UKRAINIAN JOURNAL OF VETERINARY SCIENCES

2024. Volume 15, No. 4. P. 112-127

Received: 15.07.2024 Revised: 31.10.2024 Accepted: 27.11.2024

DOI: 10.31548/veterinary4.2024.112

UDC 619:619.661

Vegetative regulation of glucose, calcium, phosphorus, and haemoglobin levels in the blood of laying hens

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Abstract. The state of mineral and energy metabolism significantly influences the growth rate and productivity of laying hens. Understanding their specific effects on physiological processes,

Suggested Citation:
Shuranova, L., Zhurenko, O., Kryvoruchko, D., Zhurenko, V., & Kulbako, O. (2024). Vegetative regulation of glucose, calcium, phosphorus, and haemoglobin levels in the blood of laying hens. *Ukrainian Journal of Veterinary Sciences*, 15(4), 112-127. doi: 10.31548/veterinary4.2024.112.

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in conjunction with the regulatory impact of varying autonomic nervous system tones, is crucial for addressing numerous issues related to improving poultry egg production. This study aimed to determine the effects of autonomic nervous system tone on the intermediary metabolism of minerals, carbohydrates, and proteins in laying hens. The research involved Hisex Brown hens. Experimental groups were formed based on the results of electrocardiography and a variational pulsometric study, followed by statistical analysis of the initial data. This approach facilitated the classification of the laying hens into three experimental groups: normotonic, sympathotonic, and vagotonic. Analysis of specific biochemical parameters in the blood serum of the laying hens revealed a significant increase ($P < 0.001$) in glucose levels in sympathotonic hens, characterised by the predominant influence of the sympathetic nervous system, compared to the other two groups – normotonic and vagotonic hens. The highest calcium concentrations were observed in the blood serum of vagotonic hens, characterised by predominant vagotonic activity ($P < 0.001$) compared to birds in other experimental groups. Phosphorus concentrations in the blood serum of normotonic hens, marked by a balanced influence of the sympathetic and parasympathetic nervous systems, were the lowest ($P < 0.01$) compared to vagotonic and sympathotonic hens. The highest haemoglobin levels were recorded in the whole blood of normotonic hens ($P < 0.001$) relative to other experimental groups. The findings demonstrated a correlation between the activity of the autonomic nervous system and the levels of phosphorus, calcium, glucose, and haemoglobin in the blood of laying hens. Identifying the individual characteristics of autonomic regulation of metabolic processes in laying hens can facilitate the development of scientifically grounded approaches to ration balancing, thereby enhancing productivity in poultry production

Keywords: poultry; normotonic; sympathotonic; vagotonic; mineral substances; carbohydrates

Introduction

Laying hens, during periods of intensive growth, development, and peak productivity, utilise significant quantities of minerals and energy-rich substances. The absorption of minerals in poultry is influenced by a multitude of factors, including age, diet, housing conditions, and neurohumoral regulation. Therefore, considering the specific molecular mechanisms maintaining homeostasis in laying hens under the influence of various exogenous and endogenous factors will contribute to their effective use in production to stimulate the growth and development of poultry and ensure consistently high productivity.

S. Pirzado *et al.* (2024) demonstrated that a significant amount of minerals, particularly calcium and phosphorus, are expended during the period of active egg-laying, which begins after 150 days. To regulate metabolic mechanisms, sources of readily available energy, such as carbohydrates, and oxygen, which are used for the formation of adenosine triphosphate, are necessary. V. Grechkina *et al.* (2023) found that for the sustainable development of poultry and to ensure high productivity, it is necessary to consider the specific impact of several leading factors on individual links of metabolism for effective and timely adjustment. In this case, the first step is to adjust the feeding ration of laying hens, which for most of their lives have an intensive metabolism that is constantly changing. Moreover, upon reaching peak productivity, the intensity of metabolism in the bird's body gradually decreases not only due to changes in

internal processes but also as a result of a decrease in the efficiency of nutrient absorption in the intestine, which further progresses with increasing age (Anene, 2023).

The content of phosphorus and calcium in the tissues and fluids of laying hens undergoes the most significant changes during the period of active egg-laying, particularly during the production phase. M. Sinclair-Black *et al.* (2023) established that approximately 20-40% of calcium is mobilised from the bird's body stores for the formation of eggshells. It is during the nighttime period that the expenditure of calcium and phosphorus increases, which is explained by the lower consumption of feed. Therefore, the deficiency of minerals is compensated for at the expense of the medullary bone with highly organised hydroxyapatite crystals, which accelerates the conversion of hydroxyapatite during egg formation. O. Umoren *et al.* (2024) found that hydroxyapatite consists primarily of 20% calcium and 80% phosphorus. As a result of the mobilisation of minerals for eggshell formation, a significant amount is expended, and the remainder is utilised to prevent the body from becoming intoxicated.

C. Zhang *et al.* (2023) and Z. Cao *et al.* (2023) noted that any processes of synthesis and breakdown of nutrients, their absorption and metabolism, consume energy. The most efficient and rapid method of obtaining energy for the organism is the breakdown of carbohydrates, which are more accessible and less costly in terms of energy expenditure for their oxidation compared to lipids. For poultry, which is characterised by a high intensity of metabolism, especially mineral metabolism, the glucose content indicator is quite important. It is also worth noting that the process of carbohydrate utilisation by the organism is closely related to the level of oxygen supply, which significantly depends on the haemoglobin content in the blood. The results of these scientists' research showed that an energy deficit in Rugao and Hyline Brown laying hens during the growth phase led to a delay in their sexual maturation and the development of reproductive organs compared to a group of birds that received a balanced diet. A significant role in the reproductive ability of poultry is played by the factor of the intensity of follicle formation. Moreover, the energy value of feeds in the diet affects the regulation of follicle maturation and egg formation, and with the help of sex hormones and their metabolites, the high reproductive function of poultry is maintained.

Y. Xiao *et al.* (2023) established a link between mineral metabolism and the individual characteristics of the nervous system. Their study describes the mechanisms of interoception in the regulation of homeostasis in bone tissue, which includes central modulation, primarily associated with the activation of osteocytes, osteoblasts, and osteoclasts. The hypothalamus has a significant impact on nervous processes in the body, which occurs indirectly through the autonomic nervous system by releasing neuropeptides and specific neuroendocrine mechanisms. This regulates the process of differentiation of mesenchymal stem cells, activates osteoclasts, and ensures the functionality of bone cells. Through specific signals, the central nervous system maintains stability in the bird's body by influencing numerous molecular processes (Bahuti *et al.*, 2023). Signals from osteoblasts are important in skeletal interoceptive pathways, which facilitate the conversion of substances in bones through sensory nerve axes and maintain hypothalamic-sympathetic tone.

A. Studenok *et al.* (2021) demonstrated that sympathotonic poultry exhibit higher levels of free amino acids in blood serum compared to normotonic and vagotonic birds. Conversely, vagotonic chicks showed significantly elevated levels of serine and glycine relative to those in normotonic chicks. The researchers also

observed a strong correlation between the concentrations of the studied free amino acids in normotonic birds. Vagotonic poultry displayed the highest correlation values between heart rate, autonomic nervous system tone, and free amino acid levels. A relationship was identified between the activity of the autonomic nervous system, body weight in chickens, and the total protein content in blood serum (Ekinci *et al.*, 2023). Furthermore, the degree of correlation between serum total protein levels and body weight in chicks was influenced by autonomic nervous system tone. Fisher's test confirmed a significant effect of a balanced sympathovagal balance on serine, valine, glycine levels, and body weight dynamics in chickens. The authors highlighted the significant impact of autonomic nervous system tone on the body weight of vagotonic poultry. This study aimed to identify the regulatory patterns of the autonomic nervous system's influence on haemoglobin levels and the indicators of mineral and carbohydrate metabolism in laying hens.

Literature Review

A. Abun *et al.* (2023) and S. Elnesr *et al.* (2024) determined that calcium and phosphorus are crucial dietary micronutrients for skeletal development in animals. They rank third among other known feed nutrients alongside crude protein and energy components. The requirements for these minerals depend on the bird's age, body structure, and genetics. In particular, calcium is known for its role in ensuring neurohumoral regulation of molecular processes and significantly influences chondrocyte maturation. Phosphorus affects chondrocyte apoptosis and is important for anabolic processes in bone tissue, specifically being directly involved in the synthesis of phospholipids and nucleotides, ensuring bone integrity during egg formation in hens and the maturation of growth plates.

Q. Zhang *et al.* (2023) determined the relationship between calcium and phosphorus content in the diet and their absorption rate in chickens. The authors found that with a calcium content of 2% in the diet, its absorption was significantly better compared to a diet where its concentration was 3.45%. The researchers also proved that chickens aged 13 to 20 weeks better absorbed calcium at a concentration of 1.1% and phosphorus at a concentration of 0.55%. P. Du *et al.* (2023) investigated the relationship between glucose content in the bird's body and breed. As a result, it was established that Arbor Acres broiler chickens, which develop intensively, had a lower concentration of glucose in the blood and a higher clearance rate compared to breeds of traditional Chinese black-skinned chicken and Silky chickens, as well as slower growth. In addition, Arbor Acres broiler chickens showed a slower increase in blood glucose concentration after oral administration of a glucose solution than in Silky chickens at both 21 and 42 days of age ($P < 0.05$).

Researchers linked this to decreased pancreatic insulin expression and increased transcription of glucose transporter 2 and phosphoenolpyruvate carboxykinase 1 in the liver of Arbor Acres broiler chickens ($P < 0.05$). As a result of feed restriction in Arbor Acres broiler chickens from 7 to 21 days, fasting blood glucose levels and the rate of response to oral glucose administration increased at 21 days ($P < 0.05$). Improvement in serum glucose concentration after three weeks of compensatory growth occurred due to early feed restriction in Arbor Acres broiler chickens. Feed restriction in broiler chickens contributed to increased blood glucose levels against the background of changes in pancreatic insulin production at 21 and 42 days and also reduced the expression of catalytic glucose-6-phosphatase, phosphoenolpyruvate carboxykinase 1, and glucose transporter 2 in hepatocytes at 21 days ($P < 0.05$).

I. El-Ratel *et al.* (2024) demonstrated that increasing the retinol content in broiler chicken feed is a factor influencing blood parameters and the body's antioxidant defence. In newly hatched chicks, all dietary supplements with varying levels of vitamin A caused an increase in the blood ($P < 0.05$) chromoprotein content, red blood cell count, and an increase in total protein and IgA levels in the serum, which was simultaneously accompanied by a decrease in the percentage of eosinophils and aspartate aminotransferase activity. Dietary vitamin A supplements from 4,000 to 8,000 IU/kg also contributed to positive changes in lymphocyte count, total antioxidant activity of blood serum, IgM content, which caused a decrease in the number of heterophils, the heterophil/lymphocyte ratio, and creatinine concentration in the blood serum of hatched chicks. The authors noted that adding a dietary supplement with vitamin A (6,000 IU/kg) to feed significantly improved the reproductive performance and antioxidant status of laying hens and the haematological, biochemical, antioxidant, and immune status of their offspring. Therefore, research into parameters such as phosphorus, calcium, glucose, and haemoglobin in laying hens is highly relevant. During the period of active growth and peak productivity of birds, the role of these feed components is quite significant, and their participation in metabolism and the synthesis of organic substances is one of the most important. It should be noted that the correction of phosphorus, calcium, glucose, and haemoglobin levels in the bird's blood should be based not only on an assessment of feed components but also on factors that can affect the absorption processes of these key elements and the intensity of carbohydrate and protein synthesis in the organism.

Materials and Methods

The experiments were conducted during 2023-2024 on Hisex Brown hens at the facilities of LLC "MP-Dobrobut" in Snovsk, Chernihiv Region, Ukraine. All procedures involving the birds were carried out in strict accordance with the principles of bioethics, as outlined in the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Convention..., 1986), and the General Ethical Principles for Experiments on Animals adopted by the First National Congress on Bioethics (Procedure..., 2012).

Experimental groups were established using electrocardiographic analysis with the Heart Mirror IKO device (Innomed, Hungary), which records three-minute electrocardiograms by detecting the electrical potentials of the heart. The data were subsequently analysed using variational-pulsometric methods to determine individual characteristics of autonomic nervous system tone. Based on the results, three experimental groups of five birds each were formed: normotonic hens (with a balanced sympathovagal tone), sympathotonic hens (characterised by the predominance of sympathotonic tone over parasympathetic tone), and vagotonic hens (characterised by the predominance of parasympathetic tone over sympathetic tone).

Blood samples were collected in the morning following a two-hour fasting period. The site for blood collection was pre-treated with a 70% ethanol solution and anaesthetised using a lidocainebased gel. Blood was obtained via puncture of the wing vein. To prepare serum, blood samples were incubated for one hour at 37°C in a thermostat, followed by settling at room temperature. Biochemical analysis of the blood serum was performed using a LabLine-010 spectrophotometer (Austria). Glucose levels in the biological material were determined using a test system provided by Granum Laboratory LLC (Kharkiv, Ukraine).

Measurements were conducted under the following conditions: wavelength 505 nm (range: 490-550 nm), cuvette optical path length 10 mm, and temperature range 15-25°C. The glucose concentration in the test samples was calculated using the formula:

$$C_{exp} = \frac{E_{exp}}{E_{std}} \times C_{std}, \quad (1)$$

where C_{exp} is the glucose concentration in the test sample (mmol/L), E_{exp} is the extinction of the test sample, E_{std} is the extinction of the standard sample, and C_{std} is the glucose concentration in the standard sample (10.0 mmol/L).

Haemoglobin levels in whole blood were determined using a reagent kit provided by Granum Laboratory LLC (Kharkiv, Ukraine). The measurements were performed under the following conditions: wavelength range 520-560 nm, cuvette optical path length 10 mm, and temperature range 15-25°C. The haemoglobin concentration was calculated using the formula (1), where C_{exp} is the haemoglobin concentration in the test sample (g/L), E_{exp} is the extinction of the test sample, E_{std} is the extinction of the standard sample, and C_{std} is the haemoglobin concentration in the standard sample (150.0 g/L).

Calcium content was determined using a reagent kit from Granum Laboratory LLC (Kharkiv, Ukraine). Measurements were performed under the following conditions: wavelength 570 nm (range: 550-590 nm), cuvette optical path length 10 mm, and temperature range 15-25°C. Data were calculated using the formula (1), where C_{exp} is the calcium concentration in the test sample (mmol/L), E_{exp} is the extinction of the test sample, E_{std} is the extinction of the standard sample, and C_{std} is the calcium concentration in the standard sample (2.5 mmol/L).

Phosphorus content was determined using a reagent kit from Granum Laboratory LLC (Kharkiv, Ukraine). The conditions for measurement included a wavelength of 340 nm, a cuvette optical path length of 10 mm, and a temperature range of 15-25°C. Data were calculated using the formula (1), where C_{exp} is the phosphorus concentration in the test sample (mmol/L), E_{exp} is the extinction of the test sample, E_{std} is the extinction of the standard sample, and C_{std} is the phosphorus concentration in the standard sample (1.45 mmol/L).

Statistical analysis was performed using the data analysis toolkit in Microsoft Excel. Differences in biochemical parameters were calculated according to the t-Student's test. Statistical significance was evaluated at levels of $P < 0.001$, $P < 0.01$, and $P < 0.05$. Quantitative differences in biochemical parameters were assessed relative to the normotonic hens, as this group exhibited a balanced sympathovagal tone, serving as a reference for comparison.

Results and Discussion

Based on the results of the study of individual blood parameters in poultry, using the spectrophotometry method, the content of the following biochemical markers of carbohydrate, protein, and mineral metabolism was established: glucose, calcium, phosphorus, and haemoglobin in laying hens with a balanced sympathovagal balance, reflecting the normotonic tone of the autonomic nervous system (Table 1).

Table 1. Content of glucose, phosphorus, calcium, and haemoglobin in the blood of normotonic laying hens (n = 5)

Parameter	NVO	SE	M	SD	Asym	Min	Max
Glucose (mmol/L)	5	0.23	11.58	0.38	0.19	11.00	12.20

Table 1. Continued

Parameter	NVO	SE	M	SD	Asym	Min	Max
Calcium (mmol/L)	5	0.08	3.10	0.11	1.22	2.91	3.40
Phosphorus (mmol/L)	5	0.05	3.72	0.07	-1.17	3.54	3.83
Haemoglobin (g/L)	5	0.35	71.29	0.55	0.59	70.54	72.40

Note: *NVO – number of valid observations; SE – standard error; M – mean; SD – standard deviation; Asym – asymmetry; Min – minimum value; Max – maximum value*
Source: *authors' development*

Based on the laboratory results (Table 1), the glucose content in the blood serum of birds with a balanced autonomic nervous system was found to range from 11.00 to 12.20 mmol/L, with an average of 11.58 ± 0.38 mmol/L. The variation in glucose levels among normotonic laying hens was relatively small at 1.20 mmol/L. The calcium content in the blood serum of normotonic laying hens ranged from 2.91 to 3.40 mmol/L, with an average of 3.10 ± 0.11 mmol/L. The variation in calcium content among normotonic laying hens was 0.49 mmol/L, indicating a small individual variation in the content of this parameter in biological samples of the studied birds in this group. The concentration of phosphorus in the blood serum of laying hens with a balanced sympathovagal tone of the autonomic nervous system ranged from 3.54 to 3.83 mmol/L, with a mean of 3.72 ± 0.07 mmol/L. Thus, the phosphorus content in normotonic laying hens showed a variation of 0.29 mmol/L, indicating some deviations in the values of the variation series of the studied parameter in biological samples. At the same time, the haemoglobin content in the blood of birds with a balanced sympathovagal tone of the autonomic nervous system varied from 70.4 to 72.40 g/L, with a mean of 71.29 ± 0.55 g/L. Thus, the content of chromoprotein in biological samples of laying hens with a balanced sympathovagal tone showed differences in values of 2.14 g/L, indicating a small discrepancy in the results of the laboratory study of the corresponding parameter in samples of native blood.

Table 2 presents the results for glucose, calcium, phosphorus, and haemoglobin content in biological samples from birds with a predominance of parasympathetic over sympathetic nervous system influence, reflecting a vagotonic tone of the autonomic nervous system.

Table 2. Glucose, phosphorus, calcium, and haemoglobin content in the blood of vagotonic laying hens (n = 5)

Parameter	NVO	SE	M	SD	Asym	Min	Max
Glucose (mmol/L)	5	0.16	10.59	0.27	-0.32	10.13	11.00
Calcium (mmol/L)	5	0.06	4.12	0.08	-1.96	3.91	4.20
Phosphorus (mmol/L)	5	0.23	5.13	0.36	-0.75	4.37	5.67
Haemoglobin (g/L)	5	0.33	65.12	0.45	0.58	64.20	66.24

Note: *NVO – number of valid observations; SE – standard error; M – mean; SD – standard deviation; Asym – asymmetry; Min – minimum value; Max – maximum value*
Source: *authors' development*

According to the research data (Table 2), the glucose content in biological samples from vagotonic laying hens ranged from 10.13 to 11.00 mmol/L, with an average of 10.59 ± 0.27 mmol/L. The variation in glucose levels in the blood serum of these birds was 0.87 mmol/L, indicating minor fluctuations in the initial results of this biochemical parameter in biological samples from the studied birds. The calcium level in biological samples from vagotonic birds ranged from 3.91 to 4.20 mmol/L, with an average of 4.12 ± 0.08 mmol/L. The values of this parameter showed a variation of 0.29 mmol/L, indicating the stability of the studied parameter values in the biological samples of the experimental birds. The phosphorus level in biological samples from vagotonic birds ranged from 4.37 to 5.67 mmol/L, with an average of 5.13 ± 0.36 mmol/L. The variation in phosphorus content in the blood serum of vagotonic birds was 0.30 mmol/L, indicating no significant differences in the initial data from this study. The results of the haemoglobin level study in the native blood of vagotonic laying hens corresponded to a range from 64.20 to 66.24 g/L, with an average of 65.12 ± 0.45 g/L. The parameters of this chromoprotein in the native blood of the studied birds showed differences of 2.04 mmol/L, indicating insignificant deviations in its initial data.

Table 3 presents the results of studies of individual biochemical profile parameters of blood: glucose, calcium, phosphorus, and haemoglobin in laying hens, which were characterised by a predominant influence of the sympathetic nervous system over the parasympathetic, reflecting the tone of the autonomic nervous system – sympathotonia (Table 3).

Table 3. Glucose, phosphorus, calcium, and haemoglobin content in the blood of sympathotonic laying hens (n = 5)

Parameter	NVO	SE	M	SD	Asym	Min	Max
Glucose (mmol/L)	5	0.23	15.06	0.33	-0.50	14.30	15.70
Calcium (mmol/L)	5	0.04	2.74	0.07	-1.26	2.60	2.80
Phosphorus (mmol/L)	5	0.11	4.54	0.16	0.73	4.29	4.89
Haemoglobin (g/L)	5	0.34	66.56	0.58	0.38	65.86	67.44

Note: *NVO – number of valid observations; SE – standard error; M – mean; SD – standard deviation; Asym – asymmetry; Min – minimum value; Max – maximum value*
Source: *authors' development*

The glucose concentration in serum samples of laying hens with sympathotonia was found to range from 14.30 to 15.70 mmol/L, with a mean of 15.06 ± 0.33 mmol/L (Table 3). The variation in glucose content in biological samples from this group of birds was 1.40 mmol/L, indicating no significant differences in the initial results of the studied parameter. The calcium content in the blood serum of birds with sympathotonia ranged from 2.60 to 2.80 mmol/L, with a mean of 2.74 ± 0.07 mmol/L. The variation in calcium content in the blood serum of birds with sympathotonia was 0.20 mmol/L, indicating a small discrepancy in the initial results of the studied parameter.

The phosphorus concentration in the blood serum of laying hens with sympathotonia

ranged from 4.29 to 4.89 mmol/L, with an average of 4.54 ± 0.16 mmol/L. The variation in phosphorus content in biological samples from sympathotonic birds was 0.60 mmol/L, indicating a small variation in the results for this parameter. The haemoglobin content in biological samples from sympathotonic birds varied from 65.86 to 67.44 g/L, with an average of 66.56 ± 0.58 g/L. The variation in chromoprotein content in the native blood of this group of birds was 1.58 g/L, also indicating small fluctuations in the absolute values of this parameter. The glucose concentration in biological samples of sympathotonic laying hens (15.06 ± 0.33 mmol/L) was significantly increased by 30.1% (P<0.001) compared to birds in the normotonic control group (11.58 ± 0.38 mmol/L), as shown graphically and also applies to the assessment of individual results obtained from birds in three groups, with five individuals in each (Fig. 1).

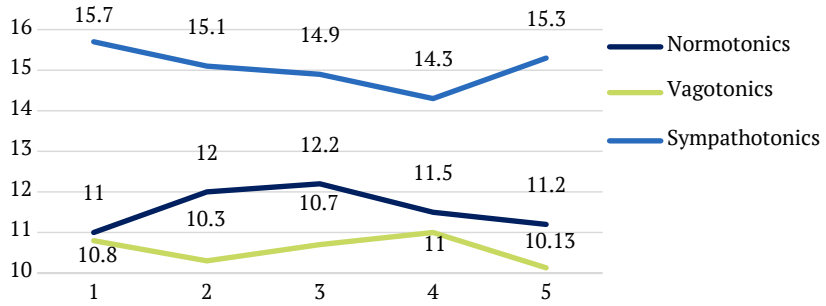

Figure 1. Glucose content (mmol/L) in the blood serum of birds in experimental groups (n = 5)
Note: *different colours represent variations in the autonomic nervous system tone in the experimental birds*
Source: *authors' development*

At the same time, the glucose content in biological samples from vagotonic laying hens (10.59 ± 0.27 mmol/L) was significantly decreased by 8.5% (P <0.05) compared to normotonic hens (11.58 ± 0.38 mmol/L), as shown in Figure 1. The calcium level in laying hens with sympathotonia (2.74 ± 0.07 mmol/L) was significantly decreased by 11.6% (P <0.05) compared to normotonic hens (3.10 ± 0.11 mmol/L), as shown graphically, demonstrating the characteristics of individual changes in the parameters of the studied indicator when assessing the results obtained from birds in three groups, with five individuals in each (Fig. 2).

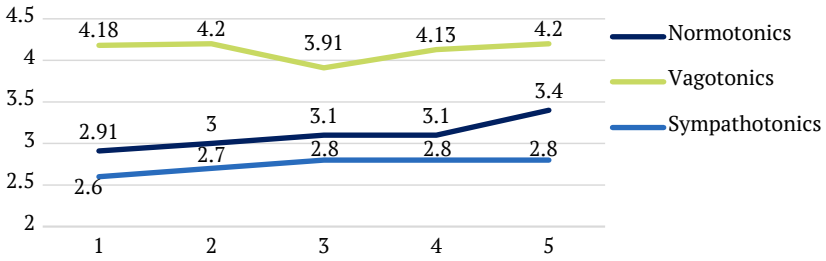

Figure 2. Calcium content (mmol/L) in the blood serum of birds in experimental groups (n = 5)
Note: *different colours represent variations in the autonomic nervous system tone in the experimental birds*
Source: *authors' development*

However, the calcium content in biological samples from vagotonic laying hens (4.12 ± 0.08 mmol/L) was significantly increased by 32.9% (P <0.001) compared to normotonic hens (3.10 ± 0.11 mmol/L), as shown graphically in Figure 2. The phosphorus concentration in sympathotonic laying hens (4.54 ± 0.16 mmol/L) significantly increased by 22.0% (P <0.01) compared to normotonic hens (3.72 ± 0.07 mmol/L), as shown graphically when assessing individual values obtained from birds in three groups, with five individuals in each (Fig. 3).

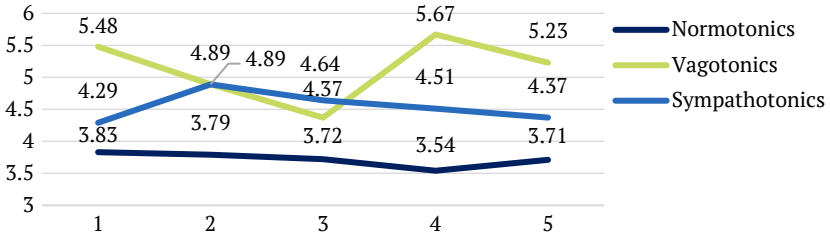

Figure 3. Phosphorus content (mmol/L) in the blood serum of birds in experimental groups (n = 5)

Note: *different colours represent variations in the autonomic nervous system tone in the experimental birds*
Source: *author's development*

The phosphorus content in biological samples from vagotonic laying hens (5.13 ± 0.36 mmol/L) also significantly increased by 37.9% (P <0.01) compared to normotonic birds, as shown graphically in Figure 3. The haemoglobin concentration in biological samples from sympathotonic laying hens (66.56 ± 0.58 g/L) was significantly lower by 6.6% (P <0.001) compared to normotonic birds (71.29 ± 0.55 g/L), as shown graphically and is important for the analysis of individual results obtained from birds in three groups, with five individuals in each (Fig. 4). The chromoprotein content in biological samples from vagotonic laying hens (65.12 ± 0.45 g/L) was also significantly decreased by 8.7% (P <0.001) compared to normotonic birds, as shown graphically in Figure 4.

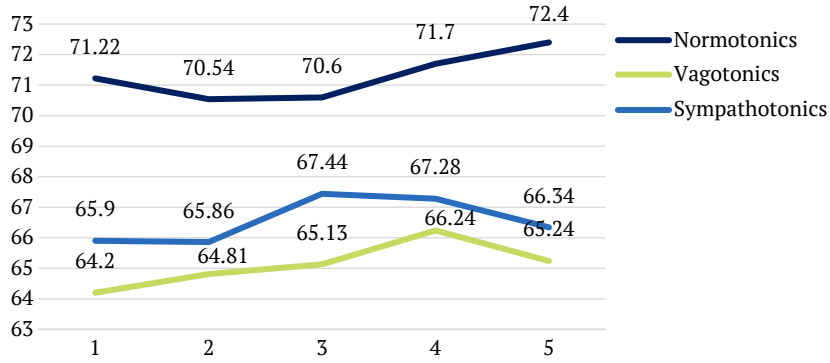

Figure 4. Haemoglobin content (mmol/L) in the blood serum of birds in experimental groups (n = 5)

Note: *different colours represent variations in the autonomic nervous system tone in the experimental birds*
Source: *authors' development*

M. Sinclair-Black *et al.* (2023) demonstrated that mineral metabolism in poultry is crucial for the productive growth and development of laying hens. This is particularly true for calcium and phosphorus, especially during periods of active laying. It is also worth noting that the physiological course of metabolic reactions always consumes a certain amount of energy, which correlates with the level of carbohydrates in the tissues and bloodstream of poultry. The metabolism of these key elements depends on many exogenous and endogenous factors. S. Pirzado *et al.* (2024) noted that the best way to monitor the state of metabolism and determine the level of phosphorus, calcium, and glucose in the bird's body is to conduct a combined assessment of these indicators in the bird's blood and their quantity consumed with feed. The trend towards studying the metabolism of these substances in laying hens by artificially changing the concentration of these components and assessing their impact on the metabolic profile of the organism is becoming increasingly popular.

In their study, Y. Ren *et al.* (2023) investigated phosphorus metabolism in poultry, focusing on the influence of diet on phosphorus content in the organism. To achieve more accurate results, the authors adjusted the phosphorus content in the diet by adding a feed supplement based on cornsoybean meal without inorganic phosphates, which contained 0.12% phytic phosphorus. Five experimental diets were created (with non-phytate phosphorus levels of 0.10%, 0.15, 0.20, 0.25, and 0.30%). Additionally, the researchers adjusted the calcium carbonate content to ensure all experimental diets contained the same percentage of this macroelement, specifically 3.81%. The experiment was conducted on chickens aged 69-78 weeks and lasted for 10 weeks. Such a dietary change had a negligible impact on the content of phosphorus, calcium, iron, copper, manganese, and zinc in biological samples.

R. Sanmiguel *et al.* (2023) investigated calcium metabolism in poultry and assessed changes in calcium levels by examining the impact of dietary factors. Specifically, they analysed the effects of adding humic substances and calcium carbonate ($CaCO_3$) to the diet. The study was conducted using 55-week-old Hy-Line Brown laying hens, which were divided into four groups. The first group received a diet without humic substances or $CaCO_3$, the second group received an additional 2.00 g/bird per day of $CaCO_3$, the third group received 0.20% humic substances, and the fourth group received both 0.20% humic substances and 2.00 g/bird per day of $CaCO_3$. The authors concluded that dietary adjustments were an effective way to influence calcium and phosphorus concentrations in the bird's body, as changes in the dietary content of these elements significantly improved the overall condition of the birds, increasing their stress resistance and productivity.

N. Esenbuga & O. Ekinci (2023) conducted a study to identify factors influencing glucose concentration in laying hens and to evaluate methods for correcting blood glucose levels. The impact of various factors on glucose concentration was assessed by adjusting the birds' diet. To achieve this, three groups of laying hens were formed and fed diets supplemented with extracts of thyme, anise, and black cumin. Results showed that the lowest serum glucose levels were observed in the group fed a diet supplemented with anise extract, while the highest levels were found in the group fed a diet supplemented with thyme extract. Additionally, there was a significant interaction effect ($P < 0.0001$) between the plant extracts on glucose concentration in biological samples.

J. Alagbe (2024) evaluated the impact of a dietary supplement, *Cordyline fruticosa* leaf meal, on the performance and haematological parameters of laying hens. Different concentrations of the supplement (10g, 20g, 30g, and

40g) were added to the hens' diet over a 90-day period. Results showed a negligible impact of the supplement on bird performance, such as weight gain and egg production. However, significant differences were observed in haemoglobin levels and red blood cell counts between the experimental and control groups ($P < 0.05$). S. Alsherify & A. Hassanabadi (2024) conducted a study to correct metabolism, immune status, and haematological parameters through dietary adjustments. Different levels of fructooligosaccharide (1g/kg, 2g/kg, and 3g/kg) were added to the diet of commercial Hy-Line W-36 laying hens. However, no significant changes were observed in haemoglobin levels or red blood cell counts in the laying hens.

Having analysed the scientific studies of other researchers and compared them to the results of their own study, the authors noted that the issue of regulating mineral and organic matter metabolism in poultry remains relevant. The bird's organism, especially during the laying period, undergoes significant metabolic shifts. At the same time, mineral metabolism is much more intense, as a large amount of minerals is used for egg formation. It is also worth noting the global trend of extending the laying period of laying hens. However, information from scientific sources regarding the use of various feed additives to improve bird performance yields ambiguous results. This is primarily due to the lack of a consistent pattern in forming experimental groups of birds. The authors also note that a preliminary analysis of metabolic components, the same minerals and energy-rich compounds, is not carried out, and the assessment is conducted only after the application of feed additives in poultry feeding. Considering the obtained results, the authors noted that the distribution of laying hens according to their autonomic nervous system tone indicates differences in the metabolism of minerals, carbohydrates, and proteins that do not go beyond physiological parameters, without the use of dietary changes. The established patterns of the influence of the sympathovagal balance on the intensity of individual links of metabolism in laying hens should be taken into account for scientifically sound correction of the bird's diet.

Conclusions

The experiment revealed specific effects of the sympathovagal balance on quantitative parameters of certain biochemical blood indicators in laying hens, which characterise the intensity of the corresponding links of carbohydrate, mineral, and protein metabolism. Certain differences were observed in the blood content of glucose, calcium, phosphorus, and haemoglobin compared to their values in the group of normotonic laying hens, but these did not exceed physiological parameters. In particular, compared to normotonic hens with a balanced sympathovagal balance, in sympathotonic hens with a predominance of the sympathetic nervous system over the parasympathetic, the glucose concentration in serum was significantly increased by 30.1% ($P < 0.001$), while in vagotonic birds with a predominance of the parasympathetic nervous system activity over the sympathetic, the level of this carbohydrate decreased by 8.5% ($P < 0.05$). Furthermore, calcium content in biological samples from sympathotonic birds decreased significantly by 11.6% ($P < 0.05$), while in vagotonic birds it increased significantly by 32.9% ($P < 0.001$) compared to normotonic laying hens. The level of phosphorus in biological samples from sympathotonic birds increased significantly by 22.0% ($P < 0.01$), and in vagotonic birds, it also increased by 37.9% ($P < 0.01$) compared to normotonic individuals. However, haemoglobin content in biological samples from laying hens decreased significantly by 6.6% ($P < 0.001$), and in vagotonic birds, it similarly decreased by 8.7% ($P < 0.001$) compared to normotonic birds.

The established features of the studied marker biochemical parameters in blood, depending on the activity of the autonomic nervous system divisions, should be considered for scientifically sound correction of the bird's diet, which will significantly improve the analysis of the initial data and the accuracy of calculations. The perspective of further research lies in the application of preparation based on nano-aqua-chelates, such as germanium and iron, to correct individual links of metabolism in the organism of laying hens with an assessment of individual characteristics, namely the activity of the autonomic nervous system.

Acknowledgements

None.

Conflict of Interest

None.

References

[1] Abun, A., Supriadi, D.N., Haetami, K., & Setiatwan, H. (2023). Blood haematology of laying hens given fermented shrimp waste extract. *GSC Biological and Pharmaceutical Sciences*, 24(1), 193-201. doi: 10.30574/gscbps.2023.24.1.0269.

[2] Alagbe, J.O. (2024). Impact of *Cordyline fruticosa* leaf meal supplemented diet on growth performance, egg production, egg quality, and some heamatological indices of laying hen. *Brazilian Journal of Science*, 3(9), 19-32. doi: 10.14295/bjs.v3i9.635.

[3] Alsherify, S.M., & Hassanabadi, A. (2024). Effects of adding different levels of fructooligosaccharide to diet on productive performance, egg quality traits, immune response and blood metabolites in commercial laying hens. *Veterinary Medicine and Science*, 10(5), article number e1550. doi: 10.1002/vms3.1550.

[4] Anene, D.O., Akter, Y., Thomson, P.C., Groves, P., & O'Shea, C.J. (2023). Effect of restricted feeding on hen performance, egg quality and organ characteristics of individual laying hens. *Animal Nutrition*, 14, 141-151. doi: 10.1016/j.aninu.2023.05.001.

[5] Bahuti, M., Junior, T.Y., Fassani, É.J., Ribeiro, B.P.V.B., de Lima, R.R., & Campos, A.T. (2023). Evaluation of different light intensities on the well-being, productivity, and eggs quality of laying hens. *Computers and Electronics in Agriculture*, 215, article number 108423. doi: 10.1016/j.compag.2023.108423.

[6] Cao, Z., Liu, Z., Zhang, N., Bao, C., Li, X., Liu, M., Yuan, W., Wu, H., & Shang, H. (2023). Effects of dietary dandelion (*Taraxacum mongolicum* Hand.-Mazz.) polysaccharides on the performance and gut microbiota of laying hens. *International Journal of Biological Macromolecules*, 240, article number 124422. doi: 10.1016/j.ijbiomac.2023.124422.

[7] Du, P., Wang, H., Shi, X., Zhang, X., Zhu, Y., Chen, W., Zhang, H., & Huang, Y. (2023). A comparative study to determine the effects of breed and feed restriction on glucose metabolism of chickens. *Animal Nutrition*, 13, 261-269. doi: 10.1016/j.aninu.2023.02.005.

[8] Ekinci, O., Esenbuga, N., & Dagdemir, V. (2023). The effects of body weight and age on performance, egg quality, blood parameters, and economic production of laying hens. *Journal of the Hellenic Veterinary Medical Society*, 74(2), 5799-5806. doi: 10.12681/jhvms.30324.

[9] Elnesr, S.S., Mahmoud, B.Y., da Silva Pires, P.G., Moraes, P., Elwan, H.A.M., El-Shall, N.A., El-Kholy, M.S., & Alagawany, M. (2024). Trace minerals in laying hen diets and their effects on egg quality. *Biological Trace Element Research*, 202, 5664-5679. doi: 10.1007/s12011-024-04121-8.

[10] El-Ratel, I.T., *et al.* (2024). Effects of supplemental vitamin A on reproduction and antioxidative status of aged laying hens, and growth, blood indices and immunity of their offspring. *Poultry Science*, 103(3), article number 103453. doi: 10.1016/j.psj.2024.103453.

[11] Esenbuga, N., & Ekinci, O. (2023). Dietary effects of some plant extracts on laying performance, egg quality, and some blood parameters in laying hens at different cage densities. *Animals*, 13(24), article number 3866. doi: 10.3390/ani13243866.

[12] European convention for the protection of vertebrate animals used for research and other scientific purposes. (1986, March). Retrieved from https://zakon.rada.gov.ua/laws/show/994_137#Text.

[13] Grechkina, V., Lebedev, S., Frolov, A., Petrusha, Y., & Silin, D. (2023). Redistribution of chemical elements in the body of laying hens in different periods of ontogenesis. *AIP Conference Proceedings*, 3011(1), article number 020008. doi: 10.1063/5.0161178.

[14] Law of Ukraine No. 249 "On the Procedure for Carrying Out Research and Experiments on Animals by Scientific Institutions". (2012, March). Retrieved from https://zakon.rada.gov.ua/laws/show/z0416-12#Text.

[15] Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty". (2006, February). Retrieved from https://zakon.rada.gov.ua/laws/show/3447-15#Text.

[16] Pirzado, S.A., Liu, G., Purba, M.A., & Cai, H. (2024). Enhancing the production performance and nutrient utilization of laying hens by augmenting energy, phosphorous and calcium deficient diets with fungal phytase (*Trichoderma reesei*) supplementation. *Animals*, 14(3), article number 376. doi: 10.3390/ani14030376.

[17] Ren, Y., Liu, Y., Jiang, K., Li, L., Jiao, N., Zhu, Z., Zhang, K., Jiang, S., Yang, W., & Li, Y. (2023). Effects of low-phosphorus diets supplemented with Phytase on the production performance, phosphorus-calcium metabolism, and bone metabolism of aged Hy-line Brown laying hens. *Animals*, 13(6), article number 1042. doi: 10.3390/ani13061042.

[18] Sanmiguel, R.A., Dedousi, A., López, J.D., Sierra, D.A., & Sossidou, E.N. (2023). Effects of feeds, supplemented with humic substances and calcium carbonate, on performance, egg quality and heart rate variability in laying hens. *Veterinary Research Forum*, 13(4), 461-467. doi: 10.30466/vrf.2022.533902.3210.

[19] Sinclair-Black, M., Garcia, R.A., & Ellestad, L.E. (2023). Physiological regulation of calcium and phosphorus utilization in laying hens. *Frontiers in Physiology*, 14, article number 1112499. doi: 10.3389/fphys.2023.1112499.

[20] Studenok, A.A., Shnurenko, E.O., Karpovskyi, V.I., Zhurenko, O.V., Kryvoruchko, D.I., Gutyj, B.V., & Trokoz, V.O. (2021). Interactions of productivity, content of separate amino acids, general protein in blood serum of chickens and tone of the autonomic nervous system. *Regulatory Mechanisms in Biosystems*, 12(3), 564-570. doi: 10.15421/022177.

[21] Umoren, O.D., Olanipekun, J.T., Osifeso, O.O., Adewoyin, A.A., Alani, O.A., & Adetoro, O.S. (2024). Comparative study of sero-haematological profile of three strains of laying hen. *Nigerian Journal of Animal Science and Technology*, 7, 102-108.

[22] Xiao, Y., Han, C., Wang, Y., Zhang, X., Bao, R., Li, Y., Chen, H., Hu, B., & Liu, S. (2023). Interoceptive regulation of skeletal tissue homeostasis and repair. *Bone Research*, 11(1), article number 48. doi: 10.1038/s41413-023-00285-6.

[23] Zhang, C., Li, C., Shao, Q., Wang, X., Chen, W., Li, Y., Huang, S., & Ma, Y. (2023). Effects of dietary *Glycyrrhiza* polysaccharide on growth, serum biochemistry, immunity, and egg laying in quail. *Animal Biotechnology*, 34(3), 520-528. doi: 10.1080/10495398.2021.1979024.

[24] Zhang, Q.Q., Chang, C., Chu, Q., Wang, H.H., Zhang, J., Yan, Z.X., Song, Z.G., & Geng, A.L. (2023). Dietary calcium and non-phytate phosphorus levels affect the performance, serum biochemical indices, and lipid metabolism in growing pullets. *Poultry Science*, 102(2), article number 102354. doi: 10.1016/j.psj.2022.102354.

Вегетативна регуляція вмісту глюкози, кальцію, фосфору та гемоглобіну в крові курей-несучок

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Анотація. Стан мінерального та енергетичного обмінів істотно впливає на інтенсивність росту і продуктивність курей-несучок. Визначення специфічної їх дії на фізіологічні процеси у поєднанні з регуляторним впливом різного тонусу автономної нервової системи важливо враховувати для вирішення численних питань із покращення яйценосності птиці. Метою цього дослідження було визначення особливостей впливу тонусу автономної нервової системи на проміжний обмін мінеральних речовин, вуглеводів та білків в організмі курей-несучок. У дослід залучали курей породи Хайсекс коричневі. Формування дослідних груп птиці здійснювали з урахуванням результатів електрокардіографії та застосування варіаційно-пульсометричного дослідження з подальшим статистичним аналізом отриманих вихідних даних. Це дозволило

розподілити курей-несучок за трьома дослідними групами: нормотоніки, симпатотоніки, ваготоніки. Завдяки проведенню аналізу окремих показників біохімічного профілю сироватки крові курей-несучок визначено, що рівень глюкози у симпатотоніків із переважним впливом симпатичної нервової системи достовірно зростав ($P < 0,001$) порівняно з птицею інших двох дослідних груп – нормотоніками і ваготоніками. Найбільші значення щодо вмісту кальцію відмічали у сироватці крові курей ваготоніків, для яких характерна переважна активності ваготонії ($P < 0,001$), відносно птиці інших дослідних груп. Концентрація фосфору в сироватці крові курей-несучок нормотоніків зі збалансованим впливом симпатичної і парасимпатичної нервової систем відзначався найменшими значеннями ($P < 0,01$) порівняно з птицею дослідних груп ваготоніків і симпатотоніків. Найвищі значення гемоглобіну встановлено в нативній крові курей-несучок нормотоніків ($P < 0,001$) відносно птиці інших дослідних груп. Відповідно до отриманих результатів виявлено залежність вмісту фосфору, кальцію, глюкози і гемоглобіну в крові курей-несучок від активності вегетативної нервової системи. Визначення індивідуальних особливостей вегетативної регуляції обмінних процесів в організмі курей-несучок сприятиме розробці науково обґрунтованих підходів до балансування раціону, що забезпечить підвищення їх продуктивності на виробництві

Ключові слова: птиця; нормотоніки; симпатотоніки; ваготоніки; мінеральні речовини; вуглеводи