The role of corticosterone in the regulation of the cellular composition of chicken blood during the stress reaction

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Abstract. The influence of hen layer density on the variability of the number of red blood cells, heterophiles and lymphocytes in the blood, the secretory activity of adrenal glands, estimated by the level of corticosterone and cortisol, as well as the presence of interrelations between hormones and blood cells by calculating complex indices, were studied. Chickens, as the research object, were kept in cages, under conditions of standard layer density and increased by 1.5 and 2.0 times. We found that chickens adapt to an increase in layer density by one and a half times, provided that egg production decreases to 33.33%; two times exceed of the regulatory requirements for laying does not correspond to the adaptive abilities of birds. Depending on the level of layer density excess (stress factor) in chicken blood, the concentration of corticosterone and cortisol increases, determining a decrease in the number of lymphocytes and an increase in heterophiles against the background of the preservation of red blood cells, reflecting the "energy price" of adaptation. Corticosterone affects the relationship of red blood cells with lymphocytes and heterophiles, determining the variability of the values of the indices reflecting the ratio of red blood cells and lymphocytes (ISEL), red blood cells and heterophiles (ISEG), red blood cells, lymphocytes and corticosterone (ISELC), red blood cells, heterophiles and corticosterone (ISEGC) and the integral index of red blood cells-heterophiles-lymphocytes and corticosterone (IIEGLC).

1 Introduction

In the conditions of industrial poultry farming, the body of bird is often exposed to technological stress factors, among which a special place is occupied by heat stressors [1]. Although the effects of heat stress are described in the scientific literature [2, 3], the main mechanisms of its development are still not fully understood. This is due to the fact that it is initiated not by one, but, most often, by several factors or their complex. Their impact cre-

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ates conditions in which the requirements for the bird body exceed its natural regulatory capabilities [4]. This affects the productive qualities of poultry, including growth processes, reproductive abilities and egg production, and product quality. [1, 3, 5, 6], as a result of the predominant direction of the body's energy resources to the course of adaptation processes [7]. In addition, heat stress leads to an increase in the morbidity and mortality of poultry [8].

Glucocorticoids play an important role in the implementation of the stress response in animals [9]. Under the action of stressors, their level increases sharply in the blood, as a result of the activation of the sympathetic and hypothalamic-pituitary-adrenal axes [10, 11]. It is established that the effectiveness of the implementation of the biological effects of these hormones plays a crucial role in the formation of the physiological pathway of adaptation. This is associated with their ability to control the volume of catabolic processes in the metabolism, and thus not only to determine the amount of released energy, but also the adequacy of its use in restoring homeostasis [12, 13].

Of the glucocorticoids, corticosterone plays an important role in the stress response in birds [6, 8, 9], which controls the level of variability of neuroendocrine and immune responses. In particular, the immunosuppressive effects of corticosterone in birds are realized both at the level of humoral and cellular components of immunity [4], determining the effectiveness of their responses. According to [14-16], the severity of the primary response of birds to stressor action reflects the ratio between heterophiles and lymphocytes. This is the result of glucocorticoid-induced rapid apoptosis of lymphocytes [4]. In studies [17], it was noted that corticosterone in birds during a stress reaction regulates the number of white blood cells in the blood by controlling their antioxidant status and, as a result, the state of plasma membranes, as well as affecting the proliferation of cells in the hematopoietic organs [8]. At the same time, the reactivity of the immune response is interrelated with the physiological state of white blood cells [18].

Most aspects of the adaptive response of birds under heat stress have been studied under conditions of a sharp increase in ambient temperature, which is most significant in hot countries [1-3, 5-7]. At the same time, in industrial conditions, one of the frequently violated technological parameters is the "bird laying density", a change in which also initiates the appearance of heat stress signs in the bird due to the absence of sweat glands, the insulating properties of feathers and the production of a large amount of metabolic heat [19-23], especially if the recommended humidity is not observed in the poultry house [15]. However, very little is known about the immune and hormonal reactions in the body of chickens under the stress effect of technological factor - laying density (crowding). Therefore, the purpose of our study was to assess the effect of the density of laying hens in a cage on the reactivity of the blood cell composition (red blood cells, heterophiles, lymphocytes) and the secretory activity of the adrenal glands for corticosterone and cortisol, as well as the presence of interrelations between these parameters, estimated by the variability of the values of complex hemato-hormonal indices [10, 11].

2 Materials and methods

The experimental scheme and the research implementation plan were reviewed and approved by the Bioethics Committee of the FSBEI HE South Ural State Agrarian University (city of Troitsk, Russia) (protocol number 4 dated 15.11.2018). When working with poul-

try, we were guided by the principles of humanity set out in the European Community Directives (86/609/EEC).

The experimental part of the work was carried out on the basis of PJSC "Chelyabinsk Poultry Farm" in 2018-2019. The object of the study was chickens of the industrial herd of the cross Lomann LSL-classic at 52 weeks of age with a live weight of 1716-1769 g, which were kept in four-tier cage batteries. The width of one cage was 600 mm, depth - 605 mm, facade height - 455 mm, rear side height - 380 mm, capacity - 8 heads. The cages are equipped with a chain feed dispenser with a one-way approach and two nipple drinkers (water temperature 20°C). The cage floor is slatted with a slope and an egg collection system. The humidity in the poultry house ranged 67-68%, the air temperature was 21.1-21.3°C. Three meals a day (7.30; 10.30; 14.30) mixed feed of own production, balanced in basic nutrients and biologically active substances, the consumption rate per head is 118 g/day. The productivity of chickens in the industrial herd was 90.5% (according to the cross passport, 85.3%) [23, 24].

To model the experimental stress, the density of birds laying in a cage, which determines the amount of metabolic mass per unit area, the level of thermal regulation of the body and muscle activity, was used as a stress factor. For this purpose, three groups were formed on the 2nd tier of the cage battery: group I - control, laying density of birds was 8 heads, corresponding to the standard; in the II experimental group, the laying density was increased by 1.5 times (n=12), in the III experimental group - by 2 times (n=16). When the laying density changed, the feeding front was increased accordingly, considering the daily norms of feed consumption per head and the number of drinkers to ensure access to water [23, 24].

The research material was blood, which was collected from the subcutaneous axillary vein by vacuum method using sterile double-sided needles 21Gx1.5 (Trainer Bio-OneGmbh, Austria), a disposable holder of vacuum systems (CJSC Firm Domain, city of St. Petersburg, Russia) in 2 ml (13x75 mm) tubes with K3 EDTA filler (Trainer Bio-OneGmbh, Austria). The place of blood collection is closer to the elbow joint, was cleaned from dawn, the skin was disinfected with 70% alcohol, and the vein was pierced, directing the needle along the vein with a bevel up. Blood from the birds of the control and experimental groups was taken before the experiment (background indicators), as well as 2, 4 and 24 hours after the change in laying density. The resulting biomaterial was delivered to the laboratory in a special refrigerator and used: 1) to determine morphological parameters on the hematological analyzer MEK 6510 (Japan); 2) to obtain blood plasma by centrifugation at 1000g for 15 minutes.

The concentration of cortisol and corticosterone in the blood plasma was determined by the enzyme immunoassay using the reagent kits "CORTISOL – IFA - BEST" (city of Novosibirsk, Russia) and "ELISA" (LDN GmbH & Co, Germany) in accordance with the attached instructions. The method is based on a solid-phase competitive enzyme immunoassay with the use of monoclonal antibodies. The strips were incubated in a thermostatically controlled shaker "ELMI Sky Line Shaker ST-3" (ELMI Ltd., Latvia), followed by optical density measurement with a microplate reader – "MINDRAY MR-96A Elisa Microplate Reader" (MINDRAY Ltd., China).

To assess the effect of glucocorticoids on the cellular composition of the blood of birds, hemato-hormonal indices were calculated, expressing their value in conventional units [10, 11]:

1. Index of ratio of red blood cells to lymphocytes (ISEL):

$$ISEL = \frac{E}{Lim} \quad (1)$$

2. Index of ratio of red blood cells to heterophiles (ISEG):

$$ISEG = \frac{E}{G}, \quad (2)$$

3. Index of ratio of red blood cells, lymphocytes, and corticosterone (ISELC):

$$ISELC = \frac{E \cdot K}{Lim} \quad (3)$$

4. Index of red blood cells, heterophiles and corticosterone ratio (ISEGC):

$$ISEGC = \frac{E \cdot K}{G} \quad (4)$$

5. Integral index of erythrocytes, heterophiles, lymphocytes and corticosterone (IIEGLC):

$$IIEGLC = (\frac{E+G}{Lim}) \cdot K \quad (5)$$

where E is the number of red blood cells in the blood, 10^{12}/l; Lim is the number of lymphocytes in the blood, 10^{9}/l; G is the number of heterophiles in the blood, 10^{9}/l; K is the concentration of corticosterone in the blood plasma, nmol/l.

Statistical data processing was performed in the program "Microsoft Excel 2010", using the add-ons "Data Analysis Package". It provided for the determination of the average value and its error, the range of trait variation in the interval X_{min}-X_{max}, and the calculation of the coefficient of trait variation.

3 Results

In the II experimental group of birds, in the first four hours after an increase in the landing density of 1.50 times, we observed a struggle between individuals for free space (social conflict). At the control point "after 4 hours", the leading chickens in the amount of 4 individuals were identified in the cage. They could move freely in the cage space. Eight chickens were grouped with their relatives in one part of the cage, which limited their ability to move. Despite some crowding in the cage, all the chickens could take food and water. At the same time, they had a normal state of feather cover. Egg production during the experiment was 33.33% for the cage.

Table 1. Assessment of the secretory activity of the adrenal glands for corticosterone and cortisol in the body of chickens

Indicator	Group	Background			Blood test time after stress initiation, h								
					2			4			24		
		X±Sx	X_{min}-X_{max}	C_V, %	X±Sx	X_{min}-X_{max}	C_V, %	X±Sx	X_{min}-X_{max}	C_V, %	X±Sx	X_{min}-X_{max}	C_V, %
Corticosterone, nmol/l	I	29.29±1.41	27.00-31.00	4.81	29.21±1.60	26.00-31.00	5.48	28.00±1.31	26.00-30.00	4.68	30.50±1.21	28.50-32.00	3.61
	II	27.00±0.95	26.00-29.00	3.53	43.00±1.35*	41.00-45.00	3.14	92.33±1.87*	90.00-95.00	2.03	57.70±0.96*	56.20-59.00	1.67
	III	29.71±1.68	27.00-32.00	5.65	124.00±2.10*	120.00-127.00	1.69	119.00±0.97*	118.00-121.00	0.81	117.00±1.03*	116.00-119.00	0.87
Cortsol, nmol/l	I	5.36±0.70	4.20-6.30	13.09	5.64±0.52	5.00-6.30	9.31	5.56±0.82	4.58-7.12	14.72	5.84±0.67	5.12-7.12	11.48
	II	5.71±0.48	4.80-6.30	8.40	10.49±1.96*	10.49-7.20	18.71	17.33±2.95*	12.30-22.80	17.05	9.56±2.11*	7.10-12.90	22.10
	III	4.64±0.37	4.64-4.13	7.96	16.62±1.56*	15.00-19.60	9.39	15.15±8.69*	13.60-17.20	8.69	14.40±1.38*	12.80-17.20	9.60

Note: * - $p<0.05$

In the III experimental group of birds, in which the laying density exceeded the regulatory requirements by 2 times, "excessive overpopulation" was observed. The birds could not move freely around the cage because of overcrowding. Although the cage was additionally equipped with drinkers and the feeding front was increased, but the chickens were in conflict with each other, both for the ability to receive food and water, and to move around the cage. In conditions of crowded housing, the chickens gradually increased their breathing, the feather became wet and ruffled, and the general condition was depressed. The maximum severity of these signs was recorded during the experiment. In conditions of constant "anxiety", the laying hens stopped laying eggs.

Table 2. Changes in blood cell composition of chickens during the development of a stress reaction

Indicator	Group	Background			Blood test time after stress initiation, h								
					2			4			24		
		X±Sx	X_{min}-X_{max}	C_V, %	X±Sx	X_{min}-X_{max}	C_V, %	X±Sx	X_{min}-X_{max}	C_V, %	X±Sx	X_{min}-X_{max}	C_V, %
Red blood cells, 10^{12}/l	I	3.13±0.38	2.75-3.70	12.02	3.01±0.33	2.70-3.70	10.90	3.02±0.27	2.60-3.60	10.93	3.08±0.26	2.50-3.75	12.41
	II	3.20±0.28	2.80-3.70	8.64	2.95±0.08	2.85-3.10	2.70	3.25±0.38	2.90-3.55	3.79	3.35±0.35	2.95-3.45	3.32
	III	3.13±0.25	2.85-3.50	7.99	2.90±0.12	2.70-3.00	3.27	3.23±0.12	3.07-3.40	3.62	3.07±0.14	2.95-3.20	3.59
Lymphocytes, 10^9/l	I	14.85±0.45	13.75-16.23	6.36	14.51±0.31	13.10-16.11	7.09	14.94±0.26	13.67-16.09	5.00	14.97±0.23	14.62-15.41	4.76
	II	15.18±0.20	14.21-16.72	4.55	13.90±0.29*	12.00-15.00	7.71	12.47±0.19*	10.08-14.26	10.09	14.60±0.16	11.84-15.96	7.27
	III	15.46±0.12	15.26-16.11	2.05	13.24±0.19*	12.87-14.09	7.94	12.74±0.11*	11.41-14.52	7.10	11.97±0.09*	10.52-13.31	4.19
Hetero-	I	9.38±0.29	8.80-10.11	5.57	9.34±0.33	8.74-10.10	5.34	9.06±0.26	8.58-9.65	4.72	9.29±0.25	8.81-9.91	3.68

philes, 10^9/l	II	9.40± 0.24	8.91-10.86	5.71	12.56± 0.14*	10.85-14.46	3.22	13.29± 0.23*	10.02-14.80	15.35	10.60± 0.34	8.54-12.66	12.88
	III	9.63± 0.34	9.04-10.46	5.58	11.23± 0.25*	9.59-10.73	10.73	12.91± 0.31*	10.05-13.82	13.53	13.93± 0.26*	11.69-15.22	15. 28

Note: * - $p<0.05$

When assessing the effect of the technological factor on the secretory activity of adrenal glands, estimated by corticosterone and cortisol concentration, it was found that their level in the II and III experimental groups increased (Table 1). In conditions of exceeding the regulatory requirements for laying density by 1.50 times, the maximum of "stress" hormones was detected 4 hours after the start of the experiment, exceeding the background and control group levels by 3.42 (corticosterone) and 3.04 (cortisol) times ($p<0.05$). At the same time, the individuals in the group had practically no differences in the concentration of corticosterone in the blood, since the value of variation coefficient fluctuated in the range of 2.03-3.14%. At the end of the experiment (24 hours after the initiation of stress), the concentration of glucocorticoids decreased, but still exceeded the background values by 2.13 and 1.24 times, reflecting the activity of the hypothalamus-pituitary-adrenal axis.

With an increase in the laying density of birds by 2.00 times, in comparison with the regulatory requirements, the stress effect of the technological factor persisted throughout the entire study period (Table 1). Therefore, in the blood of chickens, the concentration of corticosterone and cortisol constantly increased, reaching a maximum at the point "after 24 hours". The variability of corticosterone in the body of chickens of this group (C_V=0.81-1.69%), compared with cortisol (C_V=8.69-9.39%), was also less pronounced, as a result of the dominance of its effects among glucocorticoids.

Table 3. Variability of hemato-hormonal indices during stress reaction development

Indicator	Group	Background			Blood test time after stress initiation, h								
					2			4			24		
		X±Sx	X_{min}-X_{max}	C_V, %	X±Sx	X_{min}-X_{max}	C_V, %	X±Sx	X_{min}-X_{max}	C_V, %	X±Sx	X_{min}-X_{max}	C_V, %
ISEL, c.u.	I	0.21± 0.02	0.05-0.06	9.18	0.21± 0.02	0.04-0.06	57.91	0.20± 0.01	0.05-0.06	8.15	0.21± 0.03	0.05-0.08	18.90
	II	0.21± 0.01	0.05-0.07	13.14	0.21± 0.01	0.05-0.06	6.74	0.26± 0.02*	0.05-0.08	19.24	0.23± 0.03	0.05-0.07	13.60
	III	0.20± 0.02	0.05-0.06	9.80	0.22± 0.03	0.04-0.06	13.71	0.25± 0.01*	0.05-0.06	6.68	0.26± 0.01*	0.05-0.09	22.84
ISEG, c.u.	I	0.33± 0.05	0.27-0.35	17.54	0.32± 0.03	0.30-0.34	11.66	0.33± 0.03	0.30-0.37	9.98	0.33± 0.00	0.30-0.41	30.07
	II	0.34± 0.02	0.31-0.35	4.99	0.25± 0.03*	0.20-0.26	11.72	0.23± 0.01*	0.23-0.26	8.45	0.32± 0.07	0.29-0.35	18.81
	III	0.33± 0.04	0.30-0.36	63.17	0.26± 0.02*	0.24-0.29	9.79	0.25± 0.02*	0.24-0.26	6.35	0.22± 0.02*	0.19-0.24	7.21
ISELC, c.u.	I	6.11± 0.17	5.66-6.69	21.38	6.06± 0.10	5.33-6.56	10.69	5.65± 0.12	5.13-5.87	7.05	6.27± 0.16	5.82-6.48	8.89
	II	5.69± 0.15	5.13-6.17	6.96	9.13± 0.27*	8.13-9.68	8.10	24.06± 0.23*	23.04-25.07	13.98	13.24±0.20*	12.02-14.04	18.98
	III	6.02± 0.09	5. 13-6.72	26.06	27.61 ±0.18*	26.05-28.79	16.20	30.07± 0.29*	28.62-32.07	24.93	30.17± 0.32*	28.06-32.00	8.48
ISEGC, c.u.	I	9.77± 0.21	8.02-10.03	18.30	9.41± 0.18	8.34-10.39	7.10	9.33± 0.12	8.32-10.11	14.79	10.11± 0.17	9.02-11.03	7.38
	II	9.19± 0.11	8.41-9.83	13.02	10.10± 0.11	9.02-10.65	11.98	22.57± 0.17*	20.05-24.13	40.11	18.23± 0.25*	16.01-20.07	74.52

	III	9.65± 0.16	8.23-10.15	12.52	32.02± 0.33*	31.09-34.11	7.03	29.77± 0.22*	27.09-31.10	7.91	25.78± 0.21*	23.09-27.10	5.24
IIEG LC, c.u.	I	24.67± 0.28	22.17-26.23	11.54	24.86± 0.11	21.16-26.22	14.46	22.63± 0.16	20.16-24.21	9.29	25.20± 0.25	21.19-26.23	6.65
	II	22.90± 0.36	20.16-25.22	12.55	47.98± 0.32*	43.30-49.46	16.50	122.46±0.57*	120.42-125.74	28.10	53.69± 0.24*	50.30-56.54	27.33
	III	24.52± 0.47	21.06-27.23	9.85	132.33±0.46*	130.69-135.11	16.55	150.75±0.83*	145.75-153.01	9.40	166.16±0.63*	160.77-171.04	9.99

Note: * - $p<0.05$

When assessing the blood cell composition, the following was revealed. The amount of red blood cells in the control and experimental groups did not significantly change during the study period and fluctuated in the interval 2,90 – 3,35 10^{12}/l. However, under conditions of increasing laying density, the value of variation coefficient sharply decreased, amounting to C_V=2.70-3.79 and C_V=3.27-3.62% in the II and III groups of birds, compared with C_V=10.90-12.41% in the control (Table. 2). That is, the variability of the parameter became more uniform.

When the laying density changed, the level of lymphocytes in the blood of birds decreased. In group II, the minimum of their amount was detected at the point "after 4 hours", differing from the background value by 17.85% ($p<0.05$), but by the end of the experiment it was almost restored to the original values. In the III group of birds, the number of lymphocytes steadily decreased from 15.46±0.12 to 11.97±0.09 10^9/l ($p<0.05$) by the end of the study period (Table 2). The dynamics of changes in the number of heterophiles in the blood of birds of the experimental groups had opposite orientation. Their level, on the contrary, increased. At the same time, in II group of chickens, the maximum number of cells in the blood was detected at the point "4 hours" after the start of the experiment, exceeding the background by 41.38%; in III group – "24 hours", that is, at the end of the experiment (by 44.65%).

The value of red blood cells and lymphocytes ratio (Table. 3) with an increase in the laying density by 1.50 times had one maximum peak, recorded 4 hours after the initiation of stress. Under conditions of the 2-time excess of the laying density, the value of the ISEL steadily increased, reaching the highest level by the end of the experiment and exceeding the background by 30.00% ($p<0.05$).

The level of the index of red blood cells to heterophiles ratio, on the contrary, decreased, reaching a "critical" minimum in group II at the control point "after 4 hours", and in group III - "after 24 hours", differing from the background values by 32.35 and 33.33% (Table 3).

The effect of corticosterone on the interrelation between red blood cells and lymphocytes, red blood cells and heterophiles reflects the indices of the ratio of red blood cells, lymphocytes and corticosterone (ISELC) and the ratio of red blood cells, heterophiles and corticosterone (ISEGC) (Table 3). With an increase in the laying density by 1.50 times, their value had a peak detected 4 hours after stress initiation, and when the regulatory requirements for chicken cage occupancy were exceeded by 2.00 times, the values of ISELC reached a maximum at the end of the experiment (exceeded the background by 5.01 times, $p<0.05$), and ISEGC – at the control point "after 2 hours" (increased by 3.32 times, $p<0.05$).

The dynamics of the integral index of erythrocytes-heterophiles-lymphocytes and corticosterone (IIEGLC) during the stress reaction in the body of chickens corresponded to the patterns of adaptive process formation revealed with the help of ISEL, ISEG, and ISELC.

The greatest change in IIEGLC in group II of chickens at the point "after 4 hours" correlates with the "lymphocytic minimum" and "heterophilic peak", which determines the response of cellular immunity to the release of corticosterone by the adrenal glands (Table 3). The planned increase in IIEGLC during the stress response in group III of birds and reaching the maximum at the end of the experiment reflected the slow rate of formation of an adaptive response in the body to the influence of an extreme factor, as a result of the predominance of the immunosuppressive properties of corticosterone over the immunoprotective ones [10, 11].

4 Discussion

In the present study, we studied the hormone-hematological features of the stress response in the body of laying hens under conditions of an excessive increase in the density of bird laying in the cage. This initiated the appearance of heat stress signs in them, as a result of the absence of sweat glands, the presence of feathers, and the dependence of thermal regulation on the respiratory rate [15].

It should be emphasized that the laying density is either the number of chickens or the body weight of a chicken per unit area [22-24]. The standard size of laying determines the ability of each chicken in the cage to occupy a certain area [25]. When a bird is given a smaller space relative to the area of its body, not only its freedom of movement decreases, but the ability to disperse the metabolic body, which increases the temperature of the body and the environment [20].

We found that in the conditions of exceeding the standard density of birds laying in the cage by 1.50 times, only during the first 4 hours of the stress reaction, the chickens showed activity to restore their social status, and then a certain zone of "conditional comfort" was reached between the individuals in the cage, allowing them to maintain thermal regulation of the body, take food and water. However, the conditions of existence were not favorable for the manifestation of productive qualities. The same type of social activity of chickens in the conditions of "close existence" was revealed in the study [21].

When the bird laying density in the cage was exceeded by 2.00 times, the metabolic mass per unit area was significantly exceeded. Under these conditions, the amount of heat produced by the birds body significantly exceeded the dispersive capacity of the environment [7], determining its functioning in the "extreme mode".

The bird bodies reacted to the impact of the technological stress factor by activating the hypothalamus-pituitary-adrenal axis, as well as when exposed to other stressors [10, 11, 20, 21], which was aimed at restoring homeostasis. This was shown by the dynamics of corticosterone and cortisol in the blood of chickens during the stress reaction. Moreover, the response to stress varied depending on the deviation of the technological factor from the regulatory requirements, reflecting the energy requirements of the bird body. At the same time, a 1.5-fold increase in density was within the adaptive capabilities of chickens, as it was accompanied by a decrease in the concentration of corticosterone and cortisol in the blood by the end of the experiment. With a twofold increase in density, the secretory function of the adrenal glands increased during the development of the stress reaction, indicating that the value of the technological factor did not correspond to the adaptive capabilities of the body. Similar conclusions were made in the studies of [21], noting that the value of the laying density is associated with the corticosterone level in the blood, which determines the speed of adaptation processes.

In the blood, the most mobile cells are those whose level in the bloodstream is determined by the balance between the processes of proliferation and elimination. Although the amount of red blood cells in the blood of chickens of the control and experimental groups practically did not change, but under the conditions of an increase in the laying density, which was established by us earlier [23, 24], their population became more heterogeneous due to an increase in the proportion of aging and degeneratively altered cells. According to [26], with an increase in the blood flow of red blood cells with a transformed plasma membrane, their physiological interrelations with "adaptive" white blood cells (lymphocytes) are disrupted. In particular, red blood cells cannot control the rate of apoptosis and lymphocyte proliferation induced by glucocorticoids under conditions of a stressful state of the body [4]. It is possible that the modification of the interrelationship between red blood cells and immune cells is a mechanism by which, under the conditions of a stress reaction, the amount and ratio between lymphocytes and heterophiles is redistributed in the body of birds.

The influence of stressors of any nature on the amount of "adaptive" immune cells in the blood of birds has been confirmed by numerous studies. At the same time, the amount of lymphocytes decreases, and the amount of heterophiles increases [8, 23, 24, 27]. In this trend, the direction of changes is important, not their quantitative shifts, since they are associated with the direction of poultry productivity, breed, gender, etc. [11, 23, 24, 28].

We have already noted that corticosterone (glucocorticoid) under stress conditions provides the body of birds with the necessary amount of energy due to the economical consumption of glucose in muscle and fat cells and the stimulation of gluconeogenesis [8], which forms the direction of adaptation processes. Corticosterone, as the main "energy regulator" of the body, also affects the cellular composition of the blood, the ratio of individual white blood cells. According to [11], the hormone has not only an immunosuppressive, but also an immune-protective role, providing the restoration of homeostasis under stress conditions. To assess the effect of corticosterone on the formation of the blood cell pool under stress reaction conditions, we calculated the hemato-hormonal indices, using them as a criterion of adaptive processes.

The dynamics of changes in the values of ELI and EGI confirms the correlation of red blood cell level with the amount of lymphocytes and heterophiles in the blood, which allows to confirm the results [4] on the ability of red cells to influence the processes of proliferation and migration of immune cells. We assume that this is associated with the oxygen transport function of red blood cells, which determines not only the possibility of aerobic (energetically beneficial) processes in the body of birds, but also their ability to be a source of pro-oxidant molecules and thereby have an effect on the life expectancy and functions of lymphocytes and heterophiles [26].

The correlation of the population of red blood cells and white blood cells in bird blood with the secretory activity of the adrenal glands and, as a result, with the concentration of corticosterone is indicated by the variability of the values of ELCI, EGCI and IIEGLC. At the same time, their variability depends on the degree of laying density deviation from the regulatory requirements and the compliance of the stressor force with the adaptive capabilities of the body. This determines the direction of the formation of relations between red blood cells, lymphocytes and heterophiles during the stress reaction, depending on the reactivity of the hypothalamic-pituitary-adrenal axis. In this regard, an increase in the concentration of corticosterone can be considered as a protective reaction of the body of chickens [10, 11], which determines the adaptation strategy of the body.

5 Conclusion

1. The laying density of hens in the cage is the technological factor that affects the adaptive potential of the body and its productive qualities. Chickens adapt to an increase in planting density by one and a half times, but the "price of adaptation" is a reduction in egg production to 33.33%. The twofold increase in laying density does not correspond to the adaptive capabilities of the body, which affects their immune status and productive qualities.
2. The density of bird laying, as a stress factor, initiates the increase in the concentration of corticosterone and cortisol in the blood, which determine a decrease in the number of lymphocytes in the pool of white blood cells and an increase in heterophiles, depending on the correspondence of stressor strength to adaptive abilities of the body.
3. Corticosterone affects the relationship of red blood cells with lymphocytes and heterophiles, determining the variability of the values of the indices of the ratio of red blood cells and lymphocytes (ISEL), red blood cells and heterophiles (ISEG), red blood cells, lymphocytes and corticosterone (ISELC), red blood cells, heterophiles and corticosterone (ISEGC) and the integral index of red blood cells-heterophiles-lymphocytes and corticosterone (IIEGLC).

The results of the research show that it is necessary to further study the long-term consequences for the body of chickens of a one-and-a-half and two-fold excess of the density of birds in the cage, compared with regulatory requirements, as well as "adaptation costs".

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