TRANSFERABILITY OF CERTAIN HEAVY METALS FROM HENS FEED TO TABLE EGGS LAID WITHIN DIFFERENT REARING SYSTEMS

Cristina Gabriela Radu-Rusu[1*], I.M. Pop[1], Aida Albu[1], Maria Bologa[1], R.M. Radu-Rusu[1]

[1]*University of Agricultural Sciences and Veterinary Medicine, Iasi, Romania*

Abstract

Conversion from conventional to alternative farming systems in aviculture, especially in the table eggs producing sector, imposed various studies to be carried out, related to the effect given by technological changes on the nutritional and sanogenic quality of these animal originated products. Within a wider study, there were monitored the alterations related to mixed feed content in certain pollutants, such as heavy metals and the proportion of their transfer in eggs. As biological materials, we used feed and samples and eggs laid by 500 Lohmann Brown hens, randomly allocated in two groups, on the housing system criteria: IC group-improved cages (250 hens), FR group-free range farm (250 hens). 100 eggs produced during the 42nd week of fowl life (laying peak) were sampled from each group. Yolks and albumens were submitted to atomic absorption spectrometry to assess heavy metals. Pb was below detection limit (0.012 ppm). For other metals, there were found slightly higher levels in the yolks produced in the free range system: Cd from 0.018 ppm (IC group) to 0.023 ppm (FR group); Cu from 2.591 ppm in improved cages vs. 2.734 ppm in free range farm ($P<0.01$, statistically distinct significant differences). Zn levels also exceeded in free range system, varying thus between 5.386ppm (IC) to 5.522 ppm (FR). Lower levels of heavy metals were found in albumens, compared to yolks. All levels did not exceed the toxicity limits for humans.

Key words: laying hens, improved cages, free range, eggs, heavy metals

INTRODUCTION

Certain studies on poultry products favour organic products, especially for the sensorial qualities, while others favour the ones produced in the conventional technology systems as they can be easily managed against risk factors on food safety and for the nutritional value of the finished product. In successive studies [1; 2], there were not noticed major differences related to eggs inner quality, under the influence of conventional and alternative farming systems for laying hens. However, free range eggs appear to have less internal unconformities [12]. Besides commercial quality, food safety is a high priority factor which should be in depth analysed, when several rearing systems for laying hens are compared. Chemicals that could threaten consumers' safety usually come from feedstuffs or fowl exposition to the organic persistent pollutants, pesticides and heavy metals from environment. These substances bio-accumulate across the food chain and are not totally inactivated in the polluted organisms. Chronic exposure to these products lead, on long term, to undesirable effects for consumers' health and organic resistance. Free range eggs were found more concentrated in heavy metals, compared to the conventionally produced ones, due to the intense soil contamination with those pollutants [17]. Higher exposure to indoor pollutants (especially medical treatments and decontaminants residues) was reported in the fowl reared in conventional cages [14]. Therefore, the topic remains controversial, so it could not be automatically assumed that a certain technological system will produce more or less contaminated eggs. This paper emphasis on some results issued from wider

*Corresponding author: radurazvan@uaiasi.ro
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researches which aimed to identify the ways in which the alternative farming systems used in laying hens rearing (compulsory in the EU since January 1st, 2012) [7] induce pollutants bioaccumulations in fowl organisms or their excretion into eggs.

MATERIAL AND METHOD

Hypothesis: The technological system used in laying hens rearing does not affect heavy metals inner content of laid eggs. *Experimental factor:* Hens rearing system (conventional cages, improved cages, free range). *Rationing criterions:* lead (Pb), Cadmium (Cd), Cooper (Cu) and Zinc (Zn) levels in feed and eggs compounds.

Biological material: Five hundred Lohmann Brown classic hens, aged 42 weeks (laying plateau), were reared in three different systems, approved by EU regulations on poultry welfare [7] and were randomly allocated 250 individuals per each group: IC group-Specht furnished cages-at least 800 cm^2/hen (approved as alternative system); FR-free range group, up to maximum 9 hens/m^2 (approved as alternative system). The material introduced in analysis consisted in feed and eggs from each system. Same feed recipe was used, based on a corn-soymeal mixture.

Sample collection and preparation, method of analysis: Feed was sampled in 20 repetitions of 100 g, from each group. Eggs were collected (100 per group), then broken, yolks and albumens separated and mixed apart. 1000 ml were collected from yolks mixture and from albumens mixture. Then, the remnants of yolks and albumens were re-mixed into the so called "melange", from which 1000 ml were also used. Samples of feed and eggs components were dried 48 hours into a forced air convection oven at 60°C, in order to reach a known level of moisture/dry matter, then grinded. The subsequent powders were preserved through freezing, till investigated for heavy metals content. GBC-AVANTA atomic absorption spectrometer was used to identify and quantify the investigated pollutants in samples, using the atomic absorption spectrometry method (AAS), in accordance with the SR EN 14082/2003 standard [16]. The spectrometer was endorsed with a flame burner oven for analysis. The principle of the method consists in calcinations of samples, dissolving of the resulted ash in hydrochloric acid and evaporation to dryness of the solution obtained. The residue was then treated with nitric acid 0.1 mol / L. The standard involves two stages: separation of metal cations from analyzed samples (metal phase transition in ionic state) and the assessment itself (instrumental analysis at spectrometer). The procedure comprised 320 repetitions which meant: 4 samples (feed, dried yolks, dried albumens, dried melange) x 4 metals x 20 repetitions). Metal separation during 1st stage was achieved by calcinating the samples in crucibles, at 450ºC, inducing the passage of metals in ionic or ionized form. This process allowed the overall assessment, gravimetric, of mineral content because residue from burning contains all mineral substances as salts form. The amount of ash produced was calculated using the simplified equation:

$$Ash\,\% = (Mi/M0) \times 100$$

in which: M_i = *mass of ash from the sample (g);* M_0 = *mass of the analyzed sample (g)*

After complete calcination, 5 mL of hydrochloric acid (6 mol/L) were added. The resulting mixture was subjected to evaporation on marine-bath and subsequent dissolution in a defined volume of nitric acid (0.1 mol / L). Crucibles and the resulting solution were let 2 hours to rest, then were transferred into a plastic container. The solutions prepared were introduced into the device to be read from the calibration curve, priory achieved. Thus, the construction of calibration curve initially assumed the achievement of optimal operating parameters as specified by spectrometer manufacturer in official documentation. This step was followed by measuring the absorbance of each successive standard solutions. The achieved results were marked in 5 points on calibration curves for the following metals: lead (0,2; 0,4; 0,6; 0,8; 1 ppm, at λ = 217 nm), cadmium (0,1; 0,3; 0,5; 0,7; 1 ppm at λ = 228.8 nm), copper (1; 1,5; 2,5; 3,5; 5 ppm at λ = 324.8 nm) and zinc (0,5; 1; 1,5; 2,0; 2,5 ppm at λ = 213.9 nm). The detection limits were of 0.012 ppm (mg/kg) for Pb; 0.016 ppm (mg/kg) for Cd, 0.006 ppm (mg/kg) for Cu and 0.020 ppm (mg/kg) for Zn, respectively. The results were processed and calculated by the AAS-AVANTA bundled software, according to the equation:

$$E \text{ mg/kg (ppm)} = (C \times V \times 1000) / M \times 1000^n$$

in which: *E = content of analyzed element; C = quantity taken from the standard curve (µg/mL); V = total volume of the sample solution (50 mL); M = quantity of sample analyzed (g); 1000 = reporting factor content in 1000 g;* 1000^n *= conversion factor of µg in mg.*

Statistical analysis: Data collected were subjected to computation, then presented as mean ± standard deviation and variation coefficient. The one-way ANOVA algorithm was applied to identify the significance threshold for each means comparison, using SPSS software, followed by post-hoc detailing. Data were also compared to other findings in scientific literature and legislation articles related to food and consumer safety.

RESULTS AND DISCUSSIONS

The average values of heavy metals contents in combined feed, yolks, albumens and whole eggs are presented in table 1, for both studied systems.

In the furnished cages system, the highest heavy metals concentrations were analytically identified in the combined feed. Pb value reached 0.079±0.007 mg/kg, but its transferability in eggs could not be quantified, using the spectrophotometer detection limit. Thus, it was fulfilled the compliance with the EC Regulation no. 1881/2006 [9], which stipulates a 0.020 mg/kg tolerable limit for aliments intended for human consumption. For Cadmium, although the detected level in feed was 2.5 folds lower (0.031±0.0008 mg/kg), compared to that measured for Pb, approximately 6.5 % passed into albumen composition (0.002±0.001 mg/kg), and environ 51.6 % in yolk (0.016±0.001 mg/kg) or, overall, 61.29% in melange-whole egg (0.019±0.001 mg/kg).

In all situations, Cd level from eggs or edible compounds were situated below the tolerable limit, specified by the EC Regulation no. 1881/2006 (0.050 mg/kg) [9]. Levels of 5.258±0.444 mg/kg were found for Cu, respectively of 20.003±1.836 ppm for Zn.

The transfer of these contaminants in egg or in its edible compartments apart was realised, for Cu, almost integrally (86.05%) or partially (33.74% in albumen and 49.37% in yolk). As absolute levels, there were excreted 4.525 mg/kg Cu, that meant 0.25 mg Cu/egg of 60 g. Thus, for an adult consumer weighing 75 kg which consumes such an egg, Cu dosage would be 0.0033 mg/kg body weight, meaning approximately 33% of the daily toxicity level (0.01 mg/kg body weight/day), calculated for a continuous aliment ingestion throughout 14 consecutive days. Zinc values reached 5.683±0.374 mg/kg in whole egg, most of it through the yolk (5.452±0.345 mg/kg). A similar calculus for an adult consumer weighing 75 kg reveal a Zn uptake of 0.32 mg/egg of 60 g and consequently 0.004 mg/kg body weight, which represents 1.33 % of daily toxicity limit (0.03 mg Zn/kg body weight/day). In conclusion, the highest transfer from feed to egg was recorded for the Cooper.

In the free range rearing system, the highest concentrations in heavy metals were assessed for Cu (5.216±0.450 ppm) and Zn (19.972±1.851 ppm), hence higher heavy metals transferability into eggs. Despite these facts, heavy metals in white, yolk and whole eggs were detected under the upper tolerable limits, accordingly to EC Regulation no. 1881/2006 [9]. Lead, which presented 0.083±0.014 ppm mean level in feed, did not reach the egg. It was noticed that the highest transfer rate from feed to egg was met for Cu (89.55%), followed by Cd (77.70%) and Zn (22.52%). In both rearing systems, the avian organism acted as a veritable filter for lead residues, the excretion rate through eggs being practical null.

Lead concentration in feed represented 6.5-11.6% (group IC) or 8.03-14.41% (group FR) of the maximum tolerable level, as specified by other authors [3; 4] or 0.79-0.98 % of the limit imposed by European regulations (EC Reg 574/2011) [10], respectively.

Table 1 Heavy metals in the diet used in feeding laying hens reared in improved cages free range system and their transfer in laid eggs

Sample	Analysed heavy metal	IC group (furnished cages)			FR group (free range system)		
		$\bar{x}$	±StDev	CV%	$\bar{x}$	±StDev	CV%
Combined feed	Pb ppm (mg/kg)	0.079	0.007	9.05	0.098	0.008	8.61
	Cd ppm (mg/kg)	0.031	0.002	7.21	0.036	0.003	7.39
	Cu ppm (mg/kg)	5.258	0.444	8.44	5.216	0.450	8.62
	Zn ppm (mg/kg)	20.003	1.836	9.18	19.972	1.851	9.27
Egg white	Pb ppm (mg/kg)	BDL	-	-	BDL	-	-
	Cd ppm (mg/kg)	0.002	0.0001	5.19	0.003	0.0002	5.28
	Cu ppm (mg/kg)	1.774[a]	0.086	4.87	1.883[c]	0.095	5.06
	Zn ppm (mg/kg)	0.058	0.004	6.25	0.067	0.005	7.13
Egg yolk	Pb ppm (mg/kg)	BDL	-	-	BDL	-	-
	Cd ppm (mg/kg)	0.016	0.001	5.21	0.022	0.001	6.08
	Cu ppm (mg/kg)	2.596[a]	0.128	4.93	2.728[c]	0.139	5.09
	Zn ppm (mg/kg)	5.452[a]	0.345	6.32	5.508[b]	0.410	7.44
Whole egg	Pb ppm (mg/kg)	BDL	-	-	BDL	-	-
	Cd ppm (mg/kg)	0.019	0.001	5.24	0.028	0.002	5.9100
	Cu ppm (mg/kg)	4.525[a]	0.228	5.03	4.671[c]	0.239	5.12
	Zn ppm (mg/kg)	5.683	0.374	6.58	5.694	0.417	7.32

*BDL – below analytical detection limit
[ab]– different superscripts within row: statistically significant differences ($p<0.05$)
[ac]– different superscripts within row: statistically significant differences ($p<0.01$)

The same EU regulation specifies a maximal tolerable level of 1 ppm Cd in combined feed, while the analytical findings indicated a content of 0.031– 0.036 ppm CD, thus 3.1-3.6 % of the critical limit.

Cu average content in feed reached 65-21-65.73% of the maximal admitted limit for this microelement (8 ppm) [3; 4].

The Zn level was assessed close to the maximal tolerated limit [3; 4] (20.003 vs. 20 ppm, thus 100% in the cages system 19.972 vs. 20 ppm, thus 99.86% in the alternative farm).

For eggs white, the literature indicates 0.315 ppm Pb [13], while our analyses did not reveal this heavy metal occurrence.

It is known that most eggs pollutants have tropism for yolks because they (including here heavy metals) are more soluble and addictive for a lipids rich environment, easily oxidable, favouring thus the chelating of highly reactive metallic ions within the organic catena of phospholipids [5]. Thus, in literature, value of 0.12ppm Pb [5]; 0,036ppm Pb [15] and 0.397 ppm Pb [13] were reported in yolk, while in the own findings, the heavy metal was found below the detection limit, for both investigated systems.

For Cadmium, yolks analytical findings revealed double concentration in N group (0.016 ppm) and more than double in C group (0.022 ppm), compared to literature (0.009 ppm) [5]. However, these levels did not pass over the upper admitted level for human consumers (2.5 µg Cd/kg body weight) [11]. Same situation occurred for Zn content, the assessed values (5.452-5.508 ppm) passing 1.5 folds the ones reported by other authors [5].

Overall, heavy metals transfer in eggs was lower in the furnished cages system, due to certain statistically significant differences noticed in comparison with the free range system ($p<0.01$ for Cu levels in whites, yolks and whole eggs and $p<0.05$ for Zn levels-in yolks). This fact indicates that the exposure of fowl to the outer environment could have additive effect, through the accumulation of pollutants in soil then in vegetation, these being consumed by hens. Other pollutants (such as certain pesticides) [6] could also affect fowl organism and metabolism, reduced capability in “cleaning” certain pollutants from eaten feed, therefore higher levels of heavy metals in eggs.

CONCLUSIONS

Trace elements known as heavy metals were transferred from feed to eggs mere intense in the hens accommodated in free range system, compared to those accommodated in furnished cages.

Under all circumstances, the heavy metals content of the eggs was situated below the upper tolerable level of toxicity for human consumers.

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