Received: 15.08.2024 | *Accepted: 22.08.2024*

Veterinary Sciences

doi: 10.31210/spi2024.27.03.14
UDC 636.52/.58:577.122:591.48
ORIGINAL ARTICLE

Scientific Progress & Innovations
https://journals.pdaa.edu.ua/visnyk

27 (3)
2024

Interrelation of protein metabolism and autonomic nervous system in laying hens

O. Kulbako[1] | V. Karpovsky[1] | O. Zhurenko[1] | I. Hryshchuk[1] | P. Karpovsky[1] | D. Krivoruchko[1] | A. Hryshchuk[2]

Article info

Correspondence Author
V. Karpovsky
E-mail:
karpovskiy@meta.ua

[1] National University of Life and Environmental Sciences of Ukraine,
Heroiv Oborony Str., 15, building 3, Kyiv, 03041, Ukraine

[2] Luhansk Taras Shevchenko National University,
3, Kovalia Str.,
36000, Poltava,
Ukraine

Citation: Kulbako, O., Karpovsky, V., Zhurenko, O., Hryshchuk, I., Karpovsky, P., Krivoruchko, D., & Hryshchuk, A. (2024). Interrelation of protein metabolism and autonomic nervous system in laying hens. *Scientific Progress & Innovations*, 27 (3), 89–94. doi: 10.31210/spi2024.27.03.14

The study of factors influencing the processes of protein metabolism in the poultry body will help to better balance the diet, which will help to more effectively stimulate the growth of animal productivity. The aim of the work was to investigate the role of the autonomic nervous system in protein metabolism and to take into account the individual characteristics of the poultry organism, characterized by different tone of the autonomic nervous system. The formation of experimental groups of animals was carried out by electrocardiographic examination using the Baevsky method, on the basis of which three experimental groups of animals were formed: normotonics, vagotonics, and sympathotonics. The blood serum was studied using a LabLine-010 spectrophotometer (Austria) and test systems from Laboratory Granum LLC, Kharkiv. Kharkiv. According to the results of the biochemical study, it was found that the content of total protein, which was compared with the experimental group of normotonics (46.10±1.35 g/l) with a balanced sympathovagal balance, was 14.8 % lower than that of the experimental group of sympathotonics (54.10±2.60 g/l) (P<0.01) and 18.1 % lower than that of the experimental group of vagotonics (56.30±1.90 g/l) (P<0.01). The albumin content, which was compared with the experimental group of normotonics (4.56±0.55 g/l) with a balanced sympathovagal balance, was 40.55 % lower than that of the experimental group of sympathotonics (6.96±0.49 g/l) (P<0.001) and 1.5 times lower than that of the experimental group of vagotonics (7.67±0.38 g/l) (P<0.01). The creatinine content compared to the normotonic group (47.12±0.77) with a balanced sympathovagal balance was lower than that of the experimental group by 6.77 % compared to the experimental group of vagotonic poultry (7.67±0.38 g/l) (P<0.001). Taking into account the individual characteristics of the poultry organism and establishing the tone of the autonomic nervous system, it was determined that the autonomic nervous system has an effect on protein metabolism in poultry. The prospect of further research is to study the use of nanoaquahelate preparations to improve productivity and metabolic processes, taking into account the tone of the autonomic nervous system.

Keywords: poultry, protein, autonomic regulation, blood, productivity.

Взаємозв'язок білкового обміну та автономної нервової системи у курей-несучок

О. В. Кульбако[1] | В. І. Карповський[1] | О. В. Журенко[1] | І. А. Грищук[1] | П. В. Карповський[1] | Д. І. Криворучко[1] | А. В. Грищук[2]

[1] Національний університет біоресурсів і природокористування України,
м. Київ, Україна

[2] Луганський національний університет імені Тараса Шевченка, м. Полтава, Україна

Вивчення факторів впливу на процеси обміну білків у організмі птиці допоможе краще збалансовувати раціон, що допоможе більш ефективно стимулювати зріст продуктивності тварин. Метою роботи було дослідити роль автономної нервової системи у білковому обміні та врахувати індивідуальні особливості організму птиці, що характеризувалися різним тонусом автономної нервової системи. Формування дослідних груп тварин виконувалося завдяки електрокардіографічному дослідженню з методикою Баєвського, на підставі якої було сформовано три дослідні групи тварин: нормотоніки, ваготоніки, симпатотоніки. Дослідження сироватки крові виконувалося за допомогою спектрофотометра LabLine-010 (Австрія) та тест систем від ТОВ «Лабораторія Гранум» м. Харків. За результатами біохімічного дослідження встановлено, що вміст загального білку, що порівнювався відносно дослідної групи нормотоніків (46,10±1,35 г/л) із збалансованим симпатовагусним балансом був меншим відносно дослідної групи симпатотоніків (54,10±2,60 г/л) на 14,8 % (P<0,01) та на 18,1 % менший відносно дослідної групи ваготоніків (56,30±1,90 г/л) (P<0,01). Вміст альбумінів, що порівнювався відносно дослідної групи нормотоніків (4,56±0,55 г/л) із збалансованим симпатовагусним балансом був меншим відносно дослідної групи симпатотоніків (6,96±0,49 г/л) на 40,55 % (P<0,001) та у 1,5 рази менший відносно дослідної групи ваготоніків (7,67±0,38 г/л) (P<0,01). Вміст креатиніну, що порівнювався відносно дослідної групи нормотоніків (47,12±0,77) із збалансованим симпатовагусним балансом був меншим відносно дослідної групи менший на 6,77 % відносно дослідної групи ваготоніків (7,67±0,38 г/л) (P<0,001). Врахувавши індивідуальні особливості організму птиці та встановивши тонус автономної нервової системи, визначено, що автономна нервова система має вплив на білковий обмін у птиці. Перспективою подальшого дослідження є вивчення питання застосування препаратів наноаквахелатів для покращення продуктивності і метаболічних процесів із врахування тонусу автономної нервової системи.

Ключові слова: птиця, білок, автономна регуляція, кров, продуктивність.

Бібліографічний опис для цитування: *Кульбако О. В., Карповський В. І., Журенко О. В., Грищук І. А., Карповський П. В., Криворучко Д. І., Грищук А. В.* Взаємозв'язок білкового обміну та автономної нервової системи у курей-несучок. *Scientific Progress & Innovations*. 2024. № 27 (3). С. 89–94.

Introduction

Protein metabolism plays a significant role in the development of laying hens, which will significantly affect their productivity [3]. It has been established that 4–6 weeks before the first egg-laying, the development of secondary reproductive organs and follicle growth in the ovary occurs in birds. The highest consumption of energy organic compounds and protein is used during the period of active egg-laying. Thanks to the successful adjustment of these components, it is possible to maintain a stable flock productivity, especially in the first period of egg-laying [7, 9]. It has been established that protein and poultry productivity are interdependent, as evidenced by the fact that the increase in protein in the diet increases productivity. Taking this feature into account, the need for protein-rich plant feeds in poultry farms is growing [2, 8].

The use of large amounts of protein to improve poultry performance is not a one hundred percent solution. This is because this issue combines additional factors [5, 20]. The first of which is the issue of protein metabolism, it is worth considering that the body has systems for regulating metabolic processes. The second issue that arises when improving protein metabolism is that excessive protein consumption in poultry farms causes an environmental problem [16, 18]. Returning to the issue of systems that correct metabolic processes, it is worth noting neurohumoral regulation. It is the combined work of the nervous system and hormones that maintains the body's homeostasis. It is worth noting that each animal has individual characteristics of metabolic processes. Since each organism is not identical to the other, identifying these differences is important to help better balance the animal's diet to improve metabolic processes [17, 19].

Determining the tone of the autonomic nervous system will facilitate the analysis of individual animal characteristics, which will help to better study metabolic processes in the body. Further, understanding this issue provides scientists with a foundation for improving the course of metabolic processes in the animal body, which will become a source of necessary information for production, which will improve productivity [1, 12].

The purpose of the study

The aim of the work was to investigate the role of the autonomic nervous system in protein metabolism and to take into account the individual characteristics of the poultry organism, characterized by different tone of the autonomic nervous system.

Materials and methods

An electrocardiographic study was performed with the recording of electrical potentials of the bird's heart for at least 100 cardiac intervals. The electrodes of the cardiograph were placed at the site of the humerus and tibia. Blood samples were taken at the age of 4.5 months from the saphenous vein of the shoulder, after a fasted diet.

To obtain serum, the samples were incubated in a thermostat at 37°C. Total protein was determined using a LabLine-010 spectrophotometer (Austria). To determine total protein, a test system from Laboratory Granum LLC (Kharkiv) was used. Kharkiv. Measurement conditions: wavelength 540 (530–650) nm cuvette with an optical layer thickness of 1 cm, temperature 15–25 °C. Before using the reagents, they were kept at room temperature for 30 minutes. After that, the following samples were prepared for analysis according to the ***table 1***:

Table 1
Scheme of reagents application for the study

Parameters	Blank sample	Standard sample	The prototype
Reagent 1 (ml)	1,0	1,0	1,0
Standard, (ml)	–	0,025	–
Sample (ml)	–	–	0,025

The samples were mixed and placed in a thermostat at 37 °C for 5 min. After that, measurements were made on a photocolorimetry in cuvettes with an optical layer thickness of 10 mm relative to the blank sample at a wavelength of 540 (530–650) nm.

The results were calculated according to the formula:

$$C_t = \frac{E_t}{E_{st}} \times C_{st} \quad (1)$$

C_t – is the concentration of total protein in the test sample, g/l.

E_t – is the optical density of the test sample, optical density units.

E_{st} – optical density of the standard, optical density units.

C_{st} – is the content of total protein in the standard, 70.0 g/l.

For the determination of albumin, a test system from Laboratory Granum LLC was used. Kharkiv. Measurement conditions: wavelength 630 (600–650) nm cuvette with an optical layer thickness of 1 cm, temperature 15–25 °C. Before using the reagents, they were kept at room temperature for 30 minutes. After that, the following samples were prepared for analysis according to the ***table 2***:

Table 2
Scheme of reagents application for the study

Parameters	Blank sample	Standard sample	Prototype
Reagent 1 (ml)	1.0	1.0	1.0
Standard, (ml)	–	0.005	–
Sample (ml)	–	–	0.005

The samples were mixed and placed in a thermostat at 37 °C for 5 min. After that, measurements are made on a photocolorimetry in cuvettes with an optical layer thickness of 10 mm relative to the blank sample at a wavelength of 630 (600–650) nm.

The albumin concentration is calculated by the formula:

$$C_t = \frac{E_t}{E_{st}} \times C_{st} \quad (1)$$

C_t – is the concentration of total protein in the test sample, g/l.

E_t – is the optical density of the test sample, optical density units.

E_{st} – optical density of the standard, optical density units.

C_{st} – is the content of total protein in the standard, 50 g/l.

For the determination of creatinine, a test system from Laboratory Granum LLC was used. Kharkiv. Measurement conditions: wavelength 500 (490–520) nm cuvette with an optical layer thickness of 1 cm, temperature 15–25 °C. Before using the reagents, they were kept at room temperature for 30 minutes. After that, the following samples were prepared for analysis according to the ***table 2***:

Table 3
Scheme of reagents for the study

Parameters	Experimental sample	Standard sample	Blank sample
Prototype ml	0.5	–	–
Distilled water ml	1.0	1.0	1.5
Standard ml	–	0.5	–
Working solution 3	0.5	0.5	0.5
Stir for 5 minutes. Then centrifuge the sample for 10 min at 3000 rpm			
Supernatant ml	1.0	1.0	1.0
Working reagent 1 ml	0.5	0.5	0.5
Working reagent 2 ml	0.5	0.5	0.5

The samples were mixed and placed in a thermostat at 37 °C for 5 min. After that, measurements are performed on a photocolorimetry in cuvettes with an optical layer thickness of 10 mm relative to the blank sample at a wavelength of 500 (490–520) nm.

The globulin concentration is calculated by the formula:

$$C_t = \frac{E_t}{E_{st}} \times C_{st} \quad (3)$$

C_t – is the concentration of total protein in the test sample, µmol/L..

E_t – is the optical density of the test sample, optical density units.

E_{st} – optical density of the standard, optical density units.

C_{st} – is the content of total protein in the standard, 166 µmol/L.

Statistical analysis of the results was calculated using Microsoft Excel software. The probability of the difference between the obtained indicators was calculated using the Student's method. Differences between the compared indicators were considered significant at the level of significance $P<0.05$, $P<0.01$, $P<0.001$.

Results and discussion

During the biochemical analysis of blood plasma of poultry at the age of 60 days, the following indicators were found for the experimental group of normotonics (***Table 4***).

Table 4
Indicators of the protein fraction in the blood serum of poultry of the experimental group of normotonics at the age of 4.5 months (n=5)

Indicators	NVB	SE	M	SD	A	Min	Max
Total protein, g/l	5	0.61	46.10	1.35	0.07	44.44	47.90
Albumin, g/l	5	0.25	4.56	0.55	-1.98	3.60	4.90
Creatinine, µmol/l	5	0.34	47.12	0.77	1.53	46.40	48.40

Note: NVB – number of valid observations, SE – standard error, M – mean value, SD – standard deviation, A – asymmetry, Min – minimum value, Max – maximum value.

According to the results of biochemical studies of poultry blood plasma, it was found that the content of total protein in normotonics on day 60 ranged from 44.44 to 47.90 g/l with an average value of 46.10±1.35 g/l. The albumin content ranged from 3.60 to 4.90 g/l and had an average value of 4.56±0.55 g/l. The creatinine content in the experimental group of normotonics ranged from 46.40 to 48.40 µmol/L with an average baseline value of 47.12±0.77 µmol/L.

According to the results of biochemical analysis of blood plasma, the experimental group of sympathotonics had differences in the content of total protein and albumin with creatinine in contrast to other experimental groups (***Table 5***).

Table 5
Indicators of the protein fraction in the blood serum of poultry of the experimental group of sympathotonics at the age of 4.5 months (n=5)

Indicators.	NVB	SE	M	SD	A	Min	Max
Total protein, g/l	5	1.17	54.1	2.60	-1.70	49.7	56.1
Albumin, g/l	5	0.22	6.96	0,49	-0.26	6.40	7.50
Creatinine, µmol/l	5	2.53	47.92	5,66	0.96	41.20	56.90

Note: NVB – number of valid observations, SE – standard error, M – mean value, SD – standard deviation, A – asymmetry, Min – minimum value, Max – maximum value.

Based on the biochemical study of blood plasma of the experimental group of sympathotonics, it was found that total protein ranged from 49.70 to 56.10 g/l with an average value of 54.10±2.60 g/l. The albumin content in this bird ranged from 6.40 to 7.50 g/l and averaged 6.96±0.49 g/l. In this experimental group of sympathotonics, the creatinine content in the blood plasma was in the range of 41.20–56.90 µmol/L with an average value of 47.92±5.66 µmol/L.

The experimental group of vagotonics according to the results of biochemical studies had differences in the content of total protein, albumin and creatinine in the blood plasma (***Table 6***).

Table 6

Indicators of the protein fraction in the blood serum of poultry of the experimental group of vagotonics at the age of 4.5 months (n=5)

Indicators.	NVB	SE	M	SD	A	Min	Max
Total protein, g/l	5	0.86	56.30	1.90	-0.14	53.70	58.70
Albumin, g/l	5	0.17	7.67	0.38	0.50	7.25	8.20
Creatinine, µmol/l	5	0.34	50.54	0.76	0.19	49.60	51.50

Note: NVB – number of valid observations, SE – standard error, M – mean value, SD – standard deviation, A – asymmetry, Min – minimum value, Max – maximum value.

It was determined that in the experimental group of vagotonics, total protein values ranged from 53.70 to 58.70 g/l, which amounted to an overall average value of 56.30±1.90 g/l. According to the results of biochemical analysis, the albumin content ranged from 7.25 to 8.20 g/l and had an average value of 7.67±0.38 g/l. In the experimental group of vagotonics, the obtained globulin values ranged from 49.60 to 51.50 µmol/L and had an average value of 50.54±0.76 µmol/L.

The results of the analysis of total protein content revealed differences in the indicators among the experimental groups of animals with different tone of the autonomic nervous system. The experimental group of normotonics, whose indicators were the basis for comparison with other experimental groups of poultry, since they had a balanced effect of the sympathetic and parasympathetic nervous system, determined that the content of sympathotonics was 23.19 % higher (P<0.01), and in vagotonics the content was 18.38 % (P<0.01) (***Fig. 1***).

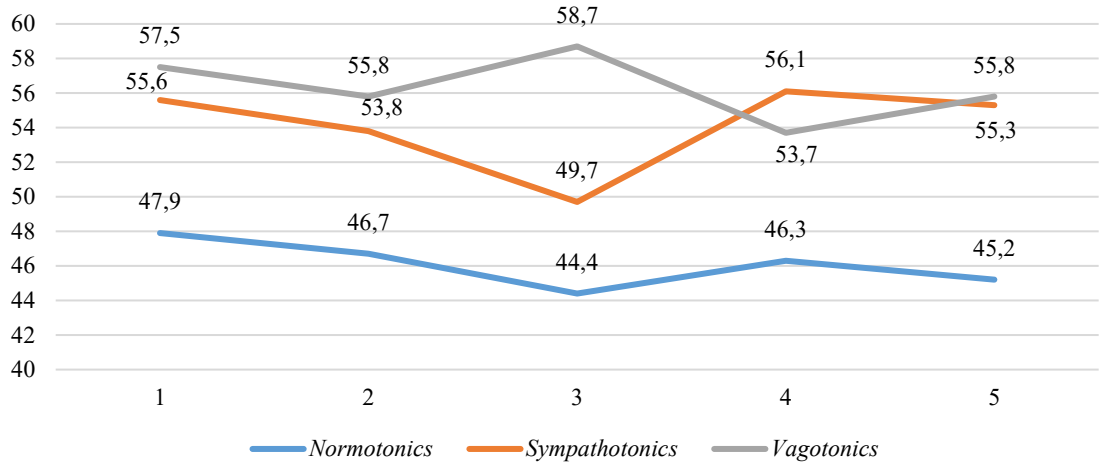

Figure 1. Total protein content in blood plasma of experimental poultry groups

The statistical analysis of the results of biochemical analysis of albumin content in poultry blood plasma revealed differences in the protein fraction. It was determined that the indicators in the experimental group of normotonics were 40.55 % lower than in the experimental group of sympathotonics (P<0.001). The experimental group of vagotonics had a higher content of albumin compared to normotonics by 1.5 times (P<0.01) (***Fig. 2***).

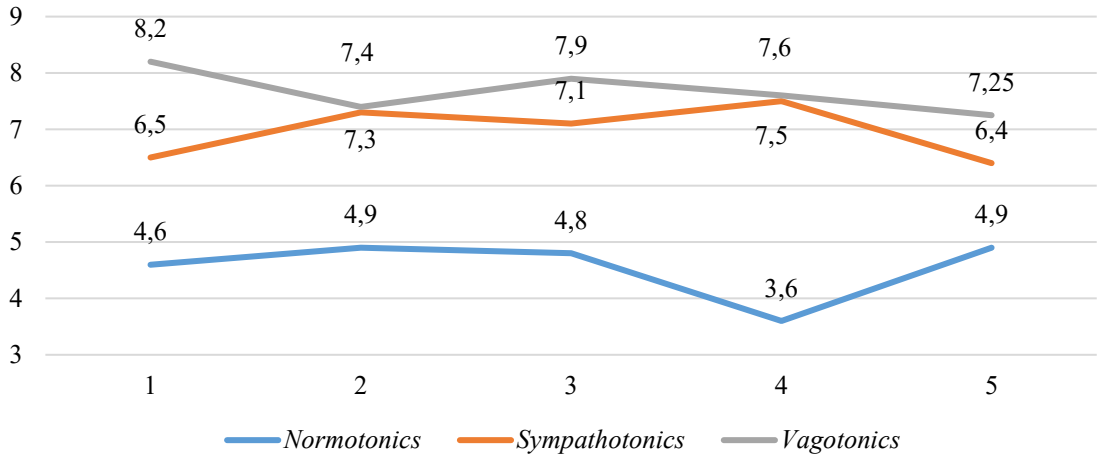

Figure 2. Albumin content in blood plasma of experimental groups of poultry

During the biochemical analysis of creatinine content, differences in indicators were found among the experimental groups of poultry with different tone of autonomic nervous regulation. Thus, in the experimental group of normotonics, creatinine levels at the age of 4.5 months were 6.77 % lower than in the experimental group of vagotonics (P<0.001) (***Fig. 3***).

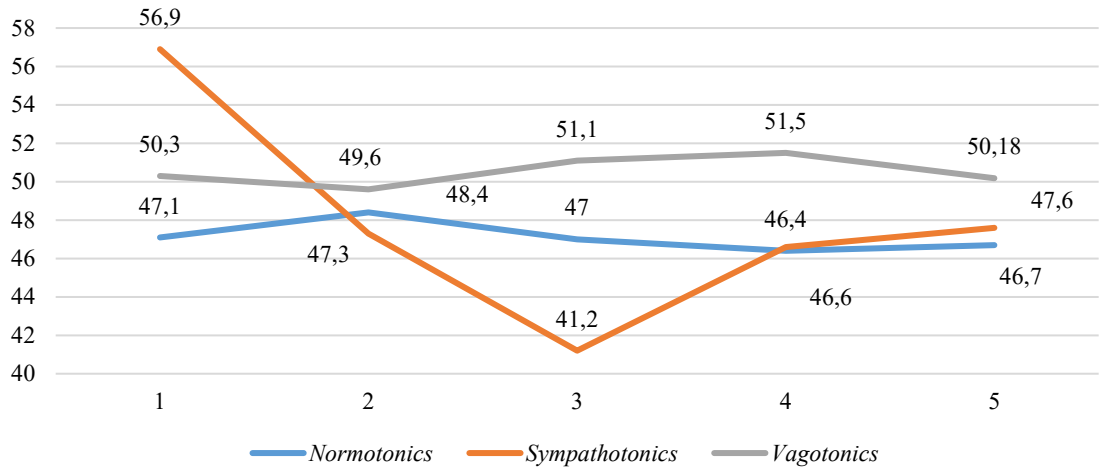

Figure 3. Globulin content in blood plasma of experimental poultry groups

Protein metabolism is very important for the poultry body. A sufficient amount of protein ensures a stable increase in muscle mass and productivity [14]. To assess protein metabolism, the best method is to evaluate biochemical parameters. Thanks to them, it is possible to better assess anabolic and catabolic processes, which will provide a clearer forecast in predicting poultry productivity [4, 6].

According to research, protein supplements with lower crude protein levels are often used to improve protein metabolism. The need to reduce the level of crude protein is necessary to prevent nitrogen imbalance, which is quite important for growing poultry that is growing rapidly [13, 15]. Also, due to the intensive metabolism of proteins in poultry and their significant accumulation in a small space, it causes the development of an environmental disaster. The reason for this is the growth of a large number of gases that negatively affect the ecological state of the planet [10, 11].

Summarizing the results, it was determined that depending on the individual characteristics of the animal's body, protein metabolism differs. This is evidenced by differences in the content of total protein, albumin, and creatine. Based on this, we can assert that the tone of the autonomic nervous system and protein metabolism processes are interdependent. Taking this into account, when studying protein metabolism and introducing new food additives into the poultry diet, it is necessary to divide animals according to the tone of the autonomic nervous system for better results.

Conclusions

It has been established that the tone of the autonomic nervous system has an effect on protein metabolism in the poultry body. The differences in the content of protein fractions of blood between experimental groups of animals, namely between normotonics, vagotonics and sympathotonics, were determined. Normotonics have the lowest levels of total protein, albumin and creatinine. Vagotonics have the highest protein content among the experimental groups of poultry. Taking into account the individual characteristics of the poultry organism, laying hens farms will be able to balance the feed ration more effectively, which will increase the productivity of the entire flock.

The prospect of further research is to study methods of correcting protein metabolism and the use of nano-aquahelates taking into account the tone of the autonomic nervous system.

Conflict of interest

The authors declare no conflict of interest.

References

1. Adeniyi, M. (2022). Impacts of Environmental Stressors on Autonomic Nervous System. *Autonomic Nervous System - Special Interest Topics*. https://doi.org/10.5772/intechopen.101842
2. Barzegar, S., Wu, S.-B., Choct, M., & Swick, R. A. (2020). Factors affecting energy metabolism and evaluating net energy of poultry feed. *Poultry Science*, 99 (1), 487–498. https://doi.org/10.3382/ps/pez554
3. Castro, F. L. de S., & Kim, W. K. (2020). Secondary functions of arginine and sulfur amino acids in poultry health: Review. *Animals*, 10 (11), 2106. https://doi.org/10.3390/ani10112106
4. Chrystal, P. V., Greenhalgh, S., McInerney, B. V., McQuade, L. R., Akter, Y., de Paula Dorigam, J. C., Selle, P. H., & Liu, S. Y. (2021). Maize-based diets are more conducive to crude protein reductions than wheat-based diets for broiler chickens. *Animal Feed Science and Technology*, 275, 114867. https://doi.org/10.1016/j.anifeedsci.2021.114867
5. Dal Bosco, A., Mattioli, S., Cartoni Mancinelli, A., Cotozzolo, E., & Castellini, C. (2021). Extensive rearing systems in poultry production: the right chicken for the right farming system. a review of twenty years of scientific research in Perugia University, Italy. *Animals*, 11 (5), 1281. https://doi.org/10.3390/ani11051281
6. Dankevych, N. I., Kovbasenko, V. M., Tarasenko, L. O., & Kushch, M. M. (2020). Effect of feed additives from marine hydrobionts on the protein metabolism condition in broiler chickens. *Ukrainian Journal of Ecology*, 10 (1), 339–343. https://doi.org/10.15421/2020_53

7. Geng, S., Huang, S., Ma, Q., Li, F., Gao, Y., Zhao, L., & Zhang, J. (2021). Alterations and correlations of the gut microbiome, performance, egg quality, and serum biochemical indexes in laying hens with low-protein amino acid-deficient diets. *ACS Omega*, 6 (20), 13094–13104. https://doi.org/10.1021/acsomega.1c00739
8. Greenhalgh, S., Chrystal, P. V., Selle, P. H., & Liu, S. Y. (2020). Reduced-crude protein diets in chicken-meat production: justification for an imperative. *World's Poultry Science Journal*, 76 (3), 537–548. https://doi.org/10.1080/00439339.2020.1789024
9. Gu, Y. F., Chen, Y. P., Jin, R., Wang, C., Wen, C., & Zhou, Y. M. (2021). Age-related changes in liver metabolism and antioxidant capacity of laying hens. *Poultry Science*, 100 (12), 101478. https://doi.org/10.1016/j.psj.2021.101478
10. Harlap, S. Y., Kadirov, N. N., Gorelik, L. S., Meshcheryakova, G. V., & Mukhamedyarova, L. G. (2021). Age-related variability of indicators of protein metabolism in the blood of laying hens. *IOP Conference Series: Earth and Environmental Science*, 677 (4), 042019. https://doi.org/10.1088/1755-1315/677/4/042019
11. Kim, C.-H., & Kang, H.-K. (2022). Effects of energy and protein levels on laying performance, egg quality, blood parameters, blood biochemistry, and apparent total tract digestibility on laying hens in an aviary system. *Animals*, 12 (24), 3513. https://doi.org/10.3390/ani12243513
12. LeBouef, T., Yaker, Z., & Whited, L. (2023). Physiology, Autonomic Nervous System. In *StatPearls*. StatPearls Publishing. Retrieved from: https://www.ncbi.nlm.nih.gov/books/NBK538516/
13. Liu, S. Y., Macelline, S. P., Chrystal, P. V., & Selle, P. H. (2021). Progress towards reduced-crude protein diets for broiler chickens and sustainable chicken-meat production. *Journal of Animal Science and Biotechnology*, 12 (1). https://doi.org/10.1186/s40104-021-00550-w
14. Nikravesh-Masouleh, T., Seidavi, A., Solka, M., & Dadashbeiki, M. (2021). Using different levels of energy and protein and their effects on bodyweight and blood chemistry of ostriches. *Veterinary Research Communications*, 45 (2–3), 129–139. https://doi.org/10.1007/s11259-021-09792-5
15. Qaid, M. M., & Al-Garadi, M. A. (2021). Protein and Amino acid metabolism in poultry during and after heat stress: a review. *Animals*, 11 (4), 1167. https://doi.org/10.3390/ani11041167
16. Sedgh-Gooya, S., Torki, M., Darbemamieh, M., Khamisabadi, H., Karimi Torshizi, M. A., & Abdolmohamadi, A. (2020). Yellow mealworm, *Tenebrio molitor* (Col: Tenebrionidae), larvae powder as dietary protein sources for broiler chickens: Effects on growth performance, carcass traits, selected intestinal microbiota and blood parameters. *Journal of Animal Physiology and Animal Nutrition*, 105 (1), 119–128. https://doi.org/10.1111/jpn.13434
17. Tomaszewska, E., Świątkiewicz, S., Arczewska-Włosek, A., Wojtysiak, D., Dobrowolski, P., Domaradzki, P., Świetlicka, I., Donaldson, J., Hułas-Stasiak, M., & Muszyński, S. (2020). Alpha-ketoglutarate: an effective feed supplement in improving bone metabolism and muscle quality of laying hens: a preliminary study. *Animals*, 10 (12), 2420. https://doi.org/10.3390/ani10122420
18. Wan, Y., Ma, R., Khalid, A., Chai, L., Qi, R., Liu, W., Li, J., Li, Y., & Zhan, K. (2021). Effect of the pellet and mash feed forms on the productive performance, egg quality, nutrient metabolism, and intestinal morphology of two laying hen breeds. *Animals*, 11 (3), 701. https://doi.org/10.3390/ani11030701
19. Wu, G. (2021). Nutrition and Metabolism: foundations for animal growth, development, reproduction, and health. *Recent Advances in Animal Nutrition and Metabolism*, 1–24. https://doi.org/10.1007/978-3-030-85686-1_1
20. Xin, Q., Ma, N., Jiao, H., Wang, X., Li, H., Zhou, Y., Zhao, J., & Lin, H. (2022). Dietary energy and protein levels during the prelay period on production performance, egg quality, expression of genes in hypothalamus-pituitary-ovary axis, and bone parameters in aged laying hens. *Frontiers in Physiology*, 13. https://doi.org/10.3389/fphys.2022.887381

ORCID

O. Kulbako https://orcid.org/0009-0003-3019-7748
V. Karpovsky https://orcid.org/0000-0003-3858-0111
O. Zhurenko https://orcid.org/0000-0002-4933-0372
I. Hryshchuk https://orcid.org/0000-0003-2571-6876
P. Karpovsky https://orcid.org/0009-0002-8113-423X
D. Krivoruchko https://orcid.org/0000-0003-1788-6090
A. Hryshchuk https://orcid.org/0000-0002-4608-337X