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Changes in haematological parameters in hens under short-term exposure to adverse environmental factors

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Abstract. Short-term exposure to adverse factors is a common phenomenon in industrial egg production. An urgent problem is to understand the impact of environmental changes on poultry use. A comparative analysis of changes in haematological parameters in hens under short-term exposure to adverse environmental factors of different natures was performed. For this purpose, a control and 3 experimental groups of hens were established in a modern complex for the production of food eggs. Within 24 hours, the hens of the 2nd group were deprived of fodder, the 3rd group – of light, and the 4th group – were kept in significant overcrowding. The smallest changes in the blood system of hens were observed under the influence of the factor of lack of fodder, namely an increase, within the physiological standard, in the content of leukocytes and erythrocyte sedimentation rate, a decrease in haemoglobin concentration, hematocrit, erythrocytes, platelets, and a violation of the ratio of different forms of leukocytes – an increase in the concentration of heterophils (3.3% > normal) against a decrease in the concentration of monocytes (1.6% < normal), lymphocytes and basophils. Therewith, under the influence of the factor of the absence of light, a higher content of leukocytes in the blood by 10.6%, a lower concentration of haemoglobin by 22.4%, hematocrit – by 4.2%, platelets – by 9.8%, and a higher erythrocyte sedimentation rate by 9.8%, a higher concentration of heterophils by 5.9% and a lower concentration of lymphocytes – by 4.6% were identified compared to the factor of the absence of fodder. The most significant changes in the blood system were noted under the influence of the factor of significant over-consolidation of hens, namely, a higher content of leukocytes in the blood by 17.1 and 5.9%, a lower concentration of haemoglobin by 29.6 and 9.2%, hematocrit – by 5.9 and 1.7%, erythrocytes – by 10.3%, platelets – by 35.8 and 28.8%, and higher erythrocyte sedimentation rate by 4.9%, a higher concentration of heterophils by 11.3 and 5.4 % and lower concentration of monocytes by 0.8 and 0.4%, lymphocytes by 9.4 and 4.8% and eosinophils by 0.7% compared to the factor of lack of fodder and lack of light,

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respectively. Thus, in production conditions, it is necessary to avoid the over-compaction of poultry, as this factor has the greatest adverse effect

Keywords: hens, adverse environmental factors, haematological parameters, stress

Relevance

Intensive management of the poultry industry includes several technological operations that cause excessive stress on the adaptive systems of the body of hens and the development of stress in them (Scanes, 2016; Zhuchaev *et al.*, 2019). The effect of adverse environmental factors or technological stressors, such as high density of housing, changes in the microclimate of production facilities, housing conditions and diet composition, vaccination, transportation and movement, reduce the level of the immunological reactivity of the poultry body (Reber *et al.*, 2007; Sloan *et al.*, 2010; Hall *et al.*, 2014), which decreases its productivity (Lara & Rostagno, 2013; Stoianovskiy *et al.*, 2018; Sakhatsky *et al.*, 2020). In conditions of intensive production, it is impossible to avoid completely the influence of stressors, however, determining the degree of influence of technological factors depending on their nature on the physiological state of poultry is a prerequisite for the development of new methods of stress prevention in the selection of optimal ways of keeping hens.

Analysis of Recent Studies and Publications

It is known that during stress in hens, the activity of all body systems is strained, which is designed to protect themselves and adapt to new living conditions (Dhabhar *et al.*, 2012; Kang *et al.*, 2018; Gorelik *et al.*, 2020). A prerequisite for the development of the stress response is the enhancement of the function of the endocrine glands, particularly the hypothalamus-anterior

pituitary-adrenal cortex system (Olubodun *et al.*, 2015). The main role in the development of stress, according to Selje, is performed by the adrenal cortex, which, under the influence of the pituitary gland, increases the secretion of steroid hormones involved in the adaptation process (Selje, 1979). Therefore, it is believed that the main mechanisms in the implementation of the stress state in the body of poultry are the sympathoadrenal and hypothalamic-pituitary-adrenal-corticotropic systems, i.e. the development of adaptive responses to the effects of various nonspecific environmental factors on the body occurs by a common mechanism: through the hypothalamic-pituitary-adrenal system and the sympathoadrenal system with the participation of catecholamines (Gavreliuk & Chykina, 2017; Infante *et al.*, 2017). Due to biological effects, catecholamines ensure the transition of the body from a state of rest to a state of excitement and allow it to remain in this state for a long time. Therewith, the emergence and course of physiological reactions in the body of poultry under the action of hormones of the medullary layer of the adrenal glands and mediators of the sympathoadrenal system are accompanied by an increase and qualitative change in metabolic processes in immunocompetent tissues (Stoianovskiy *et al.*, 2018), which is reflected in their blood system.

Changes in the blood system in response to stressors such as lymphopenia, eosinopenia and neutrophilia were first described by Hans Selye in 1936 (Selye, 1936). Modern studies

demonstrate that acute stress primarily causes significant changes in the quantitative and qualitative composition of leukocytes in hens (Nicol *et al.*, 2006; Sekeroglu *et al.*, 2011; Nwaigwe *et al.*, 2020). Leukemoid blood reaction in hens has been described under the influence of stressors such as starvation (Najafi *et al.*, 2015), temperature (Prieto & Campo, 2010), light (Huth & Archer, 2015), contamination with microorganisms (Redmond *et al.*, 2011), transportation (Al-Murrani *et al.*, 1997), movement restriction (Bedanova *et al.*, 2007), etc. However, the vast majority of studies are devoted to the effects on the body of hens, usually only one stressor. In turn, the issue of physiological changes in the body of poultry under short-term exposure to stressors of different natures has been understudied.

The purpose of the research was to compare changes in haematological parameters in hens under short-term exposure to adverse environmental factors of different natures.

Materials and Methods of Research

As the object of research were used egg hens of the industrial flock “Hy-Line W-36”. Experiments with experimental animals were conducted following the rules of the European Convention

for the Protection of Vertebrate Animals (Official Journal of the European Union L276/33, 2010).

In the conditions of a modern complex for the production of food eggs in poultry houses with an area of 2915 m², 3 groups of hens (101 heads in each) were established at the age of 52 weeks, each of which was kept in a separate cage-analogous in area and equipment manufactured by “Big Dutchman” (Germany). The control group was established from 10 representatives of each of the 3 experimental groups before the simulation of acute technological stress. Blood samples were taken from them, from which a control group of 30 heads was established. Subsequently, each group was exposed to short-term adverse environmental factors of different natures (acute stressors). In particular, hens of the 2nd group were deprived of fodder, the 3rd group – of light, and the 4th group – were kept with significant overcrowding (Table 1). Modelling the effects of acute stressors in hens of the 2nd and 3rd groups was achieved by turning off the corresponding systems in the poultry house – the fodder distribution line and lighting. In the 4th group, 60 hens were planted in the cage to ensure significant over-compaction of the livestock.

Table 1. Experiment scheme

Characteristic	Group of hens			
	1	2	3	4
Environmental factor	control (before the factor influence)	absence fodder light		increased density of content
Duration of the factor exposure, hours	24			
Number of cages per floor	1176			
Number of birds per cage	101		161	
Planting density, birds / m ²	24.9		39.7	
Area coverage, cm ² /birds	401.4		251.8	
Cage area, cm ²	40544			
Number of nipples per cage, pcs.	12			
Feeding front, cm	7.2		4.5	
Poultry house area, m ²	2915			

Exposure to the factor in all groups was 24 hours, after which haematological parameters of hens were determined. For this purpose, 1.0-1.5 ml of blood was taken from the subwing vein of 30 hens of each group into a test tube with EDTA. Haematological parameters of laying hens were determined on a Micros 60 haematological analyser (Horiba Ltd.) in the laboratory "Bald" (certificate No. LB/02/2016).

The obtained digital results were processed by methods of variation statistics. The significance of differences between the mean values was determined by Student's t-test, differences were considered significant at $p < 0.05$.

Research Results

Haematological parameters of hens of all experimental groups at the beginning of the research were within the physiological standards for each parameter (group 1). No significant differences were identified between the groups. According to the results of the research, after 24 hours of exposure to adverse environmental factors, regardless of their nature, the content of haemoglobin, erythrocytes, hematocrit, platelets and erythrocyte sedimentation rate in the blood of hens were within the physiological standard, and the leukocyte content exceeded them (Table 2).

Table 2. Haematological parameters of laying hens

Indicator	Group				Reference values, (Jain, 1993)
	1	2	3	4	
Leukocytes, thousand/ μ l	26.9 \pm 0.46	36.9 \pm 0.61***	40.8 \pm 0.39*****	43.2 \pm 0.37*****	20-40
Haemoglobin, g/dl	11.8 \pm 0.20	9.8 \pm 0.11**	7.6 \pm 0.23*****	6.9 \pm 0.18*****	7-13
Hematocrit, %	32.9 \pm 0.68	31.1 \pm 0.37*	26.9 \pm 0.53*****	25.2 \pm 0.52*****	22-35
Erythrocytes, mln/ mm^3	3.2 \pm 0.08	2.9 \pm 0.06**	2.9 \pm 0.08**	2.6 \pm 0.07*****	2.5-3.5
Platelets, thousand/ mm^3	73.8 \pm 0.82	69.3 \pm 0.47***	62.5 \pm 0.36*****	44.5 \pm 0.42*****	32-100
The erythrocyte sedimentation rate, Mm/hour	5.4 \pm 0.04	6.1 \pm 0.09***	6.1 \pm 0.06***	6.4 \pm 0.03*****	4.0-6.5

Notes: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ – in comparison with the first group; $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.01$, $^{\circ\circ\circ}p < 0.001$ – in comparison with the second group; $^{\prime}p < 0.05$, $^{\prime\prime}p < 0.01$, $^{\prime\prime\prime}p < 0.001$ – in comparison with the third group

In particular, there was an increase in the content of leukocytes in the blood of hens under the influence of adverse environmental factors. In hens of the 2nd group, the leukocyte content was higher by 37.2% ($p < 0.001$) compared to the 1st group but was within the physiological standard. Therewith, in hens of group 3, the blood leukocyte content exceeded the physiological standard by 2.0% and was higher by 51.7% ($p < 0.001$) and 10.6% ($p < 0.001$) compared to groups 1 and 2, respectively. In hens of group 4, the leukocyte content exceeded the physiological standard by 8.0% and was higher by 60.6% ($p < 0.001$) compared to group 1 and by 17.1% ($p < 0.001$) and 5.9% ($p < 0.001$) compared to

groups 2 and 3, respectively. Thus, the highest content of leukocytes in the blood of hens was observed under the influence of the factor of significant overcompaction, and then in descending order – in the absence of light and deprivation of fodder. In addition, when hens were deprived of fodder, the content of leukocytes in their blood did not exceed the physiological standard, although it was close to its upper limit.

Haemoglobin content and hematocrit in hens of all groups were within the physiological standard. However, there was a decrease in haemoglobin content under the influence of adverse environmental factors. Thus, in hens of group 2, the haemoglobin content in the

blood was lower by 16.9% ($p<0.001$) compared to group 1. Therewith, haemoglobin content in hens of the 3rd group was lower by 35.6% ($p<0.001$) and 22.4% ($p<0.001$) compared to the 1st and 2nd groups, respectively. Therewith, the haemoglobin content in hens of group 4 was higher by 41.5% ($p<0.001$) compared to group 1 and by 29.6% ($p<0.001$) and 9.2% ($p<0.05$) compared to groups 2 and 3, respectively.

In addition, there was a decrease in hematocrit under the influence of adverse environmental factors. In hens of group 2, hematocrit was lower by 1.8% ($p<0.001$) compared to group 1. Therewith, hematocrit in hens of group 3 was lower by 6.0% ($p<0.001$) and 4.2% ($p<0.001$) compared to groups 1 and 2, respectively. While in hens of group 4 hematocrit was lower by 7.7% ($p<0.001$) compared to group 1 and by 5.9% ($p<0.001$) and 1.7% ($p<0.001$) compared to groups 2 and 3, respectively.

The influence of adverse environmental factors during the keeping of hens was accompanied by a decrease in the concentration of red blood cells in their blood within the physiological standard. Thus, in hens of the 2nd and 3rd groups, the concentration of red blood cells in the blood was the same and, therewith, lower than in the 1st group by 9.4% ($p<0.01$). Therewith, in hens of the 4th group, the erythrocyte concentration was lower by 18.8% ($p<0.001$) compared to the 1st group and by 10.3% ($p<0.01$) compared to the 2nd and 3rd groups, respectively.

The concentration of platelets in the blood of hens decreased under the influence of adverse environmental factors. Thus, in hens of the 2nd group, the platelet content in the blood

was lower by 6.1% ($p<0.001$) compared to the 1st group. In hens of group 3, the platelet content in the blood was lower by 15.3% ($p<0.001$) compared to group 1 and by 9.8% ($p<0.001$) compared to group 2. Whereas in hens of group 4, the platelet concentration was lower by 39.7% ($p<0.001$) compared to group 1 and by 35.8% ($p<0.001$) and 28.8% ($p<0.001$) compared to groups 2 and 3, respectively.

The parameters of erythrocyte sedimentation rate in the blood of hens of all groups, regardless of the presence and nature of the factor of influence, were within the physiological standard. Therewith, there was an increase in the erythrocyte sedimentation rate under the influence of environmental factors. In particular, in hens of the 2nd and 3rd groups, the erythrocyte sedimentation rate was higher by 13.0% ($p<0.001$) compared to the 1st group. Therewith, in hens of the 4th group the erythrocyte sedimentation rate was higher by 18.5% ($p<0.001$) compared to the 1st group and by 4.9% compared to the 2nd ($p<0.05$) and 3rd ($p<0.01$) groups, respectively.

The influence of adverse environmental factors during the keeping of hens was reflected in the ratio of leukocytes in their blood (Table 3), namely accompanied by an increase in the number of heterophile in the blood. In hens of groups 2-4, which were kept in the absence of fodder, light and significant overcompaction, the content of heterophile in the blood exceeded the physiological standard by 3.3%, 9.2% and 6.8%, respectively. In particular, the concentration of heterophile in hens of the 2nd group was higher by 12.3% ($p<0.001$) compared to the 1st group.

Table 3. Leukogram of laying hens, %

Indicator	Group				Reference values, (Jain, 1993)
	1	2	3	4	
Monocytes	7.3±0.22	3.4±0.26***	3.0±0.18***	2.6±0.10****	5-10

Table 3. Continued

Indicator	Group				Reference values, (Jain,1993)
	1	2	3	4	
Lymphocytes	64.3±0.48	57.0±0.48***	52.4±0.89*****	47.6±0.33*****	45-70
Eosinophils	4.5±0.34	3.9±0.14	3.4±0.31*	3.2±0.20***	1.5-6.0
Basophils	2.8±0.17	2.3±0.13*	2.0±0.13***	2.0±0.18**	1-3
Heterophiles	21.0±0.53	33.3±0.61***	39.2±0.27*****	44.6±0.28*****	15-30

Notes: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ – in comparison with the first group; ° $p<0.01$; °° $p<0.001$ – in comparison with the second group; ‘ $p<0.05$, ‘° $p<0.001$ – in comparison with the third group

In hens of group 3, the number of heterophile was higher by 18.2% ($p<0.001$) and 5.9% ($p<0.001$) than in groups 1 and 2, respectively. Thus, the content of heterophile in hens of group 4 was the highest and exceeded the indicators of group 1 by 23.6% ($p<0.001$), group 2 – 11.3% ($p<0.001$) and group 3 – 5.4% ($p<0.001$).

The increase in the level of heterophile occurred against the background of a decrease in the number of other forms of leukocytes. In particular, in hens of the 2nd group the concentration of monocytes did not reach the physiological standard by 1.6% and was lower by 3.9% ($p<0.001$), and in hens of the 3rd group – by 2.0% and was lower by 4.3% ($p<0.001$) compared to the 1st group. Therewith, in hens of the 4th group, the concentration of monocytes did not reach the physiological standard by 2.4% and was lower by 4.7% ($p<0.001$) compared to the 1st group and by 0.8% ($p<0.01$) and 0.4% ($p<0.05$) compared to the 2nd and 3rd groups, respectively.

The number of lymphocytes in the blood of hens decreased under the influence of adverse environmental factors, but within the physiological standard. In particular, in hens of the 2nd group the concentration of lymphocytes was lower by 7.3% ($p<0.001$) compared to the 1st group, and in hens of the 3rd group – by 11.9% ($p<0.001$) and 4.6% ($p<0.001$) compared to the 1st and 2nd groups, respectively. While in hens of group 4, the concentration of lymphocytes was lower by 16.7% ($p<0.001$) compared to group 1 and by 9.4% ($p<0.001$) and 4.8% ($p<0.001$) compared to groups 2 and 3, respectively.

In addition, a decrease in the content of eosinophils and basophils in the blood of hens within the physiological standard was observed under the influence of environmental factors. In particular, the content of eosinophils in hens of the 2nd group was at the same level as in the 1st group. In hens of the 3rd group, the concentration of eosinophils was lower by 1.1% ($p<0.05$) compared to the 1st group and did not differ from the 2nd group, and in hens of the 4th group – by 1.3% ($p<0.01$) and 0.7% ($p<0.05$) compared to the 1st and 2nd groups, respectively, and did not differ from the 3rd group.

The content of basophils in hens of group 2 was lower by 0.5% ($p<0.05$), in hens of groups 3 and 4 – by 0.8% ($p<0.01$) compared to group 1. There were no differences between 2-4 groups, i.e. depending on the nature of the factor of influence, in the content of basophils.

Thus, the short-term effect of adverse environmental factors on the body of hens was accompanied by changes in their blood system, which were reflected in an increase in the content of leukocytes by increasing the number of heterophile and decreasing the level of monocytes and depending on the nature of the factor of influence. An increase in leukocyte levels is a characteristic response of immunocompetent tissues to the action of glucocorticoids and catecholamines, the concentration of which in the blood of hens increases under the influence of various stress factors (Sapolsky, 2000; Jiang *et al.*, 2017). In general, the development of leukocytosis is based on an increase in the content of

heterophile in the blood of hens, which was noted in the research (Table 3). According to several authors (Christopher and Link, 2007; Dhabhar *et al.*, 2012), the increase in leukocyte content due to heterophile occurs due to hypercortisolemia and hypercatecholamanemia caused by stress, which increases their number and mobilisation in the blood. Increasing the pool of circulating heterophile is the result of preparing the body for a protective response in response to possible damage (Kubes, 2018; Liew & Kubes, 2019).

Conclusions and perspectives

The short-term impact of adverse environmental factors on the body of hens was accompanied by changes in their blood system, which were reflected in an increase in the content of leukocytes, increasing the number of heterophile and decreasing the level of monocytes, and depending on the nature of the factor of influence. The smallest changes in the blood system of hens were observed under the influence of the factor of lack of feed, namely an increase, within the physiological standard, in the content of leukocytes by 37.2% and erythrocyte sedimentation rate – by 13.0%, a decrease in haemoglobin concentration by 16.9%, hematocrit – by 1.8%, erythrocytes – by 9.4%, platelets – by 6.1%, and violation of the ratio

of different forms of leukocytes – increase in the concentration of heterophils by 12.3% (3.3%>normal) against the background of a decrease in the concentration of monocytes – by 3.9% (1.6%<normal), lymphocytes – by 7.3% and basophils – by 0.5%. Therewith, under the influence of the factor of the absence of light, a higher content of leukocytes in the blood by 10.6%, a lower concentration of haemoglobin by 22.4%, hematocrit – by 4.2%, platelets – by 9.8%, and a higher erythrocyte sedimentation rate by 9.8%, a higher concentration of heterophils by 5.9% and a lower concentration of lymphocytes – by 4.6% were identified compared to the factor of the absence of fodder.

The most significant changes in the blood system were noted under the influence of the factor of short-term significant over-consolidation of hens, namely, a higher content of leukocytes in the blood by 17.1 and 5.9%, a lower concentration of haemoglobin by 29.6 and 9.2%, hematocrit - by 5.9 and 1.7%, erythrocytes - by 10.3%, platelets - by 35.8 and 28.8%, and higher erythrocyte sedimentation rate by 4.9 %, a higher concentration of heterophils by 11.3 and 5.4 % and lower concentration of monocytes by 0.8 and 0.4 %, lymphocytes by 9.4 and 4.8 % and eosinophils by 0.7 % compared to the factor of lack of fodder and lack of light, respectively.

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Зміни гематологічних параметрів у курей за короткотермінового впливу негативних факторів навколишнього середовища

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Анотація. Короткочасний вплив негативних факторів, поширене явище під час промислового виробництва яєць. Актуальною проблемою є зрозуміти вплив змін зовнішнього середовища на використання птиці. Проведено порівняльний аналіз змін гематологічних параметрів у курей за короткотермінового впливу негативних факторів навколишнього середовища різної природи. Для цього в умовах сучасного комплексу з виробництва харчових яєць сформували контрольну і 3 дослідні групи курей. Впродовж 24 годин кури 2-ї групи були позбавлені корму, 3-ї – світла, а 4-ї – утримувались за значного переущільнення. Найменші зміни в системі крові курей спостерігались за впливу фактора відсутності корму, а саме підвищення, в межах фізіологічної норми, вмісту у крові лейкоцитів та швидкості осідання еритроцитів, зниження концентрації гемоглобіну, гематокриту, еритроцитів, тромбоцитів, а також порушення співвідношення різних форм лейкоцитів – підвищення концентрації гетерофілів (3,3 % > норми) на тлі зниження концентрації моноцитів (1,6 % < норми), лімфоцитів та базофілів. Тоді як за впливу фактора відсутності світла було виявлено вищий вміст у крові лейкоцитів на 10,6 %, нижчу концентрацію гемоглобіну на 22,4 %, гематокриту – на 4,2 %, тромбоцитів – на 9,8 %, а також вищу швидкість осідання еритроцитів на 9,8 %, вищу концентрацію гетерофілів на 5,9 % та нижчу концентрацію лімфоцитів – на 4,6 % у порівнянні з фактором відсутності корму. Найсуттєвіші зміни в системі крові відмічені за впливу фактора значного переущільнення курей, а саме вищий вміст у крові лейкоцитів на 17,1 і 5,9 %, нижчу концентрацію гемоглобіну на 29,6 і 9,2 %, гематокриту – на 5,9 і 1,7 %, еритроцитів – на 10,3 %, тромбоцитів – на 35,8 і 28,8 %, а також вищу швидкість осідання еритроцитів на 4,9 %, вищу концентрацію гетерофілів на 11,3 і 5,4 % та нижчу концентрацію моноцитів – на 0,8 та 0,4 %, лімфоцитів – на 9,4 і 4,8 % та еозинофілів – на 0,7 % у порівнянні з фактором відсутності корму та відсутності світла відповідно. Таким чином у виробничих умовах необхідно уникати переущільнення птиці, оскільки цей фактор має найбільший негативний ефект

Ключові слова: кури, негативні фактори навколишнього середовища, гематологічні параметри, стрес