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Evaluation of the effect of enzymatic plant hydrolysate on the organoleptic, biochemical and microbiological parameters of laying hens' eggs

V.M. Bachinskaya[1*], *F.I.* Vasilevich[2], *D.V.* Gonchar[2], *N.A.* Bachinskaya[2] and *A.V.* Sharkova[1]

[1]Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, 49, Timiryazevskaya st., Moscow, 127434, Russia
[2]Moscow State Academy of Veterinary Medicine and Biotechnology – MVA named after K.I. Skryabin, 23, Academician Scriabin st., Moscow, 109472, Russia

Abstract. The paper presents the results of a study investigating the effects of a feed additive based on an enzymatically hydrolyzed soy protein solution on the morphometric, amino acid, and microbial parameters of laying hen eggs under industrial rearing conditions. Changes in egg weight, albumen, yolk mass, and shell thickness in both experimental and control groups over the course of the experiment were assessed along with the amino acid composition of chicken eggs. A positive yet non-statistically significant ($p > 0.05$) trend towards increased morphometric indices influenced by the additive was observed. These findings align with the hypothesized mode of action of the additive, potentially reflecting enhanced anabolic metabolism through easily accessible peptides and amino acids. Statistically significant increases ($p < 0.05$) were noted in the overall levels of essential, semi-essential, and dispensable amino acids, such as asparagine, glutamine, and cystine, directly indicating elevated biological value of egg products. Enrichment of the amino acid profile was attributed to the high bioavailability of the additives' peptide components, which are quickly absorbed in the digestive system and efficiently incorporated into biosynthetic pathways, circumventing energy-intensive enzyme-mediated degradation steps. Application of the Abiopeptide supplementation resulted in improvements in egg quality without adversely affecting sensory attributes or microbial safety. Conclusively, it is suggested that this type of feed additive may serve as an efficient means to optimize protein-mineral balance in laying hens, enhancing nutritional value while minimizing economic losses associated with egg production.

* Corresponding author: bachinskaya1980@mail.ru

1 Introduction

Enhancing product quality remains a top priority in contemporary poultry farming. Modern layer strains under intensive industrial conditions demonstrate high annual productivity, where balanced rations become critical factors ensuring sustained egg yield and product quality [1,2].

Increasing prices of conventional feed ingredients prompt the need for alternative feeding strategies focused on reducing production expenses while preserving poultry performance [3]. One promising direction lies in optimizing amino acid nutrition via feed supplements designed to enhance protein utilization [3–5].

Egg quality relies heavily on both husbandry practices and the nutritional composition of feeds, specifically amino acid and mineral balances [6, 7]. Common challenges in poultry management involve dietary deficiencies in protein and minerals necessary for optimal metabolic functioning. High-producing hybrids exhibit heightened sensitivity to these imbalances given their higher nutrient demands. Additionally, intense egg production imposes greater strain on the bird's physiology, requiring adequate supplies of nutrients beyond mere energy sources [8].

Amino acids are central to avian nutrition, directly impacting growth rates, reproduction capabilities, and general health status. Supplementation with indispensable amino acids like methionine, lysine, threonine, and tryptophan supports enhanced egg output and immune response stimulation. Despite overall benefits from optimized amino acid profiles, certain studies report no substantial alterations in individual egg component weights (albumen and yolk) [3–5]. This discrepancy might arise from differential absorption efficiencies between intact dietary proteins versus free-form amino acids. Traditional methods of amino acid optimization often overlook morphometric parameters, whereas employing enzymes capable of improving amino acid accessibility could present new possibilities. Modifying the amino acid profile within the diet can subsequently influence the amino acid composition of the egg itself, thereby elevating its biological worth as a food source. Enhancements in egg protein quality, alongside maintenance of stable morphometric traits, hold considerable practical relevance since they boost nutritional values without sacrificing productivity—a crucial consideration for large-scale commercial poultry operations [9].

Deficiency in essential amino acids or reliance on indigestible protein sources impedes protein synthesis and diminishes the biological efficacy of the final product. Protein synthesis rates in birds depend largely upon amino acid availability rather than gross dietary protein concentrations [4]. Nitrogenous compounds delivered either as whole proteins, free amino acids, or shorter peptides significantly influence how efficiently nutrients are utilized [10].

Modern feeding paradigms emphasize the "ideal protein" model, focusing on essential amino acid sufficiency while permitting reductions in crude protein levels. Feed additives rich in readily absorbable peptides, amino acids, and biologically active molecules facilitate diet adjustment and improve protein utilization efficiency [3,4]. Among promising solutions are enzymatically-hydrolyzed protein derivatives providing amino acids in highly digestible formats [7]. Notably, the feed supplement "Abiopeptid," developed by A-BIO, incorporates a blend of low-molecular-weight peptides and free amino acids with a reported hydrolysis level near 45%. As per manufacturers' specifications, the formulation comprises roughly 20% free amino acids, about 30% dipeptides, another 30% tripeptides, and the remaining fraction (~20%) constitutes larger polypeptide chains.

Enzyme-assisted processing preserves all essential amino acids while transforming much of the protein matrix into smaller di- and tripeptides. Such low-molecular-weight peptides undergo rapid intestinal uptake facilitated by dedicated transport mechanisms, enabling direct entry into enterocytes without prior complete hydrolysis into free amino acids. Consequently, these compounds expedite incorporation into tissue-building and egg-synthesis processes, thus augmenting overall protein synthesis efficiency [9].

The use of such supplements can contribute to regulating protein and mineral metabolism. Enzyme-based additives can enhance the absorption not only of protein but also of minerals, notably calcium and phosphorus. This potential improvement affects egg productivity, shell integrity, and biological value. It is important to assess not just productive metrics but also the quality characteristics of the resultant product, its consumer safety, and the economic feasibility of supplement application [2].

Although there exists extensive research on the roles of amino acids and minerals in determining egg quality, the influence of low-molecular-weight peptide complexes on the amino acid composition of eggs and shell strength measures remains inadequately explored. Hence, it becomes relevant to investigate the impact of a feed supplement derived from an enzymatic plant (soy protein) hydrolysate solution (“Abiopeptid”) on the morphometric, amino acid, and microbiological parameters of laying hens' eggs.

2 Materials and methods

The experimental section of the work took place at the Department of Parasitology and Veterinary-Sanitary Expertise of the K.I. Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology.

Forty clinically healthy laying hens belonging to the SP-789 breed, aged 120 days old, were used in the study. These hens were raised in cages that fully complied with industry-specific requirements for keeping birds intended for egg production. Unlimited access to feed and water was ensured, and environmental conditions in the enclosure were maintained according to established veterinary and zoological standards.

For feeding purposes, the hens received a complete mixed pelletized feed known as PK-1, suitable for broodstock chickens (complying with Russian standard GOST 18221-2018). The composition of the feed comprised mainly wheat (63.922%), sunflower meal (17.55%), limestone as a source of calcium ions (7.88%), full-fat soybeans (5.43%), meat-and-bone meal (4.0%), agro-premix (0.5%), lysine sulfate (0.273%), sodium chloride (table salt, 0.264%), methionine (0.086%), choline chloride (0.08%), and fecord (0.015%).

Using a random allocation technique, the birds were divided into two equal groups of twenty individuals each: one serving as the control group and the other designated as the experimental group. During the 30-day trial period, the experimental group received a novel feed additive, "Abiopeptid," dissolved in their drinking water at a dosage of 1.0 milliliter per liter every day. Meanwhile, the control group continued receiving plain water without any added substances.

Prior to commencing the trial and again immediately afterward, detailed evaluations of various morphometric characteristics of the laid eggs were undertaken, focusing particularly on assessing egg weight and shell thickness. All recorded data were presented in absolute numerical terms and as comparative ratios against those from the control group. Furthermore, the veterinary-sanitary assessment of the collected eggs strictly conformed to the technical regulations stipulated in Russian Standard GOST 31654-2012.

To analyze the statistical significance of the gathered information, we applied the widely recognized Student's t-test methodology for comparing independent samples. Only variations demonstrating p-values below 0.05 were regarded as statistically meaningful.

3 Results

Organoleptic studies of chicken eggs are presented in table 1.

Table 1. Results of organoleptic studies of chicken eggs, n=10.

Indicator	Experimental group	Control group
Air chamber condition and its height	Fixed, air chamber height 3.0 mm	
Condition and position of the yolk	Durable, barely visible, contours are not visible, occupies a central position and does not move	
Protein density and color	Dense, light, transparent	
Tasting assessment of chicken eggs, points	5.0	5.0
Weight of one egg, g	58.02±3.21	57.01±2.58
% to control	101.8	100
Weight of egg without shell, g	49.79±1.99	48.11±1.34
% to control	103.5	100
Protein weight, g	34.98±2.25	33.09±3.32
Yolk weight, g	16.05±1.05	15.03±1.64
Eggshell thickness, µm	0.382±0.044	0.370±0.037

Based on the organoleptic assessment of chicken eggs, all examined samples satisfied the criteria set forth for fresh dietary eggs: the air chamber remained stationary, the yolk was centrally positioned, and the albumen exhibited proper firmness. Taste evaluation scores for hard-boiled eggs from both groups yielded maximum ratings of five points, suggesting that the tested feed additive "Abiopeptid" did not negatively influence the flavor qualities of the product.

Morphometric parameter analyses indicated a tendency toward increased egg weights among the experimental group hens. Specifically, mean egg weight rose by 1.8%, shell-free egg weight increased by 3.5%, albumen weight grew by 5.7%, and yolk weight augmented by 6.8%. Moreover, shell thickness surpassed control values by 3.2%.

In particular, the average egg weight in the experimental group was 58.02 ± 3.21 g versus 57.01 ± 2.58 g in the control, protein weight was 34.98 ± 2.25 g versus 33.09 ± 3.32 g, yolk weight was 16.05 ± 1.05 g versus 15.03 ± 1.64 g, and shell thickness was 0.382 ± 0.044 mm versus 0.370 ± 0.037 mm. The absence of statistically significant differences despite a steady positive trend may be related to the relatively small sample size and warrants further investigation with a larger population.

To further evaluate the influence of the feed additive on protein quality and the biochemical composition of the eggs, an analysis of amino acid composition was conducted (see table 2).

Table 2. Results of the amino acid composition of chicken eggs.

Indicator	Content, %	
	Experimental group	Control group
Essential amino acids		
Valin	0.8±0.3	0.8±0.3
Leucine, Isoleucine	1.6±0.5	1.4±0.5
Lysine	1.0±0.3	0.9±0.3
Methionine	0.4±0.01	0.4±0.2
Phenylalanine	0.9±0.3	0.7±0.3
Threonine	0.6±0.2	0.6±0.2
Tryptophan	0.14±0.04	0.13±0.03
Total amount	5.44	4.93
Partially replaceable amino acids		
Arginine	1.0±0.4	1.0±0.4
Histidine	0.7±0.02	0.5±0.1
Total amount	1.7	1.5
Replaceable amino acids		

Alanine	0.7±0.2	0.7±0.2
Glycine	0.4±0.1	0.4±0.1
Proline	0.6±0.1	0.5±0.1
Tyrosine	0.5±0.1	0.5±0.1
Serene	0.8±0.2	0.8±0.2
Cystine	0.3±0.1	0.1±0.01
Mass fraction of asparagine and aspartic acid	1.8±0.7	менее 0.5
Mass fraction of glutamine and glutamic acid	1.5±0.6	менее 0.5
Total amount	6.6	4.0

Analysis of the amino acid composition of chicken eggs showed that the use of the feed additive "Abiopeptid" contributed to an increase in the total amino acid content. The total amount of essential amino acids in the eggs from the experimental group was 5.44% compared to 4.93% in the control group. The highest increase was observed for leucine and phenylalanine. The total amount of semi-essential amino acids was 1.7% in the experimental group and 1.5% in the control group, primarily due to an increase in histidine content. A significant increase was noted among non-essential amino acids: 6.6% in the experimental group versus 4.0% in the control group. In addition, a notable increase in aspartic and glutamic acid levels was recorded in the experimental group: in the control group, their content was below 0.5%, while in the experimental group, it reached 1.8% and 1.5%, respectively. Thus, the most pronounced effect of the supplement was manifested in relation to replaceable amino acids, the increase in which was 65% relative to the control.

Microbiological and hygienic parameters of the eggs were also assessed (Tables 3 and 4) to confirm the safety of the products.

Table 3. Results of microbiological studies of chicken eggs.

Indicator	Experimental group	Control group
The number of mesophilic aerobic and facultative anaerobic microorganisms, CFU/g (cm^3)	100	100
Coliform bacteria (g/cm^3) in the product mass	0.1	0.1

Table 4. Results of hygienic studies of chicken eggs for toxic elements and pesticides.

Indicator	Content, mg/kg	
	Experimental group	Control group
Toxic elements		
Lead	0.3	0.3
Arsenic	0.1	0.1
Cadmium	0.01	0.01
Mercury	0.02	0.02
Pesticides		
HCH	0.1	0.1
DDT	0.1	0.1
Dioxins (in terms of fat)	0.000003	0.000003

Microbiological and hygienic indicators for toxic elements and pesticides in chicken eggs fell within the limits specified by TR CU 021/2011. The count of mesophilic aerobic and facultatively anaerobic microorganisms did not exceed 100 colony forming units (CFU)/g, and coliform bacteria were absent in 0.1 g of the product, conforming to the requirements of the Technical Regulation. Concentrations of toxic elements (lead, arsenic, cadmium, mercury) and pesticides (hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT), dioxins) in all samples were below the maximum allowable levels.

4 Discussion

Changes in egg parameters under the influence of the feed additive "Abiopeptid" can be explained by its composition and mechanism of action. Short peptides formed during enzymatic hydrolysis are absorbed in the intestine more efficiently than free amino acids and can enhance the bioavailability of trace elements, especially calcium ions (Ca^{2+}), which are vital for shell formation. This facilitates synthetic processes in the bird's organism.

Analysis of morphometric parameters showed a trend toward increased albumen, yolk weight, and shell thickness in the experimental group compared to the control group. Although these differences did not reach statistical significance ($p > 0.05$), the observed trends indicate the involvement of peptides in protein metabolism. The lack of statistical significance in the context of a clear positive trend suggests that the sample size and duration of the experiment were limited, warranting further research with larger flocks and extended periods.

Improved nutrient absorption likely contributed to the formation of a stronger shell, reducing brittleness and enhancing storage stability and microbiological safety.

Moreover, the amino acid analysis demonstrated a statistically significant increase ($p < 0.05$) in the content of both essential (leucine, phenylalanine) and non-essential amino acids (asparagine, glutamine, cystine). This indicates enhanced protein metabolism and activation of synthetic processes, driven by the easy-to-digest precursor molecules supplied by the additive. Incorporating low-molecular-weight peptides and free amino acids into the diet ensures their rapid absorption and subsequent integration into egg protein synthesis, leading to superior protein quality and increased biological value of the product.

Thus, eggs acquired a more balanced amino acid composition, characterized by an optimal ratio of essential amino acids, which raises their biological value and digestibility. The enhancement of amino acid content has implications not only for nutritional value but also for technological applications, as protein with an improved amino acid profile can possess desirable functional properties for the food-processing industry [5].

These findings align with previous studies showing that optimizing amino acid nutrition in laying hens leads to improved protein utilization without detrimentally affecting egg quality [3, 9]. Use of the "Abiopeptid" supplement therefore represents a valuable strategy for simultaneously improving the amino acid profile of eggs and maintaining their marketable qualities, thus offering additional advantages for raising the biological value of the product and reducing nitrogen waste.

Such modifications carry both consumer and economic benefits for producers, as they minimize losses and enhance the commercial attractiveness of the product. Strengthening the shell structure alone can decrease egg breakage and surface damage, delivering tangible economic gains in industrial settings by reducing wastage of finished goods. Furthermore, positioning eggs enriched with amino acids as a premium-quality product provides marketing opportunities and bolsters competitive edge.

Finally, microbiological and hygienic parameters of the eggs remained within the limits prescribed by TR CU 021/2011, underscoring the safety of the additive. Absence of adverse effects on organoleptic and sanitary characteristics is essential when introducing any feed additive into industrial poultry farming practice.

Thus, administration of the "Abiopeptid" supplement to laying hens stimulates protein and mineral metabolism, as evidenced by a significant improvement in the amino acid profile of eggs and favorable trends in morphometric parameters. Its use not only enhances the biological value and safety of the product but also increases the efficiency of feed utilization by the birds. The safety data obtained are consistent with the manufacturer's claims. However, interpretation of the results must take into account that the effectiveness of feed additives may vary depending on the composition of the diet.

Future research efforts could focus on evaluating the long-term effects of the supplement on poultry productivity, as well as optimizing dosages and feeding regimes to maximize its beneficial impact.

5 Conclusion

The study demonstrated that the feed additive "Abiopeptid" exerts a comprehensive positive effect on the quality of laying hens' eggs, encompassing both physical-chemical and biological characteristics of the product. This effect is substantiated by a statistically significant improvement in the amino acid composition ($p < 0.05$), chiefly manifested by increased levels of asparagine, glutamine, and cystine, indicative of enhanced protein synthesis in the bird's body. Concurrently, a noticeable upward trend was observed in egg, albumen, and yolk weight, coupled with increased shell strength, attributable to improved absorption of calcium and other micronutrients mediated by low-molecular-weight peptides. Importantly, all samples fulfilled the stringent microbiological safety requirements mandated by the Customs Union Technical Regulation (TR CU 021/2011), assuring the safety of the product for human consumption.

These changes translate into an elevation of the biological and nutritional value of the product: the eggs are fortified with essential amino acids while retaining their organoleptic properties (flavor, odor, texture of whites and yolks). A sturdier shell minimizes breakage and cracks during handling, transportation, and storage, thereby mitigating product losses. Enhanced quality features, such as elevated amino acid content and an optimal mineral composition, qualify the eggs as a nutritious commodity, bolstering their market competitiveness and establishing grounds for premium pricing.

Consequently, a feed supplement formulated from a solution of enzymatic plant (soy protein) hydrolysate ("Abiopeptid") emerges as an effective and secure instrument for optimizing protein and mineral metabolism in laying hens. Its implementation concurrently tackles two pivotal objectives in modern poultry farming: enriching the nutritional value of eggs for consumers and amplifying production profitability by curbing losses and refining product quality.

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