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Bromine in chicken eggs, feed, and water from different regions of Ukraine

Oleksandr Orobchenko, Yuliia Koreneva, Anatoliy Paliy, Kateryna Rodionova, Mikola Korenev, Nataliya Kravchenko, Olena Pavlichenko, Svetlana Tkachuk, Oleksandr Nechyporenko, Svitlana Nazarenko

ABSTRACT

The purpose of these studies was to analyse and compare the content of bromine in samples of chicken eggs, feed, and water from different regions of Ukraine in the dynamics of 2016 – 2020: with an increased risk of bromine in products (Kharkiv, Poltava, Dnipropetrovsk and Mykolaiv regions) and outside the risk zone (Volyn, Vinnytsia and Zaporizhzhia). Studies of bromine content in eggs, feed, and water were performed in the laboratory of toxicological monitoring of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv) using X-ray fluorescence analysis. As a result of the conducted researches, the increase of the bromine content in chicken eggs in the dynamics of 2016 – 2020 was established: the bromine content increased regardless of the region of the poultry farm location. The highest bromine concentration in chicken eggs was found in Kharkiv, Dnipropetrovsk, Mykolaiv, and Zaporizhia regions. Bromine source in poultry products is the excessive intake of bromine in the poultry body with alimentary environmental factors (feed and water). Bromine content in feed for chickens increased in the research dynamics (from 35.1% in the Poltava region to 2.5 times in the Zaporizhzhia region). It exceeded the established EFSA (4.4% of the total) and the average in Ukraine (51.2% of the total number of samples). In addition, the average bromine content in feed from poultry farms of the studied regions of Ukraine correlated with the number of registered and approved bromine-containing pesticides. The average bromine concentration in water sources in the studied regions of Ukraine had no significant differences compared to the beginning of the study but exceeded the maximum allowable concentration by 21.7% in 2016, 34.8% in 2018 and 39.1% in 2020. The maximum bromine concentration was in water sources in Mykolayiv, Kharkiv, and Dnipropetrovsk regions.

Keywords: eggs, bromine, feed, water, laying hens.

INTRODUCTION

Eggs are a valuable food product used directly for food or frozen egg products and dry powders production. Chicken eggs are one of the most useful products in the daily diet of a person, especially children. They contain all the necessary nutrients and biologically active substances easily absorbed by the human body **[19]**, **[20]**, **[28]**. In addition to protein, fats (fatty acids), enzymes, and vitamins, egg white and yolk also contain minerals (macro- and micronutrients). Chicken eggs are a source of iron, phosphorus, sulphur, calcium, chlorine, potassium, magnesium, and sodium. In small quantities contain silver, aluminium, boron, barium, bromine, cobalt, chromium, copper, fluorine, iodine, lithium, manganese, molybdenum, rubidium, selenium, silicon, strontium, titanium, vanadium, and zinc. Eggs may also contain heavy metals such as arsenic, bismuth, cadmium, mercury, lead, thallium and others in small concentrations. **[21]**, **[30]**, **[43]**.

However, inorganic elements that are necessary for the proper functioning of the body, as well as heavy metals, can become harmful to the human body by receiving them in high doses or in low, but for a long period **[16]**, **[33]**. It has been proven **[5]** that the introduction of such elements as silver, barium, beryllium, bismuth, cobalt, iron, gallium, mercury, potassium, magnesium, nickel, sulphur, antimony, silicon, zinc and zirconium into the diet of chickens causes increase in eggs of the following elements: bismuth, cobalt, nickel, sulphur, iron, potassium, antimony. When iodine is added to the diet of laying hens, the high content of the element in eggs is noted **[31]**,

[36]. The quality of eggs also depends on the sanitary conditions of productive poultry **[24]**. In recent years, scientists have been interested in an element such as bromine, as it is widely used in various industries (especially in agriculture) and can be included in the food chain **[6]**, **[10]**. It should be noted that today the mechanism of action of bromine on the body is insufficiently studied, and its positive physiological function is not fully proven **[25]**, **[38]**. Given that the WHO recommends human consumption of bromine in the amount of 0.4 $mg.kg^{-1}$ body weight per day **[47]**, we consider it necessary to ensure a safe level of the element in the human body, as eggs and products are widely used, and according to our previous studies **[14]**. When introduced into the diet of laying hens sodium bromide at a dose of 250.0 $mg.kg^{-1}$ of feed in egg white observed a significant increase in bromine (243.52 ± 4.39 $mg.kg^{-1}$) compared with the control group (9.06 ± 0.54 $mg.kg^{-1}$), the bird which received "background" amount of bromine 2.0 $mg.kg^{-1}$ of feed. Also, our previous studies **[13]**. found that bromine is a fairly common element in Ukraine. Its substantive content in the body of animals comes with both water and feed. Water sources with a bromine content of more than 1.8 $mg.dm^{-3}$ and feed concentrators of the element, which are components of feed for laying hens, are dangerous: barley and sunflower oilcake, the element content in which is 8 – 40 $mg.kg^{-1}$. In addition, the accumulation of bromine in feed has certain ecogeographical features: an increase in the content of bromine was mainly found in feed from the southern and eastern regions of Ukraine, especially in areas with developed industrial mining. Given the importance and scale of human consumption of eggs and egg products, this work aimed to analyse and compare the presence of bromine in samples of chicken eggs, feed, and water from different regions of Ukraine.

Scientific Hypothesis

The bromine content in chicken eggs does not significantly depend on the location of the poultry farm and its intake with feed and water gradually increases over time due to anthropogenic stress but does not exceed the WHO recommended dose of 0.4 $mg.kg^{-1}$body weight per day.

MATERIAL AND METHODOLOGY

Samples

Samples of eggs, feed and water were selected in poultry farms with increased risk of bromine contamination in four regions of Ukraine, namely Kharkiv, Poltava, Dnipropetrovsk and Mykolayiv. In addition, samples were collected in Volyn, Zaporizhzhia and Vinnytsia regions, which are outside the zone of this risk. These regions were chosen on the basis of the hydrogeological data from the State Service for Geology and Subsoil of Ukraine, as well as the cartographic data of the elevated bromine groundwater in Ukraine (Figure 1) **[35]**, **[13]**. Monitoring studies were conducted at two-year intervals in 2016, 2018 and 2020. Twelve egg samples (10 pieces per sample) and 12 samples of feed and water (once a month) were taken from each farm during the year. Thus, 252 egg samples, 252 fodder samples and 252 water samples were collected in total during the study period.

Chemicals

Standard sample of bromide ion SSSU 022.66-96, manufactured by SDTB RP IPC NASU, Ukraine;
Gallium (III) oxide, ABCR GmbH & Co, Germany, 99,999% (metals basis);
Silicium (IV) oxide, ABCR GmbH & Co, Germany, pure for analysis.

Animals and Biological Material

Laboratory and farm animals were not used directly during the studies.

Instruments

Studies of bromine content in eggs feed and water were performed in the laboratory of toxicological monitoring of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv) on X-ray fluorescence spectrometer (XRF) "Spectroscan-Max-G" research and production facility Spectron" (St. Petersburg, Russia) according to the developed methodology: Determination of inorganic elements in biological substrates by X-ray fluorescence analysis (guidelines) **[12]**.

Laboratory Methods

The main parameters of the device for measuring spectral parameters: the first display - from 950 mÅ to 3150 mÅ, the second display – from 315 mÅ to 1575 mÅ. The step size of the device and the exposure time were 4.

The method use of X-ray fluorescence of elements with subsequent analysis of the spectra on the device "Spectroscan-MAX". When the sample is irradiated with X-rays, the sample, which is previously subjected to dry mineralization, begins to emit (fluoresce) in the X-ray range.

The spectrum of this secondary fluorescence adequately reflects the elemental composition of the analysed sample. The atom of each element has its characteristic spectral lines. The presence of certain characteristic lines in the recorded spectrum indicates the presence of the corresponding elements in the sample. The concentration of the element is determined by the change in the number of pulses along the characteristic line. The depth of penetration of X-rays into the irradiated sample (matrix) depends on its structure (material). The method provides measurements with a relative error not exceeding 15% with a confidence level of 0.95. The limit for determining the bromine content by this method is: for feed – 0.27 mg.kg^{-1}, biological material (including egg yolk and egg white) – 0.18 mg.kg^{-1}, for water – 0.014 mg.mL^{-1}.

Description of the Experiment

Sample preparation: To prepare samples of eggs and feed for analysis, porcelain crucibles were selected with the volume 5 times exceeding the sample volume. For the analysis of chemical elements by X-ray fluorescence method, we took the average sample of eggs 25.00 – 30.00 g and feed weighing 10.00 – 15.00 g (with an accuracy of weighing up to 0.01 g). Water samples were prepared by 300 – 400 mL evaporation and porcelain crucibles.

Number of samples analyzed: 252 egg samples (10 pieces per sample), 252 feed samples and 252 water samples.

Number of repeated analyses: 12

Number of experiment replication: 3

Design of the experiment:

We incinerated the test material (ashing to black or gray ash) for X-ray fluorescence analysis. The crucibles were placed in a muffle furnace with an adjustable and controlled heating system. When burning samples, the optimal amount of ash is formed at a temperature of 350 – 400 °C for 4 – 6 hours, and from water samples, the dry residue is formed in 2 – 3 hours at a temperature of 250 °C.

To correct the individual fluorescence intensity of each matrix, we applied an internal standard in the form of gallium silica powder to each ash sample.

The weight of the internal standard was selected taking into account the weight of ash and the concentration of the gallium element in the standard. It was weighed to the nearest 0.01 g (usually 0.07 – 0.08 mg of the internal standard – gallium concentration of 5.0 mg.g^{-1} was added to the ash samples of feed and eggs).

The mixture of ash and internal standard was stirred with a glass rod and ground in a mortar. The mass of ash was then determined in the sum of the weight of the introduced standard by weighing to 0.001 g. The crushed material was transferred to glass vials with a volume of 10 – 20 cm^3, closed with stoppers and stored for analysis on the device.

We performed measurements on the device "Spectroscan-MAX", following the instructions for its use. The ash samples prepared for the study were placed in the cuvette of the instrument and sealed. The radiation intensity of an element in the sample depends on each specific test sample's qualitative and quantitative composition. Therefore, this shortcoming was eliminated when quantifying the element by introducing the coefficient (K1) into the basic formula, calculated by the fluorescence intensity of the internal standard (gallium) in each sample and the fluorescence intensity of gallium in the standard on silicon oxide. The total calculation of bromine in the samples was performed by formula (1):

$$X = \frac{ME_{\mathrm{Inst}} \times \mathrm{M}_{stGa} \times 5000}{\mathrm{Ga}_{\mathrm{Inst}} \times \mathrm{M}} \tag{1}$$

Where:

X is the amount of the element in the test product, mg.kg^{-1}; ME_{Inst} - the concentration of the element in the test sample, obtained using the device, mg.kg^{-1}; M_{stGa} - mass of the internal standard in the sample, g; 5000 – concentration of gallium in the internal standard, mg.kg^{-1}; Ga_{Inst} - the concentration of gallium in the test sample obtained by the device, mg.kg^{-1}; M is the mass of the sample of the test material, g.

Statistical Analysis

The obtained results were processed by methods of variation statistics using the software package for analysis of variance (ANOVA) StatPlus 5 (6.7.0.3) (AnalystSoft Inc., USA). Correlations between the data groups were evaluated by the Pearson coefficient, the probability of the obtained results was evaluated by the Tukey criterion (HSD mean difference) at a probability level of 95.0% (p <0.05).

RESULTS AND DISCUSSION

It should be noted that the data on the content of bromine in chicken eggs is not enough: the content of bromine from 4 different regions of China, found its average content (5.51 mg.kg^{-1}), the minimum value was 1.66 mg.kg^{-1} and a maximum of 10.7 mg.kg^{-1}, and the average bromine content in chicken eggs from Pakistan was of 7.3 ± 0.5 mg.kg^{-1} **[26]**. However, in our opinion, it is best to interpret the data obtained by us in relation to the European data given in the EFSA technical report, hereinafter EFSA (max 2.6 mg.kg^{-1}) and established in Ukraine, hereinafter UA (max 4.79 mg.kg^{-1}) **[23]**. The content of bromine in chicken eggs from Kharkiv region in 2016 and 2018 exceeded EFSA by 43.5% and 54.6%, but was within the UA indicator, while in 2020 the content of bromine in eggs exceeded both indicators: EFSA – almost 2 times, and UA – by 7.9%. Compared to the beginning of the research, the content of bromine in chicken eggs from the Kharkiv region tended to increase (by 7.8%) in 2018, while in 2020 it reliably exceeded both the initial indicator by 38.6% (p <0.05) and the indicator in 2018 – by 28.6% (p <0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Kharkiv region in 2016 were 3.23 – 4.21 mg.kg^{-1}; in 2018 – 3.45 – 4.56 mg.kg^{-1} and in 2020 – 4.86 – 5.54 mg.kg^{-1}.

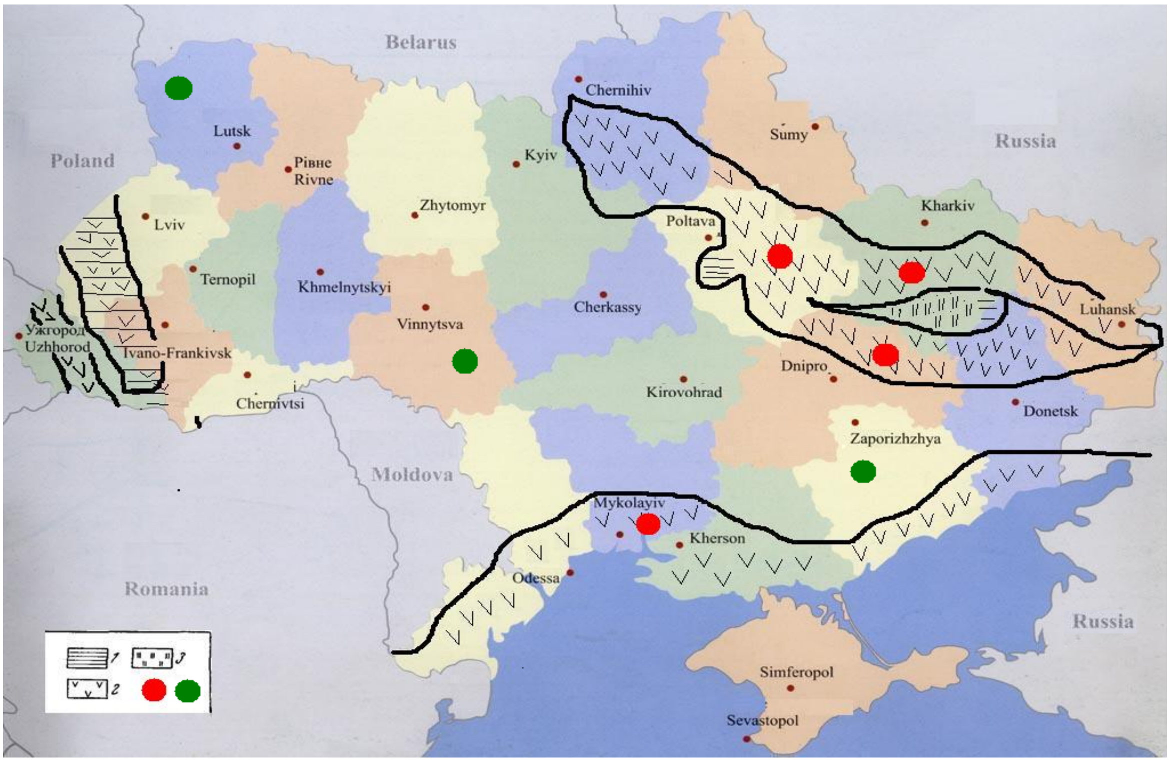

Figure 1 Schematic map of groundwater in Ukraine **[35]**, **[13]**. Note: 1 – 3 – areas of distribution of waters with industrial content (1 – Iodine, 2 – Bromine, 3 – Potassium, red (with the risk of increased bromine) and green (without risk) circle – sampling areas).

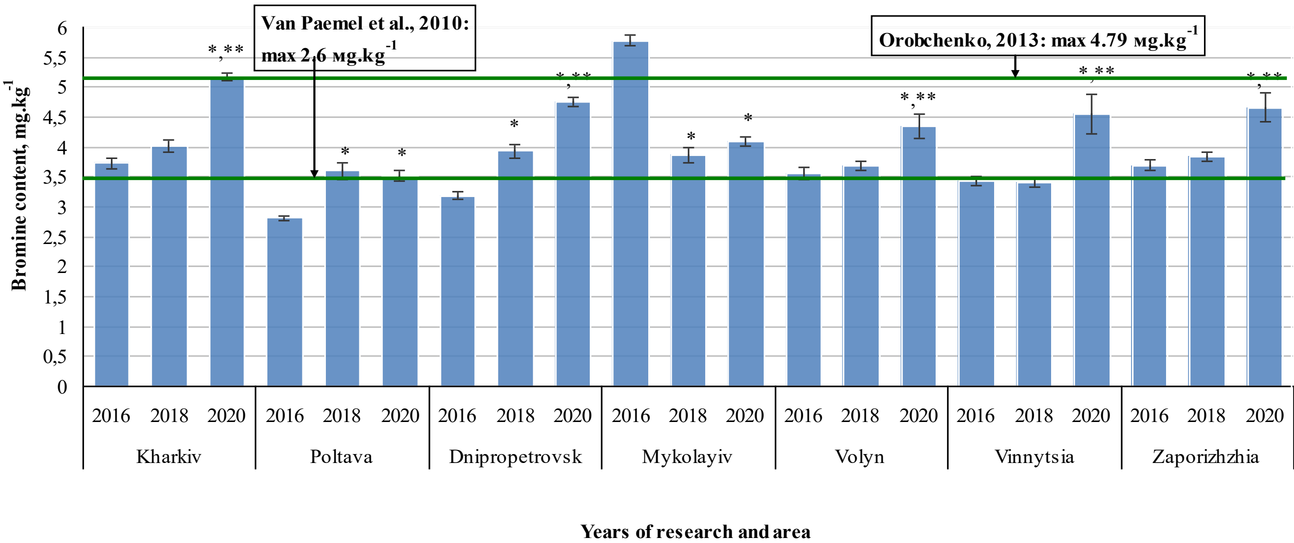

Figure 2 The results of the study of chicken eggs from different regions of Ukraine for bromine content in 2016, 2018 and 2020 (M ± m, n = 12). Note: * – p <0.05 – relative to 2016, ** – p <0.05 – relative to 2018.

Bromine content in chicken eggs from the Poltava region in 2016, 2018, and 2020 exceeded the EFSA technical report by 8.1%; 38.5% and 35.4%, respectively, but in all 3 terms of the research was within the established UA indicator. Relative to the beginning of the research, the content of bromine in chicken eggs from Poltava region in 2018 and 2020 exceeded the initial indicator by 28.1% and 25.3% (p <0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Poltava region in 2016 was 2.51 – 3.04 mg.kg^{-1}; in 2018 – 2.98 – 4.19 mg.kg^{-1} and in 2020 – 2.91 – 4.01 mg.kg^{-1}.

Bromine content in chicken eggs from the Dnipropetrovsk region in 2016, 2018 and 2020 exceeded the EFSA technical report by 22.7%, 51.2% and 83.1%, respectively, but in all 3 terms of the research was within the established UA indicator. Compared to the beginning of the research, the content of bromine in chicken eggs from the Dnipropetrovsk region in 2018 and 2020 exceeded the initial indicator by 23.2% and 49.2% (p <0.05). In addition, in 2020 the bromine content in eggs exceeded the indicator of 2018 by 17.4% (p <0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Dnipropetrovsk region in 2016 was 2.82 – 3.70 mg.kg^{-1}; in 2018 3.45 – 4.71 mg.kg^{-1} and in 2020 4.27 – 5.13 mg.kg^{-1}.

The content of bromine in chicken eggs from the Mykolayiv region in 2018 and 2020 exceeded the EFSA indicator by 48.8% and 57.3%. Still, it was within the UA indicator, while in 2016, the bromine content in eggs exceeded both indicators: EFSA – 2.2 times and UA – by 20.7%. Compared to the beginning of the research, the content of bromine in chicken eggs from the Mykolayiv region decreased by 33.0% in 2018, and in 2020 the decrease was by 29.2% (p <0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Mykolayiv region in 2016 were 5.24 – 6.25 mg.kg^{-1}; in 2018 – 3.12 - 4.47 mg.kg^{-1} and in 2020 – 3.84 – 4.54 mg.kg^{-1}.

Bromine content in chicken eggs from the Volyn region in 2016, 2018 and 2020 exceeded the EFSA technical report by 36.9%; 41.9% and 67.3%, respectively, but all three research terms were within the research established UA indicator. Compared to the beginning of the study, the content of bromine in chicken eggs from the Volyn region tended to increase (by 3.7%) in 2018, while in 2020, it reliably exceeded both the initial indicator by 22.0% (p <0.05) and the indicator in 2018 – by 15.2% (p <0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Volyn region in 2016 were 3.06 – 4.09 mg.kg^{-1}; in 2018 – 3.35 – 4.13 mg.kg^{-1} and 2020 – 3.45 – 5.62 mg.kg^{-1}.

Bromine content in chicken eggs from the Vinnytsia region in 2016, 2018 and 2020 exceeded the EFSA technical report by 31.9%; 31.2% and 74.6%, respectively, but in all 3 terms of the research was within the established UA indicator. Compared to the beginning of the research, the content of bromine in chicken eggs from the Vinnytsia region did not have significant deviations in 2018, while in 2020 it reliably exceeded both the initial indicator by 24.4% (p <0.05) and the indicator in 2018 – by 24.9% 0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Vinnytsia region in 2016 were 3.02 – 3.82 mg.kg^{-1}; in 2018 – 3.16 – 3.91 mg.kg^{-1} and in 2020 – 3.07 – 5.96 mg.kg^{-1}.

Bromine content in chicken eggs from the Zaporizhzhia region in 2016, 2018 and 2020 exceeded the EFSA technical report by 41.9%; 47.7% and 79.2%, respectively, but in all 3 terms of the research was within the established UA indicator. Compared to the beginning of the research, the content of bromine in chicken eggs from the Zaporizhzhia region did not have significant deviations in 2018, while in 2020 it reliably exceeded both the initial indicator by 26.3% (p <0.05) and the indicator in 2018 – by 21.4% (p <0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Vinnytsia region in 2016 were 3.12 – 4.11 mg.kg^{-1}; in 2018 – 3.44 – 4.27 mg.kg^{-1}, and 2020 – 3.56 – 5.91 mg.kg^{-1}.

From research results, we can see the tendency to increase bromine content in chicken eggs in almost all areas (except the Mykolayiv region), even in those which are out of a risk zone. It should also be noted the higher concentration of bromine in eggs from Kharkiv, Dnipropetrovsk, and Zaporizhzhia regions, which is due to more developed industrial activities and, accordingly, the technogenic load on the ecosystems of the areas. Despite the above, the average bromine content in eggs from poultry farms of the studied regions of Ukraine had no significant differences compared to the beginning of the study and in 2016 was 3.74 ± 0.10 mg.kg^{-1}, in 2018 – 3.77 ± 0.04 mg.kg^{-1}, and in 2020 – 4.44 ± 0.09 mg.kg^{-1}, i.e., there was a tendency to increase by 18.7% and 17.8%, respectively, compared to 2016 and 2018).

The ingress of inorganic elements (including bromine) in products can occur mainly due to excessive entry into the body of animals (poultry) with alimentary environmental factors (feed and water) **[11]**, **[37]** which became the subject of our further research.

In Ukraine, the bromine content in the feed is not regulated. The monograph **[1]** gives the maximum allowable level of inorganic bromides in feed, 35.0 mg.kg^{-1}. In the EFSA technical report **[41]**, the bromine content in complete feeds is up to 9 mg.kg^{-1}. The maximum tolerable level of bromine in poultry feed, according to **[22],** is 2500.0 mg.kg^{-1}, which also contributes to the accumulation of bromine in the products, as there are no symptoms of poisoning due to large amounts of the element in the body of poultry.

The EFSA report **[41]** in addition to the content of bromine in the product also indicates its content in feed (max 9.0 mg.kg^{-1}), but our previous studies showed that the average content of bromine in feed for poultry in Ukraine is 3.65 mg.kg^{-1} **[13]**.

Thus, compared to EFSA data, no excess bromine content was found in the feeds of all poultry farms in the studied areas, with the exception of bromine content in feeds from Kharkiv region for the period of 2020, which exceeded EFSA data by 18.7% (Figure 3).

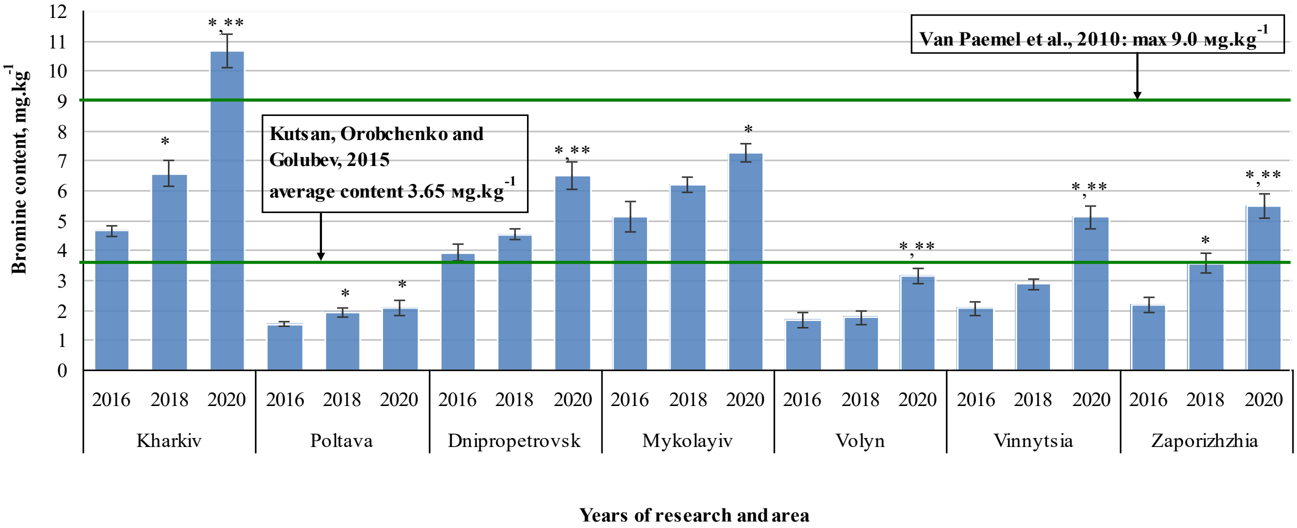

Figure 3 The results of the study of feed for chickens from different regions of Ukraine for bromine content in 2016, 2018 and 2020 (M ± m, n = 12). Notes: * – *p* <0.05 - relative to 2016, ** – *p* <0.05 - relative to 2018.

Compared to the average indicator in Ukraine, the content of bromine in feed from Kharkiv region exceeded it by 27.7% in 2016, by 80.3% in 2018, and in 2020 the excess was 2.9 times (Figure 3). Fluctuations in the content of bromine in feed for chickens from the Kharkiv region in 2016 was 4.04 – 5.75 $mg.kg^{-1}$; in 2018 – 5.04 – 9.18 $mg.kg^{-1}$ and in 2020 – 7.89 – 13.49 $mg.kg^{-1}$.

In Poltava and Volyn regions, the content of bromine in feed did not exceed the average in Ukraine. Fluctuations in the bromine content in compound feeds for chickens in 2016 were 1.02 – 1.84 and 0.84 – 3.16 $mg.kg^{-1}$; in 2018 – 1.24 – 3.05 and 0.89 – 2.98 $mg.kg^{-1}$ and in 2020 – 1.01 – 3.62 and 1.67 – 4.89 $mg.kg^{-1}$, respectively.

In the Dnipropetrovsk region, the bromine content exceeded the average in Ukraine by 7.9%; 24.4% and 78.1% in 2016; In 2018 and 2020, respectively, a similar situation was found in the Mykolayiv region: the average indicator was exceeded by 40.5%; 69.9% and 99.5% respectively. Fluctuations in the bromine content in compound feeds for chickens in 2016 were 3.08 – 6.12 and 3.29 – 8.69 $mg.kg^{-1}$; in 2018 – 3.73 – 5.78 and 4.44 – 7.47 $mg.kg^{-1}$ and in 2020 – 4.43 – 9.62 and 5.27 – 8.59 $mg.kg^{-1}$, respectively.

In Vinnytsia and Zaporizhzhia oblasts, the bromine content exceeded the average in Ukraine only in 2020 by 40.5% and 50.1%, respectively (Figure 3). Fluctuations in the bromine content in compound feeds for chickens in 2016 were 0.89 – 3.33 and 1.05 – 3.81 $mg.kg^{-1}$, in 2018 – 2.14 – 3.85 and 2.30 – 6.78 $mg.kg^{-1}$ and in 2020 – 3.87 – 8.26 and 3.20 – 8.23 $mg.kg^{-1}$, respectively.

When comparing the content of bromine in feed relative to the beginning of research (2016) it was found that in the Kharkiv region in 2018 the content of bromine increased by 41.2% (*p* <0.05), and in 2020 – 2.3 times, which compared to 2018 was 62.3% (*p* <0.05).

In the feed from Poltava region in comparison with 2016, the bromine content reliably (*p* <0.05) increased by 24.0% and 35.1% in 2018 and 2020, respectively.

In compound feeds from the Dnipropetrovsk region in 2018, relative to the beginning of research, only a tendency to increase the bromine content (15.2%) was established, while in 2020 the bromine content in feeds exceeded the initial indicator (*p* <0.05) by 65.0%, which compared to 2018 was 43.2% (*p* <0.05).

In compound feeds for poultry from the Mykolaiv region in 2018, a tendency to increase the bromine content (20.9%) was established, while in 2020 the bromine content in the feed exceeded the initial indicator (*p* <0.05) by 41.9%.

In compound feeds from Volyn region in 2018, relative to the beginning of the research, only a tendency to increase the bromine content (6.0%) was established, while in 2020 the bromine content in feeds exceeded the initial indicator (*p* <0.05) by 89.2%, which compared to 2018 was 78.5% (*p* <0.05).

In feed for poultry from Vinnytsia region in 2018 compared to the beginning of the study only a tendency to increase the bromine content (39.6%) was found, while in 2020 the bromine content in the feed exceeded the initial indicator (*p* <0.05) by 2.5 times, which compared to 2018 was 77.5% (*p* <0.05).

In the Zaporizhzhia region in 2018 the bromine content in compound feeds for poultry increased by 63.5% (p <0.05), and in 2020 – 2.5 times, which compared to 2018 was 53.1% (p <0.05).

The average content of bromine in compound feeds from poultry farms of the studied regions of Ukraine had no significant differences from the beginning of the study, but there was a tendency to increase: in 2016 the content was 3.03 ± 0.18 mg.kg^{-1}, in 2018 – 3.92 ± 0.22 mg.kg^{-1}, and in 2020 – 5.76 ± 0.32 mg.kg^{-1}, which was 29.4% and 90.1%, respectively, compared to 2016 (Figure 3).

It should be noted that excessive amounts of bromine can be released into the environment with brominated flame retardants. They are widely used in the manufacture of electronics, cars, furniture, building materials, etc. to slow down the ignition of combustible materials in case of fire.

Thus, in 2000, 38% of world production of bromine was used as flame retardants. During operation or after the end of service life and improper disposal of such products, brominated substances may leach or evaporate and thus contaminate the environment and food **[4]**, **[45]**, **[48]**. Due to the detection of these compounds in samples of various environmental objects on the recommendation of the European Commission **[2]**, Member States of the European Union should monitor their presence in food. According to Fernandes AR et al. **[6]** the highest ranges of flame retardant concentrations were found in fish, fish products and fish feed. Similar data were obtained by other authors **[3]**, **[7]**, **[27]**. Wang et al. **[42]** noted in their studies the content of polybrominated biphenyl ethers in feed and meat of various Chinese producers. In the 1980s, bromine compounds were widely used as pesticides, while today most European countries have abandoned the use of bromine-containing pesticides due to their negative impact on the Earth's ozone layer **[39]**.

In Ukraine, on the contrary, the number of such drugs is growing: according to **[18]**, at the beginning of 2012 in Ukraine, only three drugs were registered and allowed to use; at the beginning of 2016 – already 23, at the beginning of 2018 – 40, while at the beginning of 2021 – 52 drugs with active substances that contain bromine (mainly diquat dibromide, bromadiolone, bromoxynil octanoate, metobromuron, bromuconazole).

It should be noted that the average bromine content in compound feeds from poultry farms of the studied regions of Ukraine correlated with the number of registered and permitted for use bromine-containing pesticides (r = 0.96). Our data can be confirmed by the fact established on the example of methyl bromide in 1981, the possibility of accumulating about 10 – 30% of the pesticide in the soil with subsequent cleavage into bromide and inclusion in vegetable raw materials for the manufacture of feed **[44]**.

In Ukraine, there are no regulations on the rationing of bromine in water used for watering animals and poultry. In contrast, bromine's maximum permissible concentration (MPC) in human drinking water is 0.23 mg.L^{-1} [34]. The data analysis showed that in the sources from the Kharkiv region, the bromine content exceeded the MPC during the whole research period: in 2016 by 17.4%, in 2017 by 1.9 times and in 2020 by 2.5 times (Figure 4).

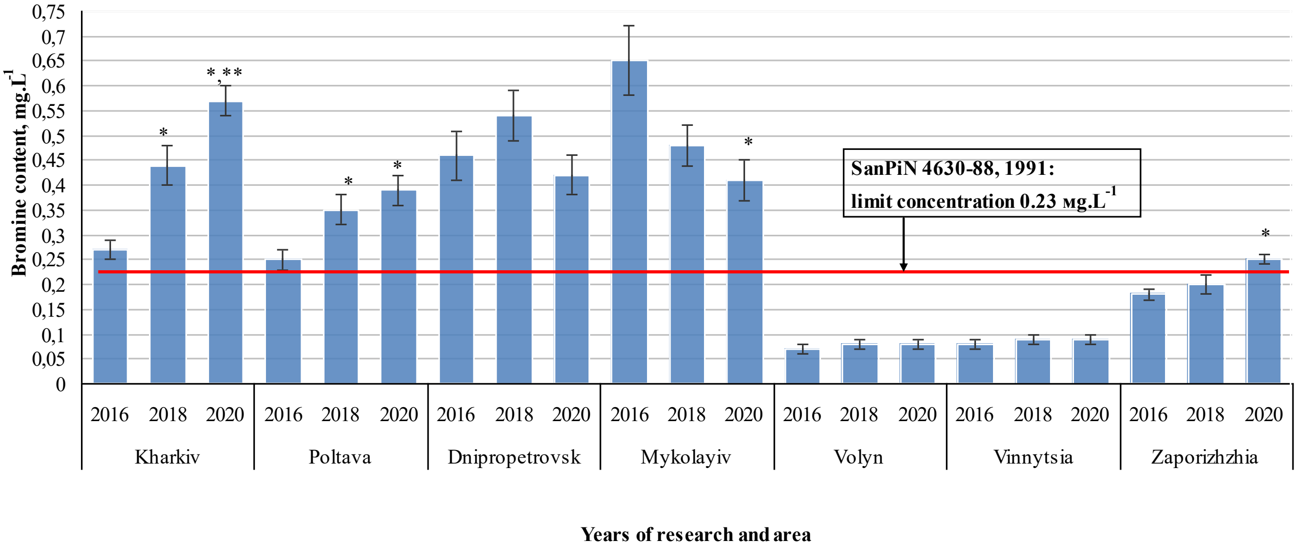

Figure 4 The results of the study of water for chickens from different regions of Ukraine for bromine content in 2016, 2018 and 2020 (M ± m, n = 12). Note: * – p <0.05 – relative to 2016, ** – p <0.05 – relative to 2018.

Compared to the beginning of research (2016), the concentration of bromine in water increased by 63.0% (p <0.05) in 2018 and 2.1 times in 2020 (p <0.05), which exceeded the indicator of 2018 by 30.0% (p <0.05)

(Figure 4). Fluctuations in the content of bromine in water from poultry farms in the Kharkiv region in 2016 was 0.17 – 0.35 mg.L^{-1}; in 2018 – 0.19 – 0.61 mg.L^{-1} and in 2020 – 0.44 – 0.82 mg.L^{-1}.

In sources from poultry farms of the Poltava region, the bromine content exceeded the maximum concentration limit throughout the study period: in 2016 by 8.7%, in 2018 by 52.2% and in 2020 by 69.6% (Figure 4). Compared to the beginning of research (2016), the concentration of bromine in water increased by 40.0% (p <0.05) in 2018 and by 56.0% in 2020 (p <0.05) (Figure 4). Fluctuations in the bromine content in water from poultry farms in the Poltava region in 2016 were 0.14 – 0.33 mg.L^{-1}; in 2018 – 0.15 – 0.51 mg.L^{-1} and in 2020 – 0.24 – 0.52 mg.L^{-1}.

The concentration of bromine in water sources for poultry from the Dnipropetrovsk region was consistently high throughout the study period (2016 – 2020) and had no significant deviations from the start of research: in 2016 the maximum concentration limit was exceeded 2 times, in 2018 – 2.3 times and in 2020 year – 1.8 times. Fluctuations in the content of bromine in water from poultry farms in the Dnipropetrovsk region in 2016 was 0.23 – 0.67 mg.L^{-1}; in 2018 – 0.25 – 0.78 mg.L^{-1} and in 2020 – 0.20 – 0.62 mg.L^{-1}.

In the sources of water for poultry from the Mykolayiv region, the reverse dynamics of bromine concentration was established: in 2018 there was a tendency to decrease (by 26.2%), and in 2020 a decrease was reliable (p <0.05) and amounted 58.5%, despite this, the concentration of bromine was higher than the MPC by 2.8; 2.1 and 1.8 times respectively in 2016; 2018 and 2020. Fluctuations in the bromine content in water from poultry farms in the Mykolaiv region in 2016 were 0.41 – 1.13 mg.L^{-1}; in 2018 – 0.24 – 0.73 mg.L^{-1} and in 2020 – 0.23 – 0.56 mg.L^{-1}. We connect the received data in the Mykolayiv region with commissioning of a new well in the poultry farm **[13]**.

In water sources from Vinnytsia and Volyn regions, the bromine content did not exceed the MPC nor differ statistically in research dynamics. Fluctuations in the bromine content in water from poultry farms in the Volyn region in 2016 were 0.02 – 0.12 mg.L^{-1}; in 2018 – 0.04 – 0.11 mg.L^{-1} and in 2020 – 0.04 – 0.12 mg.L^{-1}. Fluctuations in the content of bromine in water from poultry farms in Vinnytsia region in 2016 was 0.05 – 0.15 mg.L^{-1}; in 2018 – 0.05 – 0.12 mg.L^{-1} and in 2020 – 0.04 – 0.15 mg.L^{-1}.

In sources from poultry farms in the Zaporizhzhia region, the concentration of bromine in 2016 and 2018 did not exceed the MPC, while in 2020 the excess was 8.7%, which was 38.9% relative to the beginning of the study (p <0.05) (Figure 4). Fluctuations in the bromine content in water from poultry farms in the Zaporizhzhia region in 2016 were 0.12 – 0.28 mg.L^{-1}; in 2018 – 0.12 – 0.28 mg.L^{-1} and in 2020 – 0.18 – 0.31 mg.L^{-1}.

The average concentration of bromine in water sources from poultry farms of the studied regions of Ukraine had no significant differences from the beginning of the study, but there was a tendency to increase: in 2016 the concentration was 0.28 ± 0.02 mg.L^{-1}, in 2018 – 0.31 ± 0.02 mg.L^{-1} and in 2020 year – 0.32 ± 0.02 mg.L^{-1}, which was 10.7% and 14.3%, respectively, relative to the beginning of the study (Figure 4). It should also be noted that the average concentration of bromine in sources from the studied poultry farms of Ukraine exceeded the MPC by 21.7% in 2016, by 34.8% in 2018 and by 39.1% in 2020.

Excess bromine can get into drinking water from open sources of water supply (rivers, lakes) as a result of pollution by waste from thermal power plants (coal) and products of incinerators, disinfectants, flame retardants, herbicides **[8]**, **[17]**, **[40]**, **[46]**. However, poultry farming is a complex closed process, and each farm has its own water supply system mainly due to drilling wells, so in our opinion, there is no such tendency to increase bromine in water as for feed. However, drilling a well is not a guarantee as there is a possibility of bromine contamination of groundwater due to the extraction of minerals (especially oil and natural gas). **[9]**, **[29]**, **[32]**, **[35]** which is confirmed by the increase of bromine in the waters of Kharkiv, Poltava, Dnipropetrovsk and Zaporizhzhia regions, where gas fields are being developed.

The consumption rate of chicken eggs in Ukraine is 275 – 310 eggs per person per year, which is an average of 17.5 kg **[15]**, and in EU countries the consumption rates of chicken eggs in some countries – members of the community have significant differences: from 9.3 kg in the Czech Republic and Ireland to 29.3 kg – in the Netherlands. Based on the results of our research, the average estimated amount of bromine consumption per person (with an average weight of 60 kg) with eggs is: in the Kharkiv region - from 0.0026 mg.kg^{-1} in 2016 to 0.0044 mg.kg^{-1} in 2020, in the Poltava region – from 0.0020 mg.kg^{-1} in 2016 to 0.0032 mg.kg^{-1} in 2020, in the Dnipropetrovsk region – from 0.0023 mg.kg^{-1} in 2016 to 0.0041 mg.kg^{-1} in 2020, in the Mykolayiv region – from 0.0050 mg.kg^{-1} in 2016 to 0.0036 mg.kg^{-1} in 2020, in the Volyn region – from 0.0024 mg.kg^{-1} in 2016 to 0.0045 mg.kg^{-1} in 2020, in the Vinnytsia region – from 0.0024 mg.kg^{-1} in 2016 to 0.0048 mg.kg^{-1} in 2020 and in the Zaporizhzhia region – from 0.0025 mg.kg^{-1} in 2016 to 0.0047 mg.kg^{-1} in 2020.

The obtained data indicate that human consumption of bromine from different regions of Ukraine with chicken eggs increased 1.6 – 2.0 times compared to 2016 but did not exceed the WHO recommended human consumption of bromine 0.4 mg.kg^{-1} body weight per day. However, if the trends we have established continue, then approximately in 150 years the intake of bromine only with eggs will be half of the recommended dose.

Research perspectives: In the future, we plan to study the impact of products with high bromine content on the body of laboratory animals, as well as residual amounts of organic bromine in feed and poultry products.

CONCLUSION

The bromine content increased regardless of the region of location of the poultry farm, and the bromine content in chicken eggs from all surveyed farms at all study dates exceeded the established EFSA (in 99.6% samples of the total quantity) and the average in Ukraine (17.1% of the total quantity). Bromine enters poultry products mainly due to excessive entry into the body of birds with alimentary environmental factors (feed and water). The bromine content in feed for chickens increased in the dynamics of research (from 35.1% in the Poltava region to 2.5 times in the Zaporizhzhia region) and exceeded the established EFSA (4.4% samples of the total quantity) and the average in Ukraine (51.2% samples of the total quantity). The average content of bromine in compound feeds from poultry farms of the studied regions of Ukraine correlated with the number of registered and permitted for use bromine-containing pesticides ($r = 0.96$). The average concentration of bromine in water sources from poultry farms in the studied regions of Ukraine did not have significant differences compared to the beginning of the research, but exceeded the MPC by 21.7% in 2016, 34.8% in 2018 and 39.1% in 2020. Consumption of bromine with chicken eggs increased 1.6 – 2.0 times compared to 2016 but did not exceed the WHO recommended human consumption of bromine 0.4 $mg.kg^{-1}$ body weight per day.

Recommendations: Excess bromine in poultry diets can lead to iodine deficiency due to antagonism of these elements, as well as to excessive accumulation in poultry products, which may be one of the causes of iodine deficiency in humans. To solve this problem, it is necessary to conduct more extensive research, which would include: a comparative study of the content of bromine and iodine in soils, water, feed used in Ukraine to establish the so-called biogeochemical provinces; determination of toxicodynamics and kinetics of various bromine compounds in animals and the behaviour of the element in the environment; establishment of a scientifically based maximum permissible level of bromine in water, soil, feed and products of animal origin; determination of biological "markers" of the effect of bromine on the body and the establishment of physiological values of the element in the organs and tissues of animals; and depending on the results of research to create drugs for the correction of pathological conditions caused by excessive intake of bromine in the body.

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Contact Address:
Oleksandr Orobchenko, National Scientific Center, Institute of Experimental and Clinical Veterinary Medicine, Laboratory for Toxicological Monitoring, Pushkinska St., 83, 61023, Kharkiv, Ukraine,
Tel.: 0973797213
E-mail: toxy-lab@ukr.net
ORCID: https://orcid.org/0000-0002-0885-7776
Yuliia Koreneva, National Scientific Center, Institute of Experimental and Clinical Veterinary Medicine, Laboratory for Toxicological Monitoring, Pushkinska St., 83, 61023, Kharkiv, Ukraine,
Tel.: 0996005371
E-mail: k.17.nk08@gmail.com
ORCID: https://orcid.org/0000-0001-9401-7732
Anatoliy Paliy, National Scientific Center, Institute of Experimental and Clinical Veterinary Medicine, Laboratory of Veterinary Sanitation and Parasitology, Pushkinska St., 83, 61023, Kharkiv, Ukraine,
Tel.: 0662253434
E-mail: paliy.dok@gmail.com
ORCID: https://orcid.org/0000-0002-9193-3548
*Kateryna Rodionova, Odesa State Agrarian University, Faculty of Veterinary madicine, Department of Veterinary Hygiene, Sanitary and Expertise, Panteleimonovskaya Str., 13, 65012, Odesa, Ukraine,
Tel.: 0662486856
E-mail: katerina.rodionova@ukr.net
ORCID: https://orcid.org/0000-0002-7245-4525
Mikola Korenev, State Biotechnological University, Department of internal diseases and clinical diagnostics of animals, Mala Danylivka, 62341, Derhachi district, Kharkiv region, Ukraine,
Tel.: 0958010659
E-mail: korenevnikolayivanovich@gmail.com
ORCID: https://orcid.org/0000-0002-1198-7301
Nataliya Kravchenko, Kharkiv State Zooveterinary Academy, Department of clinical diagnostics and clinical biochemistry, Mala Danylivka, 62341, Derhachi district, Kharkiv region, Ukraine,
Tel.: 0509838531

E-mail: diagnost839@gmail.com
ORCID: https://orcid.org/0000-0002-9268-5304
Olena Pavlichenko, State Biotechnological University, Department of Sanitation, Hygiene and Forensic Veterinary Medicine, 44 Alchevskih Str., 61002, Kharkiv, Ukraine,
Tel.: 050-026-35-30
E-mail: pavlichenkoelena777@gmail.com
ORCID: http://orcid.org/0000-0002-6577-6577
Svetlana Tkachuk, National University of Life and Environmental Sciences of Ukraine, Department of Veterinary Hygiene, 16 Colonel Potekhin Str., 03041, Kyiv, Ukraine,
Tel.: 067-592-09-00
E-mail: ohdin@ukr.net
ORCID: https://orcid.org/0000-0002-6923-1793
Oleksandr Nechyporenko, Sumy National Agrarian University, Department of Therapy, Pharmacology, Clinical Diagnostics and Chemistry, 160 Herasym Kondratiev Str., 40021, Sumy, Ukraine,
Tel.: 099-251-22-92
E-mail: f_vet@ukr.net
ORCID: https://orcid.org/0000-0001-9915-5915
Svitlana Nazarenko, Sumy National Agrarian University, Department of Veterinary Examination, Microbiology, Zoohygiene and Safety and Quality of Livestock Products, 160 Herasym Kondratiev Str., 40021, Sumy, Ukraine,
Tel.: 099-141-75-29
E-mail: nazarenko.sveta2014@gmail.com
ORCID: https://orcid.org/0000-0001-6733-8565

Corresponding author: *