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Leukocyte profile of laying hens’ blood under subchronic intake of sodium bromide with feed

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The optimal physiological development and productivity of poultry depends on the intake of essential macro- and microelements at levels that ensure the safety and quality of the resulting products (meat and eggs). It is important to note that both a lack and an excess of elements can lead to organ and system dysfunction. For instance, even small doses of bromine can cause hepatocyte dysfunction and disruptions to the endocrine and genitourinary systems. Additionally, the ability of these elements to accumulate in eggs and meat makes the products potentially dangerous for human consumption. Our work aimed to investigate the blood leukocyte profile of laying hens under subchronic intake of sodium bromide with feed and calculate integral leukocyte indices that allow assessing the state of the immune system and the degree of endogenous intoxication of the body. We also aimed to determine the strategy for further therapeutic or preventive measures. It was found that the intake of sodium bromide (by bromine) in the body of laying hens for 28 days in doses of 10.0 mg/kg, 50.0 mg/kg, and 250.0 mg/kg of feed leads to a significant increase in the Krebs index, leukocyte intoxication index, leukocyte shift index. This indicates changes in the functional activity of the immune system with the activation of the humoral immune system and decompensation of endogenous intoxication caused by the toxic effects of bromine. The significant decrease in the leukocyte index, neutrophil-monocyte ratio index, lymphocyte-monocyte ratio index, allergy index, and immunoreactivity index indicates a disruption in the interaction between the affective and effector links of immunity. This could potentially lead to cytokine deficiency, a reduction in the level of phagocytic defense, and an increased risk of immediate hypersensitivity.
Key words: laying hens; sodium bromide; blood; leukocyte formula; integral leukocyte indices.

INTRODUCTION

Poultry farming is a rapidly developing sector of agriculture. When raising poultry, it is important to ensure the optimal levels of nutrients, particularly microelements, in its body, because this affects not only the physiological state of the bird itself but also the quality and safety of the products obtained from it [1, 2]. When raising laying hens, the greatest attention is paid to such essential elements as calcium, potassium, phosphorus, sodium, sulfur, chlorine, magnesium, iron, copper, zinc, selenium, manganese, and iodine [3, 4].

Despite the 2014 research by American scientists McCall et al. [5], who proved the importance of bromine for all living organisms, this element can be dangerous due to its ability to replace other halogens. As an antagonist of iodine and chlorine, bromine causes endocrine disorders, including a decrease in thyroxine content in thyroid tissue, an increase in the amount of thyroid-stimulating and adrenocorticotropic hormones in the pituitary gland, a reduction in the serum content of thyroxine, testosterone, and corticosterone, and an increase in follicle-stimulating hormone and insulin. Furthermore, there are indications of hypothyroidism and slowed spermatogenesis in the testes. Bromine can cross the transplacental barrier, accumulating more in fetal tissues than in the mother [6]. Pavelka et al. [7] reported thyroid dysfunction in newborns after lactating rats consumed 5 g Br/l of water. In addition to milk, bromine can also be excreted in egg whites [8], and bromine administration into fertilized eggs at concentrations higher than 0.01 mg/l leads to delayed development of chicken embryos, and concentrations of >1 mg/l were lethal.

It is important to note that even small doses of this halogen can be toxic due to its ability to accumulate [9]. A study in rats found that feeding them eggs and meat from poultry that consumed feed containing 250 mg/kg of bromine decreased total thyroxine and total triiodothyronine levels [10]. It is worth noting that the bromine content in feed and water for laying hens in Ukraine is gradually increasing. This is reflected in the accumulation of bromine in poultry products (eggs and meat). According to data from 2020, the bromine level in chicken eggs increased almost 2.0 times compared to 2016 [11]. The lack of observable clinical symptoms in laying hens when consuming bromine at a dose of 250 mg/kg of feed, coupled with the presence of elevated levels of the element in eggs and meat of such poultry [8], suggests a need for a more comprehensive investigation into the physiological parameters of poultry, particularly the status of the leukocyte blood system. Accordingly, our study aimed to examine the leukocyte blood profile of laying hens under subchronic sodium bromide intake via feed.

METHODS

The experiment was conducted on 60 laying hens of the *Highsex White* crossbreed, which were previously kept in the equalization period for 15 days in the vivarium of the NSC “IECVM”. During this period and throughout the experiment, the birds were kept in typical laboratory facilities in cages following the Order of the Ministry for Development of Economy, Trade and Agriculture of Ukraine dated February 8, 2021, No. 224, “Requirements for the welfare of laying hens during their keeping”. The microclimate in the poultry houses, daylight hours, lighting intensity, and all other technological parameters met the regulatory requirements (VNTP-APK-04.05, 2005). The temperature was within the specified range of 18-20°C, humidity was 60-70%, and the daylight hours day/night were 16/8 h/day. The poultry was fed a complete feed for laying hens, specifically the KK 1-18 product. The birds had free access to water. The background bromine content in the chicken feed was 2.0 mg/kg of feed. After the equalization period, three experimental and one control group of animals (n = 15) were formed according to the principle of analogs. The laying hens in the experimental groups were of the *Highsex White* cross, 365 days old, and weighed (1.4 ± 0.2) kg following the generally accepted requirements of the experiment [12, 13].

The experimental design followed the principles of toxicological experimental design [14]. An aqueous sodium bromide solution was added to the feed of the experimental groups. For 28 days, the first experimental group received a daily dose of 10.0 mg/kg of bromide in their feed, while the second group received 50.0 mg/kg, and the third group received 250.0 mg/kg. Over the following 14 days, all chicken groups, including the control and experimental groups, were maintained on a standard diet and continued to be monitored. The total duration of the experiment was 42 days.

Blood samples were collected on days 14, 28, and 42 (14 days after termination) of the experiment for further determination of clinical parameters. Blood was taken from 5 birds from each group under conditions of euthanasia by total exsanguination during inhalation chloroform anesthesia. The leukogram was derived by counting leukocytes in blood smears stained by the Romanowsky-Giemsa method [15].

The experiments on animals were conducted under the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 “On the Protection of Animals from Cruelty”) as amended on 04.08.2017. The “General Ethical Principles for Animal Experiments”, as adopted by the First National Congress on Bioethics (Kyiv, 2001), as well as international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Strasbourg, 1985)) [16], were considered. All procedures involving experimental animals were approved by the Bioethics Committee of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Protocol No. 2-23 of November 24, 2023).

Results were processed using the MS Excel toolkit with the Student-Fisher method. Descriptive statistics were expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

Analyzing the data of the blood leukocyte formula of laying hens at different periods of the experiment (Table 1), a significant decrease

Table 1. Blood leukocyte formula of laying hens in the dynamics of subchronic oral sodium bromide intake (M ± m, n = 5)

Poultry group	Study period		After cessation
	14 day	28 day	14 day
Pseudo-eosinophils			
Control	25.33±0.88	27.67±1.45	25.00±0.58
I (10 mg/kg)	26.00±0.58	41.33±0.88**	26.67±0.33
II (25 mg/kg)	31.67±0.88**	47.33±1.45***	30.67±0.67**
III (250 mg/kg)	15.00±1.53**	32.67±0.88*	26.33±1.20
Eosinophils			
Control	7.33±0.67	8.00±0.58	6.33±0.33
I (10 mg/kg)	6.33±0.33	5.00±0.58*	6.33±0.33
II (25 mg/kg)	8.67±0.88	4.67±0.33**	6.67±0.33
III (250 mg/kg)	7.67±0.33	3.67±0.33**	9.00±0.58*
Monocytes			
Control	5.33±0.33	4.00±0.00	6.67±0.67
I (10 mg/kg)	9.00±0.58**	4.67±0.33	9.67±0.33*
II (25 mg/kg)	5.33±0.33	6.33±0.33**	6.67±0.33
III (250 mg/kg)	6.00±0.58	7.33±0.33***	5.67±0.33
Basophils			
Control	0	0.67±0.67	0.33±0.33
I (10 mg/kg)	0.33±0.33	1.00±0.58	0.33±0.33
II (25 mg/kg)	0.33±0.33	0.33±0.33	0.33±0.33
III (250 mg/kg)	0.33±0.33	0	0
Lymphocytes			
Control	62.00±0.11	59.67±1.45	61.67±0.33
I (10 mg/kg)	58.33±0.67**	55.67±0.67	57.00±1.15
II (25 mg/kg)	54.00±2.31*	31.33±0.88***	55.67±1.33*
III (250 mg/kg)	71.00±0.58***	45.00±2.65**	58.00±0.58*

*P ≤ 0.05 - relative to the control; **P < 0.01 - relative to the control; ***P < 0.001 - relative to the control.

of 40.8% in the number of pseudo-esinophils in the blood of group III hens was found on day 14 of the experiment. Instead, an increase of 25.0% in the number of these cells was observed in group II during this period. The maximum increase in the number of pseudo-eosinophils of 49.3%, 71.0% and 18.1%, respectively, was observed on day 28 of the experiment in all experimental groups. After cessation of sodium bromide exposure, the number of cells returned to the control level, except for group II - the increase remained at 22.7%. Such dynamics of the number of pseudo-eosinophils indicate the development of endogenous intoxication or allergy [17].

The number of eosinophils decreased significantly by 37.5%, 41.6%, and 54.1% in the laying hens of the I, II, and III experimental groups, respectively, on day 28 compared to the control. However, 14 days after the cessation of sodium bromide administration, the number of eosinophils in groups I and II returned to levels comparable to the control, except in group III, where the number of these cells increased by 42.2%. A reduction in the number of eosinophils may indicate stress, diminished resistance to external and internal factors, and other factors [18]. This provides insight into the observed fluctuations in cell levels in the chickens in groups I and II. The initial decline in eosinophil numbers, followed by an increase over time, suggests the emergence of allergic reactions in the body, as observed in the birds in the III experimental group.

The percentage of monocytes increased significantly in birds of group I on day 14 and at the end of the experiment, with an increase of 68.8% and 45.0%, respectively. Similarly, in birds of groups II and III, an increase of 58.2% and 83.2% was observed on day 28 of the experiment, respectively. Given those monocytes are involved in the formation and regulation of the immune response, and perform the function of presenting antigens to lymphocytes, as well as being a source of biologically active substances (including regulatory cytokines, interleukins, interferons, and complement components), it can be concluded that monocytosis under toxicant exposure leads to impaired immunoreactivity and increased endogenous intoxication [19].

Lymphocytopenia (of 6.0% and 12.9%) was recorded in birds of the I and II experimental groups, and in the III group - lymphocytosis by 14.5% on day 14 of the experiment. On day 28, the number of lymphocytes decreased by 47.5% and 24.6% in the blood of laying hens of groups II and III, respectively, was observed. On day 14 after the cessation of exposure to the toxicant, the direction of changes remained the same for poultry in these groups (9.7% and 6.0%, respectively). It should be noted that the lymphocytopenia indicates intoxication of the organism, as it is accompanied by an increased number of pseudo-eosinophils [20].

The quantitative indices of the blood leukocyte formula of laying hens under conditions of bromine intake revealed significant changes, indicating the development of intoxication, particularly on day 28 of the experiment. These changes included lymphocytopenia, monocytosis, eosinopenia, and pseudo-eosinophilia.

Table 2 presents the results of the leukocyte index calculation in the blood of laying hens at various stages of the experiment. The calculation of integral leukocyte indices (ILI) is a valuable tool for studying immunological resistance, differentiating the severity of acute conditions and endogenous intoxication, and determining the effectiveness of treatment and prevention [21].

The ratio of neutrophils to lymphocytes (Krebs index) is an indicator of the body's nonspecific and specific defense capabilities [19]. This index demonstrates a notable increase in laying hens, reaching 43.9% on day 14 in group II, 1.6, 3.2, and 1.6 times on day 28 in groups I, II, and III, and 17.5%, 37.5%, and 12.5% in all experimental groups, respectively, 14 days after cessation. This indicates the activity of nonspecific defense cells. This is due to an increase in the proliferative activity of the bone

Table 2. Blood leukocyte indices of laying hens in the dynamics of subchronic oral administration of sodium bromide (M ± m, n = 5)

Poultry group	Study period		After cessation
	14 day	28 day	14 day
Krebs index			
Control	0.41±0.01	0.46±0.02	0.40±0.01
I (10 mg/kg)	0.44±0.02	0.74±0.04*	0.47±0.02*
II (25 mg/kg)	0.59±0.03*	1.51±0.06*	0.55±0.03*
III (250 mg/kg)	0.21±0.01*	0.72±0.03*	0.45±0.01*
Leukocyte intoxication index			
Control	0.34±0.01	0.38±0.02	0.33±0.01
I (10 mg/kg)	0.35±0.01	0.63±0.03*	0.36±0.01
II (25 mg/kg)	0.46±0.02*	1.12±0.06*	0.44±0.02*
III (250 mg/kg)	0.18±0.01*	0.58±0.03*	0.36±0.01
Leukocyte shift index			
Control	0.48±0.02	0.57±0.02	0.46±0.02
I (10 mg/kg)	0.48±0.02	0.78±0.04*	0.40±0.01
II (25 mg/kg)	0.68±0.03*	1.38±0.07*	0.60±0.03*
III (250 mg/kg)	0.30±0.01*	0.69±0.03*	0.55±0.02
Leukocyte index			
Control	2.45±0.08	2.16±0.07	2.47±0.09
I (10 mg/kg)	2.24±0.09	1.34±0.05*	2.14±0.04
II (25 mg/kg)	1.70±0.04*	0.66±0.03*	1.81±0.06*
III (250 mg/kg)	4.73±0.06*	1.37±0.04*	2.24±0.08
Lymphocyte to monocyte ratio index			
Control	11.63±0.35	14.92±0.39	9.24±0.27
I (10 mg/kg)	6.48±0.15*	11.92±0.26*	5.89±0.19*
II (25 mg/kg)	10.10±0.28	4.95±0.13*	8.35±0.15
III (250 mg/kg)	11.83±0.17	6.14±0.22*	10.40±0.14*
Neutrophil to monocyte ratio index			
Control	4.75±0.08	6.92±0.07	3.75±0.11
I (10 mg/kg)	2.89±0.11*	8.85±0.05*	2.76±0.13*
II (25 mg/kg)	5.94±0.09	7.47±0.08	4.60±0.15*
III (250 mg/kg)	2.51±0.07*	3.92±0.11*	4.64±0.12*
Allergization index			
Control	1.78±0.06	1.63±0.05	1.75±0.06
I (10 mg/kg)	1.48±0.07*	1.10±0.02*	1.39±0.07*
II (25 mg/kg)	1.22±0.02*	0.49±0.01*	1.31±0.05*
III (250 mg/kg)	1.86±0.08	1.03±0.03*	1.56±0.02
Immunoreactivity index			
Control	13.00±0.41	16.92±0.28	10.19±0.16
I (10 mg/kg)	7.18±0.24*	12.99±0.37*	6.55±0.18*
II (25 mg/kg)	12.32±0.63	5.68±0.16*	9.35±0.22
III (250 mg/kg)	13.11±0.55	6.64±0.22*	11.81±0.36

*$P \leq 0.05$ - relative to control

marrow, which is expressed in an increase in the number of heterophils [20, 22]. However, in poultry of group III on day 14 of the experiment, the Krebs index significantly decreases by 48.7%, indicating a decrease in the activity of phagocytic reactions and factors of nonspecific immunity [23].

The leukocyte intoxication index (LII) also showed a significant increase in laying hens (35.3% on day 14 in group II; 1.6, 2.9, and 1.5 times on day 28 in groups I, II, and III, respectively; 33.3% on day 14 after cessation only in group II), which is a moderate hyperergic type of systemic response to the etiologic factor [24]. However, there was a significant decrease in LII of 47.0% in group III hens on day 14 of the experiment. Such a significant difference from the control values can be explained by the formation of an endogenous intoxication syndrome. LII is an accessible and informative index and, based on the assessment of general conditions and laboratory parameters, allows timely selection of methods of prevention and treatment [18, 25].

The blood leukocyte shift index (LSI) is a marker of the body's reactivity during acute inflammation that characterizes the ratio of granulocytes to agranulocytes and is independent of the number of leukocytes in the blood [23, 26]. In laying hens, LSI increased significantly by 41.6% in group II on day 14 of the study, the maximum increase was 1.3, 2.4, and 1.2 times on day 28 in groups I, II, and III, and at the end of the study, the difference was 13.0, 30.4 and 19.5%, respectively, compared to the control. The observed direction of changes indicates a shift in the leukocyte blood count to the left, which suggests a violation of immunological reactivity and the entry of a significant number of "young" leukocyte forms into the peripheral blood. However, a 37.5% decrease in LSI in group III birds on day 14 and a 13.0% decrease in group I birds indicates a rightward shift in the leukocyte count, as well as an increase in the proportion of segmented neutrophils, a phenomenon observed in megaloblastic anemia, kidney, and liver dysfunction [15, 24].

The leukocyte index (LI) dynamics reflect the relationship between humoral and cellular immunity, indicating the dominance of cellular immune system activation [26]. Therefore, this index was reduced by 30.6% in group II birds on day 14; by 37.9%, 69.4%, and 36.5% in all experimental groups on day 28; and by 26.7% in group II hens compared to the control group. Group II exhibited a lower LI compared to the control group, while group III demonstrated an elevated LI by 93.0% on day 14 of the study compared to the control. This may be attributed to immunosuppression resulting from the toxic effects of bromine on blood cells and leukemogenesis organs [27].

Calculation of the neutrophil/monocyte ratio index (NMRI), which reflects the ratio of components of the micro-macrophage system, showed that the neutrophilic reaction is predominant (a reliable increase in this index by 25.0% and 22.6% and 27.8%, respectively) under conditions of bromine intake in chickens at a dose of 25 mg/kg feed for 14 days and 14 days after its cessation, as well as in birds receiving 10 mg/kg feed for 28 days. On the other hand, analyzing the dynamics of NMRI, we found its reliable decrease (in chickens of groups I and II by 39.1% and 47.1% on day 14; group III - by 43.3% on day 28 and the group I at the end of the observation period - by 26.4%), which is due to an increase in monocytes, which in our case reflects poisoning [23].

In addition, we calculated the lymphocyte-to-monocyte ratio index (LMRI), which reflects the relationship between the affective and effector links of the immunological process [17], which was also significantly reduced compared to the control. Thus, on day 14, a significant decrease of 44.3% was found in birds of group I, and 14 days after the cessation of intake - by 36.2%; on day 28, the decrease was 20.1%, 66.8%, and 58.8% for chickens of groups I, II and III, respectively. According to G. Ponti [28], this hematological index can be considered a prognostic parameter reflecting the functional state of cardiac activity and its decrease of more than 50.0% (day 28 of the experiment) indicates the need for detoxification therapy, as various complications may develop, including multiple organ failure syndrome [26].

Our calculations of integral hematological indices of non-specific reactivity indicate a significant decrease in the allergy index (AI). In the first stages of intoxication, the delayed-type hypersensitivity reaction prevails over the immediate-type hypersensitivity reaction, which leads to the activation of allergic mechanisms of the immune response [19]. Thus, on day 14, a decrease of 16.8% and 31.4% were observed in birds of groups I and II, on day 28 - by 32.5%, 70.0%, and 36.8% in all experimental groups, and 14 days after the cessation of bromine intake - by 20.5% and 25.1% in birds of groups I and II, respectively, compared to the control. The use of allergy indices can be recommended both to determine the feasibility of conducting or intensifying elimination therapy and to evaluate its effectiveness [17].

Our results of calculating the immune reactivity index (IRI), which reflects the balance of lymphokines and monokines [22], indicate a deficiency of the detoxification component and an increase in the number of monokines, which in turn can lead to angiogenesis and proliferation of fibroblasts and endothelium [25], which is confirmed by a decrease in the allergy index. Under the conditions of our experiment, the immune reactivity index decreased significantly by 44.7% in chickens of group I on the 14th day of the experiment, by 23.2%, 66.4%, and 60.7% in birds of groups I, II, and III, respectively, on the 28th day, and by 35.7% in group I at the end of the observation compared to the control.

Investigating the impact of certain biotic and abiotic factors on the quality and safety of poultry products is a perspective for further research.

CONCLUSIONS

The intake of bromine into the body of chickens for 28 days has been found to result in a significant increase in the Krebs index, leukocyte intoxication index, and leukocyte shift index. This indicates changes in the functional activity of the immune system, with activation of the humoral immune system and decompensation of endogenous intoxication caused by the toxic effects of bromine. The reliable decrease in leukocyte index, neutrophil-monocyte ratio index, lymphocyte-monocyte ratio index, allergy index, and immunoreactivity index indicate a disruption in the interaction between the affective and effector links of immunity. This can lead to cytokine deficiency, a decrease in the level of phagocytic defense, and an increased risk of immediate hypersensitivity. Integral leukocyte indices provide valuable insights into the immune system and the body's response to subchronic bromine (sodium bromide) administration in laying hens. These indices also inform the development of targeted therapeutic or preventive measures.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ЛЕЙКОЦИТАРНИЙ ПРОФІЛЬ КРОВІ КУРЕЙ-НЕСУЧОК ЗА УМОВ СУБХРОНІЧНОГО НАДХОДЖЕННЯ З КОРМОМ НАТРІЮ БРОМІДУ

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Фізіологічний розвиток та продуктивність птиці залежить від багатьох чинників, зокрема від надходження до організму оптимального вмісту макро- та мікроелементів, що є підґрунтям для отримання безпечної та якісної продукції (м›ясо, яйця). Адже як нестача, так і надлишок елементів може призводити до порушення функції органів та їх систем. Метою нашої роботи було дослідити стан лейкоцитарного профілю крові курей-несучок за умов субхронічного надходження натрію броміду з кормом і

розрахувати інтегральні лейкоцитарні індекси, що дають змогу оцінити стан імунної системи та ступінь ендогенної інтоксикації організму, а також визначити стратегію подальших лікувальних або профілактичних заходів. Було встановлено, що надходження в організм курей-несучок натрію броміду (за бромом) протягом 28 діб у дозах 10,0 , 50,0 та 250,0 мг/кг корму призводить до вірогідного збільшення індексу Кребса, лейкоцитарного індексу інтоксикації, індексу зсуву лейкоцитів, що вказує на зміни функціональної активності імунної системи з активацією гуморальної ланки імунітету і декомпенсацію ендогенної інтоксикації, яка зумовлена токсичним впливом брому. Отримане вірогідне зниження лейкоцитарного індексу, індексу співвідношення нейтрофілів та моноцитів, індексу співвідношення лімфоцитів та моноцитів, індексу алергізації, індексу імунореактивності характеризує порушення взаємодії афекторних і ефекторних ланок імунітету, яке може призводити до дефіциту цитокінів, зниження рівня фагоцитарного захисту та посилення розвитку гіперчутливості негайного типу.
Ключові слова: кури-несучки; натрію бромід; кров; лейкоформула; інтегральні лейкоцитарні індекси.

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