

Effect of Dietary Humic Preparations on the Content of Trace Elements in Hens' Eggs

¹Zbigniew Dobrzanski, ²Henryk Górecki, ²Katarzyna Chojnacka, ²Helena Górecka and
¹Marzena Synowiec

¹Department of Animal Hygiene and Environment, Wrocław University of Environmental and Life Sciences, Chelmonski st. 38 C, 51-630 Wrocław, Poland

²Institute of Inorganic Technology and Mineral Fertilizers; Wrocław University of Technology, Smoluchowski st. 25, 50-372 Wrocław, Poland

Abstract: Mineral-humic preparations Humokarbowit (HKW) and Humobentofet (HBF) were used in feeding of reproductive laying hens Lohmann Brown. The effect of these preparations on microelement composition of eggs was investigated. The content of microelements in hens diet, egg albumen and eggshell was determined by ICP-MS technique and relative bioaccumulation coefficient (RBC) was evaluated. The humic preparations significantly increased selenium and decreased molybdenum concentration in egg albumen. Increase of selenium and iron content in egg yolk and increase of iron concentration in eggshells was observed. The preparations did not influence the content of such elements as Cr, Co, Cu, I, Mn, Zn. The highest levels of RBC were found for selenium and iodine and the lowest for manganese.

Key words: humic preparation, hens, eggs, chemical composition

INTRODUCTION

Bioelements play significant physiological role in laying hens, in particular in embryogenesis and organism development. They have advantageous influence on health state of birds, laying and meat yield and on the quality of meat and eggs.

Among microelements, the mostly significant physiological role is played by: Fe, Mn, Cu, Zn, I, Se which are standardized in poultry feeding^[1,2]. Also a role in metabolism is attributed to such elements as: Al, As, Co, Cr, Li, Mo, Ni, Si, Sb and V^[3]. However, the mostly significant role in birds' embryogenesis is played by 9 microelements: Fe, Zn, Cu, I, Mn, Se, Cr, Ni and Co^[4].

Various organic compounds of egg bind trace elements. For instance, ovoalbumin easily binds selenium and mercury^[5] and possesses affinity towards di- and trivalent metal ions, such as zinc, copper and manganese^[6]. However, conalbumin (ovotransferin) binds iron depending on albumen pH^[7] and also other metals, such as Cu, Zn, Al, Cd, Co, Mn, Cr^[8]. Phosphoprotein, such as egg yolk phosvitin binds almost the whole iron in egg content^[9]. Trace elements, such as Cr, Ni, I, Zn can be bound by some lipid compounds of egg yolk^[8,10] and many others can be incorporated in eggshells or eggshell membranes^[11,12]

and these processes are very complicated biochemically^[13,14].

In poultry feeding, beside premixes (mineral, mineral-vitamin) increasing variety of preparations is used, in particular if in EU countries, meat and bone meals were banned at the end of 2004 (beside meals of fish origin) and at the beginning of 2006, the use of feeding antibiotics was prohibited.

Humic preparations as the source of various mineral salts and other biologically active components, are used in Poland in laying hens feeding^[15], in swine fattening^[16], and also in small ruminants feeding^[17,18]. There is no information on efficiency of their application in feeding of reproductive poultry flocks, and in particular, on the effect on chemical composition of eggs.

The aim of the present work was investigation of experimental feeding of humic preparations (Humokarbowit and Humobentofet) to reproductive flocks on elemental composition of eggs, in particular, the main microelements.

MATERIALS AND METHODS

Birds and their management: Birds were chosen randomly for the experiment from the flock of ca. 6000 heads of hens and cocks of laying line Lohmann

Corresponding Author: Z. Dobrzanski, Department of Animal Hygiene and Environment, Wrocław University of Environmental and Life Sciences, Chelmonski st., 51-630 Wrocław Poland, Tel./Fax +48 71 3205 866

Brown. The birds were divided into two groups (C and E), 150 hens each. Birds were kept on straw litter in separated, netted boxes with the dimensions 4.5x5.5 m each (0.15 m² of surface area per a bird) with manual feeding and supplementation of preparations. The boxes were equipped with typical nipple drinkers (18 pieces in each box) and individual feeders (located on the whole length of the coop) and nest boxes. The object was equipped with mechanical ventilation and artificial lighting with automatic regulation (lighting program), according to the instructions of Lohmann Tierzucht company.

In both groups (C and E), the birds were fed *ad libitum* with the standard diet DJR. The birds in the group E were additionally fed with humic preparations Humokarbowit (HKW) and Humobentofet (HBF), *ad libitum* in special trays, complemented daily. The experimental period was from 19th to 62nd week of birds' life (300 days). Consumption of feed and preparations, laying, egg mass, deaths of hens was recorded.

Diet and preparations: The composition of complete diet DJR is reported in Table 1. The diet contained 17.6 % total protein, 11.54 MJ of metabolic energy, 0.93 % lysine and 0.32 % methionine, 4.15 % calcium and 0.64 % total phosphorus. The content of nutrients and nutritive value generally corresponded to the requirements of nestlings' producer (Lohmann Brown) and Polish poultry feeding standards^[2].

The composition of humic preparations is available in patent descriptions Nr PL-172908 (HKW) and PL-315211 (HBF). The preparations contain high levels of macroelements and trace elements. The materials used in the production of these supplements originated from mining minerals, such as peat, humodetrinite, dolomite, bentonite and other organic supplements. The presence of over 40 elements was detected in the preparation..

Table 1: The content of the main nutrients in the poultry diet DJR*)

Nutrients	Content
Dry mass, %	89.5
Total protein, %	17.58
Raw fat, %	3.14
Raw fiber, %	3.72
Ash, %	8.62
Metabolic energy, MJ/kg	11.54
Lysine, %	0.930
Methionine, %	0.321
Methionine+Cystine %	0.630
Tryptophan, %	0.203
Vitamin A m.u.	10000
Vitamin D ₃ m.u.	2 600
Vitamin E mg	30
Folic acid, mg	0.85
Linoleic acid, %	0.75
Total calcium, %	4.15
Total phosphorus, %	0.64
Available phosphorus, %	0.41
Total sodium, %	0.145
Total magnesium, %	0.137

*)according to the calculation of producer of the diet

Collection of samples: Before the experiment, the samples of feed DJR and humic preparation (HKW and HBF) were taken for chemical analyses. The eggs were sampled 3 times from both groups, 30 pieces in 30th, 45th and 60th day of life. The eggs were knocked out, the material from 3 eggs was combined, separately egg albumen and yolk from each group. Similarly, in the case of eggshells (with eggshell membranes), preliminary drying and milling was carried out to obtain dry eggshell powder. In this way, 30 samples of egg yolks, albumens and shells were sampled from each group.

The samples of the material (feed, preparations, eggs) were analyzed at accredited by ILAC-MRA Laboratory of Multielemental Analyses (nr AB 696) for the content of the following elements Co, Cr, Cu, Fe, I, Mn, Mo, Se and Zn by ICP-MS Varian UltraMass-700 instrument. The samples were mineralized by microwave digestion method by microwave Milestone MCS-2000 oven^[19].

Statistical analysis: The results were elaborated statistically. Mean values (\bar{x}), standard deviations (SD), significance of differences were ($p < 0,05$) determined between the groups C and E with the use of Statgraphics ver. 5.0 software. For determination of differences, t-test was carried out.

Also, relative bioaccumulation coefficient (RBC_x) was determined for each element, as well as quotient of the total content of a given element in the mass of layed eggs (in mg) to its total concentration in the consumed diet and humic preparations (in mg), calculated per head during 30 days of laying production.

RESULTS AND DISCUSSION

Performance: Production results from the whole period of hens' management are presented in Table 2. The preparations used did not influence significantly the number of layed eggs, egg yield and mass. Also, the consumption of feed calculated per head, the mass of layed eggs and eggs mass did not differ between the groups, although a tendency of increased consumption of DJR diet in the group E was observed, Hens consumed during 300 days of laying production, averagely 119.19 g of diet in the group C and 120.54 g in the group E. In the latter groups, hens consumed additionally HKW preparation in the quantity 2.94 and HBF – 3.33 g/hen/day. Production yield corresponded to the general technological assumption for reproductive flocks of Lohmann Brown hens.

Table 2: Performance of hens (300 days of production)

Item	Group- C	Group-E
No of hens initial/final (head)	150/146	150/140
No of eggs (pcs)	36110	34890
No of eggs per medium-stock layer (pcs)	240.0	240.6
Laying rate (%)	81.33	80.21
Total weight of eggs (kg)	2176.7	2117.5
Mean egg weight (g)	60.28	60.69
*Feed intake of:		
• layer/day (g)	119.19	120.54
• initial-stock layer (kg)	35.76	36.16
• egg (g)	146.6	150.3
• kg eggs' mass (kg)	2.432	2.476

Intake of mineral nutrients: The results of analyses of the content of microelements in the diet, humic preparations with their daily consumption by hens are shown in Table 3. Among microelements, the intake of iron was the highest (averagely 54.83 and 88.63 mg/hen/day, respectively), manganese (23.48 and 24.79), zinc (21.69 and 22.08) and copper (2.217 and 2.260 mg/hen/day). Other elements (Co, Cr, I, Mo, Se) were absorbed in the range 0.038 – 0.231 mg/hen/day. Supplementation of humic preparations in feeding in the group E caused increased absorption of iron (61.6 %), selenium (39.5 %) and chromium (15.0 %) when compared with the group C.

Chemical evaluation of eggs: The results of analyses of the content of microelements in eggs components and calculated relative bioaccumulation coefficient (RBC) are shown in Tables 4 and 5.

Cobalt: The content of Co in particular elements of egg was found to be in the range 0.0015 – 0.166 mg/kg. Cobalt was the mostly accumulated by eggshells and in the lowest extent in albumen. No statistically significant differences between the average content of Co in particular elements of eggs between the group C and E were determined. The average value of RBC was 0.5 and 0.6, respectively.

In the literature, the ranges for the concentrations of this element are diversified. Richards^[4] reported the content of Co in egg yolk on the level 0.0084 and in egg albumen only 0.012 mg/kg fresh mass. Kato et al.^[20] determined the concentration in egg content as 0.0877 mg/kg d.m. and other authors^[21,22] in egg content from hens kept in free range system, reported the average cobalt content in the range 0.0061 – 0.01 mg/kg d.m. It was found that the supplementation of cobalt salts or vitamin B₁₂ only slightly increased the level of Co in egg content^[20]. In shells of hens eggs (without eggshell membranes), the content of Co was found to be in the range 0.70–0.82 mg/kg^[11].

This element occurred in the mixture in both preparations at very low concentrations (0.61–1.87

mg/kg). Its daily absorption was 0.224 mg/hen in the group C and in the group E increased only 3 %. It was found that humic preparations did not influence accumulation of Co in albumen, egg yolk or in egg shells.

Chromium: The content of Cr in various components of egg was in the range 0.021 -1.173 mg/kg. The highest level of chromium was accumulated in eggshells and the lowest – in egg albumen. No statistically significant difference between the average content of Cr in various elements of eggs between the group C and E was determined. The value of RBC was high and was evaluated as 5.7 and 53, respectively.

The ranges of the concentrations of this element reported in the literature are wide. Richards^[4] reports the content of Cr in egg yolk on the level 0.0084 and in egg albumen – only 0.029 mg/kg fresh mass. Elmadfa and Muskat^[23] reported the range of Cr concentration in egg content 0.05-0.30 and in yolk itself 0.20 mg/kg fresh mass. After supplementation of chromium-enriched yeasts it was possible to increase 17 % the concentration of Cr in egg content (from 0.0106 to 0.0124 mg/kg fresh mass) and in eggshells 26,7 % (from 1.129 to 1.431 mg/kg d.m.)^[24].

In DJR diet, the concentration of this element was on the level 1.68 mg/kg and daily absorption was 0.20 mg/hen. Chromium was present in both preparations in the concentration 4.68 mg/kg (HBF) and 5.37 g/kg (HKW), which increased 15 % daily consumption of this element. This increase however did not result in changes in accumulation of this element in eggs.

Copper: The content of Cu in various egg components was in the range 0.237 - 2.42 mg/kg. Eggshells accumulated the highest quantities of copper, and in the lowest extent this element was accumulated in egg albumen. No statistically significant differences between the average levels of Cu in various egg components between the group C and E were determined. The value of RBC was ca. 2.0 in both groups.

Literature reports various ranges of the concentration of this element. Richards^[4] reports the content of Cu in egg yolk on the level 1.95 and in egg albumen only 0.294, according to American data^[25], the average copper content in egg albumen was 0.002 mg and in egg yolk 0.004 mg, which after recalculation yields the concentration 0.060 mg/kg fresh mass (albumen) and 0.25 mg/kg fresh mass (yolk). Other authors^[23] report the average range for Cu concentration in egg content in the range 0.5-2.3 mg/kg fresh mass^[26]. In shells of hens' eggs (without eggshell membrane), the reported concentrations of copper were in the following range: 0.92 – 3.72 mg/kg^[11].

In the diet DJR, the concentration of Cu was on the level 18.60 mg/kg which corresponded to the absorption 2.22 mg/hen/day. Copper in both preparations was present in the concentration lower than 4 mg/kg, which caused only 2 % increased absorption of this element in the group E. This did not influence accumulation of Cu in various egg components.

Iron: The content of Fe in various elements of egg was in the range 0.301 - 72.02 mg/kg. Bioaccumulation of iron was the highest in egg yolk and the lowest in egg albumen. Statistically significant differences ($p < 0.05$) between the groups were observed in the content of Fe in egg yolk and shell. The value of RBC was in the group C 2.34 and in the group E 1.52.

Literature reports the following concentration of this element: in egg yolk 53.53 and in albumen 1.56 mg/kg fresh mass^[4]. Other authors report the concentration of Fe in egg content in the range 18.45 – 23,20 mg/kg fresh mass. In eggshell (without eggshell membrane) the content of this metal was 69.3-71 mg/kg and in eggshell membranes 26.8 mg/kg^[11].

In the diet, the concentration of this element was on the level 460 mg/kg and its daily absorption was 54.8 mg/hen and in the group E was 61.6 % higher which resulted from high Fe content in HBF and HKW preparations. For this reason, increased Fe intake from the preparations and significant increase of the content in egg yolk and eggshells showed high bioavailability of this metal from humic preparations.

Iodine: The content of I in various egg components was in the range 0.175 – 1.091 mg/kg. The highest levels of iodine were accumulated by eggshells and in the case of yolk bioaccumulation was slightly lower. No statistically significant differences between the average content of I in various egg components were determined between the group C and E. The value of RBC was in both groups similar (11.5).

In the literature, the reported ranges of concentrations of this element are as follows: in egg yolk 1.68, albumen 0.059 mg/kg fresh mass^[4], however Elmadfa and Muskat^[23] reported the level in egg yolk 0.12 and in albumen only 0.07 mg/kg fresh mass. According to American data^[27], the average content of iodine in egg albumen was 0.001 mg, in yolk 0.002 mg, which after recalculation gives the concentration 0.030 mg/kg fresh mass (albumen) and 0.0125 mg/kg fresh mass (yolk). Dobrzanski et al.^[28] by increasing the content of iodine in premix, obtained 52.2 % increase of the concentration of this element in egg content (from 0.693 to 1.055 mg/kg fresh mass). In eggshell

(without eggshell membranes), the content of this element was 0.34 – 0.50 mg/kg^[11] and in eggshells with membrane, in the range 1.31 – 1.44 mg/kg^[15].

Iodine was present in both used preparations in the concentration below 0.7 mg/kg, however in the mixture, the concentration of this element was on the level 1.79 mg/kg and the daily absorption was 0.213 mg/hen. Minimally higher (2.8 %) absorption of iodine in the group E did not cause any changes in the average content of I in egg content as well as in eggshell.

Manganese: The content of manganese was in the range 0.044 – 1.39 mg/kg in various elements of egg. The highest accumulation of manganese was observed for eggshells and the lowest for albumen. No statistically significant differences between the average content of Mn in various egg components between the group C and E were observed. The value of RBC was similar in both groups (0.08 and 0.075, respectively).

Literature reports the following concentrations: in egg yolk 0.526 and in albumen 0.0015 mg/kg fresh mass^[4]. However data of Elmadfa and Muskat^[23] showed that the content in egg yolk was 0.5-2.0 and in albumen 0.4 mg/kg fresh mass. According to American data^[25], the average concentration of manganese in albumen was 0.001 mg and in yolk 0.012 which after recalculation yields the concentration 0.03 mg/kg fresh mass (albumen) and 0.73 mg/kg fresh mass (yolk). Dobrzanski et al.^[28], by increasing the content of iodine in the diet, decreased the concentration of Mn in egg content: 14.2 % (from 0.274 to 0.235 mg/kg fresh mass). In eggshells of hens' eggs (without membranes) the concentration of this metal was 0.4-1.1 mg/kg^[11].

This element is present in both preparations as well as in the diet in similar concentrations (159 -197 mg/kg). Its daily intake was 23.48 mg/hen in the group C and in the group E was 5.6 % higher. However, this did not produce any changes in availability and accumulation of this element in eggs.

Molybdenum: The content of molybdenum was in the range 0.0028 – 0.131 mg/kg. The highest accumulation of molybdenum was observed for eggshells and the lowest for albumen. The differences in the concentration of this element were not statistically significant between the groups in egg yolk and shells and were confirmed ($p < 0.05$) in albumen (decrease was observed). The value of RBC was slightly diversified in the groups C and E (0.613 and 0.562, respectively).

In the literature, the reported ranges of the concentration of this element were present in egg content in the range 0.030 – 0.054 mg/kg fresh mass depending on the system of hens breeding^[22]. In hens'

eggshells (without membrane) the content of this metal was 0.28 – 0.39 mg/kg and only in shell membranes 0.19 mg/kg^[11].

This element was found to be present in both preparations in the concentration below 1 mg/kg. In the diet, the content was 1.72 mg/kg. Daily absorption of Mo was in the group C 0.205 mg/hen and in the group E was ca. 2 % higher. No significant changes in its concentration in egg yolk or shells were determined and even significant (p<0.05) decrease of the concentration in egg albumen was found, which probably results from interactions (antagonism) of this element with other elements^[3].

Selenium: The content of Se in various egg components was in the range 0.078 – 0.354 mg/kg. The highest levels of selenium were accumulated in yolk and the lowest in albumen. Statistically significant differences (p<0.05) occurred in the content of Se in egg yolk and albumen between the control and the experimental group. It is worthy to point out high value of RBC which was evaluated as 19.15 and 15.88, respectively.

In the available literature there are many data on this element. The values reported for egg yolk were 0.474 and albumen 0.059 mg/kg fresh mass^[4] or in yolk 0.19 and in albumen 0.05 mg/kg fresh mass^[23]. According to other data, the average concentration of selenium in egg content, depending on the system of breeding of hens was from 0.117 to 0.344 mg/kg fresh mass. By increasing the content of selenium in feed from 0.2 to 0.8 mg/kg, it was possible to increase its concentration from 18.04 to 43.35 µg/egg^[29]. In eggshells, the reported range of concentrations of Se was wide: from 0.1 to 2.23 mg/kg^[24,30].

Selenium was present in the diet in the concentration of 0.32 mg/kg which gives daily absorption 0.038 mg/hen. Significant concentration of Se – 3.72 mg/kg was detected in HKW which caused 39.5 % increased intake of this element in the group E. The consequence was significantly increased (p<0.05) concentration of this metal in the content of eggs from this group, which suggests good absorption of Se from humic preparations.

Zinc: The content of Zn in various elements of egg was in the range 0.169 – 43.13 mg/kg. The highest levels of Zn were accumulated in egg yolk, and the lowest – in albumen. No statistically significant differences in the concentration of this metal in egg components between the control group (C) and experimental (E) were observed. The value of RBC was similar in both groups (averagely 2.7).

In the available literature there are various reports on this element, although there are not many

differences. The values reported for egg yolk are: 30, albumen 0.147 mg/kg fresh mass^[4] or in yolk 38 and in albumen 0.20 mg -kg fresh mass^[23]. According to other papers, the average content of zinc in egg matter, depending on the system of hens breeding was 9.77 – 13.75 mg/kg fresh mass.^[21,22,31] By increasing the content of Zn in the feed (zinc enriched yeasts) it is possible to slightly increase its concentration in egg content^[24]. In eggshells, the concentration of zinc is diversified 2.36 – 8.11 mg/kg^[24,30].

In the preparations used, zinc was present in increased concentrations, only in HBF the level was 32.35 mg/kg. However, in the mixture, the concentration of this element was 182 mg/kg, which gives daily absorption 21.69 mg/bird. Zinc taken up by hens from humic preparations did not influence general absorption and accumulation of this element in eggs.

Table 3: The content of microelements in the diet and in the preparations, as well as daily consumption by hens

Element	Diet mg/kg	HKW mg/kg	HBF mg/kg	Intake - mg/hen/day*)		Δ%
				C - group	E - group	
Co	1.87	1.43	0.61	0.224	0.231	3.1
Cr	1.68	5.37	4.68	0.200	0.230	15.0
Cu	18.6	1.74	4.06	2.217	2.260	1.9
Fe	460	5900	4800	54.83	88.63	61.6
I	1.79	0.69	0.35	0.213	0.219	2.8
Mn	197	179.7	159.0	23.48	24.79	5.6
Mo	1.72	0.29	0.74	0.205	0.210	2.4
Se	0.32	3.72	0.92	0.038	0.053	39.5
Zn	182	10.20	32.35	21.69	22.08	1.8

*)daily intake was calculated with the assumption that in a given group, the consumption of the diet was 119.19 g, in the group E – 120.54 g/head and the consumption of HKW was 2.94 and HBF was 3.33 g/head/day.

Table 4: The content of microelements in egg elements (mg/kg fresh mass)

Microelement	Yolk		Albumen		Eggshell*	
	Group C	Group E	Group C	Group E	Group C	Group E
Co	0.0040	0.0049	0.0015	0.0017 ±	0.162	0.166
	± .0006	± .0005	± 0.0002	0.0004	± 0.010	± 0.044
Cr	0.227	0.240	0.024	0.021	1.173	1.298
	± 0.061	± 0.045	± 0.005	± 0.003	± 0.145	± 0.167
Cu	1.593	1.756	0.244	0.237	2.201	2.420
	± 0.320	± 0.236	± 0.061	± 0.043	± 0.462	± 0.558
Fe	68.56a	72.02b	0.301	0.347	59.62a	63.58b
	± 4.405	± 6.120	± 0.140	± 0.128	± 5.764	± 4.231
I	0.952	1.015	0.177	0.175	1.051	1.091
	± 0.234	± 0.289	± 0.017	± 0.005	± 0.289	± 0.478
Mn	0.642	0.658	0.050	0.044	1.390	1.385
	± 0.132	± 0.167	± 0.017	± 0.013	± 0.436	± 0.279
Mo	0.025	0.025	0.0033b	0.0028a	0.131	0.120
	± 0.004	± 0.003	± 0.0008	± 0.0007	± 0.041	± 0.020
Se	0.301a	0.354b	0.078b	0.096a	0.164	0.162
	± .0335	± .0685	± 0.0061	± 0.0072	± .0164	± .0118
Zn	43.13	42.77	0.169	0.187	4.888	5.071
	± 3.43	± 3.67	± 0.048	± 0.036	± 1.013	± 1.287

a-b - p<0.05
* - in dry mass

Table 5: Mean values of Relative Bioaccumulation Coefficient (RBC) for trace elements

Element	Group - C	Group - E
Co	0.503	0.600
Cr	5.70	5.30
Cu	1.89	2.09
Fe	2.34	1.52
I	11.43	11.56
Mn	0.08	0.075
Mo	0.613	0.562
Se	19.15	15.88
Zn	2.67	2.74

CONCLUSIONS

The humic preparations used in the experiment significantly influenced the increase of selenium and decrease of molybdenum in egg albumen, increase of selenium and iron in yolk and increase of iron in eggshells. The preparations did not influence the content of such elements as Co, Cu, I, Mn, Zn. The highest RBC was found for selenium and iodine and the lowest for manganese.

The average concentrations of trace elements in yolks of settable eggs were in the following decreasing order: Fe < Zn < Cu < I < Mn < Se < Cr < Mo < Co.

The average concentrations of trace elements in albumen of settable eggs were in the following decreasing order: Fe < Cu < I < Zn < Se < Mn < Cr < Mo < Co.

The average concentrations of trace elements in eggshells of settable eggs were in the following decreasing order: Fe < Zn < Cu < Mn < Cr < I < Se < Co < Mo.

The average concentrations of nine elements studied, in the content of settable eggs did not differ significantly from the values reported in the literature for the consumption (commercial) eggs.

REFERENCES

- Jongbloed, A.W., P.A. Kemme, G. De Groote, M. Lippens and F. Meschy, 2002. Bioavailability of major and trace minerals. EMFEMA International Association of the European (EU) Manufacturers of Major, Trace and Specific Feed Mineral Materials, Brussels.

- Smulikowska, S. and A. Rutkowski, 2005. Normy żywienia drobiu. Wyd. IFiZZ PAN Jabłonna k. Warszawy.
- Jamroz, D. (red.), 2004. Żywnienie zwierząt i paszoznawstwo. Wyd. Nauk. PWN, Warszawa.
- Richards, M.P., 1997. Trace mineral metabolism in the avian embryo. *Poultry Sci.*, 76: 152-164.
- Latshaw, J.D. and M.D. Biggert, 1981. Incorporation of selenium into egg proteins after feeding seleno-methionine or sodium selenite. *Poultry Sci.*, 60: 1309-1313.
- Goux, W.J. and P.N. Venkatasubramanian, 1986. Metal ion binding properties of hen ovalbumin and S-ovalbumin: characterization of the metal ion binding site by ³¹P NMR and water proton relaxation rate enhancements. *Biochemistry*, 25(1): 84-94.
- Palmer, B.D. and L.J. Guillette, 1991. Oviductal proteins and their influence on embryonic development in birds and reptiles. In: *Egg Incubation: its effects on embryonic development in birds and reptiles*. D.C. Deeming and M.W.J. Ferguson (eds.). Cambridge Univ. Press, Cambridge, p. 29-46.
- Burley, R.W. and D.V. Vadehra, 1989. *The Avian Egg Chemistry and Biology*. John Wiley and Sons, New York.
- Castellani, O., C. Guerin-Dubiard, E. David-Briand and M. Anton, 2004. Influence of physicochemical conditions and technological treatments on the iron binding capacity of egg yolk phosvitin. *Food Chem.*, 85: 569-577.
- Piva, A., E. Meola, P.P. Gatta and et al., 2003. The effect of dietary supplementation with trivalent chromium on production performance of laying hens and the chromium content in the yolk. *Anim. Feed Sci. Technol.*, 106: 149-163.
- Konieczna, L., 1993. Skorupy jaj źródłem związków mineralnych. *Biul. Inf. Drob.*, 31, 2: 21-23.
- Trziszka, T. (red.), 2001. *Jajczarstwo*, Wyd. AR Wrocław.
- Nys, Y., M.T. Hincke, J.L. Arias, J.M. Garcia-Ruiz and S.E. Salomon, 1999. Avian eggshell mineralization. *Poultry and Avian Biology Reviews*, 10: 142-166.
- Tsai, W.T., J.M. Yang, C.W. Lai, Y.H. Cheng, C.C. Lin and C.W. Yeh, 2006. Characterization and adsorption properties of eggshells and eggshell membrane. *Biores. Technol.*, 97: 488-493.

15. Rudnicka, A., Z. Dobrzanski and J. Wisniewski, 2000. Wplyw preparatów humusowo-mineralnego i mineralno-tluszczowego na jakosc jaj od kur Lohmann Brown w drugim okresie produkcyjnym. Proc. XI Int. Congr. Anim. Hyg. (ISAH), Maastricht, Holand, Vol. 1: 247-250.
16. Korniewicz, A., Z. Dobrzanski, R. Kolacz and D. Korniewicz, 2004. Efektywnosc stosowania preparatów humicowych w zywnieniu tuczników. Acta Sci. Pol. Zoot., 3(1): 35-48.
17. Korniewicz, A., B. Paleczek, H. Czarnik-Matusiewicz and B. Sieradzka, 1999. Preparat mineralno-tluszczowy Humobentofet w tuczu jagniat. Rocz. Nauk. Zoot., 26, 3: 199-214.
18. Patkowska-Sokola, B., R. Bodkowski and S. Tronina, 2001. Wplyw podawania preparatu mineralno-tluszczowego Humobentofet na sklad tluszczu siary koziej. Rocz. Nauk. Zoot., supl., 11: 263-271.
19. Górecka, H., J. Górecki and Z. Dobrzanski, 2001. An application of plasma spectrometry ICP-OES and ICP-MS for metal content analysis in biological and environmental samples. Chem. Agric., vol. 3: 359-365.
20. Kato, R.K., A.G. Bertechini, E.J. Fassani, C.D. Santos, M.A. Dionizio and E.T. Fialho, 2003. Cobalt and vitamin B₂ in diets for commercial laying hens on the second cycle of production. Rev. Bras. Cienc. Avic., 5, 1.
21. Fakayode, S.O. and I.B. Olu-Owolabi, 2003. Trace metal content and estimated daily human intake from chicken eggs in Ibadan, Nigeria. Arch. Environ. Health., 58(4): 245-251.
22. Dobrzanski, Z., H. Górecka, T. Trziszka and H. Górecki, 1999. Concentration of macro- and microelements in the eggs of hens housed in the three different system. Proc. VIII Europ. Symp. Quality Eggs and Egg Products. Bologna, Italy, p. 283-287.
23. Elmadfa, I. and E. Muskat, 2003. Wielkie tabele kalorii i wartosci odzywczych. Wyd. MUZA S.A., Warszawa.
24. Dobrzanski, Z., D. Jamroz, H. Górecka and S. Opalinski, 2003. Bioavailability of selenium and zinc supplied to the feed for laying hens in organic and inorganic form. EJPAU, ser. Animal Husbandry, vol. 6, issue 2.
25. Eggyclopedia. American Egg Board. Park Ridge, Illinois USA, 1994.
26. Uzieblo, L., M. Ligocki, B. Hapanowicz and K. Romaniszyn, 1993. Zawartosc niektórych metali ciezkich i fluoru w jajach o różnym pochodzeniu. Bromat. Chem. Toksykol., 2: 91-96.
27. Cotterill, O.I. and J.L. Glauert, 1979. Nutrient values for shell liquid/frozen and dehydrate eggs derived by linear regression analysis and conversion factors. Poultry Sci., 58: 131-134.
28. Dobrzanski, Z., H. Górecka, G. Strzelbicka, J. Szczypel and T. Trziszka, 2001. Study on enrichment of hen eggs with selenium and iodine. EJPAU ser. Animal Husbandry, vol. 4, issue 2.
29. Surai, P.F. and N.H. Sparks, 2001. Designer eggs: from improvement of egg composition to functional food. Trends Food Sci. Technol., 12: 7-16.
30. Dobrzanski, Z., A. Rudnicka and T. Trziszka, 2000. Wplyw kredy „humicowej” na jakosc i sklad chemiczny jaj kurzych. Zesz. Nauk. AR Wroclaw, ser. Zoot., 47: 35-43.
31. Zmudzki, J., T. Juskiewicz, A. Niewiadomska, J. Szkoda, S. Semeniuk, A. Golebiowski and K. Szyposzynski K, 1992. Chemiczne skazenia bydla, mleka i jaj w regionie zgorzelecko-bogatynskim. Medycyna Wet., 48(5): 213-216.