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Effect of monochrome light with different wavelengths on biochemical parameters of hens

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Abstract Artificial light, as one of the environmental factors, plays a significant role in regulating the synthesis and secretion of hormones related to the coordination of parameters of life, growth, immunity, and reproductive functions of hens. The article aims to study the influence of monochrome light with different wavelengths on the biochemical parameters of hens` blood serum. Four groups of "Hy-Line W-36" crossbred hens were formed. Hens of the 1st group were kept using monochrome light with different wavelength lamps with a wavelength of ~ 460 nm, the 2nd group ~ 600 nm, the 3rd group ~ 630 nm, and the 4th group ~ 650 nm. It was found that the use of light with different wavelengths for keeping hens in cages of multilevel batteries affects hen' biochemical parameters, according to the research results. It was established that when using light with a wavelength of ~ 630 and ~ 650 nm, the indicators of clinical biochemistry of hens' blood serum were within the normal physiological values. Whereas, with the use of light with a wavelength of ~ 600 nm, an increase in the level of glucose, creatinine, total protein, total bilirubin, and phosphorus, a decrease in the ratio of calcium to phosphorus, in the activity of alkaline phosphatase, aspartate aminotransferase, and lactate dehydrogenase, were observed in the hens' blood serum. The use of light with a wavelength of ~ 460 nm was accompanied by a further increase in the level of glucose, creatinine, total protein, urea, total bilirubin, phosphorus, the activity of alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, and gamma-glutamyltransferase, a decrease in the ratio of calcium and phosphorus.

Keywords: biochemistry, blood serum, enzymes, laying hens, light wavelength

1. Introduction

The egg industry is one of the most important poultry industries, attracting substantial investment worldwide. Consequently, there is an urgent need to increase productivity and improve poultry welfare (El-Sabrout et al 2022). Lighting is an important practical strategy that can potentially be used in layer farms to improve hens' behavior and egg production. Light is an important exogenous factor that regulates physiological and behavioral processes and involves the circadian rhythms of hormones and immune cells in hens (Hofmann et al 2020). Since hens are always kept indoors, their body is usually exposed to artificial rather than natural light. The latest generation of artificial light sources in hens farming includes light-emitting diode (LED) lamps. LEDs are characterized by a long service of life, a specific spectrum (in a comparative aspect), lower thermal power, high energy efficiency, and reliability compared to incandescent lamps and fluorescent lamps (Pattison et al 2018). LED lamps require lower maintenance costs (Sultanana 2013; Yang et al 2016), leading to broader use in hens' farms (Shi et al 2019).

LEDs are a special type of semiconductor diodes that can emit monochromatic light. Light wavelength is one of the primary light components that influence lighting quality (El-Sabrout et al 2022). Color is determined by the wavelength of the visible spectrum while monochrome light is characterized by only one peak wavelength (Yenilmez et al 2021). Unlike mammals with three monochrome photoreceptors, the hens have four types and distinguish wavelengths from 350 to 700 nm, meaning they also perceive the light in the infrared (longer wavelengths) and ultraviolet (shorter wavelengths) spectra (Barros et al 2020). Correctly selected light color positively affects the hen's welfare and reduces stress (Sayin et al 2022). Thus, a lower concentration of corticosterone in the blood of hens is observed when red light is used during their confinement compared to white light (Shi et al 2019; Archer 2019) or when exposed to ultraviolet radiation (Sobotik et al 2020; House et al 2020). Therefore, the light's color determined by the light wavelength's length can be an additional tool for managing the light regime in hen farming to prevent and reduce stress in hens, improve immune responses and increase productivity (Wei et al 2022; El-Sabrout et al 2022).

The spectral composition of light influences the behavior, physiological state, and parameters of hens' productivity (Fernandes et al 2021; Yenilmez et al 2021; Wu et al 2022). However, the analysis of studies conducted by various authors shows that the data on the effect of monochromatic light on the hens' body are rather controversial. Thus, the research of some authors (Li et al 2015; Li et al 2019) showed that the use of blue light, compared to white, green and red, stimulates laying hens. Blue light stimulates follicle-stimulating hormone, while red

light stimulates luteinizing hormone (Mudhar and Tabeekh 2016). According to the data of other researchers, the use of the red spectrum of light contributes to the increase in laying hens (Zhang et al 2017) and the increase in the thickness of the eggshell (Kim et al 2010), and the use of blue and green light - to increase the mass of eggs (Hassan et al 2013). In addition, changes in hematological parameters (a decrease in the ratio of heterophils to lymphocytes) were noted when using red light compared to white light (Archer 2019). There are also reports that using red light causes a significant reduction in egg mass, while using green light positively correlates with egg quality indicators (Eret al 2007). According to this, a number of researchers have shown that monochrome blue, yellow, green, white, and red light do not affect the laying hens and the quality of their eggs (Borille et al 2015). In general, the influence of light wavelength on poultry's welfare, behavior, and productivity is described in detail in several studies (Senaratna et al 2015; Khaliq et al 2017; Çapar and Onbaşılar 2018; Barros et al 2020; Soliman and El-Sabrout et al 2020).

Based on the above, the question remains open about the effect of monochrome light on the hens' body, the markers of which are the clinical and biochemical parameters of the blood serum of hens, as an indicator system of the general physiological state of the body, which is determined by the main goal of our work.

2. Material and Methods

2.1. Experimental design

Four groups of laying hens of an industrial flock at the age of 18 weeks with a live weight of 1260–1300 g was formed in the conditions of a modern complex to produce edible eggs, each of which was kept in a separate analogue poultry house. Each aviary with an area of 2915 m^2 was equipped with 12-tiered cage batteries "Big Dutchman" (Germany), consisting of 4704 cages with an area of 40544 cm^2 (362×112 cm). The provision of hens with cage area was 401.4 cm^2/bird with a feeding front of 7.2 cm/bird. Thus, 475,104 hens were planted in each aviary. The differences between the birdhouses concerned only the LED lights. For keeping hens of the 1st group, LED lamps (TH LEDLIFE, LLC) with a peak wavelength of ~ 460 nm (blue), 2nd group ~ 600 nm (yellow), 3rd group ~ 630 nm (orange) and 4th group ~ 650 nm (red) were used. The intensity of illumination of the poultry house was maintained at 30 lux with the duration of the light day of 12 hours from the 18-week age of the hens, which was gradually increased to 16 hours by 30 weeks. This mode of illumination of the poultry house - illumination intensity of 30 lux with a light day duration of 16 hours, was maintained until the end of laying hens.

2.2. Poultry keeping

The hens were provided with drinking water, full-ration compound feed of the same composition and kept following the requirements (VNTP-АПК-04.05) during the experiment (Table 1).

The poultry house's lighting regime during the rearing of young hens and after transfer to an adult flock corresponded to the recommendations of the developer of the cross (Guide to the content of the final hybrid Hy-Line W-36 2019).

2.3. Data Collection

The value of the peak wavelength of each of the monochrome LED lamps was determined using a spectrometer («MK 350 UPRtek», UPRtek).

Samples of whole blood (1.0-1.5 ml from the pterygoid vein) were taking 30 times from laying hens of each group at the age of 18 weeks (at the beginning of the research) and 52 weeks. Reference values were determined by Nasonov et al (2014).

2.4. Biochemical data

Biochemical parameters and enzyme activity of blood serum of hens, the content of total protein, albumin, glucose, creatinine, urea, bilirubin, cholesterol, phosphorus, calcium, the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (AP) and lactate dehydrogenase (LDH) were determined on a BioChem FC-360 biochemical analyzer (Hightechnology Inc., USA) in the Bald laboratory (certificate No. LB/02/2016). Commercial diagnostic kits (High Technology Inc., USA) - HT-G242 (method: oxidase, endpoint; wavelength - 500 nm), total protein - HT-T251 (method: biuret, endpoint; wavelength - 540 nm), albumin - HT-A203 (method: bromocresol green, endpoint; wavelength - 630 nm), creatinine - HT-C225 (method: Jaffe, kinetics; wavelength - 510 nm), urea - HT-U254 (method: Trinder / uricase, end point; wavelength - 520 nm), cholesterol - HT-C218 (method: enzymatic, end point; wavelength - 500 nm), phosphorus - HT-P244 (method: ammonium molybdate, end point; wavelength - 340 nm), calcium - HT-C216 (method: OKF, end point; wavelength - 570 nm); concentration of alanineaminotransferase - HT-A206 (method: IFCC, kinetics; wavelength – 340 nm), aspartateaminotransferase - HT-A109 (method: IFCC, kinetics; wavelength - 340 nm), alkaline phosphatase - HT-A205 (method: kinetics; wavelength - 405 nm), lactate dehydrogenase - HT-L236 (method: modified Wacker/Tris method, kinetics; wavelength - 340 nm), gamma-glutamyltransferase - HTI-G7571-120 (method: modified Szasz method, kinetics; wavelength - 405 nm) (Kaplan 1984; Murry 1984). The concentration of biochemical components was calculated according to the manufacturing instructions.

2.5. Statistical analysis

The methods of variational statistics processed the obtained digital results. The significance of differences between groups was assessed using a one-way analysis of variance (ANOVA) and the Tukey-Kramer multiple comparisons test as a post-hoc testing tool. Checking the distribution of sample data for normality was performed

using the Kolmogorov-Smirnov test. If the data distribution was likely to differ from normal, the non-parametric Mann-Whitney U-test was used. Differences between groups were considered significant at $P < 0.05$.

Table 1 The composition of feed for laying hens in the productive period.

Component	The intensity 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	3.000	0
Soybean oil	0.959	0.661	0	0.500
Shell 0–3 mm	10.701	9.922	10.25	11.088
Salt	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
Loxidan TD 100	0	0.010	0	0
Millersheim	0.013	0.015	0.011	0
Globamax 1000	0.100	0	0	0
ProActive	0	0	0.150	0.150
Enteronormin Detox	0.150	0.150	0	0
Mastersorb	0.150	0.130	0.130	0
Mycocide Pro	0	0	0	0.090
Choline chloride	0.050	0.050	0.040	0.035
Cronozyme	0	0	0	0.011
Yellow carnation	0.003	0.003	0.003	0.003
Red carnation	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
Together	100.000	100.000	100.000	100.000

3. Results

Biochemical parameters and enzyme activity of hens' blood serum of all experimental groups at the beginning of the research at the age of 18 weeks were within physiological norms for each parameters. No significant differences were found between the groups. It was found that the differences in the wavelength values of keeping hens in cages of multilevel batteries did not affect the content of total protein, albumin, urea, cholesterol, and calcium in their blood serum according to the results of the research at the 52nd week of life (Table 2). However, within the physiological norm, an increase in the total protein content in blood serum was observed depending on the wavelength of light. The content of total protein in hens blood serum of the 1st group was higher by 4.3% compared to the 2nd group and by 6.8% and 6.0% compared to the 3rd and 4th groups. The content of total protein in hens blood serum of the 2nd group was higher by 2.4% and 1.6% compared to the 3rd and 4th groups. No differences in this parameter were found in the 3rd and 4th groups of hens.

In addition, the urea content in the hen's blood serum of the 1st group was higher by 17.4% compared to the 2nd group and by 25.6% and 24.1% compared to the 3rd and 4th groups. Whereas the content of urea in hens blood serum of 2-4 groups was at the same level.

Table 2 Biochemical parameters of hens` blood serum (x ± SE, n = 30/group).

Parameters	Group				Reference intervals	Signi-ficance
	1	2	3	4		
Total protein, g/l	58,1±1,04[a]	55,7±0,12[b]	54,4±0,46[c]	54,8±0,16[cd]	37,8-59,0	P<0.05
Albumin, g/l	18,0±0,13[a]	19,2±0,24[b]	18,8±0,21[b]	18,7±0,21[b]	15,0-25,0	P<0.05
Glucose, mmol/l	19,2±0,19[a]	17,2±0,39[b]	15,9±0,24[c]	15,1±0,09[d]	10,0-16,5	P<0.05
Creatinin, µmol/l	29,2±0,24[a]	27,0±0,45[b]	24,6±0,35[c]	22,5±0,32[d]	22,0-27,0	P<0.05
Urea, mmol/l	1,08±0,015[a]	0,92±0,055[b]	0,86±0,010[b]	0,87±0,01 [b]	0,7-2,4	P<0.05
Bilirubin, µmol/l						
– general	2,16±0,075[a]	1,69±0,022[b]	1,58±0,015[c]	1,44±0,043[d]	1,7	P<0.05
– straight	0,26±0,002[a]	0,25±0,021[a]	0,18±0,023[b]	0,12±0,032[b]	0,5	P<0.05
– indirect	1,90±0,007[a]	1,44±0,008[b]	1,40±0,013[c]	1,32±0,055[cd]	*	P<0.05
Cholesterol, mmol/l	3,5±0,02[a]	3,4±0,12[a]	3,1±0,12[b]	3,3±0,17[ab]	2,0-4,0	P<0.05
Phosphorus, mmol/l	2,54±0,044[a]	2,25±0,027[b]	1,42±0,058[c]	1,43±0,062[cd]	1,15-2,2	P<0.05
Calcium, mmol/l	4,22±0,008[a]	4,34±0,028[b]	4,28±0,034[c]	4,50±0,025[d]	2,8-4,6	P<0.05
Calcium / phosphorus	1,7±0,03[a]	1,9±0,02[b]	3,1±0,10[c]	3,3±0,16 [cd]	3-3,8:1	P<0.05

Note: [a, b, c, d] - indicate values that significant differed in one row of the table (P < 0.05);
* - reference values are not available for laying hens.

Also, a lower albumin content was observed within the physiological norm in the 1st group of hens blood serum, namely by 6.7% compared to the 2nd group and by 4.4% and 3.9% compared to the 3rd and 4th groups. The albumin content in hens' blood serum was at the same level in 2-4 groups.

The glucose concentration in the hens' blood serum of the 1st and 2nd groups exceeded the physiological norm by 16.4 and 4.2%. The glucose content was higher by 11.6% in the 1st group of hens and by 20.8% in the 2nd group, and 27.2% in the 3rd and 4th groups. The glucose level was higher by 8.2% in the 2nd group and by 13.9% in the 3rd and 4th groups. The glucose concentration was higher by 5.3% in the 3rd group of hens compared to the 4th group. The obtained data are consistent with the results of other studies (Mert and Yildirim 2016; Gupta et al 2017; Kraus et al 2021), which described hyperglycemia as a reaction of the hen's body to chronic (Gupta et al 2017; Kraus et al 2021) and acute stress (Mert and Yildirim 2016).

An increase in the level of creatinine was observed in the 1st group of hens with an excess of the physiological norm by 8.1%, as well as by 8.1% of the parameters of the 2nd group and by 18.7% and 29.8% - of the 3rd and 4th groups. The concentration of creatinine in the 2nd group of hens reached the upper limit of the physiological norm and at the same time was higher by 9.8% and 20.0% compared to the 3rd and 4th groups. The creatinine content in the 3rd group of hens was higher by 9.3% compared to the 4th group.

There was also a tendency to increase the total bilirubin concentration in the hens` blood serum depending on the wavelength of light. The content of total bilirubin in the 1st group of hens exceeded the physiological norm by 27.1% and was higher by 27.8% compared to the 2nd group and by 36.7% and 50.0% compared to the 3rd and the 4th groups. The bilirubin content in the 2-4 groups of hens fluctuated within the physiological norm: in the 2nd group, the content of total bilirubin in hens` blood serum was higher by 7.0% and 17.4% compared to the 3rd and 4th groups. The content of total bilirubin in the 3rd group was higher by 9.7% compared to the 4th group. Direct bilirubin of all groups was within the physiological norm, with some increase depending on the wavelength of light. In particular, hens of the 1st group are characterized by a higher concentration of this indicator by 44.4% and 116.7%, and hens of the 2nd group by 38.9% and 108.3% compared to the 3rd and 4th groups. No similar tendency was found for hens of the 3rd and 4th groups. Thus, the increase in total bilirubin by the group occurred due to indirect bilirubin. In particular, the content of indirect bilirubin was higher in the 1st group of hens by 31.9% compared to the 2nd group and by 35.7% and 43.9% compared to the 3rd and 4th groups. The content of indirect bilirubin was higher in the 2nd group of hens by 2.9% and 9.1% compared to the 3rd and 4th groups. The value of this indicator was almost at the same level in the 3rd and 4th groups of hens.

Notably, the phosphorus content in the hens` blood serum of the 1st and 2nd groups exceeded the physiological norm by 15.5% and 2.3%. The phosphorus content in hens` blood serum in the 1st group was higher by 12.9% compared to the 2nd group and by 78.9% and 77.6% compared to the 3rd and 4th groups. Phosphorus content in the 2nd group was higher by 58.5% and 57.3% compared to the 3rd and 4th groups. No differences in the parameter of phosphorus concentration in hens` blood serum in the 3rd and 4th groups were found.

The calcium content in the hens` blood serum of all experimental groups was within the physiological norm, but its decrease was observed depending on the wavelength of light. A decrease in calcium content was established in the 1st group of hens by 2.8% compared to the 2nd group and by 6.2% compared to the 4th group. The calcium content was lower by 3.6% in the 2nd group of hens and in the 3rd group by 4.9% compared to the 4th group.

The ratio of calcium and phosphorus in the hens' blood serum of birds of the 3rd and 4th groups was within the physiological norm, and in the 1st and 2nd - it did not reach the normative level. A low ratio of calcium and phosphorus and the greatest deviation from the physiological norm (by 43.3%) was found in the hens of the 1st group, which is 10.5% lower compared to the 2nd group and by 45.2% and 48.5% compared to the 3rd and 4th groups. The ratio of calcium and phosphorus (36.7%) did not reach the physiological norm in the 2nd group of hens and was lower by 38.7% and 42.4% compared to the 3rd and 4th groups. Differences were not found in the ratio between the 3rd and 4th groups.

Violation of the metabolism of macroelements especially important for laying hens - calcium and phosphorus - is also confirmed by the change in the activity of alkaline phosphatase in their blood serum (Table 3). Exceeding the physiological norm of alkaline phosphatase activity was detected in the 1st and 2nd groups of hens. The alkaline phosphatase activity exceeded the physiological norm by 20.7% in the 1st group and was higher by 19.7% compared to the 2nd group and by 33.4% and 42.2% compared to the 3rd and 4th groups. The alkaline phosphatase activity exceeded the norm by 0.9% in the 2nd group and was higher by 11.5% and 18.8% compared to the 3rd and 4th groups. The differences between the 3rd and 4th groups were 6.6% and were not statistically confirmed. The obtained data confirm the results of other researchers (Onbaşılar Ebru et al 2016), which described a stress-induced increase in the alkaline phosphatase level in the hens` blood serum.

According to the results of the research, it was found the activity of aspartate aminotransferase (AST) also increased in the hens' blood serum that were kept under shorter wavelengths of light. Exceeding the upper limit of the physiological norm (25.6% and 3.0%) was observed in the 1st and 2nd groups of hens. In particular, in the 1st group of hens AST activity increased by 21.9% compared to the 2nd group and by 26.7% and 27.7% compared to the 3rd and 4th groups. At the same time, AST activity in the 2nd group of hens was higher by 3.9% and 4.7% compared to the 3rd and 4th groups. There were practically no differences between the 3rd and 4th groups; the difference in parameter values was 1.6 units and was within the statistical error. Many researchers obtained similar results (Park et al 2018; Abo Ghanima et al 2020; Kraus et al 2021), who described the increase in AST activity as a reaction of the hens` and ducks body to the effect of technological stressors. Obviously, the increase in AST activity under the influence of light wavelength on the hens' body is caused by their constant state of neuromuscular tension. A constant stress load leads to an increase in the activity of AST, according to (Everds et al 2013), and at the same time, an increase in the concentration of glucose in the hens` blood serum, which is confirmed by the data of the study.

Scientists explain the increased activity of lactate dehydrogenase (LDH) by muscle destruction due to neuromuscular tension (Sandercock et al 2006). An increase in LDH activity was observed in the hens` blood serum kept under shorter wavelengths of light. Exceeding the physiological norm was found in the 1st and 2nd groups - by 43.5% and 6.9%. At the same time, LDH activity in hens` blood serum of the 1st group was higher by 34.2% compared to the 2nd group and by 46.9% and 47.6% compared to the 3rd and 4th groups. LDH activity was higher by 9.4% in the hens` blood serum of the 2nd group and 10.0% compared to the 3rd and 4th groups. Differences between the 3rd and 4th groups were within statistical error.

Gamma-glutamyltransferase activity (GGT) increased in the hens` blood serum depending on the light wavelength parameters. The maximum activity of GGT was found in the 1st group with an excess of 36.4% of the activity of the 2nd group and by 47.3% and 48.6% – of the 3rd and 4th groups. At the same time, GGT activity in the 2-4 groups of hens was almost identical, without statistical confirmation of differences.

The preservation of hens' livestock in all groups, regardless of the light wavelength parameters, was lower than the level (97.4%) recommended by the company by the cross developer of (Guide to the content of the final hybrid Hy-Line W-36, 2019), which may be related to the peculiarities of keeping large numbers of birds in multi-tiered cage batteries of new structures (Sakhatsky et al 2020; Osadcha et al 2021). As a result, a decrease in the hens' preservation was observed with a decrease in the length of the light wave (Table 4). The largest difference - 12.2%, with the recommended level of preservation was noted in hens of 1st group, laying hens of the 2nd group did not reach the standard by 9.0%, while in the laying hens of the 3rd and 4th groups the preservation was almost at the same level and did not reach the norm by 2.6–2.3%. At the same time, the survival rate of hens in the 1st group was lower by 3.2% compared to the 2nd group and by 9.6% and 9.9% compared to the 3rd and 4th groups, respectively. In hens of 2nd group, survival was lower by 6.4% and 6.7% compared to the 3rd and 4th groups, and in the hens of the 3rd group - by 0.3% compared to the hens of the 4th group.

A decrease live weight of with a decrease the wavelength was also observed. In particular, the hens' live weight of groups 2–4 corresponded to the standard (Guide to the content of the final hybrid Hy-Line W-36, 2019) and decreased within its limits (1.54–1.58 kg), and in the 1st group - did not reach the norm by 5.1%. At the same time, hens of the 1st group were characterized by a lower live weight by 5.2% compared to the 2nd group and by 5.7% and 6.5% compared to the 3rd and 4th groups, respectively. In turn, the hens of 2nd group had a lower live weight by 0.5% and 1.3%

compared to the 3rd and 4th groups, and hens of 3rd group - by 0.8% compared to the 4th group.

Laying per initial laying also decreased with decreasing light wavelength. According to the regulatory requirements, the laying capacity for the initial layer at the age of 52 weeks should vary between 204.1-209.6 eggs, and for the average one - 206.9-212.5 eggs (Guide to the content of the final hybrid Hy-Line W-36, 2019). In fact, for the initial laying hen, the laying capacity of none of the groups reached the normative level. The lowest egg-laying rate and, accordingly, the largest deviation from the norm - 16.8%, was observed in hens of the 1st group. At the same time, their laying capacity per initial laying hen was lower by 6.4% compared to the 2nd group, and by 13.1% and 15.8% compared to the 3rd and 4th groups, respectively. In hens of the 2nd group, the laying rate per initial laying hen was lower than the norm by 11.0% and by 7.1% and 10.0% compared to the 3rd and 4th groups, respectively. Hens of the 3rd group did not reach the norm by 4.2% and were inferior to the hens of the 4th group by 3.1%. In turn, hens of the 4th group did not reach the norm by only 1.2%.

Table 3 Enzyme activity of hens` blood serum (units/l, x ± SE, n = 30/group).

Parameters	Group				Reference interval
	1	2	3	4	
Alanineaminotransferase	1,0	0,8	0,8	0,8	13,0-26,5
	±0,02	±0,03	±0,08	±0,07	
Aspartateaminotransferase	263,8	216,4	208,2	206,6	125-210
	±2,01^{a}	±2,18^{b}	±1,07^{c}	±1,81^{cd}	
Gamma-glutamyltransferase	33,0	24,2	22,4	22,2	*
	±0,52^{a}	±0,74^{b}	±1,03^{cb}	±1,10^{bcd}	
Alkaline phosphatase	1002,2	837,6	751,2	704,8	350-830
	±16,10^{a}	±14,98^{b}	±20,42^{c}	±21,85^{cd}	
Lactate dehydrogenase	2812,4	2095,6	1914,8	1904,8	636-1960
	±6,17^{a}	±11,07^{b}	±9,21^{c}	±2,56^{cd}	

Note: $^{a, b, c, d}$ - indicate values that significant differed in one row of the table (P < 0.05);
* - reference values are not available for laying hens.

Table 4 Preservation, live weight and productivity of hens (x ± SE, n = 475104/group).

Parameters	Group			
	1	2	3	4
Livestock preservation, %	85,2±0,10 ^{a}	88,4±0,09^{b}	94,8±0,06^{c}	95,1±0,06^{d}
Live weight, g	1462±0,28 ^{a}	1543±0,12^{b}	1551±0,26^{c}	1563±0,11^{d}
Egg laying, eggs				
– to the initial laying hen	169,9±0,14^{a}	181,6±0,17^{b}	195,5±0,11^{c}	201,7±0,19^{d}
– on an average laying hen	199,4±0,16^{a}	205,4±0,10^{b}	206,2±0,07^{c}	212,1±0,11^{d}
Egg mass, g	63,2±0,05^{a}	63,0±0,06^{b}	63,1±0,07^{ab}	63,3±0,04^{ac}

Note: $^{a, b, c, d}$ - indicate values that significant differed in one row of the table (P < 0.05)

Only hens of the 4th group reached the standard level of laying per average laying hen. The lowest egg-laying rate per average layer and, accordingly, the largest deviation from the standard - 3.6%, was observed in hens of the 1st group. At the same time, their average egg laying capacity was lower by 2.9% compared to the 2nd group and by 3.3% and 6.0% compared to the 3rd and 4th groups, respectively. In hens of the 2nd group, the laying rate was lower than the norm by 0.7%, as well as by 0.4% and 3.2% compared to the 3rd and 4th groups. Hens of the 3rd group did not reach the standard by only 0.1% and were inferior to laying hens of the 4th group by 2.8%.

The data obtained in the work on the light wavelength effect on the hens' egg-laying are consistent with the results of other researchers (Hassan et al 2013; Svobodova et al 2015; Zhang et al 2017), which describe an increase in the hens' egg-laying under the influence of red light. This effect is caused by the sensitivity of extraretinal photoreceptors of the hypothalamus to long-wave radiation, and not by the perception of light color through the retina (Renema et al 2001; Mobarkey et al 2010).

The eggs weight of laying hens "Hy-Line W-36" cross at the age of 52 weeks should be 62.9 g (Guide to the content of the hybrid Hy-Line W-36, 2019). As can be seen from the experimental data, the mass of eggs of laying hens of all groups corresponded to the norm. Due to this, the hens of the 2nd group had a lower mass of eggs by 0.3% and 0.5% compared to the 1st and 4th groups, respectively, and the hens of the 3rd group - by 0.3% compared to the 4th group However, the difference in egg mass between groups was insignificant and did not reflect a decrease in light wavelength.

4. Discussion

The use of light with a wavelength of ~ 460 and 600 nm was accompanied by an increase in the level of several biochemical parameters. What are the main mechanisms that could be responsible for this effect? In birds, the retina includes photoreceptors responsible for vision, as well as extraretinal photoreceptors responsible for detecting photoperiods and synchronizing the body's physiology with the environment through an endocrine response (Johnston 2005). Photoreceptors are located in the retina, pineal gland and hypothalamus. The retina contains three types of photoreceptors, including rods, cones, and double cones (Perry 2004). Cones are responsible for color vision and are tetrachromic in birds, that is, they contain four different photoreceptor cones (Prescott and Wathes 1999). In addition to recognizing the color of light, hens are able to perceive the ultraviolet part of the spectrum due to the presence of an extraretinal cone in the eye, which allows the transmission of radiation at a wavelength of less than 400 nm (Bowmaker et al 1997). The photoreceptors of the pineal gland receive ultraviolet light perceived by the retina and transmit it to oscillators that control the circadian rhythm of hens through the synthesis and release of melatonin (Kumar et al 1999).

The length of the light wave affects the secretion of melatonin (Ma et al 2018). In particular, many researchers have described the beneficial effect of green light on melatonin secretion (Li et al 2015). The pineal gland contains a special light-sensitive pigment that is sensitive to short waves of light (Faluhelyi and Csernus 2007). This feature, namely the presence of photopigment, can be one of the factors explaining the sensitivity of the hens' body to shorter wavelengths of light. However, short wavelengths must be accompanied by a higher intensity of light to affect the hypothalamus, while long waves directly penetrate the brain, even at low intensity, and reach the hypothalamus (Baxter et al 2014).

Another possible mechanism of the effect of light on the immune system of chickens is the action of stress hormones. In birds and mammals, the introduction of melatonin is associated with a decrease in the secretion of corticosterone (Gehad et al 2008; Singh et al 2010), a decrease in the regulation of glucocorticoid receptors (Sainz et al 1999) and a weakened negative effect of glucocorticoids on the immune system (Singh et al 2010). Thus, the increased concentration of the stress hormone due to a certain color of light can impair immune functions (Hofmann et al 2021).

Correctly selected light color has a positive effect on the well-being of animals and reduces their stress load (Sayin et al 2022). Thus, in hens, a lower concentration of corticosterone in the blood is observed when red light is used during their detention compared to white light (Shi H., 2019; Archer G.S., 2019) or under the influence of ultraviolet radiation (Sobotik et al 2020; House et al 2020). According to Xie D. et al. (Xie et al 2008), the level of stress in chickens decreases under the influence of blue light. Similarly, chickens show a lower fear response, that is, a tonic immobility response, under ultraviolet light (Sobotik et al 2020; House et al 2020) or under green and blue light compared to red light (Baxter et al 2014; Sultana et al 2013) or white light (Shi et al 2019). Therefore, the color of light determined by the length of the light wave can be an additional tool for managing the light regime in poultry farming for the prevention and reduction of stress in hens, as well as improving their physiological condition (Wei et al 2022).

As for the influence of the light regime on hens' productivity, the endocrine system is also directly involved here. Melatonin stimulates an increase in the concentration of growth hormone in the hens' blood plasma and is responsible for the activation of the inhibitory pathway of the reproductive axis (Ubuka et al 2005). One of the places of light perception in birds is the suprachiasmatic nucleus of the hypothalamus. Light of certain wavelengths can penetrate the feathers, bones and tissues of the skull and reach the hypothalamus, and this, together with light received from the retina via signals from the nervous system, is used to regulate the sexual activity of the bird by controlling the secretion of gonadotropin-releasing hormone and gonadotropin-inhibiting hormone (Wang et al 2022).

It is known that red light has an increased ability to stimulate the photosexual response in hens compared to white, blue or green light (Huber-Eicher et al 2013; Baxter et al 2019). This is due to the fact that light waves with a length of 400–500 nm are more absorbed by cranial tissues, which leads to a decrease in their stimulating effect on the hypothalamus (Foster and Follett 1985). Whereas light with a longer wavelength (red light) is better able to penetrate through the skull and brain tissue to stimulate the hypothalamus (Mobarkey et al 2010). However, some studies have shown that the inability of light with shorter wavelengths to stimulate the photosexual response is most likely caused by the suboptimal intensity of the light, rather than its specific color, i.e., wavelength (Benoit 1964). Therefore, shorter wavelengths (blue and green light) require greater intensity for the possibility of stimulating hypothalamic photoreceptors (Pang et al 1974).

The hen's reproductive system is regulated by two antagonistic neuropeptides, including stimulatory (gonadotropin-releasing hormone) and inhibitory (gonadotropin-inhibitory hormone) neuropeptides. In particular, gonadotropin-inhibitory hormone acts on the

anterior lobe of the hypothalamus, suppressing the development and maintenance of gonads by reducing the release and synthesis of gonadotropin (Tsutsui et al 2010; Wyk et al 2021), while gonadotropin-releasing hormone stimulates the release of gonadotropins (follicle-stimulating and luteinizing hormones) from the anterior lobe of the pituitary gland (Ciccone et al 2004; Pineda et al 2010). This triggers the development of gonads and the synthesis of steroid hormones, namely progesterone from granulosa cells of large follicles and estradiol from small follicles (Dunn et al 2009; Tsutsui et al 2010).

Estradiol stimulates the development of the reproductive system and participates in ovulation (Rangel et al 2014). In addition, it stimulates the hepatic synthesis of the main components of the yolk and increases the activity of calcitriol, which increases the level of calcium in the blood, making it available for the synthesis of the eggshell (Etches 1996). Progesterone is involved in the regulation of ovulation (Ottinger and Bakst 1995; Rangel et al 2014).

5. Conclusions

The wavelength of light affects preservation, productivity, the biochemicalprofile, and enzyme activity of hens` blood serum when keeping them in cages of multilevel batteries. The biochemical parameters and enzyme activity of hens` blood serum was within the physiological norm when light waves with a length of ~ 650 and ~ 630 nm were used to keep them. However, the use of a light wave of ~ 630 nm was accompanied by a decrease in the hens' preservation by 0.3% (0.3% < normal), their live weight by 0.8%, the initial laying rate by 3.1% (4, 2% < normal) and for the average laying hen – by 2.8% (0.1% < normal). The use of light waves with a length of ~ 600 nm was accompanied by an increase in the level of glucose in the hens` blood serum by 8.2-13.9% (4.2% > normal), creatinine by 9.8-20.0%, total protein by 1.6-2.4%, total bilirubin - 7.0-17.4%, phosphorus - by 57.3-58.5% (2.3% > normal), a decrease in the ratio of calcium and phosphorus by 38.7-42.4% (36.7% < normal), which was confirmed by an increase in the activity of alkaline phosphatase by 11.5-18.8% (0.9% > normal), aspartate aminotransferase by 3.9-4.7% (3 .0% > normal) and lactate dehydrogenase - by 9.4-10.0% (6.9% > normal), as well as a decrease in hens' preservation by 6.4–6.7% (9.0% < normal), body weight – by 0.5–1.3%, initial laying rate – by 7.1–10.0 % (11.0% < normal) and the average laying - by 0.4–3.2% (0.7% < normal). The use of light with a wavelength of ~ 460 nm was accompanied by an increasing increase in the hens` blood serum in the level of glucose by 11.6-27.2% (16.4% > normal), creatinine by 8.1-29.8% (8.1% > normal), total protein - by 4.3-6.8%, urea - by 17.4-25.6%, total bilirubin - 27.8-50.0% (27.1% > normal), phosphorus - by 12.9–78.9% (15.5% > normal), a decrease in the ratio of calcium and phosphorus by 10.5-48.5% (43.3% < normal), which was confirmed by an increase in the activity of alkaline phosphatase by 19, 7-42.2% (20.7% > normal), aspartate aminotransferase by 21.9-27.7% (25.6% > normal), lactate dehydrogenase - by 34.2–47.6% (43.5 % > normal) and gamma-glutamyltransferase - by 36.4–48.6%, as well as a decrease in hens' preservation by 3.2–9.9% (12.2% < normal), body weight – by 5.2–6.5% (5.1% < normal), laying capacity at the initial by 6.4–15.8% (16.8% < normal) and for the average layer – by 2.9–6.0% (3.6% < normal).

Ethical considerations

Laying hens of the industrial cross breed "Hy-Line W-36" were used as the object of research. Experiments with experimental animals were performed following the rules of the European Convention for the Protection of Vertebrate Animals (Official Journal of the European Union L276/33, 2010) and the Order of the Ministry of Economy of Ukraine «On approval of the requirements for the welfare of farm animals during their housing» of February 18, 2021, were organized. The local Commission approved the experimental protocol on Bioethics of the National University of Life and Environmental Sciences of Ukraine

Conflict of interest

The authors declare that they have no conflict of interest.

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