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Adaptive Dynamics of Blood Cell Parameters in Hens upon Changes in the Lighting Conditions

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Abstract: We evaluated the physiological and biochemical status of chickens' organism in the process of their adaptation to light regimes. We studied the dynamics of several morphological and functional parameters of red blood cells and white blood cells by means of atomic force microscopy. We have found that the hen house lighting change and the chickens' adaptation were accompanied with significant changes in the surface area, volume, height, diameter, width and depth of the perinuclear space, modulus and surface potential of the cells. The adaptation reactions that occur in response to the new lighting mode were identified. We defined the set of changes of several morphometric and functional parameters of red blood cells and white blood cells, characteristic of different adaptive reactions of the organism. Chronic stress in chickens showed increased surface area and volume of lymphocytes, reducing elastic modulus of lymphocytes, neutrophils and erythrocytes. Antistress reactions of training result in reducing the surface area of neutrophils, lymphocytes decrease in the surface potential and the elastic moduli of all the studied cells.

Key words: Blood cells · Morphofunctional parameters: surface area · Volume · Elasticity modulus · The surface potential · Chickens · Lighting modes · Stress · Adaptive responses

INTRODUCTION

Numerous studies demonstrated that the housing conditions of agricultural animals often act as stressing factors decreasing the efficiency of agricultural production [1-3]. Lighting conditions are among such factors; they control the motion activity, eating behavior, immunological status and the state of health [4]. Different lighting conditions are used currently in poultry farms; however, the effect of these conditions on the functional state of poultry is still little studied.

The body's response depends on the exposure intensity and duration. Too intense irritating factors induce the development of stress, while medium-intensity irritants cause anti-stress response and pre-stress conditions, thus increasing the natural resistance of the organism [5]. Evaluation of adaptive responses including their intensity and staging may promote the development of efficient methods for diagnosis of stress conditions and preventive measures.

The blood cell composition reflects the neuroendocrine, immune and metabolic changes occurring in the body during adaptation [6]. Studies of Renaudeau, D., A. Collin and S. Yahav, 2012 indicate that more detailed characteristics of adaptive mechanisms requires elucidation of cell responses [7]. Determination of morphometric and morphofunctional parameters of blood cells may provide information necessary for this purpose [8]. This possibility is provided by atomic force microscopy [9].

The goal of the work is to study the variation of morphofunctional parameters of blood cells during adaptation of hens to lighting conditions.

Procedure: The experimental part of the work was carried out in the vivarium of the Belgorod Agricultural Academy on 18 month-old hens of the Highsex brown cross, which were divided, according to the analogue principle, into 4 groups, each comprising 16 hens.

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The hens were housed in cages, the floor space per bird and feeder space è watering space being in line with zootechnical standards and the technology of breeding for this cross. For the control group hens (1st group), the lighting schedule included a 12-hour day and a 12-hour night (conditions 1), which resembles most closely the outdoor lighting; the 2nd group hens were housed with a 7 hour day and a 1 hour night (conditions 2), which is often used in industry, in particular, at the OJSC Lopanskoye of the Belgorod Agricultural Holding "BEZRK-Belgrankorm"; the third group had a 2-hour day and a 3-hour night, a 5-hour day, a 1-hour night, a 4-hour day and a 9-hour night (conditions 3), which corresponds most fully to the poultry biological rhythms and for the 4th group continuous illumination for three days (conditions 4) followed by the transfer to conditions 1 was employed.

The blood for investigation of morphofunctional cell parameters (surface area, volume, height, diameter, elastic modulus and surface potential) was taken from the hen axillary vein, 5 ml each time, on the 6th, 16th and 30th day of adaptation. Heparin, 20 units/mL was used as the anticoagulant. The cells (n=10) were scanned on an INTEGRA VITA atomic force microscope (NT MDT, Zelenograd) at the Physiology of Adaptive Processes research laboratory of the Belgorod National Research University by the semicontact method; the results were treated by Nova 1.0.26 Build 1397 software (NT MDT). The cell surface potential was estimated by Kelvin probe force microscopy [10]. The validity of the differences was determined by the Student t-criterion.

Main Part: The adaptive responses in hens to various housing factors including the conditions of lighting were revealed and investigated based on evaluation of the functional status of the body, analysis of biochemical and hematological blood parameters [11-13]. As a result, it was found that the 12-hour day and a 12-hour night, which resembles most closely the outdoor conditions, is the schedule of choice. The housing of 1st group hens under these conditions did not cause statistically significant changes in the functional status of the body and blood cell parameters being measured. The use of other lighting conditions that are used in poultry production practice ultimately results, according to our data, in chronic stress appearing by the 30th day of experiments. For different adaptive responses, characteristic sets of changes in the morphofunctional parameters of blood cells were distinguished. Tables 1, 2 and 3 summarize the parameters that showed statistically significant changes during the adaptation.

Table 1: Morphofunctional parameters of hen blood lymphocytes at stress reaction

	Groups			
Cell parameters	1	2	3	4
Surface area, mkm²	109.2±8.2	157.3±1.6**	145.2±2.3**	137.1±8.2*
Volume, mkm³	56.2±0.8	92.3±0.5**	96.4±2.5**	96.8±3.2**
Elastic modulus, mPa	12.6±3.6	4.9±0.5*	3.5±0.8*	2.6±0.8*
Surface potential, mV	-8.2±3.2	-8.2±2.6	-25.2±3.1**	-6.9±2.6

Note: statistical significance of the differences from the data for the 1st group * – at Ð<0.05, ** – at Ð<0.01

Table 2: Morphofunctional parameters of hen blood heterophils at stress reaction

	Groups			
Cell parameters	1	2	3	4
Surface area, mkm²	172.8±8.2	120.5±0.9**	131.1±1.2**	109.3±9.3**
Volume, mkm³	65.8±5.2	69.1±1.5	70.3±2.0	79.5±7.1
Elastic modulus, mÐà	9.2±0.6	3.4±0.7**	2.9±0.2**	3.7±0.5**
Surface potential, mV	-6.9±1.1	-9.7±1.3	-16.8±1.7**	-11.9±2.7

Table 3: Morphofunctional parameters of red cells in hens at stress reaction

	Groups			
Cell parameters	1	2	3	4
Surface area, mkm²	90.2±3.2	101.1±5.1	92.5±6.3	108.1±9.2
Volume, mkm³	64.2±1.8	67.7±2.5	76.6±7.8	71.8±5.4
Elastic modulus, mÐà	6.8±1.4	3.5±0.1*	3.9±0.1*	3.8±0.7*
Surface potential, mV	-7.8±1.2	-3.5±0.7**	-5.7±0.9	-11.4±1.2*

Thus, for all test groups, the average surface area and volume of lymphocytes increased with respect to the control group, while the cell elastic modulus decreased. In the 3rd group, the surface potential of the cell membrane in hens under stress decreased.

The cell volume is an important physiological characteristic that affects the shape, the intracellular osmolarity, migration processes, cellular growth and metabolism regulation. Even short-term changes in the volume may cause rather pronounced changes in cell functions and excessive oscillations may deteriorate the cell membrane integrity and cytoskeleton architectonics [14]. Presumably, the cell surface area increases due to the fuller utilization of the membrane reserve, the cell content being distributed uniformly, thus reducing the elasticity, which probably improves the blood circulation in microvessels [15].

The average values of the surface area and elastic modulus of heterophils (neutrophils) decreased in all test groups, while the surface potential decreased significantly only in the 3rd group.

The study of red blood cell characteristics during the adaptation of hens to lighting conditions showed a decrease in the average values of the elastic modulus in all test groups, while the surface potential increased in the 2nd group and decreased in the 4th group as compared with control values. According to some data, changes in the red cell elastic modulus may be due to conformational rearrangements of hemoglobin in the cell and its positioning at the centre [14]. The variation of the morphofunctional parameters of red cells is an important characteristic of adaptation processes, as it can affect the respiratory function of blood and reflect the cellular adaptation mechanisms.

The presented data on the variation of morphofunctional characteristics of white and red blood cells were obtained by the 30th day of experiments when chronic stress had been detected in all test groups of hens considering a whole set of parameters of the functional status of the body [13]. Characteristic and similar changes were noted, namely, an increase in the lymphocyte surface area and volume and a decrease in the lymphocyte elastic modulus and surface potential, a decrease in the segmented neutrophil surface area, elastic modulus and surface potential and a decrease in the red cell elastic modulus and surface potential.

In the 4th group, the lighting-induced desynchronizes brought about the development of stress reaction as soon as on the 6th day of experiments and chronic stress was detected in this group by the 16th and 30th day.

Development of the chronic stress in hens upon different conditions of lighting occurred gradually and stage by stage. For characterization of the stress stages

Table 4: Morphofunctional parameters of blood of 2nd group hens

	Groups			
		2		
Cell parameters	1	6th day	16th day	30th day
Lymphocytes				
Surface area, mkm²	109.7±13.6	107.7±6.2	85.9±2.8**	157.3±1.6**
Volume, mkm³	57.1±13.6	59.1±6.5	53.8±5.6	92.3±0.5**
Elastic modulus, mPa	11.5±0.9	10.8±2.6	16.6±3.3	4.9±0.5*
Surface potential, mV	-6.9±1.2	-6.2±2.6	-8.2±2.6	-8.2±2.6
Heterophils				
Surface area, mkm²	176.1±27.2	146.3±3.8	132.1±6.9**	120.5±0.9**
Volume, mkm³	65.3±10.3	70.9±7.6	65.1±8.4	69.1±1.5
Elastic modulus, mPa	9.2±0.8	0.8±0.1**	9.5±5.1	3.4±0.7**
Surface potential, mV	-6.9±1.2	-10.8±1.9	-8.2±2.6	-9.7±1.3
Red cells				
Surface area, mkm²	90.8±2.1	83.2±2.5*	92.7±4.5	101.1±5.1
Volume, mkm³	64.1±1.8	57.9±3.1	55.7±2.1*	67.7±2.5
Elastic modulus, mPa	6.8±1.5	1.4±0.3**	7.8±1.1	3.5±0.1*
Surface potential, mV	-7.6±1.4	-10.4±1.9	-7.4±2.6	-3.5±0.7**

Table 5: Morphofunctional parameters of blood cells in 3rd group hens

	Groups			
		3		
Cell parameters	1	6th day	16th day	30th day
Lymphocytes				
Surface area, mkm²	109.7±13.6	109.7±5.9	56.1±2.3**	145.2±2.3**
Volume, mkm³	57.1±13.6	66.2±6.5	49.8±7.9	96.4±2.5**
Elastic modulus, mPa	11.5±0.9	1.9±0.5**	2.9±0.4**	3.5±0.8*
Surface potential, mV	-6.9±1.2	-25.3±2.3**	-25.8±2.6**	-25.2±3.1**
Heterophils				
Surface area, mkm²	176.1±27.2	110.2±4.1*	128.2±1.7**	131.1±1.2**
Volume, mkm³	65.3±10.3	73.5±6.6	75.5±9.1	70.3±2.0
Elastic modulus, mPa	9.2±0.8	1.4±0.8**	2.9±0.5**	2.9±0.2**
Surface potential, mV	-6.9±1.2	-25.3±2.3**	-10.2±3.5	-16.8±1.7**
Red cells				
Surface area, mkm²	90.8±2.1	70.4±3.5**	100.6±6.1	92.5±6.3
Volume, mkm³	64.1±1.8	41.5±2.1**	67.2±3.3	76.6±7.8
Elastic modulus, mPa	6.8±1.5	2.4±0.1*	2.9±0.1*	3.9±0.1*
Surface potential, mV	-7.6±1.4	-5.5±1.4	-5.0±1.7	-5.7±0.9

and adaptive responses, the changes of morphofunctional parameters of blood cells for the 2nd and 3rd group hens were considered. Table 4 summarizes the data on the morphofunctional parameters of blood cells for 2nd group hens during adaptation to the lighting schedule.

Study of the stress reaction stages indicated that the alarm stage (6th day) was accompanied by a decrease in the surface area of red cells and a decrease in the elastic moduli of heterophils and red cells. The resistance stage (16th day) was accompanied by a considerable decrease in the lymphocyte and heterophil surface areas and the red cell volume. The exhaustion stage of chronic stress (30th day) was accompanied by increase in the surface area and the volume of lymphocytes, a decrease in the surface potential of red cells, a decrease in the surface area of heterophils and a decrease in the elastic moduli of heterophils and red cells.

Table 5 presents the variation of the morphofunctional parameters of blood cells for 3rd group hens during adaptation to the lighting schedule. In this group, the anti-stressor training reaction was distinguished by the 6-16th days and stress reaction was noted on the 30th day.

A somewhat different set of changes was observed in this case, namely, a decrease in the surface area of the studied cells and a decrease in the surface potential of lymphocytes and the elastic moduli of all studied cells.

On going to the stress reaction, which was detected by the 30th day, an increase in the lymphocyte surface area and volume was detected in hens subjected to all of the studied lighting conditions.

Summary: The performed research provides the conclusion that the lighting conditions are among the factors of industrial poultry production that can disturb the body homeostasis, cause adaptive response and induce chronic stress. Study of the pattern of variation of morphometric and morphofunctional parameters of blood cells allows one to evaluate the cell adaptation mechanisms. Among the studied morphofunctional parameters of blood cells in hens during adaptation to lighting conditions, the most pronounced changes were found for elastic moduli, which decrease statistically significantly during the training reaction and in all stages of stress development.

CONCLUSIONS

The surface area, volume, elastic modulus and surface potential of red and white blood cells are of diagnostic significance for evaluation of stages of stress reaction and adaptive responses of the body. These parameters experience statistically significant changes during the organism adaptation and can be applied for characterization of a current state of blood cells and adaptive mechanisms.

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