 mg·kg^{-1}) was accompanied by a lower level of TG in the plasma. This is a linear effect of Cu and fat accumulating in the liver. Conversely, Dmoch and Polonis (2007) using 30 mg·dm^{-3} Cu and lysine chelate with water in chickens' nutrition noted a significant increase in the level of cholesterol and TG concentration in the blood. Studies by Güçlü et al. (2008) demonstrated an elevated concentration of total cholesterol with an addition of 300 to 400 mg of Cu/kg in the form of Cu protein, while an addition of 450 mg·kg^{-1} resulted in a higher level of HDL. Konjufca et al. (1997) report that an addition of Cu in an organic form to feed leads to a higher content of cholesterol and the HDL fraction, while LDL is reduced. However, Lee et al. (2001) do not confirm these results.

The activity of ALP, ALT, AST and LDH enzymes, irrespective of the amount of organic Cu, did not differ from reference values (Winnicka 2008). An addition of a premix in the form of Cu-Gly to mixtures for Ross 308 chickens significantly enhanced the activity of ALT, AST and LDH in chickens' blood compared to the activity of these enzymes when inorganic Cu is applied. An increased concentration of LDH in blood may suggest damage to internal organs, such as pathological changes in the birds' livers. For this particular reason, after slaughter the chickens were subjected to a detailed anatomopathological analysis which did not reveal any changes in these organs, and the level of LDL determined in the author's own studies did not exceed physiological norms. It may thus be concluded that the increase in LDH activity was caused just by intensified glycogenesis processes. The activity of this enzyme may reveal great diversity, depending on the birds' age (Szabó et al. 2005). Similarly, in studies by Makarski et al. (2009a) the use of lysine-and-copper chelate amounting to 50 mg·dm^{-3} resulted in a higher activity of ALT, AST and LDG in the blood of male turkeys, which seems to confirm the intensification of endogenous amino acids necessary to synthesize proteins. The studies by Güçlü et al. (2008) showed that an addition of 450 mg·kg^{-1} of Cu resulted in higher activity of ALP and lower activity of ALT and LDH in laying hens.

The hematological parameters of blood

An important source of information on the health status of an animal is the concentration of haemoglobin and the value of haematocrit. The values of the blood's haematological indices were within reference values (Winnicka 2008). An addition of organic Cu led to a significant increase in the number of white blood cells and the haematocrit value. Haemoglobin biosynthesis is directly related to two elements, namely Fe and Cu, because Cu present in ceruloplasmin regulates the transport and metabolism of iron. The lowest concentration of haemoglobin was observed for mixtures supplemented with Cu limited to 50%, in the form of CuSO$_4$. On the other hand, the highest number of red blood cells was recorded when the chickens' diet was supplemented with copper in amounts fully satisfying the requirement. Copper has a stimulating effect on weight gain in chickens due to its participation in the process of haemoglobin synthesis. The increase in haematological parameters, noted in the study, may result from a lower concentration of Fe in the plasma, which suggests it is better utilized in the process of heme synthesis. Improved haematological indicators were observed in studies by Makarski et al. (2002), following an addition of Cu in the form of Cu-lysine chelate and Cu-methionine chelate. Later studies performed by Makarski and Zadura (2006) showed that supplementing turkeys' diet with Cu-lysine chelate did not significantly affect the level of haematological indices of blood.

Conclusion

Summing up the obtained results, it may be concluded that adding lower levels of Cu (8 and 4 mg·kg^{-1}) in comparison with recommended doses (16 mg·kg^{-1}) to the mixtures for broiler chickens, in the form of highly assimilable organic sources did not reduce the content of minerals in the chickens' livers. The biochemical and haematological parameters of the chickens' blood, which are among the indicators defining the birds' health status, remained within the range of physiological norms when the analyzed elements were added at lower levels. Adding Cu at lower levels is also supported by ecological aspects since such levels are more environmentally friendly (reduced excretion of Cu, Zn and Fe with droppings). The results indicate that the use of organic Cu did not decrease the examined parameters and since they are better assimilable in comparison to CuSO$_4$, they must be taken into account when preparing feed mixture recipes for chickens.

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Acknowledgements

References

Aksu, D.S., Aksu, T. & Őzsoy, B. 2010. The effects of lower supplementation levels of organically complexed minerals (zinc, copper and manganese) versus inorganic forms on hematological and biochemical parameters in broilers. *Kafkas Universitesi Veteriner Fakultesi Dergisi* 16: 553–559.

AOAC 2000. *Official Methods of Analysis*. Horwitz W. (ed.). 17th ed. AOAC International, Gaithersburg, MD, USA.

Arias, V.J. & Koutsos, E.A. 2006. Effects of copper source and level in intestinal physiology and growth of broiler chickens. *Poultry Science* 85: 999–1007.

Arredondo, M. & Nunez, M.T. 2005. Iron and copper metabolism. *Molecular Aspects of Medicine* 26: 313–327.

Aviagen, 2013. *Ross 308 parent stock:* Nutrition specification. en.aviagen.com.

Aydin, A., Pekel, A.Y., Issa, G., Demirel, G. & Patterson, P.H. 2010. Effects of dietary copper, citric acid, and microbial phytase on digesta pH and ileal and carcass microbiota of broiler chickens fed a low available phosphorus diet. *Journal of Applied Poultry Research* 19: 422–431.

Banks, K.M., Thompson, K.L., Rush, J.K. & Applegate, T.J. 2004. Effects of copper source on phosphorus retention in broiler chicks and laying hens. *Poultry Science* 83: 990–996.

Bao, Y.M., Choct, M., Iji, P.A. & Bruerton, K. 2007. Effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues. *The Journal of Applied Poultry Research* 16: 448–455.

Chowdhury, S.D., Paik, I.K., Namkung, H. & Lim, H.S. 2004. Responses of broiler chickens to organic copper fed in the form of copper-methionine chelate. *Animal Feed Science and Technology* 115: 281–293.

Dmoch, M. & Polonis, A. 2007. Influence of biopleks-Cu on hematological and biochemical indices and content of mineral components in blood of chicken broilers. *Zootechnica - Acta Scientarum Polonorum* 6: 11–18.

Dobrzański, Z., Korczyński, M., Chojnacka, K., Górecki, H. & Opaliński, S. 2008. Influence of organic forms of copper, manganese and iron on bioaccumulation of these metals and zinc in laying hens. *Journal of Elementology* 13: 309–319.

Dozier, W.A., Davis, A.J., Freeman, M.E. & Ward, T.L. 2003. Early growth and environmental implications of dietary zinc and copper concentrations and sources of broiler chicks. *British Poultry Science* 44: 726–731.

EC 2009. Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. *The Official Journal of the European Union* L 303: 1–30.

Ettle, T., Schlegel, P. & Roth, X. 2008. Investigations on iron bioavailability of different sources and supply levels in piglets. *Journal of Animal Physiology and Animal Nutrition* 92: 30–45.

Feng, J., Ma, W.Q., Xu, Z.R., Heb, J.X., Wang, Y.Z. & Liu J.X. 2009. The effect of iron glycine chelate on tissue mineral levels, fecal mineral concentration, and liver antioxidant enzyme activity in weanling pigs. *Animal Feed Science and Technology* 150: 106–113.

Feng, J., Maa, W.Q., Xua, Z.R., Wang, Y.Z. & Liu, J.X. 2007. Effects of iron glycine chelate on growth, haematological and immunological characteristics in weanling pigs. *Animal Feed Science and Technology* 134: 261–272.

Friedewald, W.T., Levy, R.I. & Frederickson, D.S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry* 18: 499–502.

Ganong, W. 2005. *Reviev of Medical Physiology*. 12th ed. McGraw-Hill Medical, New York, USA. 928 p.

Güçlü, B.K., Kara, K., Beyaz, L., Uyanik, F., Eren, M. & Atasever, A. 2008. Influence of dietary copper proteinate on performance, selected biochemical parameters, lipid peroxidation, liver, and egg copper content in laying hens. *Biological Trace Element Research* 125: 160–169.

Konjufca, V.H., Pesti, G.M. & Bakalli, R.I. 1997. Modulation of cholesterol levels in broiler meat by dietary garlic and copper. *Poultry Science* 76: 1264–1274.

Kwiecień, M., Winiarska-Mieczan, A., Zawiślak, K. & Sroka, S. 2014. Effect of copper glycinate chelate on biomechanical, morphometric and chemical properties of chicken femur. *Annals of Animal Science* 14: 127–139.

Kwiecień, M., Smolińska, W. & Bujanowicz-Haraś B. 2015. Effects of iron glycine chelate on growth, carcass characteristic, liver mineral concentrations and haematological and biochemical blood parameters in broilers. *Journal of Animal Physiology and Animal Nutrition.* DOI: 10.1111/jpn.12322.

Lee, S.H., Choi, S.C., Chae, B.J., Lee, J.K. & Acda, S.P. 2001. Evaluation of metal-amino acid chelates and complexes at various levels of copper and zinc in weanling pigs and broiler chicks. *Asian-Australasian Journal of Animal Sciences* 14: 1734–1740.

Luo, X.G., JI, F., Lin, Y.X., Steward, F.A., Lu, L., Liu, B. & Yu, S.X. 2005. Effects of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and oxidation stability of vitamin E in feed. *Poultry Science* 84: 888–893.

Makarski, B. 2002. Wpływ kompleksu chelatowego miedzi z lizyną oraz fitazy na reakcje biologiczne indyków. Rozprawy Naukowe AR Lublin. 256. (in Polish).

Makarski, B., Kwiecień, M. & Zadura, A. 2009a. The influence of copper in the form of a lysine chelate and lactic acid on biological reactions of turkeys. I. Hematological and biochemical indices of blood and production effects of turkeys. In: Pasternak K. (ed.) *Elements, the environment and human life*. p. 184–192. (Monographs, in English)

AGRICULTURAL AND FOOD SCIENCE

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Makarski, B., Kwiecień, M. & Zadura, A. 2009b. The influence of copper in the form of a lysine chelate and lactic acid on biological reactions of turkeys. II: The shares of mineral elements in the tissues and the contents of the large intestine in turkeys. In: Pasternak K. (ed.) *Elements, the environment and human life*. p. 193–198. (Monographs, in English)

Makarski, B., Polonis, A., Czech, A. & Sembratowicz, I. 2002. Effect of copper deriving from inorganic form and chelats on the components of blood and performance of turkeys. *Annales Universitatis Mariae Curie-Skłodowska (sectio EE)* 41: 287–293.

Makarski, B. & Zadura, A. 2006. Influence of copper and lysine chelate on hematological and biochemical component levels in turkey blood. *Annales Universitatis Mariae Curie-Skłodowska (sectio EE)* 48: 357–363.

Makarski, B., Zadura, A. & Kwiecień, M. 2006. The effect of Cu-Lysine chelate in turkeys diets on the results of slaughter analysis, chemical composition and the fatty acids profile in tissues. *Zootechnica - Acta Scientarum Polonorum* 5: 57–66.

Männer, K., Simon, O. & Schlegel, P. 2006. Effects of different iron, manganese, zinc and copper sources (sulphates, chelates, glycinates) on their bioavailability in early weaned piglets. In: Rodehutscord, M. (ed.) 9. *Tagung Schweine- und Geflügelernährung.* Institut für Agrar- und Ernährungswissenschaften, Universität Halle-Wittenberg, Deutschland. p. 25–27.

Mondal, M.K., Das, T.K., Biswas, P., Samanta, C.C. & Bairagy, B. 2007. Influence of dietary inorganic and organic copper salt and level of soybean oil on plasma lipids, metabolites and mineral balance of broiler chickens. *Animal Feed Science and Technology* 139: 212–233.

Nollet L., Wakeman, W. & Belyavin, C. 2005. Replacement of inorganic Cu, Mn, Fe and Zn with Bioplex on growth performance and faecal mineral excretion in broilers. *Proceedings of the 15th European Symposium on poultry nutrition*, Balatonfüred, Hungary. p. 183–185.

Nollet, L., van der Klis, J.D., Lensing, M. & Spring, P. 2007. The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion. *The Journal of Applied Poultry Research* 16: 592–597.

Nollet, L., Huyghebaert, G., & Spring, P. 2008. Effect of different levels of dietary organic (bioplex) trace minerals on live performance of broiler chickens by growth phases. *The Journal of Applied Poultry Research* 17: 109–115.

NRC 1994. *National Research Council.* Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, USA. 176 p.

Nys, Y. 2001. Trace elements as related to growth and health in chickens. *Productions Animales* 14: 171–180.

Pierce, J.L., Pescatore, A.J., Ford, M.J. & Cantor, A.H. 2005. The effects of source and level of dietary copper on copper and zinc metabolism in broiler chicks. *Alltech-University of Kentucky Nutrition Research Alliance*. Lexington, Abstracts M 30, 10.

PN-76/R-64781, 1976. Polska Norma. Pasze. Oznaczenie zawartości fosforu. (in Polish).

Predieri, G., Elviri L., Tegoni, M., Zagnoni, I., Cinti, E., Biagi, G., Ferruzza, S. & Leonardi, G. 2005. Metal chelates of 2-hydroxy-4-methylthiobutanoic acid in animal feeding. Part 2: Further characterizations, in vitro and in vivo investigations. *Journal of Inorganic Biochemistry* 99: 627–636.

Reeves, P.G. & DeMars, L.C.S. 2004. Copper deficiency reduces iron absorption and biological half-life in male rats. *Journal of Nutrition* 134: 1953–1957.

Ruiz, J.A., Pérez-Vendrell, A.M., & Esteve-Garcia, E. 2000. Effect of dietary iron and copper on performance and oxidative stability in broiler leg meat. *British Poultry Science* 41: 163–167.

Ševčíková, S., Skřivan, M., Skřivanová, V., Tůmová, E. & Koucký, M. 2003. Effect of supplementation of copper in copper sulphate and Cu-glycine on fatty acid profile in meat of broiler chickens, cholesterol content and oxidation stability of fat. *Czech Journal of Animal Science* 48: 432–440.

Shelton, J.L. & Southern, L.L. 2006. Effects of phytase addition with or without a trace mineral premix on growth performance, bone response variables, and tissue mineral concentration in commercial broilers. *The Journal of Applied Poultry Research* 15: 94–102.

StatSoft Inc. 1997. *Statistica for Windows*. Tulsa: StatSoft.

Świątkiewicz, S., Koreleski, J. & Zhong, D.Q. 2001. The bioavailability of zinc from inorganic and organic sources in broiler chickens as affected by addition of phytase. *Journal of Animal Feed Sciences* 10: 317–328.

Szabó, A., Mézes, M., Horn, P., Sütö, Z., Bázár, G.Y. & Romvári, R. 2005: Developmental dynamice of some blond biochemical parameters in growing turkey (*Meleagris Gallopavo*). *Acta Veterinaria Hungarica* 53: 397–409.

Vieira, S.L. 2008. Chelated minerals for poultry. *Revista Brasileira de Ciência Avícola* 10: 73–79.

Waldroup, P.W., Kersey, J.H., Saleh, E.A., Fritts, C.A., Yan, F., Stilborn, H.L., Crum Jr., R.C. & Raboy, V. 2000. Nonphytate phosphorus requirement and phosphorus excretion of broilers fed diets composed of normal or high available phosphate corn with and without microbial phytase. *Poultry Science* 79:1451–1459.

Wang, Z., Cerrate, S., Coto, C., Yan, F. & Waldroup, P.W. 2007. Evaluation of MINTREX® copper as a source of copper in broiler diets. *International Journal of Poultry Sciences* 6: 308–313.

Winiarska-Mieczan, A. & Kwiecień M. 2015. The effects of copper-glycine complexes on chemical composition and sensory attributes of raw, cooked and grilled chicken meat. *Journal of Food Science and Technology* DOI 10.1007/s13197-014-1510-8.

Winnicka, A. 2008. Wartości referencyjne podstawowych badań laboratoryjnych w weterynarii. SGGW, Warszawa. 122 p. (In Polish).

Xia, M.S., Hu, C.H. & Xu, Z.R. 2004. Effects of copper-bearing montmorillonite on growth performance, digestive enzyme activities, and intestinal microflora and morphology of male broilers. *Poultry Science* 83:1868–1875.

Žilic, S.M., Božovic, I.N., Savic, S. & Šobajic, S. 2006. Heat processing of soybean kernel and its effect on lysine availability and protein solubility. *Central European Journal of Biology* 1: 572–583.

Ziołecki, J. & Doruchowski, W. 1989. Metoda oceny wartości rzeźnej drobiu. COBRD, Poznań. p. 1–22. (in Polish).