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Innovative feed additives in the optimization of laying hen diets

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The relevance of this research is determined by the need to improve the efficiency of protein utilization and reduce the cost of poultry products through the use of highly bioavailable protein and amino acid components, which corresponds to modern trends in sustainable and energy-efficient feeding. The study aimed to evaluate the effectiveness of complex enrichment of compound feeds with high-protein feed yeast (46%) and the "Progloat" additive (19%), which contains a balanced complex of amino acids, vitamins, and microelements, in ensuring optimal protein nutrition and maintaining physiological homeostasis in laying hens. This article presents a fragment of comprehensive research on the intensity of metabolic processes in laying hens under conditions of quantitative and qualitative modification of dietary protein, including the use of bioelements in the form of nanoaquacitrates and enzyme supplements to maximize digestible protein absorption and minimize feed conversion and nutrient emissions. The data presented focus on the effects of dietary correction on selected biochemical indicators in the blood and liver. It was found that the optimal feeding regime for Lohmann Brown laying hens included 19% of the innovative protein concentrate "Progloat" and 3.5% of high-protein (46%) feed yeast, with a simultaneous replacement of inorganic mineral premixes by citrate forms of trace elements (Zn, Mn, Fe, Cu, Co, Se, I) at 25% of their control diet content. Under such dietary correction, no negative dynamics of the serum biochemical profile were observed. The total protein content in the blood of all groups remained stable and depended mainly on protein quality rather than its quantity. The use of innovative protein supplements enhanced protein utilization and increased total hepatic protein and proteolytic enzyme activity. Additionally, the calcium concentration in the blood correlated with increased eggshell strength, which rose by 4.1% compared to the control. The practical value of this study lies in the scientific justification for introducing innovative protein additives into production diets to increase the biological value of feeds, improve product quality, and reduce resource consumption.

Keywords: blood; protein concentrate; feed yeast; microelement citrates.

Introduction

For the maximum realization of the genetic potential of high-yielding breeds and crosses of laying hens, all factors related to management, feeding, environment, and general health must operate at an optimal level (Underwood et al., 2021). It is well established that feed plays a decisive role in achieving this goal and has a substantial impact on production costs (Shim et al., 2013).

The modern poultry feeding system is based on balancing diets according to the content of essential nutrients (proteins, fats, carbohydrates) and biologically active substances (micro- and macroelements, vitamins, amino acids) (Weber, 2009; Mousavi et al., 2018). For laying hens, as for other types of poultry, dietary protein is a key nutrient (Bryden et al., 2021). The quality of protein nutrition depends not only on the total amount of protein consumed but also on the quantitative and proportional composition of amino acids in the diet, which is crucial for providing the bird with the plastic material required for normal synthesis of body, egg, and feather proteins (Bunchasak et al., 2005).

The amino acid profile of a diet can be balanced in several ways: by increasing the proportion of major protein feed components to the required level; by selecting and combining feed ingredients based on their amino acid composition; or by supplementing feed mixtures with synthetic or microbiologically produced amino acids (Liu et al., 2024). Overall, the poultry industry is interested in novel, easily digestible protein substrates for use in laying hen nutrition, as they contribute to higher productivity, improved feed conversion efficiency, and reduced nitrogen emissions to the environment.

At present, the evaluation of dietary protein value and the regulation of protein nutrition in poultry are still commonly based on crude protein (CP) content, i.e., the total nitrogen compounds per 100 g of complete feed (Pirgozliev et al., 2024). Crude protein remains one of the primary components of poultry diets and is essential for maintaining health, growth, productivity, and vital physiological processes. Compared with growing birds, laying hens have lower dietary CP requirements; thus, determining the optimal CP level in their diets to maximize performance or economic efficiency requires a better understanding of the protein needs of laying hens (Alagawany et al., 2016; Kralik et al., 2018).

The effect of dietary CP levels on egg production remains controversial. Some studies have reported that hens fed diets containing more than 16% CP produced more eggs than those receiving 14% or less (Meluzzi et al., 2001; Bunchasak et al., 2005; Abd El-Maksoud et al., 2011). Other studies, however, found no significant effect of CP content on egg production in diets containing 12–16% (Zeweil et al., 2011), 14–17% (Hsu et al., 1998), or even 16–20% CP (Junqueira et al., 2006). The feasibility of reducing dietary CP levels in laying hens to 15% is also supported by our previous studies. We found that lowering CP, while supplementing the diet with the innovative high-protein concentrate "Progloat" as a partial replacement for soybean meal, resulted in higher egg production and improved eggshell strength (Hunchak et al., 2023).

The level of crude protein in poultry diets can be reduced by supplementing feeds with crystalline amino acids. This approach enables the application of the "ideal protein" concept in feed formulation, thereby improving amino acid utilization and reducing nitrogen excretion (Keshavarz & Austic, 2004). However, when formulating such diets, it is essential to consider the potential influence of these additives on feed intake intensity in hens (Tavernari et al., 2013; Lelis et al., 2014). Of particular interest are studies investigating the effectiveness of incorporating high-protein (46%) feed yeast containing various amino acid profiles into the diets of laying hens (Fathima et al., 2023).

Mineral nutrition also plays an important role in poultry production. Certain bioelements directly or indirectly participate in vital biological processes in the body of laying hens, provide protection against stress factors, and affect both productivity and egg quality. To achieve the full

beneficial potential of mineral supplementation in laying hen diets, attention must be paid to the sources, dosages, and bioavailability of the added elements (Zafar & Fatima, 2018; Byrne et al., 2023).

For many years, micronutrient deficiencies in poultry diets have been compensated by the inclusion of bioelements in inorganic forms such as sulfates, chlorides, or carbonates within premixes. However, these inorganic compounds are characterized by relatively low absorption rates. Increasing their concentrations to achieve optimal assimilation in the organism may lead to toxicity in birds (Bao & Choct, 2009; Zafar & Fatima, 2018), as well as to excessive mineral excretion and environmental accumulation (Byrne & Murphy, 2022).

Therefore, finding ways to introduce biogenic elements into poultry diets in easily assimilable forms is of great practical importance. A promising direction is the use of organic mineral compounds produced via electro-explosive dispersion technology within the framework of nanotechnology. Microelements in aquacitrate form possess high biological activity, are better absorbed, and are more efficiently utilized in metabolic processes. Our previous studies confirmed the effectiveness of replacing inorganic mineral salts in guaranteed mineral premixes used in poultry feeds with a mixture of the same elements in nano-citrate form (Hunchak et al., 2018; Medvid et al., 2018; Hunchak et al., 2021). The amounts of organic forms of Fe, Co, Mn, Zn, Cu, I, and Se introduced into the diets were several times lower than the quantities of these same elements in inorganic form within traditional mineral premixes.

The aim of this study was to determine the effect of modifying the quantitative and qualitative composition of dietary protein, combined with the replacement of the inorganic mineral premix by a mixture of the same bioelements in nano-citrate form of nanotechnological origin, on the biochemical parameters of blood in laying hens.

Materials and methods

The housing, feeding, care, and all experimental manipulations with animals were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the General Ethical Principles of Experiments on Animals approved by the First National Congress on Bioethics (Kyiv, 2001). All experimental procedures were conducted in compliance with the principles of humanity stated in the Directive of the European Community.

The experiments were performed under vivarium conditions at the Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine (Lviv, Ukraine), taking into account results obtained in our previous studies. In particular, we previously confirmed the feasibility of including the sunflower-based protein concentrate "Progloat", an innovative product developed by specialists of the Dnipro Plant Potoky (Ukraine), in the diets of laying hens, and demonstrated its feeding efficiency compared to soybean meal (Hunchak et al., 2023). However, the potential for using this high-protein concentrate in combination with citrate forms of trace elements (as a replacement for the inorganic mineral premix) remains of scientific interest.

Laying hens of the Lohmann Brown breed, 150 days of age, were divided into three groups: one control (C) and two experimental groups (E1 and E2). The housing and feeding conditions complied with the technological requirements for cage rearing of laying hens. All birds received a complete balanced compound feed (CF) formulated to meet their nutrient and bioactive substance requirements. The diets differed in both the quantity and quality of crude protein (CP). Hens of the control group received feed containing 17% CP, whereas hens of the experimental groups were fed diets containing 15% CP. In groups E1 and E2, soybean oil and sunflower meal were partially replaced with the sunflower protein concentrate "Progloat" at 19.0% and 17.2%, respectively, and supplemented with high-protein (46%) feed yeast at 3.5% and 5.0%, respectively.

At the same time, the control group received a mineral premix (MP) containing Zn, Mn, Fe, Cu, Co, Se, and I in inorganic salt form, whereas in the experimental groups, this premix was replaced by a mixture of the same elements in citrate form, added at a level corresponding to 25% of their total content in the control diet. Phytase was added to the feed of all groups to enhance mineral utilization.

"Progloat" is a novel innovative product developed by specialists of the Dnipro Plant "Potoky" (Ukraine) and produced using nanotechnological processing of sunflower seeds. This technology ensures gentle denaturation without disrupting the primary protein structure or destroying amino acids. According to the developers, the digestibility coefficients of the sunflower protein concentrate are 90.6% for protein, 85.9% for fat, 9.7% for fiber, and 60% for nitrogen-free extractive substances (Hunchak et al., 2023).

The composition of the high-protein yeast included (g/kg): threonine – 3.99; serine – 4.19; proline – 3.44; glycine – 4.08; alanine – 4.70; valine – 4.93; isoleucine – 4.23; leucine – 7.65; tyrosine – 2.90; lysine – 7.24; phenylalanine – 4.06; cystine – 1.11; histidine – 1.94; arginine – 4.06; methionine – 2.11.

The mixture of citrate forms of bioelements was developed and kindly provided for this study by the researchers of LLC "Nanomaterials and Nanotechnologies" (Kyiv, Ukraine). The microelements, produced using nanotechnology, possess enhanced bioavailability and demonstrate efficiency at significantly lower doses compared with their inorganic and chelated analogs.

Throughout the experimental period, the physiological state of hens, feed intake, livability, and egg production were monitored. At the end of the experiment, the birds were slaughtered and biological material was collected for laboratory analysis. Serum samples were analyzed for total protein, albumin, cholesterol, triglycerides, urea, calcium, phosphorus, creatinine, and the enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) using a Humalyzer 2000 biochemical analyzer.

Statistical analysis of blood parameters was performed using a personal computer with the Statistica 7.0 software package (StatSoft Inc., USA). Graphical representations were created in Statistica 7 according to standard algorithms. The results are presented as arithmetic means and standard deviations ($x \pm SD$). Differences between control and experimental groups were evaluated using the Tukey test, with statistical significance accepted at $P < 0.05$.

Results

The concentration of total protein in the blood serum is a relatively stable indicator, which was also confirmed by the results of our study (Table 1). In the experimental groups, there was a clear trend toward an increase in total protein content compared with the control group: by 8.6% ($P < 0.05$) in the first experimental group and by 5.2% in the second. This indicates the activation of protein synthesis as a result of the improved amino acid composition of the feed and enhanced protein digestibility. All values remained within physiological limits, confirming the balanced nature of the diets. It can also be assumed that with a higher proportion of digestible protein in the diet, its utilization by the birds improves. Despite the fact that a certain portion of protein was excreted through egg production, the overall assimilation efficiency remained high; during the experimental period, the egg production of hens in groups E1 and E2 exceeded that of the control by 2.8% and 1.9%, respectively.

The albumin level in the first experimental group was slightly lower than in the control, which may be due to more intensive utilization of albumins as a plastic material for egg synthesis. In contrast, in the second experimental group, its concentration increased significantly ($P < 0.05$), indicating more efficient plasma protein synthesis and adequate amino acid supply.

Creatinine (57.82–59.17 µmol/L) and urea (2.09–2.12 mmol/L) concentrations did not differ statistically from the control group and remained within physiological ranges. This suggests the absence of any disturbances in liver or kidney function and confirms that the protein supplements used did not cause toxic metabolic load.

Triglyceride levels remained virtually unchanged, whereas the concentration of cholesterol in the blood of hens in both experimental groups was lower than in the control – by 9.0% and 12.7% ($P < 0.05$), respectively. This may indicate an intensification of lipid metabolism and more active utilization of lipids for yolk formation, which has a positive effect on the reproductive performance of laying hens. The content of inorganic phosphorus in the blood decreased from 5.31 to 4.62 mmol/L

(P < 0.05), while the calcium concentration, on the contrary, increased from 6.03 to 6.92 mmol/L (P < 0.05). The elevated calcium level corresponded with the improvement in eggshell strength observed in the experimental groups, indicating more efficient absorption of macroelements and a balanced calcium–phosphorus metabolism.

Table 1
Biochemical parameters of blood serum of laying hens under changes in the quantitative and qualitative composition of dietary protein (x ± SD, n = 5)

Indicator	Group of hens		
	control	experimental 1	experimental 2
Total protein, g/L	54.9 ± 2.0^{a}	59.7 ± 1.6^{b}	57.8 ± 2.5^{ab}
Albumin, g/L	22.50 ± 0.64^{ab}	19.93 ± 1.17^{a}	23.89 ± 0.64^{b}
Creatinine, µmol/L	57.5 ± 1.7^{a}	57.8 ± 1.3^{a}	59.2 ± 1.2^{a}
Urea, mmol/L	2.10 ± 0.07^{a}	2.12 ± 0.05^{a}	2.09 ± 0.07^{a}
Triglycerides, mmol/L	10.27 ± 0.15^{a}	10.35 ± 0.12^{a}	10.23 ± 0.11^{a}
Cholesterol, mmol/L	4.01 ± 0.15^{b}	3.65 ± 0.12^{a}	3.50 ± 0.16^{a}
Phosphorus, mmol/L	5.31 ± 0.07^{b}	4.90 ± 0.10^{ab}	4.62 ± 0.08^{a}
Calcium, mmol/L	6.03 ± 0.12^{a}	6.92 ± 0.09^{b}	6.63 ± 0.11^{ab}
Alanine aminotransferase, U/L	18.20 ± 1.01^{b}	16.87 ± 0.92^{a}	18.14 ± 0.84^{b}
Aspartate aminotransferase, U/L	303.4 ± 9.3^{b}	270.3 ± 8.0^{a}	289.5 ± 9.3^{ab}
Alkaline phosphatase, U/L	498 ± 21^{a}	589 ± 27^{b}	522 ± 20^{ab}

Note: letters indicate significant differences between the groups within one line (P < 0.05) according to the Tukey test.

The activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) reflects the intensity of protein and energy metabolism, as well as the functional state of the liver in hens. According to the obtained data, ALT activity in the blood remained stable across all groups and did not differ significantly from the control values, indicating normal transamination processes. At the same time, AST activity in hens of the first experimental group decreased by 20% (P < 0.05) compared with the control, which may suggest a reduction in the intensity of catabolic processes in the liver and a more efficient utilization of amino acids in anabolic pathways. In the second experimental group, AST activity was also lower than in the control, although the difference did not reach statistical significance.

However, the De Ritis ratio did not change significantly and remained within the reference range. It was 16.67 in the control group and 16.02 and 15.96 in hens of the first and second experimental groups, respectively.

The activity of serum alkaline phosphatase (ALP) is determined by the intensity of metabolic processes occurring in various organs, from which the enzyme is released into the bloodstream. An increase in ALP activity within physiological limits is often associated with enhanced calcium and phosphorus metabolism between bone tissue and the organism. The activity of this enzyme typically rises in animals during periods of intensive growth and development, and in laying hens – during egg formation and oviposition.

In our study, a significant increase in ALP activity (P < 0.01) was observed in the blood of hens from the first experimental group compared with the control. This may be due to an adaptive physiological response associated with increased egg production and improved eggshell strength. Alkaline phosphatase plays an essential role in eggshell formation, facilitating the transport of calcium ions. Therefore, the elevated ALP activity observed in laying hens likely reflects the activation of calcium metabolism processes occurring during the period of eggshell mineralization and egg laying.

It was found that the simultaneous inclusion of the "Progloat" additive (19%) and high-protein feed yeast (3.5%) in the diets of hens in the first experimental group resulted in an increase in total protein content in liver tissues by 6.7% (P < 0.05) compared with the values observed in the control group (Table 2).

Similar results were obtained in the study of proteolytic enzyme activity in the liver. In particular, these findings indicate the absence of protease inhibitors in the experimental compound feeds. It was established that proteolytic activity was significantly higher (P < 0.01–0.05) – by 16.5% and 14.8%, respectively – in the liver of hens from both experimental groups compared with the control group.

Table 2
Individual indicators of protein metabolism in the liver tissues of laying hens (x ± SD, n = 5)

Indicator	Group of hens		
	control	experimental 1	experimental 2
Soluble proteins, g/kg	63.5 ± 1.2^{a}	67.8 ± 1.1^{b}	65.9 ± 1.9^{ab}
Proteolytic enzyme activity, µkat/g protein	3.44 ± 0.08^{a}	4.01 ± 0.14^{b}	3.95 ± 0.20^{b}
Amino nitrogen, mg/g	2.78 ± 0.05^{ab}	2.63 ± 0.03^{a}	2.89 ± 0.04^{b}
Alanine aminotransferase, U/g tissue	3.90 ± 0.20^{a}	$4,10 \pm 0.20^{b}$	4.10 ± 0.18^{b}
Aspartate aminotransferase, U/g tissue	15.97 ± 0.16^{a}	16.03 ± 0.22^{a}	16.23 ± 0.16^{b}

The increase in protein content in the liver tissues of experimental birds may also be attributed to the elevated proteolytic activity, as hydrolytic digestive enzymes themselves are proteins. In our study, a decrease in the content of amino nitrogen in the liver tissues of laying hens from the first experimental group by 5.4% (P < 0.05) was observed. Such results may be associated with the intensive transport of amino acids from the liver to the oviduct, where they participate in the synthesis of specific egg proteins.

The total content of free amino acids in the blood and tissues of birds is used to assess the intensity of digestion and the breakdown of feed nutrients. It is evident that the changes observed in the studied parameters are related to the composition of the diet, rather than to the overall crude protein content in the compound feed.

Discussion

Blood serves as a mediator in all metabolic processes and maintains constant contact (through interstitial fluid) with all organs and tissues, reflecting all internal physiological changes both quantitatively and qualitatively (Ostapyuk & Gutyj, 2018). The study of changes in the biochemical parameters of blood serum is therefore of great importance, particularly in relation to feed quality and the productivity of laying hens. Alterations in protein metabolism are a key objective indicator of the physiological state of an organism, both under normal and pathological conditions, as proteins play an essential role in the formation of enzymatic and hormonal systems. Consequently, any changes in their concentration in the blood affect the overall metabolism of the animal (Kim & Kang, 2022).

The results of the present study indicate that the use of innovative protein-based feed additives contributed to the optimization of protein metabolism and the maintenance of metabolic homeostasis in laying hens. The concentration of total serum protein remained within physiological limits and showed no significant intergroup differences, which is consistent with the findings of other researchers (Gutyj et al., 2019; Ostapyuk & Gutyj, 2020). This confirms the stability of protein metabolism under conditions of increased digestible protein in the compound feed. The improvement in protein utilization is likely associated with a more balanced amino acid profile of the diet, particularly through the optimization of lysine, methionine, and threonine ratios – amino acids known to be limiting in the nutrition of laying hens.

The increased total protein content and higher proteolytic enzyme activity in liver tissues suggest an activation of anabolic processes in hepatocytes. The absence of protease inhibitors in the compound feed confirms the high biological value of the protein components used. In contrast, the observed decrease in amino nitrogen levels in the liver may result from the intensive transport of amino acids to the oviduct, where they participate in the synthesis of specific egg proteins. Thus, hens in the experimental groups exhibited a well-balanced interaction between hepatic protein metabolism and reproductive organ function.

The final products of protein catabolism in the avian organism are ammonia, uric acid, and nitrogen-containing compounds such as creatine, creatinine, and others. Ammonia, being toxic to the body, is detoxified in the liver, where it is converted into urea that is excreted with urine. The concentration of urea in the blood serum of birds indicates the functional state of the kidneys' excretory capacity and the liver's detoxification activity (Simoyi et al., 2002). Among the key diagnostic indicators of the lipid profile are serum triglyceride concentrations.

The synthesis of adipose tissue, fat deposition, and yolk formation in domestic birds depend on their levels in the bloodstream.

The intensity of protein metabolism in various tissues can be evaluated by studying the activity of aminotransferases, which transfer amino groups from amino acids to keto acids, and alkaline phosphatase, which participates in protein synthesis. It is known that rapid mobilization of protein components to meet energy demands – under conditions such as insufficient or imbalanced feeding and various stress factors – is associated with adaptive, hormone-stimulated biosynthesis of specific aminotransferases, primarily those involved in gluconeogenesis, including aspartate and alanine aminotransferases, as well as aminotransferases of aromatic amino acids. Fluctuations in enzyme activity in the blood serum of laying hens are thought to be associated with the physiological state of the organism, its structural features, and even the level of egg production. Therefore, determining aminotransferase activity is important for an objective assessment of the physiological state of birds and, consequently, the balance of their feeding.

The content and ratio of essential amino acids (lysine, methionine, tryptophan, threonine, arginine, leucine, isoleucine, phenylalanine, cystine) – which cannot be synthesized by birds – are critical for protein synthesis in their bodies. According to various authors, the most deficient or limiting amino acids in modern poultry diets are lysine, methionine, and threonine, or alternatively, cystine, tryptophan, and arginine (Frikha et al., 2009; Duarte et al., 2010; Duarte et al., 2013). It should be noted that feeds of animal origin contain more essential amino acids than plant-based ones. A deficiency, absence, or imbalance of essential amino acids in poultry diets leads to impaired protein utilization, disruption of metabolic processes, and reduced productivity (Goulart et al., 2011; Ostapyuk & Gutyj, 2020; Kidd et al., 2021).

Thus, the combined inclusion of high-protein feed yeast (46%) and the "Progloat" additive in the diets of laying hens had a beneficial effect on metabolic processes, hepatic enzyme activity, and reproductive function. The obtained results are consistent with modern research data (Bashchenko et al., 2010; King et al., 2013; Gutyj et al., 2019) and confirm the feasibility of using amino acid–enriched feed additives to improve productivity and the biological efficiency of poultry feeding.

Conclusion

It was established that the optimal feeding strategy for Lohmann Brown laying hens involves the inclusion of the innovative protein concentrate "Progloat" at a level of 19% and high-protein feed yeast (46%) at 3.5%, along with the replacement of the mineral (inorganic) premix by a citric form of the same trace elements (Zn, Mn, Fe, Cu, Co, Se, I) at 25% of their content compared to the control group's diet. Under such dietary correction, no negative changes were observed in the biochemical profile of the hens' blood serum. At the same time, it was shown that the total protein content in the blood of hens across all groups remained at a similar level and depended not on the quantity of consumed protein but rather on its quality. Thus, an intensification of protein assimilation by the birds was observed, even alongside increased egg production, as confirmed by the elevated levels of total proteins and proteolytic enzyme activity in the liver. An increase in calcium concentration ($P < 0.05$) in the blood serum of hens correlated with the eggshell strength index, which rose by 4.1% ($P < 0.01$) compared with the control, indicating enhanced mineral metabolism and improved eggshell quality.

The authors declared no conflicting interests.

References

Abd El-Maksoud, A., El-Sheikh, S. E. M., Salama, A. A., & Khidr, R. E. (2011). Performance of local laying hens as affected by low protein diets and amino acids supplementation. Egyptian Poultry Science Journal, 31, 249–258.

Alagawany, M., El-Hack, M. E. A., Farag, M. R., Ruchi, T., Swati, S., Kumaragurubaran, K., & Kuldeep, D. (2016). Positive and negative impacts of dietary protein levels in laying hens. Asian Journal of Animal Sciences, 10(2), 165–174.

Alkhalf, A., Alhaj, M., & Al-Homidan, I. (2010). Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens. Saudi Journal of Biological Sciences, 17(3), 219–225.

Bao, Y. M., & Choct, M. (2009). Trace mineral nutrition for broiler chickens and prospects of application of organically complexed trace minerals: A review. Animal Production Science, 49(4), 269–282.

Bashchenko, M. I., Boiko, O. V., Honchar, O. F., Gutyj, B. V., Lesyk, Y. V., Ostapyuk, A. Y., Kovalchuk, I. I., & Leskiv, K. Y. (2020). The effect of milk thistle, metiphen, and silimevit on the protein-synthesizing function of the liver of laying hens in experimental chronic cadmium toxicosis. Ukrainian Journal of Ecology, 10(6), 164–168.

Bryden, W., Li, X., Ruhnke, I., Zhang, D., & Shini, S. (2021). Nutrition, feeding and laying hen welfare. Animal Production Science, 61(10), 893–914.

Bunchasak, C., Poosuwan, K., Nukraew, R., Markvichitr, K., & Choothesa, A. (2005). Effect of dietary protein on egg production and immunity responses of laying hens during peak production period. Journal of Poultry Science, 4(9), 701–708.

Byrne, L., Ross, S., Taylor-Pickard, J., & Murphy, R. (2023). The effect of organic trace mineral supplementation in the form of proteinates on performance and sustainability parameters in laying hens: A meta-analysis. Animals, 13, 3132.

Duarte, F. D., Lara, L. J., Baião, N. C., Cançado, S. V., & Teixeira, J. L. (2010). Efeito da inclusão de diferentes fontes lipídicas em dietas para frangos de corte sobre o desempenho, rendimento e composição da carcaça. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 62(2), 439–444.

Duarte, K. F., Junqueira, O. M., Filardi, R. S., Siqueira, J. C., Puzotti, M. M., Garcia, E. A., & Laurentiz, A. C. (2013). Digestible tryptophan requirements for broilers from 22 to 42 days old. Revista Brasileira de Zootecni, 42(10), 728–733.

Fathima, S., Shanmugasundaram, R., Sifri, M., & Selvaraj, R. (2023). Yeasts and yeast-based products in poultry nutrition. Journal of Applied Poultry Research, 32(2), 100345.

Frikha, M., Safaa, H. M., Serrano, M. P., Arbe, X., & Mateos, G. (2009). Influence of the main cereal and feed form of the diet on performance and digestive tract traits of brown-egg laying pullets. Poultry Science, 88(1), 994–1002.

Goulart, C. C., Costa, F. G. P., Silva, J. H. V., Souza, J. G., Rodrigues, V. P., & Oliveira, C. F. S. (2011). Require-ments of digestible methionine + cystine for broiler chickens at 1 to 42 days of age. Revista Brasileira de Zootecnia, 40(4), 797–803.

Gutyj, B., Ostapiuk, A., Kachmar, N., Stadnytska, O., Sobolev, O., Binksevych, V., Petryshak, R., Petryshak, O., Kulyaba, O., Naumyuk, A., Nedashkivsky, V., Nedashkivska, N., Magrelo, N., Golodyuk, I., Nazaruk, N., & Binkevych, O. (2019). The effect of cadmium loading on protein synthesis function and functional state of laying hens' liver. Ukrainian Journal of Ecology, 9(3), 222–226.

Hsu, J. C., Lin, C. Y., & Chiou, P. W. S. (1998). Effects of ambient temperature and methionine supplementation of a low protein diet on the performance of laying hens. Animal Feed Science Technology, 74(4), 289–299.

Hunchak, A., Medvid, S., Stefanyshyn, O., Sirko, Y., & Koretchuk, S. (2021). Microelement (Mn, Zn, Cu, Fe, Co, I) levels in quails and their eggs according to their forms and dosage in poultry diet. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Agricultural Sciences, 23(94), 50–55.

Hunchak, A. V., Sirko, Y. M., Kyryliv, B. Y., Stefanyshyn, O. M., Ratych, I. B., Hunchak, V. M., & Gutyj, B. V. (2023). Intensity of metabolic processes in laying hens due to changes in the qualitative and quantitative composition of protein in the diet. Colloquium-Journal, 163, 40–46.

Hunchak, A. V., Medvyd, S. M., & Syrko, Y. M. (2018). Intensyvnist proteinvoho obminu v orhanizmi perepilok ta yikhnia nesuchist za vykorystannia mikroelementnykh dobavok do ratsioniv [Intensity of protein metabolism in quails and their egg production under the application of micro-element supplements]. Taurida Scientific Herald, 102, 94–99 (in Ukrainian).

Junqueira, O. M., de Laurentiz, A. C., da Silva Filardi, R., Rodrigues, E. A., & Casartelli, E. M. C. (2006) Effects of energy and protein levels on egg quality and performance of laying hens at early second production cycle. Journal of Applied Poultry Research, 15, 110–115.

Keshavarz, K., & Austic, R. E. (2004). The use of low-protein, low-phosphorus, amino acid- and phytase-supplemented diets on laying hen performance and nitrogen and phosphorus excretion. Poultry Science, 83(1), 75–83.

Kidd, M. T., Maynard, C. W., & Mullenix, G. J. (2021). Progress of amino acid nutrition for diet protein reduction in poultry. Journal of Animal Science and Biotechnology, 12(1), 45.

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.

King, E. J., Witt, F. H., Merwe, H. J., Hugo, A., & Fair, M. D. (2013). The effect of lipid saturation on nutrient di-gestibility of layer diets. South African Journal of Animal Science, 43(5), 130–134.
Kralik, G., Kralik, Z., Grčević, M., Kralik, I., & Gantner, V. (2018) Enrichment of table eggs with functional ingredients. Journal of Central European Agriculture, 19(1), 72–82.
Lelis, G. R., Albino, L. F. T., Tavernari, F. C., Calderano, A. A., Rostagno, H. S., Barros, V. R. S. M., & Maia, R. C. (2014). Digestible valine-todigestible lysine ratios in brown commercial layer diets. Journal of Applied Poultry Research, 23, 683–690.
Liu, M., Xia, Z.-Y., Li, H.-L., Huang, Y.-X., Refaie, A., Deng, Z.-C., & Sun, L.-H. (2024). Estimation of protein and amino acid requirements in layer chicks depending on dynamic model. Animals, 14(5), 764.
Medvid, S. M., Hunchak, A. V., Stefanyshyn, O. M., & Pashchenko, A. G. (2018). The impact of microelement nanoparticle citrates on protein metabolism intensity in tissues of broiler chicken and their productivity. The Animal Biology, 20(2), 58–64.
Meluzzi, A., Sirri, F., Tallarico, N., & Franchini, A. (2001) Nitrogen retention and performance of brown laying hens on diets with different protein content and constant concentration of amino acids and energy. British Poultry Science, 42(2), 213–217.
Mousavi, S. N., Afsar, A., Khalaji, S., & Abbasi, M. (2018). Estimation of digestible tryptophan:lysine ratios for maximum performance, egg quality and welfare of white-egg-laying hens by fitting the different non-linear models. Journal of Applied Animal Research, 46(1), 411–416.
Ostapyuk, A. Y., & Gutyj, B. V. (2020). Influence of milk thistle, methifene and sylimevit on the morphological parameters of laying hens in experimental chronic cadmium toxicosis. Ukrainian Journal of Veterinary and Agricultural Sciences, 3(1), 42–46.
Ostapyuk, A., & Gutyj, B. (2018). Influence of cadmium loading on morphological parameters of blood of the Laying Hens. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, Series: Veterinary Sciences, 20(88), 48–52.
Pirgozliev, V. R., Whiting, I. M., Mansbridge, S. C., Abdulla, J. M., & Rose, S. P. (2024). Consideration of dietary protein level for laying hen production and egg quality during storage. Journal of Central European Agriculture, 25(1), 47–54.
Shim, M., Song, E., Billard, L., Aggrey, S., Pesti, G., & Sodsee, P. (2013). Effects of balanced dietary protein levels on egg production and egg quality parameters of individual commercial layers. Journal of Poultry Science, 92(10), 2687–2696.
Simoyi, M. F., Van Dyke, K., & Klandorf, H. (2002). Manipulation of plasma uric acid in broiler chicks and its effect on leukocyte oxidative activity. American Journal of Physiology – Regulatory, Integrative and Comparative Physiology, 282(3), R791–R796.
Tavernari, F. C., Lelis, G. R., Vieira, R. A., Rostagno, H. S., Albino, L. F. T., & Neto, A. R. O. (2013). Valine needs in starting and growing Cobb (500) broilers. Journal of Poultry Science, 92(1), 151–157.
Tóthová, C., Sesztáková, E., Galiková, B., Glembová, V., Oršuľaková, V., & Nagy, O. (2024). Changes in serum protein profile in laying hens housed in a cage-free system. Veterinary Medicine International, 2024, 4135744.
Underwood, G., Andrews, D., & Phung, T. (2021). Advances in genetic selection and breeder practice improve commercial layer hen welfare. Animal Production Science, 61(10), 856–866.
Weber, G. M. (2009). Improvement of flock productivity through supply of vitamins for higher laying performance and better egg quality. World's Poultry Science Journal, 65(3), 443–458.
Zafar, M. H., & Fatima, M. (2018). Efficiency comparison of organic and inorganic minerals in poultry nutrition: A review. PSM Veterinary Research, 3(2), 53–59.
Zeweil, H. S., Abdalah, A. A., Ahmed, M. H., & Marwa, R. S. A. (2011). Effect of different levels of protein and methionine on performance of Baheij laying hens and environmental pollution. Egyptian Poultry Science Journal, 31, 621–639.