



Hematological parameters of domestic chickens as stress reaction markers derived from different etiologies

S. V. Sokolenko*, Y. V. Sokolenko**, Y. L. Van***, M. O. Ozymok***, I. V. Kobal*, V. L. Sokolenko*

*Bohdan Khmelnytsky National University of Cherkasy, Cherkasy, Ukraine

**Northwestern University, Chicago, USA

***Municipal Organization "Cherkasy City Zoological Park "Roshen", Cherkasy, Ukraine

Article info

Received 04.09.2024

Received in revised form

01.10.2024

Accepted 16.10.2024

Bohdan Khmelnytsky National
University of Cherkasy,
Shevchenko st., 81,
Cherkasy, 18031, Ukraine.
Tel.: +38-067-869-17-91.
E-mail: sokolenko@ukr.net

Division of Pulmonary
and Critical Care Medicine,
Department of Medicine,
Northwestern University,
North st., 676, Clair Street
Arkes Suite, 2330,
Chicago, 60611, USA.
Tel.: +1-312-926-68-95.
E-mail:
domweb@northwestern.edu

Municipal Organization
"Cherkasy City Zoological Park
"Roshen", Smilianska st., 132,
Cherkasy, 18000, Ukraine.
Tel.: +38-047-263-46-42.
E-mail: zoocityck@ukr.net

Sokolenko, S. V., Sokolenko, Y. V., Van, Y. L., Ozymok, M. O., Kobal, I. V., & Sokolenko, V. L. (2024). Hematological parameters of domestic chickens as stress reaction markers derived from different etiologies. *Regulatory Mechanisms in Biosystems*, 15(4), 767–775. doi:10.15421/0224111

We studied the stress reaction markers among blood parameters from three groups of broiler chickens (*Gallus gallus domesticus*) of Ross-308 breed aged 5 weeks, with a total number of 60 animals. There were 20 chickens from a private farm in an ecologically clean area; 20 chickens from a private farm in a radiation-contaminated territory; and 20 chickens were brought from a poultry farm to feed animals in the zoo. We determined numbers of erythrocytes, thrombocytes, leukocytes and their individual populations, and heterophils and lymphocytes ratio (H/L ratio). We identified morphological features of blood cells in broilers from a radiation-contaminated territory: an increased number of medium and large lymphocytes and heterophils in half-destroyed or destroyed forms ("shadow cells"). It was shown that the morphological and quantitative features of blood in broilers raised on the private farm from an ecologically clean territory met the normal parameters. Typical features of stress reaction were observed in broilers brought from the poultry farm to the zoo: the relative and absolute numbers of lymphocytes were significantly lower, and the relative and absolute numbers of heterophils were significantly higher than in chickens from the other two analyzed groups. As a result, the leading marker of stress in birds, which is the ratio of heterophils/lymphocytes, was significantly higher in broilers from the poultry farm than in the other two groups. The indicators of monocyte number were significantly lower in broilers from the radiation-contaminated territory than in birds from the private farm in an ecologically clean area. Moreover, the indicators of basophil number were significantly higher, which was an additional sign of a stress reaction. This group also revealed certain morphological features of blood cells such as the tendency of heterophils to destruction, the formation of platelet clusters, and the presence of a significant number of medium and large lymphocytes. It was found that these denoted signs of stress in chickens appeared in cases of acute stress through capture and transportation, which, presumably, was potentiated by previous rearing in industrial conditions. Stress reactions of chickens from areas with an increased radiation background require additional research. The taming effect had a positive influence on the birds' blood parameters, reducing indications of stress. It is recommended to minimize stress in chickens whose meat is to be used in human and animal nutrition.

Keywords: *Gallus gallus domesticus*; rearing peculiarities; emotional factor; background radiation; morphology of leukocytes; heterophils/lymphocytes ratio; animals' nutrition.

Introduction

Stress is a factor that can significantly affect the productivity of farm birds, disrupt the immune response, and increase susceptibility to diseases (Viriden & Kidd, 2009; Vizzier-Thaxton et al., 2016; Abo-Al-Ela et al., 2021; Nielsen et al., 2023). In 1937, it was shown that a general adaptive syndrome in birds, similarly to mammals, is formed in response to stress, where adrenal hormones play a crucial role (Selye, 1937). Activation of the hypothalamic-pituitary-adrenocortical axis in a stressful situation leads to an increase in the concentration of corticosteroids. Corticosterone, which is the dominant stress hormone in birds, contributes to physiological and behavioral adaptive reactions with the provision of allostasis. However, the hormone is necessary in low concentration for a constant body energy balance. Diversity in the adrenocortical response is important in the process of reproduction and growth of young individuals, contributing to the evolutionary strategy (Blas, 2015). A chronic increase in the concentration of corticosterone leads to an earlier response to subsequent stressors (Maxwell, 1993).

However, the estimation of corticosteroid concentration has certain problems. In addition to the cost of the analysis, it is necessary to calculate

clearly the moment of blood sampling during the acute phase of stress. The exact timing of the rise in corticosterone concentration during stress is unknown for most bird species (Romero & Reed, 2005). Therefore, to characterize the presence of stress in animal populations, the identification of blood indicators is attracting more attention. Estimation of white blood cell populations has certain advantages over direct measurement of adrenal hormone concentrations. First, the effect persists for a relatively long time and does not require immediate analysis. Second, the method is quite cheap and available to various laboratories. Third, the hematological response to stress is similar in different taxonomic groups of vertebrates and makes it possible to adequately compare the results (Davis et al., 2008). *In vitro*, it has been demonstrated that bird leukocytes can be stimulated by corticotropin-releasing factor and are capable of producing ACTH themselves. It means, that the participation of the hypothalamic-pituitary-adrenal axis in the production of corticosteroids in birds has its features associated with leukocyte factors (Hendricks III et al., 1991; 1995; Viriden & Kidd, 2009).

The analysis of studies related to the influence of non-infectious stress factors (nutritional peculiarities, climatic, physical, or psychological-social factors) on the blood parameters of birds shows variable data. Undoubtedly,

ly, the optimal marker of hormone-induced reactions is indicated by the ratio of heterophils (similar to mammalian neutrophils) and lymphocytes (H/L ratio), as this indicator increases under conditions of stress (Prieto & Campo, 2010; Redmond et al., 2011; Anand Laxmi, 2019; Maness et al., 2023). Moreover, the reaction was most clearly observed under the influence of environmental and climatic factors. It is even considered to be a more indicative and accessible marker than the assessment of the concentration of corticosteroids in the plasma. On the other hand, such changes depend on the intensity and duration of the stressor (Maxwell, 1993).

One of the first studies of the influence of stress factors on the heterophils and lymphocyte ratio in chickens is the work of Gross and Siegel (1983). The presence of physiological stress in broilers characterized by an increase in the H/L ratio, was detected during transportation (Mitchell et al., 1992; Al-Aqil et al., 2013; Anand Laxmi, 2019), and a change in living conditions or a social group (Gross et al., 1984; Anthony et al., 1988; Tainika et al., 2023).

Unlike other stress indicators, the H/L ratio does not differ significantly among chicken breeds (Campo et al., 2000). There are known cases when the variability of the H/L ratio was observed in chickens with different reactions to emotional stress (the indicator was higher in birds with a high expression of fear), but there were no differences in the concentration of corticosterone (Beuving et al., 1989). This confirms the effectiveness of using the heterophils and lymphocytes ratio in birds as a leading marker in a chronic stress situation (Scanes, 2016).

Ionizing radiation is indicated as one of the physical stress factors (Nylund et al., 2014; Moreels et al., 2020). Research in populations of people who have been living in radiation-contaminated territories for a long time demonstrates both an increased concentration of cortisol and a moderate redistribution of the neutrophils/lymphocytes ratio with a prevalence of polymorphonuclear cells, and the level of such indicators increased with additional influence of emotional factors (Sokolenko, 2016). Accordingly, a problematic fact is the lack of data on the state of health of agricultural birds which are raised on territories with enhanced radioecological control and the possibility of the formation of chronic stress signs in them. Some publications about the effects of radiation on domestic chickens present the data from the experiments on the assessment of the consequences of artificial irradiation and indicate the critical role of damage to hematopoiesis under acute irradiation (Malhotra & Rana, 1988). There are also publications on the intensity of accumulation of radionuclides in the body of birds and the speed of their removal (Pöschl et al., 1997; Vicentijevic et al., 2013), and about quantitative changes of blood cells in chickens during the first week of life after low-dose irradiation of eggs (Pejaković et al., 2021).

The relevance of such research is because even 30 years after the accident at the Chornobyl nuclear power plant, animals in the zone of the "Chornobyl footprint" in Europe have an increased content of radionuclides (Beňová et al., 2016). This indicates the continued circulation of radionuclides in the circulation of substances and the possibility of their entering the body of animals and humans with food products (Vicentijevic et al., 2013; Cannon & Kiang, 2022; Klyuchko et al., 2023). A possible criterion of danger in the case of farm birds could be signs of stress.

Hormonal drugs used in animal households are the subject of active discussions regarding possible negative effects on human health (Jeong et al., 2010). At the same time, there is no analysis of the effect of corticosteroids, which can be accumulated in meat products due to stress. There is data on the negative impact of high corticosterone concentration on the quality of poultry meat (Xing et al., 2019). Cases where live birds or fresh carcasses are taken after or during acute stress, in particular, when feeding animals in a zoo, deserve additional attention. Meat food forms the basis of the predators' diet, and an important requirement in zoos is feeding with whole carcasses or even live animals. Young poultry is an important element of feeding predators in captivity, while not only nutrients and biologically active substances of muscle tissue are assimilated, but also internal substances, contents of the stomach and intestines, etc. (Burlaka et al., 2012).

The purpose of the work is to analyze markers of stress reaction within hematological parameters in broiler chickens that were reared in different ecological conditions or were exposed to different socio-emotional influences.

Materials and methods

All procedures were performed following the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the rules of bioethics when working with biological objects, regulated by the International Guideline for the Care and Use of Laboratory Animals (2011), the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments; <http://arriveguidelines.org>). The research plan was approved by the Bioethics Commission of Cherkasy National University (Examination Report No. 5 dated March 11, 2021).

Broilers received all scheduled vaccinations during the study. At least 2 weeks had passed since the last vaccination. The clinical condition of the birds was checked by veterinarians by external examination and temperature measurement before blood sample collection. None of the broilers had signs of infectious diseases.

Peripheral blood was obtained from 60 broiler chickens (*Gallus gallus domesticus*) of the Ross-308 breed (Ross-308 cross), aged 5 weeks and weighing 490–515 g. There were 20 chickens from private farms in a relatively ecologically clean area (Zolotonosha district of Cherkasy province); 20 chickens were raised on private farms on territory that had had the status of enhanced radioecological control for a long time (Zvenyhorod district of Cherkasy province); 20 broilers were brought to the zoo from a poultry farm (Cherkasy district of Cherkasy province) to feed the animals. The feeding regime for birds on private farms was similar to that used on poultry farms. Compound feed of a similar composition, produced by regional manufacturers, was used. Broilers from private farms were kept in closed enclosures, wood shavings were used as bedding, as on poultry farms. The research was conducted at the beginning of summer. Weather and climate conditions in the analyzed districts did not differ. The density of birds per unit area on private farms was twice as low as on the poultry farm (5 head per m² versus 10 head per m²).

Biomaterial was collected by veterinarians from the brachial vein (wing vein) of birds using the following methods: Campbell (1994) and Kelly & Alworth (2013). The procedure was conducted 5 hours after the transportation of the broilers from the poultry farm. Birds from private farms were handed over to the veterinarians by farmers to whom the birds were adapted and of whom they were not afraid.

Work with the obtained biomaterial was performed according to the recommendations from the source: Boiko et al. (2013).

20 μ L of the collected blood was added to a test tube with 4 mL of physiological solution and carefully mixed. The mixture was loaded with a pipette into the prepared Goryaev chamber. Cells were counted in five large (80 small) squares, moving the chamber diagonally.

The formula $X = A \times 4000 \times 200 / 80$ was used for the calculation, where X is the number of cells in 1 μ L of blood, A is the number of cells counted in the chamber (for 80 small squares), 4000 is the multiplier for converting the value to 1 μ L; 200 is the blood dilution factor. In the tables, the data was presented per 1 liter.

Blood smears were prepared on previously degreased and disinfected glass slides. Staining of smears was made after their drying according to the Pappenheim method. Smears were covered with May-Grünwald solution (for 3–5 minutes). The paint was washed off with distilled water, and then covered with a diluted Romanovsky-Giemsa solution (for 20–30 minutes). After staining, the smears were washed with running water and dried.

The smears were analyzed under high magnification with immersion oil (MICROmed Evolution ES-4130 microscope, Plan 100x/1.25 Oil objective, WF 10x/22 eyepiece). Imaging was made using a 5.0 Mpix USB 2.0 CCD video camera compatible with the microscope. The number of individual populations of blood cells per 200 examined cells was determined, and the obtained values were divided by 2 to obtain the percentage. To determine the absolute number of erythrocytes, leukocytes, and platelets, their percentage content was multiplied by the number of cells found in the Goryaev chamber. To evaluate different populations of leukocytes, their total cell number was considered as 100% and the relative number of individual populations was proportionally calculated, and the total number was determined by multiplying the population percentage by the total number of leukocytes.

The H/L ratio was determined by the percentage ratio of heterophils and lymphocytes according to the examples of the analyzed parts of the literature (Abdi-Hachesoo et al., 2011; Gottstein et al., 2015).

Morphological analysis of blood cells was performed according to the illustrative material given by Campbell (1994) and Boiko et al. (2013).

Comparison of data in samples was performed using the ANOVA method with Bonferroni's correction. Data in the tables are presented as mean \pm standard error ($\bar{x} \pm SE$). The statistical significance of the difference in indicators between groups is indicated with three values: $P < 0.05$; $P < 0.01$; $P < 0.001$.

Results

Morphological analysis of smears of broilers from a private farm in a relatively ecologically clean area showed the dominance of classical forms of blood cells of birds. Most heterophils had a well-defined visible nucleus, usually with 2 blade-shaped segments of chromatin (Fig. 1). There were mostly small lymphocytes in the cell population (Fig. 1, 2a, 2c) and medium (Fig. 2b) lymphocytes with different densities of nuclear chromatin. There were typical changes in the shape of cells from round to oval. The presence of small pseudopodia was noted in individual lymphocytes.

Monocytes had a classical appearance. They were large cells with a well-defined bright cytoplasm and nuclei of an oval or concave (kidney-shaped) shape (Fig. 3).

Eosinophils and basophils were relatively rare in the field of vision and had typical morphological features (Fig. 4).



Fig. 1. Erythrocytes (1), heterophil (2) and small lymphocyte (3) in peripheral blood of broilers from a private farm in an ecologically clean area

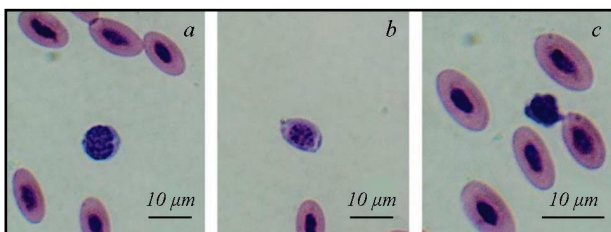


Fig. 2. Modification of lymphocytes' morphology in peripheral blood of broilers from a private farm in an ecologically clean area

Morphological analysis of smears from the broilers raised in the poultry factory showed a significant dominance of heterophils among blood leukocytes. The heterophils had small, dense granularity, which masked the nucleus and did not allow a clear view of its shape. Small forms dominated among lymphocytes. Their nucleus occupied almost the entire cell, and the chromatin was packed very tightly. Erythrocytes and platelets had a typical form without pathological features (Fig. 5). An important characteristic of many heterophils was morphological changes that indicated the probable formation of heterophil extracellular traps (HETs) (Fig. 6).

Eosinophils and basophils had well-defined granularity and were found in the field of view relatively often between cell populations in the birds' blood (Fig. 7). At the same time, monocytes were relatively rare.

They were relatively small in size, and some cells were in the process of active phagocytosis (Fig. 8).

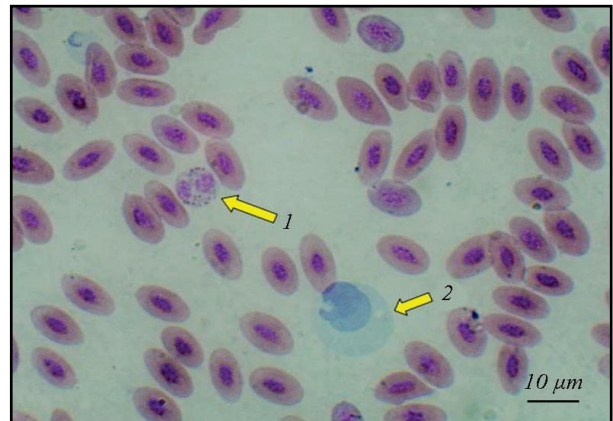


Fig. 3. Heterophil (1) and monocyte (2) in peripheral blood of broilers from a private farm in an ecologically clean area

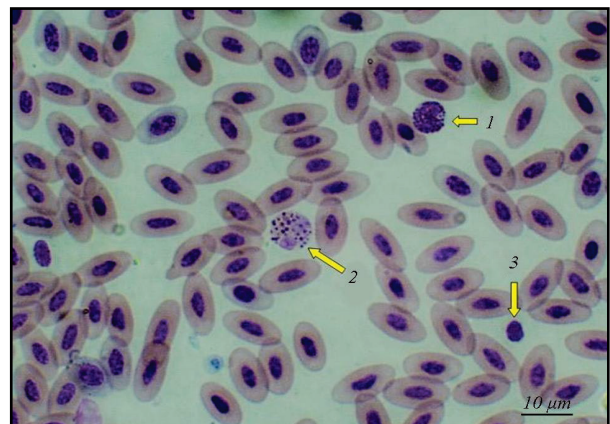


Fig. 4. Basophil (1), eosinophil (2) and thrombocyte (3) in peripheral blood of broilers from a private farm in an ecologically clean area

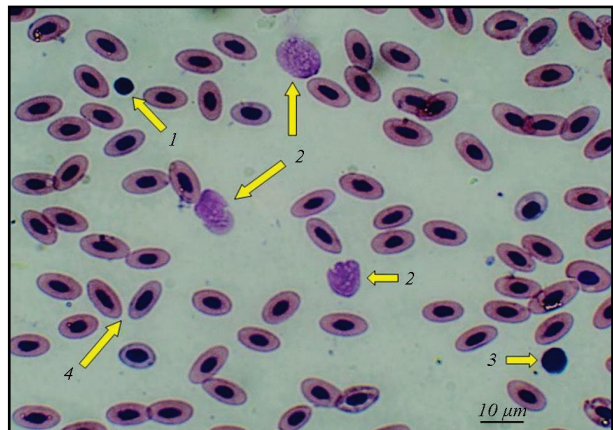


Fig. 5. Thrombocyte (1), heterophil (2), small lymphocyte (3), and erythrocytes (4) in peripheral blood of broilers from a poultry farm

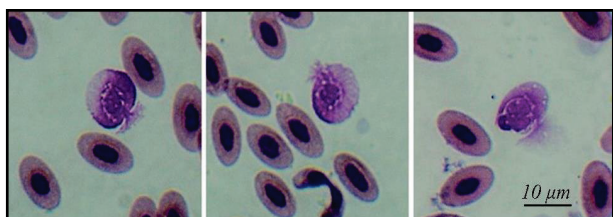


Fig. 6. Heterophils with morphological features of extracellular net formation in peripheral blood of broilers from a poultry farm

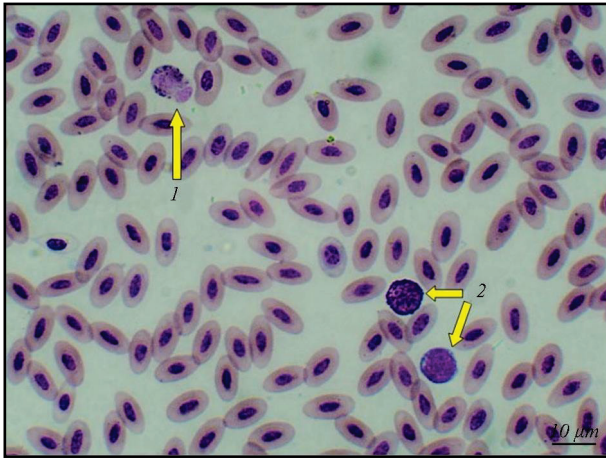


Fig. 7. Eosinophil (1) and basophils (2) in broilers' peripheral blood from a poultry farm

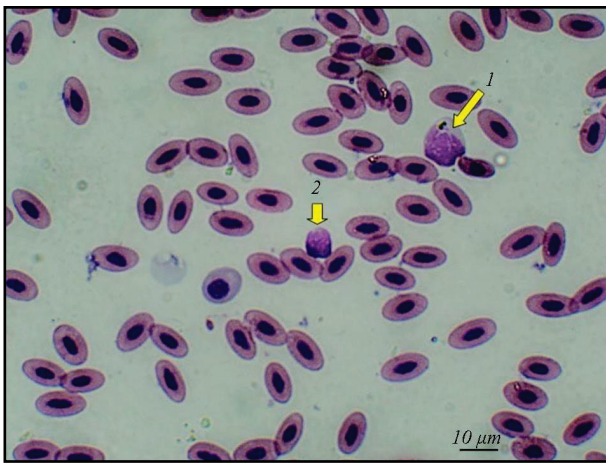


Fig. 8. Monocyte (1) and lymphocyte (2) in peripheral blood of broilers from a poultry farm

For many smears obtained from broilers from the territory with the long time status of enhanced radioecological control we detected the presence of large (Fig. 9) and medium (Fig. 10, 11) lymphocytes, the formation of platelets clusters (Fig. 9, 12) and the presence of "shadows" that are fragments of nuclei of partially destroyed leukocytes, mainly heterophils (Fig. 9).

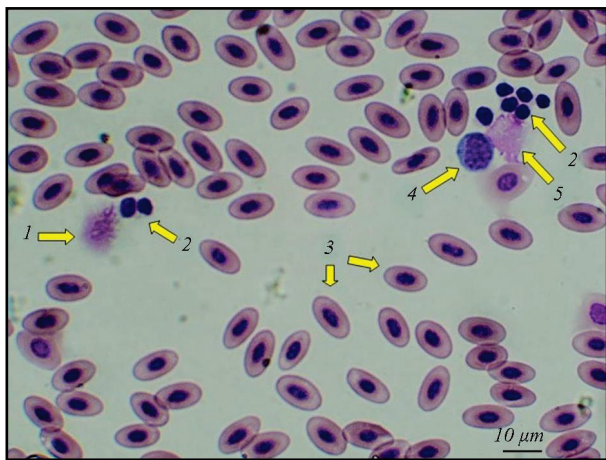


Fig. 9. Heterophilic "shadow" (1), thrombocytes (2), erythrocytes (3), large lymphocytes (4) and heterophil (5) in peripheral blood of broilers from a private farm on the territory with long time enhanced radioecological control status

The majority of the heterophils were morphologically similar to those observed in broilers from the poultry farm, with fine dense grain and a "hidden" nucleus (Fig. 10). Individual lymphocytes were imaged during the period of proliferation (Fig. 12). Eosinophils and basophils had a typical morphology, however, basophils were seen relatively often. Monocytes, on the contrary, were rarely found (Fig. 11).

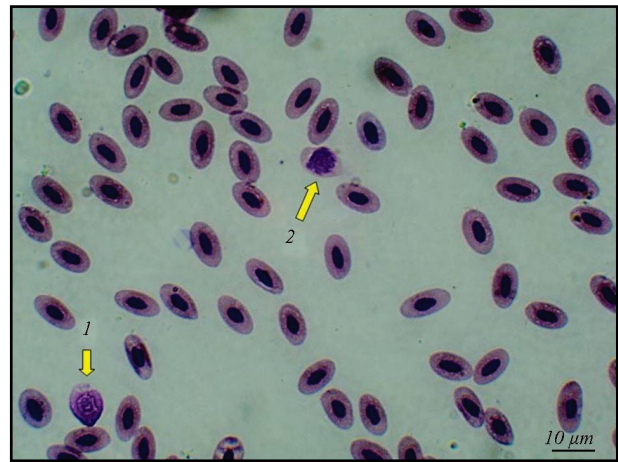


Fig. 10. Heterophil (1) and middle lymphocyte (2) in peripheral blood of broilers from a private farm on the territory with long time status of enhanced radioecological control

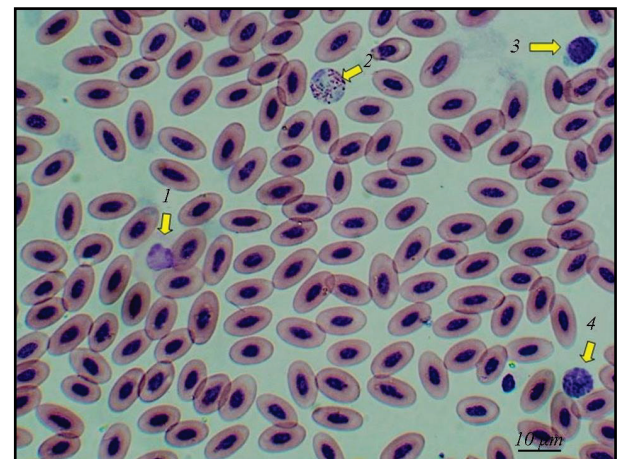


Fig. 11. Monocyte (1), basophil (2), middle lymphocyte (3) and basophil (4) in peripheral blood of broilers from a private farm on the territory with long time status of enhanced radioecological control

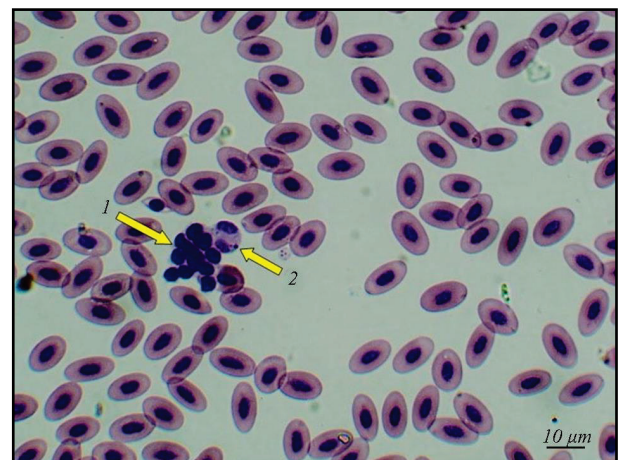


Fig. 12. A cluster of thrombocytes (1) and a lymphocyte during proliferation (2) in peripheral blood of broilers from a private farm on the territory with long time status of enhanced radioecological control

Quantitative analysis showed that the number of erythrocytes and platelets, the total and absolute numbers of monocytes, eosinophils and basophils, the absolute number of lymphocytes and heterophils in broilers from the private farm (in the relatively ecologically clean areas) were within the parameters indicated in the literature. The relative measurements of lymphocytes were shifted to the lower limit of the indicated values, and the relative measurements of heterophils were shifted to the upper limit.

In broilers from the poultry farm, the number of erythrocytes was within the recommended values, the number of platelets was shifted to the upper limit of the data specified in the literature, and in some birds it even exceeded it. The total number of leukocytes, relative and absolute number of eosinophils and basophils were also shifted to the upper limit. The rela-

tive and absolute number of monocytes shifted to a lower level. Parameters for lymphocytes significantly exceeded the lower limit of the recommended values, and heterophils exceeded the upper limit (Table 1).

In broilers raised in territories that had been in the status of enhanced radioecological control for a long time, the parameters for lymphocytes and heterophils showed trends similar to those found in birds from the private farm in a relatively ecologically clean territory. The relative number of heterophils was at the upper recommended values, and the number of lymphocytes was at the lower ones. The relative number of monocytes was lower than the recommended values, and the relative number of basophils was higher (Table 1).

Table 1

Blood parameters in broilers raised under different conditions and exposed to certain environmental or social factors ($x \pm SE$; $n = 20$)

Components	Blood parameters in broilers from a private farm from the relatively ecologically clean territory	Blood parameters in broilers from a private farm from the territory that was in the status of enhanced radioecological control	Blood parameters in broilers from a poultry farm
Erythrocytes, $\times 10^{12}/L$	3.64 ± 0.81	3.59 ± 0.88	3.54 ± 0.89
Leukocytes, $\times 10^9/L$	24.21 ± 6.06	31.01 ± 6.22	33.99 ± 5.10
Thrombocytes, $\times 10^9/L$	41.05 ± 4.36	48.10 ± 6.67	45.01 ± 6.11
Lymphocytes, %	49.98 ± 6.10	47.25 ± 6.02	$16.28 \pm 4.97^{***,###}$
Lymphocytes, $\times 10^9/L$	12.02 ± 0.94	14.10 ± 0.80	$4.69 \pm 0.61^{***,###}$
Heterophils, %	43.98 ± 5.91	46.31 ± 6.02	$78.35 \pm 5.24^{***,###}$
Heterophils, $\times 10^9/L$	10.55 ± 1.05	14.31 ± 1.89	$26.41 \pm 2.80^{***,##}$
Heterophils and lymphocytes ratio, H/L	0.86 ± 0.10	1.01 ± 0.29	$4.78 \pm 0.40^{***,##}$
Monocytes, %	2.29 ± 0.26	$0.54 \pm 0.30^{***}$	$0.99 \pm 0.29^{**}$
Monocytes, $\times 10^9/L$	0.51 ± 0.09	$0.19 \pm 0.10^*$	0.36 ± 0.10
Eosinophils, %	2.01 ± 0.82	2.55 ± 0.90	2.70 ± 0.80
Eosinophils, $\times 10^9/L$	0.48 ± 0.10	0.78 ± 0.30	1.01 ± 0.28
Basophils, %	0.99 ± 0.41	$3.01 \pm 0.55^{**}$	2.06 ± 0.83
Basophils, $\times 10^9/L$	0.24 ± 0.18	$0.91 \pm 0.19^*$	0.72 ± 0.30

Notes: * – $P < 0.05$ parameters compared to the parameters of broilers from a private farm from a relatively ecologically clean territory; ** – $P < 0.01$ parameters compared to the parameters of broilers from a private farm from a relatively ecologically clean area; *** – $P < 0.001$ parameters compared to the parameters of broilers from a private farm from a relatively ecologically clean area; ## – $P < 0.01$ parameters compared to the parameters of broilers from the private farm from the territory that was in the status of enhanced radioecological control; ### – $P < 0.001$ parameters compared to parameters in broilers from a private farm from the territory that was in the status of enhanced radioecological control.

At the same time, there was no statistically significant difference in the number of erythrocytes in birds raised in different conditions (Table).

The average value of the absolute number of leukocytes and platelets in birds from the private farm in an ecologically clean territory was lower than in broilers from the poultry farm and from the private farm in the territory that was in the status of enhanced radioecological control, however, due to a significant spread of parameters and a large standard error, statistically, there was no significant difference in these indicators, as well as in the number of erythrocytes (Table).

Statistical analysis confirmed the presence of trends noted in the morphological analysis of blood smears. Broilers from the poultry farm had significantly lower relative and absolute numbers of lymphocytes than chickens from the other two analyzed groups and significantly higher relative and absolute numbers of heterophils. Also, these birds had a significantly lower relative number of monocytes and a tendency to an increased number of eosinophils and basophils, compared to broilers from the private farm in a relatively ecologically clean area. Moreover, the relative and absolute number of monocytes was significantly lower in broilers from the private farm in a territory that has been under enhanced radioecological control for a long time than in birds from a private farm in a relatively ecologically clean territory. Parameters of basophils were significantly higher too (Table 1).

Analysis of the heterophils/lymphocytes ratio revealed a significantly higher indicator in broilers from the poultry farm than in birds from private farms (Table 1).

Discussion

Evaluating the leukogram of birds can be an important diagnostic indicator in veterinary medicine and environmental research. Methods for collecting, processing, and analyzing avian blood samples have been developed in detail (Campbell, 1994; Davis et al., 2008; Kelly & Alworth, 2013; Jones, 2015). However, birds' blood smears are significantly different from blood smears obtained from mammals. The main difference is

the presence of three types of true nuclear cells: erythrocytes, leukocytes, and platelets. Nuclei in these cells are preserved throughout the life of the cell. This feature complicates morphological studies of bird blood even for experienced hematologists (Boiko et al., 2013).

The literature describes quite variable data regarding the normal parameters of chickens' blood in different periods of their development. Gottstein et al. (2015) report a higher number of heterophils in newborn chickens (on average, about 62% versus 37% of lymphocytes). By the age of 3 weeks, parameters change with the increase of lymphocytes (about 18% vs. 81%), and this pattern is kept throughout the following life (Abdi-Hachesoo et al., 2011; Boiko et al., 2014; Gottstein et al., 2015). Our obtained data was compared during the analysis with the above information and recommendations for the normal parameters (Jain, 1993; Simarakas et al., 2004; Kongroi & Likitdacharote, 2019).

The obtained results indicate that birds from the private farm in a relatively ecologically clean area have initial signs of stress (there is a shift in the number of lymphocytes to the lower limit of the norm and heterophils to the upper limit). However, they do not have a clear manifestation. The presence of small pseudopodia (Fig. 2c) detected in individual lymphocytes is a common phenomenon in birds (Boiko et al., 2013). In broilers from a poultry farm, there is a clear dominance of granulocytic fractions of leukocytes and suppression of mononuclear ones.

The increase in the H/L ratio in birds is considered to be a sign of the response of the immune system to chronic stress caused by a persistent rise in the concentration of circulating corticosterone (Gross & Siegel, 1983; Maxwell, 1993; Davis et al., 2008; Shini et al., 2008; Weimer et al., 2018; Maness et al., 2023). The stress-induced decrease in the number of circulating lymphocytes occurs as a result of processes of redistribution of different populations of leukocytes. Circulating lymphocytes adhere to endothelial cells of blood vessel walls in response to the influence of glucocorticoids, then migrate from the circulation to the lymph nodes, spleen, bone marrow, etc. (Dhabhar, 2002). At the same time, the migration of neutrophils into the bloodstream from the bone marrow is stimulated and their exit from the blood is inhibited (Bishop et al., 1968).

Research experiments show that a typical stress leukocyte reaction can be caused by the addition of corticosteroids to the drinking water for broilers (Weimer et al., 2018). The stress effect of corticosterone treatment persists 7 days after finishing the injection course (Mehaisen et al., 2017). Accordingly, the H/L ratio is considered an important parameter of stress intensity in domestic chickens (Huth & Archer, 2015; Najafi et al., 2015; Pejaković et al., 2021).

The combination of several stress factors increases both the H/L ratio and corticosteroid concentrations. Social stress, infections, lack of nutrition and lighting, and transportation are considered to be possible factors that might cause stress effects (Scaney, 2016).

The increase in the heterophils/lymphocytes ratio in broilers from the poultry farm can be caused by two factors, which are social factors of housing density and subsequent capture and transportation to the zoo. The factory production of broiler chickens makes them sensitive to potential stress factors and can cause a constant production of stress hormones (Mehaisen et al., 2017; Nielsen et al., 2023). Accordingly, we can assume that changes in blood parameters in broilers should be expected during factory farming. However, some studies show the absence of such an effect, which may mean the absence of clear stress factors in chickens from poultry farms. Furthermore, some authors believe that birds have generally relatively low adrenal gland reaction to social stress influence, and its variability depends on species, genetic factors, and age (Beuving & Vonder, 1977; Mormède et al., 2007). The diversity of genetically determined reactions to stress factors of different natures has been confirmed for various animals and humans (King et al., 2001; Melnyk et al., 2005; Muráni et al., 2010).

The detected increase in the H/L ratio in our case could be caused by the transportation process. Acute stress caused by 30-minute or longer transportation creates a significant increase in the concentration of corticosteroid in chickens (Kober et al., 2011; Gou et al., 2021). The available data shows that usually the rise of corticosterone during acute stress can begin 2 minutes after the capture of birds (Romero & Reed, 2005), and the following increase in the number of heterophils and decrease in the number of lymphocytes occurs in one to two hours (Cirule et al., 2012). Blood samples were taken from broilers 5 hours after transportation to the zoo. Thus, potentiation of moderate chronic stress during cultivation and acute during transportation is possible.

The presence of monocytes in the process of phagocytosis and heterophils with signs of the extracellular traps formation and increased granularity indicate the mobilization and possible stress-induced reaction of phagocytic leukocytes (Chuammitri et al., 2009; Nidamanuri et al., 2017; Anand Laxmi, 2019; Abo-Al-Ela et al., 2021). Considering that monocytes are the precursors of macrophages, which are involved in numerous immunopathological processes (Guttenberg et al., 2024), the detected signs require additional analysis.

A chronic increase in concentration of corticosterone causes an earlier response to following stressful effects. A two-phase cellular reaction can be observed in birds during some types of stress, which is characterized by a change in the redistribution of blood cells. The first phase appears under light and moderate stress loads and is characterized by heterophilia and an increase in the H/L ratio. The second phase occurs under severe stress associated with life-threatening conditions and is characterized by basophilia (Maxwell et al., 1992; Maxwell, 1993). Indeed, broilers from the poultry farm showed a tendency to increase in the number of basophils. However, another tendency is the increase of the eosinophil number, which is not typical for a stressful situation (Davis et al., 2008; Cirule et al., 2012). Perhaps such an effect is caused by the peculiarities of nutrition. There are data on probiotic-stimulated increase of the number of eosinophils. Eosinophils play an active role in the regulation of allergic reactions and parasitic invasions, respectively, they can react to any atypical food components (Yudiarti, 2017). Moreover, some studies show a multidirectional response of basophils to different types of stress factors (Nwaigwe et al., 2020).

An important element of maintaining the health of predators in zoos is their nutrition with chicken carcasses and, in some cases, live birds. This contributes to the provision of their physical, psychological, and social needs (Cooper, 1983; Mellor, 2015), and the implementation of the principle of "natural feeding" (Mellen, 1991; Kurtz, 2002), which, as a result,

creates a satisfaction effect with food consumption (Lindburg, 1988; Carlstead et al., 1991). Many animals need to make some effort to obtain food, rather than having easy access to it (Siegford, 2013; Wolfensohn et al., 2018). On the other hand, the situation of animals living in captivity is complicated by stress factors, which are limited opportunities for movement, lack of camouflage, unfamiliar food, interrupted circadian rhythms, etc. It is difficult to measure the level of stress reaction both by monitoring vegetative reactions and by analyzing biochemical parameters, in particular, the concentration of cortisol in biological material (Line et al., 1989; Wolfensohn et al., 2018; Tallo-Parra et al., 2023). At the same time, numerous studies show that social stress factors, especially when combined with other types of stress, can significantly affect the immune system and, accordingly, the state of health of both humans and animals (Sapolsky, 2004; Cavigelli & Chaudhry, 2012; Sokolenko, 2016). Feeding such animals with chicken carcasses or live birds, whose body contains a high concentration of corticosteroids (a sign of which is an increased number of heterophils), can cause the effect of hypercortisolemia, which will negatively affect their physiological status. The discovered phenomenon should be carefully studied by the veterinary services of zoos and requires further research accordingly. It is desirable to keep chickens for a certain time in zoo conditions before using them as food for predators considering the duration of the stress effect.

Among the analyzed groups of chickens were broilers from a private farm on the territory under long term status of enhanced radioecological control following the accident at the Chernobyl nuclear power plant. An increased activity of ¹³⁷Cs is observed in this area nowadays. Radiation disasters create involuntary conditions for studying the immediate and distant consequences of the impact of ionizing radiation on various populations of living organisms. Despite the long time that has passed since 1986, the expression of the radioadaptive response in wild populations living in contaminated areas remains poorly understood (Galván et al., 2014; Webster et al., 2016; Murphy, 2018; Cannon & Kiang, 2022; Klyuchko et al., 2023). It is necessary to consider the species and individual variability of the potential for the formation of adaptive reactions (Luckey & Lawrence, 2006). The impact of radiation on the population of living organisms in Chernobyl, particularly on birds, is characterized generally as negative (Møller et al., 2012). Activation of oxidative processes and suppression of antioxidant protection during chronic irradiation of various biological objects, including humans, is indicated (Sokolenko et al., 2018; Sokolenko & Sokolenko, 2019). However, the growth of many bird populations in contaminated areas confirms the potential presence of an adaptive response to chronic exposure and the oxidative processes induced by it. The thesis proposes that birds not only successfully adapt to the negative effects of chronic exposure, but can also demonstrate hormonal effects beneficial to the population. Adaptive responses are often correlated with pigment concentration (Galván et al., 2011, 2014). In particular, albinism in bird populations is correlated with tumor formation and background radiation doses in polluted areas (Møller et al., 2013).

Ionizing radiation is both a physical and psychological stress factor (Nylund et al., 2014; Contis & Foley, 2015; Samaila et al., 2024). It is possible to predict a redistribution of leukocyte populations in favor of heterophils and an increase in the H/L ratio in the group of chickens with an increased radiation background. However, the blood parameters of these broilers were more similar to those of birds from private farms in ecologically clean territory. They significantly differed in many parameters from broilers grown in the poultry factory after their transportation. Perhaps the determining factor was the social conditions of cultivation, especially the adaptability of birds to contact with humans. Birds from private farms were in constant contact with their owners, as they were often picked up. Regular gentle handling of chickens reduces physiological stress responses (Zulkifli & Siti Nor Azah, 2004). This is expressed both in the features of corticosterone production and in the ratio of heterophils/lymphocytes (Al-Aqil et al., 2013). Fear of humans is one of the strongest socio-emotional stress factors for many animals, particularly birds. It is an adaptive psychophysiological response to potential or real danger (Jones et al., 1988). It has been shown that psychological stress in chickens caused by fear can lead to morphological changes in lymphocytes and heterophils (Mauldin et al., 1979). The morphological features of lymphocytes and heterophils in broilers from the radiation-contamina-

ted territory that we discovered were not caused by emotional factors. The blood collection procedure can be ignored as a stress factor in this case. Indeed, the concentration of corticosteroids increases already during the capture of the bird and blood collection, especially if the duration of the procedure exceeds 2 minutes (Mormède et al., 2007; Chloupek et al., 2011; Blas, 2015), and if the stress is increased by the fear of humans and temporary restriction of movement (Hemsworth et al., 1994; Kannan & Mench, 1996). However, considering data on quantitative changes in the H/L ratio during stress (at least after one hour (Círule et al., 2012)), short-term blood collection would not take long enough to cause either quantitative or morphological changes.

Changes in cell morphology can be caused by stress factors of a physical nature, and one of the consequences of physical stress effects is indicated as an increase in the number of basophils (Maxwell et al., 1992), which is noted in broilers from a number of areas with increased ^{137}Cs activity and can be explained as a radiation-induced effect (Table 1). Chickens are characterized by rapid accumulation of ^{137}Cs (especially in meat) when consuming contaminated food. However, more than half of these radionuclides (55%) are removed from an organism during the 1st day after receiving a dose, and in two weeks more than 90% of radionuclides are removed. Small animals, especially birds, are characterized by intensive removal of radionuclides (Pöschl et al., 1997; Vicentijevic et al., 2013). Thus, the existing effect can be explained only by the regular use of contaminated feed. On the other hand, it is unrealistic to talk about high concentrations of radionuclides in compound feed. Perhaps, in this case, the effect of chronic radiation appears over several generations. The consequences can be observed as a tendency of many heterophils to destruction, accumulation of platelets, and dominance among lymphocytes of medium and large forms. Medium lymphocytes are often reactive, and large lymphocytes indicate potential oncological processes in the blood system. The reactivity of lymphocytes was confirmed by the detected proliferative processes in birds from the radiation-contaminated territory (Fig. 12). Thrombocytosis can characterize the presence of existing inflammatory processes (Jones, 2015).

Thereafter, birds raised in areas with a high radiation background have certain signs of stress. In addition to an increased number of basophils, another stress marker is a reduced number of monocytes. This effect also can be caused by the treatment of broilers with glucocorticoid hormones (Vicuna et al., 2015; Scanes, 2016). However, the stress reaction is quite specific. The absence of changes in the H/L ratio may be an allostatic adaptive response, which, however, did not work for all blood parameters. Additional information can be provided by the following research related to indicators of lipid metabolism and oxidative processes. Stress leads to significant changes in the metabolism of chickens, and it is characterized by changes in the concentration of cholesterol (Virden & Kidd, 2009). The concentration of different fractions of cholesterol, especially in combination with the condition of the oxidant-antioxidant system, is a reflection of the presence of various types of stress in people who have lived in radiation-contaminated areas for a long time (Sokolenko & Sokolenko, 2019). In this case not only the adrenal glands but also other endocrine factors are involved in the formation of radiation-induced stress processes (Sokolenko & Sokolenko, 2019a), which should be taken under consideration in future studies of agricultural animal populations.

Variability in the interpretation of data becomes a problem when assessing stressful situations caused by extreme social and environmental factors. However, it is generally accepted that corticosteroids lead to an increase in the number and percentage of neutrophils in mammals or heterophils in birds and a decrease in the number and percentage of lymphocytes. This feature can be suggested as a marker of the presence of stress (Davis et al., 2008; Maness et al., 2023). The hormonal condition of farm animals is quite stable during chronic stressful situations, so it requires dynamic testing of blood parameters (Mormède et al., 2007). Research has not yet explained the mechanisms that enable effective minimization of the harmful effects of physiological stress on poultry farms (Virden & Kidd, 2009; Vizzier-Thaxton et al., 2016). Early detection of stress load activation through blood analysis can help maintain bird health and prevent significant economic losses. Considering the significant variability of indicators, it is recommended to determine various parameters of stress load with a comparison of interlaboratory standards. This ap-

proach provides the maximum reliability of the obtained results (Scanes, 2016; Tainika et al., 2023). The radiation stress factor is particularly difficult to study (Pejaković et al., 2021). There was an issue with the analysis of ancestor broilers that produced the current generation of chickens (they were purchased by the owners at the local market) from the territory under long-term enhanced radio-ecological control and the young age of the birds. It is known that the age factor is important in the sensitivity of birds to radiation and the efficiency of recovery processes (Malhotra & Rana, 1988). It is necessary to investigate the blood parameters of chickens that have lived in contaminated areas for several generations and reached a mature age.

Conclusions

There are potentially diverse marker expressions of quantitative and morphological features in broilers that were stressed by factors of different natures. Morphological changes prevailed in birds raised on the private farm on the territory with an increased radiation background. In the same sample, the main quantitative expression of the stress reaction was an increase in the number of eosinophils and a decrease in the number of monocytes. In birds raised in relatively ecologically clean areas, typical stress reactions, characterized by a significant increase in the heterophils and lymphocytes ratio, were developed after acute transport stress. As the H/L ratio is considered to be a sign of chronic stress, the effect found in broilers from the poultry farm could be a sign of the potentiation of various stress factors. The effect of taming had a positive effect on stress reactions in birds, reducing their expression. It is desirable to minimize stress in broilers whose meat is intended for human and animal nutrition. Additional research is needed to clarify the assessment of the adaptive processes of domestic birds to various eco-social effects of a stressful nature and to understand their mechanisms.

The research was conducted within the framework of the grant topic "Immunological and microbiological parameters of allostatic load formation" (registration number 0121U107531) at the request of the Ministry of Education and Science of Ukraine.

The authors declare that they have no competing interests (in research performance, obtained results, and their presentation).

References

- Abdi-Hachesoo, B., Talebi, A., & Asri-Rezaei, S. (2011). Comparative study on blood profiles of indigenous and Ross-308 broiler breeders. *Global Veterinaria*, 7(3), 238–241.
- Abo-Al-Ela, H. G., El-Kassas, S., El-Naggar, K., Abdo, S. E., Jahejo, A. R., & Al Wakeel, R. A. (2021). Stress and immunity in poultry: light management and nanotechnology as effective immune enhancers to fight stress. *Cell Stress and Chaperones*, 26(3), 457–472.
- Al-Aqil, A., Zulkifli, I., Hair Bejo, M., Szilvi, A. Q., Rajion, M. A., & Somchit, M. N. (2013). Changes in heat shock protein 70, blood parameters, and fear-related behavior in broiler chickens as affected by pleasant and unpleasant human contact. *Poultry Science*, 92(1), 33–40.
- Anand Laxmi, N. (2019). The chicken heterophil – A short review. *Open Access Journal of Veterinary Science and Research*, 4(1), 168.
- Anthony, N. B., Katanbaf, M. N., & Siegel, P. B. (1988). Responses to social disruption in two lines of White Leghorn chickens. *Applied Animal Behaviour Science*, 21(3), 243–250.
- Beňová, K., Dvořák, P., Tomko, M., & Falis, M. (2016). Artificial environmental radionuclides in Europe and methods of lowering their foodstuff contamination – A review. *Acta Veterinaria Bmo*, 85(1), 105–112.
- Beuving, G., & Vonder, G. A. (1977). Daily rhythm of corticosterone in laying hens and the influence of egg laying. *Reproduction*, 51(1), 169–173.
- Beuving, G., Jones, R. B., & Blokhuis, H. J. (1989). Adrenocortical and heterophil/lymphocyte responses to challenge in hens showing short or long tonic immobility reactions. *British Poultry Science*, 30(1), 175–184.
- Bishop, C. R., Athens, J. W., Boggs, D. R., Wamer, H. R., Cartwright, G. E., & Wintrobe, M. M. (1968). Leukokinetic studies: XIII. A non-steady-state kinetic evaluation of the mechanism of cortisone-induced granulocytosis. *The Journal of Clinical Investigation*, 47(2), 249–260.
- Blas, J. (2015). Stress in birds. In: Scanes, C. G. (Ed.). *Sturkie's avian physiology*. Academic Press, Cambridge. Pp. 769–810.
- Boiko, N. I., Boiko, Y. V., Konakhii, R. V., & Mykolaichuk, R. P. (2013). Doslidzhennia morfolohiyi klitny krovi u kurey [Investigation of the morphology of

- blood cells in chickens]. *Suchasne Ptakhivnytsvo*, 12(133), 18–22 (in Ukrainian).
- Boiko, Y. V., Dukhnytskyi, V. B., Boiko, H. V., & Boiko, N. I. (2014). Morfolohichnyi sklad krovi kurchat-broileriv za sumisnoyi diyi okhratoksynu A i dezoksynivalenolu [Morphological composition of the blood of broiler chickens with the combined action of ochratoxin A and deoxynivalenol]. *Ukrainskyi Chasopys Vetrynarnamykh Nauk*, 201, 15–20 (in Ukrainian).
- Burlaka, V. A., Kryvyi, M. M., Skoromna, O. I., Chudak, R. V., Goralski, L. P., Borshchenko, V. V., Pavlyuk, N. V., & Suknenko, T. M. (2012). Hodivlia ekzotychnykh tvaryn [Feeding exotic animals]. Polissia, Zhytomyr (in Ukrainian).
- Campbell, T. W. (1994). Hematology. In: Ritchie, B. R., Harrison, G. J., & Harrison, L. R. (Eds.). *Avian medicine: Principles and applications*. Wingers Publishing, Lake Worth. Pp. 176–198.
- Campo, J. L., Gil, M. G., Munoz, I., & Alonso, M. (2000). Relationships between bilateral asymmetry and tonic immobility reaction or heterophil to lymphocyte ratio in five breeds of chickens. *Poultry Science*, 79(4), 453–459.
- Cannon, G., & Kiang, J. G. (2022). A review of the impact on the ecosystem after ionizing irradiation: Wildlife population. *International Journal of Radiation Biology*, 98(6), 1054–1062.
- Carlstead, K., Seidensticker, J., & Baldwin, R. (1991). Environmental enrichment for zoo bears. *Zoo Biology*, 10(1), 3–16.
- Cavigelli, S. A., & Chaudhry, H. S. (2012). Social status, glucocorticoids, immune function, and health: Can animal studies help us understand human socioeconomic-status-related health disparities? *Hormones and Behavior*, 62(3), 295–313.
- Chloupek, P., Bedanova, I., Chloupek, J., & Vecerek, V. (2011). Changes in selected biochemical indices resulting from various pre-sampling handling techniques in broilers. *Acta Veterinaria Scandinavica*, 53(1), 31.
- Chuammitri, P., Ostojic, J., Andreasen, C. B., Redmond, S. B., Lamont, S. J., & Palić, D. (2009). Chicken heterophil extracellular traps (HETs): Novel defense mechanism of chicken heterophils. *Veterinary Immunology and Immunopathology*, 129(1–2), 126–131.
- Čirule, D., Krama, T., Vrublevska, J., Rantala, M. J., & Krams, I. (2012). A rapid effect of handling on counts of white blood cells in a wintering passerine bird: A more practical measure of stress? *Journal of Ornithology*, 153(1), 161–166.
- Contis, G., & Foley Jr., T. P. (2015). Depression, suicide ideation, and thyroid tumors among Ukrainian adolescents exposed as children to Chernobyl radiation. *Journal of Clinical Medicine Research*, 7(5), 332.
- Cooper, M. E. (1983). The Zoo Licensing Act 1981. *The Veterinary Record*, 112(24), 564–567.
- Davis, A. K., Maney, D. L., & Maerz, J. C. (2008). The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. *Functional Ecology*, 22(5), 760–772.
- Dhabhar, F. S. (2002). A hassle a day may keep the doctor away: stress and the augmentation of immune function. *Integrative and Comparative Biology*, 42(3), 556–564.
- Galván, I., Bonisoli-Alquati, A., Jenkinson, S., Ghanem, G., Wakamatsu, K., Mousseau, T. A., & Möller, A. P. (2014). Chronic exposure to low-dose radiation at Chernobyl favours adaptation to oxidative stress in birds. *Functional Ecology*, 28(6), 1387–1403.
- Galván, I., Mousseau, T. A., & Möller, A. P. (2011). Bird population declines due to radiation exposure at Chernobyl are stronger in species with pheomelanin-based coloration. *Oecologia*, 165, 827–835.
- Gottstein, Ž., Ciglar Grozdanić, I., Mazija, H., Shek Vugrovečki, A., & Milinković-Tur, S. (2015). Changes in blood cell count in chickens vaccinated as newly-hatched against Marek's disease using HVT FC 126 by means of nebulisation. *Veterinarski Arhiv*, 85(1), 11–22.
- Gou, Z., Abouelezz, K. F. M., Fan, Q., Li, L., Lin, X., Wang, Y., Cui, X., Ye, J., Masoud, M. A., Jiang, S., & Ma, X. (2021). Physiological effects of transport duration on stress biomarkers and meat quality of medium-growing Yellow broiler chickens. *Animal*, 15(2), 100079.
- Gross, W. B., & Siegel, H. S. (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Diseases*, 27(4), 972–979.
- Gross, W. B., Dunnington, E. A., & Siegel, P. B. (1984). Environmental effects on the wellbeing of chickens from lines selected for responses to social strife. *Archiv fuer Gefluegelkunde*, 48, 3–7.
- Guttenberg, M. A., Vose, A. T., Birukova, A., Lewars, K., Cumming, R. I., Albright, M. C., Mark, J. I., Salazar, C. J., Swaminathan, S., Y. Z., Sokolenko, Y. V., Bunyan, E., Yaeger, M. J., Fessler, M. B., Que, L. G., Gowdy, K. M., Misharin, A. V., & Tighe, R. M. (2024). Tissue-resident alveolar macrophages reduce ozone-induced inflammation via MerTK mediated efferocytosis. *American Journal of Respiratory Cell and Molecular Biology*, 70(6), 493–506.
- Hemsworth, P. H., Coleman, G. J., Barnett, J. L., & Jones, R. B. (1994). Behavioural responses to humans and the productivity of commercial broiler chickens. *Applied Animal Behaviour Science*, 41(1–2), 101–114.
- Hendricks III, G. L., Mashaly, M. M., & Siegel, H. S. (1995). Validation of an assay to measure adrenocorticotropin in plasma and from chicken leukocytes. *Poultry Science*, 74(2), 337–342.
- Hendricks III, G. L., Siegel, H. S., & Mashaly, M. M. (1991). Ovine corticotropin-releasing factor increases endocrine and immunologic activity of avian leukocytes *in vitro*. *Proceedings of the Society for Experimental Biology and Medicine*, 196(4), 390–395.
- Huth, J. C., & Archer, G. S. (2015). Comparison of two LED light bulbs to a dimmable CFL and their effects on broiler chicken growth, stress, and fear. *Poultry Science*, 94(9), 2027–2036.
- Jain, N. C. (1993). *Essential of veterinary hematology*. Lea & Febiger, Philadelphia.
- Jeong, S. H., Kang, D., Lim, M. W., Kang, C. S., & Sung, H. J. (2010). Risk assessment of growth hormones and antimicrobial residues in meat. *Toxicological Research*, 26(4), 301–313.
- Jones, M. P. (2015). Avian hematology. *Clinics in Laboratory Medicine*, 35(3), 649–659.
- Jones, R. B., Beuving, G., & Blokhuis, H. J. (1988). Tonic immobility and heterophil/lymphocyte responses of the domestic fowl to corticosterone infusion. *Physiology and Behavior*, 42(3), 249–253.
- Kannan, G., & Mench, J. A. (1996). Influence of different handling methods and crating periods on plasma corticosterone concentrations in broilers. *British Poultry Science*, 37(1), 21–31.
- Kelly, L. M., & Alworth, L. C. (2013). Techniques for collecting blood from the domestic chicken. *Lab Animal*, 42(10), 359–361.
- King, J. A., Abend, S., & Edwards, E. (2001). Genetic predisposition and the development of posttraumatic stress disorder in an animal model. *Biological Psychiatry*, 50(4), 231–237.
- Klyuchko, O. M., Lizunov, G. V., & Beloshitsky, P. V. (2023). Radiation phenomena: Some natural sources, mechanisms of effects, ways of biological organisms protection and rehabilitation. *Biotechnologia Acta*, 16(3), 24–44.
- Kober, H. A. K. M., Aoyama, M., Tsukahara, N., & Sugita, S. (2011). The effects of acute transportation stress on the adrenal gland of the domestic chicken (*Gallus domesticus*). *Animal Behaviour and Management*, 47(3), 97–103.
- Kongroi, K., & Likitdacharote, B. (2019). The relationship of hematological values with Newcastle disease antibody in Thai indigenous chicken: Strain Leung Hang Khao. *Journal of Agricultural Technology*, 15(2), 309–318.
- Kurtz, J. (2002). Feeding animals nature's way. *Safari!*, 10(4), 4–5.
- Lindburg, D. G. (1988). Improving the feeding of captive felines through application of field data. *Zoo Biology*, 7(3), 211–218.
- Line, S. W., Morgan, K. N., Markowitz, H., & Strong, S. (1989). Influence of cage size on heart rate and behavior in rhesus monkeys. *American Journal of Veterinary Research*, 50(9), 1523–1526.
- Luckey, T. D., & Lawrence, K. S. (2006). Radiation hormesis: The good, the bad, and the ugly. *Dose-Response*, 4, 169–190.
- Malhotra, N., & Rana, K. (1988). Effect of gamma radiation on haematology of chick (*Gallus gallus domesticus*). *Radiobiologia Radiotherapia*, 29(1), 119–132.
- Maness, T. J., Grace, J. K., Hirschak, M. R., Tompkins, E. M., & Anderson, D. J. (2023). Circulating corticosterone predicts near-term, while H/L ratio predicts long-term, survival in a long-lived seabird. *Frontiers in Ecology and Evolution*, 11, 1172904.
- Mauldin, J. M., Siegel, P. B., & Gross, W. B. (1979). Interfacing genetics, behavior and husbandry in White Leghorns presented with *E. coli* challenge. *Applied Animal Ethology*, 5(4), 347–359.
- Maxwell, M. H. (1993). Avian blood leucocyte responses to stress. *World's Poultry Science Journal*, 49(1), 34–43.
- Maxwell, M. H., Robertson, G. W., Mitchell, M. A., & Carlisle, A. J. (1992). The fine structure of broiler chicken blood cells, with particular reference to basophils, after severe heat stress. *Comparative Haematology International*, 2(4), 190–200.
- Mehaisen, G. M., Eshak, M. G., Elkaiaty, A. M., Atta, A. R. M., Mashaly, M. M., & Abass, A. O. (2017). Comprehensive growth performance, immune function, plasma biochemistry, gene expressions and cell death morphology responses to a daily corticosterone injection course in broiler chickens. *PLoS One*, 12(2), e0172684.
- Mellen, J. D. (1991). Factors influencing reproductive success in small captive exotic felids (*Felis* spp.): A multiple regression analysis. *Zoo Biology*, 10(2), 95–110.
- Mellor, D. J. (2015). Positive animal welfare states and encouraging environment-focused and animal-to-animal interactive behaviours. *New Zealand Veterinary Journal*, 63(1), 9–16.
- Mel'nyk, T. O., Sokolenko, V. L., & Sokolenko, S. V. (2005). Vplyv normobarychnoy hipoksii na deiakii pokaznyky klitynnoho imunitetu v osib, shcho mayut' rizni hrupy krovi systemy ABO [Normobaric hypoxia effects on some parameters of cellular immunity of persons with different blood groups]. *Fiziolohichniy Zhurnal*, 51(5), 61–64 (in Ukrainian).
- Mitchell, M. A., Kettlewell, P. J., & Maxwell, M. H. (1992). Indicators of physiological stress in chicken chickens during road transportation. *Animal Welfare*, 1, 91–103.
- Möller, A. P., Bonisoli-Alquati, A., & Mousseau, T. A. (2013). High frequency of albinism and tumours in free-living birds around Chernobyl. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 757(1), 52–59.

- Møller, A. P., Bonisoli-Alquati, A., Rudolfson, G., & Mousseau, T. A. (2012). Elevated mortality among birds in Chernobyl as judged from skewed age and sex ratios. *PLoS One*, 7, e35223.
- Moreels, M., Baselet, B., Van Hoey, O., Vanhavere, F., & Baatout, S. (2020). Stress and radiation responsiveness. In: Choukèr, A. (Ed.). *Stress challenges and immunity in space: From mechanisms to monitoring and preventive strategies*. Springer, Cham. Pp. 373–404.
- Mormède, P., Andanson, S., Aupérin, B., Beerda, B., Guémené, D., Malmkvist, J., Manteca, X., Manteuffel, G., Prunet, P., van Reenen, C. G., Richard, S., & Veissier, I. (2007). Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. *Physiology and Behavior*, 92(3), 317–339.
- Muráni, E., Ponsuksili, S., D'Eath, R. B., Turner, S. P., Kurt, E., Evans, G., Tholkng, L., Klont, R., Foury, A., Mormede, P., & Wimmers, K. (2010). Association of HPA axis-related genetic variation with stress reactivity and aggressive behaviour in pigs. *BMC Genetics*, 11(1), 74.
- Murphy, L. (2018). Ionizing radiation in veterinary medicine. In: Gupta, R. C. (Ed.). *Veterinary Toxicology*. Academic Press, Cambridge. Pp. 327–337.
- Najafi, P., Zulkifli, I., Soleimani, A. F., & Kashiani, P. (2015). The effect of different degrees of feed restriction on heat shock protein 70, acute phase proteins, and other blood parameters in female broiler breeders. *Poultry Science*, 94(10), 2322–2329.
- Nidamanuri, A., Murugesan, S., & Mahapatra, R. (2017). Effect of heat stress on physiological parameters of layers: A review. *International Journal of Livestock Research*, 7(4), 1–17.
- Nielsen, S. S., Alvarez, J., Bicout, D. J., Calistri, P., Canali, E., Drewe, J. A., Garin-Bastuji, B., Rojas J. L. C., Schmidt, C. G., Herskin, M. S., Chueca, M. A. M., Padalino, B., Pasquali, P., Roberts H. C., Spooler H., Stahl K., Velarde, A., Viltrop, A., Winckler, C., Tiemann, I., de Jong, I., Gebhardt-Henrich S. G., Kee-ling, L., Riber, A. B., Ashe, S., Candiani, D., Matas, R. G., Hempen, M., Mosbach-Schulz, O., Gimeno, C. R., Van der Stede, Y., Vitali, M., Bailly-Caumette, E., Velarde, A., & Michel, V. (2023). Welfare of broilers on farm. *EFSA Journal*, 21(2), e07788.
- Nwaigwe, C. U., Ihedioha, J. I., Shoyinka, S. V., & Nwaigwe, C. O. (2020). Evaluation of the hematological and clinical biochemical markers of stress in broiler chickens. *Veterinary World*, 13(10), 2294.
- Nylund, R., Lemola, E., Hartwig, S., Lehr, S., Acheva, A., Jahns, J., Hildebrandt, G., & Lindholm, C. (2014). Profiling of low molecular weight proteins in plasma from locally irradiated individuals. *Journal of Radiation research*, 55(4), 674–682.
- Pejaković Hlede, J., Vince, S., Žura Žajca, I., Majer, M., & Vilić, M. (2021). Effect of low dose gamma irradiation on number and ratio of some peripheral blood cells in hatched chicks. *Veterinaria*, 70(1), 27–36.
- Pöschl, M., Borkovec, V., & Zelenka, J. (1997). Dynamics and distribution of radio-caesium in broiler chicken. *Radiation and Environmental Biophysics*, 36(3), 169–174.
- Prieto, M. T., & Campo, J. L. (2010). Effect of heat and several additives related to stress levels on fluctuating asymmetry, heterophil: Lymphocyte ratio, and tonic immobility duration in White Leghorn chicks. *Poultry Science*, 89(10), 2071–2077.
- Redmond, S. B., Chuammitri, P., Andreasen, C. B., Palić, D., & Lamont, S. J. (2011). Proportion of circulating chicken heterophils and CXCL12 expression in response to *Salmonella enteritidis* are affected by genetic line and immune modulating diet. *Veterinary Immunology and Immunopathology*, 140(3–4), 323–328.
- Romero, L. M., & Reed, J. M. (2005). Collecting baseline corticosterone samples in the field: Is under 3 min good enough? *Comparative Biochemistry and Physiology, Part A: Molecular and Integrative Physiology*, 140(1), 73–79.
- Samaila, B., Tijjani, A. M., Abdul-Azeez, M. A., & Olasoji, O. W. (2024). Implications of ionizing radiation exposure to patients' mental health during diagnosis and treatment: A systematic review. *Cancer Research and Cellular Therapeutics*, 8(3), 2640–1053.
- Sapolsky, R. M. (2004). Social status and health in humans and other animals. *Annual Review of Anthropology*, 33, 393–418.
- Scanes, C. G. (2016). Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. *Poultry Science*, 95(9), 2208–2215.
- Selye, H. (1937). Studies on adaptation. *Endocrinology*, 21(2), 169–188.
- Shini, S., Kaiser, P., Shini, A., & Bryden, W. L. (2008). Differential alterations in ultrastructural morphology of chicken heterophils and lymphocytes induced by corticosterone and lipopolysaccharide. *Veterinary Immunology and Immunopathology*, 122(1–2), 83–93.
- Siegförd, J. M. (2013). Multidisciplinary approaches and assessment techniques to better understand and enhance zoo nonhuman animal welfare. *Journal of Applied Animal Welfare Science*, 16(4), 300–318.
- Simarak, S., Chinrasri, O., & Aengwanich, W. (2004). Hematological, electrolyte and serum biochemical values of the Thai indigenous chickens (*Gallus domesticus*) in Northeast Thailand. *Songklanakarin Journal of Science and Technology*, 26(3), 425–430.
- Sokolenko, V. L. (2016). Vplyv psykhoemotsiynoho navantazhennia na pokaznyky immunoyi systemy v osib, shcho prozhyvaly na terytoriyakh, zabrudnennykh radionuklidamy [Impact of emotional stress on the immune system indices among residents of radiation contaminated areas]. *Fiziolohichniy Zhurnal*, 62(4), 53–59 (in Ukrainian).
- Sokolenko, V. L., & Sokolenko, S. V. (2019a). Interdependence of oxidative/anti-oxidant system indicators and thyroid status under conditions of prolonged exposure to small doses of radiation. *Regulatory Mechanisms in Biosystems*, 10(2), 219–227.
- Sokolenko, V. L., & Sokolenko, S. V. (2019b). Lipid profile parameters and oxidative processes intensity in the persons who have been affected by low doses of radiation. *The Ukrainian Biochemical Journal*, 91(3), 99–106.
- Sokolenko, V. L., Sokolenko, S. V., Sheiko, V. I., & Kovalenko, O. V. (2018). Interconnection of the immune system and the intensity of the oxidative processes under conditions of prolonged exposure to small doses of radiation. *Regulatory Mechanisms in Biosystems*, 9(2), 167–176.
- Tainika, B., Şekeröglü, A., Akyol, A., & Waithaka Ng'ang'a, Z. (2023). Welfare issues in broiler chickens: Overview. *World's Poultry Science Journal*, 79(2), 285–329.
- Tallo-Para, O., Salas, M., & Manteca, X. (2023). Zoo animal welfare assessment: Where do we stand? *Animals*, 13(12), 1966.
- Vicentijevic, M. C., Pantelić, G. K., Vukovic, D. Z., Vukovic, V. J., Mitrovic, B. M., & Zivanov, D. Z. (2013). The effect of radioprotectors protection ability in pheasants after alimentary radio-contamination with Cs-137. *Nuclear Technology and Radiation Protection*, 28(2), 232–236.
- Vicuna, E. A., Kuttappan, V. A., Galarza-Seeber, R., Latorre, J. D., Faulkner, O. B., Hargis, B. M., Tellez, G., & Bielke, L. R. (2015). Effect of dexamethasone in feed on intestinal permeability, differential white blood cell counts, and immune organs in broiler chicks. *Poultry Science*, 94(9), 2075–2080.
- Virden, W. S., & Kidd, M. T. (2009). Physiological stress in broilers: Ramifications on nutrient digestibility and responses. *Journal of Applied Poultry Research*, 18(2), 338–347.
- Vizzier-Thaxton, Y., Christensen, K. D., Mench, J. A., Rumley, E. R., Daug Herty, C., Feinberg, B., Parker, M., Segel, P., & Scanes, C. G. (2016). Symposium: Animal welfare challenges for today and tomorrow. *Poultry Science*, 95(9), 2198–2207.
- Webster, S. C., Byrne, M. E., Lance, S. L., Love, C. N., Hinton, T. G., Shamovich, D., & Beasley, J. C. (2016). Where the wild things are: Influence of radiation on the distribution of four mammalian species within the Chernobyl Exclusion Zone. *Frontiers in Ecology and the Environment*, 14(4), 185–190.
- Weimer, S. L., Wideman, R. F., Scanes, C. G., Mauromoustakos, A., Christensen, K. D., & Vizzier-Thaxton, Y. (2018). An evaluation of methods for measuring stress in broiler chickens. *Poultry Science*, 97(10), 3381–3389.
- Wolfensohn, S., Shotton, J., Bowley, H., Davies, S., Thompson, S., & Justice, W. S. (2018). Assessment of welfare in zoo animals: Towards optimum quality of life. *Animals*, 8(7), 110.
- Xing, T., Gao, F., Tume, R. K., Zhou, G., & Xu, X. (2019). Stress effects on meat quality: A mechanistic perspective. *Comprehensive Reviews in Food Science and Food Safety*, 18(2), 380–401.
- Yudiarti, T. (2017). Biochemical and blood leukocytes descriptions of 25 days age of Kampong chicken feed with fungi *Rhizopus oryzae*. *Jurnal Veteriner*, 18(1), 46–50.
- Zulkifli, I., & Siti Nor Azah, A. (2004). Fear and stress reactions, and the performance of commercial broiler chickens subjected to regular pleasant and unpleasant contacts with human being. *Applied Animal Behaviour Science*, 88, 77–87.