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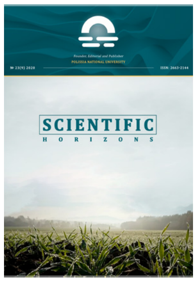

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Biochemical parameters of chicken blood under the influence of technological stimuli of various etiologies

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Abstract. Violation of the main technological parameters of keeping and feeding poultry is the main factor in reducing egg productivity and health in the industrial production system. Therefore, the research aims to determine biochemical markers in acute technological stress caused by factors of various etiologies. In this study, serum

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biochemical parameters were evaluated for the effects of stimuli in egg-laying chickens. The study was conducted on several Hy-Line W-36 cross chickens in the conditions of a production line. Four groups of chickens (101 heads each) aged 52 weeks were formed to conduct the study. The control group was formed from 10 representatives of each of the groups. Further, each group was exposed to a corresponding technological stimulus. Birds of group 2 were not fed for 24 hours, those of group 3 were kept in a darkened place, and 60 additional birds were placed in a cage with the chickens of group 4. The highest effect on the dynamics of biochemical parameters was an increase in their concentration by 1 m^2 cage space. The studies identified a substantial increase in the concentration of glucose and creatinine in the blood serum of chickens during over-compaction – by 24-28% ($p<0.05$) compared to the control group, regardless of the cause of the stressful situation, all birds exposed to acute stress had an increase in these indicators in the blood serum. The main part of the examined enzymes, such as aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase, tended to increase their activity depending on the strength of the influence of the technological factor on the body of birds. Dynamic characteristics of glucose and creatinine levels were distinguished among the examined biochemical parameters by rapid and differentiated reactions within the physiological norm, and considering the simplicity of their determination, they may have prospects of becoming the main markers of early diagnosis of stress in industrial poultry farming

Keywords: glucose level; creatinine; aspartate aminotransferase; lactate dehydrogenase; stress; marker

INTRODUCTION

Poultry farming is one of the actively developing branches of animal husbandry not only in Ukraine but also around the world. However, according to K. El-Sabrout *et al.* (2022), the economic attractiveness of this area of animal husbandry is based on the short period of poultry rearing, rapid turnover of invested funds, and intensive development due to the variability of production. Such prerequisites contributed to the emergence of large, specialised poultry enterprises in Ukraine. Modern scientific findings in the field of animal husbandry and zoo hygiene have allowed egg poultry farming to a new level of production. Specialised hybrid chickens produce 300 or more eggs per year. Therewith, due to artificial light control and optimisation of feeding rations, it was possible to level out the seasonal decrease in egg productivity during moulting and get a constant egg laying throughout the year. This was made possible by the introduction of the results of studies into the main production processes: S. Molnár & L. Szőllősi (2020) on increasing the concentration of animals in the room through the use of high-tech cage equipment, Y. Ouchi *et al.* (2022) and M. Madkour *et al.* (2022) on the use of modern air conditioning and temperature control systems during maintenance, and W.L. Bryden *et al.* (2021) on ration feeding. However, industrial poultry farming, despite its high profitability, achieved by reducing logistics and labour costs, also remains the most vulnerable industry in terms of the large crowding of birds in a relatively small area and the impact of constant technological stress on them.

I.A. Fisenko *et al.* (2021) are convinced that the productivity of an adult bird is largely determined by the conditions of its rearing and keeping. Due to the targeted influence on the zootechnical parameters of the microclimate, which is artificially created in poultry houses where laying hens are kept, a substantial increase in egg productivity is achieved, compared to an extensive type of production, while maintaining the quality of commercial eggs. However, such substantial physical and feed loads on the bird's body correspondingly affect its physiological state. According to L. Yan *et al.* (2022), this is accompanied by stressful changes in the blood system and hormone balance and leads to a decrease in productivity and leads to substantial economic losses. Therefore, ensuring optimal microclimate parameters, comfortable conditions for keeping, and proper feeding of birds is the basis of industrial technology for the production of egg and meat products in poultry farming.

To date, according to K. Olejnik *et al.* (2022), monitoring the condition of animals in the conditions their productive use, with intensive egg production technology in Ukraine, is conducted by monitoring the behaviour of animals and constant observation of the level of productivity, feed consumption, bird safety, and their general condition, in particular, the development of aggression, cannibalism, or eating feathers. Such approaches allow stating the harmful effect of stress factors on animals only when the clinical picture develops from negative effects and forces the measures to eliminate the consequences of stressors. A more promising method of monitoring the condition of animals in an industrial system of keeping is laboratory methods that allow the identification of the negative technological impact of one or more factors on animals in advance of the manifestation of a decrease in productivity. This will allow the prevention of short-term losses and maintain production at a high level.

Based on the importance of monitoring the productive well-being of poultry in industrial egg production, the research aims to determine biochemical markers of poultry blood under acute technological stress caused by factors of various etiologies.

LITERATURE REVIEW

Stress is the response of the body to an internal or external stimulus that causes anatomical, physiological, and behavioural changes. Among the most common stresses during cage keeping, the primary stress source is temperature fluctuations, high humidity, lack of light, high crowding of animals, insufficient air exchange, noise, and fear (Bulent & Niyazi, 2018). According to A. Usturoi *et al.* (2023), high egg production in poultry balances on the edge of the physiological norm and the constant risk of stress. Therefore, industrial poultry farming is constantly looking for methods to control the occurrence of stress and prevent it.

According to most authors, the most common stress factors are heat stress (Ghoname *et al.*, 2022) and high crowding of caged birds (Osadcha *et al.,* 2022; Herbert *et al.*, 2021). According to J.A.C. Sáenz (2021), heat stress in the United States alone causes economic damage of 1.128-165 million annually and a 0.5-7.2% decline in egg production. M. Ghoname *et al.* (2022) determined acute heat stress to reduce feed intake in all egg-laying chicken crosses, a direct correlation between the intensity of temperature stress and quantitative indicators of egg productivity was identified. Losses from over-compaction of poultry in cages, calculated by G.T. Herbert *et al.* (2021) in the UK egg industry, amounted to 6.5 million pounds a year as were result of suffocation alone, excluding the effects of sublethal injuries such as bone fractures and stress from physical restriction. Birds kept in cages showed signs of fear, decreased faecal immunoglobulin A levels, and increased corticosterone concentrations in their feathers (Campbell *et al.*, 2022).

A study by E.E. Onbasilar & F.T. Aksoy (2005) confirms the conclusions of previous authors on the deterioration of both egg productivity and the condition of the birds themselves with an increase in their concentration. In addition, it was shown that the higher the ceiling level of the cage, the better the performance indicators. The difference in egg quality was substantially better in animals from higher levels compared to lower ones. Antibody titers were lowest on compacted planting. The optimal number of birds was 3 per cage. L. Hedlund and P. Jensen (2022) proved the sustained effect of stress on the subsequent productivity of chickens. Thus, productive poultry raised under stressful conditions substantially ($p<0.001$) had the worst productivity and egg quality. In addition, according to L. Yan *et al.* (2022), birds exposed to stress have impaired reproductive capacity. Corticosterone, which is actively produced during stress, inhibits the development of follicles and prevents the deposition of yolk in them.

Control of stress levels in birds is conducted by invasive and non-invasive methods. Non-invasive methods based on observation and analysis are most often used. The following parameters are monitored: egg production percentage, average egg weight, average feed intake per bird per day, average feed conversion expressed in grams of feed/egg, average chicken weight, cumulative mortality rates, and the number of suffocation cases that resulted in mortality. In addition, a mandatory element in determining stress is monitoring the bird's behavioural responses – a test for a stationary person, a new object, and feather damage. Among invasive methods, several biochemical parameters of blood serum are used (Nwaigwe *et al.*, 2020), which, unlike non-invasive ones, allow for determining in more detail the physiological state of the body, adaptive responses, and metabolic disorders.

MATERIALS AND METHODS

The study was conducted in the branch of one of the largest producers of chicken eggs in Ukraine on the number of chickens in the egg productivity area of the cross "Hy-Line W-36". All manipulations with controlled poultry were conducted following the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986). During the experiments, the company operated normally in compliance with all sanitary and hygienic, veterinary, and technological requirements. The study was conducted in the conditions of a technological line of an industrial-type poultry complex for the production of commercial eggs. The bird was kept in standard poultry houses with a controlled microclimate, equipped with cage equipment from the company "Salmet" (Germany). The microclimate parameters corresponded to the recommendations for breeding chickens of the commercial cross "Hy-Line W-36" (W-36 Commercial Layers..., 2020). Feeding of birds was conducted with complete mixed feeds using automatic cup feeders following the standards specified in the recommendations of breeders. 4 groups of chickens (101 heads each) aged 52 weeks were formed to conduct the study. Each group was kept in separate cage analogues by area and equipment (Table 1).

Table 1. *Scheme of conducting experiments to examine the effect of technological stimuli of various etiologies on the body of chickens*

Feature	Groups of egg-laying chickens			
	1	2	3	4
Irritant	Control	Food stress	Lighting stress	Over-compaction
Duration of exposure to the stimulus, h	24			
Number of heads in a cage	101			161

Table 1, Continued

Feature	Groups of egg-laying chickens			
	1	2	3	4
Irritant	**Control**	**Food stress**	**Lighting stress**	**Over-compaction**
Concentration, heads/m^2		24.9		39.7
Area availability, cm^2/head		401.4		251.8
Feeding front, cm		7.2		4.5

Source: *compiled by the authors*

The control group was formed from 10 representatives of each of the 4 groups before the start of the acute technological stress simulation. In the future, each group was exposed to a corresponding technological stimulus of different etiologies. Birds of group 2 were not fed for 24 hours, those of group 3 were kept in a darkened place, and 60 additional birds were placed in a cage for chickens of group 4 to ensure substantial over-compaction. Modelling of the effect of acute stimuli in chickens of groups 2 and 3 was achieved by turning off the corresponding systems in the poultry house – the feed distribution line and lighting. Exposure to the factor in all groups was 24 hours, after which the biochemical parameters and activity of enzymes in the blood serum of chickens were determined. Blood samples were taken from randomly selected 30 laying hens aged 52 weeks from each of the experimental groups. 1-1.5 ml of blood was taken from the axillary vein and stabilised with 2-3 drops of ethylenediaminetetraacetic acid solution (EDTA, trilon-B), according to the guidelines for collecting biopsies in veterinary practice for the diagnosis of small pets in the laboratory where biochemical studies were conducted (Guidelines for collecting..., 2021).

Biochemical parameters and enzyme activity in the blood serum of laying hens were determined using a BioChem FC-360 biochemical analyser (High Technology Inc., USA) in the laboratory of the Centre for Animal Diagnostics "Bald" (Kyiv). Biochemical parameters (glucose, total protein, albumin, creatinine, urea, cholesterol, phosphorus, and calcium) and the activity of enzyme complexes (Alanine aminotransferase (ALT), Aspartate transaminase (AST), Lactate dehydrogenase (LDH), Alkaline phosphatase (ALP), and Gamma-glutamyltransferase (GGT) were determined using commercial diagnostic kits from High Technology Inc. (USA) following the guidelines for their application. The results of the examination were subjected to mathematical analysis using the software product TIBCO Statistica v14.0.0.15 (USA). Indicators of descriptive statistics and the influence of technological factors on the biochemical parameters of laying hens' blood were determined using a one-factor analysis of variance. The probability of differences between the groups was estimated by determining the Student's t-criterion.

RESULTS

Technological stress is the main factor in reducing egg productivity in poultry at the enterprise, which is caused by many factors. Therefore, to reduce the factors not considered that could affect the results of research, poultry was fed with recommended diets. Table 2 shows the composition of complete mixed feeds, as their elements can also cause certain biochemical changes in blood parameters in the body.

Table 2. *Composition of mixed feed for laying hens during the productive period, %*

Component	Mixed feed				
	Guaranteed performance level, %				
	100	**93**	**88**	**85**	**Molting period**
Grain group					
Wheat	20.42	19.34	12	10.57	10
Corn	37.05	45.4	54.33	52.33	52.84
Sunflower meal	20.75	22.28	18.17	23.53	19.39
Soy meal	7	-	3	-	8.07
Dietary supplements					
Soy oil	0.96	0.66	-	0.5	-
Crushed feed shell	10.7	9.92	10.25	11.09	7.61
Salt	0.21	0.2	0.2	0.21	0.23
Monocalcium Phosphate	1.19	0.81	0.81	0.53	1.06
Sodium sulfate	0.16	0.12	0.12	0.09	0.16
Methionine	0.19	0.1	0.09	0.08	0.04

Table 2, Continued

Component	Mixed feed				
	Guaranteed performance level, %				
	100	93	88	85	Molting period
Lysine sulfate	0.64	0.59	0.52	0.58	0.18
Threonine	0.13	0.09	0.06	0.06	-
LOXIDAN TD 100 (preservative)	-	0.01	-	-	-
Millertime (enzyme preparation)	0.01	0.02	0.01	-	0.02
Globamax 1000 (microflora activator)	0.1	-	-	-	-
Proactive Poultry (microflora activator)	-	-	0.15	0.15	-
Enteronormin Detox (probiotic)	0.15	0.15	-	-	0.15
Mastersorb (mycotoxin inactivator)	0.15	0.13	0.13	-	-
Mycocide Pro (mycotoxin inactivator)	-	-	-	0.09	0.09
Choline Chloride	0.05	0.05	0.04	0.04	0.04
Kronozyme (enzymatic preparation)	-	-	-	0.01	-
Yellow aerophilous (dye)	0.003	0.003	0.003	0.003	-
Karofil red (dye)	0.003	0.003	0.003	0.003	-
Mineral complex	0.1	0.1	0.1	0.1	0.1
Vitamin complex	0.03	0.03	0.03	0.03	0.03
Total	100	100	100	100	100

Source: *compiled by the authors*

In these diets, the main part of the feed is represented by high-energy cereals and various biologically active additives, in particular, amino acids, vitamins, macro-and microelements, antioxidants and other useful elements that increase the digestibility and absorption of nutrients. In addition, the composition of mixed feeds, regardless of the level of productivity, did not include pharmacological preparations with adaptogenic properties that could reduce or distort the influence of the examined factors on the body's response. After considering most of the factors that could affect the results of the study in one way or another, a series of controlled stress stimuli of a technological nature was conducted on experimental groups of birds. Biochemical parameters of the blood of controlled laying hens after the action of technological factors are given in Table 3.

Table 3. *Biochemical parameters of chicken blood serum under the influence of technological stimuli of various etiologies, n=30/group*

Attributes and units of measurement	Group				Reference values*
	1	2	3	4	
Total protein, g/L	54.42±0.41[a]	57.02±0.78[b]	57.46±0.55[b]	58.54±0.56[b]	41-65
Albumin, g/L	18.5±0.19[a]	18.61±0.22[ab]	19.38±0.08[c]	19.18±0.22[c]	19-24
Glucose, mmol/l	15.17±0.21[a]	16.31±0.11[b]	16.82±0.16[c]	18.12±0.13[d]	11.6-275
Creatinine, mmol/L	21.86±0.2[a]	22.82±0.24[b]	25.22±0.35[c]	27.02±0.28[d]	20.3-28.6
Urea, mmol/L	1.1±0.05	1.04±0.06	1.02±0.07	1.12±0.01	0.31-0.73
Bilirubin, mmol/L - total - direct - indirect	 1.36±0.03[a] 0.12±0.03[a] 1.24±0.05	 1.46±0.11[a] 0.38±0.02[b] 1.08±0.1	 1.54±0.02[b] 0.26±0.02[c] 1.28±0.11	 1.6±0.04[b] 0.26±0.02[c] 1.34±0.06	 1.2-1.7 0.2-0.5 0.8-1.4
Cholesterole, mmol/L	2.7±0.13	2.72±0.11	2.91±0.08	2.78±0.09	3.3-12.2
Phosphorus, mmol/L	1.43±0.05	1.46±0.04	1.59±0.08	1.58±0.07	1.3-2.2
Calcium, mmol/L	4.12±0.01[a]	4.38±0.02[b]	4.54±0.02[c]	4.56±0.04[c]	3.5-6.8
Calcium/phosphorus ratio	3.01±0.15	3.01±0.11	3.02±0.14	3.03±0.13	3-3.6:1

Note: *a, b, c – differences are reliable (p <0.05) within the indicator; * Reference values according to I.V. Nasonov et al. (2014)*
Source: *compiled by the authors*

As a result of the conducted studies, it was established that only indicators of glucose and creatinine in the blood serum had a prognostic value for the manifestation of acute stress on the bird's body, while other indicators in the first hours of exposure to technological stimuli almost did not change. The first, under the action of stress factors, are the biochemical parameters associated with carbohydrate and energy metabolism, which is a manifestation of the compensatory phase of the body's response to stimuli. Most often, this is manifested by an increase in the concentration of glucose and creatinine in the blood since these compounds are indicators of increased energy consumption by the body. Studies have identified a substantial increase in the concentration of glucose in the blood serum of chickens under the influence of various technological stimuli. In laying hens of group 2, underfeed stress, the glucose content increased by 15.1% ($p<0.05$) compared to birds of the control group. The highest stress factor for birds was a decrease in the area of keeping per chicken. It was in the birds of group 4 that were exposed to over-compaction that the glucose content was highest – 27.9% ($p<0.05$) higher compared to the control group, and by 11.1% ($p<0.05$) and 7.7% ($p<0.05$) with groups 2 and 3, respectively.

Similar dynamics were characteristic of the level of creatinine in the blood serum of birds, which corresponds to the activation of energy metabolism in the compensatory period to the effect of a technological stimulus. Regardless of the cause of the stressful situation, all birds exposed to acute stress had elevated serum creatinine levels. As was the case with glucose levels, its maximum increase was in laying hens of group 4, which, due to over-compaction, had creatinine content 23.6% ($p<0.05$) higher compared to the control group, and 18.4% ($p<0.05$) and 7.1% ($p<0.05$) compared to groups 2 and 3, respectively. In birds to which feed and light stressors were applied, the level of creatinine, although elevated, was still within the physiological norm, in particular, in chickens of group 2, the content of creatinine in the blood serum during fasting was higher by 4.4% ($p<0.05$), and in group 3 – by 10.5% ($p<0.05$) compared to animals that were not exposed to stress.

Under the influence of technological stimuli, an increase in the total protein content in the blood serum occurred within the physiological norm. Thus, in chickens of group 2, under food stress, the total protein content in the blood serum was higher by 4.8% ($p<0.05$), in chickens of group 3 – by 5.6% ($p<0.05$), and in chickens of group 4 – by 7.6% ($p<0.05$) compared to poultry of the control group. While differences in protein content between different groups of animals exposed to stress of different etiologies ranged from 0.8-2.7% and were not statistically confirmed. A similar pattern was observed in terms of albumin content. A notable increase in the level of albumin fractions of the protein under the action of various technological factors was not observed, all of them were within the reference intervals. Slightly higher was the increase in the number of albumins in the blood serum of chickens of the third group by 4.1-4.8% ($p<0.05$) and the fourth – 3.1-3.7% ($p<0.05$), which were kept in over-compacted cages and under the condition of darkening, compared with groups 1 and 2, respectively. There was no substantial difference in serum albumin content between the laying hens of the control and first experimental groups.

The highest effect on the dynamics of biochemical parameters of blood from technological stressors was an increase in their concentration by 1 m^2 cage spaces. It was this factor that caused the fastest and greatest reaction of the body to the action of the stress factor. In addition to the above-mentioned effect of the over-compaction factor on several blood serum parameters, an increase in such indicators as the content of total bilirubin and the level of calcium ions was also detected. Other technological factors also had a substantial impact on the bird's body, but their effect was substantially lower compared to the stress of over-compaction, even though the "Hy-Line W-36" cross is considered the most adapted to caged breeding. Thus, the content of total bilirubin in animals of group 4 exceeded the level by 3.9-9.6% of its concentration in animals of other groups that were subjected to technological exposure and 17.6% ($p<0.05$) of chickens of the control group. A slightly different picture was observed for other bilirubin fractions. Thus, the highest level of direct bilirubin was observed in chickens of group 2, which were exposed to feed stress, the content of direct bilirubin in them substantially exceeded the indicators in animals of other groups. This may be due to liver activity due to prolonged starvation of animals. The indirect bilirubin content was at the same level in chickens of all groups. Thus, the increase in total bilirubin under the influence of technological stimuli occurred due to direct bilirubin.

The calcium content in the blood serum of chickens of all groups was within the physiological norm, but its increase was observed under the influence of technological stimuli. In particular, in group 2, its content was higher by 6.3% ($p<0.05$) compared to group 1. The differences between groups 3 and 4 were only 0.4% and were not statistically confirmed. The effect of technological stimuli did not affect the content of cholesterol and phosphorus in the blood serum of chickens, which were at the same level in chickens of all groups and were within the physiological norm, which also affected the ratio of calcium and phosphorus. No fewer substantial markers of the action of acute stress in poultry may be the activity of certain enzymes. As part of the study, the activity of several serum enzyme systems after 24 hours of exposure to individual technological stressors was analysed (Table 4).

***Table 4.** The activity of several enzymes in the blood serum of laying hens under the influence of technological factors, n=30/group*

Indicator, units/L	Group				Control value*
	1	2	3	4	
Alanine aminotransferase (ALT)	1.2±0.15	1.04±0.02	0.84±0.06	1.02±0.01	1.5-3.3
Aspartate transaminase (AST)	164.61±3.74[a]	211.4±5.84[b]	224.42±1.09[c]	259.8±2.31[d]	118-298
Gamma-glutamyltransferase (GGT)	22.19±1.11	22.42±1.03	22.24±0.49	22.64±1.07	13.9-27.6
Alkaline phosphatase (ALP)	654.86±11.85[a]	686.41±10.42[b]	717.62±12.4[bc]	732.02±16.26[c]	350-830
Lactate dehydrogenase (LDH)	1368.82±63.68[a]	1694.84±64.49[b]	2019.05±26.41[c]	2358.04±41.91[d]	1794-2372

***Note:** a, b, c – differences are reliable (p <0.05) within the indicator; *Reference values according to I.V. Nasonov et al. (2014)*
***Source:** compiled by the authors*

The main part of the examined enzymes, such as aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase, tended to increase their activity depending on the strength of the influence of the technological factor on the body of birds. An increase in the activity of these enzymes in the blood is associated with partial destruction of cages. Therefore, the study showed a proportional increase in the activity of enzymes to the strength of the stress factor. As in previous studies, the strongest stressor for birds, based on the dynamics of growth in enzyme activity, was the over-compaction of animals. Thus, for the main enzymes – AST, ALP, and LDH, the increase was from 13 to 73% and substantially exceeded the corresponding values in animals of the control group. Animals exposed to feed or lighting stress occupied an intermediate place and slightly differed both from each other (2-4.5%) and from animals of the control group (4.8-9.6%). GGT in animals of all groups, both experimental and control, was at the same level and was within the physiological interval, so as a marker of stress in poultry farming, it will not have practical importance. The results obtained regarding the decrease in ALT activity in animals after the negative effect of technological factors require a more in-depth study. Ultimately, even in animals of the control group, ALT activity was at the lower limit of the physiological norm, and after stress load, it generally decreased below the permissible interval, which is more similar to the genetic feature of the bird population of the cross "Hy-Line W-36".

Summarising the results obtained, it can be stated that individual biochemical parameters of blood serum and the activity of enzyme complexes can be used for early diagnosis of the negative impact of technological stimuli of various etiologies.

DISCUSSION

The biggest challenge of modern industrial poultry farming is the constant search for a compromise between high bird productivity and balancing on the edge of stress. Stress reduces the birds' feed intake, the intensity of their growth and productivity, reduces the immune response and function, which leads to the high susceptibility of laying hens to diseases. Therefore, the actual area of research in poultry farming is the search for informative systems for early diagnosis of stress development in laying hens, as a precursor to reduced productivity. Several cytological techniques (Lee *et al.*, 2022) and humoral approaches (Osadcha *et al.*, 2021) have already been used to diagnose stress. However, they have substantial drawbacks: cytological methods have a long-hidden period, caused by the accumulation of appropriate cellular elements in the blood, and humoral methods are rather expensive and resource-intensive. These shortcomings are devoid of methods for determining most of the biochemical characteristics of animal blood and, the main thing is that changes in the blood develop within a short time after the action of the stimulus, which allows consideration of these methods as markers of acute stress in poultry.

From the results of the study on the influence of some technological factors of various etiological nature on the biochemical parameters of the blood of productive laying hens, it was determined that such characteristics as an increase in the concentration of glucose and creatinine, and the activity of aspartate aminotransferase and lactate dehydrogenase are fast and reliable markers of stress in poultry. The changes in these biochemical parameters were manifested already in the first hours after the action of stressors, so they can become markers of the development of a pathological reaction of the bird body to the action of technological factors. Moreover, the methods for determining the levels of these indicators are simple, and the use of ready-made kits for conducting tests generally makes the duration of the study no longer than a few minutes.

Mechanisms of increasing the level of glucose and creatinine in the serum of animals exposed to stress are a consequence of the destruction of part of the body's cells, and the intensity of this process is directly proportional to the strength of the stressor. Similar conclusions were made by M.I. Sakhatsky & and Yu.V. Osadcha (2021), when analysing the influence of such a technological parameter as the height of cages on the biochemical parameters of the blood of laying hens in an industrial egg production system. An increase in certain biochemical parameters of the blood is associated with the active work of the adrenal glands and the expression of corticosteroids. The latter, as proven in the study by Y. Wang *et al*. (2020), trigger the mechanism of activation of the enzymes creatine kinase and lactate dehydrogenase, which is observed in the results obtained in the framework of this study. An increase in blood glucose in animals exposed to stress occurs due to increased gluconeogenesis and glycogenolysis under the influence of epinephrine and norepinephrine, released during the development of the acute phase of the body's response to stimuli (Chu *et al.*, 2022).

A similar pattern was observed in the blood serum of experimental chickens. In particular, feeding stress resulted in only a slight increase in glucose levels within the physiological norm, 16.3 mmol/L. While due to other factors, its concentration increased substantially higher than the reference values for poultry. In addition, the concentration of glucose in the blood was directly proportional to the strength of the stimulus. In the conducted studies, the highest glucose level was observed in chickens exposed to technological stress of over-compaction – 18.2 mmol/L. Analysis of other marker indicators – creatinine levels and blood enzyme activity confirmed that this factor is the greatest stress for industrial birds involved in the study. Similar conclusions were obtained by Yu. Osadcha *et al.* (2022), identified that with an increase in the planting density of laying hens up to 26.7 head/m^2 resulted in a decrease in the safety of chickens by 8.9-9%, and egg production – by 4-5.8%. That is, in their opinion, clinical signs similar to those that occur under the influence of chronic stress were obtained. This is assumably why most farmers are considering replacing the conventional cage-keeping system with other, less stressful approaches.

Informative marker was the concentration of creatinine in the blood of experimental birds. Creatinine, like glucose, is a high-energy compound involved in the body's energy metabolism, namely, it provides energy to muscle tissue. Therefore, an increase in the level of creatinine in the blood serum of chickens indicates neuromuscular tension in their body, which is confirmed by the conducted studies. In particular, when the body of chickens was exposed to technological stresses, creatinine concentrations fluctuated from 21.9 mmol/L in the control group to 27.2 mmol/L in the group of chickens that were subjected to over-compaction. That is, this indicator had a wider range of fluctuations compared to glucose levels under appropriate conditions and also had a straight-line correlation with the intensity of stress. The main reason for its use as a future marker is that all creatinine values were within the physiological norm. That is, it allows determining the effect of the stress factor on the body more accurately, after minor analytical studies with a change in the intensity of one technological stimulus, without causing pathological changes or a decrease in productivity. S.C. Huang *et al.* (2018), confirmed the use of prognostic value of creatinine as a marker of the effect of temperature stress in chickens. Therefore, it is planned to examine in more detail the dynamics of changes in creatinine concentration depending on the intensity of the technological stimulus in the future, and this is the goal of subsequent studies.

As for other biochemical parameters, there were no substantial changes in their levels that could recommend making them markers of the effect of stress on the body. S. Tang *et al.* (2018) identified prognostic levels of urea in the blood of chickens after exposure to temperature stress, while in a study by C. Nwaigwe *et al.* (2020), this indicator was the dynamics of changes in albumin levels. The results of the analysis of biochemical parameters of blood performed in the framework of this study did not confirm these assumptions, which may have been due to the different periods of laboratory tests.

Changes in the activity of enzymes, in particular, aspartate aminotransferase and lactate dehydrogenase, were equally informative markers. The increased activity of these enzymes is due to the overexpression of corticosteroids and epinephrine (Wang *et al.*, 2020). Like other biochemical parameters that have been identified as potential markers, the activity of AST and LDH increases in proportion to the strength of the stress factor, and this can be used for prognostic purposes since the activity of both enzymes increased within the physiological norm. The results obtained are quite consistent with those of most researchers (Park *et al.*, 2018; Abo Ghanima *et al.*, 2020), which note that the increase in aspartate aminotransferase activity is the reaction of the bird's body to the action of technological stimuli. An increase in the level of the enzyme above the physiological norm is associated with damage to the function of internal organs, which is already a sign of a pathological condition and is not quite suitable as a clinical tool. Similar dynamics are characteristic of the enzyme lactate dehydrogenase. LDH is an enzyme involved in carbohydrate metabolism, catalysing energy reactions, so it undergoes similar changes along with other products of energy metabolism. According to R. Klein *et al.* (2020), due to the branched structure of LDH isoenzymes, they can become the most informative systems since they enter the serum during tissue damage. Therefore, when determining the model of LDH isoenzyme in serum, it is possible to determine which organ damage prevails, which is also quite an attractive area for future research, provided that the necessary equipment and reagents are available.

Even though the recommendations for breeding chickens of the commercial cross "Hy-Line W-36" did not indicate the biochemical profile of birds and individual studies that were conducted with animals of this genotype were not informative enough, the results obtained in the framework of this study are of scientific and practical importance, which can be used when working in egg poultry farming.

CONCLUSIONS

From the results obtained in the framework of the conducted studies and based on the data obtained by researchers in previous papers on the investigation of the influence of technological factors of various etiologies on the biochemical parameters of blood in laying hens in the industrial egg production system, several conclusions can be drawn. In the conditions of industrial production of commercial eggs, the main factor in reducing the productivity of birds is the effect of technological factors that cause them stress. Therefore, the search for informative systems that can become early markers of this condition in the animal body will have scientific and practical importance. The strongest stress factor used in the study was the factor of over-compaction of animals, while others – such as the feed factor and the factor of changing the lighting mode had substantially less impact on the birds' body and were approximately at the same level. Among the biochemical parameters of blood serum, glucose and creatinine concentrations were a rapid and differentiated response to the action of technological factors of different intensities. The range of glucose fluctuations under stress was from 15.2 to 18.1 mmol/L and for creatinine-from 21.9 to 27.2 mmol/L. Given the simplicity of determining these indicators and fluctuations in their levels within the physiological norm, they may have prospects of becoming the main markers of early diagnosis of stress in industrial poultry farming. Among the enzyme complexes, such enzymes as LDH and AST, which are involved in carbohydrate metabolism, had similar qualities. A substantial range of changes in the activity of these enzymes depending on the intensity of the stress factor also allows them to be used as markers of the influence of technological factors on the body of birds. The following research aims to examine the effect of a stress factor of varying intensity on the level of creatinine concentration in the body of birds and develop a scale of the negative impact of the technological factor on the egg productivity of birds in industrial production based on this.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Abo Ghanima, M.M., Abd El-Hack, M.E., Taha, A.E., Tufarelli, V., Laudadio, V., & Naiel, M.A.E. (2020). Assessment of stocking rate and housing system on performance, carcass traits, blood indices, and meat quality of French Pekin Ducks. *Agriculture*, 10(7), article number 273. doi: 10.3390/agriculture10070273.

[2] Bryden, W.L., Li, X., Ruhnke, I., Zhang, D., & Shini, S. (2021). Nutrition, feeding and laying hen welfare. *Animal Production Science*, 61, 893-914. doi: 10.1071/AN20396.

[3] Bulent, E., & Niyazi, B. (2018). Importance of stress factors in poultry. *Juniper Online Journal of Case Studies*, 7(5), article number 555723. doi: 10.19080/JOJCS.2018.07.555723.

[4] Campbell, A.M, Johnson, A.M, Persia, M.E., & Jacobs, L. (2022). Effects of housing system on anxiety, chronic stress, fear, and immune function in Bovan Brown laying hens. *Animals*, 12(14), article number 1803. doi: 10.3390%2Fani12141803.

[5] Chu, B., Marwaha, K., Sanvictores, T., & Ayers, D. (2022). *Physiology, stress reaction.* Treasure Island: StatPearls Publishing.

[6] El-Sabrout, K., El-Deek, A., Ahmad, S., Usman, M., Dantas, M.R.T., & Souza-Junior, J.B.F. (2022). Lighting, density, and dietary strategies to improve poultry behavior, health, and production. *Journal of Animal Behaviour and Biometeorology*, 10(1), 2212. doi: 10.31893/jabb.22012.

[7] European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. (1986). Retrieved from https://rm.coe.int/168007a67b.

[8] Fisenko, I.A., Pustova, O.G., & Tkachenko, D.V. (2021). *Comfortable keeping of farm poultry*. Mykolaiv: MNAU.

[9] Ghoname, M., Elnaggar, A., Hassan, S., & Habashy, W. (2022). Effect of acute heat stress on production performance and egg quality in four strains of chickens. *South African Journal of Animal Science*, 52(2), 168-176. doi: 10.4314/sajas.v52i2.6.

[10] Guidelines for collecting biopsies in veterinary practice for the diagnosis of small domestic animals. (2021). Retrieved from http://surl.li/liqvg.

[11] Hedlund, L., & Jensen, P. (2022). Effects of stress during commercial hatching on growth, egg production and feather pecking in laying hens. *PLoS One*, 17(1), article number e0262307. doi: 10.1371/journal.pone.0262307.

[12] Herbert, G.T., Redfearn, W.D., Brass, E., Dalton, H.A., Gill, R., Brass, D., Smith, C., Rayner, A.C., & Asher, L. (2021). Extreme crowding in laying hens during a recurrent smothering outbreak. *Veterinary Record*, 188(12), article number e245. doi: 10.1002/vetr.245.

[13] Huang, S.-C., Fu, Y. F., Lan, Y.F., Rehman, M., & Tong, Z.X. (2018). Histopathological and biochemical evaluations of the kidney in broiler chickens under acute heat stress conditions. *Indian Journal of Animal Research*, 52(4), 637-639. doi: 10.18805/ijar.v0iOF.7652.
[14] Klein, R., Nagy, O., Tóthová, C., & Chovanová, F. (2020). Clinical and diagnostic significance of lactate dehydrogenase and its isoenzymes in animals. *Veterinary Medicine International*, 2020, article number 5346483. doi: 10.1155%2F2020%2F5346483.
[15] Lee, C., Kim, J.H., & Kil, D.Y. (2022). Comparison of stress biomarkers in laying hens raised under a long-term multiple stress condition. *Poultry Science*, 101(6), article number 101868. doi: 10.1016/j.psj.2022.101868.
[16] Madkour, M., Salman, F.M., El-Wardany, I., Abdel-Fattah, S.A., Alagawany, M., Hashem, N.M., Abdelnour, S.A., El-Kholy, M.S., & Dhama. K. (2022). Mitigating the detrimental effects of heat stress in poultry through thermal conditioning and nutritional manipulation. *Journal of Thermal Biology*, 103, article number 103169. doi: 10.1016/j.jtherbio.2021.103169.
[17] Molnár, S., & Szőllősi, L. (2020). Sustainability and quality aspects of different table egg production systems: A literature review. *Sustainability*, 12(19), article number 7884. doi: 10.3390/su12197884.
[18] Nasonov, I.V., Buyko, N.V., Lizun, R.P., Volykhina, V.E., Zakharik, N.V., & Yakubovsky, S.M. (2014). *Guidelines for hematological and biochemical studies in chickens of modern crosses*. Minsk: Institute of Experimental Veterinary Medicine named after S. N. Vyshelesky of the National Academy of Sciences of Belarus.
[19] Nwaigwe, C.U., Ihedioha, J.I., Shoyinka, S.V., & Nwaigwe, C.O. (2020). Evaluation of the hematological and clinical biochemical markers of stress in broiler chickens. *Veterinary World*, 13(10), 2294-2300. doi: 10.14202%2Fvetworld.2020.2294-2300.
[20] Olejnik, K., Popiela, E., & Opalinski, S. (2022). Emerging precision management methods in poultry sector. *Agriculture*, 12(5), article number 718. doi: 10.3390/agriculture12050718.
[21] Onbasilar, E.E., & Aksoy, F.T. (2005). Stress parameters and immune response of layers under different cage floor and density conditions. *Livestock Production Science*, 95(3), 255-263. doi: 10.1016/j.livprodsci.2005.01.006.
[22] Osadcha, Yu., Bazyvoliak, S., & Paskevych, G. (2022). Influence of the conditions of keeping laying hens on their productivity and efficiency of food egg production. *Modern Poultry*, 5-6, 8-13. doi: 10.31548/poultry2022.05-06.008.
[23] Osadcha, Yu.V., Sakhatsky, M.I., & Kulibaba, R.O. (2021). Serum clinical biochemical markers of Hy-Line W-36 laying hens under the influence of increased stocking densities in cages of multilevel batteries. *Regulatory Mechanisms in Biosystems*, 12(3), 425-429. doi: 10.15421/022158.
[24] Ouchi, Y, Chowdhury, V.S., Cockrem, J.F., & Bungo T. (2022). Thermal conditioning can improve thermoregulation of young chicks during exposure to low temperatures. *Frontiers in Animal Science*, 3, article number 919416. doi: 10.3389/fanim.2022.919416.
[25] Park, B.S., Um, K.H., Park, S.O., & Zammit, V.A. (2018). Effect of stocking density on behavioral traits, blood biochemical parameters and immune responses in meat ducks exposed to heat stress. *Archives Animal Breeding*, 61(4), 425-432. doi: 10.5194/aab-61-425-2018.
[26] Sáenz, J.A.C. (2021). *Heat stress in laying hens: Impact and prevention*. Retrieved from https://www.veterinariadigital.com/en/articulos/heat-stress-in-laying-hens-impact-and-prevention/.
[27] Sakhatsky, M.I., & Osadcha, Yu.V. (2021). Clinical-biochemical status of hens due to changes of battery cages height location. *Theoretical and Applied Veterinary Medicine*, 9(3), 130-134. doi: 10.32819/2021.93020.
[28] Tang, S., Zhou, S., Yin, B., Xu, J., Di, L., Zhang, J., & Bao, E. (2018). Heat stress-induced renal damage in poultry and the protective effects of HSP60 and HSP47. *Cell Stress Chaperones*, 25, 1033-1040. doi: 10.1007%2Fs12192-018-0912-3.
[29] Usturoi, A., Usturoi, M.G., Avarvarei, B.V., Pânzaru, C., Simeanu, C., Usturoi, M.I., Spătaru, M., Radu-Rusu, R.M., Doliş, M.G., & Simeanu, D. (2023). Research regarding correlation between the assured health state for laying hens and their productivity. *Agriculture*, 13(1), article number 86. doi: 10.3390/agriculture13010086.
[30] W-36 Commercial Layers. Management Guide. (2020). Retrieved from https://www.hyline.com/filesimages/Hy-Line-Products/Hy-Line-Product-PDFs/W-36/36%20COM%20ENG.pdf.
[31] Wang, Y., Xia, L., Guo, T., Heng, C., Jiang, L., Wang, D., Wang, J., Li, K., & Zhan, X. (2020). Research note: Metabolic changes and physiological responses of broilers in the final stage of growth exposed to different environmental temperatures. *Poultry Science*, 99(4), 2017-2025. doi: 10.1016/j.psj.2019.11.048.
[32] Yan, L., Hu, M., Gu, L., Lei, M., Chen, Z., Zhu, H., & Chen, R. (2022). Effect of heat stress on egg production, steroid hormone synthesis, and related gene expression in chicken preovulatory follicular granulosa cells. *Animals*, 12(11), article number 1467. doi: 10.3390/ani12111467.

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Анотація. Порушення основних технологічних параметрів утримання та годівлі птиці є основним чинником зниження яєчної продуктивності та здоров'я птиці в системі промислового виробництва. Тому метою дослідження є визначення біохімічних маркерів при гострому технологічному стресі, спричиненому чинниками різної етіології. У цьому дослідженні оцінювали біохімічні показники сироватки крові за дії подразників у курей-несучок. Дослідження проводили на декількох курчатах кросу Hy-Line W-36 в умовах виробничої лінії. Для проведення дослідження було сформовано чотири групи курей (по 101 голові в кожній) віком 52 тижні. Контрольна група була сформована з 10 представників кожної з груп. Далі кожна група піддавалася впливу відповідного технологічного подразника. Птицю групи 2 не годували протягом 24 годин, птицю групи 3 утримували в затемненому місці, а ще 60 голів додатково помістили в клітку до курей групи 4. Найбільший вплив на динаміку біохімічних показників мало збільшення їх концентрації на 1 $м^2$ кліткового простору. Дослідженнями встановлено суттєве підвищення концентрації глюкози та креатиніну в сироватці крові курчат при переущільненні – на 24-28 % ($p<0,05$) порівняно з контрольною групою, незалежно від причини виникнення стресової ситуації, у всієї птиці, що зазнала впливу гострого стресу, спостерігалося підвищення цих показників у сироватці крові. Основна частина досліджуваних ферментів, таких як аспартатамінотрансфераза, лужна фосфатаза та лактатдегідрогеназа, мали тенденцію до підвищення своєї активності залежно від сили впливу технологічного чинника на організм птиці. Динамічні характеристики рівнів глюкози та креатиніну вирізнялися серед досліджуваних біохімічних показників швидкими та диференційованими реакціями в межах фізіологічної норми, а з огляду на простоту їх визначення, можуть мати перспективу стати основними маркерами ранньої діагностики стресу в промисловому птахівництві

Ключові слова: рівень глюкози; креатинін; аспартатамінотрансфераза; лактатдегідрогеназа; стрес; маркер