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New Biological Dietary Feed Supplement for Laying Hens with Microelements Based on Duckweed (*Lemna minor*)

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ABSTRACT

In this study the applicability of enriched duckweed (*Lemna minor*) as a dietary supplement witch microelements is reported. In our previous studies, the technology of new feed additives with microelements based on duckweed biomass was elaborated. Here, we report the evaluation of the properties of a new product. The effect of duckweed enriched with microelements on the productivity parameters of laying hens was studied in zootechnical research. Birds feed was supplemented with duckweed enriched by biosorption process with microelements (Cu(II), Zn(II), Co(II), Cr(III)). In the feeding experiment, laying hens were divided into four experimental groups and one control group. The feeding experiment was conducted for 41 days. Samples of egg yolk, albumen, eggshells, blood, feathers and droppings were collected and the content of metal ions was determined by Inductively Coupled Plasma-Optical Emission Spectrometer with ultrasonic nebulizer. The amount of a given microelement transferred into the egg yolk and egg white was calculated. The eggshells thicknesses were measured with micrometer screw. The research showed that enriched *Lemna minor* improved the egg quality parameters. In all experimental groups, the increase of eggshell thickness was observed. In three of four experimental groups of hens, fed with diet containing biological form of microelements (Co(II), Zn(II), Cr(III)), the quantity of given microelement in the egg content increased. Therefore, the biosorption process can be applied not only for the supplementation of microelements in hens feed, but also to produce eggs biofortified with microelements-new functional food for human.

Keywords: *Lemna Minor*, Laying Hens, Biosorption, Feed Additives, Functional Food

1. INTRODUCTION

Biosorption, which uses the materials of biological origin, is metabolically independent process, which relies on the passive binding of microelements from aqueous solutions to the functional groups present on the cell surface of non-living biomass (Davis *et al*., 2003). Traditionally, biosorption is investigated as the process used in wastewater treatment. The mechanism of biosorption process has been described recently. Researchers have shown that biosorption occurs by several mechanisms, among which ion exchange dominates: the ions of alkali and alkaline earth metals, naturally bound with the functional groups on the surface of cells, are released into the solution and in their places other metal ions are bound (Chojnacka, 2010). Other mechanisms of biosorption that have much smaller contribution in the process, are microprecipitation, physical adsorption, complexation and chelation (Schiewer and Volesky, 1997).

Recently, a new perspective on the use of biosorption has been offered-not as a method for removing heavy metals from solutions, but as a method of biological materials enrichment in desirable elements (Chojnacka,

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2007; 2010). Some of the elements play a role of micronutrients that are important to the health and growth, both for animals and for plants (i.e., zinc, copper, iron, chromium, manganese) (Gupta *et al*., 2008). The biological material edible to animals can be enriched with microelements in a simple process of biosorption and by that biological feed additives with microelements can be obtained (Chojnacka, 2010). Biosorption represents a potentially powerful tool to increase dietary intake of microelements in foods (Johns and Eyzaguirre, 2007) and it gives the opportunity to increase the content of microelements several fold, i.e., the content of Cu(II) in the biomass of macroalga *Pithophora varia* Wille after biosorption process increased 498 times (Michalak and Chojnacka, 2008), in the biomass of an aquatic plant *Lemna minor* (duckweed) 55 times (Chojnacka, 2006) and in the biomass of another aquatic freshwater plant *Riccia fluitans* 53 times (Chojnacka, 2007).

Nowadays, a special attention is being paid to the chemical form of microelements in animal diet. There has been a considerable interest in the application of chelated or organic form of microelements (i.e., zinc methionine, manganese methionine, iron methionine, zinc lysine, copper lysine) (Spears, 1996) instead of conventionally used mineral salts such as: sulfates (of manganese, copper, zinc, cobalt, iron), carbonates (of copper, zinc, cobalt), chlorides (of copper, zinc, cobalt) and oxides (of manganese, copper, zinc, iron) (McDowell, 1996). In the literature there are several reports, which showed the advantages of the organic over inorganic form of micronutrients (Dobrzanski *et al*., 2003; 2008; Spears, 1996), but on the other hand the main disadvantage of the organic form is high price (McDowell, 1996). Another option considers the application of the process of biosorption, whereby a biological material is enriched with microelements and used in animal diet, i.e., enriched by biosorption marine macroalgae-*Enteromorpha* sp. and *Cladophora* sp. (Michalak *et al*., 2011). In the literature it is reported that bioavailability of microelements from yeasts-*Saccharomyces cerevisiae* enriched with Se and Zn was higher than from inorganic forms such as sodium selenite and zinc oxide. Also, the selenium content in eggs from hens fed with enriched yeast was higher (10.4%) in comparison with the eggs from hens fed with the diet supplemented with sodium selenite (Dobrzanski *et al*., 2003).

In this study, the application of organic form of microelements (*Lemna minor* enriched via biosorption process) as biological feed additive with microelements for laying hens is proposed. The main aim of the present work was to investigate the effect of biomass enriched with Cu (II), Zn(II), Co(II) and Cr(III) on the transfer of microelements to albumen, egg yolk, eggshell, blood, feathers and droppings. The choice of duckweed as a natural carrier of microelements in animal feed was justified by its good biosorption properties and also ubiquity. This aquatic plant is found floating on the surface of many ponds and slow-moving streams, as well as in rice paddies. Duckweed grows under a variety of climatic conditions in most parts of the world (Wahaab *et al*., 1995). Moreover, *Lemna* sp. with a natural property of binding metal ions from aqueous solutions, was found to be a very effective and cheap biosorbent (Chojnacka, 2006; Saadet *et al*., 2005) and bioaccumulator (Chojnacka, 2006; Wahaab *et al*., 1995; Prasad *et al*., 2001) of metal ions. Some attention was also paid to the application of duckweed in animal feeding. Previously it was shown that duckweed has the potential to be used as mineral feed additive in livestock nutrition (Chojnacka, 2006). Apart from high mineral content, duckweed is rich in protein (20-40% of dry weight), vitamins, carotenoids and fiber and can be successfully used as a soybean substitute in the feeding of laying hens and cattle (Hanczakowski *et al*., 1995; Haustein, 1990). In the literature it is reported that duckweed could replace a part of the traditional sources of protein (soybean and fish meal) (Men *et al*., 1997; Samnang, 1999) and that the supplementation of feed improved reproductive performance of sows (Men *et al*., 1997). It was also indicated that the feed supplementation with duckweed was much cheaper than the supplementation with ground soya beans and additionally introduced small amounts of fresh duckweed (30-40 g day^{-1}) to chickens diet, improved their growth rate (Samnang, 1999). Moreover, it was reported that addition of duckweed to laying hens diet significantly increased yolk pigmentation (Haustein, 1990). Of concern could be the content of toxic metals and microbial contamination. For this reason, chemical and microbiological tests are indispensable. Moyo *et al*. (2003) showed that *Lemna minor* is microbiologically safe.

The objective of the present work was to investigate the applicability of duckweed enriched with microelements by biosorption process as feed supplements with microelements for laying hens. This condensed form of essential elements could be used not only in the supplementation of deficiencies of microelements in animal diet, but also in the biofortification of eggs or meat with microelements. This kind of biofortified products could be applied as a new type of functional food, which would supply microelements not as inorganic salts, but in a highly bioavailable form.

2. MATERIALS AND METHODS

2.1. Preparation of Biosorbent

Aquatic plant *Lemna minor* was collected from the pond near Opatowek in Poland. Collected biomass was washed with tap water several times to remove foreign matter.

2.2. Biosorption Experiments

Lemna minor biomass (Lm) was separately enriched with the following microelements: Cobalt (II) (Lm-Co), Chromium (III) (Lm-Cr), Copper (II) (Lm-Cu) and Zinc(II) (Lm-Zn) via biosorption. The enrichment processes were performed in 40 dm^3 containers with metal ion solutions in tap water at ambient temperature. The solutions were prepared by dissolving appropriate amounts of inorganic salts: Co $(NO_3)_2 \cdot 6H_2O$, Cr $(NO_3)_3 \cdot 9H_2O$, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$ (POCH, Gliwice, Poland) accepted as a mineral supplement for laying hens. The initial concentrations of metal ions in the solution were as follows: $5.77.10^{-3}$ M, $5.10.10^{-3}$ M, $4.72.10^{-3}$ M and 4, 59.10^{-3} M for Cr (III), Co (II), Cu (II) and Zn(II), respectively. The biomass was exposed to microelement ions solutions for 4 h. pH was adjusted with NaOH/HCl (POCH, Gliwice, Poland) to 5. pH measurements were conducted with pH-meter equipped with an electrode InLab413 with the compensation of temperature (Mettler-Toledo, Seven Multi, Switzerland). The biomass concentration was 1.6% (16.3 g of fresh mass/L). Moisture of fresh plant biomass was evaluated as 94.3%.

2.3. Analytical Methods

Multielemental analysis of the biomass was undertaken. The appropriate mass of biological samples (0.1 g, 0.25 g, 0.5 g, 1.5 and 2.5 g in the case of eggshells, droppings, feathers, albumen or egg yolk and blood, respectively) was digested in 5 mL 65% HNO_3 supra-pure grade (Merck, Germany) in Teflon vessels (microwave oven Milestone MLS-1200). After mineralization, all samples were diluted to 50 mL. Inductively Coupled Plasma-Optical Emission Spectrometer with ultrasonic nebulizer (Varian VISTA-MPX ICP-OES, Victoria, Australia) was used to determine the content of metal ions in fresh plant and in all digested and diluted biological samples in the Chemical Laboratory of Multielemental Analysis at Wrocław University of Technology, which is accredited by ILAC-MRA and Polish Centre for Accreditation (Nr AB 969) (Michalak and Chojnacka, 2009).

To determine the eggshell thickness, micrometer screw (TSS Technical Services and Supplies, Germany) was used. The thickness of eggshell was measured in the central part of an eggshell, where the perimeter was the highest. The arithmetic mean values ($\bar{x}$), Standard Deviations (SD) and significance of differences between the groups were examined with the use of computer software *Statistica* ver. 9.0. Normality of distribution was assessed by Shapiro-Wilk test. Data were found to be non-parametric, so the Tukey test was applied. Statistical significance between the groups was accepted for $p \leq 0.05$.

Assuming that an egg is composed of yolk (26%), albumen (60%), eggshell (12%), membrane (2%), it was possible to calculate the mass of a given microelement (mg), which was transferred to the albumen or yolk from the Equation 1:

$$c_{microelement(albumen/yolk)} \cdot m_{albumen/yolk} = m_{microelement(albumen/yolk)} \quad (1)$$

Where:

$c_{microelement(albumen/yolk)}$ = The content of a given microelement in albumen or yolk (mg/kg)
$m_{albumen/yolk}$ = The mass of albumen or yolk (kg)
$m_{microelement(albumen/yolk)}$ = The mass of microelement in albumen or yolk (mg)

The Equation 2 was used to calculate the mass of microelement (mg), which was transferred from the feed to the egg content.

$$c_{microelement(albumen)} \cdot m_{albumen} + c_{microelement(egg\ yolk)} \cdot m_{egg\ yolk} = m_{microelement(egg)} \quad (2)$$

Where:

$c_{microelement(albumen)}$ = The content of a given microelement in albumen (mg/kg)
$m_{albumen}$ = The mass of albumen (kg)
$c_{microelement(egg\ yolk)}$ = The content of a given microelement in egg yolk (mg/kg)
$m_{egg\ yolk}$ = The mass of egg yolk (kg)
$m_{microelement(egg)}$ = The mass of microelement in the egg (mg)

2.4. Feeding experiments

2.4.1. Feed

Duckweed (*Lemna minor*) biomass was investigated as the biological carrier for microelements bound via biosorption process. Four experimental groups and one control group of laying hens were distinguished. The control group was fed with the standard feed (TASOMIX Company, Poland), which was composed of: corn, wheat, triticale, barley, soybean meal, sunflower meal, rape cake, feed fat, methionine hydroxy analog acid, $CaCO_3$, $CaHPO_4 \cdot 2H_2O$, NaCl, Na_2SO_4. In the standard feed, all microelements were provided in the form of inorganic salts, with the exception of chromium, which is currently not added to feeds (AMARD, 2004; Jamroz, 2004).

Table 1. The recommended content of microelements in feed and experimental supplementation of microelements

Lemna minor enriched with supplemented microelement	Requirement for supplementation of microelement (g/kg of feed)	Content of microelement in enriched *Lemna minor* (g/kg)	Mass of enriched *Lemna minor* additive (mg/kg of feed)
Lm-Co	1*	42.862	0.0234
Lm-Cu	10*	48.768	0.2060
Lm-Cr	2*	49.207	0.0406
Lm-Zn	50**	60.067	0.8314

*; Jamroz (2004) **; (Smulikowska and Rutkowski, 2005)

Table 2. Mineral composition of natural *Lemna minor* and the standard feed

Element		Natural *Lemna minor* (mg/kg dry mass, ± SD)	Standard feed (mg/kg dry mass, ± SD)	Maximum content in the feed
Microelements	Mn	333±49.9	110±16.5	150.00*
	Zn	106±0.24	89.6±13.4	150.00*
	Cu	5.82±0.873	21.2±3.18	25.00*
	Co	1.68±0.423	1.40±0.21	2.00*
	Fe	543±81.4	247±37.0	750.00*
	Cr	26.5±3.97	1.97±0.295	-
Alkali and alkaline earth metals	K	10.6±1.59	8080±1616	0.25**
	Ca	11180±2236	27021±5404	3.50**
	Mg	2115±423	2302±460	0.05**
	Na	3260±3260	1565±0.313	0.15**
	Ba	30.8±4.62	5.62±0.843	-
Toxic elements	Cd	<0.0045	<0.0045	1.00***
	Ni	2.6±0.39	0.801±0.12	-
	Pb	<0.0044	5.07±0.760	10.00***
Other	Ti	4.98±2.49	5.26±0.789	-
	Al	124±18.6	221±33.15	-

*; mg/kg, AMARD (2004) **; %, (Smulikowska and Rutkowski, 2005) ***; mg/kg, European (Commission, 2002)

The experimental groups were fed with the same feed, but a given microelement in the form of inorganic salt was replaced by *Lemna minor* enriched with this microelement by biosorption. In the case of Lm-Cr group, the feed was supplemented with soybean meal enriched with Cr (III) ions and the standard feed remained unchanged since no chromium supplement was added to the standard feed. In experimental groups, supplementations with single microelement bound with the *Lemna minor* were undertaken. Remaining microelements were supplemented by inorganic salts (except of chromium). On the basis of the equilibrium biosorption capacity obtained from biosorption experiments the appropriate masses of enriched biomass of plant were evaluated (**Table 1**). Doses of micronutrients were established on the basis of legislation and nutritional recommendations (**Table 1 and 2**).

2.5. Animals

Laying hens (50 heads, 30 weeks old, Löhmann Brown) were divided into one control group and four experimental groups (10 hens per group) and were housed in pairs (5 replicates (N) per group). The study was performed in three-tier battery system in the vivarium with controlled microclimate. Inside the vivarium the mean temperature was 20.8±1.45°C, in the middle tier of the cages 21.4±1.80°C and outside the vivarium 15.7±1.45°C. The mean relative humidity was 58.2±6.56% inside the vivarium, 62.4±7.17% in the middle tier of the cages and 58.8±9.73% outside the vivarium. Feed and water were available *ad libitum*.

2.6. Sampling

The feeding experiment was conducted for 41 days and was divided into three series of sampling: after 13 (series I), 27 (series II) and 41 (series III) days of

feeding. After each series, all eggs from the experimental groups were weighed and the thickness of the eggshells was measured on the equator at 3 points in five randomly selected eggs from every cage. The mass of one egg was calculated as a quotient of mass of all eggs from given group per number of eggs. After series I and III, all hens from each group were weighed. Samples of albumen, egg yolk, eggshells, blood, feathers and droppings were collected at the end of the experiment. Blood and feathers were sampled from the vein under the wing from randomly selected hens from each group. Before blood sampling, heparin was added to the probe in order to prevent blood coagulation. Moreover, from all eggs collected after each series, albumens, yolks and eggshells were separated. Samples of albumens, yolks, eggshells, blood, droppings and feathers were homogenized. All samples, with the exception of eggshells, were kept in the freezer before multielemental analysis.

3. RESULTS

In **Table 2** the mineral composition of natural biomass of *Lemna minor* is presented, as well as the mineral composition of standard feed used in these experiments. **Table 3** presents the results of measurements of eggs masses and eggshell thickness and **Fig. 1**. presents the change of eggshell thickness during the experiment in a given group. Mineral content of samples taken during the feeding experiment is presented in **Table 4 and 5**, while **Table 6** shows the amounts of microelements (mg) which were transferred to albumen or yolk from the feed, calculated according to Equation 1 and the amounts of microelements, which were transferred from the feed to the egg content (albumen and yolk), calculated according to Equation 2.

Table 3. Average mass of one egg (g), the arithmetic mean ($\bar{x}$) and Standard Deviation (SD) of eggshell thickness

	Mass of egg (g)			
Group	Series I	Series II	Series III	$\bar{x} \pm SD$
Control	62.2	63	64.1	63.1±0.954
Lm-Co	64.5	65	63.5	64.3±0.764
Lm-Cr	61.9	62.5	63.8	62.7±1.015
Lm-Cu	62.5	62.7	63	62.8±0.278
Lm-Zn	63.9	64.1	61.8	63.3±1.274
Mean eggshell thickness (± SD, N = 5, mm)				
Group	Series I	Series II	Series III	$\bar{x} \pm SD$
Control	0.391±0.340[a]	0.416±0.032[b,1]	0.484 ± 0.071[a,b,3]	0.430±0.046
Lm-Co	0.417±0.047[c]	0.450±0.048	0.457 ± 0.058[c]	0.441±0.051
Lm-Cr	0.421±0.070	0.427±0.017	0.437 ± 0.041	0.428±0.043
Lm-Cu	0.383±0.032[d,e]	0.412±0.025[d,2]	0.414 ± 0.048[e,3]	0.403±0.035
Lm-Zn	0.399±0.038[f,g]	0.462±0.069[f,1,2]	0.454 ± 0.074[g]	0.438±0.060

Letters ‘a’, ‘b’,…: statistically significant differences between the series in one group, $p \leq 0.05$ Numbers ‘1’, ‘2’, …: statistically significant differences between the groups in one series, $p \leq 0.05$

Table 4. Mineral content of microelements, macroelements and toxic elements in egg yolk, albumen and eggshell

		Microelements (± SD, N = 3, mg/kg)		
Element	Group	Egg yolk	Albumen	Eggshell
Co	Control	LLD<0.0009	LLD<0.0009	LLD<0.0009
	Lm-Co	0.0057±0.0014	LLD<0.0009	3.32±0.50
	Lm-Cr	0.0083±0.0021	LLD<0.0009	LLD<0.0009
	Lm-Cu	0.0095±0.0024	LLD<0.0009	0.8197±0.1230
	Lm-Zn	0.0076±0.0019	LLD<0.0009	2.14±0.32
Cr	Control	0.0731±0.0183	LLD<0.00035	0.327±0.477
	Lm-Co	0.118±0.018	LLD<0.00035	2.48±0.37
	Lm-Cr	0.0779±0.0195	LLD<0.00035	LLD<0.00035
	Lm-Cu	0.0678±0.0169	LLD<0.00035	0.242±0.036
	Lm-Zn	0.0790±0.0197	LLD<0.00035	LLD<0.00035
Cu	Control	1.66±0.25	0.250±0.037	3.83±0.57
	Lm-Co	1.74±0.26	0.299±0.045	1.77±0.27
	Lm-Cr	1.79±0.27	0.322±0.048	2.93±0.44
	Lm-Cu	1.44±0.22	0.331±0.050	3.51±0.53
	Lm-Zn	1.68±0.25	0.309±0.077	1.15±0.17
Mn	Control	0.694±0.104	0.0106±0.0027	0.9015±0.1350
	Lm-Co	0.686±0.103	0.0220±0.0055	1.46±0.22
	Lm-Cr	0.834±0.125	0.0137±0.0034	1.71±0.26

Table 4. Continued

	Lm-Cu	0.732±0.110	0.0362±0.0091	0.456±0.068
	Lm-Zn	0.755±0.113	0.0419±0.0105	0.603±0.091
Zn	Control	31.0±4.7	0.3497±0.0524	11.5±1.7
	Lm-Co	32.2±4.8	0.269±0.040	59.8±9.0
	Lm-Cr	33.7±5.1	0.384±0.058	LLD<0.0651
	Lm-Cu	31.8±4.8	0.531±0.798	LLD<0.0651
	Lm-Zn	33.3±5.0	0.589±0.088	LLD<0.0651
Fe	Control	41.3±6.2	0.127±0.019	4.08±0.61
	Lm-Co	48.6±7.3	0.258±0.387	11.7±1.8
	Lm-Cr	49.2±7.4	0.290±0.043	0.725±0.109
	Lm-Cu	40.8±6.1	0.219±0.033	LLD<0.0072
	Lm-Zn	45.5±6.8	0.242±0.036	LLD<0.0072
Macroelements (± SD, N = 3, mg/kg)				
Ca	Control	596±89	69.0±10.4	380001±76000
	Lm-Co	629±94	58.6±8.8	376740±75348
	Lm-Cr	651±98	77.7±11.6	369664±73933
	Lm-Cu	533±80	73.7±11.1	367345±73469
	Lm-Zn	564±85	60.4±9.1	374855±74971
Mg	Control	75.3±11.3	104±16	3582±716
	Lm-Co	86.0±12.9	89.6±13.4	3884±777
	Lm-Cr	97.4±14.6	112±17	4204±841
	Lm-Cu	55.3±8.3	115±17	3850±770
	Lm-Zn	76.9±11.5	103±15	3891±778
Na	Control	463±69	1590±318	2497±499
	Lm-Co	456±68	1468±294	1897±379
	Lm-Cr	503±75	1521±304	2611±522
	Lm-Cu	348±53	1575±315	2408±482
	Lm-Zn	421±63	1549±310	2922±584
K	Control	817±128	1019±204	728±109
	Lm-Co	939±141	994±149	915±137
	Lm-Cr	965±145	1054±211	942±141
	Lm-Cu	638±96	1036±207	804±121
	Lm-Zn	853±128	1061±212	1070±214
Toxic elements (± SD, N = 3, µg/kg)				
Cd	Control	9.74±1.95	6.63±1.33	LLD<1.8
	Lm-Co	LLD<0.45	5.02±1.00	LLD<1.8
	Lm-Cr	1.74±3.48	LLD<0.45	LLD<1.8
	Lm-Zn	8.87±1.77	5.39±1.08	LLD<1.8
	Lm-Cu	5.72±1.44	LLD<0.45	LLD<1.8
Pb	Control	113±15	554±72	14100±1830
	Lm-Co	61.3±8.0	50.6±10.1	26800±3480
	Lm-Cr	LLD<4.40	933±121	1780±231
	Lm-Cu	LLD<4.40	113±15	29900±3890
	Lm-Zn	335±44	133±17	27800±3610
As	Control	227±29	LLD<23.7	LLD<23.7
	Lm-Co	296±39	117±15	LLD<23.7
	Lm-Cr	208±27	143±19	LLD<23.7
	Lm-Cu	448±58	LLD<23.7	LLD<23.7
	Lm-Zn	221±29	183±24	60331±7843

LLD; Low Limit Detection

Table 5. Mineral content of microelements, macroelements and toxic elements in blood, feathers and droppings

	Microelements (± SD, N = 3, mg/kg)			
Element	Group	Blood	Feathers	Droppings
Co	Control	0.0077±0.0019	0.0783±0.0196	0.311±0.047
	Lm-Co	0.0043±0.0011	0.0725±0.0181	0.339±0.051
	Lm-Cr	0.0078±0.0019	0.1638±0.0246	0.709±0.106
	Lm-Cu	0.0076±0.0019	0.1386±0.0208	0.492±0.074
	Lm-Zn	LLD<0.0009	0.1088±0.0163	0.447±0.067
Cr	Control	0.0664±0.0166	0.182±0.027	0.271±0.041
	Lm-Co	0.0665±0.0166	0.142±0.021	0.290±0.043
	Lm-Cr	0.0644±0.0161	0.315±0.048	0.314±0.047
	Lm-Cu	0.0608±0.015	0.00042±0.00053	0.258±0.039
	Lm-Zn	0.0679±0.0169	0.2590±0.0388	0.883±0.132
Cu	Control	0.4602±0.0690	4.052±0.608	13.3±2.0
	Lm-Co	0.453±0.068	5.75±0.86	20.6±3.1

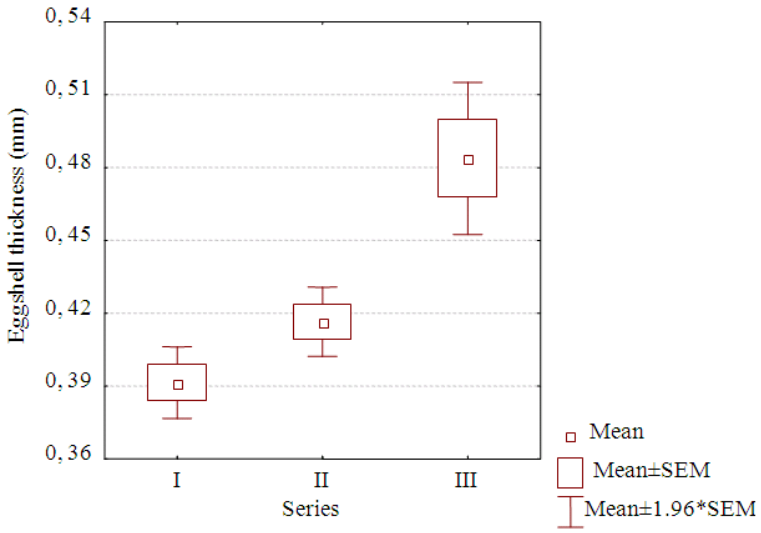
Table 5. Continued

	Lm-Cr	0.401±0.060	6.44±0.97	24.2±3.6
	Lm-Cu	0.428±0.064	3.76±0.56	14.9±2.2
	Lm-Zn	0.461±0.069	5.27±0.79	25.2±3.8
Mn	Control	0.138±0.021	8.1337±1.2200	68.8±10.3
	Lm-Co	0.154±0.023	11.4577±1.7200	81.9±12.3
	Lm-Cr	0.167±0.025	16.438±2.470	108.9±16.3
	Lm-Cu	0.174±0.021	12.9118±1.9400	89.3±13.4
	Lm-Zn	0.194±0.029	11.3774±1.7100	87.4±13.1
Zn	Control	7.622±1.140	245±37	71.2±10.7
	Lm-Co	6.48±0.97	292±44	69.8±10.5
	Lm-Cr	6.99±1.05	356±38	60.5±9.1
	Lm-Cu	7.55±1.13	259±39	93.9±14.1
	Lm-Zn	7.83±1.17	251±38	108±16
Fe	Control	251±38	39.9±6.0	182±27
	Lm-Co	233±35	40.0±6.0	123±18
	Lm-Cr	252 ±38	47.6±7.1	191±29
	Lm-Cu	247± 37	32.2±4.8	137.9±20.7
	Lm-Zn	246±37	47.8±7.2	130.6±19.6
Macroelements (± SD, N = 3, mg/kg)				
Ca	Control	138±21	1317±263	5902±1180
	Lm-Co	152±23	1199±240	4797±959
	Lm-Cr	135±20	1837±367	7356±1471
	Lm-Cu	135±20	1256±251	4051±810
	Lm-Zn	153±23	1436±287	4372±874
Mg	Control	38.9±5.8	189±28	1150±230
	Lm-Co	39.1±5.9	183±27	1489±298
	Lm-Cr	36.9±5.5	249±37	1892±378
	Lm-Cu	37.8±5.7	149±22	1424±285
	Lm-Zn	42.6±6.4	167±25	1212±242
Na	Control	1925±385	2002±400	862±129
	Lm-Co	1971±394	1337±267	737±110
	Lm-Cr	1846±369	1472±294	1026±205
	Lm-Cu	1894±379	1188±237	1098±219
	Lm-Zn	1899±380	1660±332	773±116
K	Control	902±135	721±108	4715±943
	Lm-Co	895±134	970±145	5138±1028
	Lm-Cr	873±130	1309±262	5218±1043
	Lm-Cu	897±134	864±130	4676±935
	Lm-Zn	913±137	1072±214	4386±877
Toxic elements (± SD, N = 3, µg/kg)				
Cd	Control	33.9±6.8	17.5±3.5	79.0±15.8
	Lm-Co	31.8±6.4	11.0±2.2	94.7±18.9
	Lm-Cr	30.7±6.1	22.0±4.4	143±19
	Lm-Zn	34.9±7.0	39.6±7.9	83.2±16.6
	Lm-Cu	35.3±7.1	LLD<0.45	84.2±16.8
Pb	Control	229±30	939±122	362±47
	Lm-Co	219±29	1320±172	469±61
	Lm-Cr	109±14	907±118	607±78.9
	Lm-Cu	92.4±18.5	617±80	1450±188
	Lm-Zn	181±23	713±93	601±78
As	Control	129±17	1300±169	1040±135
	Lm-Co	194±25	924±120	1120±146
	Lm-Cr	133±17	1045±136	LLD<23.7
	Lm-Cu	116±15	878±114	1630±212
	Lm-Zn	124±16	678±88	957±124

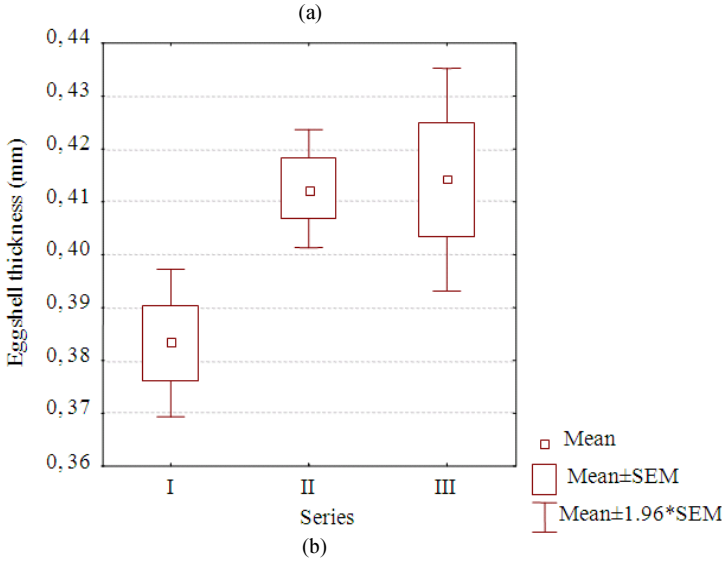
LLD - Low Limit Detection

Table 6. The amount of microelements in albumen, yolk and in the egg content (albumen + yolk)

		The amount of microelements					
Microelement	Group	Yolk (mg)	Increase(↑)/ Decrease(↓) (%)	Albumen (mg)	Increase(↑)/ Decrease(↓) (%)	Egg content (mg)	Increase(↑)/ Decrease(↓) (%)
Co(II)	Control	$<1.50 \cdot 10^{-5}$	>527 (↑)	$<3.46 \cdot 10^{-5}$	>0.867 (â)	$<5.0 \cdot 10^{-5}$	>156 (↑)
	Lm-Co	$9.41 \cdot 10^{-5}$		$<3.43 \cdot 10^{-5}$		$<1.28 \cdot 10^{-4}$	
Cr(III)	Control	$1.22 \cdot 10^{-3}$	5.73 (↑)	$<1.34 \cdot 10^{-5}$	-	$<1.23 \cdot 10^{-3}$	>6.50 (↑)
	Lm-Cr	$1.29 \cdot 10^{-3}$		$<1.34 \cdot 10^{-5}$		$<1.31 \cdot 10^{-3}$	
Cu(II)	Control	0.0277	14.8 (↓)	0.01	30.0 (↑)	0.037	3.22 (↓)
	Lm-Cu	0.0236		0.013		0.036	
Zn(II)	Control	0.517	3.48 (↓)	0.013	69.2 (↑)	0.53	5.01 (↑)
	Lm-Zn	0.535		0.022		0.557	



(a)



(b)

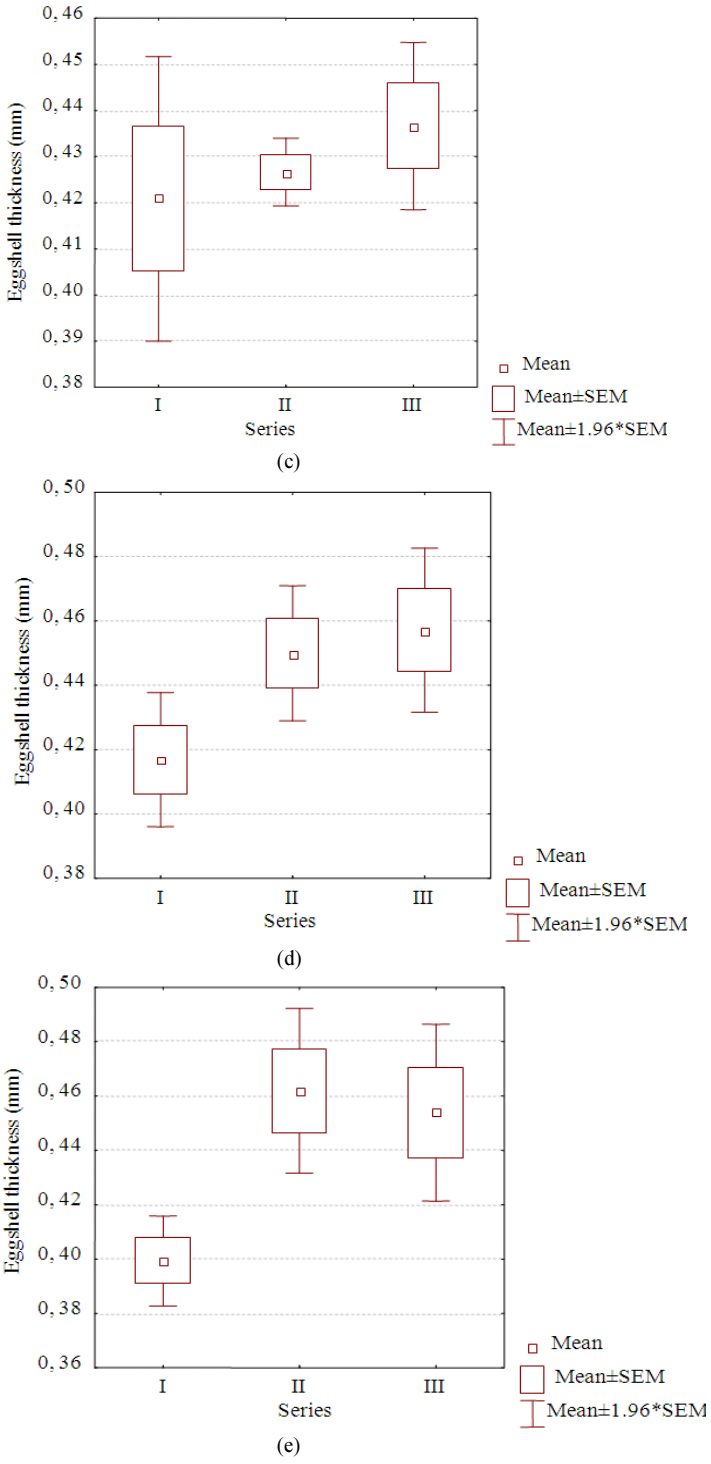


Fig. 1. Eggshell thickness during the experiment for control and experimental groups: (a) control group, (b) Lm-Cu group, (c) Lm-Cr group, (d) Lm-Co group, (e) Lm-Zn group

4. DISCUSSION

Previously, new technology of biological feed supplements with microelements bound to the biomass of *Lemna minor* was elaborated (Chojnacka, 2006). The bioresource was found to be effective in concentrating microelement ions from the solution (i.e., over 68, 127 and 534 times for Cr (III), Zn (II) and Cu (II), respectively) by ions exchange mechanism. Cations were bound to carboxyl groups present on the surface of cells. In the present work utilitarian properties of new biological dietary supplement with microelements were investigated in zootechnical studies. Bioavailability of microelements (zinc, iron, copper and chromium) bound with the biomass of duckweed for laying hens was tested and compared with an inorganic dietary supplements. It was hypothesized that biological form of microelements whereby microelements are bound, similarly as in chelates, to carboxyl group should be a valuable and cost-effective alternative.

Here we report the effect of supplementation of feed with enriched aquatic plant on hens body weight, eggshell thickness and mineral content of albumen, egg yolk, eggshells, blood, feathers and droppings. The known amount of a given microelement bound with *Lemna minor* was supplemented and its level was determined in the collected samples using multielemental analysis to assess the influence of this new kind of mineral feed additive on health condition of animals.

The tested material was almost free of toxic metals (**Table 2**), their levels were below the restricted levels accepted by the obligatory law (DPMARD, 2012). Content of both lead and cadmium in natural *Lemna minor* was very low (below the lower detection limit). In many cases, the content of elements was higher than in the standard feed (Mn, Zn, Co, Fe, Cr, K, Na, Ba).

4.1. The effect of Supplementation of Enriched with Microelements *Lemna Minor* on Egg Quality Parameters and Laying Hens Body Weight

The increase of the mass of eggs was observed in the case of two experimental groups and the control group (**Table 3**). The highest egg mass increase was noticed in the Lm-Cr group, by 3.12%. In the control group the increase by 2.96% was noticed. The average weight of eggs from groups fed with Lm-Co and Lm-Zn slightly decreased, 1.55 and 3.21% respectively. There was no statistically significant difference in the weight of hens, which was measured at the beginning and at the end of the experiment.

The increase of eggshell thickness increase was observed in the case of all groups, both experimental and control. The highest increase of eggshell thickness during the experiment (between series I and III) was observed for the control group, by 28.3%. In the experimental groups the highest increase was found for the Lm-Zn group, by 13.8%. For Lm-Co, Lm-Cu and Lm-Cr the eggshell thickness increase was by 9.59, 8.09 and 3.80%, respectively. The mean eggshell thickness was the highest in the Lm-Co group, higher by 2.33% in comparison with the mean eggshell thickness of the control group.

It was observed that in the case of all experimental groups, except for Lm-Cr, the highest increase of eggshell thickness was observed between the series I and II, while for the control group the highest increase was observed between the series II and III (**Fig. 1**). The strongest trend can be observed for Lm-Cu and Lm-Zn group, where statistically significant differences ($p \leq 0.05$) between the series I and II were found. For example, in Lm-Cu group, the eggshell thickness increase between the series I and II was 7.57% and between the series II and III only 0.485%. In the control group the eggshell thickness increase between the series I and II was found 6.4%, while between the series II and III by 16.3%. Also, in the series II statistically significant differences were observed in eggshell thickness between the control group and the groups of Lm-Zn and Lm-Cu. This can indicate that eggshells from hens fed with biological supplement became thicker sooner than eggshells from the hens fed with the feed with inorganic salts.

4.2. The Influence of Supplemented Microelements on their Content in Eggs

In Lm-Co group the content of Co in egg yolk was 533% higher in comparison with the control group (**Table 4**). In eggshells, significant increase of Co content was observed, over 3500 times. Considering Cr content in collected samples in the Lm-Cr group it can be seen that the duckweed supplement increased Cr content in egg yolk by 6.57%. In eggshells the content of Cr was found lower by over 99.2%. In Lm-Cu group the content of Cu in albumen was 32.4% higher. In the Lm-Zn group, Zn content in egg yolks was 7.4% higher. Also, in albumen the increase of Zn was found, 68.4%. In eggshells, Zn content significantly decreased, over 99.9%, in comparison with the control group.

Supplementation of feed with enriched *Lemna minor* increased the amount of given microelement in albumen and yolk. Supplements increased Co and Cr amounts in egg yolk, Cu amount in albumen and Zn amount in albumen and egg yolk. The highest increase of the total amount of given microelement in the egg content was found in Lm-Co group, 156%.

4.3. The Influence of Supplemented Microelements on Their Content in Blood, Feathers and Droppings

In droppings in every experimental group the content of microelement supplemented in biological form was higher than in the control group: 9.0, 12.015.9 and 51.7% in the Lm-Co, Lm-Cu, Lm-Cr and Lm-Zn group, respectively (**Table 5**). In the case of Lm-Co and Lm-Cu groups the content of supplemented microelement in blood and feather samples was lower than in the control group. In Lm-Zn group the increase of Zn content in feathers and blood was observed, while in the Lm-Cr group the Cr content in blood remained unchanged and in feathers the increase by 73.1% was found.

4.4. Interactions (Antagonism and Synergism) Between Elements

Synergism interactions were observed between all experimental groups.

4.5. Other Microelements

The increase of Mn content was observed in all albumen samples. The highest increase was for Lm-Zn group, 295%. In egg yolks the increase of Mn was found for every experimental group with the exception of Lm-Co group. The highest increase was observed for Lm-Cr group, 20.2%. The Mn content in droppings, feathers and blood was higher in every experimental group in comparison with the control group.

Fe content in albumen increased in all experimental groups. In comparison with the control group. Fe content was higher by at least 72.4% in the case of Lm-Cu, the highest increase was in sample taken from Lm-Cr group, by 128%. The increase was also observed in egg yolks with the exception of samples from Lm-Cu group. The highest increase was found in sample from Lm-Cr group, 19.1%. In blood and in droppings Fe content was lower in every experimental group except Lm-Cr group.

4.6. Macroelements

In all experimental groups slight increase of magnesium content and slight decrease of Ca content in eggshells was observed. Na content was lower in comparison with the control group in albumen and feathers in all experimental groups. K content in eggshells and feathers was higher in comparison with the control group in all experimental groups.

4.7. Toxic Metal Ions

Cd was excreted with droppings in higher amounts in all experimental groups when compared with the control group. Both in egg yolks and albumen, Cd content was lower in all experimental groups. In egg yolks the highest decrease was observed for Lm-Co group, at least by 95.4%, in albumen the lowest content of cadmium were found in Lm-Cr and Lm-Cu groups, at least by 93.2% lower in comparison with the control group. Pb content in droppings in all experimental groups was higher, in samples from Lm-Cu group the increase of Pb was observed by 300%. The Pb content was lower when compared with the control group in blood samples in all experimental groups. It can be concluded that feed supplementation with enriched *Lemna minor* resulted in cadmium and lead excretion into droppings and causes the decrease of Cd content in eggs and Pb content in blood.

5. CONCLUSION

In this research the applicability of duckweed enriched with microelements by biosorption process as the feed supplement for laying hens was investigated. Enriched *Lemna minor* improved egg quality parameters. The eggshell thickness increased in all experimental groups. The total amount of microelements in the egg content increased in three of four experimental groups. The new biological supplement increased Co content in egg yolk and eggshells, Cr content in egg yolk and also Cu and Zn content in albumen and yolk. In all experimental groups the Cd content in egg yolk and albumen samples was lower, as well as the Pb content in blood samples. Therefore, biological form of microelements could be a valuable and cost-effective alternative to inorganic dietary supplements and expensive chelates. Moreover, enriched duckweed could be used not only in animal diet supplementation, but also in the biofortification of eggs with microelements-new functional food for human.

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