J. Elementol. 2008, 13(3): 309-319

INFLUENCE OF ORGANIC FORMS OF COPPER, MANGANESE AND IRON ON BIOACCUMULATION OF THESE METALS AND ZINC IN LAYING HENS

Zbigniew Dobrzański[1], Mariusz Korczyński[1], Katarzyna Chojnacka[2], Henryk Górecki[2], Sebastian Opaliński[1]

[1]Depertment of Animal Hygiene and Environment
Wroclaw University of Environmental and Life Sciences
[2]Institute of Inorganic Technology and Mineral Fertilisers
Wroclaw University of Technology

Abstract

The paper presents results of research concerning an assessment of bioaccumulation of copper, manganese and zinc in Lohmann Brown layer hens (5 groups of 12 hens in each). Using ICP-MS method the concentration of these elements was determined in the content and shell of eggs, whole blood and in feathers of hens.

Feeding was based on all-mash feed mixture J-297 type with a content of Cu – 21.8, Fe – 200.8, Mn 140.5 mg·kg^{-1}, but in particular groups the contribution of organic and inorganic forms of these 3 microelements was different. Content of Zn in the mixture was 86 mg·kg^{-1} (zinc oxide). Microelements in the amounts of: Cu – 10, Fe – 40 and Mn – 80 mg·kg^{-1} were separately introduced to the control and to the test mixtures by using special premixes. In the control version, inorganic forms of these elements were used in a premix (copper sulfate, iron sulfate and manganese oxide), while in the experimental version they appeared in the organic form, i.e. *Saccharomyces cerevisiae* yeasts enriched with the three elements. In the experimental mixtures the contribution of organic forms of microelements was for Cu – 47, Fe – 20 and Mn – 58%. Content of the premix in a mixture was 0.5%. Yeasts contained: Fe – 33.9, Mn – 35.4, and Cu – 22.7 mg·kg^{-1} d.m. Content of yeasts in the mixtures did not exceed 0.4%.

Application of organic forms of copper caused a significant increase in copper concentration in the egg content and shell, in blood and in feathers in the group receiving organic-Cu, which proves better availability of copper from organic forms compared to copper sulfate.

Zbigniew Dobrzański, Depertment of Animal Hygiene and Environment, Wroclaw University of Environmental and Life Sciences

Introduction of organic forms of iron and manganese to feed did not cause any significant changes in the content of these metals in eggs, blood and feathers of hens, except the organic-Mn group (the level of Mn in feathers was significantly higher in feathers compared to the control group).

Organic forms of copper, manganese and iron did not result in any interactions with respect to Zn although an antagonistic influence of Cu (organic-Cu group) and synergistic of Mn (organic-Mn group) in the egg content was observed.

Key words: copper, iron, manganese, zinc, hen egg, blood, feathers.

WPŁYW ORGANICZNYCH FORM MIEDZI, MANGANU I ŻELAZA NA BIOAKUMULACJĘ TYCH PIERWIASTKÓW ORAZ CYNKU U KUR NIEŚNYCH

Abstrakt

W pracy przedstawiono wyniki badań dotyczących określenia biakumulacji miedzi, manganu i żelaza oraz cynku u kur nieśnych Lohmann Brown (5 grup po 12 kur). Żywienie opierało się na pełnoporcjowej mieszance typu J-297 o zawartości Cu – 21,8, Fe – 200,8, Mn 140,5 $mg \cdot kg^{-1}$, z tym że w poszczególnych grupach różny był w udział organicznych i nieorganicznych form tych 3 mikroelementów. Zawartość Zn w mieszance wynosiła 86 mg□kg^{-1} (tlenek cynku). Mikroelementy w ilościach: Cu – 10, Fe – 40 i Mn – 80 $mg \cdot kg^{-1}$ wprowadzono osobno do paszy kontrolnej i do pasz doświadczalnych, stosując specjalne premiksy. W wersji kontrolnej w premiksie zastosowano formy nieorganiczne tych pierwiastków (siarczan miedzi, siarczan żelaza oraz tlenek manganu), natomiast w wersji doświadczalnej formy organiczne, tj. drożdże *Saccharomyces cerevisiae* wzbogacone w te trzy pierwiastki. W mieszankach doświadczalnych udział organicznych form mikroelementów wynosił: Cu – 47, Fe – 20 i Mn – 58%. Udział premiksu w mieszance wynosił 0,5%. Drożdże zawierały: Fe – 33,9, Mn – 35,4, Cu – 22,7 $mg \cdot kg^{-1}$ s.m. Udział drożdży w mieszankach nie przekroczył 0,4%.

Zastosowanie organicznych form miedzi spowodowało istotny wzrost stężenia miedzi w treści i skorupach jaj, we krwi i piórach w grupie otrzymującej Cu-org., co świadczy o lepszej przyswajalności miedzi w formach organicznych w porównaniu z siarczanem miedzi.

Wprowadzenie do paszy organicznych form żelaza i manganu nie powodowało istotnej zmiany w kształtowaniu się zawartości tych metali w jajach, krwi i piórach kur, z wyjątkiem grupy otrzymującej Mn-org. (istotnie więcej było Mn w piórach w porównaniu z grupą kontrolną).

Organiczne formy miedzi, manganu i żelaza nie spowodowały interakcji w odniesieniu do Zn, chociaż w treści jaja zaznaczył się antagonistyczny wpływ Cu (grupa Cu-org.) oraz synergistyczny Mn (grupa Mn-org.).

Słowa kluczowe: miedź, żelazo, mangan, cynk, jajo kurze, krew, pióra.

INTRODUCTION

Copper, iron and manganese, beside zinc, belong to the most important, basic microelements that are standardized in poultry feeding (SMULIKOWSKA, RUTKOWSKI 2005). They are accumulated in tissues and organs of birds, and also in the content and egg shells in quite different concentrations, dependent on a dose and form of these elements as well as on many other factors, including physiological ones. According to JAMROZ (2001) absorption of copper, iron and manganese from commercial mixtures for poultry reaches 10-15% on average (zinc – 30%). In turn, JONGBLOED et al. (2002) reveal that in poultry (and other livestock species) a relative biological value for Cu, Fe, Mn or Zn is higher from organic sources (e.g. Metal Amino Acid Complex, Metal Amino Acid Chelate, Metal Proteinate) comparing to oxides or chlorides of these metals.

A good source of organic forms of microelements are *Saccharomyces cerevisiae* yeasts (DOBRZAŃSKI et al. 2006, VASUDEVAN et al. 2002). There is a biotechnological possibility of yeasts enrichment with Cu, Fe and Mn and their application in the form of dry feed yeasts in poultry feeding (DOBRZAŃSKI et al. 2008). Little information is available on how organic forms of these elements influence their bioaccumulation in poultry, mainly in eggs, and if there is any interaction with zinc that plays important metabolic functions in poultry (PARK et al. 2004).

The aim of the study was to evaluate the influence of organic forms of copper, iron and manganese from *Saccharomyces cerevisiae* yeasts on the content of Cu, Fe, Mn and Zn in eggs, blood and feathers of laying hens.

MATERIAL AND METHODS

An eight-week experiment was conducted on 60 Lohmann Brown layers, divided into 5 groups and kept in vivarium conditions (cage housing). A standard lighting programme was applied, and thermal-humid conditions were controlled. Birds had constant access to water and feed. The level of egg laying by hens was high and similar in all the groups (95% on average; phase – peak and after a peak of egg laying). Feed consumption was similar per one layer (127.9-130.3 g^{-1} per day) or production of one egg and did not differ significantly between the groups (DOBRZAŃSKI et al. 2008).

Feeding was based on all-mash standard mixture of J-297 type, with crude protein content of 16.5 % and 11.6 $MJ \cdot kg^{-1}$ of metabolizable energy; an overall content of calcium was 3.65%, of available phosphorus 0.30%, while Cu – 21.8, Fe – 200.8, Mn 140.5 $mg \cdot kg^{-1}$, and in particular groups the contribution of organic and inorganic form of these 3 microelements was different (Table 1). Content of Zn in mixtures was similar: 86 $mg \cdot kg^{-1}$ (zinc

Table 1

Experiment design – content of Cu, Fe and Mn in feed mixture for laying hens ($mg \cdot kg^{-1}$ d.m.)

Micro-element	Source	Group				
		I inorg. Cu+Fe+Mn	II Cu-org.	III Fe-org.	IV Mn-org.	V org. Cu+ Fe+Mn
Cu	feed*	11.2	11.2	11.2	11.2	11.2
	premix**	10	-	10	10	-
	yeast	-	10	-	-	10
Calculated values		21.2	21.2	21.2	21.2	21.2
Determined values		21.8	23.4	22.7	19.9	22.5
Fe	feed *	160.8	160.8	160.8	160.8	160.8
	premix**	40	40	-	40	-
	yeast	-	-	40	-	40
Calculated values		200.8	200.8	200.8	200.8	200.8
Determined values		226.0	215.4	203.2	192.3	198.3
Mn	feed *	60.5	60.5	60.5	60.5	60.5
	premix**	80	80	80	-	-
	yeast	-	-	-	80	80
Calculated values		140.5	140.5	140.5	140.5	140.5
Determined values		138.5	144.6	136.9	143.0	157.8

* feed mixture without premix
** premix contains $CuSO_4$, $FeSO_4$, MnO_2, ZnO providing the following concentrations of elements: Cu – 2000, Fe – 8000, Mn – 16000, Zn – 10 000 $mg \cdot kg^{-1}$

oxide was used in a premixe). The above concentrations of microelements correspond to the feeding recommendations for laying hens (SMULIKOWSKA, RUTKOWSKI 2005).

Microelements in the following amounts: Cu – 10, Fe – 40 and Mn – 80 $mg \cdot kg^{-1}$ were separately introduced to the control and experimental mixtures by an application of special premixes. In the control version inorganic forms of these elements were used in the premix (copper sulfate, iron sulfate and manganese oxide), while in the experimental trials they occurred in the organic form, i.e. *Saccharomyces cerevisiae* yeasts enriched with these three elements. The contribution of the organic form of microelements in the experimental mixtures was for Cu – 47, Fe – 20 and Mn – 58%. The share of the premix in the mixture was 0.5%.

Saccharomyces cerevisiae yeast enriched with microelements (Cu, Fe, Mn) was produced on a laboratory scale according to an original technology based on whey (DOLIŃSKA et al. 2006). The yeast contained almost 40% of protein, about 1% of crude fat, a little more than 10 MJ·kg^{-1} of metabolizable energy, and the concentrations of the elements were: Fe – 33.9, Mn – 35.4, and Cu – 22.7 mg·kg^{-1} d.m. The yeast share in the mixtures did not exceed 0.4%.

Birds were divided into 5 groups (12 hens in a group), one control and four experimental one, with different contribution of organic and inorganic forms of microelements introduced to the premix:

- I group (control C) – inorganic forms of Cu, Fe and Mn;
- II group – organic forms of Cu and inorganic forms of Fe and Mn;
- III group – organic forms of Fe and inorganic forms of Cu and Mn;
- IV group – organic forms of Mn and inorganic forms of Cu and Fe;
- V group – organic forms of Cu, Fe and Mn.

Eggs for the analysis (12 from each group) were collected three times, i.e. after 25, 40 and 55 days of the experiment. Mean mass of an egg was from 62.4 to 65.0 g. After breaking, the content of an egg was separated from an egg shell. Eggs from the same groups were pooled for 2, thus 18 samples of egg content and 18 samples of egg shell for chemical analysis were obtained in total from each group. On day 55 blood from the wing vain (n=12) and also feathers from the back area (n=12) were collected from hens from each group. Albumen and yolk were carefully mixed, and egg shells (with under-shell membranes) were dried, while feathers were washed with warm water (about 50°C) and detergent, and then rinsed off with distilled water. Egg shells were ground and feathers were crushed. Also samples of the mixtures, premix and yeasts were collected for analysis before the experiment to determine their content of Cu, Mn, Fe and Zn. Mass spectrometry method (ICP-MS) with the use of a Varian Ultramass-700 apparatus was applied. Before the analyses, the samples were mineralized by the microwave method using an MCS-2000 microprocessor station (Górecka et al. 2001).

The results were elaborated statistically using analysis of variance, and significance of differences between particular groups was assessed by Duncan's test (Statgraphics software ver. 5.1).

RESULTS AND DISCUSSION

The content of microelements in eggs, blood or feathers of hens, i.e. in the biological material that may be collected without birds' decapitation, is to a high degree conditioned by feeding factors (chemical content of feed) and by the maintenance system. For instance, Skřivan et al. (2005) using different concentrations of Cu, Fe and Zn in diet of hens, obtained variious concentrations of these elements in eggs' content, liver and droppings of these birds. In turn, Kołacz et al. (2003) reveal different concentrations of Cu and Zn in muscles and liver of hens from small scale and farm husbandry, while DOBRZAŃSKI et al. (2004) demonstrated significant differences in bioaccumulation of these metals in the content of eggs laid by hens from industrialized and agricultural regions.

CONTENT OF MICROELEMENTS IN EGGS

Results of the determinations of the elements in the content and shells of eggs are presented in Tables 2 and 3. Mean concentration of Cu in egg content oscillated within 0.699 – 0.784 $mg \cdot kg^{-1}$ of fresh mass and was significantly higher ($p<0.05$) in group II (organic-Cu supplement) than in the other groups. The mean content of Cu in the egg shell was 2.273--3.034 $mg \cdot kg^{-1}$ d.m. with an increasing tendency in groups II and IV. Thus, copper from Y-Cu was better available to hens than from copper sulfate.

Table 2

Mean concentration of microelement in egg content ($mg \cdot kg^{-1}$ f.m.)

Element	Group				
	I	II	III	IV	V
Cu	0.735^{b}	0.784a	0.703^{b}	0.699^{b}	0.715^{b}
Fe	23.76	20.34	21.43	23.34	22.46
Mn	0.392	0.460	0.413	0.482	0.409
Zn	16.48	15.97^{a}	16.51	16.93^{b}	16.12

a-b, $p<0.05$

Table 3

Mean concentration of microelement in egg shell ($mg \cdot kg^{-1}$ d.m.)

Element	Group				
	I	II	III	IV	V
Cu	2.273^{a}	2.905^{b}	2.384	3.034^{b}	2.368
Fe	2.104	2.464	2.068	1.984	2.187
Mn	1.329	1.776	1.417	1.465	1.384
Zn	3.448	2.914	3.397	2.779	2.792

a-b, $p<0.05$

Quite wide ranges of the concentrations of this element are given in literature. ELMADFA and MUSKAT (2003) report a mean Cu range in the egg content on the level of 0.5-2.3, while UZIĘBŁO et al. (1993) claimed it was 0.49-0.70 $mg \cdot kg^{-1}$ of fresh mass. SKŘIVAN et al. (2006) give Cu values of 1.98-

-2.62 in hens' egg shells, depending on a copper dose in a diet, while DOBRZAŃSKI et al. (2007a) observed 2.2-2.42 mg of Cu·kg^{-1} d.m. in shells of hatching eggs.

The mean concentration of Fe in the egg content oscillated from 20.34 to 23.76 mg·kg^{-1} of fresh mass, and in the egg shell – from 1.984 to 2.464 mg·kg^{-1} d.m. The differences demonstrated between the groups were not statistically significant. Thus, Fe from Y-Fe was no more available than that from iron sulfate.

In literature, ranges of Fe concentration are given in narrow limits. FAKAYODE and OLU-OWOLABI (2003) give the mean concentration iron in the content of eggs from hens maintained in the free range system as 23.2, and according to some American data (*Eggcyclopedia* 1994) – the mean value is 14.4 mg·kg^{-1} of fresh mass. The content of this element in the egg shell given by SKŘIVAN et al. (2005) is between 13.2 and14.7 mg·kg^{-1} d.m. depending on the level of Cu, Fe and Zn in a diet. The latter values are thus a few-fold higher that the results of the present study.

The mean concentration of Mn in the egg ranged from 0.392 to 0.482 mg·kg^{-1} of fresh mass and was the highest in group IV, but the differences with respect to the other groups were not statistically significant. An average content of Mn in the egg shell was within 1.329-1.776 mg·kg^{-1} d.m. Thus, Mn from Y-Mn was not available better than that from manganese oxide although there was a tendency towards higher Mn accumulation in the egg content in group IV (organic-Mn supplement).

Ranges of Mn concentrations given in literature are in quite narrow. ELMADFA and MUSKAT (2003) give a mean concentration of Mn in the egg content of 0.3 mg·kg^{-1} of fresh mass, while DOBRZAŃSKI et al. (2001) demonstrated a 14.2% decrease in the Mn concentration of the egg content (from 0.274 to 0.235 mg·kg^{-1} of fresh mass) following an increased content of iodine and selenium in a feed premix. In shells of hens' eggs (without membranes) the content of this element is 0.4-1.1 mg·kg^{-1} (KONIECZNA 1993) or, according to MABE et al. (2003), 2.74-2.81 mg·kg^{-1} d.m.

The average concentration of Zn in the egg content oscillated within 15.97-16.93 mg·kg^{-1} of fresh mass, and was significantly higher (p<0.05) in group IV comparing to group II (organic-Cu supplement). The mean content of Zn in the egg shell was 2.779-3.448 mg·kg^{-1} d.m. Thus, introduction of organic forms of Cu, Fe and Mn did not cause any interactions with respect to Zn, even though an antagonistic influence of Cu (group II) and synergistic of Mn (group IV) were observed in the egg content.

There are many data concerning this element in literature. For example, depending on a system of hens husbandry, the concentration of Zn in the egg content was from 9.77 to 13.11 mg·kg^{-1} of fresh mass (DOBRZAŃSKI et al. 1999, ŻMUDZKI et al. 1992). ELMADFA and MUSKAT (2003) give an even higher mean concentration: 13.5 mg·kg^{-1} of fresh mass. Values of Zn in hens' egg shell are within a wide range of 4.2-5.47 mg·kg^{-1} of fresh mass (MABE et al. 2003) to 7.8-8.7 mg·kg^{-1} d.m. (SKŘIVAN et al. 2005).

Generally, it should be stated that organic forms of Cu, Fe and Mn applied in the form of enriched *Saccharomyces cerevisiae* yeasts caused exclusively an increase in the copper concentration in the content and shell of eggs. However, the concentration of Fe, Mn and Zn in the egg content and in shells was similar in all groups.

CONTENT OF MICROELEMENTS IN BLOOD

Results of the whole blood analysis of hens are presented in Table 4. The content of copper was 0.32-0.343 mg·l^{-1}, being significantly higher in group II (p<0.05) versus groups II, III and IV. The iron concentration was 272.4-305.7, manganese 0.174-0.21, and zinc 8.312-8.931 mg·l^{-1}, without any significant differences between the groups. The organic forms of Cu, Mn and Fe introduced to the hens' diet did not cause any interactions with respect to zinc, an important element in hens' nutrition.

Table 4

Mean concentration of microelements in whole blood of laying hens (mg·l^{-1})

Element	Group				
	I	II	III	IV	V
Cu	0.326^{b}	0.343^{a}	0.317^{b}	0.320^{b}	0.333
Fe	305.7	302.6	280.3	272.4	302.3
Mn	0.181	0.210	0.195	0.174	0.179
Zn	8.534	8.931	8.312	8.770	8.730

a-b, p<0.05

Table 5

Mean concentration of microelements in feathers of hens (mg·kg^{-1} d.m.)

Element	Group				
	I	II	III	IV	V
Cu	7.098^{b}	8.349^{a}	6.735^{b}	6.922^{b}	7.750
Fe	89.27	93.03	95.40	87.02	85.66
Mn	54.31^{a}	65.18	66.67	68.92^{b}	59.68
Zn	60.53	64.30	61.19	58.44	59.57

a-b, p<0.05

The concentrations of these elements revealed by our tests are difficult to comment on, as there is lack of reference values for poultry in literature. MONDAL et al. (2007) in blood plasma of 42-day-old chickens observed the concentration of Cu of 0.45 or 0.51 $\mu g \cdot l^{-1}$ when the animals were given a supplement of Cu-proteinate or Cu-sulphate, respectively. Other microelements like Zn, Mn and Fe did not undergo any significant changes. In turn, after introduction of organic Mn (Mn-amino acid complex) to a diet of chickens, JI et al. (2006) observed a significant increase in the Mn concentration in blood serum (maximum concentration 26.16 $\mu g \cdot l^{-1}$) compared to manganese sulfate.

Thus, blood of poultry is quite a good indicator of bioavailability of microelements. It is probable that higher doses of organic forms of copper, manganese of iron would give a wider differentiation of results in our present study although the level of these microelements is quite changeable in blood of poultry (SAHIN et al. 2007).

CONTENT OF MICROELEMENTS IN FEATHERS

Results of hens' feathers analysis are presented in Table 5. The content of copper was 6.922-8.349 $mg \cdot kg^{-1}$ d.m., iron concentration was 85.66-95.4 $mg \cdot l^{-1}$, manganese 54.31-68.92, while zinc 58.44-64.3 $mg \cdot kg^{-1}$ d.m. The differences we observed, with the exception of group II (Y-Cu) and group IV (Y-Mn), were not statistically significant between the groups. The organic forms of Cu, Mn and Fe added to the feeds did not cause any interactions with respect to zinc.

In the case of poultry, concentrations of these elements in feathers are important because birds can eat feathers (JENSEN et al. 2006). Moreover, there are technologies which enable us to use feathers for production of feed components (BERTSCH, COELLO 2005). Nevertheless, feather meals in animals feeding are not allowed in EU countries.

In wild birds living in an urban environment, examined in Belgium, the given values of Zn in feathers are 176-244, while these of Cu are 7.6-88 $\mu g \cdot g^{-1}$ (DAUWE et al. 2002). In pigeons in Israel the following contents of elements were observed in feathers: Cu – 5.45 – 11.8, Mn – 0.42 – 36.9, Zn – 28.7--146 $\mu g \cdot g^{-1}$ depending on the region (urban, industrial, agricultural regions) (ADOUT et al. 2007).

It seems, however, that feathers of birds are not a good index of elemental availability in a diet, since they are exposed to a risk of environmental pollution. Besides, their structure is not uniform.

CONCLUSIONS

1. Application of organic forms of copper in nutrition of layer hens caused a significant increase in copper in the content and shell of eggs, in blood and feathers in the group receiving organic-Cu, which proves better availability of organic forms of copper comparing to copper sulfate.

2. Introduction of organic forms of iron and manganese to feed for hens did not cause any significant changes in the concentration of this element in eggs, blood and feather of hens, with the exception of the group receiving organic-Mn (significantly higher content of Mn in feathers relative to the control group).

3. Organic forms of copper, manganese and iron did not cause any interactions with respect to Zn, although an antagonistic influence of Cu (organic-Cu group) and synergistic effect of Mn (organic-Mn group) were observed in the egg content.

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