Journal of Elementology ISSN 1644-2296

Bombik E., Bombik A. 2021.
Assessment of accumulation of fluorine compounds in selected tissues of laying hens depending on the distance from sources of pollutant emissions in Central Pomerania, Poland.
J. Elem., 26(1): 75-85. DOI: 10.5601/jelem.2020.25.4.2041

RECEIVED: 22 July 2020
ACCEPTED: 28 November 2020

ORIGINAL PAPER

ASSESSMENT OF ACCUMULATION OF FLUORINE COMPOUNDS IN SELECTED TISSUES OF LAYING HENS DEPENDING ON THE DISTANCE FROM SOURCES OF POLLUTANT EMISSIONS IN CENTRAL POMERANIA, POLAND*

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Abstract

The research aim was to evaluate the levels of contamination with fluorine compounds in selected tissues of laying hens kept in a free range system in Central Pomerania (Poland) in relation to the distance from contaminant emission sources. The study covered 60, randomly selected, two-year-old laying hens on 6 farms. Fluorine levels in tissues of laying hens were determined with the potentiometric method using an ion-selective electrode from ORION. The results for the tissues and organs, such as pectoral muscles, livers and breastbones, were investigated with an analysis of variance, the Tukey's test and linear correlation coefficients. The analyses revealed a higher mean fluorine compound content in the pectoral muscles, livers and breastbones of laying hens in zone 1, located 50 km to the west of the contaminant emission sources, than in zone 2, situated between 80 and 100 km to the west of the pollution sources. Fluorine compound levels in the pectoral muscles of zone 1 birds were almost twice as high in hens from experimental zone 2 (respectively: 1.835 mg kg^{-1} of DM and 0.978 mg kg^{-1} of DM). A statistically significantly higher average content of fluorine compounds (4.92 mg kg^{-1} of DM) was found in the liver of laying hens in zone 1, nearer to the sources of fluorine compounds emission compared to zone 2 (3.34 mg kg^{-1} of DM). The mean fluorine compounds content in the breastbones of hens in zone 1 was more than 4 times as high as the level of this element identified in the bones of hens kept in zone 2 (respectively: 765 mg kg^{-1} of DM and 183 mg kg^{-1} of DM). A significant positive correlation was confirmed between fluorine compound levels in the breastbones and pectoral muscles. The lower level of fluorine compounds in the examined tissues of laying hens in zone 2 could have been influenced by the dominant west and south wind, which limited the spread of pollution. This may also be due to the lower level of fluorine compounds in soil and feed

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* The research was financed by the Ministry of Science and Higher Education, from funds for statutory activity No 473/16/S.

for laying hens in this zone determined in previous studies. The study showed that the closer the sources of emissions of fluorine compounds, the more fluoride in hard tissues of hens' bodies.

Keywords: fluorine compounds, laying hens, pectoral muscles, liver, breastbones.

INTRODUCTION

Fluorine compounds are toxic substances. Therefore, their increasing presence in the environment requires constant monitoring. Fluorine compounds have been officially recognised as a carcinogenic agent (Machoy-Mokrzyńska 2000). The levels of this element in animal tissues and organs depend on the presence of fluorine compounds in the water, soil and air (Kurzeja 2015, Szostek, Ciećko 2017). Fluorine compounds are unnecessary for the proper functioning of the body, as fluoride is not metabolised and no physiological processes in which fluorides would be necessary are known. When assimilated in excess, its effects are noxious. Elevated fluorine compounds levels in water, already at 2 mg l^{-1}, cause adverse changes in enzyme activities and negatively affect some biochemical parameters of blood serum and tissues of living organisms (Pasternak, Truchliński 1999). When chronically exposed or acutely poisoned, human and animal soft tissues accumulate fluorine compounds. Biological fluids and soft tissues contain negligible amounts of this element (Floriańczyk 1999, Machoy-Mokrzyńska 2000). A safe fluorine compound level for farm animals should not exceed 1.8 mg kg^{-1} of body weight. Even at the lowest concentrations, long-term exposure to fluorine compounds changes cellular homeostasis (Kurzeja et al. 2015). High levels of fluorine compounds were detected in the livers and kidneys, i.e. organs directly responsible for detoxication and removal of final metabolic products from the system. It was confirmed that lung and liver functions are the first to become impaired during long-term exposure of the analysed animals to small doses of fluorine compounds contained in the air (Ogoński et al. 1996). Fluorine compounds levels in the meat of mammals range from 0.2 to 2 mg kg^{-1}, generally amounting to 0.6 mg kg^{-1} in the muscle tissue of healthy animals and approximately 0.9 mg kg^{-1} in those with fluorosis (Wędzisz 1994). The content of fluorine compounds in meat generally does not exceed 8 mg kg^{-1} (Pasternak, Truchliński 1999). The main defence mechanism is the excretion of fluorine compounds in urine and most of the absorbed dose is removed very quickly. Bone fluorine compound content increases as the organism grows older. Fluorine compound accumulation in bones has been confirmed in studies performed by a number of authors (Czerwiński et al. 1981, Papierkowski et al. 1996, Górecki et al. 2006). The body deposits fluoride in hard tissues to protect vital organs from the effects of toxic substances present in the environment. Bone fluorine compound content is a good indicator in an evaluation of an animal's exposure

to excessive amounts of the element present in the environment (Telesiński, Śnioszek 2009).

Fluorine compounds can enter the food chain of poultry with industrial pollution, feeds and water (Maurice et al. 2002). Research conducted by Bombik et al. (2020) showed that the average fluorine content in feed given to chickens in a zone located in northern Poland, in Central Pomerania, up to 50 km from pollutant emission sources, was 3.5 times higher than the average fluorine content in feed in the experimental zone located at a distance of 80 to 100 km west of pollutant emission sources (respectively 17.29 mg kg^{-1} of DM and 4.92 mg kg^{-1} of DM). Statistically, a significantly higher average fluorine content was found in soils in the zone near pollution sources (67.63 mg kg^{-1} of DM) compared to soils from the experimental zone (60.80 mg kg^{-1} of DM). The authors also found a significantly higher average level of fluoride in chicken eggshells near pollutant emission sources (17.52 mg kg^{-1} of DM) compared to the more distant test zone (5.47 mg kg^{-1} of DM). This study also showed an almost twice higher statistically significant mean fluorine content in chicken eggs collected on farms located within 50 km (1.488 mg kg^{-1} of DM) compared to the content of chicken eggs in the experimental zone (0.640 mg kg^{-1} of DM)). Normally, birds consume small amounts of fluorine compounds with food, without unwanted effects. Increased intake of fluorine compounds with feed can cause anaemia, impair erythrocyte membrane integrity, disrupt oxygen and oxygen dioxide transport capacities in animals, as well as affect immunoglobulin levels in broiler chicks (Deng et al. 2013, Liu et al. 2013). Excessive fluorine compound content in a diet, ranging from 800 to 1200 mg kg^{-1}, has been demonstrated to cause marked oxidative stress and disturb the elimination and retention of fluorine compounds in chicks (Bai et al. 2010, Chen et al. 2010). High fluorine compounds content in broiler chick diet can also disrupt the natural balance and structure of intestinal microflora (Luo et al. 2016). Miao et al. (2017) showed that fluorine compounds concentration in the soft tissues of laying hens was positively correlated with F levels in their diet. In laying hens receiving a 3% phosphogypsum supplement in the diet, the highest levels of fluoride compounds were found in muscles, liver, bones and eggs and eggshells (Górecki et al. 2006).

The available literature lacks information on the content of fluorine compounds in the tissues and organs of laying hens on farms located at different distances from pollution emission sources. This study was aimed at assessing the levels of fluorine compounds contamination of selected tissues of laying hens kept in a free range system in Central Pomerania in relation to a distance from contaminant emission sources.

MATERIAL AND METHODS

The analyses were conducted in the area of Central Pomerania (Poland) at different distances from the emission sources of contaminants, including fluorine compounds, constituted by Rafineria Gdańska (Gdansk Refinery), Gdańskie Zakłady Nawozów Fosforowych (Gdańsk Phosphorus Fertilizer Plant), Elektrociepłownia Wybrzeże (Wybrzeże Combined Heat and Power Plant), Keramzyt Gniew (Gniew Expanded Clay Aggregate Plant) and industrial waste dumps, particularly phosphogypsum, slag and ash landfills. Winds from the western and southern directions predominate in this area (https://armaag.gda.pl/wykresy_meteo.htm). The wind velocity varies, the lowest speeds occur in the summer (May - September) – below 3 $m\ s^{-1}$, which is important for the reduced ability of the atmosphere to self-clean.

The study covered 60 randomly selected two-year-old Karmazyn laying hens maintained in a free-range system on 6 farms: 3 of them were located in zone 1, near the Vistula estuary, at a distance of up to 50 km west of the pollution sources, and 3 were situated in zone 2, near the Łeba and Słupia estuaries, between 80 km and 100 km to the west of the emission sources. The hens were extensively reared (in free-range coops). The hens were kept outdoors in flocks of 20-35 birds. Their diet was chiefly based on wholegrain cereals (barley, wheat and oats), green feed and, periodically, on cooked potatoes. No mineral and vitamin additives or prophylactic preparations were applied. The average egg yield of these hens was approximately 180 eggs per year.

10 laying hens were collected from each farm and subjected to decapitation. The hens were dissected to obtain the following tissue and organ samples for analysis: pectoral muscles, livers and breastbones. All steps were carried out in accordance with relevant guidelines and regulations on animal welfare (*Regulation of the Minister of Agriculture and Rural Development …* 2010). This study was carried out in strict accordance with the recommendations in the Directive 63/2010/EU and the Journal of Laws of the Republic of Poland of 2015 on the protection of animals used for scientific or educational purposes. The study was approved by the Polish Local Ethical Commission, Warsaw, Poland (Number: 51/2015).

The samples underwent pressure microwave digestion with the use of a Milestone MLS-1200 microwave oven (Rodushkin et al. 1998). Samples of 0.5 g of tissue (pectoral muscles, liver, breastbones) were obtained for analysis. All the samples were digested in 5 mL concentrated supra-pure grade HNO_3 from Merck.

Fluorine compound levels in the tissues and organs of laying hens were determined with the potentiometric method using an ion-selective electrode from ORION and fluorine levels were presented in mg kg^{-1} of DM.

The numerical results were profiled using: the arithmetic mean ($\bar{x}$),

extreme values (min., max.) and the coefficient of variation (V%). The results for the tissues and organs: pectoral muscles, livers and breastbones were investigated with analysis of variance, i.e. two-way hierarchical classification according to the following model:

$$y_{ijl} = m + a_i + b_{ij} + e_{ijl},$$

where:

y_{ijl} – values of the investigated parameter for the i^{th} level of factor A (zone) and the j^{th} level of factor B (farms within the zones);

m – population mean;

a_i – the effect of the i^{th} level of factor A (zone);

b_{ij} – the effect of the j^{th} level of factor B (farms within the zones);

e_{ijl} – random effect.

The means were compared in detail on the basis of Tukey's test, assuming significance at 0.05 with Statistica 12.5 software (StatSoft Inc., 2015). Additionally, coefficients of linear correlation between fluorine levels in the selected laying hen tissues and organs were identified. The significance of the correlations was assessed at 0.05 and 0.01.

RESULTS AND DISCUSSION

The mean fluorine compounds content in chicken breastbones statistically significantly ($p<0.05$) differed between the experimental zones, with the result for zone 1 almost twice as high in comparison with zone 2 (Table 1).

Table 1

Fluorine compound content in pectoral muscles of laying hens (mg kg^{-1} of DM)

Zone	Farm	Basic parameters			Mean
		arithmetic mean ($\bar{x}$)	extreme values (min.-max.)	coefficient of variance (V%)	
	1	1.825 *b*	1.632 - 1.928	5.3	
1	2	1.565 *a*	1.361 - 1.833	12.4	1.835 *b*
	3	2.114 *a*	1.928 - 2.250	5.4	
	4	1.153 *b*	0.968 - 1.475	16.5	
2	5	0.850 *a*	0.735 - 0.944	8.1	0.978 *a*
	6	0.930 *a*	0.815 - 1.112	8.7	
Overall mean					1.406
$LSD_{0.05}$ for the zones 0.069					
$LSD_{0.05}$ for the farms within the zones 0.144					

a, *b*, *c* – the means in the columns marked with the same letters do not significantly differ ($P \leq 0.05$).

As regards experimental zone 1, statistically significant differences in the mean fluorine compounds content in the pectoral muscles of laying hens were identified between all the analysed farms. The highest level of this element was found in the pectoral muscles of hens on farm 3 (2.114 mg kg^{-1} of DM). In zone 2, a significantly higher fluorine compounds content (1.153 mg kg^{-1} of DM) was identified on farm 4, as opposed to farms 5 and 6, where the differences were not significant. The coefficient of variance in fluorine compounds content in the pectoral muscles of the hens was diverse and ranged between 5.3 and 16.5%. The highest value of the coefficient of variance in fluorine compounds content (in excess of 16%) was observed for the muscles of laying hens on farm 4 in zone 2, with a slightly lower value (above 12%) on farm 2 in zone 1.

Muscle tissue fluorine compounds content did not exceed the 8 mg kg^{-1} recognised as a limit value for mammalian meat (Pasternak, Truchliński 1999). The higher content of fluorine compounds in the pectoral muscles of laying hens in zone 1 could have been caused by the higher levels of fluorine compounds found in previous studies by Bombik et al. (2020) in soil and in feed given to laying hens in this zone. The muscles of laying hens that received a 3% phosphogypsum feed supplement were observed by Górecki et al. (2006) to contain fluorine compounds levels ranging from 1.47 to 3.70 mg F kg^{-1} of DM, in a similar spectrum to the present study results for zone 1. Bombik et al. (2010) identified significantly higher levels of this element in the muscles and bones of horses on farms located in the Vistula estuary area in comparison with the tissues of horses on farms situated 50 kilometres away from the contaminant emission sources.

A statistically significantly higher mean value of liver fluorine compounds content was identified for the laying hens in experimental zone 1 (4.92 mg kg^{-1} of DM) in comparison with zone 2 (3.34 mg kg^{-1} of DM), as shown in Table 2. Significantly higher fluorine compounds levels were detected in the livers of laying hens on farms 2 and 3 (5.29 and 5.65 mg kg^{-1} of DM, respectively) as compared with farm 1. Significant differences in fluorine compounds content in zone 2 were determined between farms 5 and 6 (2.94 and 3.80 mg kg^{-1} of DM, respectively). Górecki et al. (2006) observed mean liver fluorine compounds content values for laying hens at similar levels, ranging from 4.14 to 7.34 mg kg^{-1} of DM. The coefficient of variance in liver fluorine compounds content for the hens was highly diverse and ranged between 6.7 on farm 2 and 26.6% on farm 5.

The mean breastbone fluorine content for laying hens in zone 1 was almost 4 times higher than the corresponding value identified for the breastbones of hens in experimental zone 2, amounting to: 765 and 183 mg kg^{-1} of DM, respectively (Table 3). Moreover, statistically significant differences were determined between the farms located in zone 1. The highest statistically significant mean fluorine content value was identified for the breastbones of hens on farm 2 (949 mg kg^{-1} of DM). It was nearly twice as high as values cited by other authors. Górecki et al. (2006) found high fluorine

Table 2

Fluorine compound content in laying hen livers (mg kg^{-1} of DM)

Zone	Farm	Basic parameters			Mean
		arithmetic mean ($\bar{x}$)	extreme values (min.-max.)	coefficient of variance (V%)	
	1	3.82 *a*	2.65 - 4.69	21.0	
1	2	5.29 *b*	4.79 - 6.02	6.7	4.92 *b*
	3	5.65 i	4.37 - 6.01	9.0	
	4	3.28 *ab*	2.37 - 4.10	18.3	
2	5	2.94 *a*	2.08 - 4.01	26.6	3.34 *a*
	6	3.80 *b*	3.12 - 4.12	7.8	
Overall mean					4.13
$LSD_{0.05}$ for the zones 0.31					
$LSD_{0.05}$ for the farms within the zones 0.64					

a, *b* – as explained in Table 1.

Table 3

Fluorine compound content in laying hen breastbones (mg kg^{-1} of DM)

Zone	Farm	Basic parameters			Mean
		arithmetic mean ($\bar{x}$)	extreme values (min.-max.)	coefficient of variance (V%)	
	1	725 *b*	640 - 883	11.1	
1	2	949 *c*	822 - 1101	9.5	765 *b*
	3	621 *a*	458 - 723	12.4	
	4	199 *a*	149 - 293	20.2	
2	5	179 *a*	165 - 189	4.8	183 *a*
	6	172 *a*	149 - 199	9.5	
Overall mean					474
$LSD_{0.05}$ for the zones 32					
$LSD_{0.05}$ for the farms within the zones 66					

a, *b*, *c* – as explained in Table 1.

compounds levels in long bones of laying hens that received a feed supplemented with 3% phosphogypsum, ranging from 149.3 to 463.8 mg kg^{-1} of DM. The mean fluorine compounds content in bones of adult Goosander ducks was identified at 402.7 mg kg^{-1} of DM (Kalisińska et al. 2014). According to Francelino Araújo et al. (2009), the tibial fluorine compound content for laying hens maintained on diets with different fluorine-phosphorus proportions oscillated between 190 mg kg^{-1} F with the (F:P) proportion of 1:100

and 1730 mg kg^{-1} F with the (F:P) proportion of 1:40. Vikoren, Stuve (1996) observed fluorine compounds concentrations in bones of European herring gulls (*Larus argentatus*) and common gulls (*Larus canus*) in colonies exposed to emissions from two Norwegian aluminium plants located in Karmoy and Sunndal to considerably rise in the female birds. The females in the exposed location had significantly higher fluorine compounds deposits than the males. Coetzee et al. (1997) found the tibial fluorine compounds content in laying hens to linearly increase along with the rise in water fluorine concentration. They also observed that laying hens in farm conditions were able to tolerate a fluorine compound intake of 4.453 mg daily for a period of up to 74 weeks. Higher level of fluorine compounds in examined tissues and organs of laying hens from zone I could have been caused by the higher level of fluorine compounds found in own studies in feed given to laying hens on farms located in this zone (Bombik et al. 2020) and the penetration of fluorine into the body as a result of drinking water or through the respiratory system (Czerwiński et al. 1981, Karpińska et al. 1996). Analyses carried out by Bombik (2010) revealed an influence of the distance from contaminant emission sources on fluorine compounds content in the tissues and organs of cattle and sheep. Higher fluorine compounds levels were detected in the muscles and bones of cattle and sheep on farms situated near the Gdansk Refinery, Gdansk Phosphorus Fertilizer Plant and phosphogypsum landfill.

Both in zone 1 and 2, all the correlation coefficients were positive, which indicates that a rise in liver fluorine compounds levels in laying hens is accompanied with fluorine compounds content increase in the muscles and bones (with a positive, albeit insignificant, tendency) – Table 4. A signi-

Table 4

Coefficients of linear correlation between fluorine compounds levels in the selected laying hen tissues and organs

Zone	Liver – pectoral muscle	Liver – breastbone	Breastbone – pectoral muscle
1 (n=30)	0.161	0.055	0.698**
2 (n=30)	0.142	0.105	0.374*

* significant dependence ($P \leq 0.05$)
** highly significant dependence ($P \leq 0.01$)

ficantly positive dependence, in turn, was revealed between the bone and muscle fluorine levels, more pronounced in zone 1 (r=0.618**) than in zone 2 (r=0.374*).

CONCLUSION

The analyses revealed higher mean fluorine compounds content in the pectoral muscles, livers and breastbones of laying hens in experimental zone 1 located 50 km to the west of the contaminant emission sources in comparison with experimental zone 2 situated between 80 and 100 km to the west of the pollution sources. The lower level of fluorine compounds in the examined tissues of laying hens in zone 2 could have been influenced by the dominant west and south winds, which limited the spread of pollution. This may also be due to the lower level of fluorine compounds found in previous studies in soil and feed for laying hens in this zone. The study showed that the closer the sources of emissions of fluorine compounds, the more the body of hens deposits fluoride in hard tissues. High diversity of fluorine compound content was observed in the tissues and organs of laying hens on farms located in experimental zone 1. This could be due to the genetically conditioned, varied sensitivity of the body to fluorine compounds. Both in zone 1 and 2, the tendency for fluorine compound content in the analysed tissues and organs was positive. A significant positive correlation was confirmed between fluorine compound levels in the breastbones and pectoral muscles. The rise in breastbone fluorine compound content was accompanied by a corresponding increase in the pectoral muscles.

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