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ACCUMULATION OF METALS IN SELECTED TISSUES AND ORGANS OF LAYING HENS IN VARIOUS AGES

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Abstract. The aim of this study was to determine the changes of content of Cd, Zn, Cu, Ni and Fe in tissues and organs of chicks, pullets and adult laying hens. The birds were reared from the first day after hatching in the litter system and had no outdoor access. They were fed ad libitum standard diets based on concentrates for chicks, pullets and laying hens and had free access to water throughout the experiment. On day 4, in weeks: 7 and 52 of the experiment, organs and tissues (liver, stomach, breast muscle, and leg muscle) were collected to determine the level of selected metals. In addition, feed samples corresponding to the age group were tested. The contents of elements were determined using AAS. Based on the obtained results it was found that the level of each metal is different depending on the age of the birds and on the tested organ or tissue. Higher levels of Cd and certain trace elements in chicks' tissues can be explained by the rapid growth and development, and rapid biochemical changes that occur in the body and/or contamination of hatching eggs. In addition, the chicks have not yet developed system of detoxification. Moreover, the developing tissues and organs increase their volume which results in their lower elements concentrations in older birds. Our studies confirm very slight exposure of birds in this type of farming to excess elements.

Key words: chicks, hens, accumulation of metals, liver, stomach, muscle

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INTRODUCTION

Consumers' interest in the relationship between diet and health has increased substantially in Europe. They show unprecedented consciousness in the way food is produced, processed and marketed [Mariam et al. 2004, Andrée et al. 2010, Mudgal et al. 2010]. The analysis of the contents of mineral elements in tissues of farm animals is necessary both for the maintenance of animal welfare, as well as for consumers' health. This includes not only highly toxic metals, such as cadmium and lead, but also essential elements such as zinc, copper, iron and magnesium. Metals incorporated in animal tissues are consumed by humans which directly affects public health [Nriagu et al. 2009, Andrée et al. 2010, Mudgal et al. 2010, Rajaganapathy et al. 2011]. Polluted food and feed products consumption is currently the main source of human and animal exposure to toxic elements [Andrée et al. 2010, Mudgal et al. 2010].

Metals penetrate into the food from atmospheric air, soil and water [Andrée et al. 2010, Mudgal et al. 2010, Rajaganapathy et al. 2011]. Also, technological processes can be the source of toxic metals contamination of food [Mariam et al. 2004, Andrée et al. 2010, Mudgal et al. 2010]. Blood transports metals that penetrate into the body, to tissues and organs, where they are concentrated and stored [Andrée et al. 2010]. Exposure to heavy metals may negatively affect blood morphological parameters, the proper functioning of enzymes, the activity of transport proteins, and the structure and function of cells, tissues and organs. The first symptoms of disorders caused by toxic metals may be: loss of appetite, growth problems, developmental disorders and reduced resistance to infections [Kabata-Pendias and Pendias 1993, Hassan et al. 1998, Mudgal et al. 2010].

Trace elements are important components of animal organisms and their concentration and relative proportions in the tissues of the body depend on the proper development and health of all organisms. Disease and decreased animal productivity due to deficiency or excess of trace elements are important and may cause serious economic problems [Kabata-Pendias and Pendias 1993, Hossny et al. 2001, Soetan et al. 2010].

Majority of research studies concerning metal accumulation in hen tissues is conducted in adult specimens. There is a lack of literature data concerning the metal contents in tissues and organs of chicks and young birds, as well as their changes with age.

The aim of this study was to determine the changes of content of highly toxic metals, such as cadmium and other trace elements known as microelements (Zn, Cu, Ni, Fe), the toxicity of which depends on the concentration in the body, in tissues and organs of chicks, pullets and adult laying hens.

MATERIAL AND METHODS

The study was carried out with 120 commercial crossbreed laying hens – ISA Brown. The birds were reared from the first day after hatching in the litter system and had no outdoor access. They were fed ad libitum standard diets based on concentrates for chicks, pullets and laying hens (appropriate for the respective rearing periods) and had free access to water throughout the experiment. The birds were kept in standard environmental (air humidity, temperature and lighting programme) and feeding conditions appropriate for the respective rearing periods [Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 15 lutego 2010 r., DzU 2010 nr 56 poz. 344]. On day 4, in weeks: 7 and 52 of the experiment, organs and tissues (liver, stomach, breast muscle and leg muscle) were collected from 6 birds to determine the level of cadmium, zinc, copper, nickel and iron. In addition, feed samples corresponding to the age group were tested. The samples were prepared by wet digestion. The contents of elements were determined by means of Atomic Absorption Spectrometer (AAS) BUCK 200A [Hseu 2004]. Each metal was estimated 3 times in each sample of tissues, organs and feed.

The results were analyzed statistically using Statistica ver. 10. Significant differences between the experimental groups were verified by the analysis of variance and post hoc Duncan test. Statistical significance was defined at P < 0.05.

RESULTS

Trace elements content in feed samples is shown in Table 1. The concentration of cadmium in the analyzed feed samples ranged from 0.418 to 0.931 mg \cdot kg⁻¹ wet weight. The nickel content was in the range of 3.428–5.777 mg \cdot kg⁻¹ wet weight. For both elements, the highest values were found in the hens' diet, and the lowest in the pullet diet. In the studied samples of feed, the zinc concentration ranged from 75.168 to 126.211 mg \cdot kg⁻¹ wet weight. The highest value was recorded in the chick diet, and the lowest in the pullet diet. The copper content ranged from 13.094 to 21.628 mg \cdot kg⁻¹ wet weight, reaching the highest value in the pullet diet, and the lowest in the hen diet. In the analyzed samples of feed, the iron content ranged from 153.929 to 261.574 mg \cdot kg⁻¹ wet weight. The highest concentration was observed in the hen diet and the lowest in the pullet diet.

The results of the examined elements' contents in the liver of chicks, pullets and adult hens are summarized in Table 2. There were no statistically significant differences in the content of cadmium in different age groups. The highest concentration of zinc was found in the tissue of pullets, lower in the 52-week-old hens and the lowest in chicks ($P \le 0.01$). There has been a lower copper content in hens as compared with younger birds ($P \le 0.01$) and a lower nickel content in

Table 1. Content of elements in full-ration mixture (mg \cdot kg⁻¹ wet weight)

Tabela 1. Zawartość pierwiastków w mieszance pełnoporcjowej (mg·kg⁻¹ mokrej masy)

Element _ Pierwiastek	Day 4. Dzień 4.		Week 7. Tydzień 7.		Week 52. Tydzień 52.	
	mean średnia	SD	mean średnia	SD	mean średnia	SD
Cd	0.461	0.011	0.418	0.009	0.931	0.018
Zn	126.211	5.042	75.168	3.236	79.518	3.812
Cu	13.424	1.003	21.628	1.987	13.094	1.122
Ni	4.237	0.241	3.428	0.201	5.777	0.328
Fe	197.664	21.035	153.929	18.578	261.574	23.574

Table 2. Content of elements in liver tissue ($mg \cdot kg^{-1}$ wet weight)

Tabela 2. Zawartość metali w watrobie (mg · kg⁻¹ mokrej masy)

ElementPierwiastek	4-days old chicks 4-dniowe pisklęta		7-weeks old pullets 7-tygodniowe kurczęta		52-weeks old hens 52-tygodniowe kury	
	mean średnia	SD	mean średnia	SD	mean średnia	SD
Cd	0.105	0.011	0.103	0.019	0.119	0.014
Zn	13.400 A	1.104	59.578 C	2.954	21.425 B	1.353
Cu	3.299 B	0.389	3.745 B	0.572	2.219 A	0.316
Ni	1.085 B	0.170	0.721 A	0.100	0.713 A	0.046
Fe	64.346 a	13.406	68.526	18.733	93.871 b	12.191

SD – standard deviation; a, b – values in rows with different letters differ significantly ($P \le 0.05$); A, B – values in rows with different letters differ highly significantly ($P \le 0.01$).

SD – odchylenie standardowe; a, b – wartości w wierszach oznaczone różnymi literami różnią się statystycznie istotnie ($P \le 0.05$); A, B – wartości w wierszach oznaczone różnymi literami różnią się statystycznie wysoko istotnie ($P \le 0.01$).

hens and pullets as compared with chicks (P \leq 0.01). In adult hens, there were higher levels of iron compared to chicks (P \leq 0.05).

In stomach, similarly to liver, there were no statistically significant differences in the Cd content between groups (Table 3). The highest concentration of zinc has been found in pullets, lower in the 52-week-old hens and the lowest in chicks ($P \le 0.01$). Statistically significant differences were also observed in the Cu content between chicks and both, pullets and hens ($P \le 0.01$), and the Ni content between chicks and adult hens ($P \le 0.05$). The iron content in stomachs was higher in hens compared with chicks ($P \le 0.01$) and pullets ($P \le 0.05$).

The contents of all the examined elements in breast muscle (Table 4) and leg muscle (except Zn) (Table 5) were the highest in the 4-day-old chicks as compared with pullets and adult chickens (P < 0.01).

DISCUSSION

From the point of view of animal welfare and human health, at the end of the food chain both deficiency and excess of metals in the diet are dangerous. It is estimated that approximately 80–90% of the total intake of toxic metals into an organism takes place with the food, and the rest through the respiratory system. It makes the safety and quality of foods very important aspects for the consumers [Andrée et al. 2010, Rajaganapathy et al. 2011]. In the case of laying hens, polluted eggs and the birds' meat – as a so-called stewing hen or as processed products like pâté, canned meat etc. – may pose a threat for the consumers.

The harmfulness of metals that are examined in this study varies significantly. Cadmium is recognized as a highly toxic metal to the body, while Zn, Cu, Ni and Fe are microelements, which may be harmful only in excess, while smaller quantities are necessary for the proper functions of the body.

Cadmium administration may lead to decreased rate of growth, alteration in biochemical parameters, damage in kidney, liver and bursa fabricius, and accumulation in liver, kidney and muscle tissues [Akyolcu et al. 2003, Johri et al. 2010, Mudgal et al. 2010].

González-Weller et al. [2006] in a study in Spain reported the average concentration of Cd in the muscle of poultry equal to 0.0168 mg \cdot kg⁻¹ of wet weight. The highest value obtained in the products consumed by the population of Tenerife was 0.042 mg \cdot kg⁻¹ of wet weight. Much higher concentration (0.31 mg \cdot kg⁻¹ of wet weight) was reported by Mariam et al. [2004] in studies on poultry in Turkey and Iwegbue et al. [2008] in Nigeria. In the studies conducted in Bulgaria [Hallak 2007], the Cd content in the liver of chickens was 0.825 mg \cdot kg⁻¹ of wet weight and in the stomach 0.593 mg \cdot kg⁻¹ of wet weight. Muscles proved to be the least polluted tissue (0.029 mg \cdot kg⁻¹ of wet weight). In these studies, the samples of feed were also analysed, and the result of 0.38 mg \cdot kg⁻¹ of wet weight was lower than this found in the present work.

In our study, we revealed the presence of highly toxic Cd in all studied feed and organs. Xenobiotic content in the feed samples ranged from 0.418 to 0.931 mg \cdot kg⁻¹ of wet weight, exceeding the permitted norms for the Cd content in poultry feed [Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 6 lutego 2012 r., Dz.U. 2012 poz. 203]. In the case of organs, the highest level of contamination was measured in the liver of adult hens (0.119 mg \cdot kg⁻¹ of wet weight), while the lowest in the breast muscles of the same birds (0.006 mg \cdot kg⁻¹ of wet weight). Moreover, in 4-day-old chicks, statistically significantly higher level of Cd was observed in the breast and leg muscles as compared with 7-week- and 52-week-old birds. On the one hand, it may be the result of this metal accumulation in breeding hens, the Cd pollution of stamped hatching eggs and, consequently,

Table 3. Content of elements in stomach tissue (mg \cdot kg⁻¹ wet weight)

Tabela 3. Zawartość metali w żołądku (mg · kg⁻¹ mokrej masy)

Element _ Pierwiastek	4-days old chicks 4-dniowe pisklęta		7-weeks old pullets 7-tygodniowe kurczęta		52-weeks old hens 52-tygodniowe kury	
	mean średnia	SD	mean średnia	SD	mean średnia	SD
Cd	0.105	0.013	0.088	0.017	0.103	0.023
Zn	15.542 A	1.203	24.730 C	2.241	19.843 B	0.666
Cu	1.554 B	0.305	0.876 A	0.158	0.767 A	0.137
Ni	0.861 b	0.034	0.622	0.118	0.592 a	0.225
Fe	23.528 A	3.196	29.786 a	4.715	38.268 Bb	5.895

SD – standard deviation; a, b – values in rows with different letters differ significantly ($P \le 0.05$); A, B – values in rows with different letters differ highly significantly ($P \le 0.01$).

SD – odchylenie standardowe; a, b – wartości w wierszach oznaczone różnymi literami różnią się statystycznie istotnie ($P \le 0.05$); A, B – wartości w wierszach oznaczone różnymi literami różnią się statystycznie wysoko istotnie ($P \le 0.01$).

Table 4. Content of elements in breast muscle tissue (mg · kg⁻¹ wet weight)

Tabela 4. Zawartość metali w mięśniach piersiowych (mg·kg⁻¹ mokrej masy)

Element	4-days old chicks 4-dniowe pisklęta		7-weeks old pullets 7-tygodniowe kurczęta		52-weeks old hens 52-tygodniowe kury	
Pierwiastek	mean średnia	SD	mean średnia	SD	mean średnia	SD
Cd	0.037 B	0.010	0.007 A	0.001	0.006 A	0.001
Zn	19.003 B	1.519	7.625 A	0.818	6.226 A	0.934
Cu	3.614 B	1.118	0.288 A	0.082	0.331 A	0.031
Ni	3.504 B	0.682	0.538 A	0.089	0.602 A	0.031
Fe	39.019 B	3.319	7.800 A	1.627	6.286 A	1.262

SD – standard deviation; A, B – values in rows with different letters differ highly significantly ($P \le 0.01$).

SD – odchylenie standardowe; A, B – wartości w wierszach oznaczone różnymi literami różnią się statystycznie wysoko istotnie (P ≤ 0,01).

Table 5. Content of elements in leg muscle tissue (mg · kg⁻¹ wet weight)

Tabela 5. Zawartość metali w mięśniach nóg (mg · kg⁻¹ mokrej masy)

ElementPierwiastek	4-days old chicks 4-dniowe pisklęta		7-weeks old pullets 7-tygodniowe kurczęta		52-weeks old hens 52-tygodniowe kury	
	mean średnia	SD	mean średnia	SD	mean średnia	SD
Cd	0.017 B	0.003	0.008 A	0.001	0.008 A	0.002
Zn	18.048	1.973	15.305	3.325	17.558	3.850
Cu	3.855 B	0.568	0.748 A	0.185	0.645 A	0.285
Ni	2.025 B	0.508	0.568 A	0.103	0.590 A	0.055
Fe	35.778 B	3.970	16.435 A	2.165	15.025 A	1.858

SD – standard deviation; A, B – values in rows with different letters differ highly significantly ($P \le 0.01$).

SD – odchylenie standardowe; A, B – wartości w wierszach oznaczone różnymi literami różnią się statystycznie wysoko istotnie ($P \le 0.01$).

of the chicks. On the other hand, the growth of tissues with age may cause the decrease of the studied metals concentration in these organs and tissues.

Zinc is a trace element that is necessary for normal growth and maintenance, and includes, among other functions, bone development, feathering, enzyme structure and function, as well as appetite regulation for all avian species [Batal et al. 2001, Richards et al. 2010, Salim et al. 2012]. In the case of laying hens, its relevant level in feed is particularly significant as it takes part in the development of the embryo, the egg, which affects their good hatching rate. It also takes part in the eggshell mineralisation and mineral substances accumulation in bones [Batal et al. 2001]. However, high levels of zinc in the diet can result in reduced growth rates in chicks, lesions of the gizzard and pancreas in laying hens, high mortality in chicks, and reduced feed intake and egg production in laying hens [Hermayer et al. 1977, Dewar et al. 1983, Richards et al. 2010].

In Iwegbue's et al. [2008] studies, the Zn content in the muscles of chickens ranged from 6.12 to 33.21 mg \cdot kg⁻¹, while in the stomachs – from 10.19 to 37.03 mg \cdot kg⁻¹. The highest concentrations of Zn were recorded in the muscles of turkeys (4.95–48.23 mg \cdot kg⁻¹). Similar results were also obtained by Mariam et al. [2004]. In the studies conducted in India [Singh and Taneja 2010], the mean concentration of Zn obtained for poultry equaled to 129.00 mg \cdot kg⁻¹ of wet weight. In this study, we reported similar Zn levels to those presented above. The highest concentration of this microelement was noted in the liver of pullets (59.578 mg \cdot kg⁻¹ of wet weight), while the lowest in the breast muscle of adult laying hens (6.226 mg \cdot kg⁻¹ of wet weight). The breast muscles of 4-day-old chicks contained statistically more, but liver and stomach – less, Zn as compared with pullets and adult hens. It may indicate the Zn contribution in the above-mentioned processes depending on the age.

Copper is an essential trace mineral for poultry which functions in numerous physiological processes primarily as a constituent of several enzyme systems. Cu is involved in mitochondrial oxidative phosphorylation, free radical detoxification, neurotransmitter synthesis and denaturation, pigment formation, connective tissue synthesis and iron metabolism [Crisponi et al. 2010, Ajuwon et al. 2011]. Deficiencies of both iron and copper can lead to anemia.

In the studies conducted in Nigeria [Iwegbue et al. 2008], the Cu concentrations ranged from 0.01 to 5.15 mg \cdot kg⁻¹ in the muscles of chickens and from 0.46 to 2.55 mg \cdot kg⁻¹ in their stomachs. Turkeys' muscles contain from 0.01 to 3.36 mg \cdot kg⁻¹. Singh and Taneja [2010] reported an average Cu concentration equal to 2.50 mg \cdot kg⁻¹ of wet weight in poultry samples.

In our study, the Cu content in the test material was similar to the above studies. Cu, with the highest concentration, was measured in organs taken from 4-day-old chicks. The content of Cu in other age groups was significantly lower.

The highest concentration of Cu was found in the leg muscles of chicks (3.855 mg \cdot kg⁻¹ of wet weight), while the lowest – in the breast muscles of pullets (0.288 mg \cdot kg⁻¹ of wet weight). Similarly to Zn, there are many more common symptoms of Cu deficiency rather than of its excess. However, in animal nutrition, Cu is considered as a "cumulative poison" and caution has been recommended in its use in feed.

In all the analysed organs and tissues of chicks, pullets and laying hens the presence of Ni was also detected. This element in trace amounts is essential in the regulation of metabolism of living organisms. The necessity of Ni for the human and animal organisms has been shown relatively recently, and the deficiency symptoms have been found only under experimental conditions. The lack of the element lowers growth, decreases the reproduction results, leads to skin and hair damages, anemia, affects iron and zinc absorption, and shortens the lifespan. The lack of Ni also contributes to the accumulation of fat in the liver and impairs its function [Nielsen and Sauberlich 1970, Oscar et al. 1995]. The excess of Ni, however, causes changes in the metabolism of other metals, mainly reduces the level of magnesium, manganese and zinc [Kabata-Pendias and Pendias 1993].

In the studies by Iwegbue et al. [2008], the concentration of Ni in the muscles of chickens ranged from 1.20 to 9.02 mg \cdot kg⁻¹, while in the stomachs from 2.07 mg \cdot kg⁻¹ to 6.67 mg \cdot kg⁻¹. In the same studies, the Ni content in the muscles of turkeys was highly diversified, depending on the region the samples were collected from (0.13–20.78 mg \cdot kg⁻¹). These results do not significantly differ from those obtained in the present work. Ni reached the highest concentration in all the samples from the 4-day-old chicks as compared with pullets and hens. The highest content of this element was observed in the breast muscles of chicks (3.504 mg \cdot kg⁻¹ of wet weight) and the lowest in the breast muscles of pullets (0.538 mg \cdot kg⁻¹ of wet weight).

The element which occurred in the highest concentration in the analysed organs proved to be Fe. As in the case of Zn, the breast and legs muscles of the 4-day-old chicks had higher, and the liver and stomach – lower levels of Fe than the older birds. The highest concentration of Fe was noted in the liver of adult hens (93.871 mg \cdot kg $^{-1}$ of wet weight), while the lowest in their breast muscles (6.286 mg \cdot kg $^{-1}$ of wet weight). The results of research studies conducted in various parts of Nigeria by Iwegbue et al. [2008] showed the highest concentrations of Fe in chicken's muscles (22.07–97.72 mg \cdot kg $^{-1}$). Stomachs were characterized by slightly lower values (19.28–45.72 mg \cdot kg $^{-1}$). In these studies, the content of Fe in the muscles of turkeys was also analysed, which ranged from 14.14 to 35.03 mg \cdot kg $^{-1}$.

As compared with the above studies, the results obtained in the present study were similar. This may be related to the feed, which had high concentration of Fe

(153.929–261.574 mg \cdot kg⁻¹ of wet weight). The high content of this micronutrient in feed can be explained by the increased demand of iron in fast-growing animals and females. Fe and its compounds are not toxic to the human and animal bodies. Fe deficiency is common in human and is generally caused by a low content of available forms of this metal in food or disturbances in the process of absorption. Animals' liver and meat are the main source of the absorbable Fe [Kabata-Pendias and Pendias 1993].

CONCLUSION

Based on the obtained results it was found that the level of each micronutrient is different depending on the age and on the tested organ or tissue of the birds. In the case of chicks, high levels of cadmium and certain trace elements in tissues can be explained by the rapid growth and development, and rapid biochemical changes that occur in the body and/or contamination of hatching eggs. Feed could have an impact on the level of metals but to a lesser extent (chicks lived only 4 days). In addition, the chicks do not yet have a fully developed detoxification system. Adult animal organisms have a well-functioning protective mechanism that may inactivate toxic metals. There are protective barriers to prevent excessive concentrations of these elements in the tissues. They rely on reducing the absorption from the respiratory system, digestive system, or rapid excretion of metals or metabolic inactivation, which occurs on the basis of a specialized enzyme system. Moreover, the developing tissues and organs increase their volume which results in their lower elements concentrations in older birds.

Our studies confirm very slight exposure of birds in this type of farming to excess elements.

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KUMULACJA METALI W WYBRANYCH NARZĄDACH I TKANKACH KUR NIOSEK W RÓŻNYM WIEKU

Streszczenie. Celem prowadzonych badań było określenie zmian w zawartości Cd, Zn, Cu, Ni i Fe w narządach i tkankach piskląt, kurcząt i dorosłych kur nieśnych. Ptaki utrzymywano od pierwszego dnia po wylegu w systemie ściołowym bez dostępu do wybiegu. Ptaki żywiono ad libitum mieszankami standardowymi dla piskląt, kurcząt i kur nieśnych sporządzonymi na bazie koncentratów. Przez cały okres doświadczenia ptaki miały swobodny dostęp do wody. W 4. dniu oraz w 7. i 52. tygodniu eksperymentu, pobrano narządy i tkanki (watroba, żołądek, mięśnie piersiowe i mięśnie nóg) w celu zbadania poziomu wybranych metali. Pomiarów dokonano także w próbkach pasz. Zawartość pierwiastków oznaczano metodą ASA. Na podstawie uzyskanych wyników stwierdzono, iż poziom poszczególnych pierwiastków jest różny i zależy od wieku ptaków oraz badanego narządu czy tkanki. Wysoki poziom Cd i mikroelementów w tkankach piskląt można tłumaczyć szybkim tempem wzrostu i rozwoju oraz gwałtownymi zmianami biochemicznymi zachodzącymi w ciele i/lub zanieczyszczeniem jaj wylegowych. Dodatkowo pisklęta nie mają jeszcze w pełni wykształconego systemu detoksykacji. Ponadto, rozwijające się narządy i tkanki zwiększają swoją objętość, co prowadzi do zmniejszenia koncentracji zawartych w nich pierwiastków u starszych ptaków. Nasze badania potwierdzają bardzo nieznaczne narażenie ptaków w chowie tego typu na nadmiar pierwiastków.

Słowa kluczowe: pisklęta, kury, kumulacja metali, watroba, żołądek, mięśnie

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