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Mercury Bioaccumulation in Eggs of Hens Experimentally Intoxicated with Methylmercury Chloride and Detoxified with a Humic-Aluminosilicate Preparation

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■Keywords

Eggs; humic-aluminosilicate preparation; mercury.

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ABSTRACT

The aim of the study was to evaluate the effectiveness of preventive-detox preparation (P-dP) based on humic and aluminosilicate substances in the diet of laying hens (3% daily dose) previously intoxicated with methylmercury chloride (CH_3ClHg, 5 mg Hg/kg feed mixture) for six weeks. Mercury content in the whole eggs of the group intoxicated with CH_3ClHg increased compared to the control group: 488-fold after 1 wk, 622-fold after 2 wks, and 853-fold after 6 wks of intoxication. The use of P-dP in the group previously intoxicated with CH_3ClHg reduced he mercury content of whole eggs by 18.4%, on average, whereas the average was 29.9% two weeks after the discontinuation of CH_3ClHg and P-dP supply. Maximum Hg content in the whole egg was observed in group III (299.7 g), whereas the highest mercury level was obtained in the egg albumen.

INTRODUCTION

Mercury (Hg) and its compounds are commonly present in both biotic and abiotic environment. Mercury is not needed for the life processes of plants, animals and humans, and it is considered an environmental poison, especially in its organic forms. It demonstrates potent chemical and biological activity, which causes enzyme function disorders and changes in DNA phosphoric bonds that are connected with its genotoxic and cytotoxic effect (Barygina *et al.,* 2010). Mercury poisoning causes numerous metabolic disorders and diseases in humans, including neurological, immunological and cardiovascular problems (Zahir *et al.,* 2005; Virtanen *et al.,* 2007). Currently, mercury poisoning in humans or animals is observed very rarely. However, Hg application in various technological processes and considerable emissions from natural sources and industrial plants represents a potential hazard due to its excessive accumulation in the environment and food chain (Chojnacka *et al.,* 2005; Clarkson & Magos, 2006; Bykowski & Dobrzański, 2014). Extensive poultry production systems are especially vulnerable to Hg uptake from the environment. High concentrations of this metal in muscles, liver, kidneys, and eggs have been detected, especially in industrialized regions (Dobrzański*et al.,*2003; Van Overmeire *et al.,* 2006; Barej *et al.,*2009), posing a potential threat to consumers' health. It should also be mentioned that poultry embryos are particularly sensitive to methylmercury; therefore, reducing methylmercury levels transferred to the egg may be beneficial for embryos development. There are some methods limiting mercury accumulation in the body of livestock, such as the dietary addition of selenium compounds or special aluminosilicate and humic compounds; however, their efficiency is not fully determined in poultry (Marettova *et al*., 2003; Żarski *et al.*, 2003; Lukashenko & Khamidullin, 2005).

The aim of the study was to evaluate an effect of the inclusion of a preventive-detox humic-aluminosilicate preparation (P-dP) in the feed of laying hens previously intoxicated with methylmercury.

MATERIAL AND METHODS

A total of 48 Tetra SL laying hens at the age of 52 weeks were used for the study. The hens were housed in16 cages and distributed into four dietary treatments (A, B, C and D) with three replicates of 12 hens each. The experiment was conducted in an environmentally controlled room with lighting regimen of 16 h light:8 h dark. Feed and water were provided *ad libitum* during the entire experimental period. Both control and treatment diets were formulated to be isocaloric (11.6 MJ/kg) and isonitrogenous (16.1 % crude protein). The control group (A) was fed with standard feed mixture (SFM) without any additives; group B was given SFM with 3% addition of the preventive-detox preparation (P-dP); group C was intoxicated with methylmercury chloride (CH_3ClHg) at a dose of 5 mgHg/kg SFM; and group D was fed SFM with an addition of CH_3ClHg (5mg) and P-dP (3%). The experiment lasted 8 weeks, but the addition of CH_3ClHg and P-dP to diets in groups C and D were stopped during two last weeks (7-8 week of the study).

Methylmercury chloride used in the experiment was obtained from Sigma-Aldrich Corporation. It was carefully mixed with SFM by gradually adding it to a roll mixer. P-dP used in the study was obtained from the PHW Tronina Companyand included the following ingredients: humodetrinite, sedge-alder wood peat, bentonite, dolomite, beidellite, feed-grade phosphate, fodderlimestone, plant oil, feed-grade yeasts, and selenium yeast in specified amounts. This preparation, not previously used in layer feeds, is patented in Poland (No. P-209792). Animal handling as well as the experimental procedures were reviewed and accepted by the 2nd Local Ethical Committee of Experimental Procedures on Animals in Wroclaw, Poland.

In order to determine total mercury content of eggs samples, the eggs from each group were collected six times on the following days: 1 day before the beginning of the experiment (series I), day 7/8 (series II), day 14/15 (series III), day 21/22 (series IV), day 41/42 (series V), and day 55/56 (series VI) of the experiment. Six and 12 eggs were collected in series I and series II through V, respectively. In the sixth series of egg collection, only 8-10 eggs from each group were collected because low egg production and high mortality of hens were observed in groups C and D. The scheme of the experiment is presented in Table 1.

The analyses of the total mercury content in studied samples were conducted in the Accredited Research Laboratory of National Marine Fisheries Research Institute in Gdynia (Certificate No. AB 017, certified by the Polish Centre of Testing and Certification). Mercury analysis was conducted using flameless atomic absorption spectrometry on an AMA-254 spectrometer (Altec) with the vapor generation method. Previously homogenized samples were subjected to decomposition in an oxygen atmosphere and elevated temperature. After their release, mercury vapors were concentrated in a catalyst (gold amalgam) and then the concentration was measured using a calibration curve and comparing apparatus indications with a reference material (oyster tissue from National Institute of Standards and Technology, USA). Mercury was analyzed in feed samples, whole eggs, as well as in the albumen, yolk, and eggshells (on fresh weight basis).

Table 1 – Scheme of experiment

	Group *			
	A	B	C	D
Number of hens	12	12	12	12
Experimental time (weeks)	8	8	6 + 2 **	6+ 2 **
Feed and additives	Basal diet (SFM)	SFM + 3% P-dP	SFM + CH_3ClHg (5ppm)	SFM + CH_3ClHg (5ppm) + 3% P-dP
Samples (number of eggs)				
Series I	6	6	6	6
Series II-V	12/series	12/series	12/series	12/series
Series VI	10	10	8	9

*A – control group; B –addition of 3% P-dP; C – intoxication with CH_3ClHg (5 mgHg/kg SFM); D –intoxication with CH_3ClHg (5 mgHg/kg SFM) + addition of 3% P-dP.

**No addition of CH_3ClHg and P-dP for two last weeks (weeks 7-8).

SFM – standard feed mixture.

P-dP - preventive-detox preparation.

Mean values and standard deviations (SD) were calculated. Duncan's test was performed to evaluate the differences among the groups. All data were analyzed using the IBM SPSS Statistics package (Version 14PL; IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

The average content of total Hg in the SFM without addition of CH_3ClHg and P-dP was 6.25 µg/kg, while in the P-dP it was 0.052 mg/kg. Daily Hg intake was only 0.8 and 1.2 µg/hen in groups A and B, respectively. This was probably caused by an inorganic form of mercury present in the SFM and in the preparation. Mercury traces of geochemical origin can always be found in fossil fuels, humic materials, feed-grade phosphate, fodderlimestone, and aluminosilicates. The permissible content of Hg in feeds and feedstuffs (88% minimum dry matter content) ranges from 0.1 to 0.5 mg/kg (Regulation of Ministry of Agriculture and Rural Development, 2012). The Hg intake in groups A and B was likely inorganic mercury, which was present only at low levels. Layers in groups C and D received CH_3ClHg at 5 mg Hg/kg of feed, which determined and calculated an average daily intake of 625-700 µg Hg/hen. According to Fritz (1973), the tolerated mercury dose in poultry feed is 5 ppm and the toxic level is 20 ppm. According to another source (Nutrient Requirements, 1994), the toxic concentrations of various mercury compounds in poultry feeds are as follows: 400 ppm for $HgSO_4$ and $HgCl_2$; 33 ppm for CH_3Hg; 3 ppm for CH_3HgCl. At the beginning of the experiment (series I), the mean values of Hg in the albumen, yolk and eggshell were similar among the groups (Table 2). The maximum value was observed in the eggyolk of group D (0.0069 mg/kg), while the minimum value (0.0025 mg/kg) was recorded in eggshell samples of group C. The average mercury content in whole eggs ranged from 0.312 to 0.375 µg Hg/egg, and was not significantly different among the tested groups. The Hg content in various egg components (albumen, yolk, eggshell) of the treatment groups during six weeks of the experiment (series II-V) is presented in Table 3. Multifold accumulation of Hg was observed in the groups that were given CH_3ClHg after only 7-8 days. The mercury content in the whole egg was as much as 488-fold higher in group C and 458-fold in group D compared with the control group (A). The concentration of Hg in the whole egg of group B was similar to the control. In series III, additional Hg accumulation in eggs of the intoxicated groups (C and D) was observed. The mercury content in the whole egg was 622-fold higher in group C and 564-fold higher in group D compared with group A. In the next series, some stabilization of the Hg content in eggs of the intoxicated groups was noted, with a tendency of Hg level decrease in group D (intoxication and detoxification). The mercury content in the whole egg was 723-fold higher in group C and 551-fold higher in group D compared with the control one. A slight increase in Hg concentration in eggs of group B was observed, but it is still below 1 µg Hg/egg. Finally, no increase in the Hg content in eggs of the intoxicated groups was observed in series V. Mercury content in the whole egg was 853-fold higher in group C and 646-fold higher in group D compared with the control group. Similar results, with 418 to 755 Hg content, were found in groups C and D compared with group B. Comparisons of mean Hg values in the albumen, yolk and eggshell of all groups are shown in Figures 1-6. Mean mercury content during the period of six weeks of the experiment (series II-V) was 0.374, 0.397, 254.2, 207.5 µg/egg for groups A, B, C and D, respectively. The highest Hg concentration, recalculated by fresh weight unit (mg/kg), was accumulated in albumen, yolk and eggshell, respectively.

Table 2 – Mercury content (mg/kg) in the eggs of laying hens (series I)

Egg component	Group *							
	A		B		C		D	
	mean	SD	mean	SD	mean	SD	mean	SD
Albumen	0.0067	± 0.0012	0.0052	± 0.0014	0.0060	± 0.0013	0.0048	± 0.0010
Yolk	0.0051	± 0.0014	0.0045	± 0.0009	0.0054	± 0.0021	0.0069	± 0.0023
Eggshell	0.0028	± 0.0006	0.0035	± 0.0008	0.0025	± 0.0006	0.0040	± 0.0011
Whole egg (µg/egg)	0.375	± 0.052	0.312	± 0.045	0.351	± 0.039	0.343	± 0.048

*A – control group; B –addition of 3% P-dP; C – intoxication with CH_3ClHg (5 mgHg/kg SFM); D –intoxication with CH_3ClHg (5 mgHg/kg SFM) + addition of 3% P-dP. SD – standard deviation.

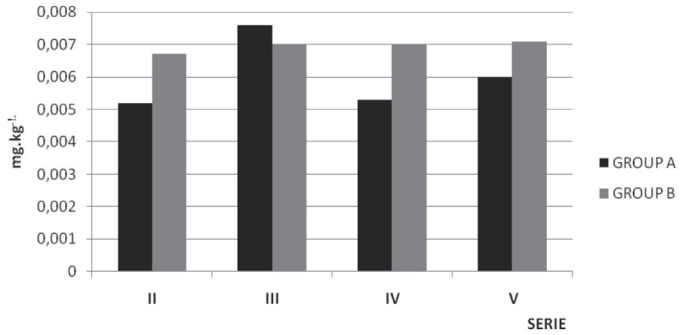

Figure 1 - Mean mercury (Hg) concentration in the egg albumen

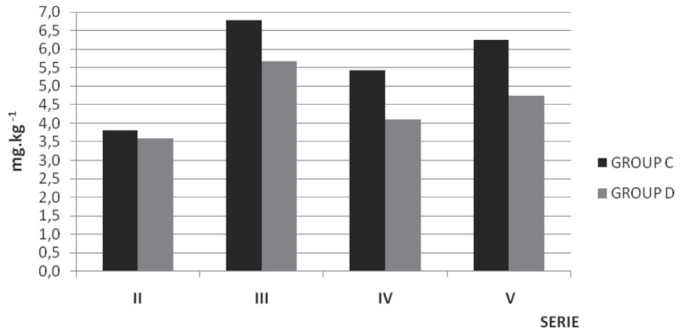

Figure 2 - Mean mercury (Hg) concentration in the egg albumen

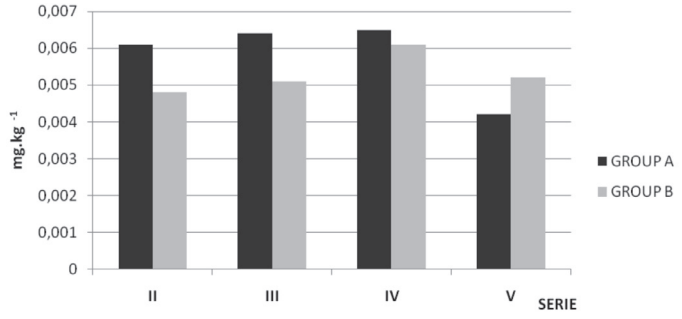

Figure 3 - Mean mercury (Hg) concentration in the egg yolk

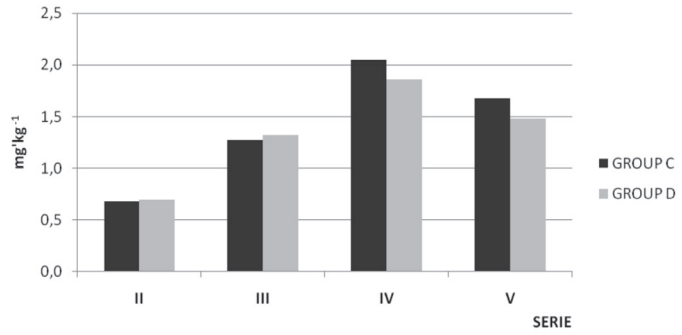

Figure 4 - Mean mercury (Hg) concentration in the egg yolk

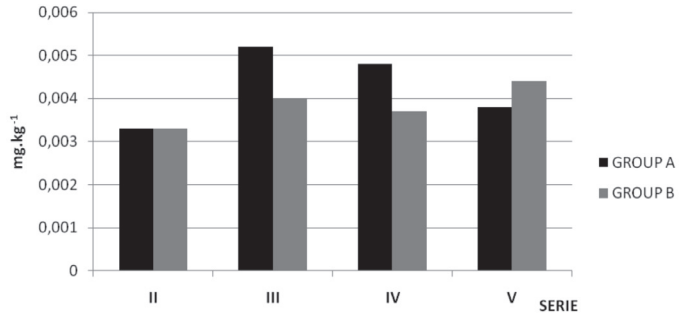

Figure 5 - Mean mercury (Hg) concentration in the eggshell

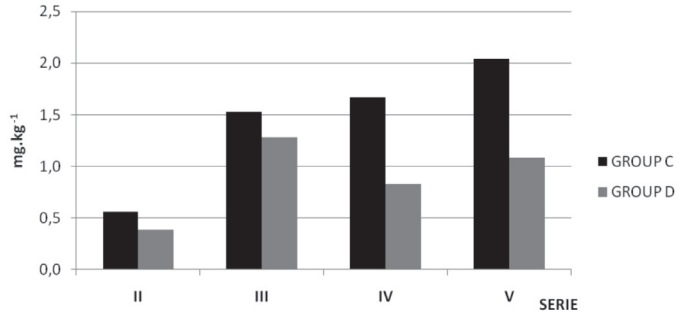

Figure 1 - Mean mercury (Hg) concentration in the egg albumen

Table 3 – Mercury content in whole eggs of the experimental series II,III, IV and V (µg/egg)

Series	Group*							
	A		B		C		D	
	mean	SD	mean	SD	mean	SD	mean	SD
II	0.339	± 0.076	0.371	± 0.045	165.4	± 34.87	155.2	± 31.55
III	0.453	± 0.065	0.397	± 0.056	299.7	± 28.50	255.6	± 43.55
IV	0.362	± 0.070	0.412	± 0.061	261.8	± 30.24	199.4	± 23.58
V	0.340	± 0.047	0.406	± 0.092	290.0	± 41.33	219.7	± 32.94
Mean	0.374^{A}	± 0.054	0.397^{A}	± 0.018	254.2^{B}	± 61.36	207.5^{B}	± 41.89

*A – control group; B –addition of 3% P-dP; C – intoxication with CH_3ClHg (5 mgHg/kg SFM); D –intoxication with CH_3ClHg (5 mgHg/kg SFM) + addition of 3% P-dP. Means with different superscript within rows ($^{A-B}$) differ significantly ($p<0.01$).

SD – standard deviation.

Table 4 – Mercury content (mg/kg) in the eggs two weeks after the end of CH_3ClHgandP-dP addition (series VI)

Egg component	Group*							
	A		B		C		D	
	mean	SD	mean	SD	mean	SD	mean	SD
Albumen	0.0061	± 0.0010	0.0059	± 0.0012	4.75	± 1.23	2.92	± 0.34
Yolk	0.0055	± 0.0011	0.0048	± 0.0010	1.79	± 0.26	1.64	± 0.29
Eggshell	0.0030	± 0.0004	0.0050	± 0.0009	1.27	± 0.31	0.48	± 0.12
Whole egg (µg/egg)	0.352^{A}	± 0.049	0.328^{A}	± 0.051	206.6^{BC}	± 24.7	144.8^{BD}	± 19.6

*A – control group; B –addition of 3% P-dP; C – intoxication with CH_3ClHg (5 mgHg/kg SFM); D –intoxication with CH_3ClHg (5 mgHg/kg SFM) + addition of 3% P-dP.

Means with different superscript within rows ($^{A-D}$) differ significantly ($p<0.01$).

SD – standard deviation.

Two weeks after the end of CH_3ClHg and P-dP application, interesting changes in egg Hg concentration were observed (Table 4). Groups A and B presented similar Hg concentrations in the albumen, yolk, and eggshell, and these were only slightly different from the values obtained in previous analyses (mean Hg concentrations in egg content was 0.352 and 0.328 µg, respectively). In the intoxicated group C, Hg content in the whole egg was still high (206.6 µg), while in group D, that value was 29.9% lower (144.8 µg) relative to group C. The decrease in Hg concentration ofin the whole egg in groups C and D was determined by a reduction in Hg content in the albumen. However, no quantitative changes in yolk Hg content were observed after two weeks. This may be explained by the fact the albumen has higher protein and amino acid content, and therefore, it has higher affinity for methylmercury. The results of this analysis confirm a positive influence of the applied P-dP preparation.

Heinz & Hoffmann (2004) fed wild ducks with feeds containing 5, 10, or 20 ppm of mercury in the form of methylmercury chloride, and observed an increased Hg egg content of up to 7, 18, and 35 ppm Hg of fresh egg weight. Mercury in the form of methylmercury in those eggs was at the level of 95-100%. In the present study, lower Hg egg levels were observed in the intoxicated groups. Similar CH_3ClHg intoxication doses were applied for laying hens by Lundholm (1995), who observed physiological disorders, including reduction of egg production on days 4-9 of the experiment and its total egg production inhibition after day 9. Additionally, some of the eggshells of the intoxicated hens were considerably thinner and had a porous surface when compared with the control group. The authors also noted a significant decrease in Ca content in the blood serum, as well as reduced synthesis of prostaglandins in the group treated with methylmercury chloride. Different doses of organic mercury were used by Kambamanoli-Dimau *et al.* (1991). They applied 500 µg CH_3ClHg /kg of body weight in group A, and 100 µg CH_3ClHg /kg in group B. The authors did not find any significant differences in birds' activity or egg production between the groups during the course of the experiment. The transfer of methylmercury chloride to the eggs started to increase on the 2nd day of methylmercury chloride application, reaching a maximum concentration in group A on the 3rdday of the study (1685 ppb), and in group B, on the 6thday (1041 ppb). According to the study of Cappon & Crispin Smith (1981) total Hg content was 5110 ng/g in the albumen and 3090 ng/g in the yolk when the laying hens were given grains contaminated with mercury (13 ppm). The proportion of methylmercury in egg albumen and yolk was 94% and 16% of the total Hg content, respectively (Rutkiewicz & Basau 2013).

After intoxication of hens with phenylmercury chloride, the preparation MESNA (sodium mercaptoethane sulfonate) was found to be useful for the detoxification process (Żarski *et al.,* 2003). Similar properties have been attributed to dimercaprol (BAL) and dimercaptosuccinic acid (DMSA) (Emanuelli *et al.,* 1996; De la Torre *et al.,* 1997). Positive preventive-detox effects may be obtained when using a TOXIPOL adsorptive preparation (Lukashenko & Khamidullin, 2005) or selenium preparations (Marettova *et al.,* 2003). Selenium lowers mercury bioavailability; however, it may be toxic in excessive amounts (Surai, 2002; Feroci *et al.,* 2005). It was demonstrated in another study (Dobrzański *et al.,* 2003) that mean Hg concentration in duck eggs in areas of industrial contamination was as high as 137.2 µg/kg, whereas in typical agricultural/ecological areas, the concentration was only 39.7 µg/kg. In goose eggs, these values were 55.1 and 10.5 µg/kg, respectively. The highest amount of Hg was found

in the yolk, and the lowest in the eggshell. On the other hand, Czaban *et al.* (2013) observed relatively lower mercury concentrations in layer eggs collected in the neighborhood of copper mine tailing ponds.

CONCLUSIONS

The P-dP preparation at 3% inclusion level in the diet of laying hens reduced mercury accumulation in the eggs of hens previously intoxicated with methylmercury chloride, but only to a limited magnitude (about 30%). The single addition of P-dP to a standard feed for layer does not reduce the natural mercury content in the eggs.

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