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The Impact of Dietary Protein Intake of Laying Hens on Production Performance, Health and Nitrogen Excretion: A Review

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ABSTRACT This review discusses the effects of dietary protein intake on production performance, health, and nitrogen excretion in laying hens. The poultry industry faces the dual challenge of improving productivity while reducing environmental impact. Dietary protein plays a central role in egg production, egg quality, and overall bird performance. Traditionally, high crude protein (CP) levels were used to meet amino acid requirements and enhance output. However, excessive CP increases feed costs and nitrogen excretion, contributing to environmental concerns. The integration of non-bound amino acids into poultry diets has allowed for reduced CP levels without compromising performance. A well-balanced amino acid profile is essential for efficient nutrient use and minimizing waste. Various protein sources differ in their amino acid composition and digestibility, influencing their suitability in poultry feed. Selecting appropriate protein sources supports both bird health and sustainable production. This review highlights the importance of optimizing protein intake and amino acid balance to enhance performance and mitigate ecological impacts. Future research should explore alternative protein sources and refine amino acid supplementation to promote environmentally responsible poultry production.

(Key words: amino acid, dietary protein, ecological impact, protein sources, laying hens)

INTRODUCTION

Dietary protein and energy are the primary nutrients that represent 90% of the cost of feedstuffs for livestock animals (Habib and Kasim, 2019). Among them, energy fuels the metabolic functions of animals whereas protein plays multiple critical roles including tissue growth and maintenance, production, immunity, and biochemical (enzymatic and hormonal) functions. Apart from this, it may influence carcass traits and abdominal fat levels in poultry, emphasizing the contribution of protein to energy metabolism in animals (NRC, 1994; Pesti, 2009). Therefore, protein and energy are the two primary considerations that feed formulators focus on during feed formulation for layer hens (Jeroch, 2011).

Ongoing controversies exist regarding using "dietary protein" and "CP" terms in feed formulations. The dietary protein content refers to the total proteins that are bioavailable to the animals while CP refers to the sum of total nitrogen content in the feed, including essential and non-essential amino acids and other nonprotein nitrogenous compounds (Pesti, 2009). Studies have shown that the protein and amino acid requirements of laying hens vary due to several factors, including the stage of egg production, body size, feed intake, and egg weight. These requirements are further influenced by variables such as breed, age, diet composition, and environmental conditions (Hurwitz and Bornstein, 1973).

CP is a crucial factor in poultry diets that may directly influence production performance. The (NRC, 1994) states that leghorn-type laying hens require 18.8%, 15.0%, or 12.5% CP, depending on daily feed intakes of 80, 100, or 120 grams per hen, respectively. For Hy-Line brown®, 16.5%, 17.80%, and 17.60% of CP requirement in laying hens in their prelay, peaking, and post-peaking stages respectively (Hy-Line, 2024). Therefore, diets must contain enough CP to provide essential amino acids as well as non-essential amino acids. Since laying hens rely on nutrients from their diet for producing eggs, the quality and composition of the feed are crucial. It is also particularly important given that feed expenses account for 60 to 70% of the total cost of egg

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production (Bell and Weaver, 2002; Oladokun and Johnson, 2012). Notably, it has become more crucial to balance feed costs and provide precise amounts of nutrients as needed throughout the laying cycle of hens to maximize profit margins (Shim et al., 2013). Hence, this review aims to provide further insight into the diverse sources of dietary protein, emphasizes the importance of optimizing protein intake for sustaining health and enhancing productivity, and explores the ecological advantages associated with efficient protein utilization.

HISTORICAL DEVELOPMENT OF PROTEIN-BASED DIETS IN POULTRY SCIENCE

In the early 20th century, the understanding of the role of protein in poultry diets began to develop, with significant progress in the chemistry of amino acids (Elwinger et al., 2016). By the 1940s, research expanded to include the study of the calorie-protein ratio and metabolizable energy in broiler diets, leading to the introduction of high-energy but protein-deficient diets (Wythe et al., 2023). The 1950s marked a turning point, with the introduction of "The Ideal Protein Concept" which could define the amino acid requirements corresponding to the maintenance and protein accretion of the birds, driving the formulation of diets tailored to specific nutrient compositions (Bryden et al., 2021). This method emerged during post－World War II research efforts aimed at refining the calorie-to-protein ratio and metabolizable energy values in broiler diets. The objective was to improve precision in estimating metabolizable energy by adjusting for zero nitrogen retention (Elwinger et al., 2016). At a similar period (in 1951), methionine was introduced as the first feed-grade crystalline amino acid. This advancement further transformed the poultry feed sector by partially replacing costly CP in poultry diets. In the modern era, innovations such as feed additives, including exogenous enzymes (i.e., introduced in the late 1980s to early 1990s), prebiotics, probiotics, and organic chelates, have enhanced the efficiency of nutrient utilization from poultry feed and hence growth performance and environmental sustainability (Ghasemi et al., 2020).

DIETARY PROTEIN SOURCES FOR LAYING HENS

Based on the origin of the ingredients, dietary protein sources can be primarily divided into two groups: animal-based and plant-based protein sources. Generally, plant-based protein sources are inferior to animal-based sources due to the lower digestibility and quality of proteins (Eagleson et al., 2018; Fanatico et al., 2018).

1. Animal-based Dietary Protein Sources

1) Meat and bone meal (MBM)

A by-product of animal slaughter, MBM is significant in nutrient cycling within agricultural production systems and ecological contexts while it is one of the most cost-effective animal protein sources available in commercial markets (Fanatico et al., 2018). Due to its higher protein and mineral content, MBM plays a crucial role in poultry nutrition. According to Parsons et al. (1997), MBM contains 48－56% of CP and it may greatly depend on processing techniques and raw material inputs. In terms of mineral content, it is an important source of calcium and phosphorus for laying hens (Dolz and De Blas, 1992; zkurt et al., 2004). Thus, the incorporation of dietary meat and bone meal (MBM) into the layer diet has been observed to enhance eggshell quality parameters (Sell and Jeffrey, 1996).

2) Blood meal

The blood meal is an animal rendering by-product that is produced at slaughterhouses. It contains 80－88% CP content and appropriate amino acid profiles. Hence, supplementation of blood meal with plant-based feedstuffs is a widely accepted approach in poultry feed formulations (Tyus et al., 2009). Among essential amino acids, methionine, cysteine, histidine, and leucine are present in beneficial amounts. Nonetheless, isoleucine and glycine contents are lower compared to meat and bone meal and fish meal. Because of this reason, isoleucine is the first limiting amino acid in blood meals (NRC, 1994; Khawaja et al., 2007). Odunsi (2003) and Onwudike (1981) found that blood meal alone cannot enhance the laying performance of hens without the addition of another protein source, such as fish meal. Inclusion rates of blood meals can vary according to the laying stage, strains, and the quality of blood meal (i.e., species and processing techniques used).

3) Fish meal

Fishmeal derives from various sources, including whole fish such as menhaden, sardines, carp, and anchovies, and fish processing waste including offal and trimmings (Fanatico et al., 2018). It contains high-quality proteins, energy, minerals (i.e., calcium and phosphorus), omega-3 fatty acids, and vitamins that are appropriate for poultry nutrition. Fishmeal protein exhibits a high biological value in animal diets. It is particularly abundant in essential amino acids, such as lysine and sulfur-containing amino acids (Karimi, 2006). The quality of fishmeal and its CP content can vary between 57% and 77%, depending on the species of fish and the processing methods used (Miles and Jacob, 1999). Fish meal production has also become an effective control measure for invasive fish species, such as Asian carp, in native aquatic ecosystems (Fanatico et al., 2018). Despite its nutritional benefits, its 10–12% fish oil content can cause a fishy odor, potential rancidity, and handling difficulties, affecting its use in poultry farming (Sellers, 2002). Excessive fish meals in diets can also lead to gizzard erosion and off-flavor transmission into eggs and meat (NRC, 1994). Additionally, high cost and limited availability can further constrain its use in feed formulations (Donkoh et al., 2001).

2. Plant-Based Protein Sources

Literature suggests that animal protein sources may lead to quality concerns and pathogenic microbial (i.e., *Salmonella*) transmission through diets. Thus, the inclusion of good quality animal-based protein sources with plant-based sources is a common practice in poultry feeding.

1) Soybean meal (SBM)

The soybean (*Glycine max*) can be fed to poultry either whole or as soybean meal, with the seed primarily consisting of 90% cotyledon and 8% hull. The cotyledon is rich in proteins and fats, which is a crucial input for the oil industry. After extracting oil, the residual material primarily serves as a high-protein feed for poultry. There are two main methods for oil extraction: expeller pressing and solvent extraction. Among these, soybean meals obtained through solvent extraction are particularly popular and widely used in poultry feed (Dourado et al., 2011; Fig. 1).

This oilseed is globally cultivated and plays a significant role in both human and animal nutrition due to its versatile applications. During the feed formulations for poultry, 44 and 49% of the CP of SBM are utilized as the major two standardized. SBM is a rich source of lysine (6.2 g/16 gN), with a low prevalence of methionine and cysteine content (2.9 g/16 gN) (Banaszkiewicz, 2011; Toomer et al., 2024).

Despite its excellent amino acid profiles and high-protein content, soybeans also contain antinutritional factors such as trypsin inhibitors, lectins (hemagglutinins), phytates, tannins, oxalates, and non-starch polysaccharides (Di et al., 2024). Processing techniques are involved in particular antinutritional factors (trypsin inhibitors) inactivation and are mostly employed by meticulously controlled thermal processing techniques (NRC, 1994). Additionally, soybean hull is an appropriate feed ingredient that can replace wheat bran (weight basis) without compromising poultry performance. Nevertheless, inclusion rates are limited due to its higher fibrous content (Dourado et al., 2011). Esonu et al. (2005) conducted a study on laying hens and found that feed conversion efficiencies were higher when their diets were supplemented with up to 20% soybean hulls and 30% cellulolytic enzymes.

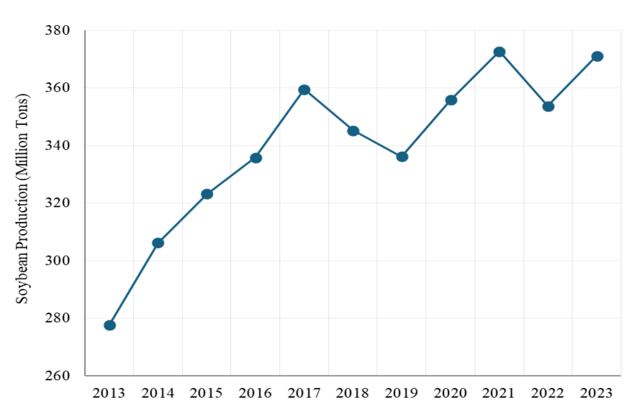

Fig. 1. Global soybean production from 2013 to 2023. Source: FAO (2024).

2) Peanut meal

Efforts to replace traditional SBM in poultry diets aim to enhance cost-effectiveness. Among alternative protein sources, peanut meals (PNM) are significantly used in poultry feed, improving growth performance and egg production in laying hens. Generally, solvent-extracted meals are considered as the preferable type for laying hens (Batal et al., 2005). It contains 2,273－3,009 kcal/kg of nitrogen-corrected true metabolizable (TMEn), 40.1－50.9% of CP, 0.68－5.97% of crude fat, and 5.77－12.6% of crude fiber content (Xia et al., 2022). On a 90% dry matter basis, the average mineral concentrations in peanut meals were measured as follows: calcium at 0.08%, phosphorus at 0.57%, sodium at 0.01%, and potassium at 1.22% (Batal et al., 2005). Nevertheless, PNM is notably low in threonine, with lysine being the first limiting amino acid whereas aflatoxin is a critical concern that can directly affect impairing production performance in chickens (Costa et al., 2001).

3) Sunflower meal

Sunflower (*Helianthus annus*) meal (SFM), a by-product of sunflower seed oil extraction, serves as an alternative protein source for broilers, layers, and waterfowl (Senkoylu and Dale, 1999). It contains substantial amounts of proteins, fiber, and minerals (Ca and P), as well as B-complex vitamins. In addition, it contains a considerable amount of α-tocopherols (608 mg/Kg seed) and antioxidants which are important to improve health of laying hens. Notably, SFM can effectively replace SBM in poultry diets owing to its lower antinutritional content. Nevertheless, its lower lysine and methionine content limits its usage as a poultry feed (Saleh et al., 2021). Baghban-Kanani et al. (2018) conducted a study with SFM inclusion up to 20%, supplemented with multi-complex enzymes for Hy-Line (39 weeks) and White Leghorn (36 weeks) hens. Their findings suggest that approximately 6% of SBM can be substituted with SFM without adversely affecting egg production or quality traits. According to the, Rao et al. (2006) the inclusion of SFM in broiler diets is limited to a maximum of 15% due to its high fiber content, which is poorly digested by poultry and may compromise nutrient utilization.

4) Cottonseed meal

Cottonseed (CSM) meal is a residual product of cottonseed (*Gossypium hirsutum*) oil manufacturing and a possible protein source (33－41 %) for poultry (Nagalakshmi et al., 2007). Nevertheless, its poor protein quality and high fibrous nature have constrained its usage in poultry diets. Conversely, free gossypol and cyclopropenoid fatty acids (CPFA) have further limited their usage by acting as antinutritional factors (ANFs) for them (Wang et al., 2020). Gossypol can inhibit pepsin and trypsin activity and bind with lysine in poultry diets and subsequently may reduce protein digestibility. Yolk molting is a one of internal quality defects occurring due to free gossypol in laying hen diets. On the other hand, CPFA may lead to a pinkish discoloration of the albumen. Feeding CSM at levels over 100 mg/kg can lower egg production, depending on the strain, age, and total protein content in the diet. However, this might be overcome by supplementing iron and lysine to layer diets (Nagalakshmi et al., 2007). Mandal et al. (2024) stated that high fibrous nature and the free gossypol presence of CSM may deleteriously affect feed intake and the body weight of the broiler chickens. For instance, incorporating 5－10% of CSM into broiler diets may significantly increase the average daily feed intake as well as the average daily gain of the broilers. Notably, this fact may work on the layer pullet development as well.

3. Non-Bound Amino Acid Supplementation

The amino acid requirements of poultry should be proportional to the CP and metabolizable energy (ME) content of the feed. In parallel, maintaining a proper balance between essential and non-essential amino acids is also vital to sustaining production performance in poultry. To promote optimal protein utilization and minimize dependence on costly CP sources, a range of feed-grade amino acids are now commercially synthesized for poultry diets. These include essential amino acids such as DL-methionine, L-lysine, L-threonine, L-tryptophan, L-arginine, L-valine, and L-isoleucine (Vieira et al., 2016), along with non-essential amino acids alanine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, proline, serine, and tyrosine which are available at industrial scale for integration into formulated feeds (Maia et al., 2024). Pesti (2009) noted that poultry feed without synthetic amino acids requires supplementation at 71% of SBM and 14% of corn to meet the minimum essential amino acid requirements. This formulation also needed a minimum of 35.6% CP content. However, the inclusion of even minute concentrations of synthetic amino acids could significantly reduce these requirements. Hence, Pesti (2009) concluded that SBM incorporation can be decreased to 29.29% with the addition of 0.27% DL-Methionine, 0.07% L-Threonine, and 0.09% L-Lysine. This proves that non-bound amino acids have a substantial impact on poultry production, exerting effect on feed ingredient replacement and cost reduction.

4. Novel Protein Sources

Higher populations growth negatively affects SBM (primary protein source) usage in poultry feed by enhancing higher demand in human consumption (Di et al., 2024). To confront this competition, numerous protein sources have been introduced as alternatives. Among them, novel protein sources such as insect meals and single cell proteins have gained attention as sustainable alternatives for laying hens. These innovative protein sources offer high nutritional value while potentially reducing the environmental impact of poultry production (Sajid et al., 2023).

1) Insect meal

Insects, such as black soldier fly larvae (Patterson et al., 2021), mealworms (Sedgh et al., 2021; Stastnik et al., 2021), and crickets (Shah and Wanapat, 2021), are rich in EAAs, fats, and micronutrients. Their proteins possess high digestibility and well-balanced amino acid profiles, compared to fish meal and meat and bone meal, positioning them as a promising alternative to conventional protein sources such as soybean meal (Sajid et al., 2023; Slimen et al., 2023). Studies show that incorporating insect protein into layer diets can maintain or even improve egg production, egg quality (e.g., shell strength, yolk color), and the overall health of hens (Slimen et al., 2023). Insects also have the added benefit of being produced using organic waste, reducing the environmental footprint of feed production. Notably, insect farming requires less land and water compared to traditional protein sources, emphasizing its efficiency in usage at global levels (Elahi et al., 2022).

2) Single cell proteins (SCP)

Recently, extensive research has explored the use of SCP as a feed ingredient in poultry diets, offering a sustainable solution to the high cost of protein supplements and the challenges of environmental pollution (Patthawaro and Saejung, 2019; Raziq et al., 2020). They are highly value-added, protein-rich sources synthesized through microbial biomass extraction. Algae (i.e., *Chlorella*, *Spirulina*), bacteria (i.e., *Cyanobacteria*, *Methylococcus*), and fungi (i.e., *Saccharomyces cerevisiae*) are under this category.

For instance, algae is a novel protein source for laying hens (Panaite et al., 2023; Madacussengua et al., 2024) and is not only rich in protein but also contains beneficial bioactive compounds such as omega-3 fatty acids, carotenoids, and antioxidants. These compounds have the potential to enhance egg quality, by improving yolk color and healthy fatty acid content in eggs (Hasan et al., 2018; Salahuddin et al., 2024). Algae can be produced on non-arable land, contributing to more sustainable feeding practices. Therefore, these sources serve as valuable ingredients in the poultry feed industry (Hombegowda et al., 2021).

Notably, the high growth rates of microorganisms and the ability for continuous harvest make SCP production advantageous, particularly when utilizing low-cost raw materials (Ritala et al., 2017). For instance, poultry waste or litter can serve as substrates for SCP-producing microorganisms, providing a potential solution to the issue of poultry waste. Jalasutram et al. (2013) conducted a study using *Candida utilis* derived from digested and undigested poultry litter. The study revealed that undigested poultry litter can effectively be used as a source of raw materials for SCP production(Table 1).

3) Role of essential amino acids in layer performance optimization

Amino acids are structural and functional units of the proteins, and they are categorized into two primary groups based on their ability to be synthesized within the animal body, named essential and nonessential amino acids (Debnath et al., 2019). Amino acid imbalances, antagonism, and toxicity can adversely affect animals when they are not provided at optimal levels in feed (NRC, 1994). Importantly, EAAs play a crucial role in optimizing the performance of laying hens by

Table 1. Comparative nutritional profiles of common protein sources used in laying hen diets

Protein source	CP (%)	Limiting amino acids	Notable nutrients	Limitations
Meat and bone meal	48−56	None	Ca & P	Quality variability, potential pathogen transmission risk
Blood meal	80−88	Isoleucine	Methionine & leucine	Low glycine limitation in incorporations
Fish meal	57−77	None	Omega-3, lysine	High cost; off-flavor in eggs
Soybean meal	44−49	Methionine, cysteine	Lysine	High demand (food vs. feed), prevalence of antinutritional factors
Peanut meal	40−51	Lysine, threonine	Crude fiber, fat	Aflatoxin contamination risk
Sunflower meal	28−41	Lysine, methionine	Fiber, antioxidants	High fiber content
Cottonseed meal	33−41	lysine	Crude fiber	Gossypol & CPFA toxicity

supporting egg production, growth, and overall health. Among EAA, methionine and lysine are particularly important. The other amino acids such as threonine, tryptophan, and arginine also contribute significantly to various physiological processes in layers (Macelline et al., 2021; Da et al., 2022).

4) Methionine

Methionine is the most prominent sulfur-containing amino acid in poultry nutrition. It is considered as the primary limiting amino acid in corn-soybean-based diets due to its limited availability in plant-based protein sources (Macelline et al., 2021). Methionine plays multiple critical roles in poultry nutrition. It functions as an essential amino acid in protein and polyamine synthesis, serves as a sulfur donor, and acts as a precursor for important metabolic intermediates such as carnitine and cysteine. For instance, two methionine molecules supply S atoms to synthesize cysteine by oxidizing sulfhydryl groups. Moreover, methionine serves as a methyl group donor required for the biosynthesis of the coenzyme S-adenosylmethionine (SAM), which plays a pivotal role in methylation reactions and metabolic regulations (NRC, 1994; Babazadeh and Simab, 2022).

Methionine is also vital for egg production performance, egg quality, and feather maintenance in laying hens (Macelline et al., 2021). For instance, a review by Elnesr et al. (2024) noted which methionine mineral chelates, such as Zn-Met, Mn-Met, and Cu-Met, can improve both internal and external quality of eggs. Furthermore, methionine contributes to enhancing egg size by improving overall protein utilization and adequate methionine levels can also help alleviate fatty liver disease, heat stress and optimize feed efficiency in layer hens (Ma et al., 2021; Liu et al., 2023). In parallel, studies indicate that low-protein diets (14−16%) supplemented adequately with methionine enhance productive performance than high-protein diets (18%) at constant methionine-to-protein ratios (Babazadesh and Simab, 2022).

5) Lysine

Lysine is often considered the second limiting amino acid in corn and soybean meal-based diets. When applying the ideal protein concept to poultry feed formulation, lysine is designated as the reference amino acid due to its distinctive attributes most notably its fundamental role in protein synthesis and its well-defined, consistent requirement across poultry species. Adequate supplementation is essential as it involves egg protein synthesis (Silva et al., 2015; Macelline et al., 2021). Lysine deficiencies may observe egg mass reduction, poor feed conversion, and impaired growth (Onimisi et al., 2012; Kakhki et al., 2016). According to the Hy-Line (2024), lysine requirement of Hy-Line brown layers at their peak should be 0.91% and gradually decrease with age up to 0.58% (at 100 weeks of age). Nevertheless, these requirements can be further differentiated with strains and health conditions of them.

6) Threonine

Threonine is the third limiting amino acid, vital for protein synthesis in the gut lining and immune tissues (barrier function), which directly support gut health and digestion. This, in turn, ensures efficient nutrient absorption and overall bird performance (Hossaninejad et al., 2021). The National Research Council (1994) recommended 0.47% of threonine supplement for the brown egg-laying hens. However, modern management guides (Hy-Line, 2024) have suggested 0.65−0.58% of threonine uptake for better performance in Hy-Line brown hens.

Notably, diet threonine levels are directly influenced by the CP levels provided. It is proved that threonine at 14% of CP diets becomes a limiting amino acid in corn soybean-based diets. Moreover, its deficiencies may reduce egg production and impaired immune function, making hens more susceptible to diseases (Macelline et al., 2021). Furthermore, Azzam et al. (2019) noted a significant decrease in total cholesterol levels at 0.66% L- threonine supplementation for Lohmann Brown hens at 28 weeks of age as well as the egg production rates(Table 2).

7) Protein quality vs. protein quantity implications for laying hens

Protein quality and quantity are critical factors in the nutrition of laying hens, affecting egg production, growth, and health (Panda et al., 2012; Kim and Kang, 2022). Protein quantity refers to the total amount of dietary protein in diets, while protein quality is defined by containing well-balanced amino acid profiles and their higher digestibility (Adhikari et al., 2022). Insufficient dietary protein intake may lead to deficiencies in essential amino acids (EAAs), such as methionine, lysine, and threonine that can negatively affect egg production and quality, feather growth and health of animals. Conversely, excessive supplementations may impair nutrient utilization due to the deamination of surplus amino acids, leading to elevated ammonia production and increased energy expenditure for its detoxification. Subsequently, this might detrimentally impact the environment.

Increasing the total protein intake may not be effective if the quality of the protein source is inadequate (Alagawany et al., 2016). Sakomura et al. (2019) stated that improving protein quality may enhance egg production and quality, even if the overall protein intake remains constant. High-quality proteins, rich in essential amino acids, support better feed efficiency and reduce nitrogen waste (by balancing amino acids), leading to more sustainable production (Attia et al., 2020). Therefore, balancing both protein quality and quantity is essential for maximizing laying hens' productivity and health, while also minimizing feed costs and environmental impact(Table 3).

8) Comparative studies on high vs. low protein diets in laying performance

Comparative studies on high vs. low protein diets in laying hens have shown significant impacts on laying hens' performance, health, and metabolic processes. For instance, low-protein diets, especially when combined with high energy levels, can lead to lipid metabolism disorders in late-phase laying hens, causing fat accumulation in the liver and nega-

Table 2. Key functions, deficiency effects and recommended inclusion levels of essential amino acids in laying hens

Amino acid	Main function in hens	Deficiency effects	Recommended inclusion (%)	References
Methionine	Egg production, liver protection, feathering	Reduced egg size, fatty liver, poor feathers	0.3−0.4	Babazadesh and Simab (2022), Macelline et al. (2021)
Lysine	Egg mass synthesis, muscle growth	Poor growth, small eggs, weak feathers	0.6−0.9	Silva et al. (2015), Kim et al. (2020)
Threonine	Gut health, immune system support	Poor digestion, lowered immunity	0.58−0.65	Azzam et al. (2019), Hossaninejad et al. (2021)
Tryptophan	Stress management, behavior modulation	Aggression, stress sensitivity	~0.18−0.20[1]	Macelline et al. (2021), Sarsour et al. (2021)
Arginine	Eggshell strength, immune cell development	Poor shell quality, weak immune function	~1.0−1.2[1]	Yuan et al. (2016), Macelline et al. (2021)

[1] Estimated values.

Table 3. Suggested CP requirements for laying hens by production stage

Production stage	CP (%)	Purpose	Reference
Pre-lay (15−18 weeks)	16.0−17.5	Supporting pullet growth and reproductive development	Hy-Line (2024)
Peak production (20−30 weeks)	17.5−18.5	Maximize egg mass and quality	NRC (1994), Hy-Line (2024)
Mid-lay (30−60 weeks)	16.0−17.0	Maintain production and health	Hy-Line (2024)
Late lay (60+ weeks)	15.0−16.0	Sustain output with lower metabolic strain	Kleyn and Ciacciariello (2023)

tively affecting egg quality and overall health (Han et al., 2023; Hu et al., 2024). Particularly, high-energy, low-protein diets can induce lipid peroxidation and inflammatory responses in both the liver and abdominal adipose tissue, suggesting a reciprocal regulation between these tissues during fatty liver development (Rozenboim et al., 2016; Du et al., 2024). Torki et al. (2015) compared different protein levels (10.5% to 16.5% CP) under high-temperature conditions and showed that diets with 12.0% to 15.0% CP maintained similar egg production and quality as the control diet (16.5% CP). However, a diet with 10.5% CP significantly decreased egg production and egg mass.

Moreover, Jahan et al. (2024) demonstrated that hens fed a low-protein diet in the morning and a high-protein diet in the afternoon showed no significant differences in egg production and size compared to hens fed a high-protein diet throughout the day. According to Hu et al. (2024), low-protein diets can also alter the caecal microbiome, affecting the metabolism of essential vitamins such as riboflavin (i.e., vitamin B2) and pantetheine (i.e., vitamin B5 derivative), which may contribute to lipid metabolism disorders and fat accumulation in the liver. These studies suggest that while low-protein diets can be beneficial in certain contexts, they must be carefully balanced with energy levels and supplemented with essential nutrients to avoid negative impacts on laying hens' health and performance(Table 4).

9) Impact of dietary protein on longevity and health of laying hens

Genetically improved or selected (by laying long clutches) layers have a production lifespan of 100≤weeks in the modern era. These "long-life layers" are predicted to lay more than 500 eggs in a particular period. The primary determinants of the economic lifespan of the layers are the progressive decline in egg production and deterioration of quality, unless they die (Kleyn and Ciacciariello, 2023). Dietary protein is crucial in determining the longevity and overall health of hens. Adequate protein intake is essential for maintaining egg production and quality, feather condition, immunity functions, and muscle maintenance of them (Shim et al., 2013). Hence, dietary protein deficiencies may deteriorate the characteristics such as egg quality traits (i.e., low-quality albumen and shell), metabolic activities, muscle nutrition, disease resistance, and feather quality (i.e., low growth and quality) (Sun et al., 2022).

Conversely, excessive protein in the diet can strain the liver and kidneys, potentially leading to metabolic disorders (Bun-

Table 4. Comparative effects of high vs. low protein diets on laying hens

Parameter	High protein diet	Low protein diet
Egg production	Often increased or sustained	Sustain with well-balanced amino acid supplementation
Egg quality	Improved albumen, yolk protein	Poor shell strength, smaller eggs (without amino acid balancing)
Health & longevity	Risk of metabolic overload	Maintained with a balanced amino acid profile
Laying persistency	Moderate to higher	Maintained
Feed cost	Higher	Lower
Nitrogen excretion	Elevated	Further reduced with synthetic amino acid inclusion
Environmental impact	Greater (ammonia, eutrophication)	Lower with proper formulation

chasak et al., 2005) and increasing the abdominal fat content of the hens (Pesti, 2009). Balancing CP levels with other nutrients, such as amino acids, ensures that hens receive the building blocks necessary for egg production, tissue repair, immune function, and optimal physiological health throughout their laying cycle (Keshavarz and Austic, 2004). A well-formulated diet with the appropriate protein content contributes to prolonged productivity, better resistance to diseases, and overall improved health for hens (Shim et al., 2013).

10) Impact of dietary protein on immune function under stress conditions

Dietary protein can regulate the oxidative stress response in laying hens. Stress conditions often lead to the overproduction of reactive oxygen species (ROS), which can damage cellular components such as proteins, DNA, and lipids (He et al., 2018; Oke et al., 2024). Sufficient dietary protein, particularly those rich in sulfur-containing amino acids such as methionine and cysteine, plays a multifaceted role in the health of laying hens. Firstly, they compensate for damaged proteins, aiding tissue repair and maintenance. Secondly, these proteins enhance the antioxidant capacity of hens by supporting the synthesis of glutathione, a major intracellular antioxidant. By bolstering glutathione production, these proteins effectively mitigate oxidative stress, contributing to overall hen well-being and potentially improving egg quality (Macelline et al., 2021). Besides, proteins are essential for producing antibodies, mucus, and immune cells (primary factors of the immune system). Thus, optimizing dietary protein levels supports immune function and helps mitigate the adverse effects of oxidative stress, contributing to better overall health and productivity in poultry (Oke et al., 2024).

11) Impact of dietary protein on growth performance and body composition

The body weight difference of the laying hens at 20 weeks persists throughout the 72 weeks of age and it has a close relation with the size and weight of the eggs (Bish et al., 1985). Dietary protein significantly influences growth and body composition in laying hens by providing the essential amino acids required for tissue development, feather growth, and protein synthesis in egg production (Shim et al., 2013). Adequate protein intake supports muscle development and overall body mass, particularly during the pullet stage when hens are still growing. At this stage, dissimilar CP recommendations could be observed depending on the breed or strain of the hens.

Dietary protein expends for production and maintenance of birds, and hence it is associated with lean tissue development and optimal body composition determination (Summers and Leeson, 1994; Srilatha et al., 2018). Insufficient protein in the diet can result in stunted growth, reduced muscle mass, and poor feather quality, which may affect the overall weight loss of the hens (Chalova et al., 2016). Thus, dietary protein level optimization is critical for achieving optimal growth rates and maintaining a healthy body composition in layer hens, contributing to their overall performance.

12) Impact of dietary protein intake on laying performance

Dietary protein intake determines egg production rates and its consistency in laying hens. Eggs contain significant amounts of protein, particularly in the egg white which consists largely of albumen (Mine, 2008). Adequate dietary protein provides the necessary amino acids which are important in the synthesis of egg components including, yolk, albumen, and the shell membranes (Macelline et al., 2021). Insufficient protein intake compromises a hen's ability to produce eggs at peak levels and its quality (Summers and Leeson, 1994).

The quantity and quality of protein in a hen's diet directly influence both the rate of egg production and the quality of the eggs (Kim and Kang, 2022). Essential amino acids, particularly methionine, lysine, and threonine, are critical for maintaining high production rates (Macelline et al., 2021). Hens fed diets with adequate protein lay more eggs and maintain better overall health, with improved muscle maintenance and body condition, allowing for consistent production (Summers and Leeson, 1994). Protein deficiencies can lead to reduced egg size, weaker shells, and erratic laying patterns, highlighting the importance of protein in sustaining both egg quantity and quality (Macelline et al., 2021). Additionally, protein requirements may vary with the hen’s laying phase, with peak protein demand occurring around 25−30 weeks of age (Thayer et al., 1974).

13) Impact of dietary protein intake on egg quality

Dietary protein levels play a crucial role in determining egg size, yolk composition, and, to a lesser extent of shell quality in chickens. Higher CP levels generally result in larger eggs due to the provision of essential building blocks for egg white and overall egg formation (Kowalska et al., 2021). Hens-fed diets with sufficient or increased protein tend to produce larger eggs with a higher proportion of albumen. In contrast, low protein levels can lead to smaller eggs, as the hens lack the necessary amino acids at optimal balance for proper egg development (Leeson, 1989; Pesti, 1991). While shell quality is more directly influenced by nutrients such as calcium and vitamin D, insufficient protein can indirectly lead to poorer shell quality by affecting the hen's overall health and nutrient absorption (Shim et al., 2013; Stefanello et al., 2014). However, exceeding requirements can also reduce eggshell quality by increasing egg size and thereby shell area.

Dietary protein also impacts yolk composition, with higher protein levels increasing yolk size and enhancing its protein content (Prochaska et al., 1996; Shim et al., 2013). Adequate protein intake ensures a more nutritionally balanced yolk, whereas a protein-deficient diet may result in smaller, less nutritious yolks. While protein levels do not have a direct negative effect on shell quality, they influence overall egg quality by supporting the hen's ability to metabolize and utilize other essential nutrients effectively (Gunawardana et al., 2008). Therefore, maintaining an appropriate balance of dietary protein, along with other key nutrients, is essential for optimizing egg size, shell strength, and yolk nutritional value.

The balance of amino acids is also critical for maintaining the reproductive health of hens, affecting processes such as follicle development and vitellogenin production in the liver, which is necessary for yolk formation (Gunawardana et al., 2008; Ma et al., 2021). Factors including hen's age, breed, and environmental stress influence their amino acid requirements. High-producing breeds, need carefully balanced diets to ensure consistent egg production and quality. Adequate amino acid intake ensures that hens can lay healthy eggs while maintaining their overall well-being (Macelline et al., 2021).

14) Impact of dietary protein intake on metabolic pathways

Protein intake in laying hens significantly affects amino acid metabolism, protein synthesis, and energy pathways (Kidd et al., 2021). The urea cycle becomes more active with higher protein intake to manage excess nitrogen, while low protein intake may lead to reduced amino acid availability, affecting overall production and growth rates (Macelline et al., 2021). In particular, amino acids derived from protein content such as cysteine, glutamine, arginine, leucine, proline and tryptophan are involved in metabolic pathways of hens (Qaid and Al-Garadi, 2021). For instance, mTOR and IGF signaling play a major role as key regulatory pathways for protein synthesis and growth regulation corresponding to protein availability within the animal body (Xu and Velleman, 2023).

Energy metabolism is also influenced by protein levels, with higher intake supporting enhanced energy production through amino acid oxidation, while lower intake shifts metabolism toward gluconeogenesis, converting amino acids into glucose to maintain energy balance (Chwalibog, 1985). Protein intake can also modulate fat metabolism, affecting the balance between fat deposition and utilization, which impacts both energy storage and reproductive health (Hu et al., 2024).

In addition, protein influences reproductive processes, bone health, and immune function. Sufficient protein supports yolk protein deposition, eggshell formation, and overall reproductive performance, while also enhancing immune responses through antioxidant defense mechanisms (Alagawany et al., 2016). Nevertheless, excessive protein may lead to inefficiencies due to increased nitrogen waste, so optimal protein intake is key for balancing these metabolic pathways for egg production and hen health (Ji et al., 2014).

15) Impact of dietary protein on environmental sustainability

In poultry, dietary proteins are broken down into amino acids, which are absorbed in the small intestine and transported to various tissues where they are used for the synthesis of muscle proteins, enzymes, and other nitrogenous compounds (Forbes and Shariatmadari, 1994). Any excess amino acids that are not used for protein synthesis undergo deamination, where the amino group is removed. This results in the formation of ammonia, which is then converted to less toxic forms such as uric acid through the uricotelic pathways, a key adaptation in birds to conserve water and manage nitrogen waste (Wright, 1995; Stern and Mozdziak, 2019). Uric acid is secreted via the kidneys and eliminated as a component of poultry excrements (Such et al., 2021). The efficiency of protein metabolism in poultry is influenced by factors such as the quality and composition of dietary proteins, the balance of essential amino acids, and the age and physiological state of the birds (Shim et al., 2013).

High levels of nitrogen excretion are associated with inefficient protein utilization and lead to increased ammonia emissions, contributing to poor air quality and potential respiratory issues in rearing environment (Giannakis et al., 2019; Such et al., 2021). Furthermore, nitrogen-rich manure can have detrimental environmental effects, such as water contamination through nitrate leaching and eutrophication of aquatic systems (Rayne and Aula, 2020). This eutrophication depletes oxygen levels when they decompose, creating "dead zones" that are unable to support aquatic life, disrupting biodiversity and fisheries (Bailey et al., 2020). Additionally, the toxins produced by some algae can pose risks to human health, wildlife, and livestock, making nitrogen runoff a major environmental concern (Pitois et al., 2001).

On land, excess nitrogen can degrade soil quality and contribute to air pollution. When poultry manure is over-applied, it may lead to soil acidification, harming plant growth and reducing soil fertility. The imbalance also disrupts microbial communities vital to maintaining healthy soils (Rayne and Aula, 2020). In the atmosphere, ammonia from manure contributes to air pollution and forms harmful particulate matter, while nitrous oxide, a potent greenhouse gas, accelerates climate change. This disruption extends to insects, birds, and other wildlife that depend on these ecosystems for survival (Tamm, 2012; Debaba et al., 2024). These emissions have serious public health implications, including respiratory problems for people living near poultry farms (Casey et al., 2006). In addition to harming ecosystems, nitrogen pollution also poses public health risks, such as nitrate contamination of drinking water, which can cause methemoglobinemia (blue baby syndrome) in infants (Ward et al., 2018). As such, optimizing dietary protein levels and improving the balance of amino acids can enhance nitrogen utilization, reduce waste excretion, and minimize the environmental risk from poultry farms.

SUMMARY

Dietary protein levels directly affect egg production and the growth performance of laying hens. Hence, precise supplementation is essential to strike a balance between productivity, bird health, and environmental sustainability. These supplementations must also remain economically viable. This review highlights the various protein sources available for laying hens, their amino acid profiles, and the role of dietary protein in influencing growth performance, egg production, and egg quality. Additionally, it emphasizes the importance of minimizing nitrogen excretion from poultry to support environmental sustainability. The “ideal amino acid balance” concept is one of the most effective approaches to achieving these goals, as it addresses the balance of protein and key nutrients in the diet. However, this concept often falls short in balancing essential and non-essential amino acids. Selecting appropriate protein sources is critical to overcoming this limitation. Novel protein sources such as single-cell proteins and insect meals show significant potential for enhancing sustainability in poultry nutrition. Future strategies should focus on innovative, highly bioavailable protein sources.

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