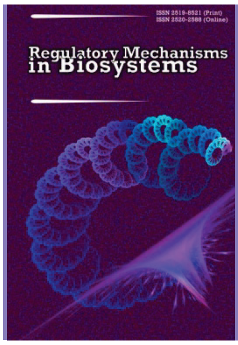

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Influence of high-bromine poultry products on clinical-biochemical blood parameters of white rats

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Bromine content in fodder and water for laying chickens in Ukraine gradually increasing, thereby accumulating in the poultry production (eggs and meat): according to the data, intake of bromine with chicken eggs in 2020 has almost doubled, compared with 2016. Taking into account the ability of bromine to accumulate and the scales of consumption of poultry products, it is relevant to study the effects of high bromine concentration on the level of thyroid hormones and clinical-biochemical parameters of blood of white rats. At the laboratory of Toxicological Monitoring of the National Scientific Center the Institute of Experimental and Clinical Veterinary Medicine and the Department of Experimental Pharmacology and Toxicology of the State Institution V.Y. Danylevskyi Institute of Problems of Endocrine Pathology of the National Academy of Medical Sciences of Ukraine, we carried out studies on white outbred male rats (n = 144). The diet of experimental groups was supplemented with eggs and meat containing high bromine concentration. We determined increase in the coefficients of liver weight and decrease in the coefficients of lung weight in the experimental rats. Moreover, we determined changes in the biochemical blood profile, in particular: after egg consumption, there occurred 20.0–22.0% decrease in the enzymatic activity of alanine aminotransferase, 23.0–48.4% in the activity of aspartate aminotransferase, and on average 21.3% decrease in alkaline phosphatase, compared with the control; after meat consumption, the level of total proteins decreased by 8.6%, glucose by 12.2–14.5%, also there were 13.8–18.5% decrease in the activity of alanine aminotransferase and 12.1–83.0% increase in the activity of aspartate aminotransferase on the 28th day, and 23.2–35.3% decrease in the activity of alkaline phosphatase. After the intake of both poultry products, the blood serum of animals was observed to have decrease in the level of overall thyroxine and overall triiodothyronine (1.3 times on average, depending on the period of study). The results of the studies can help in prediction and timely alleviation of the negative impact of poultry products with high bromine content on the human organism.

Keywords: eggs; meat; hepatotoxycity; hypothyreosis; aminotransferases; alkaline phosphatase; thyroid hormones.

Introduction

Inorganic elements such as proteins, fats and carbohydrates that are present in animals and humans have no energy value, but at the same time are absolutely necessary for the normal course of most vital processes. The range of action mechanisms of various microelements is broad and is based on the ability to influence the activities of enzymes or hormones, carrier proteins, specific receptors and cell membranes, and also interact with other elements such as synergists or antagonists (Yatoo et al., 2013; Bomko et al., 2018; Verma et al., 2018; Elmadfa & Meyer, 2019). A deficiency or excess of even one of the microelements is able to onset a cascade of metabolic impairments and lead to a disease (Magee & McCann, 2019; Kozeniecki et al., 2020). Deficiency of essential microelements and intoxication by low doses of toxic microelements is often downplayed by doctors, resulting in little attention to reasons for emergence of various microelementoses (Bailey et al., 2015; Gutyj et al., 2017; Gutyj et al., 2019; Slivinska et al., 2019; Razanova et al., 2022). According to the experts of the International initiative (Micronutrient Initiative, Canada), a correctly organized prophylaxis of micronutrient deficiency can prevent 4 of 10 child deaths, decrease maternal death by more than 1/3; increase the work capacity by 40%, increase the intellectual coefficient by 10–15 points, and increase the gross national product by 5%. Vitality and adaptation of an organism to life in an environment are to a high degree related to biogeochemical environmental factors (Barysheva, 2018; Vishchur et al., 2019; Martyshuk et al., 2020; Martyshuk et al., 2022).

As known, the development of poultry farming is one of the most economically attractive and promising directions of livestock farming because it produces precocity and high coefficients of livestock reproduction (Sobolev et al., 2019, 2020; Szőllősi et al., 2021; Vaskin et al., 2021). In order to increase mean daily increments and reduce fodder expenses, farms use vitamin and mineral fodder supplements. One of such microelements used to decrease energy expenditures for vitality support is bromine (Orobchenko et al., 2018; Kutsan et al., 2020). Therefore, intake of bromine and iodine-containing 15% *Laminaria digitata* marine algae improved the quality of meat of broiler chicken by increasing antioxidant pigments. At the same time, γ-tocopherol in meat decreased, the overall chlorophylls and carotenoids increased, and bromine and iodine in meat accumulated, and also the share of ω-3 polyunsaturated fatty acids increased (Costa et al., 2022).

Du Toit & Casey (2011) noted a much smaller amount of water and fodder consumed by the broiler groups that had been receiving 3 mg of Br/L of drinking water. Energy expenditures of animals in the form of thermal production decrease as a result of decrease in the activity of hormones, for example, thyroxine, because bromine is an antagonist to iodine. Bromine competitively inhibits the iodine transport in the thyroid gland (Marsan & Bayse, 2020). As a result, increments increase while expenses for fodder per increment unit decrease.

According to the results of our previous studies (Kutsan et al., 2020), the introduction of sodium bromide in the dose of 250.0 mg/kg to the diet of broiler chicken promoted increase in bromine in egg white (243.52 ± 4.39 mg/kg), compared with the control group (9.06 ± 0.54 mg/kg), the birds in which had been receiving 2.0 mg/kg "background" amount of bromine. At the same time, other studies (Orobchenko et al., 2022) confirmed that while the bromine in chicken eggs does not exceed the WHO-approved dose for people, equaling 0.4 mg/kg of body weight (WHO, 2018), the intake of bromine with chicken eggs in 2020 has increased by 1.6–2.0 times, compared with 2016. Taking into account bromine's ability to accumulate (Du Toit & Casey, 2011, 2012; Koreneva, 2020) and the popularity of poultry products (Nimalaratne & Wu, 2015; Nalyvayko et al., 2021), the objective of our study was determining the influence of high-bromine poultry products the level of thyroid hormones and clinical-biochemical blood parameters of white rats.

Materials and methods

The program of the studies was considered and approved by the Commission of Bioethics of the National Research Center the Institute of Experimental and Clinical Veterinary Medicine in the established order. The experiments on animals were performed in accordance with the international standards of bioethics (the materials of the 4th European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, Strasburg, 1985) (Simmonds, 2017) and in adherence to the current legislation of Ukraine (Article 26 of the Law of Ukraine as of 16.10.2012 No. 5456-VI "On the Protection of Animals against Abuse") and "General ethical principles of animal experiments", adopted by the First National Congress of Bioethics (Kyiv, 2001).

The experiment with providing poultry products (eggs and meat) with heigh bromine content was carried out on outbred white male rats (n = 144) aged 3–4 months and weighing 170–200 g respectively. According to the analogues pair principle, we formed two experimental and two control groups (n = 36).

The poultry products were obtained as a result of a previous experiment, in which carrier chickens were fed using sodium bromide for 28 days in the following doses: 10.0 (experiment I), 50.0 (experiment II), and 250.0 mg/kg of fodder (experiment III). The eggs were being gathered from chicken of experimental group III (bromine dose equaling 250.0 mg/kg of fodder) and the control group (the background parameter of bromine in mixed feed was 2.0 mg/kg of fodder) from the 4th to 30th day of the experiment. The egg yolk and white were mixed into a uniform mass and the bromine content was determined (it was 70.0 for the experimental group and 3.0 mg/kg for the control). The mixture was stored in a domestic refrigerator at the temperature of 18.0 ± 0.5 ºC for the following feeding of rats. Similarly, the muscular tissues of carcasses of the experimental-group III chicken and the control group, which had been euthanized on days 28 and 42, were processed into ground meat (the background bromine was 73.0 in the experimental group and 6.9 mg/kg in the control) and were frozen at the temperature of 18.0 ± 0.5 ºC. The egg mixture and ground meat were later de-frozen at the room temperature and mixed with grain (oats and maize), processed thermally (by boiling) and fed to the laboratory animals in the form of kasha.

Because a rat needs on average 30–32 g of food daily, they received 30.0 g of kasha every day. Rats of the control group I received 15.0 g of the egg mixture with the daily diet (with eggs from the control group of chickens), and experimental group I was given 15.0 g of the egg mixture (from eggs of experimental-group-III chickens). Similarly, rats of control group II received 15.0 g of ground meat of carcasses of the control group with the daily diet, and rats of experimental group II were given 15.0 g of ground meat from carcasses of chickens that had been receiving bromine in the dose of 250.0 mg/kg of fodder.

Bromine content in ready-to-eat diet was as follows: 5.50 ± 0.35 and 10.50 ± 0.43 mg/kg for animals of the control group with the egg mixture and ground meat, respectively, and 44.3 ± 5.17 and 46.6 ± 4.16 mg/kg for the experimental groups, respectively. They consumed the diet supplemented with eggs and meat for 28 days. For the next 21 day, rats of all the groups received kasha containing 12.0 g of oats ad 18.0 g of corn. Throughout the experiment, rats of all the groups had free access to water. During the experiment, we conducted clinical monitoring of the animals (Kotsiumbas, 2005; Karpenko et al., 2022; Sameliuk et al., 2022) and performed pathoanatomical autopsy to record any changes in the internal organs. In order to collect blood samples, 6 rats of each group were euthanized on days 14, 28, 48 and 49 of the experiment prior to feeding, with the chloroform-inhalation narcosis, by complete exsanguinations (blood serum was obtained using the generally accepted method of leaving blood undisturbed for some time (Vlizlo, 2012)). The studies of the concentration of thyroid hormones – total triiodothyronine (T_3) and total thyroxine (T_4) – were performed using the standard commercial test kits for the immunoenzymatic analysis (Granum, Ukraine) using a Stat Fax 3200 immunoenzymatic microplate analyzer (Awareness Technology inc., USA) at the State Institution the V.Y. Danylevskyi Institute of Problems of the Endocrine Pathology of the National Academy of Medical Sciences of Ukraine (Laboratory of Pharmacology of the Department of the Experimental Pharmacology and Toxicology).

Total proteins, albumin, urea, creatinine, glucose and the level of enzymatic activity in plasma were determined using the CORMAY (Poland) reagent kits as described in the manufacturer's manual (Vlizlo, 2012). Spectrophotometric measurements were made using a Shimadzu UV-1800 instrument (Japan).

The results were analyzed in the Statistica 7.0 software (StatSoft Inc., USA). The data in tables are presented as x ± SD (mean ± standard deviation). To compare the differences of mean parameters between the control and experimental groups, we used the Tukey test where the differences were considered statistically significant at $P < 0.05$ for all the data.

Results

During the experiment, animals of all the groups were observed to have no clinical signs of poisoning: the rats were active, consumed fodder and water actively. During the pathoanatomical autopsy, we observed no significant microscopic changes in the animals of both the experimental groups, as compared with the control.

However, we determined changes in the weight coefficients (Table 1). In particular, on the 14th day, the coefficients of liver weight in rats of experimental group I ($P < 0.05$) increased by 34.8%; and on the 28th day of giving the mixture to both the groups, it increased by 19.6% and 13.5% ($P < 0.05$), respectively. The coefficients of lung weight decreased on the 28th day of the intake of products by animals of experimental group II and on the 14th day after its intake had stopped in experimental group I, accounting for 50.0% and 23.4% ($P < 0.05$) respectively.

The main metabolites, including thyroid hormones, in blood serum of white rats over 49 days of the experiment were as follows. On the 14th day after the intake of eggs with fodder had stopped, the male rats that had been receiving the diet with high-bromine eggs were observed to have 13.5% decrease ($P < 0.05$) in the level of total proteins, as compared with the values in the control (Table 2). However, values of albumins, urea, glucose and creatinine in blood serum of rats in the study dynamics were not statistically different from the control. As revealed by the activity of enzymes characterizing the functional parameters of the liver (Table 2), in animals that had been receiving the diet with high-bromine eggs, the enzymatic activity of alanine aminotransferase (ALT) decreased by 21.0%; 20.0% and 22.0% ($P < 0.05$) on the 28th, 42nd and 40th days of the experiment, respectively.

Table 1
Coefficients of weight of the internal organs of white male rats that had been receiving high-bromine eggs and ground meat with their diet (x ± SD; n = 6)

Periods	Groups		Brain	Kidneys	Heart	Spleen	Liver	Lungs
Day 14	eggs	control I	0.65 ± 0.13^{b}	0.76 ± 0.03^{b}	0.45 ± 0.07^{b}	0.40 ± 0.05^{ab}	3.94 ± 0.06^{a}	0.89 ± 0.10^{a}
		experimental I	0.58 ± 0.08^{ab}	0.95 ± 0.15^{c}	0.44 ± 0.07^{b}	0.45 ± 0.08^{b}	5.31 ± 0.25^{d}	1.16 ± 0.25^{b}
	meat	control II	0.52 ± 0.02^{a}	0.82 ± 0.04^{b}	0.40 ± 0.02^{ab}	0.37 ± 0.03^{ab}	3.91 ± 0.30^{a}	1.09 ± 0.23^{ab}
		experimental II	0.52 ± 0.06^{a}	0.78 ± 0.01^{b}	0.41 ± 0.04^{ab}	0.39 ± 0.03^{ab}	3.97 ± 0.63^{a}	1.29 ± 0.17^{b}
Day 28	eggs	control I	0.56 ± 0.03^{a}	0.76 ± 0.04^{b}	0.40 ± 0.03^{ab}	0.46 ± 0.04^{b}	4.03 ± 0.11^{ab}	1.15 ± 0.13^{b}
		experimental I	0.51 ± 0.02^{a}	0.76 ± 0.06^{b}	0.34 ± 0.01^{a}	0.37 ± 0.03^{ab}	4.82 ± 0.23^{c}	1.04 ± 0.13^{ab}
	meat	control II	0.50 ± 0.03^{a}	0.79 ± 0.03^{b}	0.37 ± 0.03^{a}	0.38 ± 0.05^{ab}	3.79 ± 0.12^{a}	1.82 ± 0.32^{d}
		experimental II	0.49 ± 0.02^{a}	0.78 ± 0.02^{b}	0.34 ± 0.00^{a}	0.33 ± 0.02^{a}	4.30 ± 0.17^{b}	0.91 ± 0.07^{a}
14th day after the intake stopped	eggs	control I	0.60 ± 0.07^{ab}	0.66 ± 0.03^{a}	0.45 ± 0.04^{b}	0.46 ± 0.10^{b}	3.73 ± 0.25^{a}	1.24 ± 0.09^{b}
		experimental I	0.52 ± 0.03^{a}	0.65 ± 0.02^{a}	0.37 ± 0.02^{a}	0.34 ± 0.02^{a}	4.40 ± 0.25^{b}	0.95 ± 0.05^{a}
	meat	control II	0.52 ± 0.05^{a}	0.70 ± 0.03^{ab}	0.41 ± 0.07^{ab}	0.42 ± 0.03^{b}	4.31 ± 0.21^{b}	1.44 ± 0.49^{c}
		experimental II	0.54 ± 0.03^{a}	0.79 ± 0.04^{b}	0.43 ± 0.02^{ab}	0.40 ± 0.04^{ab}	4.34 ± 0.24^{b}	1.44 ± 0.18^{c}
21st day after the intake stopped	eggs	control I	0.57 ± 0.05^{ab}	0.92 ± 0.08^{c}	0.43 ± 0.10^{ab}	0.41 ± 0.02^{b}	3.87 ± 0.17^{a}	0.85 ± 0.03^{a}
		experimental I	0.50 ± 0.01^{a}	0.75 ± 0.04^{b}	0.36 ± 0.00^{a}	0.38 ± 0.02^{ab}	4.53 ± 0.29^{b}	0.93 ± 0.08^{a}
	meat	control II	0.51 ± 0.02^{a}	0.71 ± 0.04^{ab}	0.37 ± 0.02^{a}	0.37 ± 0.03^{ab}	4.02 ± 0.15^{ab}	1.16 ± 0.12^{b}
		experimental II	0.50 ± 0.02^{a}	0.72 ± 0.04^{ab}	0.40 ± 0.03^{ab}	0.32 ± 0.02^{a}	3.92 ± 0.20^{a}	1.42 ± 0.39^{c}

Note: letters indicate significant changes between the groups within one column ($P < 0.05$) according to the Tukey's test.

At the same time, the activity of aspartate aminotransferase (AST) decreased by 35.5% ($P < 0.05$) on the 14th day of the experiment, 48.4% on the 28th day, and 23.0% on the 14th day after feeding eggs with the diet, compared with the control values of this enzyme. Decrease in the activity of alkaline phosphatase (AP) was seen during all the periods of the study, equaling on average 21.3% ($P < 0.05$), compared with the control.

Table 2
Biochemical blood parameters of rats that had been receiving high-bromine eggs with fodder (x ± SD, n = 6)

Parameters	Groups of rats	Periods of the study			
		day 14	day 28	day 14 after the intake stopped	day 21 after the intake stopped
Total proteins, g/L	control	69.6 ± 2.0^{b}	67.1 ± 1.4^{b}	68.4 ± 1.2^{b}	67.6 ± 1.4^{b}
	experimental	68.1 ± 0.8^{b}	66.4 ± 1.0^{b}	59.1 ± 1.2^{a}	66.9 ± 1.9^{b}
Albumines, g/L	control	26.9 ± 1.8^{a}	27.0 ± 1.0^{a}	27.4 ± 0.7^{a}	27.2 ± 1.8^{a}
	experimental	27.5 ± 1.3^{a}	27.2 ± 0.7^{a}	27.4 ± 1.5^{a}	27.3 ± 1.7^{a}
Urea, µmol/L	control	5.89 ± 0.69^{a}	5.88 ± 0.46^{a}	5.98 ± 1.37^{a}	6.03 ± 0.44^{a}
	experimental	5.94 ± 0.48^{a}	5.91 ± 0.20^{a}	5.85 ± 0.34^{a}	5.92 ± 0.26^{a}
Creatinine, µmol/L	control	79.2 ± 1.1^{a}	83.9 ± 2.0^{a}	83.7 ± 2.1^{a}	84.1 ± 2.2^{a}
	experimental	78.7 ± 2.1^{a}	80.7 ± 3.2^{a}	81.5 ± 1.8^{a}	82.9 ± 1.5^{a}
Activity of alanine aminotransferase, mmol/h•L	control	5.83 ± 0.09^{b}	5.96 ± 0.58^{b}	6.04 ± 0.24^{b}	6.10 ± 0.44^{b}
	experimental	5.61 ± 0.13^{b}	4.70 ± 0.20^{a}	4.83 ± 0.14^{a}	4.76 ± 0.17^{a}
Activity of aspartate aminotransferase, mmol/h h•L	control	16.8 ± 0.8^{e}	17.1 ± 0.5^{e}	17.1 ± 0.2^{e}	16.9 ± 0.5^{e}
	experimental	10.8 ± 0.6^{b}	8.8 ± 0.6^{a}	13.1 ± 0.4^{c}	15.3 ± 0.6^{d}
Glucose, mmol/L	control	6.03 ± 0.09^{a}	5.96 ± 0.27^{a}	5.98 ± 0.42^{a}	5.92 ± 0.26^{a}
	experimental	5.85 ± 0.27^{a}	5.88 ± 0.16^{a}	5.90 ± 0.32^{a}	5.90 ± 0.16^{a}
Activity of alkaline phosphatase, nmol/s•min	control	1490 ± 9^{c}	1490 ± 10^{c}	1538 ± 11^{c}	1522 ± 10^{c}
	experimental	1220 ± 10^{b}	1209 ± 11^{b}	1219 ± 13^{b}	1103 ± 10^{a}

Note: different letters indicate values that are significantly different one from another in one line of the table according to the Tukey test ($P < 0.05$), taking into account the Bonferroni correction.

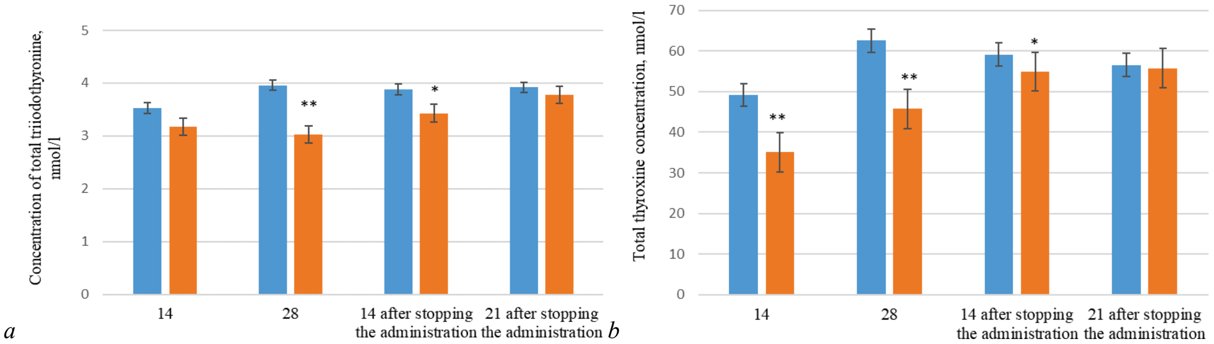

Fig. 1. Dynamics of the level of total triiodthyronine (*a*) and total thyroxine (*b*) in blood serum of male rats that had been receiving diet supplemented with eggs with high bromine content: orange – control, blue – experimental; x ± SD, n = 6; * – $P < 0.05$, ** – $P < 0.01$ compared with the control

In blood serum of rats of the experimental group, the level of total thyroxine (T_4) was also lower than in the control, but starting from the 14th day of the diet intake (Fig. 1b). Therefore, on the 14th and 28th days of the experiment, we observed 1.40-fold ($P < 0.05$) decrease in the hormone level, compared with such of the control. In case of feeding the male rats with the diet with high-bromine meat, on the 28th day of the experiment, we observed 8.6% ($P < 0.05$) decrease in the level of total proteins, compared with the control values, whereas after provision of the diet had stopped, this parameter did not statistically differ from the control. On the contrary, on the 49th day, we observed 10.1% ($P < 0.05$) increase in their level respectively (Table 3).

It has to be noted that parameters of albumins, urea and creatinine did not significantly differ from the control level during the monitoring period. However, on the 28th day of intake and 14 and 21 days after the provision of bromine with ground meat had ended, the concentration of glucose in blood serum of rats decreased on average by 13.0%, 12.2% and 14.5%

($P < 0.05$), respectively, compared with the control values. Analysis of hepatospecific enzymes revealed decrease in the activity of alanine aminotransferase and increase in the activity of aspartate aminotransferase, equaling 13.8% and 19.4% ($P < 0.05$) on the 28th day, 16.9% and 83.0% ($P < 0.05$) on the 42nd day, and 18.5% and 12.1% ($P < 0.05$) on the 49th day, compared with the control. Decreases in the activity of alkaline phosphatase were seen on days 42 and 49 of the experiment, being 35.3% and 23.2% ($P < 0.05$), respectively (Table 3).

The dynamics of concentration of thyroid hormones in blood serum of the experimental male rats that had been receiving the diet with high-bromine ground meat are indicated in Figure 2: throughout the experiment, we saw no significant changes in the level of overall triiodothyronine in the blood serum of rats of the experimental group, compared with the control (Fig. 2a). The dynamics of the level of total thyroxine in blood serum of the experimental male rats that had been receiving the diet with high-bromine ground meat are given in Figure 2b. The content of this hormone in blood serum of rats of the experimental group was also lower than in the control group. Therefore, on the 14th and 28th days of the experiment, we saw 1.3 and 1.2-fold ($P < 0.05$) decreases in the level of the hormone, compared with the control.

Table 3

Biochemical blood parameters of rats that had been receiving diet with high-bromine meat (x ± SD, n = 6)

Parameters	Groups of rats	Period of study			
		day 14	day 28	day 14 after the intake was over	day 21 after the intake was over
Total proteins, g/L	control	66.7 ± 2.2^{b}	63.0 ± 0.5^{b}	66.2 ± 1.0^{b}	66.9 ± 1.2^{b}
	experimental	61.6 ± 2.9^{b}	57.6 ± 1.0^{a}	67.0 ± 1.9^{b}	73.7 ± 0.8^{c}
Albumins, g/L	control	29.0 ± 1.0^{a}	29.0 ± 0.4^{a}	28.8 ± 0.8^{a}	29.6 ± 0.5^{a}
	experimental	28.0 ± 1.7^{a}	28.6 ± 1.1^{a}	29.3 ± 1.7^{a}	29.2 ± 1.9^{a}
Urea, µmol/L	control	5.78 ± 0.55^{a}	5.72 ± 0.54^{a}	$5.82 \pm 0{,}98^{a}$	5.79 ± 0.21^{a}
	experimental	5.52 ± 0.84^{a}	5.64 ± 0.26^{a}	$5.72 \pm 0{,}83^{a}$	5.97 ± 0.46^{a}
Creatinine, µmol/L	control	81.7 ± 0.1^{a}	82.2 ± 0.1^{a}	82.4 ± 0.1^{a}	82.2 ± 0.1^{a}
	experimental	80.1 ± 0.1^{a}	81.9 ± 0.0^{a}	82.2 ± 0.1^{a}	82.3 ± 0.0^{a}
Activity of alanine aminotransferase, mmol/h•L	control	5.70 ± 0.17^{b}	5.89 ± 0.35^{b}	6.03 ± 0.23^{b}	6.05 ± 0.31^{b}
	experimental	5.14 ± 0.32^{a}	5.08 ± 0.28^{a}	5.01 ± 0.22^{a}	4.93 ± 0.24^{a}
Activity of aspartate aminotransferase, mmol/h•L	control	8.67 ± 0.45^{a}	8.71 ± 0.67^{a}	8.67 ± 0.66^{a}	8.70 ± 0.46^{a}
	experimental	8.37 ± 0.99^{a}	10.40 ± 0.32^{bc}	15.87 ± 0.43^{c}	9.75 ± 1.35^{b}
Glucose, mmol/L	control	5.78 ± 0.10^{b}	6.00 ± 0.16^{b}	5.92 ± 0.33^{b}	6.00 ± 0.16^{b}
	experimental	5.80 ± 0.24^{b}	5.22 ± 0.26^{a}	5.20 ± 0.06^{a}	5.13 ± 0.09^{a}
Activity of alkaline phosphatase, nmol/s•L	control	1880 ± 8^{d}	1860 ± 8^{d}	1780 ± 10^{c}	1810 ± 12^{c}
	experimental	1860 ± 11^{d}	1891 ± 11^{d}	1152 ± 14^{a}	1390 ± 9^{b}

Note: different letters indicate significantly different changes between the values in one line of table according to the Tukey test ($P < 0.05$) taking into account the Bonferroni correction.

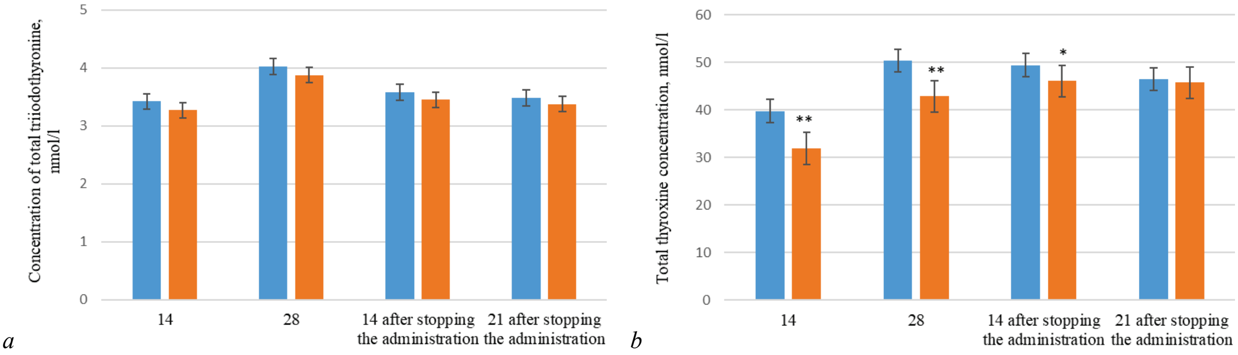

Fig. 2. Dynamics of the level of total triiodthyronine (*a*) and total thyroxine (*b*) in blood serum of the male rats that had been receiving diet with ground meat containing high content of bromine: orange – control, blue – experimental groups; x ± SD, n = 6; *– $P < 0.05$, ** – $P < 0.01$ compared with the control

Discussion

During the intake of high-bromine products (eggs and meat), the main target organ of the toxic impact in rats of experimental group I (eggs) – especially during the first periods of the studies – was the liver. In group II (meat), the liver and lungs were targeted. The hepatotoxic impact of bromine was confirmed by the studies of other researchers, indicating the negative effect of bromine potassium on the rats' organism (Dhouibi et al., 2021). Also, a study reported the hepatotoxic impact on mice, caused by 12-week action of bromine (halophenolic) side products of disinfection of drinking water (Jiang, 2022). The action of this element towards the lungs can be explained by the fact that it can accumulate in this organ in large amounts and transform into a volatile compound – interhalogen ClBr (Kohlmeier, 2003), which has irritating effects and likely causes dystrophic changes and decreases organ's weight. The latter fact was confirmed on white rats that had been inhaling bromine (Lam et al., 2016; Addis et al., 2021).

According to the results of clinical-biochemical studies of rats that had been receiving the diet with high-bromine eggs, there was hepatospecific hypoenzymemia (against the background of slowing of the activity of alanine aminotransferase; aspartate aminotransferase and alkaline phosphotase, $P < 0.05$) during all the periods of the experiment, and obviously gradual development of primary hypothyreosis (in the conditions of decrease in the level of T_3 and T_4, $P < 0.05$) and hypoproteinemia (against the background of decrease in total protein, $P < 0.05$) during the late periods of the experiment respectively, which is coherent with the dynamics of the coefficient of weight of the internal organs of rats of this experiment group, indicating that the liver was the target of the toxic action of bromine. However, the consumption of ground chicken meat with high bromine content led to temporary decrease in rats' overall proteins, hypoenzymemia of alanine aminotransferase and alkaline phosphatase, hypoenzymemia of aspartate aminotransferase and gradual hypoglycemia (with decrease in the main energy resource – glucose, $P < 0.05$). Unlike the rats on the diet with high-bromine eggs, the blood serum of the animals that had been receiving chicken ground meat was observed to have decrease only in the concentration of T_4 ($P < 0.05$) during 28 days of peroral intake of bromine. Inferring from the fact that - according to the clinical studies of coefficients of weight of the internal organs - the target organs of rats of this experimental group were the liver and lungs, we can state possible ways of the elimination (biotransformation) of bromine from the animal organism. Decrease in the overall thyroxine in blood serum was observed by Woodling et al. (2022), who studied rats during intake of bromine plant oil, which is approved by the U.S. Food and Drug Administration on temporary basis as a food supplement. Decrease in the concentrations of thyroxine and triiodothyronine was determined on days 16 and 66 of rats consuming potassium bromide in the doses of 50–400 mg/dm^3 of drinking water (Velický et al., 1997; Velický et al., 1998). It has to be noted that study of the toxicity of inorganic bromine peaked in 1980–2000. The hepatotoxic action during intake of eggs and meat with high content of bromine correlates with the studies (Jones et al., 1983): for 35 days, the rats consumed a diet containing 2% or bromine corn oil, dibromostearat monoglyceride, tetra bromstearat monoglyceride or mixture of two monoglycerides. During the autopsy, we observed the following picture: the hearts of all the animals that had been receiving bromine-containing products were yellow and had solid texture, cellular degeneration of the myocardium, light or moderate edema and some small necrotic sites (which is coherent with our data in relation to the activity of aspartate aminotransferase); moreover, the animals had an enlarged liver and its intercellular fat degeneration (which is consistent with increased alanine aminotransferase and alkaline phosphatase in our studies). However, we did not observe such dramatic changes in our experiments, because of the lower concentration of bromine in the diets and the shorter term of intake of the excessive element with poultry products.

Our study indicating some of the tendencies are coherent with the literature data regarding the pathogenetic influence of bromine on macroorganisms: during a long ingress of 1/10–1/50 of LD$_{50}$ bromine, there occurred changes in carbohydrate and protein metabolisms, slowing of the activity of cholinesterase and amylase in blood, decrease in the content of ascorbic acid in the adrenal glands; a systematic intake of bromine first promotes stimulating effect on thyroid gland, but further causes ruination of follicules, degeneration of some areas of the gland and formation of adenomas (by entering the thyroid gland, bromides cause compaction of follicular colloid of the gland, thereby slowing the ingress of thyroxine into blood) (Kutsan et al., 2014).

Our studies revealed that 4 and 12 weeks of consumption sodium bromide added to the diet in the doses of 20–19,200 mg/kg caused impairments in the endocrine system: decrease in the content of thyroxine in the tissue of thyroid gland, increase in the amounts of thyrotrophic and adrenocorticotropic hormones in hypophysis, decreases in thyroxine, testosterone and corticosterone in blood serum, and increases in follicle-stimulating hormone and insulin. There were signs of hypothyriosis and slowing of spermatogenesis in the testicles. The analysis of the reproductive system revealed that the consumption of high doses of bromine (1,200 mg/kg of fodder and higher) led to decrease in female fertility or inviability of offspring (all newborns died before the 21st day of life (Van Leeuwen et al., 1983).

The results of our studies of clinical diagnosis could be interpreted as primary hypothyreosis: a clinical symptomatic complex induced by thyroxine deficiency that causes insufficient activity of the overall triiodthyronine in the body's cells, thus totally slowing the metabolic processes and promoting the development of interstitial edema as a result of depositions in the subcutaneous adipose tissue, muscles and other tissues of fibronectin and hydrophilic glycosaminoglycans (Fabri, 2005).

The experiments carried out by Pavelka et al. (2002) on lactating rats confirmed that intake of an increased amount of bromine (5 g/L of water) decreased the concentration of iodine ions in milk and increased it in urine of the lactating rats. This led to impairments of the thyroid gland function in the newborn that had been consuming milk from those animals. As a result of a longer intake, bromides hamper the entry to the thyroid gland for iodines, causing iodine deficiency and hyperplasia of the gland. In the thyroid gland, a fraction of bromine is in protein-bound form. This allows it to enter the iodine metabolism so deeply (Rauws, 1983; Pavelka et al., 2002; Pavelka, 2004). As more bromide is consumed, its concentration in the thyroid gland increases, while the iodine concentration decreases, i.e. the total amount remains unchanged, but iodine concentration significantly decreases in relation to bromine. This indicates that iodine in thyroxine molecules is replaced by bromine (Vobecký & Babický, 1994; Vobecký et al., 1996). The abovementioned data reveal the action mechanism of those poultry products with high bromine content, because egg white (and meat) contains a large bromine concentration likely because of the biotransformation in birds that had received excessive-bromine diet (Koreneva, 2020).

Conclusions

Twenty eight-day intake of the diet with poultry products (eggs) with high content of bromine, equaling 44.3 ± 5.17 mg/kg, led to hepatospecific hypoenzymemia (against the background of slowing of the activities of ALT, AST and ALP, $P < 0.05$), primary hypothyreosis (in the conditions of level of T_3 and T_4, $P < 0.05$) and hypoproteinemia (against the background of decrease in total proteins (46.6 ± 4.16 mg/kg) caused gradual decrease in overall proteins, hypoenzymemia of ALT and ALP, hyperenzymemia of AST and gradual hypoglycemia ($P < 0.05$), and also decrease only in T_4 concentration ($P < 0.05$) during 28 days of peroral intake of bromine. During the intake of high-bromine products (eggs and meat), especially during the first periods of the study, the toxic impact mostly targeted the liver in rats of experimental group I (eggs) and the liver and lungs in group II (meat).

In the future, we are planning to study the concentration of iodine in the organs and tissues of experimental rats consuming poultry products with high bromine concentration.

The authors claim no conflict of interest.

References

Addis, D. R., Lambert, J. A., Ford, D. A., Jilling, T., & Matalon, S. (2021). Halogen gas exposure: Toxic effects on the parturient. Toxicology Mechanisms and Methods, 31(4), 272–287.

Bailey, R. L., West, K. P., & Black, R. E. (2015). The epidemiology of global micronutrient deficiencies. Annals of Nutrition and Metabolism, 66(2), 22–33.

Barysheva, E. S. (2018). Experimental simulation of the effects of essential and toxic trace elements on thyroid function. Bulletin of Experimental Biology and Medicine, 164(4), 439–441.

Bomko, V., Kropyvka, Y., Bomko, L., Chernyuk, S., Kropyvka, S., & Gutyj, B. (2018). Effect of mixed ligand complexes of zinc, manganese, and cobalt on the manganese balance in high-yielding cows during first 100-days lactation. Ukrainian Journal of Ecology, 8(1), 420–425.

Costa, M. M., Pestana, J. M., Osório, D., Alfaia, C. M., Martins, C. F., Mourato, M., Gueifão, S., Rego, A. M., Coelho, I., Coelho, D., Lemos. J. P. C., Fontes, C. M. G. A., Lordelo, M. M., & Prates, J. A. M. (2022). Effect of dietary *Laminaria digitata* with carbohydrases on broiler production performance and meat quality, lipid profile, and mineral composition. Animals, 12(8), 1007.

Dhouibi, R., Affes, H., Ben Salem, M., Charfi, S., Marekchi, R., Hammami, S., Zeghal, K., & Ksouda, K. (2021). Protective effect of *Urtica dioica* in induced neurobehavioral changes, nephrotoxicity and hepatotoxicity after chronic exposure to potassium bromate in rats. Environmental Pollution, 287, 117657.

Du Toit, J., & Casey, N. (2011). Effect of bromine and iodine in drinking water on production parameters of broilers. South African Journal of Animal Science, 40(4), 301–310.

Du Toit, J., & Casey, N. H. (2012). Iodine as an alleviator of bromine toxicity in thyroid, liver and kidney of broiler chickens. Livestock Science, 144, 269–274.

Elmadfa, I., & Meyer, A. L. (2019). The role of the status of selected micronutrients in shaping the immune function. Endocrine, Metabolic and Immune Disorders – Drug Targets, 19(8), 1100–1115.

Fabri, A. Z. (2005). Ekolohohihienichni aspekty poshyrennia endemichnoho zoba v riznykh bioheokhimichnykh zonakh Zakarpattia [Ecological and hygienic aspects of the spread of endemic goiter in various biogeochemical zones of Zakarpattya]. Endocrinology, 10, 41–50 (in Ukrainian).

Gutyj, B. V., Ostapyuk, A. Y., Sobolev, O. I., Vishchur, V. J., Gubash, O. P., Kurtyak, B. M., Kovalskyi, Y. V., Darmohray, L. M., Hunchak, A. V., Tsisaryk, O. Y., Shcherbatyy, A. R., Farionik, T. V., Savchuk, L. B., Palyadichuk, O. R., & Hrymak, K. (2019). Cadmium burden impact on morphological and biochemical blood indicators of poultry. Ukrainian Journal of Ecology, 9(1), 235–239.

Gutyj, B., Ostapiuk, A., Kachmar, N., Stadnytska, O., Sobolev, O., Binksevych, V., Petryshak, R., Petryshak, O., Kulyaba, O., Naumyuk, A., Nedashkivsky, V., Nedashkivska, N., Magrelo, N., Golodyuk, I., Nazaruk, N., & Binkevych, O. (2019). The effect of cadmium loading on protein synthesis function and functional state of laying hens' liver. Ukrainian Journal of Ecology, 9(3), 222–226.

Gutyj, B., Stybel, V., Darmohray, L., Lavryshyn, Y., Turko, I., Hachak, Y., Shcherbatyy, A., Bushueva, I., Parchenko, V., Kaplaushenko, A., & Krushelnytska, O. (2017). Prooxidant-antioxidant balance in the organism of bulls (young cattle) after using cadmium load. Ukrainian Journal of Ecology, 7(4), 589–596.

Jiang, X., Shi, P., Jiang, L., Qiu, J., Xu, B., Pan, Y., & Zhou, Q. (2022). *In vivo* toxicity evaluations of halophenolic disinfection byproducts in drinking water: A multi-omics analysis of toxic mechanisms. Water Research, 218, 118431.

Jones, B. A., Tinsley, I. J., Wilson, G., & Lowry, R. R. (1983). Toxicology of brominated fatty acids: Metabolite concentration and heart and liver changes. Lipids, 18(4), 327–334.

Karpenko, Y., Hunchak, Y., Gutyj, B., Hunchak, A., Parchenko, M., & Parchenko, V. (2022). Advanced research for physico-chemical properties and parameters of toxicity piperazinium 2-((5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-acetate. ScienceRise: Pharmaceutical Science, 36, 18–25.

Kohlmeier, M. (2003). Bromine. In: Kohlmeier, M. (Ed.). Nutrient metabolism. Academic Press. Pp. 753–756.

Koreneva, Y. (2020). The toxicokinetics of bromine in the body of laying hens under conditions of subchronic administration of sodium bromide diet. Scientific Journal of Veterinary Medicine, 2, 140–149.

Kotsiumbas, I. Y. (2005). Doklinichni doslidzhennia veterynarnykh likarskykh zasobiv [Preclinical studies of veterinary medicinal products]. Triada Plius, Lviv (in Ukrainian).

Kozeniecki, M., Ludke, R., Kerner, J., & Patterson, B. (2020). Micronutrients in liver disease: Roles, risk factors for deficiency, and recommendations for supplementation. Nutrition in Clinical Practice, 35(1), 50–62.

Kutsan, O. T., Orobchenko, O. L., & Kochergin, Y. A. (2014). Toksyko-biohimichna kharakterystyka neorganichnykh elementiv ta zastosuvannia rentgenofluorestsentnogo analizu u veterynarnij medytsyni [Toxic-biochemical characteristic of inorganic elements and application of X-ray fluorescence analysis in veterinary medicine]. Planet Print, Kharkiv (in Ukrainian).

Kutsan, O. T., Orobchenko, O. L., & Koreneva, Y. M. (2020). The quality and safety of eggs obtained from laying hens after their experimental poisoning with sodium bromide. Journal for Veterinary Medicine, Biotechnology and Biosafety, 6, 25–30.

Lam, A., Vetal, N., Matalon, S., & Aggarwal, S. (2016). Role of heme in bromine-induced lung injury. Annals of the New York Academy of Sciences, 1374(1), 105–110.

Magee, P. J., & McCann, M. T. (2019). Micronutrient deficiencies: Current issues. Proceedings of the Nutrition Society, 78(2), 147–149.

Marsan, E. S., & Bayse, C. A. (2020). A halogen bonding perspective on iodothyronine deiodinase activity. Molecules, 25(6), 1328.

Martyshuk, T. V., Gutyj, B. V., Zhelavskyi, M. M., Midyk, S. V., Fedorchenko, A. M., Todoriuk, V. B., Nahirniak, T. B., Kisera, Y. V., Sus, H. V., Chemerys, V. A., Levkivska, N. D., & Iglitskej, I. I. (2020). Effect of Butaselmevit-Plus on the immune system of piglets during and after weaning. Ukrainian Journal of Ecology, 10(2), 347–352.

Martyshuk, T., Gutyj, B., Vyshchur, O., Paterega, I., Kushnir, V., Bigdan, O., & Tkachenko, N. (2022). Study of acute and chronic toxicity of "Butaselmevit" on laboratory animals. Archives of Pharmacy Practice, 13(3), 70–75.

Nalyvayko, L., Rodionova, K., Pankova, S., Shomina, N., Katerynych, O., & Khimych, M. (2021). Comparative characteristics of eggs of chickens of domestic and foreign selection in their diverse age. Potravinarstvo Slovak Journal of Food Sciences, 15, 245–253.

Nimalaratne, C., & Wu, J. (2015). Hen egg as an antioxidant food commodity: A review. Nutrients, 7, 8274–8293.

Orobchenko, O. L., Kutsan, O. T., & Koreneva, Y. M. (2018). Toksykokinetyka bromu v orhanizmi bilykh shchuriv-samtsiv za umov odnorazovoho peroralnoho vvedennia natriiu bromidu [The toxicokinetics of bromine in the body of white male rats under conditions of sodium bromide single-dose oral using]. Bulletin of the Sumy National Agrarian University, Veterinary Medicine, 42, 177–185 (in Ukrainian).

Orobchenko, O., Koreneva, Y., Paliy, A., Rodionova, K., Korenev, M., Kravchenko, N., Pavlichenko, O., Tkachuk, S., Nechyporenko, O., & Nazarenko, S. (2022). Bromine in chicken eggs, feed, and water from different regions of Ukraine. Potravinarstvo Slovak Journal of Food Sciences, 16, 42–54.

Pavelka, S. (2004). Metabolism of bromide and its interference with the metabolism of iodine. Physiological Research, 53(1), 81–90.

Pavelka, S., Babický, A., Lener, J., & Vobecký, M. (2002). Impact of high bromide intake in the rat dam on iodine transfer to the sucklings. Food and Chemical Toxicology, 40, 1041–1045.

Rauws, A. G. (1983). Pharmacokinetics of bromide ion – an overview. Food and Chemical Toxicology, 21(4), 379–382.

Razanova, O., Yaremchuk, O., Gutyj, B., Farionik, T., & Novgorodska, N. (2022). Dynamics of some mineral elements content in the muscle, bone and liver of quails under the apimin influence. Scientific Horizons, 25(5), 22–29.

Sameliuk, Y., Kaplaushenko, A., Nedorezanıuk, N., Ostretsova, L., Diakova, F., & Gutyj, B. (2022). Prospects for the search for new biologically active compounds among the derivatives of the heterocyclic system of 1,2,4-triazole. Hacettepe University Journal of the Faculty of Pharmacy, 42(3), 175–186.

Simmonds, R. C. (2017). Chapter 4. Bioethics and animal use in programs of research, teaching, and testing. In: Weichbrod, R. H., Thompson, G. A. H., Norton, J. N. (Eds.). Management of animal care and use programs in research, education, and testing. 2nd edition. CRC Press, Taylor & Francis, Boca Raton. Pp. 1–28.

Slivinska, L. G., Shcherbatyy, A. R., Lukashchuk, B. O., Zinko, H. O., Gutyj, B. V., Lychuk, M. G., Chernushkin, B. O., Leno, M. I., Prystupa, O. I., Leskiv, K. Y., Slepokura, O. I., Sobolev, O. I., Shkromada, O. I., Kysterna, O. S., & Musiienko, O. V. (2019). Correction of indicators of erythrocytopoesis and microelement blood levels in cows under conditions of technogenic pollution. Ukrainian Journal of Ecology, 9(2), 127–135.

Sobolev, O. I., Gutyj, B. V., Darmohray, L. M., Sobolieva, S. V., Ivanina, V. V., Kuzmenko, O. A., Karkach, P. M., Fesenko, V. F., Bilkevych, V. V., Mashkin, Y. O., Trofymchuk, A. M., Stavetska, R. V., Tkachenko, S. V., Babenko, O. I., Klopenko, N. I., & Chernyuk, S. V. (2019). Lithium in the natural environment and its migration in the trophic chain. Ukrainian Journal of Ecology, 9(2), 195–203.

Sobolev, O. I., Gutyj, B. V., Sobolieva, S. V., Borshch, O. O., Nedashkivsky, V. M., Kachan, L. M., Karkach, P. M., Nedashkivska, N. V., Poroshinska, O. A., Stovbetska, L. S., Emelyanenko, A. A., Shmayun, S. S., & Guta, Z. A. (2020). Selenium in natural environment and food chains. A review. Ukrainian Journal of Ecology, 10(4), 148–158.

Szőllősi, L., Béres, E., & Szűcs, I. (2021). Effects of modern technology on broiler chicken performance and economic indicators – a Hungarian case study. Italian Journal of Animal Science, 20(1), 188–194.

Van Leeuwen, F. X. R., Den Tonkelaa, E. M., & Van Logten, M. J. (1983). Toxicity of sodium bromide in rats: Effects on endocrine system and reproduction. Food and Chemical Toxicology, 21(4), 383–389.

Vaskin, V. F., Korosteleva, O. N., Kuzmitskaya, A. A., Repnikova, V. I., & Khvostenko, T. M. (2021). Strategy of innovative development of animal husbandry in the Bryansk region. E3S Web of Conferences, 254, 08007.

Velický, J., Titlbach, M., Dusková, J., Vobecký, M., Strbák, V., & Raska, I. (1997). Potassium bromide and the thyroid gland of the rat: Morphology and immunohistochemistry, RIA and INAA analysis. Annals of Anatomy, 179(5), 421–431.

Velický, J., Titlbach, M., Lojda, Z., Dusková, J., Vobecký, M., Strbák, V. & Raska, I. (1998). Long-term action of potassium bromide on the rat thyroid gland. Acta Histochemica, 100(1), 11–23.

Verma, P. K., Sharma, A., Shankar, H., Sharma, A., & Rao, D. N. (2018). Role of trace elements, oxidative stress and immune system: A triad in premature ovarian failure. Biological Trace Element Research, 184(2), 325–333.

Vishchur, V. Y., Gutyj, B. V., Nischemenko, N. P., Kushnir, I. M., Salata, V. Z., Tarasenko, L. O., Khimych, M. S., Kushnir, V. I., Kalyn, B. M., Magrelo, N. V., Boiko, P. K., Kolotnytskyy, V. A., Velesyk, T., Pundyak, T. O., & Gubash, O. P. (2019). Effect of industry on the content of fatty acids in the tissues of the honey-bee head. Ukrainian Journal of Ecology, 9(3), 174–179.

Vlizlo, V. V. (2012). Laboratorni metody doslidzhen u biolohiji, tvarynnytstvi ta veterynarnij medytsyni [Laboratory research methods in biology, animal husbandry and veterinary medicine]. Spolom, Lviv (in Ukrainian).

Vobecký, M., & Babický, A. (1994). Effect of enhanced bromide intake on the concentration ratio I/Br in the rat thyroid gland. Biological Trace Element Research, 43, 509–516.

Vobecký, M., Babický, A., & Lener, J. (1996). Effect of increased bromide intake on iodine excretion in rats. Biological Trace Element Research, 55(3), 215–219.

Woodling, K. A., Chitranshi, P., Jacob, C. C., Loukotková, L., Von Tungeln, L. S., Olson, G. R., Patton, R. E., Francke, S., Mog, S. R., Felton, R. P., Beland, F. A., Zang, Y., & Gamboa da Costa, G. (2022). Toxicological evaluation of brominated vegetable oil in Sprague Dawley rats. Food and Chemical Toxicology, 165, 113137.

World Health Organization (2018). Alternative drinking-water disinfectants: Bromine, iodine and silver. World Health Organization, Geneva.

Yatoo, M. I., Saxena, A., Deepa, P. M., Habeab, B. P., Devi, S., Jatav, R. S., & Dimri, U. (2013). Role of trace elements in animals: A review. Veterinary World, 6(12), 963–967.