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Comparative study of metal concentration determination in albumen of hen eggs originating from industrial poultry farms, backyard and free-range hens using ICP-OES technique

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Abstract

There have been multiple types of research focusing on the relationship between feed ingredients and metal content in the egg white due to their role in human nutrition. The aim of the present study is to determine the metal concentration in hens' eggs and, in particular, to compare the metal concentration in egg albumen originating from industrial poultry farms with that of backyard and free-range hens. All samples were collected in Romania from five separate counties and 10 different farms, over a period of two weeks and, as a result, a total of 50 were collected, 10 from each housing system (batteries/cages, litter/soil, free-range, organic and backyard). The measurements of the metals were taken by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), with a wide range of elements reported. For the essential elements, we measured Cr, Cu, Fe, Mn and Zn; Al, Cd, Ni and Pb for the heavy metals and, in addition, we measured B, Ba, Sr, Ca and Mg. The present study revealed that the metals in eggs from free-range hens are richer in essential elements with mean concentrations as follows: 1.528 mg/kg for Fe, 3.278 mg/kg for Zn, 0.058 mg/kg for Mn and 1.362 mg/kg for Cu. We concluded that the egg quality is closely connected with the housing system and nutrition. Furthermore, the results demonstrate that eggs from backyard housing are no better than those from free-range hens in terms of essential metal composition. The heavy and non-essential metal contents, present in the albumen of all the examined eggs, were much lower than the maximum allowed concentration and, therefore, egg consumption does not pose any risk to human health.

Keywords
albumen, egg-white, food safety, farming systems, ICP-OES, poultry housing, poultry nutrition

Introduction

Products from the poultry industry are primary animal sources of proteins, microelements and vitamins for human nutrition. This is defined by the lower price of poultry products, compared to those from other animal sources, the easier way of production, good taste and provision of much of the recommended daily intake for microelements and vitamins (Vilà 1999; Youssef et al. 2014). During the last few years, the production of eggs in the European Union is in decline in some countries, while the demand from consumers is permanently increasing (European Parliament, Directorate General for Internal Policies 2010). Industrial development and lower environmental control in the past, pose risks for contamination of the hens' feed with different heavy metals and from there, the quality and microelement content of the eggs (Ternes and Leitsch 1997). Having knowledge of the eggs' mineral composition is required for different purposes: their nutritional value and percentage of daily requirements supplement, level of heavy metal contamination and proper poultry feeding and welfare. Contamination of poultry products could be a result of improper nutrition or dietary supplements for the laying hens. Feeds are often provided with the addition of essential supplements for the birds' growth: Cr, Cu, Fe, Mn and Zn: although heavy metals, such as Al, Cd, Ni and Pb have no important purpose for the hens' body functions, they may possess risks for human health (Li et al. 2005). Increased concentration of different metals in poultry products could be due to different sources: mainly from feed, but also from drinking water, litter, housing equipment and the living environment (Codling et al. 2008).

Despite the increasing interest in egg nutritional values for the human diet and higher expectations about organic and free-range production, the amount of information on the subject is still limited. The purpose of the present study was the analysis of the albumen of the eggs, resulting from different housing systems, using ISP-OES. The aims of our study were to establish baseline values for the metal concentration in eggs laid by hens from different production systems: conventional or intensive systems, organic and free-range and those raised in the backyard.

For simplification of the description of the housing systems, for the purpose of the study, we will use the term ecological when describing together backyard, organic and free-range and the term intensive farming, when describing soil/litter and cage housing systems.

Materials and methods

Study area and sample collection

Egg collection was undertaken during a 2-week period, between 1 and 25 February 2022. Eggs were collected from areas where the largest producers of commercially sold

Table 1. Location where collection was undertaken.

Code	Housing system	Area of collection
BkYd	Backyard	AR BH
0	Organic	DJ BH
1	Free-range	DJ DB
2	Soil	DB BH
3	Cages	DB BH

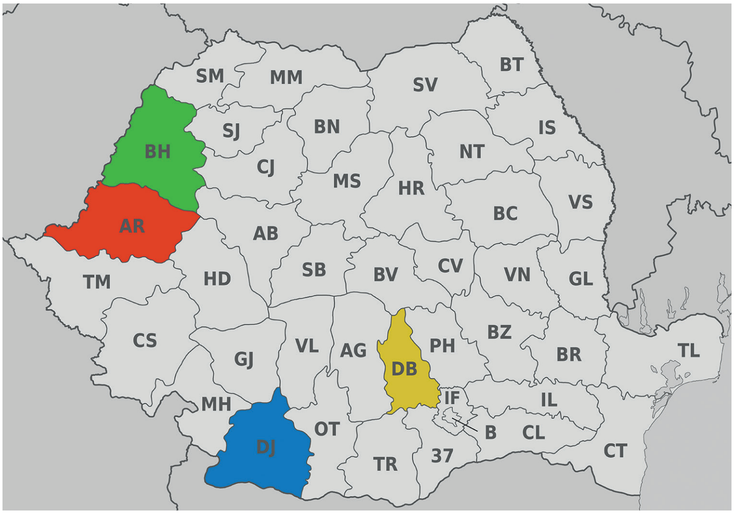

Figure 1. Map of Romania with areas of collection. (*TUBS*, *CC BY-SA 3.0*).

eggs in Romania are located. The areas from where the eggs were collected are shown in Table 1 and Fig. 1. A total of 50 samples were collected, 10 from each housing system. Forty eggs were collected from commercial producers and 10 from villages where people raise their hens in backyards for household consumption of produced eggs. All hens were fed with commercial feed with equal mineral content, composed of 2800 kcal/kg metabolisable energy, 18% crude protein, 0.72% cysteine+methionine, 0.9% lysine, 3.5% calcium and 0.35% phosphorus. Hens were also given mineral supplements on a regular basis. In addition, free-range, organic and backyard chickens had access to outdoor foraging areas. Backyard hens frequently received food leftovers from household meals with no supplementary vitamins and minerals. Organic and free-range poultry had access to yards with natural grass and soil for 5 to 6 hours per day. Backyard chickens had access to the yard and surrounding areas for more than 8 hours per day.

Sample preparation

The collected eggs were cracked and the egg white was separated from the yolk into ceramic containers. The samples were heated in ventilated ovens at 104 °C until no further weight loss was registered. All samples were evenly ground and homogenised.

The glassware used for analysis was firstly washed with detergent and rinsed, then filled with previously-prepared 6N nitric acid (HNO_3), left for 12 hours and, finally, rinsed with double-distilled water.

Using a digital analytical balance, five grams of each sample were measured and added to a glass beaker containing a 10 ml solution composed of 10 volumetric parts of concentrated HNO_3 (65%) and 3 volumetric parts of concentrated sulphuric acid (H_2SO_4). The beakers were left for 30 minutes during which time the initial reaction between the egg and the acid mixture abated. Next, the beakers were heated to 90 °C for 45 minutes, then the temperature was increased to 140 °C. During the boiling process, the volume in each container was maintained above 3 ml by the addition of concentrated HNO_3. The wet digestion process was complete at the time when the solution inside the beakers turned lighter and the released steam became white-coloured. For metal ions fixation, a mixture of 0.5% hydrochloric acid (HCl) and 2% HNO_3 was added to each solution. After filtration through ceramic filters, the samples were collected in sterile containers and double-distilled water was added until 25 ml final volume per sample was reached.

A blank sample was prepared in a similar way without the addition of the egg component.

ICP-OES

The analytical investigations were performed with a high-resolution radial viewing ICP-OES system - HORIBA JY ULTIMA 2 (Jobin Yvon, Longjumeau, France). The stock solutions of the elements of interest were prepared by using Merck mono – element standard solutions, traceable to SRM from NIST 1000 mg/l Certipur.

The ICP-OES working conditions are described in Table 2.

Table 2. CP-OES working conditions.

Parameter	Value
Rf generator power	1.0 kW
Plasma gas flowrate	12 l/min
Auxiliary gas flowrate	0 l/min
Sheath gas flowrate	0.2 l/min
Nebuliser gas flowrate	0.5 l/min
Nebuliser flowrate	2.0 bars
Sample uptake	0.8 ml/min
Argon humidifier	no
Injector tube diameter	3.0 mm

Statistical analysis

The collected data were subject to one-way variance analysis followed by the Tukey-HSD test for testing the effects of the husbandry system on the mineral content of eggs. Differences with $p < 0.05$ were considered statistically significant. All statistical analyses were performed using SPSS for Mac OS (IBM® SPSS® Statistics, version 23, IBM Corp.)

Results and discussion

The physical properties of the eggs showed no significant differences between the different housing systems. The weight of the eggs was 65.3 ± 3.0 g, albumen weight: intensive farming 36.8 ± 2.2 g, 36.2 ± 2.4 g for organic and free-range and 37.0 ± 2.8 g for backyard housing.

Essential elements

Table 3 identifies the detected values for the essential microelements. The higher values for essential microelements are closely dependent on the origin of the eggs – those from backyard, organic and free-range systems showed higher concentrations of essential elements, giving higher nutritional value to the eggs.

Chromium

Considered an essential element in the human diet, chromium functions in maintaining normal glucose tolerance primarily by regulating insulin action. It has been described that the presence of optimal amounts of biologically-active chromium resulted in much lower amounts of insulin being required. Glucose intolerance, related to insufficient dietary chromium, appears to be widespread (Anderson 1992). Although the mechanism of this action has not been clearly described, it has been proposed that chromium binds to an oligopeptide to form chromodulin, a low-molecular-weight, chromium-binding substance that binds to and activates the insulin receptor to promote insulin action (Ross et al. 2014).

In accordance with the US Institute of Medicine (2001), the recommended daily intake for chromium for an adult is 25–35 mcg/day (US Institute of Medicine, Food and Nutrition Board 2001). With the average content of chromium per egg of approx. 2 mcg/serving, a single egg provides 1% of the daily recommended dosage of the mineral.

In 2014, the European Food Safety Authority (EFSA) published a scientific opinion concluding that there is a lack of scientific evidence for chromium being an essential element and, therefore, setting chromium intake recommendations would be inappropriate (EFSA 2014).

As seen in Table 3, the medium value for chromium content is nearly double in the backyard, organic and free-range eggs in comparison with those from intensive farming: 0.05546 mg/kg vs. 0.02166 mg/kg.

Table 3. Trace concentrations of essential elements [mg/kg]. NDL – non-detectable levels.

Elements	Backyard	Organic	Free-range	Soil/Litter	Cages	min	max	Toxic level [mg/kg]
Cr	0.087377	0.033807	0.045204	0.0435248	0.0169195	0.0169195	0.087377	10
Cu	0.837978	1.361827	1.324966	1.0679699	0.6721668	0.6721668	1.361827	250
Fe	2.50444	1.528068	11.56562	0.901086	0.8495411	0.8495411	11.56562	4500
Mn	0.006639	0.057608	NDL	NDL	0.01324	0	0.057608	4000
Zn	1.088555	3.278107	1.5635	2.288861	0.8333671	0.8333671	3.278107	2000

Copper

In its role as an essential mineral, copper has an important role as a co-factor in some enzymes, related to the energy production in the body, synthesis of neurotransmitters and connective tissue, metabolism of iron and activation of neuropeptides (US Institute of Medicine, Food and Nutrition Board 2001; Li et al. 2005).

In accordance with the US Institute of Medicine (2001), the recommended daily intake of copper for an adult is 900–1300 mcg/day (US Institute of Medicine Food and Nutrition Board 2001). One egg contains approx. 10 mcg/serving, thus providing 1.1% of the recommended daily intake of copper.

In our results, the eggs from intensive systems have lower medium concentrations of copper 0.7100 mg/kg compared to 1.1749 mg/kg of those from ecological housing systems.

Iron

Iron is a highly important element in the human body. As part of the blood haemoglobin, it is required for oxygen transport and oxidative metabolism. It is also essential in the processes of cellular growth. Lack of nutritional iron can result in iron-deficient anaemia – a disorder leading to reduced performance and increased morbidity.

The current recommended daily intake for adults is between 10 and 15 mg/day (US Institute of Medicine, Food and Nutrition Board 2001). With their iron concentration, eggs could play an important role in human nutrition as a source of dietary iron. With their low energy values, eggs could be an easy choice for these requiring high iron and low-calorie nutrition. One egg provides approx. 10% of the daily intake of iron and less than 40% of the daily calories intake (for 3000 kcal diet).

In our study, the average iron content in ecological eggs was more than five times higher in comparison with the other housing systems: 5.1993 mg/kg vs. 0.8753 mg/kg. This is the result of the access that some of the hens have to iron-rich vegetation (*Urtica dioica*) and iron-rich soils.

Manganese

Manganese is an essential element with a trace presence in the human body. It acts as a co-factor in many enzymes through whose actions, manganese is involved in amino acid, cholesterol, glucose and carbohydrate metabolism and immune response (US Institute of Medicine Food and Nutrition Board 2001). With its lower concentration in different housing systems products, we cannot consider eggs as an important nutritional source for the mineral. Meeting the recommended daily intake of between 1.8 and 2.6 mg/day will require a high number of consumed eggs (US Institute of Medicine, Food and Nutrition Board 2001).

Our study showed that most of the eggs are with manganese concentrations below the concentrations detectable by ICP-OES.

Zinc

One of the most important elements in the human body, zinc is involved in the catalytic activities of hundreds of enzymes, plays a role in DNA and protein synthesis and assists with the proper functioning of the immune system (US Institute of Medicine Food and Nutrition Board 2001).

With recommended daily average intake for adults between 8 and 12 mg/day, eggs could be an easy choice for providing supplementary zinc with low calorie intake (US Institute of Medicine, Food and Nutrition Board 2001). From our study, the detected concentration of the element would provide approx. 5% of the daily intake from a single egg.

The zinc concentration in both types of housing systems had similar concentrations, with its being a little higher in the ecological systems.

Heavy metals

In addition to the essential microelements, we tested the yolk samples for the presence of some heavy metals. These metals have no biological functions and are considered contaminants in animal forages (EC 2002). Table 4 shows the levels of Al, Cd, Ni and Pb in the yolk obtained from different housing systems.

Aluminium

With high neurotoxicity for the human body, aluminium is considered a key player in the development of Alzheimer's Disease and embryotoxicity (BfR - Bundesinistitut für Risikobewertung 2014). Our study shows that, to reach the level of toxicity, an adult has to consume more than 80 eggs. Small differences between ecological and intensive farming products show that there is no contamination with aluminium in any of the egg groups.

Cadmium

An excess of cadmium in the human body could produce kidney and liver damage, enzyme inhibition, skin conditions and lung cancer. Specific for cadmium is its persistence in the organisms – the level of excretion is very low and can remain resident for years. For humans, the major pathway of exposure is smoking, followed by the consumption of contaminated food and water.

Table 4. Trace concentrations of heavy metal elements [mg/kg]. NDL – non-detectable levels.

Elements	Backyard	Organic	Free-range	Soil/Litter	Cages	min	max	Toxic level [mg/kg]
Al	0.980842	1.551223	1.14409	0.9189452	0.8907284	0.8907284	1.551223	100
Cd	0.019442	0.018028	0.033308	0.0407327	0.0518918	0.018028	0.051892	12
Ni	0.235058	0.094421	0.136302	0.1891993	0.1227397	0.0944213	0.235058	400
Pb	NDL	0.272676	NDL	0	0.1638184	0	0.272676	200

The higher concentrations of cadmium in the intensively-farmed eggs, compared to the ecological ones, can be explained by the higher use of cereal-rich feed in intensive housing systems – corn and wheat (Wang et al. 2017).

Nickel

For proper production of erythrocytes, small amounts of nickel are necessary. However, in excessive amounts, the metal can show some low levels of toxicity. Long-term exposure could result in reduced body weight, liver and heart damage and skin irritations. Short-term exposures are not known to result in any effect on the human body (EFSA 2015).

With similar concentrations of the element in eggs resulting from all the observed housing systems, we can conclude that nickel contamination is absent. The resultant presence demonstrates normal ranges of the element, delivered from feed sources – cereals and greens (Monika et al. 2019).

Lead

With its high toxicity, lead can affect almost every organ in the human body. In small children, lead poisoning produces arrested development, low IQ, CNS damage, mental impairment and hyperactivity. In people of all ages, it can produce anaemia, stomach and muscle weakness, brain damage and kidney failure. Most of the eggs in our research demonstrated lead concentration below detectable levels. Only in organic and cages production have we found concentrations with normal lead presence in forage with 1000–1300 times lower levels than the toxicity threshold.

Other minerals

Besides the essential and heavy metals, we obtained results for some other elements, as shown in Table 5.

Boron is an element naturally found in grains and mostly in leafy vegetables and greens. With an estimated daily requirement of 1–1.5 mg/day for an adult, eggs of any origin can be used as a good dietary source for the element (WHO 1996).

Higher concentrations of barium in ecologically-produced eggs result from the consumption of green vegetables by the hens when they have access to open fields. For

Table 5. Trace concentrations of other non-essential metal elements [mg/kg].

Elements	Backyard	Organic	Free-range	Soil/Litter	Cages	min	max	Toxic level [mg/kg]
B	1.451486	1.696924	1.839805	2.2440477	1.8133759	1.4514857	2.244048	
Ba	0.319478	0.158141	0.213257	0.1488283	0.0993431	0.0993431	0.319478	200
Sr	0.960984	0.524698	0.457679	0.5391281	0.4732974	0.4576793	0.960984	
Ca	125.3539	123.0285	138.6179	188.52904	145.91882	123.02849	188.529	
Mg	443.0058	489.142	496.7324	448.8178	473.33271	443.00577	496.7324	11200

backyard hens, the level of the element can be explained with the addition of lettuce and carrots to their diet.

A higher concentration of strontium in backyard eggs can be explained by richer concentrations of the element in the soils from the areas where the hens were housed. It is evident from other studies that the presence of strontium in eggs has the opposite correlation with calcium – calcium is lower in concentration in the eggs with higher strontium presence (Doberenz et al. 1969).

Magnesium is required for maintaining numerous body processes – muscle and nerve functions, heart rate, blood sugar and DNA production. With a daily recommended dosage of 310–420 mg per adult, eggs are a good source for a healthy diet (US Institute of Medicine, Food and Nutrition Board 1997). Our results demonstrated very slight differences between the eggs from all tested housing systems.

Discussion and conclusions

The eggs from ecologic and free-range systems showed that they are richer in essential elements Cr, Cu, Fe, Mn and Zn. However, the concentration of heavy metals, like Cd, was higher in intensive farming compared with the ecological systems. This may be due to contamination of the food composition used as hen feed or contamination from the surrounding equipment, used for the hens' housing.

The differences in egg quality from different housing systems suggests that consumers have the ability to improve their dietary intake by selecting eggs from ecological sources, as the ones with higher nutritional value. Furthermore, the results indicate the need for improving the husbandry practices and welfare in animal production with the direction pointing to ecological practices.

References

Anderson RA (1992) Chromium, glucose tolerance and diabetes. Biological Trace Element Research 32(1–3): 19–24. https://doi.org/10.1007/BF02784583

BfR [Bundesinistitut für Risikobewertung] (2014) Aluminiumhaltige Antitranspiranzien tragen zur Aufnahme von Aluminium bei. Stellungnahme. Nr. 007/2014 des Bundesinstituts für Risikobewertung vom 26.

Codling EE, Chaney RL, Charles L, Mulchi CL (2008) Effects of broiler litter management practices on phosphorus, copper, zinc, manganese and arsenic concentrations in Maryland Coastal Plain Soils. Communications in Soil Science and Plant Analysis 39(7): 1193–1205. https://doi.org/10.1080/00103620801925901

Doberenz AR, Weber CW, Reid BL (1969) Effect of high dietary strontium levels on bone and egg shell calcium and strontium. Calcified Tissue Research 4(1): 180–184. https://doi.org/10.1007/BF02279119

EC (2002) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed - Council statement.

EFSA (2014) Scientific opinion on dietary reference values for chromium. EFSA Journal 10(12): 3845. https://doi.org/10.2903/j.efsa.2014.3845

EFSA (2015) The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014. EFSA Journal 15.

European Parliament. Directorate General for Internal Policies (2010) The poultry and egg sectors: evaluation of the current market situation and future prospects. Retrieved from https://www.europarl.europa.eu/RegData/etudes/etudes/join/2010/438590/IPOL-AGRI_ET(2010)438590_EN.pdf

Li Y, McCrory DF, Powell JM, Saam H, Jackson-Smith D (2005) A survey of selected heavy metal concentrations in Wisconsin dairy feeds. Journal of Dairy Science 88(8): 2911–2922. https://doi.org/10.3168/jds.S0022-0302(05)72972-6

Monika M, Małgorzata R, Jacek P (2019) Food as a source of exposure to nickel. Warsaw, Poland. National Institute of Public Health-National Institute of Hygiene 70(4): 393–399.

Ross CA, Caballero BH, Cousins RJ, Tucker KL, Ziegler TR (2014) Modern nutrition in health and disease: 11th edn. Lippincott Williams & Wilkins, 2014, 248–251.

Ternes W, Leitsch S (1997) Chemistry of egg yolk. Eggs and Egg Products Quality. WPSA, 127–144.

US Institute of Medicine, Food and Nutrition Board (1997) Dietary Reference Intakes: calcium, phosphorus, magnesium, Vitamin D and fluoride. National Academy Press, Washington, DC.

US Institute of Medicine, Food and Nutrition Board (2001) Dietary Reference Intakes for Vitamin A, Vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington, DC.

Vilà B (1999) Improvement of biologic and nutritional value of eggs. Cahiers Options Méditerranéennes 37: 379–396.

Wang S, Wu W, Liu F, Liao R, Hu Y (2017) Accumulation of heavy metals in soil-crop systems: A review for wheat and corn. Environmental Science and Pollution Research International 24(18): 15209–15225. https://doi.org/10.1007/s11356-017-8909-5

WHO (1996) Boron. Trace elements in human nutrition and health. Geneva.

Youssef AA, Mohammed AA, Mohammed MS (2014) Evaluation of quality and nutrient contents of table eggs from different sources in the retail market. Italian Journal of Animal Science 13(2): 3294. https://doi.org/10.4081/ijas.2014.3294 [s.l.]