ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXXI(4) SECTIO EE 2013

Institute of Animal Nutrition and Bromatology, Faculty of Biology and Animal Breeding
University of Life Sciences, Akademicka 13, 20-950 Lublin
e-mail: malgorzatakwiecienar@wp.pl

MAŁGORZATA KWIECIEŃ

The effect of the level and form of Fe on the quality of femur bones in broiler chickens

Wpływ poziomu i formy żelaza na jakość kości udowych kurcząt brojlerów

Summary. The analyses aimed at determining the effect of administering iron in the form of glycine chelates on physical, chemical, morphometric and strength parameters of the tibia bone in broiler chickens. 200 one-day-old Ross 308 chicks were divided into 4 groups each in 5 repetitions of 10 chicks. The feed mixtures were supplemented with Fe in inorganic form ($FeSO_4$) and organic form (Fe-Gly), covering 100% or 50% of the total requirement of the component recommended for Ross 308 broiler chicks. After the chickens were sacrificed, the tibiae bones were isolated, weighed, measured and frozen for further mechanical analysis. An Instron Universal Testing Machine (Model 3369) was used to determine the bone maximum elastic strength (Wy) and the maximum force moment (Wf). The geometric properties of bones (second moment of interia – Ix, cross-section area – A, mean relative wall thickness – MRWT) and cortical indexes (thickness of cortical layer – GWK, cortical index – WK, cortical surface – PK, cortical surface index – WPK) were estimated on the basis of measuring the external and internal horizontal and vertical axes in the cross section of the bones at the site of the fracture. Degreased bones dried to constant weight were submitted to mineralization in a muffle furnace and the mineral content was determined. The addition of Fe-Gly at the recommended level increased the mass of the bones ($g \cdot 100\ g^{-1}$ of body weight) by 17.3%, in comparison with the group administered $FeSO_4$ amounting to 20 $mg \cdot kg^{-1}$. The addition of Fe-Gly at a recommended dose caused a significant increase in the content of Ca and Zn in the chickens' femur. The results of the study suggest that Fe-Gly may be used as an alternative for the $FeSO_4$, and the administration of 20 $mg \cdot kg^{-1}$ of Fe in the form of glycine compounds does not result in a lower quality of the bone.

Key words: chicken, iron, glycine chelate, bone parameters

INTRODUCTION

The quick rate of chickens' growth, as well as their welfare, apart from improved nutritional value and dietary qualities of poultry products, are the key indicators in modifying the demand for nutrients. The accomplishment of the desired objectives becomes possible thanks to balancing the diets more accurately and improving the availability of the ingredients. As far as minerals area concerned, an addition of e.g. phytase enzyme is used, which increases the availability of numerous elements. It has been proved in recent years that organic compounds including some definite minerals in their structure are utilized more efficiently by animals [Predieri *et al*. 2005, Makarski and Zadura 2006, Wang *et al.* 2007, Vieira 2008]. These compounds include chelates which contain metal ions combined with amino acids, mainly methionine. The production of some new additives started recently in which methionine has been replaced by glycine, an amino acid of lower molecular mass. Glycine chelates with lower molecular mass in relation to the chelates of the older generation may be better absorbed and offer better production results than chelates with higher molecular weight. Sparce studies suggest that chelated iron with glycine may be easily absorbed and it maintains high availability, despite the presence of Fe absorption inhibitors, such as phytic acid [Oscar and Ashmead 2001, Männer *et al*. 2006, Ettle *et al.* 2008]. So it seems that their administration will increase the accessibility of micro elements, as compared with other amino acid chelates. The optimization of the level of the additives in feeds and the use of glycine forms of the studied micro elements may bring about enhanced performance parameters, blood indicators and the adequate bone mineralization in the first place. The available literature offers few study results discussing the form of minerals in relation to bone quality. Mineral elements which directly participate in mineralization processes in the bone system include, among others, iron and copper. Fe is a micro element absorbed primarily through the digestive system, and it is the main component composing haemoglobin, mioglobin and numerous enzymes [Lieu *et al.* 2001, Andrews 2002]. Fe deficiency results in anaemia, mucose atrophy, growth inhibition and general emaciation of the organism. On the other hand, excessive amounts of Fe will be deposited everywhere, causing disturbances in the metabolism of other trace metals, and deactivating manganese in particular. Moreover, the excess of iron salts in a feed may lead to disturbances favouring formation of insoluble iron phosphates [Gfeller and Messonnier 2004, Papanikolaou and Pantopoulos 2005]. These compounds reduce phosphorus absorption, causing thus rickets. When they occur in the form of a coloid suspension they may absorb vitamins or micro elements and block their absorption at the same time. Although the effect of adding iron glycine chelates on general performance or blood parameters has been already proved, though mainly in reference to pigs [Close 1998, 1999, Yu *et al*. 2000, Feng *et al*. 2007, 2009], there are few study results regarding broiler chickens and the effect of these compounds on adequate bone mineralization. Hence, raising this particular issue seems to be fully justified.

The objective of this study was to compare the effect of two chemical forms (sulphates and glycinate chelates) of iron administered in various amounts in feed mixtures for Ross 308 broiler chickens on the biomechanical, morphometric and chemical properties of chicken femur.

MATERIAL AND METHODS

Animals and diets

All procedures used during the research were approved by the Local Ethics Committee for Animal Testing at the University of Life Sciences in Lublin, Poland. 200 one-day-old Ross 308 chicks were split into 4 groups each in 5 repetitions of 10 chicks. Ross 308 male chicks were reared in cages in a controlled temperature and humidity environment. Throughout the term of the experiment electric lighting was used to ensure lighting for 24 hours. In the first week the chicks were kept at a temperature of 33°C. Every week the temperature was reduced by 2°C and finally 24°C was reached.

Basal diets for the birds were prepared on the basis of ground corn, wheat and extracted soya meal. The composition and nutritive value of basic feed mixtures are presented in Table 1 and 2. Starter diets were administered to chicks in crumbled form and grower

Table 1. Composition of experimental mixtures
Tabela 1. Skład surowcowy mieszanek doświadczalnych

Ingredients (%) Składniki (%)	Starter (1–21 days)	Grower (22–35 days)	Finisher (36–42 days)
Maize Kukurydza	24.44	40.00	40.00
Wheat Pszenica	42.99	27.84	28.84
Soybean meal* Śruta sojowa poekstrakcyjna*	25.0	24.97	22.87
Soybean oil Olej sojowy	2.50	3.69	3.98
Monocalcium phosphate Fosforan 1-Ca	0.90	0.90	0.81
Limestone Kreda pastewna	1.40	1.13	1.09
Sodium bicarbonate Kwaśny węglan sodu	0.08	0.08	0.08
NaCl	0.29	0.25	0.26
Vitamin-mineral premix (without Fe)** Premiks wit.-min. (bez Fe)**	0.50[1]	0.50[2]	0.50[3]
Protein-fat concentrate*** Koncentrat białkowo-tłuszczowy***	1.00	–	1.00
DL-methionine 99% DL-metionina 99%	0.30	0.23	0.23
L-lysine HCl L-lizyna HCl	0.42	0.28	0.27
L-threonine 99% L-treonina 99%	0.18	0.13	0.07

[1] composition of the premix per kg of starter diet / skład premiksu w 1 kg mieszanki starter: Mn 100 mg, J 1 mg, Zn 100 mg, Se 0.15 mg, vit. A 15 000 UI, vit. D_3 5 000 UI, vit. E 75 mg, vit. K_3 4 mg, vit. B_1 3 mg, vit. B_2 8 mg, vit. B_6 5 mg, vit. B_{12} 0.016 mg, biotin 0.2 mg, folic acid 2 mg, nicotic acid 60 mg, pantothenic acid 18 mg, choline 1800 mg

[2] composition of the premix per kg of grower diet / skład premiksu w 1 kg mieszanki grower: Mn 100 mg, J 1 mg, Zn 100 mg, Se 0.15 mg, vit. A 12 000 UI, vit. D_3 5 000 UI, vit. E 50 mg, vit. K_3 3 mg, vit. B_1 2 mg, vit. B_2 6 mg, vit. B_6 4 mg, vit. B_{12} 0.016 µg, biotin 0.2 mg, folic acid 1.75 mg, nicotic acid 60 mg, pantothenic acid 18 mg, choline 1600 mg

[3] composition of the premix per kg of finisher diet / skład premiksu w 1 kg mieszanki finisher: Mn 100 mg, J 1 mg, Zn 100 mg, Se 0.15 mg, vit. A 12 000 UI, vit. D_3 5 000 UI, vit. E 50 mg, vit. K_3 2 mg, vit. B_1 2 mg, vit. B_2 5 mg, vit. B_6 3 mg, vit. B_{12} 0.011 µg, biotin 0.05 mg, folic acid 1.5 mg, nicotic acid 35 mg, pantothenic acid 18 mg, choline 1600 mg

* 46% crude protein in the dry matter / 46% białka ogólnego w suchej masie
** in experiment Fe was added to the premix (containing no Fe) in an amount of 40 or 20 mg·kg^{-1} in the form of $FeSO_4$ or in the form of Fe-Gly chelate in an amount of 40 or 20 mg·kg^{-1} / w doświadczeniu Fe dodawano do do premiksu (bez Fe) w ilości 40 lub 20 mg·kg^{-1} w formie $FeSO_4$ lub w formie chelatu Fe-Gly w ilości 40 lub 20 mg·kg^{-1}
*** 1 kg of protein-fat concentrate contain: 39% crude protein, 2% crude fat, 10,8 MJ ME / 1 kg koncentratu białkowo-tłuszczowego zawiera: 39% białka ogólnego, 2% tłuszczu surowego, 10,8 MJ ME

Table 2. Nutritional value of experimental mixtures
Tabela 2. Wartość pokarmowa mieszanek eksperymentalnych

Ingredients, % Składniki, %	Starter (1–21 days)	Grower (22–35 days)	Finisher (36–42 days)
[2]Metabolizable energy (ME), MJ kg^{-1} [2]EM, MJ/kg	12.7	13.1	13.2
[1]Crude protein, % [1]Białko surowe, %	20.9	20.4	19.5
[1]Crude fibre, % [1]Włókno surowe, %	3.06	2.99	2.99
[1]Crude fat, % [1]Tłuszcz surowy, %	4.66	6.08	6.43
[2]LYS, %	1.29	1.13	1.09
[2]MET + CYS, %	0.93	0.83	0.81
[2]Total Ca, % [2]Ca ogólny, %	0.88	0.78	0.75
[2]Total P, % [2]P ogólny, %	0.66	0.65	0.63
[2]Available P, % [2]P przyswajalny, %	0.42	0.41	0.39
[2]Total Ca/available P [2]Ca ogólny/P przyswajalny	2.12	1.90	1.92
[1]Fe, mg *			
40 mg $FeSO_4$	113.59	109.80	106.69
20 mg $FeSO_4$	93.49	89.80	86.69
40 mg Fe-Gly	110.28	107.32	104.61
20 mg Fe-Gly	90.25	89.84	85.37
[1]Cu, mg	14.03	14.09	13.83
[1]Zn, mg	99.71	98.50	98.52

[1] values analysed / wartość analizowana
[2] values calculated / wartość kalkulowana
* in experiment Fe was added to the premix (containing no Fe) in an amount of 40 or 20 mg·kg^{-1} in the form of $FeSO_4$ or in the form of Fe-Gly chelate in an amount of 40 or 20 mg·kg^{-1} / w doświadczeniu Fe dodawano do do premiksu (bez Fe) w ilości 40 lub 20 mg·kg^{-1} w formie $FeSO_4$ lub w formie chelatu Fe-Gly w ilości 40 lub 20 mg·kg^{-1}

and finisher feed mixtures as pellets. The mixes were supplemented with Fe in inorganic form ($FeSO_4$) and organic form (Fe-Gly), covering 100% or 50% of the total requirement of the component recommended for Ross 308 broiler chicks. The demand for mineral components in feed mixtures was based on the recommendations issued by the Ross Company for broiler chickens of the Ross 308 line [Aviagen 2013], amounting to 40 mg·kg^{-1} Fe, without considering its content in the components of feed mixtures. Following these recommendations, the content of Fe should be the same at each breeding stage, which was taken into account in the experiment. The basic mixes contained: starter – 73.59 mg, grower – 69.80 mg, and finisher – 66.69 $mg \cdot kg^{-1}$ Fe. In experiment Fe was added to the premix (containing no Fe) in an amount of 40 or 20 $mg \cdot kg^{-1}$ in the form of $FeSO_4$ or in the form of Fe-Gly chelate in an amount of 40 or 20 $mg \cdot kg^{-1}$. The experiment involved the use of *GLYSTAR FORTE* chelate made by ARKOP Sp. z o.o., containing 15% of Fe.

Biomechanical, morphometric and chemical properties of femur

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 dissection. After 10 h starvation the birds were slaughtered after which right leg femoral bones of chicks were prepared. Bone samples were mechanically cleared of soft tissue and their length was measured with an electronic slide calliper (accuracy up to 0.001 mm), each time in an identical measuring position, bone circumference was measured at ½ of bone length, and the samples were packed into labelled foil bags and frozen (at – 25°C) until analyses.

Mechanical properties of the bones were determined identically for both groups after thawing at room temperature using a three-point bending test in an INSTRON 4302 apparatus (Instron, Canton, USA) linked with a computer. The relationship between forces perpendicular to the longitudinal axis of the bone and the displacement were registered. The distance between bone supports was set at 40% of the total bone length and bone samples were loaded with a constant speed of 10 mm/min. The maximum elastic strength (Wy) and the ultimate strength (Wf) of the bones were determined [Ferretti *et al.* 1993b]. In addition, Young's modulus was calculated according to the formula:

$$E = \frac{Wy \cdot L}{12 dy \pi Ix}$$

(where: E – Young's modulus; L – spacing of supports in bending test, dy – yielding deformation, Ix – second moment of interia) and bone density index was determined [Bruno *et al*. 2007]: BI = mk/dk (where: BI – bone density index, mk – bone weight, dk – bone length).

The geometric properties and cortical indexes of bones were estimated on the basis of horizontal and vertical diameter measurements of the mid-diaphyseal cross section of bone. The cross-section area (A), the second moment of interia (Ix), and the mean relative wall thickness (MRWT) were determined as described previously [Ferretti *et al*. 1993a,b]. The thickness of cortical layer (GWK), the cortical index (WK), the cortical surface (PK) and the cortical surface index (WPK) were determined as described previously [Ferretti *et al.* 1993a,b].

After evaluating the strength and structural properties, the femurs were defatted, dried to constant mass and finally mineralised in a muffle furnace at 600 °C [AOAC 2000]. The content of mineral components (Ca, Mg, Cu, Fe, Zn) in bones was determined by atomic absorption spectrometry using a Unicam 939/959 apparatus, and total P content [PN-76/R-64781] with a Helios α-Unicam apparatus, using molybdenum and vanadium as the reagent (NH_4VO_3, $(NH_4)_6Mo_7O_{24} \cdot H_2O$, H_2O) was determined at λ = 430 nm. The content of Ca, P, Mg, Cu, Fe and Zn in the bone was calculated as the content of these components in crude ash. All the results obtained from the analytical laboratory were performed in three replications.

Statistical analysis

The results were statistically verified using two-way analysis of variance using General Linear Model (GLM) ANOVAs combined with Duncan's multiple range tests (the SAS statistical software, version 9.1.3.; SAS Institute Inc Cary, NC.). The model considered the constant impact of the level, sources and level-source interactions. Significant statistical differences were set at the level of $P \leq 0.05$ and $P \leq 0.01$.

RESULTS AND DISCUSSION

The administration of Fe-Gly supplementation at the recommended level increased the bone weigth ($g \cdot 100\ g^{-1}$ of BW) by 17.3%, as compared with the group administered an addition of $FeSO_4$ reduced to 50% of the demand (Tab. 3). The use of Fe-Gly at the lowest amount added, i.e. at 10 mg of Fe/kg of the diet (25% of the recommended dose of this element) did not have a negative effect on the remaining physical parameters of the bone. It seems thus that the administration of Fe in the form of Fe-Gly at lower levels is sufficient and it condtions adequate growth of the femur bone in broiler chickens.

Table 3. Physical parameters of chicken femoral bones
Tabela 3. Parametry fizyczne kości udowych kurcząt

Fe source Źródło Fe	$FeSO_4$		Fe-Gly		SEM	*p* value Wartość *p*		
Fe level, $mg \cdot kg^{-1}$ Poziom Fe, $mg \cdot kg^{-1}$*	40	20	40	20				
Item Składnik						Fe source źródło Fe	Fe level poziom Fe	Fe level × source poziom Fe × źródło
Bone weight, g Masa kości, g	16.60	16.27	17.21	16.77	0.384	0.336	0.167	0.883
$g \cdot 100\ g^{-1}$ of BW	0.77^{AB}	0.69^{B}	0.81^{A}	0.78^{AB}	0.023	0.025	0.013	0.289
Length, mm Długość, mm	84.25	83.75	84.75	84.71	0.995	0.793	0.476	0.820
Perimeter, mm Obwód, mm	27.75	27.00	28.12	28.14	0.613	0.562	0.234	0.543

BW – body weight/ masa ciała
^{AB} – values in rows with different denoted letters differ significantly at $p \leq 0.01$/ wartości w wierszach oznaczone różnymi literami różnią się istotnie przy $p \leq 0,01$
SEM – standard error of the means / błąd standardowy średniej
* addition of Fe in $mg \cdot kg^{-1}$ mixture / dodatek Fe w $mg \cdot kg^{-1}$ mieszanki

Factors which condition the strength of the bone tissue include, among others, geometric and cortical features of the bone. The value represented by the indicators of morphometric parameters of the femur in individual groups was more or less even, which suggests that the selected levels of organic and inorganic sources did not have any significant effect on the studies features (Tab. 4). Administering Fe in its both organic and inorganic forms leads to an adequate increase in the volume and cross-sectional area, without any excessive growth of this tissue. Available studies do not offer a discussion concerning the effect of Fe forms and levels on geometrical and cortical indicators in the femur of broiler chickens. Hence, our studies offer both cognitive and practical values.

Table 4. Geometric features and cortical indexes of chicken femoral bones
Tabela 4. Parametry geometryczne i wskaźniki korowe kości udowych kurcząt

Fe source Źródło Fe	$FeSO_4$		Fe-Gly		SEM	*p* value Wartość *p*		
Fe level $mg \cdot kg^{-1}$ Poziom Fe, $mg \cdot kg^{-1}$*	40	20	40	20				
Item Składnik						Fe source źródło Fe	Fe level poziom Fe	Fe level × source poziom Fe × źródło
Geometric features/Parametry geometryczne								
Ix, mm^4	105.4	104.5	107.8	111.0	4.964	0.796	0.354	0.671
A, mm^2	18.13	18.38	18.63	18.69	0.946	0.868	0.676	0.921
MRWT	0.269	0.259	0.280	0.279	0.023	0.831	0.515	0.848
Cortical indexes/Wskaźniki korowe								
GWK, mm	1.55	1.51	1.82	1.70	0.163	0.629	0.175	0.794
PK, mm^2	24.29	24.79	25.73	24.56	2.601	0.901	0.820	0.756
WK, %	8.30	8.36	8.25	8.32	0.159	0.706	0.783	0.994
WPK, %	76.39	76.23	77.21	77.83	2.566	0.930	0.646	0.883

SEM – standard error of the means / błąd standardowy średniej
* addition of Fe in $mg \cdot kg^{-1}$ mixture / dodatek Fe w $mg \cdot kg^{-1}$ mieszanki

No significant changes in the strength parameters of the femur in broiler chickens administered Fe in the form of $FeSO_4$ and Fe-Gly (Tab. 5 and 6**)** were noted, though it was possible to observe some positive tendency regarding an increase in the values of these features when Fe was introduced in its organic form. Available scientific literature does not provide any experimental data regarding the influence of the form of Fe in feeds on the quality of chickens' skeletal system, expressed with strength parameters.

An increased content of crude ash in the bones may be a good indicator of the degree of bone mineralization dependent on the availability of P, Ca and other elements from phytate complexes. Our own studies did not reveal any significant effect of Fe-Gly addition on the content of crude ash in the femur of broiler chickens (Tab. 7). However, we observed a positive tendency for increased share of ash in the bones when the element was administered in its organic form. Similarly, other authors [Banks *et al.* 2004, Abdallah *et al*. 2009] noted higher shares of crude ash in the chickens' femur with the element administered in its organic form. On the other hand, Bao *et al*. [2007] while studying chickends and Mikulski et al. [2009] while studying turkeys, did not record any significant changes in the content of ash, related to the chemical form of the component.

Table 5. Strength parameters of chicken femoral bones
Tabela 5. Parametry wytrzymałościowe kości udowych kurcząt

Fe source Źródło Fe	$FeSO_4$		Fe-Gly		SEM	*p* value Wartość *p*		
Fe level Poziom Fe mg·kg^{-1}*	40	20	40	20				
Item Składnik						Fe source źródło Fe	Fe level poziom Fe	Fe level × source poziom Fe × źródło
Wy, N·mm	149.6	148.2	159.5	153.3	4.096	0.374	0.082	0.573
dy, mm	1.21	1.23	1.23	1.26	0.040	0.560	0.535	0.841
Wf, N·mm	259.0	258.8	265.2	256.9	12.083	0.732	0.862	0.744
Wy/dy, N mm·mm^{-1}	104.2	110.5	98.7	106.5	5.002	0.175	0.364	0.887
Wf/A, N·mm·mm^{-2}	13.09	12.75	13.91	12.98	0.838	0.468	0.544	0.734
E, N m^{-2}	1.54	1.57	1.61	1.49	0.121	0.737	0.988	0.553
BI, mg·mm^{-1}	197.3	194.6	203.1	197.9	4.717	0.420	0.346	0.804

Wy/dy – load-to-deformation ratio / sztywność
Wf/A – bending point resistance / wytrzymałość na zginanie
SEM – standard error of the means / błąd standardowy średniej
* addition of Fe in mg·kg^{-1} mixture / dodatek Fe w mg·kg^{-1} mieszanki

Table 6. Maximum elastic strength (Wy) and maximum force moment (Wf) towards bone weight and body weight
Tabela 6. Wartość maksymalnej siły sprężystości (Wy) i momentu siły maksymalnej (Wf) w odniesieniu do masy kości i masy ciała

Fe source Źródło Fe	$FeSO_4$		Fe-Gly		SEM	*p* value Wartość *p*		
Fe level Poziom Fe mg·kg^{-1}*	40	20	40	20				
Item Składnik						Fe source źródło Fe	Fe level poziom Fe	Fe level × source poziom Fe × źródło
Wy/mk, N·mm·g^{-1}	9.02	9.11	9.30	9.18	0.285	0.939	0.532	0.665
Wy/mc, N·mm·1000·kg^{-1}	64.57	67.88	70.27	69.54	2.269	0.770	0.195	0.264
Wf/mk, N·mm·g^{-1}	15.59	15.92	15.47	15.52	0.831	0.850	0.740	0.842
Wf/mc, N·mm·1000·kg^{-1}	112.3	117.8	116.8	117.0	5.719	0.779	0.916	0.515

Wf/mc – maximum force moment towards body weight / moment siły maksymalnej w odniesieniu do masy ciała,
Wf/mk – maximum force moment towards bone weight / moment siły maksymalnej w odniesieniu do masy kości,
Wy/mc – maximum elastic strength towards body weight / maksymalna siła sprężysta w odniesieniu do masy ciała,
Wy/mk – maximum elastic strength towards bone weight / maksymalna siła sprężysta w odniesieniu do masy kości,
SEM – standard error of the means / błąd standardowy średniej

The significant effect of the form Fe concentrations of Ca, Mg and Zn in the femur chickens. The administration of Fe in the form of Fe-Gly resulted in a significant increase in the content of Ca in the chickens' femur, as compared with its share in the bones of chickens receiving Fe in its inorganic form (Tab. 7). It may be presumed that the organic forms of Fe enhance the absorption of minerals, since they reduce their binding with those components of the feed which could form insoluble compounds. In our own studies the administration of Fe-Gly supplementation at the recommended level and the amount reduced to 50% of the demand increased the concentration of Zn, in comparison with the group receiving Fe amounting to 50% in its inorganic form, by 6.6% on the average. Some authors suggest [Studziński *et al.* 2006] that supplementing animal feeds with minerals in the form of chelates may contribute to increasing the metabolic supply available to animals. The components of the chelates are better absorbed from the digestive system than those of inorganic origin as the latter must first be transformed into their ion form and only then can be bound with a carrier which is most often an amino acid or a protein, and only in this form are they able to overcome the barrier of protein and lipid membranes.

Table 7. The mineral composition of chicken femoral bones
Tabela 7. Skład mineralny kości udowych kurcząt

Fe source Źródło Fe	$FeSO_4$		Fe-Gly		SEM	*p* value Wartość *p*		
Fe level, $mg \cdot kg^{-1}$ Poziom Fe, $mg \cdot kg^{-1}$	40	20	40	20				
Item Składnik						Fe source źródło Fe	Fe dose dawka Fe	Fe dose × source dawka Fe × źródło
Crude ash, % Popiół surowy, %	15.90	15.64	16.00	16.36	0.304	0.864	0.196	0.326
Ca, $g \cdot kg^{-1}$	271.9^B	274.4^B	282.5^A	285.5^A	1.548	0.094	< 0001	0.885
P, $g \cdot kg^{-1}$	180.3	179.0	184.3	183.3	1.968	0.574	0.057	0.955
Mg, $g \cdot kg^{-1}$	9.42	9.12	9.71	9.59	0.144	0.166	0.014	0.537
Zn, $mg \cdot kg^{-1}$	501.4^{ab}	498.9^b	531.0^a	531.3^a	10.470	0.918	0.007	0.896
Cu, $mg \cdot kg^{-1}$	7.20	7.43	7.64	7.75	0.305	0.583	0.235	0.857
Fe, $mg \cdot kg^{-1}$	450.4	440.4	453.5	435.1	21.93	0.529	0.961	0.850

$^{A, B}$ – values in rows with different denoted letters differ significantly at P ≤ 0.01 / wartości w wierszach oznaczone różnymi literami różnią się istotnie przy p ≤ 0,01
$^{a, b}$ – values in rows with different denoted letters differ significantly at P ≤ 0.05, / wartości w wierszach oznaczone różnymi literami różnią się istotnie przy p ≤ 0,05
SEM – standard error of the means / błąd standardowy średniej

No significant changes were recorded regarding the content of P, Ca and Fe. Also, Bao *et al.* [2007] observed that the content of Cu and Fe in the tibia bone did not depend significantly on the amount of these components added in the form of chelates and thus, administering them in higher amounts would be pointless. Only in case of zinc it was noted that higher supply of this element in its organic form resulted in Zn deposits found in the bones.

CONCLUSIONS

1. Administering the chelate at the amount limited to 20 mg·kg^{-1} did not result in worse bone parameters than with the recommended dose (40 mg·kg^{-1}).
2. The diet administered in feeding broiler chickens of Ross 308 line Fe in the form of the glycine chelate may become an alternative for iron sulphate, at the dose 50% lower than the recommended value, without any negative effect on physical, chemical, strength or morphometric parameters of the femur in broiler chickens.

REFERENCES

Abdallah A.G., El-Husseiny O.M., Abdel-Latif K.O., 2009. Influence of some dietary organic mineral supplementations on broiler performance. Int. J. Poult. Sci. 8(3), 291–298.

Andrews N.C., 2002. Metal transporters and disease. Curr. Opin. Chem. Biol. 6, 181–186.

AOAC, 2000. Official methods of analysis. Intern. 17th ed. AOAC Inter., Gaithersburg, MD, USA.

Aviagen, 2013. Ross 308 BROILER. Nutrition Specification. On-line: en.aviagen.com.

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. Poult. Sci. 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. J. Appl. Poult. Res. 16, 448–455.

Bruno L.D.G., Luquetti B.C., Furlanb R.L., Macarib M., 2007. Influence of early qualitative feed restriction and environmental temperature on long bone development of broiler chickens. J. Therm. Biol. 32, 349–354.

Close W.H., 1999. Organic minerals for pigs: an update. In: Lyons, T.P., Jacques, K.A. (eds.), Biotechnology in the feed industry. Nottingham University Press, Nottingham, UK, pp. 51–60.

Close W.H., 1998. The role of trace mineral proteinates in pig nutrition. In: Lyons, T.P., Jacques K.A. (eds.), Biotechnology in the feed industry. Nottingham University Press, Nottingham, UK, pp. 469–483.

Ettle T., Schlegel P., Roth X., 2008. Investigations on iron bioavailability of different sources and supply levels in piglets. J. Anin. Phys. Anim. Nutr. 92, 1, 30–45.

Feng J., Ma W.Q., Xu Z.R., He 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. Anim. Feed Sci. Technol. 150, 106–113

Feng J., Ma W.Q., Xu Z.R., Wang Y.Z., Liu J.X., 2007. Effects of iron glycine chelate on growth, haematological and immunological characteristics in weaning pigs. Anim. Feed Sci. Technol. 134, 261–272.

Ferretti J.L., Capozza R.F., Mondelo N., Montuori E., Zanchetta J.R., 1993a. Determination of femur structural properties by geometric and material variables as a function of body weight in rats. Evidence of sexual dimorphism. Bone, 14, 256–270.

Ferretti J.L., Capozza R.F., Mondelo N., Montuori E., Zanchetta J.R., 1993b. Interrelationships between densitometric, geometric and mechanical properties of rat femora: inferences concerning mechanical regulation of bone modeling. J. Bone Miner. Res. 8, 1389–1395.

Gfeller R.W., Messonnier S.P., 2004. Iron. In: Handbook of small animal toxicology and poisonings. Mosby, St. Louis, 202–204.

Lieu P.T., Heiskala M., Peterson P.A., Yang Y., 2001. The roles of iron in health and disease. Mol. Aspects Med. 22, 1–87.

Makarski B., Zadura A., 2006. Effect of copper supplementation in various chemical forms on the utilization of mineral components and on the health status of turkeys. Pol. J. Nat. Sci. Suppl. 3, 459–465.

Männer K., Simon O., Schlegel P., 2006. Effects of different iron, manganese, zinc and copper sources (sulfates, chelates, glycinates) on their bioavailability in early weaned piglets. In: M. Rodehutscord (ed.). 9. Tagung Schweine- und Geflügelernährung, Universität Halle-Wittenberg, Germany.

Mikulski D., Jankowski J., Zduńczyk Z., Wróblewska M., Mikulska M., 2009. Copper balance, bone mineralization and the growth performance of turkeys fed diet with two types of Cu supplements. J. Anim. Feed Sci. 18, 677–688.

Oscar P., Ashmead H.D., 2001. Effectuveness of treatment of iron-deficiency anemia in infants and young children with ferrous bis-glycinate chelate. Nutrition 17, 381–384.

Papanikolaou G., Pantopoulos K. 2005. Iron metabolism and toxicity. Toxicol. Appl. Pharmacol. 202, 199–211.

PN-76/R-64781. Pasze. Oznaczenie zawartości fosforu.

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. J. Inorg. Biochem. 99, 627–636.

SAS Institute SAS/STAT User's Guide release 9.1.3. Cary, NC, Statistical Analysis System Institute, license 86636.

Studziński T., Matras J., Grela E.R., Valverde Piedra J.L., Truchliński J., Tatara M.R., 2006. Minerals: functions, requirements, excessive intake and toxicity. In: Biology of Nutrition in Growing Animals. Eds. R. Mosenthin, J. Zentek, T. Żebrowska, Elsevier, 4, 81–134.

Vieira S.L., 2008. Chelated minerals for poultry. Rev. Bras. Cienc. Avic. 10(2), 73–79.

Wang Z., Cerrate S., Coto C., Yan F., Waldroup P.W., 2007. Evaluation of MINTREX® copper as a source of copper in broiler diets. Int. J. Poult. Sci. 6(5), 308–313.

Yu B., Huang W.J., Chiou P.W., 2000. Bioavailability of iron from amino acid complex in weanling pigs. Anim. Feed Sci. Technol. 86, 39–52.

The study was financed by the Ministry of Agriculture of the Poland (Project No. N N311 543540)

Streszczenie. Badania miały na celu określenie wpływu podawania żelaza w postaci chelatów glicynowch na parametry fizykochemiczne, morfometryczne i wytrzymałościowe kości piszczelowych kurcząt brojlerów. 200 jednodniowych kogutków Ross 308 podzielono na 4 grupy, w 5 powtórzeniach, po 10 kurcząt w każdej. Do mieszanek dodawano Fe w formie nieorganicznej ($FeSO_4$) i organicznej (Fe-Gly), w ilości 100% lub 50% całkowitego zapotrzebowania składnika rekomendowanego dla kurcząt brojlerów Ross 308. Po uboju wyizolowane kości udowe zważono, zmierzono i zamrożono do dalszej analizy mechanicznej. Za pomocą aparatu Instron (model 3369) określono maksymalną siłę sprężystą (Wy) i moment gnący siły maksymalnej (Wf) kości. Właściwości geometryczne kości (wtórny moment bezwładności – Ix, pole przekroju poprzecznego – A, średnia względna grubość ścian – MRWT) oraz wskaźniki korowe (grubość warstwy korowej – GWK, wskaźnik korowy – WK, powierzchnia korowa – PK, wskaźnik powierzchni korowej – WPK) określono na podstawie pomiarów zewnętrznej i wewnętrznej średnicy horyzontalnej i wertykalnej przekroju poprzecznego trzonu kości w miejscu złamania. Odtłuszczone i wysuszone do stałej masy kości poddano mineralizacji w piecu muflowym i oznaczono zawartość składników mineralnych. Dodatek Fe-Gly w dawce rekomendowanej zwiększył masy kości ($g \cdot 100\ g^{-1}$ masy ciała) o 17,3% w porównaniu z grupą otrzymującą $FeSO_4$ w ilości 20 $mg \cdot kg^{-1}$. Dodatek Fe-Gly spowodował istotne zwiększenie zawartości Ca i Zn w kościach udowych kurcząt. Wyniki badań pozwalają stwierdzić, że Fe-Gly może być użyty jako alternatywa $FeSO_4$, a zastosowanie 20 $mg \cdot kg^{-1}$ Fe w postaci związków glicynowych nie powodowało pogorszenia jakości kości.

Słowa kluczowe: kurczęta, żelazo, chelat glicynowy, parametry kości