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Research Article

# Influence of Different Stocking Densities on Some Blood Parameters in Laying Hens

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**Abstract:** The aim of the scientific work is to study the changes in the hematological indicators of chickens when they are kept at a high stocking density. In the conditions of the current complex for the formation of eggs, 4 groups of laying hens were formed. The results showed that high stocking density to 24.0 birds $m^{-2}$ was accompanied by an increase in their blood of leukocytes by 12.2%, heterophils – by 1.8%, and a decrease in thrombocytes' concentration by 4.0%. Provided that the planting density is increased to 25.3 birds $m^{-2}$, there was an increase in the content of leukocytes by 13.7%, heterophils – by 3.1%, and a decrease in thrombocytes concentration by 10.8% with a decrease in their volume by 9.2%. Further increase in stocking density to 26.7 birds $m^{-2}$ caused an increase in the blood content of leukocytes by 22.7%, heterophils – by 13.5%, and a decrease in thrombocytes concentration by 69.0% with a decrease in their volume by 18.6%. Thus, the high stocking density of laying hens is shown by changes in their hematological parameters, which is reflected in the increase of leukocytes, due to an increase in the number of heterophils, and a decrease in thrombocytes.

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## 1. Introduction

The effect of technological stressors, such as increased planting density, the formation of new microclimate conditions of production premises, the conditions of keeping hens, and the composition of the diet, vaccination, transportation, and movement reduce the level of the immunological reactivity of poultry, which causes a productivity decrease (Hall et al., 2014; Stoyanovskyy et al., 2018; Zhuchaev et al., 2019). It is impossible to avoid the influence of stressors in the conditions of intensive production, as increased stocking density is one of the ways to save resources in the poultry industry and is often used to produce more products per 1 $m^2$ (Sakhatsky et al., 2020). However, the effect on the body of hens of high stocking density has not been studied enough. It should be noted that under the influence of stress, the activity of all physiological structures that are responsible for the process of adaptation to changed life factors is undermined. The reason for the spread of the stress reaction is an increase in the dynamics of the work of the endocrine glands, in particular on the axis of the hypothalamus-anterior lobe of the pituitary gland-adrenal cortex (Olubodun et al., 2015).

An important place in the development of stress is occupied by the cortex of the adrenal glands, because they, due to the influence of the pituitary gland, contribute to the development of secreted steroid hormones, which in turn play an important role during adaptation (Berger et al., 2019). Based on this, it is appropriate to note that the main tools for the development and maintenance of stress in a living organism are the sympathoadrenal and hypothalamic-pituitary-adrenocorticotropic systems. Thus, this process is generally affected by adaptive reactions that appear during the appearance of non-specific environmental conditions. This process can be implemented at the expense of the hypothalamic-pituitary-adrenal axis, as well as the sympathoadrenal system, based on catecholamines (Infante et al., 2017). The latter can implement such a process as the transition of the body from a state of rest to a state of excitement. Such a function is embedded in their biological actions, respectively, catecholamines contribute to ensuring the necessary condition for the body. At the same time, one should not forget about the possibility of increasing and deforming metabolic processes in immunocompetent poultry tissues, which is possible due to their implementation of physiological reactions, as well as the use of hormones of the medulla of the adrenal glands and mediators of the sympathoadrenal system (Stoyanovskyy et al., 2018; Zhanabayeva et al., 2021).

Recent studies showed that acute stress caused significant changes in the quantitative and qualitative composition of hens' leukocytes (Weimer et al., 2019; Nwaigwe et al., 2020). The leukemoid reaction of hens blood can be caused by different stressful factors such as starvation, temperature, light, contamination by microorganisms, transportation, shackling, and others (Huth and Archer, 2015; von Eugen et al., 2019; Hofmann et al., 2021; Hussein, 2021; Cellak and Babacanoğlu, 2022; Noaman et al., 2022). However, the vast majority of studies are concerned about the effects of acute stress on hens. The issue of transformations that occur in the structure of chickens under chronic stress caused by high density has not been studied enough.

The purpose of the research is to study the impact of high stocking density on the physiology and other parameters of the hens' bodies.

## 2. Material and Methods

### 2.1. Study sample

The research was based on the study of such living organisms as industrial laying hens Hy Line W-36. The main feature in the selection of hens for research was the intensity of egg laying. Hens were kept for 34 weeks in cages of multilevel batteries at different stocking densities: one group by European standards (13.3 birds $m^{-2}$) and three groups with increasing stocking densities – to 24.0; 25.3 and 26.7 birds $m^{-2}$. Laying hens were individually placed in appropriate conditions that resembled an aviary in terms of area (2640 $m^2$), in which 12-tier cage accumulators "Salmet" were placed (Table 1). Thus, this structure included 18,144 cages with an area of 7,506 $cm^2$ (120 x 62.55 cm). Experiments with animals were based on principles defined by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986).

Table 1. The structure of the appearance of changes caused by an increase in stocking density on the hematological indicators of laying hens in systems of multi-tier accumulators

| Characteristic | Group of laying hens | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| **The number of hens in the cage** | 10 | 18 | 19 | 20 |
| **Stocking density, birds $m^{-2}$** | 13.3 | 24.0 | 25.3 | 26.7 |
| **Provision of area, $cm^2$ per bird** | 750.6 | 417.0 | 395.1 | 375.3 |
| **Feeding front, cm** | 12.0 | 6.7 | 6.3 | 6.0 |

### 2.2. The environmental conditions

The stocking density of hens of the I group met the European norms and requirements of the manufacturer (Guide to the…, 2019) – 13-20 birds $m^{-2}$ (area of 490-750 $cm^2$ per bird and feeding less than 7.0 cm per bird), the II group – Ukrainian standards (VNTP-APK-04.05…, 2005) – 22-25 birds $m^{-2}$ (area – 400-450 $cm^2$ per bird), hens of the III and IV groups were held quite tightly. The housing density was regulated by the number of hens in the cage, which led to different feeding. During the experiment,

laying hens were given drinking water, as well as compound feed (Table 2), and kept in accordance with the requirements (Guide to..., 2019). The windows of the henhouse face the south side, and the door faces the east side. This is necessary in order for enough light to enter throughout the day. Because, due to the long daylight hours, the egg-laying capacity of hens increases. Artificial lighting is used early in the morning and at sunset. The henhouse has effective ventilation. The optimal temperature for keeping laying hens is +12 °C. The relative humidity of the air is 70%. Perches and nests are at a height of at least 60 cm above the floor. The floor is lined with sawdust litter in several layers. This serves not only to maintain hygiene but is also an additional source of heat.

Table 2. The content of the structure of special feed intended for laying hens in the production stage,%

| The nutrient content of the rations of experimental hens | Dynamics of egg laying, % | | | |
|---|---|---|---|---|
| | 95-100 | 93 | 88 | 85 |
| Wheat | 20.418 | 19.336 | 12.000 | 10.566 |
| Corn | 37.053 | 45.399 | 54.330 | 52.334 |
| Sunflower meal | 20.754 | 22.278 | 18.166 | 23.533 |
| Soybean meal | 7.000 | 0.000 | 3.000 | 0.000 |
| Soybean oil | 0.959 | 0.661 | 0.000 | 0.500 |
| Shell 0-3 mm | 10.701 | 9.922 | 10.25 | 11.088 |
| Salt (NaCl) | 0.210 | 0.200 | 0.200 | 0.210 |
| Monocalcium phosphate | 1.193 | 0.811 | 0.805 | 0.532 |
| Sodium sulfate | 0.160 | 0.117 | 0.120 | 0.095 |
| Methionine | 0.186 | 0.105 | 0.088 | 0.076 |
| Lysine sulfate | 0.637 | 0.585 | 0.516 | 0.579 |
| Threonine | 0.127 | 0.095 | 0.057 | 0.065 |
| 100 | 0.000 | 0.010 | 0.000 | 0.000 |
| Globamax 1000 | 0.100 | 0.000 | 0.000 | 0.000 |
| Proactive | 0.000 | 0.000 | 0.150 | 0.150 |
| Enteronormin Detox | 0.150 | 0.150 | 0.000 | 0.000 |
| Mastersorb | 0.150 | 0.130 | 0.130 | 0.000 |
| Mycocide Pro | 0.000 | 0.000 | 0.000 | 0.090 |
| Choline chloride | 0.050 | 0.050 | 0.040 | 0.035 |
| Cronozyme | 0.000 | 0.000 | 0.000 | 0.011 |
| Carophyll yellow | 0.003 | 0.003 | 0.003 | 0.003 |
| Carophyll red | 0.003 | 0.003 | 0.003 | 0.003 |
| Mineral complex | 0.100 | 0.100 | 0.100 | 0.100 |
| Vitamin complex | 0.033 | 0.030 | 0.030 | 0.030 |
| Total | 100.000 | 100.000 | 100.000 | 100.000 |

Note: The content of the vitamin complex: Vitamins A, D, E, C, K, vitamins of group B; the content of the mineral complex: phosphorus, calcium, sulfur, manganese, and sodium chloride).

### 2.3. Randomization method

At the beginning of the study, at the age of 14 weeks, and at the end, namely at 52 weeks, 30 blood samples were taken from the studied living organisms. In this case, 1.0-1.5 ml of the substance was taken from the axillary vein into a test tube with EDTA (ethylenediaminetetraacetic acid). The following indicators were determined: the content of erythrocytes, leukocytes, and thrombocytes – change of impedance in conductometric method; hemoglobin – spectrophotometric method; hematocrit – integer integration; the average volume of erythrocytes, the average content of hemoglobin in the 1 erythrocyte, the concentration of hemoglobin in erythrocytes, with the distribution of erythrocytes, the average volume of thrombocytes – calculation based on the saved results of direct measurements; trough speed (the content of monocytes, lymphocytes, eosinophiles, basophils, and heterophiles – change of impedance by the conductometric method. The ratio of heterophils to lymphocytes was determined using the action of a division, in which the divisor was the number, and the divisor was the number of lymphocytes. Reference values of hens' hematological parameters were determined by N.C. Jain (1993).

### 2.4. The laying performance and egg quality parameters

Hematological parameters of hens were determined on a Micros 60 hematology analyzer (Horiba Ltd. (Privat,e limited company)) in the "Bald" stations (certificate No. LB/02/2016). Trading assets from Horiba Ltd. (United States of America) were used for the determination of hematological parameters, namely the reagent for diluting "ABX Minidil LMG", lysing reagent "ABX Minilyse LMG", reagent for washing "ABX Cleaner", "ABX Miniclair" deproteinizer, "ABX Minipack LMG" reagent container and a set of controls 2N, 1H, 1L "Para 12 Extend".

Eggs were weighed and the optimal quality parameters were determined: the egg weight was 53.9 g; egg length was 5.1 cm; egg width was 4.09 cm; yolk weight was 16.3 g; yolk height was 1.2 cm; albumen weight was 33.1 g; albumen height was 4.8 mm; shell thickness was 0.3 mm; yolk colour was 1.1; yolk index was 39.1; egg shape index was 0.9; albumen index was 20.8; specific gravity was 1.3; haugh unit was 67.1.

### 2.5. Data analysis

One-way analysis of variance (ANOVA) and Tukey-Kramer multiple comparison testing were used to establish and investigate group differences. The latter instrument was applied in the context of a posthoc test tool. Information placed in Tables 1; 2 was presented as M ± SEM (mean ± standard error of the mean). Assessment of the distribution of sample materials for normality was carried out using the Kolmogorov-Smirnov criterion. In this case, if the data distribution was significantly different from normal, the Mann-Whitney U-test was applied. Thus, the concept of normal differences meant those that reached a value of $p<0.05$. The statistical analysis data were produced by Microsoft Excel.

## 3. Results and Discussion

The hematological indicators of the chickens of all objects of the research objects at its initial stage corresponded to the physiological qualities of the indicators. Therefore, no fundamental distinguishing features of the studied categories were found. According to the results of studies (52 weeks of life), regardless of the stocking density the hemoglobin, erythrocytes, and hematocrit parameters in the blood of hens were within the physiological values (Table 3), while the content of leukocytes increased in proportion to the stocking density and in the IV group of hens the physiological norm exceeded. In general, the content of leukocytes in the blood of the IV group of birds significantly 22.7% higher than the I group, 9.3% higher than the II group, and 7.9% greater than the III group of hens. It should be noted that the structure of leukocytes in the blood of the population of groups I-III corresponded to the norm of biological values, but reached the upper limit. The content of leukocytes in the blood birds of the II group was significantly better by 12.2%, and the III – by 13.7% compared to the I group. There was no significant difference in the content elements in the blood of the research subject of II and III groups.

Table 3. Hematological parameters of laying hens depending on stoking density (n = 30 per group)

| Parameters | Category of laying hens | | | | Reference indicators |
|---|---|---|---|---|---|
| | I | II | II | IV | |
| Leukocytes, thousand per µl | $34.4 \pm 1.07^{a}$ | $38.6 \pm 0.61^{b}$ | $39.1 \pm 0.28^{b}$ | $42.2 \pm 1.16^{c}$ | 20-40 |
| Hemoglobin, g $dl^{-1}$ | $10.8 \pm 0.33^{ab}$ | $12.3 \pm 0.12^{a}$ | $11.0 \pm 0.12^{b}$ | $11.1 \pm 0.23^{ab}$ | 7-13 |
| Hematocrit, % | 31.7 ± 0.87 | 31.9 ± 0.46 | 31.1 ± 0.26 | 30.6 ± 0.54 | 22-35 |
| Erythrocytes, million per $mm^3$ | 3.0 ± 0.08 | 3.1 ± 0.07 | 3.0 ± 0.01 | 2.9 ± 0.06 | 2.5-3.5 |
| Thrombocyte, thousand per $mm^3$ | $46.3 \pm 0.25^{a}$ | $44.5 \pm 0.09^{b}$ | $41.8 \pm 0.92^{c}$ | $27.4 \pm 0.35^{d}$ | 32-100 |

Note: $^{a, b, c, d}$ – express traits that were fundamentally different in one row of the table ($P<0.05$).

There was also a decrease in thrombocytes in hens` blood with increasing stocking density. The lowest content of thrombocytes was established in the IV group of poultry – by 69.0%; 62.4% and 52.6% less in contrast to categories I, II, and III. The structure of thrombocytes in the I-III groups of hens`

blood decreased with increasing stocking density but was within the physiological values. Thus, in the blood of hens of the II group, fewer thrombocytes were found by 4.0% than in the I group, and in the III group of hens – by 10.8% and 6.5% than in the I and II groups. Erythrocyte and thrombocyte indices were within the physiological values in all groups of hens (Table 4). At the same time, there was a noticeable increase in the level of the proportion of hemoglobin in one erythrocyte within the physiological value at a high planting density. Thus, the highest content of hemoglobin in one erythrocyte was established in the IV group of hens, and the lowest – was in the first group.

Table 4. Erythrocytes and thrombocytes indices of laying hens depending on stoking density (n = 30 per group)

| Parameters | Category of laying hens | | | | Reference indicators |
|---|---|---|---|---|---|
| | I | II | II | IV | |
| Average erythrocyte volume, mkm$^3$ | 106.0 ± 0.96$^a$ | 98.2 ± 0.91$^b$ | 104.2 ± 0.59$^a$ | 105.4 ± 0.73$^a$ | 90-140 |
| The average content of hemoglobin in the 1 erythrocyte, pg. | 34.0 ± 0.34$^a$ | 34.4 ± 0.44$^a$ | 36.6 ± 0.38$^b$ | 38.4 ± 0.37$^c$ | 33-47 |
| Concentration of hemoglobin in erythrocytes, g dl$^{-1}$ | 34.1 ± 0.32 | 34.9 ± 0.11 | 34.4 ± 0.26 | 34.1 ± 0.34 | 26-35 |
| Erythrocyte distribution width, % | 7.8 ± 0.13$^a$ | 7.9 ± 0.09$^a$ | 7.9 ± 0.07$^a$ | 8.2 ± 0.06$^b$ | 10-15 |
| Average volume of thrombocyte, mkm$^3$ | 8.3 ± 0.30$^{ab}$ | 8.7 ± 0.15$^a$ | 7.6 ± 0.12$^b$ | 7.0 ± 0.17$^c$ | 7-10 |

Note: $^{a, b, c}$ – express traits that were fundamentally different in one row of the table ($P<0.05$)

There was also a decrease in the average volume of thrombocytes within the physiological values with an increasing stocking density of hens. The average volume of platelet in the IV group of birds was at the lower limit of the physiological values, which is less by 18.6%; 24.3% and 8.6% compared to the I, II, and III groups. In addition, no fundamental distinguishing features were established between the hens of the I and II groups, while the thrombocyte of hens of the III group had a smaller volume compared to the I group by 9.2%, and from the II – by 14.5%. In groups with a high density of planting, indicators were established that characterized the dynamic development of elements such as heterophils in the blood of hens (Table 5). Thus, in the IV group of hens, the number of heterophils exceeded the physiological values by 5.8% and in comparison with other groups – by 13.5; 11.7, and 10.4% higher than in the I, II, and III groups. The content of heterophils increased in I-III categories of birds, according to physiological indicators. In particular, in the II categories of hens` blood the content of heterophils was higher by 1.8%, and in the III group – by 3.1 compared to the I group.

Table 5. Leukogram of laying hens depending on stoking density, % (n = 30 per group)

| Parameters | Category of laying hens | | | | Reference indicators |
|---|---|---|---|---|---|
| | I | II | II | IV | |
| Monocytes | 8.4 ± 0.18$^a$ | 6.2 ± 0.06$^b$ | 5.2 ± 0.23$^c$ | 3.4 ± 0.42$^d$ | 5-10 |
| Lymphocytes | 63.8 ± 0.52$^a$ | 62.0 ± 0.18$^b$ | 61.4 ± 0.42$^b$ | 52.2 ± 1.11$^c$ | 45-70 |
| Eosinophils | 2.5 ± 0.18$^a$ | 4.7 ± 0.26$^b$ | 5.0 ± 0.65$^b$ | 5.6 ± 0.58$^b$ | 1.5-6.0 |
| Basophils | 3.0 ± 0.25 | 3.0 ± 0.09 | 3.0 ± 0.22 | 3.0 ± 0.53 | 1-3 |
| Heterophils | 22.3 ± 0.70$^a$ | 24.1 ± 0.15$^b$ | 25.4 ± 1.34$^b$ | 35.8 ± 2.03$^c$ | 15-30 |

Note: $^{a, b, c, d}$ – express traits that were fundamentally different in one row of the table ($P<0.05$).

The content of eosinophils increases within the limits of physiological permissible indicators and in the IV group of hens reached its upper limit and was higher by 3.1% in contrast to the 1st group. The difference between the II and III groups was not statistically significant. At the same time, the content of eosinophils in blood was higher in the II group of hens by 2.2%, and in the III group – by 2.5% compared to the I group. The increase in the content of heterophils and eosinophils occurred due

to a decrease in the share of other types of leukocytes, namely lymphocytes, and monocytes. The number of lymphocytes in the IV categories of hens` blood was lower by 11.6; 9.8 and it was more than in the I, II, and III groups by 9.2%. The number of monocytes in the IV group of hens was 5.0% lower compared to the I group and 2.8% and 1.8 % compared to the II and III groups. In the II group of hens, it was found few monocytes by 2.2% compared to the I group, and in the hens of the III group – by 3.2 and 1.0% compared to the I and II groups. A significant crowding of laying hens was accompanied by the development and spread of the concentration of leukocytes in the blood. In particular, the concentration of leukocytes in the blood increased by 12.2% with high crowding of hens to 24.0 birds $m^{-2}$; up to 25.3 birds $m^{-2}$ – by 13.7%; for increasing the stocking density to 26.7 birds $m^{-2}$ was characterized by an increase in their number by 22.7%. The obtained data agree with the results of research by other scientists, who describe the dynamics of the distribution of leukocytes in the blood of laying hens, broiler chickens, and ducks at high stocking density (Kang et al., 2018; Nwaigwe et al., 2020; Xiong et al., 2020).

The increase in the content of leukocytes 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 stressors (Jiang et al., 2017; Gryshchenko et al., 2019). Thus, the basis for the development of leukocytosis is an increase in the heterophil content in the blood, as noted in these studies (Table 5). According to a number of authors (Dhabhar et al., 2012; Wirths et al., 2014), the increase in the content of leukocytes due to heterophils occurs due to hypercortisolemia and hypercatecholamania, which lead to an increase in their mobilization in the blood. The increase in circulating heterophiles is the result of the body's preparation for a protective response to possible damage (Liew and Kubes, 2019; Vashchyk et al., 2020). The obtained results confirm this assumption. According to the results of research, it is shown that high crowding of hens to 24.0 birds $m^{-2}$ leads to an increase in heterophils by 1.8%, to 25.3 birds $m^{-2}$ – by 3.1%; to 26.7 birds $m^{-2}$ causes an increase of their content by 13.5%. The obtained data on the increase in the content of heterophils coincide with the results obtained in similar experiments with overcrowding of laying hens, as well as in broiler chickens during stress, and stress from starvation, heat, and immobilization stress (Kang et al., 2018; Li et al., 2019; Nwaigwe et al., 2020; Gul et al., 2021).

Increased levels of heterophils in the blood can be explained by two assume mechanisms: slowing of the transition of heterophils from the blood to peripheral tissues caused by an increase in blood corticosteroid hormones due to stressors, as well as redistribution of heterophils in the vascular bed by reducing parietal and increasing circulating pools caused by hormones such as adrenaline, noradrenaline, and cortisol. At the same time, it is known that the rapid mobilization of the parietal pool of heterophils is due to adrenaline under acute stress, and the mobilization of the bone marrow pool occurs under the influence of corticosteroids during chronic stress (Ince et al., 2019; Nykonov et al., 2019). Therefore, it can be assumed that the increase in the level of heterophils in the blood during prolonged compaction of hens is due to the mobilization of the bone marrow pool of heterophils and the slowing of their transition from blood to peripheral tissues. There was also a decrease in thrombocyte concentration, with a high stocking density of hens. In particular, when creating tight conditions for laying hens up to 24.0 g $m^{-2}$, there was a decrease in thrombocyte content by 4.0%, to 25.3 birds $m^{-2}$ – by 10.8%; to 26.7 birds $m^{-2}$ was accompanied by a decrease in their concentration by 69.0%. The decrease in the volume and in the concentration of thrombocytes in the hens` blood – to 25.3 birds $m^{-2}$ – by 9.2%, and to 26.7 birds $m^{-2}$ – by 18.6%. The obtained data confirm a reduction in the number of platelets in the blood of broiler chickens in response to heat stress.

## Conclusion

Changes in hematological parameters of laying hens due to factors caused by increased stocking density were analyzed. It was established that the high stocking density of laying hens is accompanied by changes in their blood system, which is reflected in the increase in its content of leukocytes, due to an increase in the number of heterophils, and a decrease in thrombocyte count. The decrease reduction in the number of thrombocytes in the blood may be explained by the high level of activity of lysosomal enzymes of heterophils in plasma, the activity of which increases due to increased heterophils.

In particular, an increase in the density of chickens to 24.0 birds $m^{-2}$ was accompanied by a 12.2% increase in leukocytes in their blood. Heterophils – by 1.8% and a decrease in thrombocytes'

concentration by 4.0%. With a further increase in the density of planting up to 25.3 birds $m^{-2}$, there was an increase in the content of leukocytes by 13.7%, heterophils – by 3.1%, and a decrease in thrombocytes concentration by 10.8% with a decrease in their volume by 9.2%. An increase in the density of planting to 26.7 birds $m^{-2}$ was characterized by the development of leukocytes in the blood by 22.7% (5.5%>normal), heterophils – by 13.5% (19.3%>normal), and a decrease in the concentration of platelets. by 69.0% (14.4%<normal) with a decrease in their volume by 18.6%.

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