Part 2. Biosafety

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THE QUALITY AND SAFETY OF EGGS OBTAINED FROM LAYING HENS AFTER THEIR EXPERIMENTAL POISONING WITH SODIUM BROMIDE

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Summary. The purpose of this study was to determine the quality and safety of eggs obtained from laying hens after their experimental poisoning with sodium bromide. According to the principle of analogues, three experimental and one control group of laying hens (n = 15) were formed. The background bromine content of the compound feed was 2.0 mg/kg. An aqueous solution of sodium bromide was added daily to the feed of the chickens of the experimental groups for 28 days, followed by the observation of the birds for 14 days without its addition. Chickens of the 1st experimental group received bromine with feed at a dose of 10.0 mg/kg, 2nd — 50.0 mg/kg, 3rd — 250.0 mg/kg of feed. During the experiment, eggs were collected daily, their quality was determined according to the requirements of DSTU 5028:2008 'Hen's Eggs for Human Consumption. Specifications' and the rules for the veterinary and sanitary examination of poultry eggs. In addition, the bromine content was determined separately in egg white, yolk, and shell. Bromine content was determined by X-ray fluorescence analysis. Statistical processing of research results was conducted. Under the conditions of the chronic experiment, clinical manifestations of poisoning in chickens were not observed. No significant deviation from the control group was observed in productivity, egg mass, white to yolk mass ratios, and pH values of yolk and white. However, an uneven distribution of the eggs by category was established. Starting from the 2nd day of the experiment, bromine was excreted in laying hens with egg whites. The maximum value was observed on the 18th–28th days of the experiment: in the 1st experimental group a reliable excess 2.5 times of bromine content relative to the control was observed; in 2nd — 7.2 times, and in 3rd — 26.9 times. Thus, eggs from chickens of all groups conformed to DSTU 5028:2008 and the rules for the veterinary and sanitary examination of poultry eggs. However, the bromine content in the eggs of all experimental groups reliably exceeded the reference value for 28 days when sodium bromide was received with feed. Even 14 days after the experiment, the content of the element reliably exceeded the control value in the eggs of chickens from the 2nd and 3rd experimental groups, which may indicate the ability of bromine to cumulate

Keywords: egg quality and safety, bromine, laying hens, sodium bromide

Introduction. Today, there is a risk of getting excess bromine into food, since large quantities of consumer products are produced using brominated flame retardants (flame retardants). In connection with the detection of these compounds in samples of various environmental objects (Alaee et al., 2003; Luda, Balabanovich and Camino, 2002), on the recommendation of the European Commission (EC, 2014), the Member States of the European Union should monitor their presence in foodstuffs.

The highest concentration ranges of flame retardants were found in fish, fish products, and fish feeds (Fernandes et al., 2016; Poma et al., 2016).

In addition, the treatment of plants with bromine-containing pesticides can lead to increasing bromine content in food. Thus, according to Greve (1983) after methyl bromide treatment bromine content was observed: 3–7 mg/kg in strawberries, vegetables, and cereals, and over 200 mg/kg in leafy vegetables and herbs (lettuce, turnips, purslane, celery).

According to our previous studies (Kutsan, Orobchenko and Golubev, 2015), the bromine content in the compound feedstuff for poultry from Ivano-Frankivsk, Kharkiv, Poltava, Luhansk, and Donetsk regions was 0.66–14.36 mg/kg. Bromine content in water from wells in Kharkiv, Mykolaiv, Poltava, Luhansk, and Cherkasy regions was at the level of 0.011–11.08 mg/l. In Ukraine, bromine content in the feed is not normalized by any normative document. The maximum permissible level of inorganic bromides is given in the monograph by Aleksandrov (2000) and is 35.0 mg/kg of feed. In drinking water, according to Directive 98/83/EC (CEU, 1998) — 0.01 mg/l, and according to Hygienic Standard 2.1.5.1315-03 — 0.2 mg/l.

It should also be noted that the literature (Gurin, 2002; Radchikov et al., 2010) provides information on the use of bromine compounds as feed additives to improve animal meat production and reduce feed costs. Thus, when added to the diet of calves of potassium bromide at a dose of 280 mg of bromine per 100 kg of live weight, there was a tendency to decrease the active acidity of the meat and some increase in the amount of moisture retention, as well as the intensity of coloring of the meat.

Increasing the level of the element in the diets of pigs to 14.7–17.2 mg/kg of dry matter contributed to the increase in the average daily growth of animals by 9.3–16.2%, slaughter yield — by 1.9–3.2%, bromine content in muscle — by 5.9%.

Bromine affects the central nervous system (inhibitory processes) and the endocrine system, namely, it has a regulatory effect on the function of the thyroid gland, delays the flow of thyroxine into the blood, resulting in the domination of assimilation processes in the body (Petunenkov, 2000).

In addition to its effect on the thyroid gland, bromine also caused a decrease in the number of luteal bodies in the ovaries of female rats and a decrease in spermatogenesis in males (Van Leeuwen, Den Tonkelaar and Van Logten, 1983). There are no data in the literature on the use of bromine compounds in poultry, in particular laying hens and its effect on the quality and safety of eggs.

Therefore, the **purpose of this study** is to determine the quality and safety of eggs obtained from laying hens after their experimental poisoning with sodium bromide.

Materials and methods. A chronic toxicological experiment was conducted in vivarium of National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine) to study the possible effect of bromine (sodium bromide) on laying hens and their products.

The experiment was carried out on 365-day-old Hisex White cross laying hens weighing 1.2–1.6 kg. According to the principle of analogues, three experimental and one control groups of animals (n = 15) were formed.

The compound feedstuff for laying hens KK 1–18 was used.

The background content of bromine in the compound feed for chickens was 2.0 mg/kg. The feed for the experimental groups was mixed daily with an aqueous solution of sodium bromide for 28 days, for the next 14 days bird monitoring was continued without adding sodium bromide solution to the feed.

Chickens of the 1st experimental group were treated with bromine at a dose of 10.0 mg/kg, 2nd — 50.0 mg/kg, 3rd — 250.0 mg/kg of feed. Chickens of all groups had free access to water.

During the experiment, eggs were collected from the experimental poultry daily to determine their quality according to physicochemical parameters, following the requirements of DSTU 5028:2008 (DSSU, 2009) and rules for the veterinary and sanitary examination of poultry eggs.

In the dynamics of the experiment the bromine content in eggs from the experimental birds was also examined (separately examined the content of the element in white, yolk, and shell). Bromine content was determined in pooled egg samples for every two days of the experiment using X-ray fluorescence analysis on a Spectroscan max spectrometer as recommended (Kutsan, Orobchenko and Kocherhin, 2014).

Statistical analysis of the research results was performed using the Microsoft Excel 2003 application package (for Windows 7). The reliability of the obtained results was evaluated by the Student's *t*-test (Van Emden, 2019).

Results and discussion. During the experiment, clinical observations of the bird were performed. Thus, the hens of all groups were mobile, received well food and water, had a cross-like appearance: comb and earrings were brilliant, bright red, beak yellowish, plumage white, smooth and shiny, well adhered to the surface of the body.

No significant deviation from the control group was observed in productivity.

Thus, for the whole study period, 314 eggs were obtained from the control group chickens, from the 1st experimental group — 316, 2nd — 313, 3rd — 311 (Table 1).

The average weight of eggs obtained during the day from the experimental groups was also not significantly different from that in the control group.

Table 1 — Dynamics of the daily number of eggs obtained from laying hens after their experimental poisoning with sodium bromide

Egg collection period, day of the experiment	Control group	Experimental groups		
		I	II	III
1–2	22	22	22	22
3–4	23	22	24	22
5–6	24	24	22	24
7–8	21	20	21	22
9–10	22	25	24	22
11–12	25	22	23	21
13–14	22	23	22	25
15–16	15	16	15	15
17–18	17	18	18	18
19–20	18	17	17	17
21–22	17	15	18	15
23–24	15	19	17	16
25–26	18	17	15	18
27–28	15	15	15	15
29–30	7	7	7	7
31–32	6	6	6	5
33–34	5	5	5	7
35–36	5	6	5	5
37–38	7	7	6	5
39–40	5	5	6	5
41–42	5	5	5	5
Total	314	316	313	311

According to the requirements of DSTU 5028:2008 (DSSU, 2009), eggs are divided into categories according to weight: selected — 73.0 g or more, higher — 63.0–72.9 g, the 1st — 53.0–62.9 g, the 2nd — 45.0–52.9 g, and small — 35.0–44.9 g.

No eggs in the 'small' category were observed in any group during the whole study period.

In the control group, 5.4% of the eggs were of the 2nd category, 58.5% — 1st, 35.8% — higher, and 0.3% — selected.

The percentage of 2nd category eggs compared to control was slightly smaller in the 1st and 2nd experimental groups, when in the 3rd group it increased and was 6.0%. The percentage of 1st category eggs increased markedly in all experimental groups, and the higher category, on the contrary, decreased compared to control.

The largest difference was observed in the 3rd experimental group, which was 72.8% and 20.1%, respectively.

The percentage of the selected category eggs was also slightly higher in all experimental groups compared to control, the highest — in the 2nd experimental group (1.2%) (Fig. 1).

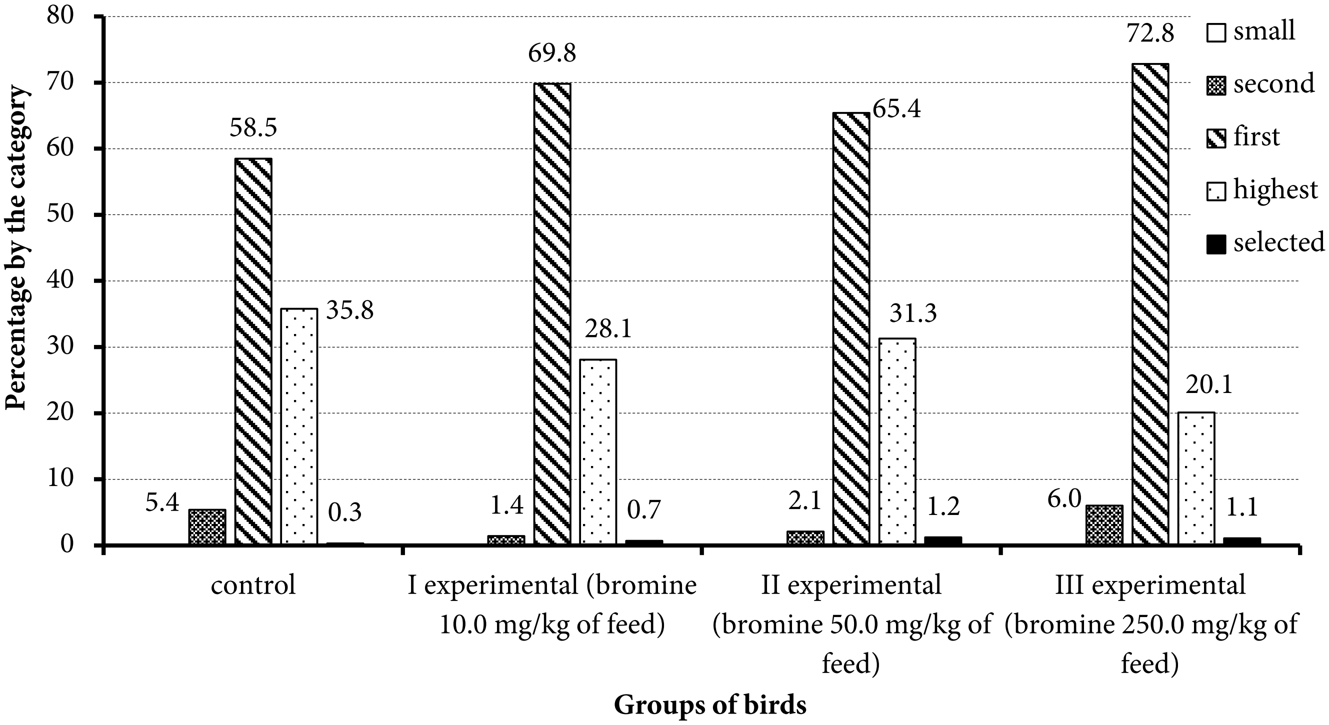

Figure 1. Distribution of eggs by category according to DSTU 5028:2008 (DSSU, 2009) during the chronic experiment

According to the results of the veterinary and sanitary examination of eggs from laying hens of the control and experimental groups, it was established that their quality met the requirements of the current DSTU 5028:2008 (DSSU, 2009). During the whole period of the experiment: the shell was whole, strong, without damage, smooth; the yolk is bright yellow, uniformly colored, elastic by consistency, retained shape; white is pure, transparent, viscous, without signs of deterioration; the smell is characteristic for fresh eggs.

The weight of the white, yolk, and shell, as well as the ratio of egg white to the yolk, under the conditions of oral administration of sodium bromide in the dynamics of the chronic toxicological experiment, were not significantly different from those in the control group (Table 2). The white weight ranged 30.88–35.15 g, the yolk — 16.70–18.90 g, the shell — 7.52–8.60 g, the ratio of the white to the yolk — 1.69–2.08.

The egg yolk and the egg white pH values in the experimental groups were also not significantly different from ones in the control group (Table 3).

Investigating the bromine content in eggs for the whole study period, it was found that in the yolk and shell of the control and all experimental groups, the bromine content was below the limit of 0.014 mg/kg for bromine. In the egg white in the control group during the experiment, the bromine content ranged from 8.01 to 10.89 mg/kg. In the 1st experimental group, on the 2nd day of the experiment, we observed a reliable excess of bromine in egg white, 1.4 times relative to control. The maximum value (23.03 ± 0.26 mg/kg) was observed on the 18th–28th days of the experiment, and on the 12th day after the experiment, its content in the experimental group was not significantly different from that in the control group (Fig. 2).

Table 2 — Dynamics of the white, yolk, and shell mass and the ratio of egg white to the yolk in eggs obtained from laying hens after their experimental poisoning with sodium bromide

Period, days	Groups		Indices			
			White mass, g	Yolk mass, g	Ratio of the white to the yolk	Shell mass, g
1–14	Control		33.40 ± 0.75	17.92 ± 0.51	1.88 ± 0.08	8.05 ± 0.20
	Experimental	I	34.42 ± 0.36	17.37 ± 0.39	1.99 ± 0.05	7.96 ± 0.24
		II	34.52 ± 0.87	17.61 ± 0.26	1.97 ± 0.07	8.24 ± 0.31
		III	32.59 ± 0.46	17.48 ± 0.23	1.87 ± 0.04	8.28 ± 0.07
15–28	Control		32.91 ± 0.62	17.89 ± 0.55	1.85 ± 0.07	8.04 ± 0.20
	Experimental	I	34.41 ± 0.54	17.18 ± 0.16	2.00 ± 0.03	8.06 ± 0.15
		II	33.20 ± 0.43	17.61 ± 0.26	1.88 ± 0.04	8.28 ± 0.16
		III	34.18 ± 0.59	17.30 ± 0.34	1.98 ± 0.06	7.93 ± 0.25
29–42	Control		33.27 ± 0.73	17.78 ± 0.42	1.87 ± 0.08	8.41 ± 0.23
	Experimental	I	32.78 ± 0.94	17.51 ± 0.45	1.88 ± 0.09	8.36 ± 0.21
		II	34.13 ± 0.77	18.11 ± 0.42	1.89 ± 0.07	8.43 ± 0.20
		III	32.99 ± 0.48	17.47 ± 0.40	1.90 ± 0.06	8.34 ± 0.19

Table 3 — Dynamics of the white and yolk pH in eggs obtained from laying hens after their experimental poisoning with sodium bromide

Period, days	Control group	Experimental groups		
		I	II	III
Egg white pH				
1–6	8.53 ± 0.09	8.70 ± 0.08	8.26 ± 0.30	8.57 ± 0.11
7–12	8.55 ± 0.05	8.58 ± 0.08	8.69 ± 0.05	8.58 ± 0.16
13–18	8.30 ± 0.12	8.09 ± 0.08	8.29 ± 0.02	8.45 ± 0.09
19–24	8.47 ± 0.04	8.58 ± 0.08	8.54 ± 0.02	8.57 ± 0.07
25–30	8.58 ± 0.03	8.56 ± 0.09	8.64 ± 0.05	8.66 ± 0.02
31–36	8.53 ± 0.07	8.56 ± 0.01	8.43 ± 0.13	8.38 ± 0.07
37–42	8.50 ± 0.01	8.46 ± 0.06	8.42 ± 0.06	8.47 ± 0.06
Egg yolk pH				
1–6	7.39 ± 0.05	7.18 ± 0.17	7.18 ± 0.11	7.27 ± 0.15
7–12	7.41 ± 0.16	7.39 ± 0.02	7.36 ± 0.08	7.21 ± 0.10
13–18	7.13 ± 0.03	7.03 ± 0.08	7.17 ± 0.07	7.11 ± 0.22
19–24	7.10 ± 0.09	7.12 ± 0.15	7.14 ± 0.18	7.05 ± 0.11
25–30	6.85 ± 0.15	7.03 ± 0.16	6.96 ± 0.04	6.94 ± 0.06
31–36	7.10 ± 0.15	6.96 ± 0.10	6.81 ± 0.10	7.09 ± 0.06
37–42	6.97 ± 0.07	6.66 ± 0.24	6.83 ± 0.08	6.76 ± 0.08

In the 2nd experimental group, the reliable excess of bromine content was observed at all study periods, and the maximum level was observed on the 18th–28th days of the experiment (65.38 ± 0.71 mg/kg), in the last study period bromine content 1.9 times exceeded the control. In the 3rd experimental group, the bromine content in egg white reached 243.52 ± 4.39 mg/kg on the 18th–28th days of the experiment, which was 26.9 times higher than the control. On the 2nd day after the experiment, its content 8.8 times exceeded the control, and on the 14th day — 3.5 times.

Analyzing the data of bromine content in eggs, we can say that with chronic sodium bromide entering the body, a significant amount of bromine is excreted from the organism with egg white, starting from the 2nd day of its supply with feed. It should also be noted that bromine tends to cumulate since the release of bromine with eggs was observed even 14 days after stopping the experiment. This is also evidenced by our previous studies (Kutsan, Orobchenko and Koreneva, 2019) concerning the toxicokinetics of bromine in the body of laboratory animals.

The uneven distribution of the percentage of eggs by category may be due to endocrine disorders caused by bromine (Petunenkov, 2000; Van Leeuwen, Den Tonkelaar and Van Logten, 1983), so this question requires a more detailed study.

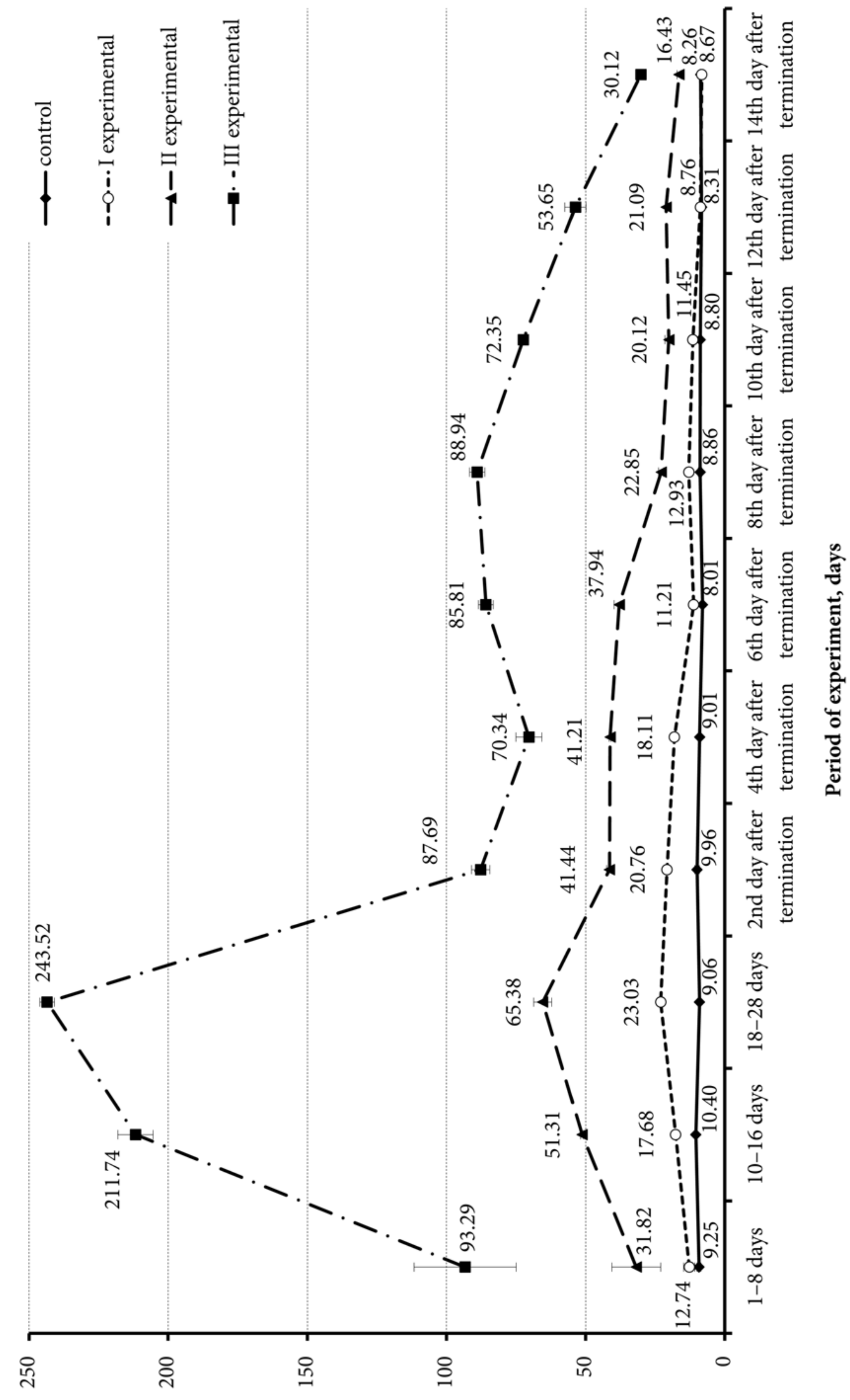

Figure 2. Dynamics of Bromine content in the white of eggs from experimental laying hens under conditions of chronic intake of sodium bromide (M ± m, n = 3)

Conclusions. Inorganic bromine under the conditions of chronic introduction to laying hens at doses of 10.0 mg/kg, 50.0 mg/kg, and 250.0 mg/kg of feed did not cause clinical manifestations of poisoning, but the uneven distribution of the percentage of egg categories may be as a result of endocrine disorders.

Eggs from chickens of all groups conformed to DSTU 5028:2008 (DSSU, 2009) and the rules for the veterinary and sanitary examination of poultry eggs.

Starting from the 2nd day of administration, bromine was excreted in laying hens with egg whites. In the birds receiving the bromine at the maximum dose, the content of the element in egg whites on the 18th–28th days of the experiment 26.9 times exceeded the control indicator. The high content of bromine in the eggs of the experimental groups after stopping its administration indicates the ability of bromine to cumulate.

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