

Laying Hens Under Low Protein Diet: Fatty Liver Bleeding Syndrome Research

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Abstract:

The egg industry in China is currently confronted with significant challenges, including a scarcity of protein feedstock resources and nitrogen that contribute to pollution in animal husbandry. To address these issues, the adoption of low-protein diets for laying hens has gained traction and evolved over the years. Research in this area frequently utilizes standardized ileal digestible amino acids as a fundamental component in developing low-protein diets for laying hens. The primary objectives of these diets are decrease the consumption of protein feedstock, reduce farming expenses, and alleviate nitrogen emissions. This paper provides a concise overview of the theoretical framework behind low-protein diet technology and advancements in research on laying hens, with the intention of offering guidance for the implementation of low-protein feeds in egg production.

Keywords: Laying hens; Low-protein diet; Amino acid balance; Energy balance; Functional amino acids

1. Low Protein Diet

1.1 The Concept of Low Protein Diet

The low protein diet (LPD) is a dietary strategy rooted in protein nutrition and amino acid balance theory. Its objective is to decrease the crude protein level (by 2%-4%) without detriment to the well-being, productivity, and quality of livestock and poultry. goal is accomplished by enriching the diet with specific amino in suitable amounts to meet the essential amino acid needs for animal development. Simultaneously, it involves decreasing reliance on soybean meal, enhancing amino acid utilization efficiency, promoting gut health, and minimizing nitrogen emissions.

1.2 Technical Background of Low Protein Diet

The feed in China is currently undergoing rapid growth; however, there is a significant deficit in the supply of protein feed, resulting in China ranking first globally in the importation of protein raw materials. Notably, in 2019, the import volume of soybeans reached 85.511 million tons. Given the strained international trade environment, the protein shortage in China is anticipated to worsen. The insufficiency of feed raw materials has emerged as a primary constraint on the advancement of China's animal husbandry sector. China heavily relies on imports for its animal husbandry industry, primarily due to the scarcity of feed raw materials. This has not only escalated the expenses of

livestock feeding but also heightened breeding risks. The fluctuating prices of imported feed raw materials expose the livestock industry to the perils of supply shortages and price surges. Furthermore, ensuring the quality and safety of animal products necessitates a focus on the quality and safety of imported feed raw materials.

Low-protein diets are characterized by a reduced content as the primary nutrient component in feed. On October 26, 2018, the China Feed Industry Association introduced the "Layer and Broiler Compound Feed" standard, with the objective of decreasing the protein levels in formulated feeds and encouraging the adoption of low-protein diets. The widespread implementation of low-protein feeds in China is anticipated in the future. The incorporation of low-protein diets in the rearing of layer chickens represents a crucial undertaking. Conventional practices in layer chicken rearing have traditionally relied on high-protein feeds, which are associated with elevated production expenses, as well as environmental and animal health concerns. Consequently, the exploration and application of low-protein feeds have emerged as a prominent area of interest in animal nutrition.

1.3 Application of Low Protein Diet in Laying Chicken Industry

Appropriately decreasing the protein content in feed has the potential to enhance egg production performance. Low-protein diets have been shown to improve egg pro-

duction rates and decrease production expenses. Moreover, reducing the protein content in eggs can lead to a reduction in nitrogen emissions from laying hens, thereby contributing to environmental conservation efforts. The adjustment of protein levels in the diet significantly influences egg production performance. A suitable reduction in protein content in feed can mitigate the accumulation of nitrogen metabolites in laying hens, reducing the burden on the kidneys and thus lowering the incidence of diseases among laying hens.

2. Fatty Liver Hemorrhage Syndrome

2.1 The Concept of Fatty Liver Hemorrhage Syndrome

Fatty Liver Hemorrhage Syndrome (FLHS) is a prevalent nutritional and metabolic disorder observed in laying hens during the peak of egg production. It is primarily characterized by liver fat accumulation, hemorrhage, and a sudden decline in egg production rate. Affected chickens typically exhibit symptoms such as lethargy, a flaccid abdomen with noticeable swelling and drooping, excessive fat deposition, changes comb color (which may lighten or turn yellow and white in severe cases), and a general lack of energy. As the condition progresses, there is a marked decrease in the egg-laying capacity of hens, with severe instances resulting in fatalities due to stress.

2.2 Etiological Analysis of Fatty Liver Hemorrhage Syndrome

2.2.1 Nutritional Factor

High-energy and low-protein feeds play a crucial role in the development of this condition. An overconsumption of calories can result in the conversion of excess energy into fat. Conversely, inadequate protein intake hinders the liver's ability to produce sufficient lipoproteins, impeding the removal of excess fat from the liver. Consequently, process leads the excessive buildup of fat within the liver.

2.2.2 Genetic Factor

The liver plays a crucial role in lipid metabolism in poultry, contributing to 70% of total fat synthesis. In contrast to humans, egg-laying chickens lack GLUT4, which is linked to hyperglycemia and insulin resistance. Moreover, birds have a blood glucose level two to four times higher than similarly weighted mammals. The presence of natural insulin resistance (IR) is a key factor in the development of hyperlipidemia and hyperglycemia syndrome in poultry. Research indicates susceptibility to this condition varies among different breeds of egg-laying chickens.

2.2.3 Feeding Method

The expansion of breeding operations has led to a notable intensification of the poultry industry, resulting in a growing number of egg-laying chickens being affected by septicemia. The prevalence of this condition has surged from around 5% to approximately 20%, leading to substantial economic losses within the poultry sector. Extensive cage rearing practices may diminish egg-laying behavior and energy utilization, and the continuous availability of feed can lead to overconsumption by egg-laying chickens.

2.2.4 Toxin Effect

The presence of moldy corn or peanut meal in feed has been shown to diminish the capacity to produce and transport lipoproteins, consequently resulting in the buildup of fat in the liver.

2.2.5 Stress Factor

During the breeding phase, particularly at the peak of egg-laying, abrupt alterations in feed, staff, ventilation, lighting, inadequate water provision, elevated water temperature, and other variables can contribute to a significant decrease in egg output, leading to the conversion of surplus energy into fat.

2.3 Clinical Manifestation and Diagnosis of Fatty Liver Hemorrhage Syndrome

Fatty liver hemorrhagic syndrome (FLHS) in laying hens is characterized by a significant decline in egg production, hepatic steatosis and rupture, and sudden death. Specific features include excessive accumulation of fat in the liver and abdomen, hepatic steatosis, liver rupture, inflammatory response, and hepatic hemorrhage. This syndrome is frequently observed in commercially caged-reared high-yielding laying hens that are overfed and overconditioned, contributing to 40% to 70% of female chicken mortality. Moreover, FLHS can result in a marked decrease in egg production rate and a shortened peak egg production period in the flock.

2.4 Pathogenesis of Fatty Liver Hemorrhage Syndrome

2.4.1 Genomics Research

Yi Xinrui et al. discovered that in fatty liver hemorrhagic syndrome (FLHS) induced by high-energy and low-protein diets, there were 443 significantly differentially expressed genes in the livers of laying hens with FLHS compared to healthy laying hens. Among these genes, 151 were up-regulated and 292 were down-regulated. Enrichment analysis revealed the differentially expressed genes, such as phosphoenolpyruvate carboxykinase (PCK1) and apolipoprotein a-1 (APOA1), were notably enriched in the PPAR signaling pathway. This pathway plays a cru-

cial role in influencing metabolic processes, thereby the development of fatty liver hemorrhagic syndrome. The study highlighted the significance of the PPAR signaling pathway in the liver and the JAK-STAT signaling pathway in abdominal fat as key regulators of lipid metabolism and inflammatory processes, offering insights for potential targeted drug therapies. Additionally, Wang Yujie et al. assessed the expression levels of IL-6 in the adipose tissue of laying hens with FLHS resulting from high-energy and low-protein diets. They observed a significant increase in the expression of the IL-6 gene in the adipose tissue of FLHS laying hens compared to the control group, indicating metabolic abnormalities in the adipose tissue of FLHS laying hens. Previous reports have indicated a significant reduction in the expression of LKB1 in the livers of FLHS laying hens. LKB1 is involved in mediating various pathways, including AMPK/mTOR, NF- κ B, and other signaling pathways that regulate essential biological processes. As an upstream gene of AMPK, LKB1 can activate AMPK through phosphorylation under conditions such as fasting, intense physical activity, and local ischemia. The activated LKB1/AMPK pathway can suppress lipid synthesis, promote fatty acid oxidation, and consequently prevent the progression of fatty liver.

2.4.2 Metabolomics Studies

Wu Yong et al. documented that the disease induces fat accumulation in the hepatocytes of affected chickens, resulting in hepatic steatosis, which markedly diminishes the egg production rate. Following the disease onset, the liver enlarges significantly, exhibits a greasy texture, and becomes highly fragile. A substantial number of hepatocytes display steatosis, with numerous lipid droplets of varying sizes evident within the hepatocytes, exerting a notable influence on the growth and blood biochemistry of laying hens. Guo Lianying et al. observed an escalation in intracellular lipid droplets and lipid vacuoles following oleic acid/palmitic acid intervention. Oil Red O staining revealed a plethora of red lipid droplets in FFA-induced cells, accompanied by lipid droplet fusion. The levels of intracellular reactive oxygen species (ROS), malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD) experienced a significant decrease, while the expression of IL-2 was markedly reduced, indicating a potential involvement in the inflammatory response. Wang Yujie et reported a substantial increase in IL-6 levels in the adipose tissue and blood of afflicted with FLHS ($P < 0.05$ and $P < 0.01$), signifying abnormal fat metabolism in FLHS patients, which could stimulate IL-6 secretion, further exacerbating symptoms such as liver enlargement and bleeding. Animal experiments conducted by the Re-

biozem team also demonstrated a higher presence of lipid vacuoles in the liver tissue sections of laying hens in the LPHF group [1].

3. Fatty Liver Bleeding Syndrome in the Application of Low Protein Diet in Laying Chicken Industry

3.1 Dietary Energy Level and Fatty Liver Hemorrhage Syndrome

In comparison to conventional diets, low-protein diets exhibit a closer alignment with the ideal amino acid pattern (IAAP) thereby reducing the energy expenditure associated with protein metabolism within the organism. Nevertheless, the insufficiency of specific amino acids, such as glutamic acid, may impede intestinal energy provision. Inadequate protein intake prior to the commencement of egg production can result in hens allocating more energy towards growth and maintenance rather than reproductive functions[2]. The liver, serving as a crucial site for lipid metabolism in laying hens, is tasked with synthesizing hydrophobic lipids like triglycerides, cholesterol esters, and free fatty acids, as well as precursor molecules for yolk proteins such as low-density lipoprotein and yolk protein source particles. These substances are then transferred to oocytes via systemic circulation to facilitate yolk formation[3]. Protein deficiency can lead to reduced production of lipophilic compounds and lipoproteins, hindering the timely transport of fats out of the liver, which can result in hepatic fat accumulation and the onset of fatty liver hemorrhagic syndrome (FLHS). Furthermore, excessive energy can be converted into fat deposits within the liver, contributing to the development of FLHS. Notably, diets high in fat and low in protein represent a primary risk factor for FLHS in laying hens.

Laying hens exhibit a high sensitivity their physiological status, and prolonged consumption of low-protein diets can significantly increase the risk of FLHS[1]. In instances where FLHS is triggered by high-energy and low-protein diets, disruption of the AMPK signaling pathway, which governs fatty acid synthesis in the liver of laying hens, results in an imbalance in energy levels exacerbates fat accumulation in the liver[4]. Imbalances in metabolic processes, such those involving carbohydrates, lipids, and amino acids, have the potential to induce insulin resistance, consequently exacerbating FLHS[5]. After laying hens develop FLHS, metabolic disorders in adipose tissue result in increased IL-6 levels in the organism, leading to symptoms such as liver enlargement and hemorrhaging. Free radicals play a crucial role in the initiation and progression of FLHS triggered by diets high in energy and low in protein. Research has also indicated the involve-

ment of the Cidea and Cidec genes in the development of FLHS induced by high-energy and low-protein diets in laying hens[6]. Consequently, the surplus of energy in low-protein diets can readily trigger FLHS, necessitating heightened vigilance.

3.2 Dietary Amino Acid Absorption and Metabolism and Fatty Liver Hemorrhage Syndrome

3.2.1 Amino Acid Balance in Low Protein Diet of Laying Hens

The promotion and implementation of low-protein diet technology can lead to a reduction in feed costs during the process of laying hen breeding, as well as an enhancement in egg production performance. When essential amino acids (AA) are balanced, decreasing the crude protein (CP) level does not have a significant impact on the growth performance, bone development, and sexual maturity of laying hens during the growing phase. However, it can notably decrease nitrogen emissions in feces, thereby aiding in cost savings on feed and environmental protection. Lowering the level in the diet from 16.5% to 12.0% and supplementing with essential AA can enhance the stress resistance and fecal quality of laying hens in high-temperature conditions while maintaining satisfactory production performance[7]. Low-protein diets have been shown to reduce plasma uric acid concentrations and methane emissions, although they may negatively affect eggshell quality due to potential calcium deficiency resulting from reduced feed intake. As the CP concentration decreases in low-protein diets for laying hens, the levels of non-essential AA also decrease. To sustain normal production performance, it is essential to increase the provision of essential AA, particularly lysine (Lys). A deficiency in AA among laying hens can lead to alterations in intestinal flora, which are closely associated with changes in critical physiological and production parameters such as uric acid levels, final body weight, abdominal fat, feed consumption, egg production rate, egg weight, egg output, and feed-to-egg ratio. Therefore, the addition of an appropriate quantity of essential AA, especially methionine (Met), lysine (Lys), isoleucine (Ile), and threonine (Thr), to low-CP diets is imperative[8].

The incorporation of an appropriate quantity of Met into low-protein diets been shown to have a significant positive impact on the intestinal microbial composition, production performance, reproductive system, and nutrient metabolism of laying hens. Studies have indicated that when the Met level reaches 0.38%, the production performance of laying hens improves [9]. Moreover, the use of encapsulated sustained-release treatment of Lys and Met can

decelerate the release rate in the intestines, enhance the balance of AA post-absorption, and decrease the required level of crystalline AAs in the diet [10]. Conversely, reducing the CP level in laying hen diets does not notably affect production performance, egg quality, or immunity; however, it may diminish the body's antioxidant capacity and alter the cecal flora structure. The supplementation of compound enzyme preparations has been found to effectively enhance production performance, egg quality, antioxidant capacity, and intestinal morphology. Particularly noteworthy is the synergistic effect observed when combined with α -monoglyceride laurate. Furthermore, the addition of β -mannanase to low-protein diets may decrease feed intake and feed conversion rate in laying hens, yet it can enhance the digestibility of essential AA in corn/soybean diets. In conclusion, the implementation of low-protein diet technology in laying hens can influence the balance of intestinal microbiota and result in modifications to the ideal amino acid pattern (IAAP) in the diet, necessitating further comprehensive investigation.

3.2.2 Functional Amino Acid Requirements of Laying Hens on a Low Protein Diet

Functional amino acids (AA) play dual roles both substrates for protein synthesis and participants in cell signaling. The supplementation of functional AA, such as threonine (Thr), tryptophan (Trp), glutamine (Gln), arginine (Arg), glycine (Gly), glutamic acid (Glu), sulfur-containing AA, and branched-chain AA (BCAA), to low-protein diets for laying hens has been shown to enhance their production performance and egg quality [11, 12]. Despite the foundation of research low-protein diets being rooted in the ideal amino acid pattern (IAAP) and the standardized ileal digestible (SID) amino acid pattern, there is a scarcity of studies focusing on the incorporation of functional AA in low-protein diets for laying hens. To further refine the IAAP and SID patterns and enhance the efficiency utilizing low-protein diets for laying hens, it is imperative to investigate the inclusion of functional AA in relevant research endeavors.

Supplementing 2% Trp in a corn-soybean meal-based low-protein diet with 15% crude protein (CP) can significantly enhance the production performance and egg quality of laying hens. This includes increasing egg production by 4.40%, reducing the feed-to-egg ratio by 7.985%, boosting serum protein content and albumen height, and decreasing nitrogen emissions that can harm the environment. Adding 0.2% Thr to a low-protein diet with 14.56% CP can notably improve egg production, feed conversion rate, eggshell quality, and promote protein deposition. Incorporating 0.57% to 0.66% Thr into a 14% CP low-protein diet during peak egg production can

enhance intestinal health and production performance by stimulating the intestinal mucosal immune system, increasing the expression levels of intestinal mucosal mucin 2 and secretory immunoglobulin A (SIgA), and enhancing intestinal microbial diversity and probiotic abundance[13, 14]. Regression analysis indicated that the optimal dietary Thr requirements for egg production, serum uric acid, and serum antioxidant capacity were 0.58%, 0.59%, and 0.56%, respectively[15]. A reduction in dietary CP level from 15.5% to 13% negatively impacted egg production, egg yield, and feed-to-egg ratio of laying hens during peak production. However, supplementing crystalline Thr, Trp, and isoleucine (Ile) alone or in combination in low-protein diets significantly enhanced production performance, reaching a level comparable to the 15.5% CP group, while reducing serum uric acid and serum ammonia concentrations[16]. This highlights that adding Trp and Thr to low-protein diets can substantially enhance the production performance and egg quality of laying hens.

BCAA, including isoleucine (Ile), leucine (Leu), and valine (Val), function as signaling molecules that control protein turnover by modulating intracellular mTOR and GCN2 signaling pathways. Ile, Val, and Leu exhibit antagonistic properties as they compete for the same transporters undergo hydrolysis by the α -ketoacid branched-chain dehydrogenase[17]. Adding Leu and Val to low-protein diets for broiler chickens can linearly reduce abdominal fat rate and significantly increase breast muscle rate and myofiber diameter[18]. Adding different doses of Ile to low-protein diets for laying hens with balanced Lys, Met, Thr, Trp, and Val did not affect egg production performance, egg quality, serum antioxidant capacity, or immunity, indicating that Ile is not a limiting AA in low-protein diets for laying hens[19]. Ile can regulate average egg weight but reduces total egg weight. Based on balancing Met, Lys, Thr, and Trp with crystalline AA, adding Ile to the diet can reduce the CP level by 2 percentage points without affecting the production performance and egg quality of laying hens, and the optimal SID Ile:Lys ratio is 82% to 88%[20]. Research suggests that the optimal BCAA balance in low-protein diets for laying hens is 1.01% Leu, 0.40% Val, and 0.29% Ile[21]. Therefore, although BCAA have little impact on production performance, their regulatory role in egg quality cannot be ignored.

After bile acids enter the small intestine, they conjugate with free Gly to form bile salts, which enhance the absorption of lipids and lipid-soluble nutrients[22]. The inclusion of Gly in laying hen diets has been shown to have a linear relationship with the enhancement of intestinal absorption of lipid-soluble substances, consequently leading to improvements in egg production and egg weight.

Supplementation of Ser in a low-protein diet containing 15.3% CP has been found to increase the average egg weight. Furthermore, the addition of 0.1% Gly to a diet with 15.00% CP does not have any detrimental effects on laying hen production performance. In cases where the CP level in the diet is decreased by 2.44%, supplementation with specific essential AA can help sustain laying performance.

4. Measures

4.1 Net Energy System

The Net Energy (NE) system incorporates dissipated energy as heat increments, thereby accounting for the actual feed available for body maintenance and production. Estimation of NE content in pig and broiler diets is determined by their nutritional components. Nevertheless, these estimations are not tailored for laying hens[23]. Shahram Barzegar et al. [24] conducted three experiments to investigate the impact of varying dietary net energy (NE) and AMEn ratio (NE:AMEn) on the production performance, egg production, and heat production rate (HP) of laying hens. The findings indicated that supplementation of a high NE:AMEn ratio (NE-based diet) to the diet improved feed utilization efficiency and reduced the mobilization of body energy for egg production. Utilizing formulas based on NE was found to positively influence the production performance and egg quality of laying hens. Previous research has suggested that diets with low protein and low energy content (crude protein: 14.29%; energy: 10.72 MJ/Kg) can enhance the production performance of Jingfen No. 2 layer breeders during the later laying phase, while not adversely affecting the hatchability of breeder eggs and reproductive performance. Moreover, the energy content of the diet was observed to have no significant impact on the diversity of cecal microbiota in Jingfen No. 2 layer breeders, with a low-energy diet leading to an increase in the abundance of *Sutterella* and a decrease in the abundance of *Romboutsia*.

Fang Shubao et al. reported that a reduction in the dietary energy level to 10.79 MJ/kg can sustain the production performance and egg quality of Jinghong laying hens during the later laying period. This adjustment significantly enhances lipid metabolism and antioxidant capacity in both serum and liver, while concurrently decreasing feeding expenses.

Wang Ziqiang discovered that decreased protein levels had a notable impact on the production performance and egg quality of laying hens, leading to a significant decrease in blood uric acid and blood sugar levels. The comparison between net energy-fitted diets and metabolic energy diets showed a significant effect on certain pro-

duction aspects during the initial phases of the study. This could be attributed to the net energy system being better aligned with the specific requirements of laying hens. Implementing a scientifically designed energy system in the early stages was found to enhance resistance and uphold consistent production performance.

4.2 Amino Acid Sustained Release

According to the findings presented by Liu Hui, a decrease of over 2% in the dietary crude protein level impacts the production performance of laying hens when supplemented crystalline amino acids. Conversely, the addition of coated amino acids leads to a notable enhancement in their production performance decline. This improvement is attributed to the balanced absorption and utilization of digestible amino acids in laying hens. In the experimental setting, lowering the dietary crude protein level and incorporating crystalline amino acids can enhance the intestinal barrier function of laying hens to a certain degree. This enhancement facilitates the secretion of digestive enzymes, boosts the expression of amino acid transporter proteins, improves the efficiency of dietary protein utilization, and decreases nitrogen emissions.

Li Jun highlighted variations in the digestion and absorption of amino acids in poultry in comparison to the ideal amino acid pattern diet, potentially impacting amino acid utilization efficiency. The approach of developing low-protein diets based on the ideal amino acid pattern overlooks the distinctions in digestion and absorption between crystalline amino acids and protein-bound amino acids in the feed, resulting in potential amino acid loss and wastage. Crystalline amino acids exhibit a faster absorption rate when added to the diet compared to synthetic absorption, leading to rapid appearance in the portal vein and potentially causing transient amino acid imbalances.

4.3 Energy Nitrogen Ratio

Relationship between the energy-to-nitrogen ratio of the diet and the occurrence of fatty liver hemorrhagic syndrome (FLHS) in high-producing laying hens is significant. Optimal production performance of laying hens is achieved when the ratio of crude protein (CP) to energy in the diet is appropriate. The energy-to-nitrogen ratio is determined by dividing the dietary metabolizable energy (MJ) by the CP content (%). Specifically, for laying hens with egg production rates of 50%, 60%, 70%, 80%, and the recommended ratios are 0.36, 0.26, 0.29, and 0.26, respectively. Research indicates the lowest incidence of FLHS is observed when the content of the laying hen diet is 11.51 MJ·kg⁻¹ and the content is 16.78%. Notably, there is a significant interplay between dietary metabolizable energy and CP levels concerning egg production rate,

egg mass, and feed-to-egg ratio in laying hens. Analysis using response surface methodology reveals optimal production performance of laying hens is achieved when the dietary metabolizable energy is 10.04 MJ·kg⁻¹ and the CP level is 16% [25]. Consequently, when utilizing low-protein it is crucial to promptly adjust the energy-to-nitrogen ratio of the diet to safeguard the health and production efficiency of laying hens.

4.4 Additive

The incorporation of 0.5% lecithin into a high-energy, low-protein diet has been shown to effectively mitigate fat accumulation in the livers of high-yielding laying hens, consequently reducing the occurrence of fatty liver hemorrhagic syndrome (FLHS). Furthermore, supplementation with 0.3 mg·kg⁻¹ biotin has demonstrated a degree of preventive efficacy. *Abrus mollis* extract exhibits a modulatory impact on the disruption of lipid metabolism in laying hens induced by a high-energy, low-protein diet. Various dosages of berberine hydrochloride have been found to alleviate lipid imbalances, mitigate liver injury, decrease liver lipid deposition through the regulation of hepatic lipid synthesis and oxidative decomposition metabolic pathways, thereby offering effective prevention against FLHS in laying hens resulting from a high-energy, low-protein diet.

During the late laying period of hens, incorporating alfalfa into their diet has been shown to decrease the occurrence of FLHS. Betaine enhances the production and discharge of yolk precursor substances in the liver by modifying the methylation status and GR binding of genes associated with lipid synthesis and transportation in liver tissue [26]. Furthermore, supplementing low-protein with sanguinarine can enhance the immune response and antioxidant capacity of laying hens, increase nutrient digestibility, and promote intestinal health [27, 28].

Resveratrol has been shown to enhance lipid metabolism disorders improve insulin signaling pathway dysfunction in FLHS laying hens induced by a high-energy and low-protein diet. This leads to a reduction in liver damage and lipid deposition associated with FLHS. When used as a feed additive, it can effectively mitigate oxidative stress and inflammation in FLHS laying hens [29, 30]. Additionally, PQQ (pyrroloquinoline quinone) can boost lipid metabolism, antioxidant capacity, mitochondrial function, the anti-apoptotic abilities of hepatocytes. It demonstrates a significant impact on the prevention of FLHS in laying hens.

5. Future Research Prospects

The role of nutrition as a significant contributing factor to development of fatty liver hemorrhagic syndrome in lay-

ing hens during peak production is crucial.

Currently, research endeavors both domestically and internationally are concentrated on examining the potential adverse effects of low-protein diets on the production performance and egg quality of broiler chickens and laying hens. Additionally, researchers are exploring the efficacy of additives in ameliorating decline in production performance and health issues in laying hens resulting from low-protein diets. The assessment parameters primarily center on superficial physicochemical indices such as blood lipids, liver lipids, and histological examinations.

The utilization of additives may have unforeseen impacts on the growth, productivity, and food safety of livestock and poultry. This study suggests that modifying the composition of low-protein diets without the introduction of new substances can be achieved by adjusting the quantity and type of existing feed components. This adjustment may involve altering the metabolizable energy levels, amino acid supplementation forms, or incorporating active enzymes to enhance nutrient digestibility and utilization rates. Through the analysis of physicochemical markers in laying hens, further investigation into gene and protein expression levels in the liver and abdominal fat can provide insights into the influence of low-protein diets on liver lipid metabolism in laying hens. Consequently, this research aims to optimize the formulation and feeding strategies of low-protein diets livestock.

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