

Review

Optimizing Reduced Protein Diets for an Efficient and Sustainable Layer Production

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Abstract

Dietary protein is essential for poultry performance, influencing egg production, growth, immunity, and physiological functions. However, the high cost and environmental impact of soybean meal has encouraged the development of reduced crude protein (CP) diets supplemented with crystalline amino acids (AAs), coupled with the reduced cost and improved availability of synthetic AA. This strategy enables precise AA balancing, improves nitrogen utilization, and reduces nitrogen excretion and ammonia emissions. Among these strategies, supplementation with arginine (Arg), an indispensable AA in chickens, is particularly important due to its critical roles in protein synthesis, reproductive function, and eggshell formation. In addition, functional Arg precursors, such as guanidinoacetic acid (GAA) and citrulline (Cit), can further enhance Arg availability while supporting energy metabolism and hormonal regulation. Despite these benefits, excessive protein reduction without adequate AA balance can impair laying performance, particularly in aged hens. Furthermore, nutrient digestibility remains a key limitation in reduced protein (RP) diets, as non-starch polysaccharides present in cereal-based feeds can reduce nutrient availability. The inclusion of exogenous enzymes, such as xylanase and β -glucanase, can improve digestibility, while phytase supplementation further enhances nutrient utilization by increasing phosphorus availability and mitigating the anti-nutritional effects of phytate; however, responses are often inconsistent. Another critical factor is maintaining an optimal energy-to-protein ratio, as imbalances may lead to increased fat deposition and reduced productive performance. While supplementation of key limiting AAs (lysine, methionine, and threonine) supports protein reduction, other AAs, such as Arg, isoleucine, and valine, may become limiting in RP diets. Hence, this review explores developments toward RP diets by highlighting three key strategies: enzyme supplementation to enhance nutrient digestibility, inclusion of functional Arg sources to improve performance and bone quality, and optimization of the energy–protein ratio in RP diets through precise AA formulation, with the overall aim to improve the sustainability of the poultry industry.

Keywords: arginine; crystalline amino acids; enzymes; laying hens; reduced protein diet

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1. Introduction

Dietary protein is a fundamental component of poultry nutrition, playing a critical role in growth, body weight maintenance, feather development, immune function, egg production, and overall physiological processes [1]. In conventional feeding systems, standard protein (SP) diets for laying hens typically contain approximately 16 to 19.8% crude protein (CP), depending on age, feed intake, body weight, and production stage [1,2]. Reduced protein (RP) diets are defined as feeding strategies in which dietary CP is reduced while maintaining adequate levels of essential AA to meet the bird's requirements [3]. This is achieved through precise formulation and supplementation with crystalline AAs, thereby reducing total nitrogen input without compromising the supply of limiting AAs such as methionine, lysine, threonine, and tryptophan. Therefore, RP diets are not simply low protein diets, but nutritionally balanced ratios designed to optimize AA supply relative to the bird's requirements. In practical terms, RP diets are formulated by reducing the inclusion of soybean meal and increasing the use of wheat and feed-grade AAs. This shift in ingredient composition results in broader changes to the diet matrix.

Specifically, the RP diet is typically characterized by higher levels of carbohydrates due to increased cereal inclusion, greater reliance on synthetic AAs, and adjustments in dietary energy sources such as fats and oils to maintain energy balance. In addition, reducing soybean meal alters the profile of other dietary components, including fiber fractions and anti-nutritional factors such as phytate and non-starch polysaccharides (NSPs) [4]. Consequently, RP diets represent a fundamentally different nutritional alternative with distinct physicochemical and digestive characteristics compared with SP diets. The adoption of RP diets has been driven by both economic and environmental considerations. Increasing costs and the market volatility of soybean meal have encouraged the poultry industry to explore strategies that reduce reliance on conventional protein sources [5]. From an environmental perspective, RP diets are associated with reduced nitrogen excretion and lower ammonia emissions, contributing to improved sustainability [6]. In addition, improvements in litter quality, reductions in footpad lesions, and better respiratory conditions have been reported, ameliorating bird welfare status [7]. For example, van Harn et al. [8] demonstrated that reducing dietary protein by up to 30 g/kg improved litter dry matter, decreased footpad lesion scores, and reduced the total nitrogen output. Despite these advantages, most research on RP diets has been conducted in broiler chickens, where rapid growth responses are often used as primary performance indicators. In contrast, comparatively fewer studies have focused on laying hens, where nutrient requirements are more complex due to the simultaneous demands of maintenance, egg production, and egg quality. As a result, translating RP feeding strategies from broilers to layers presents several challenges.

One of the key challenges associated with RP diets is maintaining optimal performance when dietary CP is reduced beyond a certain threshold. Reductions greater than approximately 30 g/kg CP have been associated with declines in feed intake and overall performance in both broilers and laying hens [9,10]. Similarly, Oluwabiye et al. [11] reported reduced body weight and increased mortality in pullets fed a 14% CP diet, while Heo et al. [12] observed decreased egg weight in hens fed 12% CP, likely due to inadequate protein supply and AA imbalance. Other studies have also reported negative effects on feed efficiency, gut health, carcass traits, and economic returns when AA balance and energy relationships are not properly optimized [13–15]. These findings highlight that the success of RP diets depends not only on reducing CP, but also on maintaining energy with appropriate AA balance and overall nutrient synchrony. Given these complexities, there is a need to better understand the nutritional and physiological factors that influence the successful implementation of RP diets in laying hens. In particular, recent research has highlighted the importance of specific AAs such as arginine (Arg), the role of exogenous

enzymes in improving nutrient utilization, and the optimization of energy to protein ratios. Therefore, this review aims to critically evaluate the application of RP diets in laying hens, with a specific focus on three key areas: (1) the role of Arg and related metabolic pathways in older laying hens, (2) the use of exogenous enzymes to enhance nutrient availability, and (3) the optimization of protein–energy ration with AA balance. The scope of this review is to synthesize current knowledge and identify key gaps to support the effective implementation of RP feeding strategies in laying hen production systems.

2. Review Methods

A literature search was conducted using Web of Science, Scopus, PubMed, and Google Scholar to identify relevant studies on reduced protein diets, amino acid supplementation, and their effects on laying hen performance and sustainability. The search terms included combinations of reduced protein diet, low crude protein, laying hens, arginine, guanidinoacetic acid, citrulline, amino acid balance, and nitrogen excretion. Additional relevant studies were identified by screening references from selected publications. Peer-reviewed studies related to poultry nutrition, reduced crude protein strategies, amino acid supplementation, nutrient utilization, production performance, egg quality, and nitrogen metabolism were included. Studies unrelated to poultry, without dietary interventions, and duplicate publications were excluded. The selected literature was evaluated to summarize current knowledge and identify future research needs for optimizing reduced protein diets in laying hens.

3. Dietary Arginine

Arginine is an indispensable AA in poultry because birds lack a functional urea cycle, specifically the enzyme carbamoyl phosphate synthetase, which is required for endogenous Arg synthesis [16]. Consequently, an adequate dietary supply is essential to support normal physiological functions. Beyond its role in protein synthesis, Arg is a central metabolic precursor involved in multiple biological pathways (Figure 1), including the synthesis of nitric oxide (NO), creatine (Cr), polyamines, proline, glutamate, and glutamine [17]. Through these pathways, Arg regulates key processes such as cell proliferation, immune function, reproductive performance, vasodilation, and nutrient partitioning [18]. Arginine and its metabolites also contribute to structural and reproductive functions. For instance, proline and polyamines are involved in collagen formation, which is critical for eggshell matrix development and calcification [19]. In addition, Arg-derived NO plays a central role in regulating reproductive hormones, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH), via gonadotropin-releasing hormone (GnRH), thereby influencing follicular development and ovulation. Arginine is also associated with enhanced antioxidant capacity and improved tolerance to heat stress [20,21]. In poultry diet formulation, Arg may act as the fourth, fifth, or sixth limiting AA depending on dietary ingredient composition, underscoring its importance in precision nutrition. When Arg is the fourth limiting AA, supplementation with feed-grade L-Arg provides an opportunity to optimize AA balance while reducing dietary CP and feed costs. However, when Arg becomes the fifth or sixth limiting AA, careful management of the digestible Arg:Lys ratio is required to prevent imbalances that may compromise performance. Although conventional soybean meal-based diets typically supply adequate Arg, its availability can be limiting in reduced CP diets, particularly when alternative ingredients such as wheat, corn gluten, or bakery meal, and corn distillers dried grains with solubles (DDGS) are included. Under these conditions, precise adjustment of Arg levels is essential to maintain performance, egg quality, and metabolic efficiency while maximizing the economic and environmental benefits of RP feeding strategies [22].

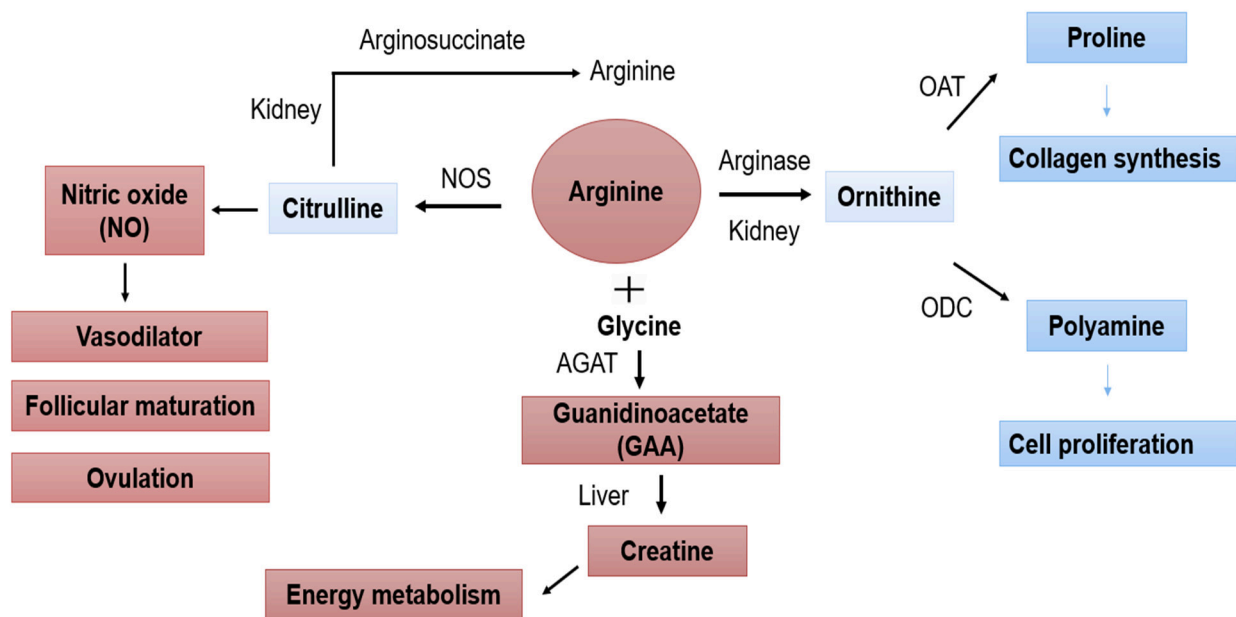


Figure 1. Metabolic pathway of Arg and its precursors [23]. NOS (nitric oxide synthase); NO (nitric oxide); AGAT (arginine glycine amidinotransferase); OAT (Ornithine aminotransferase); ODC (ornithine decarboxylase).

3.1. Arginine Supplementation

Dietary Arg supplementation has been studied as a strategy to enhance production performance in poultry, particularly in relation to egg production and reproductive efficiency. In laying and breeder systems, increasing dietary Arg has consistently been associated with improvements in egg output, egg quality, and overall reproductive performance [24–26]. For instance, elevating digestible Arg levels has been shown to increase egg production in broiler breeder hens by approximately 4% over both short- and long-term feeding periods [24,27]. These responses are largely attributed to the role of Arg in modulating reproductive physiology, particularly through NO-mediated pathways that enhance blood flow and nutrient delivery to reproductive tissues [28,29]. In addition, Arg has been implicated in the regulation of reproductive hormones, including LH and FSH, which are essential for follicular development and ovulation [27,30]. Supporting this, studies in quails have demonstrated a positive association between circulating NO levels and egg production, with higher NO concentrations observed in high-producing birds [31,32]. Conversely, inhibition of NO synthesis has been shown to reduce egg traits, further emphasizing the role of Arg-derived NO in reproductive performance.

Experimental evidence further supports the importance of adequate Arg supply for optimizing laying performance. In a dose–response study, Lima et al. [33] reported that egg production increased progressively with higher dietary Arg levels, reaching a maximum of approximately 83% at 7.11 g/kg. In contrast, birds fed low-Arg diets exhibited markedly reduced performance, producing nearly half (49%) as many eggs as those receiving optimal Arg levels (7.11 g/kg). Egg weight was also affected, with hens on low (2.37 g/kg) or marginal Arg diets (3.16–4.74 g/kg) producing eggs approximately 13 g lighter than those fed high-Arg concentrations (7.90 or 10.27 Arg g/kg). Similarly, egg output declined substantially under low-Arg diets (2.37 g/kg), highlighting the sensitivity of laying performance to dietary Arg supply [33]. Beyond female reproduction, Arg also contributes to male breeder fertility, although most evidence is derived from non-avian models. Supplementation has been associated with improved sperm quality, including increased count

and motility, which is largely attributed to its antioxidant defense mechanisms that protect sperm from oxidative damage [34].

Although research in commercial laying hens remains relatively limited, a substantial body of evidence from broilers, broiler breeders, and other poultry species provides valuable insights into the broader physiological roles of Arg. In these systems, increased dietary Arg has been linked to improvements in growth performance, feed efficiency, and muscle development. These effects are mediated through multiple pathways, including the NO-mediated pathway and the activation of growth-related signaling pathways such as growth hormone and insulin-like growth factor-1 [24,35–38]. Collectively, these findings demonstrate the multifunctional role of Arg in supporting both productive and reproductive performance across poultry species. In addition to its role in production, Arg is important in mitigating environmental and physiological stress. Heat stress, for example, induces oxidative damage and impairs productivity, whereas Arg supplementation has been shown to enhance antioxidant capacity and reduce lipid peroxidation [21]. These effects contribute to improved cellular protection and maintenance of performance under challenging conditions [39,40]. At the mechanistic level, many of the beneficial effects of Arg are mediated through its role as the sole precursor for NO. For example, in ovo Arg supplementation has been shown to improve hatchability, chick quality, and post-hatch growth by enhancing energy metabolism and tissue development [41]. Similarly, under temperature-challenge conditions such as high-altitude rearing, Arg increased the NO levels, thereby supporting vascular function and oxygen delivery and reducing the risk of pulmonary hypertension and ascites [42].

Physiological conditions tightly regulate the synthesis of NO. Under hypoxia or hypertension, endothelial NOS activity is upregulated, promoting vasodilation and improved tissue perfusion [43]. Similarly, under immune challenge, inducible NOS is activated by inflammatory mediators, leading to the increased production of NO as part of the host defense response [44]. While this is essential for pathogen control, excessive NO production can be detrimental, necessitating tight metabolic regulation [45]. Arginine metabolism is further regulated through competition between NOS and arginase, an enzyme that converts Arg into ornithine. Arginase activity, which is prominent in the liver, kidney, and immune cells, increases during later stages of inflammation, thereby limiting Arg availability for NO synthesis and preventing excessive NO accumulation. Importantly, arginase-mediated metabolism also supports tissue growth and repair. The conversion of ornithine into polyamines facilitates cell proliferation and differentiation, while its further metabolism to proline and hydroxyproline contributes to collagen synthesis, antioxidant defense, and the regulation of mammalian target of rapamycin (mTOR) cellular signaling pathways [46–48]. Overall, Arg plays a central and multifaceted role in poultry biology and developmental functions. Although direct evidence in laying hens is comparatively limited, the consistency of responses across different poultry classes underscores the importance of adequate Arg supply in optimizing performance and resilience under modern production conditions.

3.2. Dietary Arginine Within Reduced Protein Diets

In poultry, the lack of a functional urea cycle necessitates a continuous dietary supply of Arg to support normal metabolic functions. In conventional diets, protein-rich ingredients such as soybean meal, meat and bone meal, and canola meal typically provide sufficient Arg. However, as dietary CP is reduced in RP feeding strategies, often through lowering soybean meal inclusion, the supply of Arg and other AAs declines, increasing the risk of deficiency [22,49]. Given that AA profiles are closely linked to dietary CP and ingredient composition, Arg may become limiting alongside other AAs, such as valine and isoleucine, in RP diet formulations. This shift underscores the need for targeted Arg

supplementation in RP diets to mitigate adverse effects on bird performance. Evidence indicates that Arg supplementation can modulate production responses in RP systems; however, its efficacy is highly dependent on inclusion level and overall dietary context. When AA balance is precisely optimized, moderate reductions in CP can be achieved without compromising performance [50]. For instance, Sun et al. [51] reported a quadratic response in laying rate, egg mass, and feed efficiency to increasing Arg levels in low protein diets, indicating that both deficiency and excess may impair productivity. Similarly, Uyanga et al. [52] demonstrated that supplementation of 0.20% Arg in RP diets enhanced growth and reproductive performance through insulin-like growth factor-1 and NO-mediated mechanisms. These improvements were associated with the upregulation of hypothalamic GnRH1 expression, increased estrogen receptor activity, and elevated FSH secretion, highlighting the role of Arg in regulating the hypothalamic–pituitary–gonadal (HPG) axis and supporting follicular development.

Despite the recognized benefits of Arg supplementation in RP diets, responses are not always consistent (Table 1). Some studies have reported no significant effects of Arg supplementation on feed intake, egg production, or nutrient digestibility in RP diets [53]. Moreover, excessive reductions in CP, even with AA supplementation, can impair performance [54]. Conversely, inadequate Arg supply has been consistently associated with declines in growth, feed intake, egg production, and immune function [20,55]. Beyond production responses, supplementation of Arg and its metabolic compounds, such as Cit and GAA, has been shown to support skeletal development, as reflected by increased femur weight and length in laying hens [56]. Improvements in mineral utilization, including enhanced zinc digestibility, may further contribute to bone mineralization and structural integrity. Collectively, these findings underscore the critical role of Arg in RP feeding systems and demonstrate the need for precise AA formulation to sustain productivity, health, and nutrient utilization under RP conditions. Interestingly, a recent study by Nawab et al. [57] in laying hens demonstrated that supplementation of the RP diet with 0.06% Arg resulted in higher yolk weight than supplementation with the same level of GAA. Moreover, supplementation with 0.06% Arg significantly improved apparent energy digestibility compared with both the standard-protein (SP) and unsupplemented RP diets. However, increasing the supplementation level of either Arg or GAA reduced the bone breaking strength compared with their respective lower inclusion levels. Furthermore, Arg supplementation had no significant effect on either external or internal egg quality parameters at 75 weeks of age. These findings are consistent with those of Dao et al. [53], who reported that supplementation with Arg, GAA, or Cit did not affect shell strength, shell reflectivity, albumen height, yolk color, or Haugh unit in laying hens. Nawab et al. [57] reported that supplementation of RP diets with either low or high levels of Arg (0.06% or 0.12%) did not significantly affect the production performance of laying hens. These findings suggest that the optimal inclusion level of Arg has not yet been established. Consequently, further dose–response studies across different production stages are required to define practical supplementation strategies and optimize the use of Arg and its related metabolites in commercial RP diets.

Table 1. Effects of Arg supplementation on poultry birds.

Dietary Arg Levels	Species	Diet Ingredients	Consequences	References
Arginine vasotocin injection at 0.15–1 µg/0.5 mL per hen	Laying hens	Commercial basal diet	Increased uterine contractions	[58]
Arg at 1.54% and 2.04% combined with Lys at 1.05, 1.67, and 2.34%	Laying hens (Saudi breed)	Yellow corn and soybean meal	Tended to increase F1 follicle weight at 2.04%; increased mean antibody titers at 1.54% Arg with 1.05% Lys at 36 weeks of age	[59]

Table 1. Cont.

Dietary Arg Levels	Species	Diet Ingredients	Consequences	References
L-Arg at 50 g/kg basal diet orally	Laying quail (Japanese)	Commercial basal diet	Increased egg production and follicular nitrite and nitrate (NO ₂ and NO ₃) levels	[31]
Arg at 5 mg/kg with zinc 60 mg/kg	Laying quail (Japanese)	Corn, soybean meal, and fish meal	Increased total antioxidant capacity, egg weight, and blood NO levels; decreased methane dicarboxylic aldehyde (MDA) level	[21]
Digestible Arg levels at 0.943, 1.093, 1.243, 1.393, and 1.543%	Broiler breeder hens	Corn, wheat bran, soy oil and soybean meal	Egg production increased at 1.262% Arg and egg weight at 1.543% Arg, with no effect on hatchability	[24]
Starter diet (digestible Arg at 1.300, 1.450, 1.600, 1.750, and 1.900%)	Broiler breeder hens	Corn, soybean meal and soy oil	Increased cloacal bursa weight; linear decrease in Newcastle disease antibody titer at day 7	[60]
Growing diet (digestible Arg at 1.150, 1.300, 1.450, 1.600, and 1.750%)	Broiler breeder hens	Corn, soybean meal and soy oil		
Digestible Arg at 0.7, 0.8, 0.9, or 1 g/kg combined with digestible Lys at 0.7 or 0.9 g/kg of diet	Laying hens (Lohmann)	Corn, soybean meal, wheat bran and corn gluten 60%	Egg weight and egg quality parameters remained unaffected	[61]
Digestible Arg at 1.36%	Broiler breeder hens	Corn, soybean meal and soy oil	Highest laying rate and total antioxidant levels; lowest MDA levels during 60 to 69 weeks of age	[27]
Arg levels at 1.25, 1.26, and 1.27%	Laying hens (Xinyang black)	Corn, corn gluten meal based diets	Increased egg weight at 1.25%; highest feed intake, laying rate, and lowest FCR at 1.27%	[62]
Arg levels at 0.66%, 0.86%, 1.06%, 1.26% and 1.46%	Laying ducks	Corn, wheat middling, Distillers dried grains with solubles (DDGS) and corn gluten meal	Egg production unaffected; 1.26% Arg increased yolk percentage and yolk color, while 1.46% Arg improved egg weight, shell thickness, and total shell percentage	[26]
Arg levels at 0%, 0.45%, 0.90%, 1.35%, and 1.80%	Broiler chickens	Yellow corn, corn gluten meal, soybean and rapeseed meal	Increased body weight, insulin level, serum growth hormone, and lymphocyte proliferation, except at 1.80% Arg	[63]
Total Arg: 8.5, 9.7, 10.9, 12.1, and 13.3 g/kg	Chinese female chickens	Corn, soybean meal, wheat bran and corn gluten meal	Increased growth performance, intestinal antioxidant parameters, innate immunity, and gut microbiota at 12.1 g Arg	[64]
Digestible Arg levels: 0.96%, 1.16%, 1.35%, 1.55%, 1.74%, and 1.93%	Broiler breeder hens (maternal diet)	Corn, soybean meal and soy oil	1.16% Arg increased egg weight; 1.55% Arg enhanced protein synthesis and immunity of offspring during the embryonic period	[65]
Arg at 0.37% SID (standardized ileal digestibility) with 0.075% SID tryptophan (Trp), and 0.5% taurine	Laying hens	Corn, soybean meal, corn gluten meal and DDGS	Tendency to enhance egg yolk color in Arg and Trp treatments; however, productive performance, egg quality, liver characteristics, antioxidant status, and immune parameters were unaffected under heat stress	[66]
Arg levels: 0.1%, 0.2%, 0.3%, 0.4%, and 0.5%	Laying geese	Corn, rapeseed meal, wheat bran, soy oil, soybean meal and corn gluten meal	Increased egg production, average egg weight, immunity, and antioxidant parameters	[67]
Three L-Arg levels: Hy-Line recommended level (RHL), 20% above RHL, and 40% above RHL with Copper (Cu: 8 and 16 mg/kg)	Laying hens	Corn, wheat bran, soybean meal, corn gluten meal, canola oil and canola meal	Increased performance, immunity, and nutrient digestibility with 16 mg Cu/kg combined with L-Arg at 20% or 40% above the recommended level	[68]

4. Significance of Guanidinoacetic Acid and Its Role as an Alternative Arginine Supplement

Guanidinoacetic acid (GAA) is a key intermediate in Arg metabolism and the immediate precursor of creatine (Cr), a compound essential for cellular energy homeostasis (Figure 1) [23,69]. Endogenously, GAA is synthesized from Arg and glycine via arginine:glycine amidinotransferase (AGAT) in the kidney and subsequently methylated in the liver to form Cr [70]. Creatine and its phosphorylated form (phosphocreatine) play a central role in cellular energy buffering (Figure 2) through the phosphocreatine shuttle system, which facilitates rapid ATP regeneration in tissues with high energy demand, such as skeletal muscle, brain, and reproductive organs [71,72]. In poultry, where Arg is an essential AA due to the absence of a functional urea cycle [17], the diversion of Arg toward Cr synthesis can limit its availability for other physiological functions, including protein accretion, nitric oxide production, and immune responses. Supplementation of GAA provides a practical nutritional strategy to reduce this metabolic demand on Arg, thereby exerting a clear Arg-sparing effect and improving overall AA utilization [53,73]. This is particularly relevant in RP diets, where Arg supply may become limiting, especially in wheat-sorghum-based formulations lacking sufficient crystalline Arg [70]. Compared with direct Cr supplementation, GAA offers several practical and metabolic advantages. Creatine is thermally unstable and prone to degradation during feed processing, whereas GAA is highly stable and exhibits high recovery in pelleted and extruded diets [71,73]. Additionally, GAA is efficiently absorbed and converted to Cr, resulting in increased tissue Cr concentrations, improved phosphocreatine-to-ATP ratios, and enhanced energy availability at the cellular level. These properties make GAA a reliable and effective feed additive in modern poultry nutrition. Reflecting its safety and efficacy, the EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed) has approved GAA for use in laying hens at inclusion levels up to 1.2 g/kg diet [74].

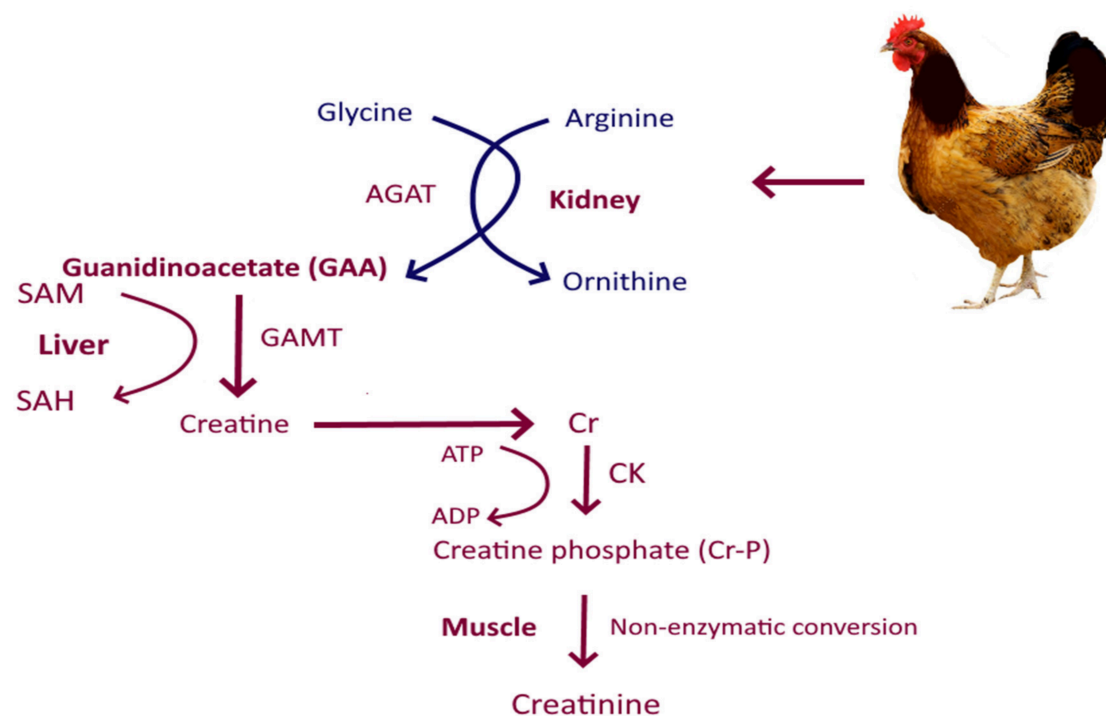


Figure 2. Metabolic pathway of GAA [75]. AGAT (arginine:glycine amidinotransferase); GAMT (guanidinoacetate N-methyltransferase ARG arginase); SAM (S-adenosylmethionine); SAH (S-adenosylhomocysteine); Cr (creatine); CK (creatine kinase).

The metabolic benefits of GAA supplementation translate into measurable improvements in production performance and physiological function (Table 2). Numerous studies have reported increased Cr concentrations in blood and tissues, accompanied by enhanced growth performance and feed efficiency in poultry [76–78]. In laying hens, dietary inclusion of 1.0–1.5 g/kg GAA has been associated with improvements in egg production, egg mass, and FCR [79]. Reproductive benefits have also been documented, with optimal inclusion levels (0.13–0.15%) improving fertility, hatchability, and embryonic survival, likely due to enhanced ATP availability supporting sperm motility and vitality [80,81]. Furthermore, GAA may be deposited in eggs and converted to Cr, thereby supporting embryonic development. Evidence from broiler studies further supports the functional role of GAA, particularly under challenging conditions. Supplementation has been shown to alleviate Arg deficiency, restore growth performance, and improve muscle energy reserves to levels comparable with Arg-adequate diets [73]. Additional benefits of GAA were also confirmed, including improved gut morphology, enhanced muscle development, and better performance under stress conditions such as heat exposure, energy restriction, and reduced CP levels [82–84]. Collectively, these responses are likely driven by enhanced energy metabolism, improved intestinal function, and more efficient utilization of available nutrients.

Despite these promising outcomes, responses to GAA supplementation in laying hens, particularly within RP diets are controversial. Some studies have reported no significant effects on egg mass, egg weight, or key egg quality parameters when GAA, Arg, or Cit were supplemented in RP diets, potentially due to limitations in other essential AAs [53,85]. While most egg quality traits appear largely unaffected, subtle changes in shell characteristics and creatine-related metabolites in eggs have been observed [53,86]. These inconsistencies highlight the complexity of nutrient interactions in RP formulations and suggest that the efficacy of GAA is highly dependent on overall diet composition, AA balance, and physiological or environmental conditions. Historically, GAA was first investigated as a therapeutic compound in the early 1950s [87], and interest in its application in poultry nutrition expanded following restrictions on meat and bone meal in Europe, which reduced dietary Cr availability and subsequently impaired the performance of farm animals [88]. Since then, GAA has gained recognition as a strategic feed additive for improving energy utilization and sustaining productive efficiency in modern poultry systems. Overall, GAA represents a practical nutritional approach to increase Cr availability, support cellular energy turnover, and reduce the metabolic demand for dietary Arg. Its application is particularly relevant in RP feeding strategies, where maximizing AA efficiency is essential for sustaining productivity in laying hens. Our recent study in 2026 demonstrated that low-dose GAA supplementation (0.06%) improved bone breaking strength compared with higher inclusion levels at 75 weeks of age, although it did not influence production performance and egg quality during the 60–75 week production period [57]. Further research is required to determine the optimal inclusion levels of GAA and to clarify its potential role in modulating production performance under commercial conditions.

Table 2. Effects of GAA supplementation on poultry birds.

Dietary GAA	Breed	Diet Ingredients	Consequences	Reference
0, 0.4, 0.8, 1.2, and 1.6 g GAA/kg	Broiler breeder hens	Starter, grower and finisher	Improved hatchability at 0.8 and 1.2 g GAA	[74]
0 or 1.2 g GAA/kg combined with 0, 3, 6, or 9 g Arg/kg	Broiler breeder hens	Corn, soybean meal, wheat and canola oil	Increased fertility and sperm penetration rate in the inner perivitelline layer; combined Arg and GAA improved overall fertility	[89]

Table 2. Cont.

Dietary GAA	Breed	Diet Ingredients	Consequences	Reference
0.57, 1.14, and 1.71 g GAA/kg	Laying hens	Corn, soybean meal, meat meal and soy oil	Increased LH and FSH levels at 1.14 g GAA; performance and NO levels were unaffected	[90]
1.5 g GAA/kg	Broiler breeder hens	Corn, wheat, soybean meal, wheat bran, sunflower meal and soapstock oil	Enhanced GAA absorption and deposition into hatching eggs	[91]
1 g and 1.5 g GAA/kg	Laying hens	Yellow maize, soybean meal and maize oil	Increased egg mass, egg production, and hepatic NO levels at 1 g and 1.5 g GAA; egg weight was unaffected	[79]
4.6 g GAA/kg in RP diets	Laying hens	Barley, wheat, sorghum, soybean meal, canola meal and canola oil	No significant effects on feed intake or production performance	[53]
0, 0.6, 1.2 and 1.8 g GAA/kg	Laying quails	Corn, soybean meal, soy oil, and corn gluten meal	Increased antioxidant status, nutrient digestibility, and intestinal morphology at 0.6 g and 1.2 g GAA; egg production increased at 1.8 g GAA	[92]
0.6 g GAA/kg under 32 °C heat stress	Broiler chickens	Corn, soybean meal and soy oil	Increased jejunal villus height, villus height: crypt depth ratio, mucus thickness, goblet cell number, and immunoglobulin A (IgA ⁺) cell density; reduced relative intestinal weight and length	[93]
0.6 g GAA/kg under 32 °C heat stress	Broiler chickens	Corn, soybean meal and soy oil	Decreased plasma corticosterone and increased immune parameters (interleukin-2 [IL-2], IgG, IgM)	[94]
0.08% GAA with ME levels (2850, 2800, and 2750 kcal/kg)	Laying hens	Yellow corn, soybean meal, wheat bran and vegetable oil	GAA increased egg production and egg mass, as well as reduced serum IL-2 at 2800 kcal/kg; no effects on egg quality traits (Haugh unit, shell strength, shell thickness, or yolk color)	[95]
0 and 0.6 g GAA/kg	Laying hens	Corn sorghum, soybean meal and soy oil	GAA increased egg mass, albumen height, Haugh unit, shell thickness, shell breaking strength, and egg production; no effects on egg weight, feed intake, or final body weight	[96]
0, 0.6, and 1.2 g GAA/kg with ME levels (2600 and 2700 kcal/kg)	Laying hens	Corn, wheat bran, soybean meal and soy oil	Improved laying performance, egg mass, and reduced FCR in low-ME diets at 0.6 g and 1.2 g GAA; no effects on feed intake, egg weight, or final body weight; no effects on albumen, yolk, shell weights, yolk color, albumen height, Haugh unit, or shell quality	[97]

5. Significance of Citrulline and Its Role as an Alternative Arginine Supplement

Citrulline is increasingly recognized as a functional non-protein AA with significant potential in poultry nutrition, particularly in the context of RP diets. As an intermediate of the Arg–NO cycle, Cit plays a central role in endogenous Arg synthesis. Unlike dietary Arg, which undergoes extensive first-pass metabolism in the intestine and liver, Cit bypasses hepatic degradation and is primarily converted to Arg in the kidneys before entering systemic circulation [98,99]. This metabolic advantage improves its efficiency as a precursor for de novo Arg synthesis, positioning Cit supplementation as a viable nutritional strategy in place of direct Arg supplementation (Figure 1) [100]. Through its involvement in the Arg–NO pathway (Figure 3), Cit supports several key physiological functions, including vascular regulation, nutrient assimilation, and thermal balance [101,102]. Notably, Cit has

been shown to conserve Arg utilization, enabling a partial reduction in dietary Arg supply without negatively affecting growth performance or intestinal integrity in broilers [100,103]. For example, supplementation of 0.50% Cit in Arg-deficient diets significantly increased circulating Arg and ornithine concentrations, confirming its *in vivo* conversion efficiency and contribution to Arg availability [100]. Beyond its role as an Arg precursor, Cit exhibits multiple functional properties that support bird health and productivity. It has been associated with enhanced antioxidant capacity, reduced lipid peroxidation, and improved immune responses [102,104–107]. Uyanga et al. [107] reported that dietary inclusion of 1% Cit increased antioxidant enzyme activities, including superoxide dismutase and catalase, while reducing malondialdehyde levels in laying hens, indicating reduced oxidative stress. In addition, Cit supplementation (1%) has been shown to elevate plasma immunoglobulin concentrations (IgA, IgG, and IgM) compared with birds fed a basal diet under the same environmental conditions, suggesting enhanced immune competence [108]. Evidence from animal models further indicates that Cit improves intestinal barrier integrity and enterocyte function, which may enhance nutrient absorption efficiency [109,110].

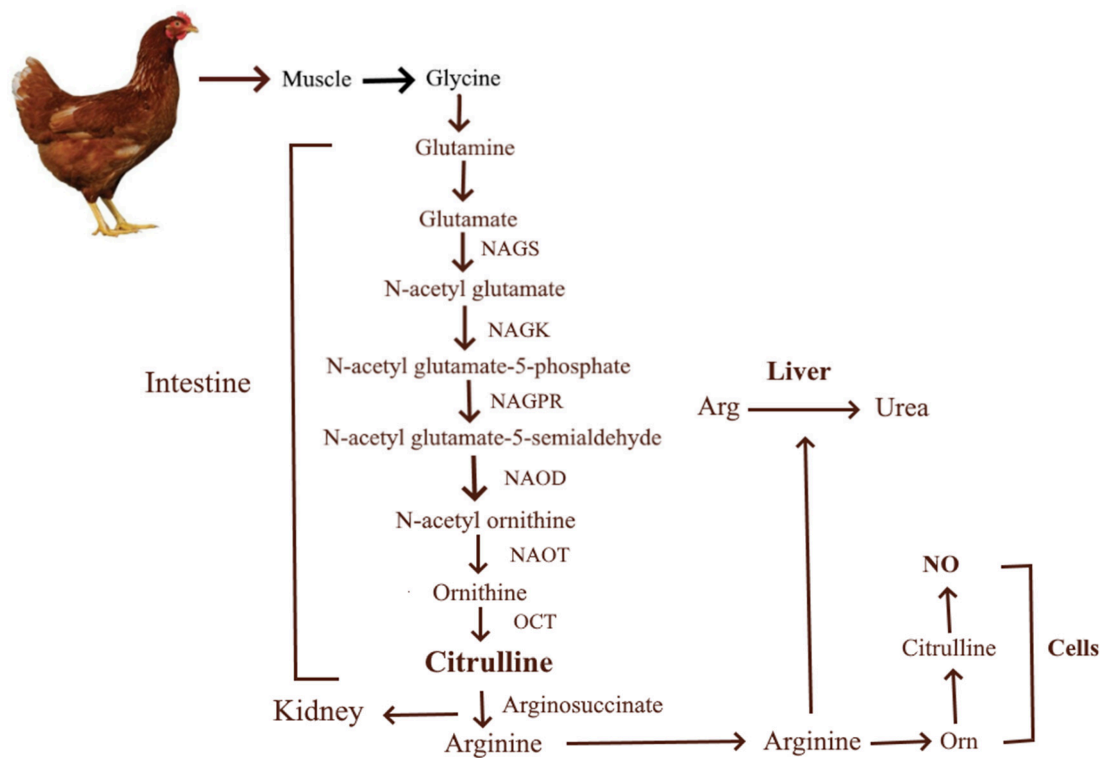


Figure 3. Metabolic pathways of Cit in chicken [111]. NAGS (N-Acetylglutamate synthase); NAGK (N-Acetylglucosamine kinase); NAGPR (N-Acetylglutamate-5-P reductase); NAOD (N-Acetylorithine deacetylase); NAOT (N-Acetylorithine transaminase); OCT (ornithine carbamoyltransferase); ORN (ornithine); NO (nitric oxide).

One of the most consistently reported benefits of Cit in poultry is its role in thermoregulation. Studies have demonstrated that Cit supplementation reduces body temperature and improves thermotolerance under both thermoneutral and heat stress conditions [102,112,113]. This hypothermic effect may be partly mediated by reductions in plasma glucose concentrations, which limit the availability of thermogenic substrates and thereby reduce metabolic heat production [113]. Given the increasing prevalence of heat stress in poultry production systems, this property is of practical relevance. Beyond thermoregulation, Cit also plays a role in metabolic and physiological regulation (Figure 3). Derived naturally from *Citrullus vulgaris*, it has been linked to improved muscle protein

synthesis, potentially via activation of the mTOR (mammalian target of rapamycin) signaling pathway [114,115]. As a pharmaconutrient, it has been investigated for therapeutic applications in metabolic disorders, urea cycle dysfunction, endothelial impairment, and muscle-related conditions [116]. Experimental evidence indicates that Cit supplementation can modulate metabolic responses, including glucose metabolism and energy balance, further supporting its functional significance [117]. In addition, Cit supplementation has been shown to improve bone strength, as evidenced by increased femur weight, length, and ash content in broilers [56]. These effects may be associated with enhanced mineral utilization, particularly zinc, which is essential for bone mineralization. Given that zinc deficiency is known to impair bone mineralization and skeletal development [118,119], these findings highlight the potential role of Cit in supporting bone health. Furthermore, Cit has been linked to improved nitrogen utilization, reflected by reduced serum uric acid levels, indicating more efficient AA metabolism in RP diets [120].

Despite these promising outcomes, most of the research on Cit supplementation has been conducted in broilers or non-avian models (Table 3), with relatively limited data available for laying hens, particularly at later stages of production. Existing studies in layers have reported inconsistent responses. Some studies observed no significant effects on egg production or thermoregulation under heat stress [107], while others reported improvements in egg quality traits such as Haugh units, although with reductions in eggshell strength [53]. These discrepancies may reflect differences in environmental conditions, diet composition, and physiological stage, all of which can influence the response to Cit supplementation. Evidence from other species further supports the metabolic significance of Cit. For instance, Cit supplementation has been reported to enhance survival and physiological resilience under stress conditions in pigs, while in ruminants, it promotes reproductive hormone secretion and more effectively enhances fetal Arg supply compared with direct Arg supplementation [121–123]. Notably, Cit is also used as an alternative to Arg supplements. In broilers challenged with necrotic enteritis, Cit supplementation improved growth performance and breast meat yield, outperforming conventional protein diets and those supplemented with Arg alone [103,124]. Overall, Cit represents a promising functional nutrient in RP feeding strategies due to its capacity as an Arg alternative, metabolic versatility, and potential to enhance thermotolerance, antioxidant status, immunity, and nutrient utilization. Clearly, in a recent study, high-level Cit supplementation (0.12%) in the RP diet increased feed intake and egg mass during 60–75 weeks of age compared with both RP and SP treatments, without affecting hen body weight [56]. Notably, only Cit supplementation increased feed intake, whereas Arg and GAA had no significant effects. The overall higher feed intake observed in this study may be attributed to birds consuming feed above the strain recommendations, potentially due to the colder climatic conditions during the experimental period. This environmental factor may represent a methodological limitation to consider when interpreting the results. Furthermore, Cit supplementation consistently improved egg mass and/or egg weight compared with Arg and GAA supplementation, suggesting that Cit may have greater potential to support laying performance under the conditions evaluated. The improvement in egg mass observed with high Cit supplementation may be partly attributed to the associated increase in feed intake. At 75 weeks of age, dietary Cit supplementation had no significant effect on internal or external egg quality parameters. Nevertheless, further research is warranted to establish the optimal dietary inclusion level of Cit and to better define its potential role in supporting the performance and egg quality parameters of laying hens under commercial production conditions.

Table 3. Effects of Cit supplementation on poultry birds and various other species.

Dietary Cit	Breed	Diet Ingredients	Consequences	Reference
L-Cit (15 mmol/10 mL/kg body weight)	Layer chicks	Maize, soybean meal, maize gluten meal, fish meal and rice bran	Feed intake was unaffected; plasma total cholesterol, triacylglycerol, and aspartate aminotransferase expression were unchanged; plasma glucose levels decreased	[113]
1% Cit	Laying hens	Corn, wheat bran, soybean meal and soy oil	Increased plasma NO, antioxidant activity, egg shape index, and serum concentrations of Arg, Cit, and ornithine	[107]
0, 0.5, 1, and 2 g ECCs/kg (ethanolic extract of <i>Citrullus colocynthis</i> seeds)	Laying hens	Yellow corn, wheat bran, soybean meal, soy oil and bone meal	Improved productive and physiological performance at 1 g and 2 g ECCs; reduced MDA and corticosterone plasma levels; decreased triglyceride and cholesterol levels in plasma, liver, and egg yolk	[125]
0.1% <i>Citrullus colocynthis</i> seed supplementation	Laying hens	Commercial basal diet	Decreased egg production; increased blood corticosterone, heterophil-to-lymphocyte ratio, total white blood cells, and B-lymphocytes in paraquat-injected hens	[126]
0, 0.5%, 1%, and 1.5% L-Cit	Broiler chickens	Corn, wheat bran, cotton seed meal and soybean meal	Increased growth rate, antioxidant status, and immunity	[127]
Control group and L-Cit at 0.5%, 0.75%, and 1.0%	Broiler chickens	Commercial starter and grower diet	Improved meat quality, organ indices (bursa, heart, and liver), and body weight gain; lowest FCR and abdominal fat observed at 0.75% Cit	[128]

6. Enzyme Supplementation (Phytase, Xylanase, and β -Glucanase)

Exogenous enzymes are widely incorporated into poultry diets to enhance nutrient availability and improve overall production efficiency, with substantial evidence supporting their efficacy under conventional feeding systems. Among the various enzyme classes, the most commonly used in poultry nutrition are phytases and carbohydrases, particularly xylanase and β -glucanase. Accordingly, this review focuses specifically on these enzymes due to their widespread application and well-documented benefits. In typical cereal-soybean meal-based diets, enzyme effectiveness is largely dependent on the presence of their respective substrates. Phytase primarily targets phytate, the principal storage form of phosphorus in plant-based ingredients, thereby improving phosphorus availability, enhancing mineral utilization, and reducing nutrient excretion [129]. In parallel, carbohydrases such as xylanase and β -glucanase act on non-starch polysaccharides (NSPs), including arabinoxylans and β -glucans, which are abundant in cereals like wheat and barley [130]. Poultry lack sufficient endogenous enzymes to effectively degrade NSP,

leading to increased digesta viscosity, impaired nutrient absorption, and reduced feed efficiency [131]. By breaking down these complex carbohydrates, carbohydrases reduce intestinal viscosity and enhance the digestibility of energy and nutrients [132–134]. Overall, supplementation with phytase and NSP-degrading enzymes improves the availability of both mineral and non-mineral nutrients by mitigating the effects of anti-nutritional factors such as phytate and indigestible fiber components [135–137]. Consequently, the strategic use of these enzymes not only enhances feed efficiency and nutrient utilization, but also supports bird health and reduces the economic and environmental costs associated with poultry production. In RP diets, changes in ingredient composition substantially alter the intestinal substrate profile for enzymes [138]. The reduction in soybean meal inclusion, combined with greater reliance on cereal grains and crystalline AAs, influences the balance of plant-derived carbohydrates and phosphorus-containing compounds. Specifically, lowering soybean meal decreases the supply of protein-associated nutrients and reduces the contribution of certain insoluble fiber fractions and phytate-bound phosphorus [139]. At the same time, increased inclusion of wheat alters the NSP profile toward a higher proportion of cereal-derived arabinoxylans [140], particularly water-extractable fractions that are more likely to increase digesta viscosity. Overall NSP concentration may decline moderately; however, the composition shifts from soybean meal-derived insoluble fiber toward more fermentable, viscosity-inducing cereal NSP fractions, which can intensify the physiological impact of remaining fiber despite lower total levels. A similar compositional shift occurs for phytate. Because soybean meal is a major contributor of phytate in conventional diets [141], its reduction in RP formulations generally leads to a lower total phytate content. However, the relative importance of cereal-derived phytate becomes more pronounced, and its interaction with minerals may still limit phosphorus and trace mineral availability. Consequently, RP diets do not simply reduce anti-nutritional substrates uniformly; rather, they restructure NSP and phytate sources, changing their chemical form, solubility, and physiological impact on the gut environment.

These substrate modifications have important implications for enzyme efficacy. For phytase, the reduced but still functionally relevant phytate pool continues to determine the magnitude of phosphorus release and mineral availability improvements [141]. For carbohydrases, although the total NSP may be lower, the increased proportion of wheat-derived soluble arabinoxylans can maintain or even enhance the functional relevance of xylanase activity due to its strong effect on digesta viscosity. Therefore, enzyme responses in RP diets depend less on total substrate quantity and more on substrate quality, solubility, and dietary origin, making their performance context-dependent compared with conventional diets [142]. Consistent with these substrate-driven dynamics, phytase supplementation remains effective in RP diets. Studies have shown that phytase improves phosphorus utilization and reduces mineral excretion without compromising performance in low protein formulations [143–145]. Similarly, carbohydrases such as xylanase and β -glucanase are increasingly relevant in RP diets due to the higher functional impact of cereal NSP fractions. Xylanase supplementation has been reported to improve egg production and reduce intestinal viscosity in wheat-based diets [146], while enzyme combinations (xylanase and β -glucanase) in NSP-rich ingredients such as DDGS (dried distillers grains with solubles) result in enhanced nutrient digestibility and performance [147]. Moreover, improvements in egg mass and feed efficiency have been observed in low energy diets supplemented with xylanase [148], and multi-enzyme complexes have further supported performance (heavier eggs) in nutrient-restricted formulations [149]. Collectively, these findings indicate that enzyme efficacy in RP diets is primarily governed by how protein reduction reshapes NSP structure and phytate availability, rather than by changes in protein alone.

In RP diets, the response to enzyme supplementation is not always consistent (Table 4), reflecting strong dependence on dietary context and substrate availability. Although reductions in CP are accompanied by changes in NSP and phytate profiles, the magnitude of response to exogenous enzymes varies considerably across studies. Phytase supplementation, for example, has shown inconsistent effects on performance traits, with some studies reporting no significant improvements in laying performance despite enhanced mineral utilization or immune responses [150,151]. Similarly, carbohydrase responses in RP diets have been variable. While several studies have reported limited or no effects of xylanase and other NSP-degrading enzymes on performance [152,153], others have demonstrated improvements in egg production, egg mass, and FCR [154]. Despite these inconsistencies, multi-enzyme strategies have shown potential benefits when dietary formulation creates favorable substrate conditions. For instance, Hahn-Didde and Purdum [151] reported improved calcium retention in birds fed low energy diets supplemented with a combination of xylanase, phytase, amylase, and protease, indicating potential synergistic effects among enzymes. Such findings suggest that enzyme efficacy in RP diets is not solely determined by the presence of a single substrate but is influenced by complex interactions among NSP structure, phytate availability, and overall diet composition. Collectively, these results highlight that enzyme responses in RP diets are highly context dependent. Variations in NSP composition, phytate concentration, and ingredient interactions all contribute to differences in performance outcomes. Therefore, the effectiveness of enzyme supplementation in RP diets should be interpreted within the framework of diet formulation and nutrient balance rather than assumed from responses observed in conventional feeding systems. In a recent study, feeding RP diets significantly reduced nitrogen excretion and enhanced apparent metabolizable energy (AME) and protein digestibility in laying hens at 57 weeks of age [155]. Similar improvements in nitrogen utilization efficiency following dietary CP reduction have been previously reported in laying hens [156–158]. The decline in nitrogen output may be related to lower dietary nitrogen intake and a reduced availability of undigested protein for microbial fermentation in the hindgut, which may help maintain a more balanced intestinal environment [159,160]. These findings support the potential of RP feeding strategies to improve nitrogen utilization and reduce the environmental burden associated with egg production. Dietary enzyme supplementation further influenced nutrient utilization responses in RP diets. In a 2026 study, supplementation with 600 FTU/kg phytase combined with 150 g/ton xylanase and β -glucanase reduced dry matter intake and excretion compared with 1200 FTU/kg PhyG at the same XB inclusion level. Additionally, the lower excreta moisture observed in RP-fed hens may be explained by reduced nitrogen intake and decreased uric acid excretion, resulting in a lower water requirement for nitrogen clearance [12,161]. In contrast, excessive dietary protein supply can increase nitrogen turnover and the amount of undigested protein entering the hindgut, potentially altering microbial populations and reducing nutrient utilization efficiency [13,162–164]. Therefore, appropriately formulated RP diets may promote a more favorable digestive environment and improve nutrient efficiency in aged laying hens.

Table 4. Effect of enzymes (phytase, xylanase and β -glucanase) on laying hens.

Enzyme's Supplement	Breed	Diet Ingredients	Consequences	Reference
Phytase (300 FTU/kg) + non-phytate phosphorus (NPP) (0.25–0.2 to 0.15%)	Laying hens	Corn and soybean meal	Performance improved at 300 FTU across NPP levels	[165]

Table 4. Cont.

Enzyme's Supplement	Breed	Diet Ingredients	Consequences	Reference
Phytase (1000 FTU/kg) + NPP (0.20, 0.25 and 0.30%)	Laying hens	Corn, soybean meal, corn germ meal, corn gluten meal, DDGS and soy oil	Increased egg production and eggshell thickness; no effect on egg weight, feed intake, egg mass, FCR, albumen height, Haugh unit, yolk, or shell color	[166]
Bacterial phytase (1500 FTU/kg)	Laying hens	Corn, soybean meal and soy oil	Increased performance, egg quality, and villus-to-crypt ratio from 44 to 64 weeks of age	[167]
Xylanase (620 IU) + β -glucanase (550 IU)	Laying hens	Maize, wheat, barley, Rye, sunflower meal and soybean meal	Improved feed efficiency and egg production, with reduced intestinal viscosity and dirty eggs	[168]
Xylanase (2000 U/kg) + 0.3% available P (phosphorus)	Laying hens	Wheat, soybean meal, canola meal, corn gluten meal and vegetable oil	Egg production was unchanged, but egg weight, albumen weight, and height were increased	[169]
Multi-enzyme complex (xylanase, β -glucanase, α -amylase, pectinase, endo- β -glucanase, pentosanase, pectinase and hemicellulose) at 1 to 2 g/kg	Laying hens	Corn, wheat, barley, wheat bran, soybean meal, sunflower oil, meat and bone meal	No effect on egg production or quality, but improved yolk index, serum albumin, and FCR	[170]
Xylanase (1500 U/kg)	Laying hens	Corn, soybean meal, bakery meal, DDGS and soy oil	Increased feed intake, egg production, egg mass, and feed efficiency (20–44 weeks)	[171]
Xylanase (0, 225, 450, and 900 U/kg)	Laying hens	Corn, wheat, soybean meal, wheat bran, corn gluten meal and animal fat	No effect on performance, but xylanase improved Haugh unit and albumen height linearly from 44–56 weeks of age	[172]
Multi-carbohydrase complex (xylanase 2200 U/g, β -glucanase 3000 U/g, and 6-phytase 1000 U/g)	Laying hens	Corn, soybean meal, soy oil, meat and bone meal	Egg production and FCR (per egg mass and per dozen eggs) were unaffected; No effects on eggshell weight, shell thickness, specific gravity, or yolk color	[173]
Xylanase (0, 45,000, and 90,000 U/kg)	Laying hens	Yellow corn, wheat, soybean meal, soy oil and sunflower meal	Increased average egg weight and feed efficiency at 45,000 U/kg; improved egg quality (albumen height and Haugh unit)	[174]
Multicarbohydrase-phytase complex (xylanase 12,500 U/g, β -glucanase 8600 U/g, α -arabinofuranosidase 46,000 U/g, and phytase 10,000 FTU/g)	Laying hens	Corn, rice bran, soybean meal, and coco oil	No significant effects on egg production, egg weight, egg mass, or livability; no effects on egg quality or egg composition	[175]
Multienzyme complex (xylanase 600,000 XU/kg, β -glucanase 500,000 BGU/kg, amylase 800,000 BAU/kg, cellulase 3,000,000 CMC/kg, mannanase 2,500,000 MNU/kg, pectinase 1,000,000 PBU/kg, protease 1,000,000 PCU/kg, and phytase 1,000,000 FTU/kg)	Laying hens	Corn, soybean meal, wheat bran and animal fat	Linearly improved egg production, egg mass, and FCR; increased Haugh unit, yolk weight, and yolk percentage; no effects on body weight, feed intake, or egg weight	[176]

7. Energy–Protein Optimization with Balanced Amino Acids in Reduced Protein Diets

Optimizing RP diets requires more than simply lowering CP; it depends on three critical factors: dietary energy balance, adequate essential Arg supply, and the inclusion of functional feed additives. While supplementation with feed enzymes and functional Arg sources can enhance nutrient availability and improve bone quality, maintaining an optimal energy to protein (AME:CP) ratio remains essential for sustaining performance and overall health in RP-fed birds. From an economic perspective, protein is one of the most expensive components of commercial poultry diets [177]. Moreover, increasing population pressure, urbanization, and climate change are constraining sustainable crop production, thereby further escalating feed ingredient costs [178]. Consequently, strategic refinement of dietary AA profiles enables the lowering of CP levels while reducing reliance on protein-rich ingredients [179] and minimizing nitrogen excretion [180]. Birds cannot synthesize several

essential AAs in sufficient quantities, making dietary protein indispensable; in RP diets, soybean meal is partially replaced by crystalline AAs and other cereal grains (e.g., wheat) to meet nutrient requirements [181,182]. Crystalline AAs are rapidly absorbed, allowing higher inclusion rates and reducing soybean meal by up to 66% [5], and their growing commercial availability supports wider adoption in feed formulations [7,183]. However, RP diets require the careful balancing of energy and protein to avoid performance and health issues. For instance, Jiang et al. [184] observed reduced egg production, increased liver and abdominal fat deposition, and altered serum biomarkers (leptin-like protein, osteocalcin, estrogen, etc.) in hens fed low protein and high energy diets (3040 kcal AME/kg, 11.3% CP) compared with control hens fed a 2735 kcal AME/kg diet containing 15.8% CP, highlighting metabolic disruption when protein supply is insufficient relative to energy intake. Similarly, Keshavarz and Austic [143] demonstrated that low CP diets supplemented with AAs can sustain laying hen performance; however, they often remain inferior to conventional CP diets due to limitations in accurately defining AA requirements, ingredient composition, digestibility, and optimal AA balance. Novak et al. [156] further showed that White Leghorn hens fed a corn soybean meal-based low protein diet with the recommended energy level (2871 kcal AME/kg, 14% CP) had reduced feed efficiency compared with hens fed the recommended protein and energy levels (17% CP, 2871 kcal AME/kg) or low protein, low energy diets (14% CP, 2785 kcal AME/kg) between 39 and 50 weeks of age.

Meluzzi et al. [185] also indicated that Hy-Line brown hens offered corn soybean meal-based low protein diets with ME (metabolizable energy) level (13 or 15% CP, 2847 kcal/kg ME) experienced significant reductions in laying performance relative to control hens fed 17% CP with equivalent energy levels between 33 and 40 weeks of age. Importantly, both Meluzzi et al. [185] and Novak et al. [157] formulated RP diets based only on a limited set of AAs (Lys, Met, Cys, Thr, and/or Trp), suggesting that deficiencies in other essential AAs, such as Arg, Leu, Val, and Ile, likely contributed to the performance decline rather than the energy–protein ratio itself. In particular, Arg imbalance relative to Lys can negatively affect laying performance [186,187], emphasizing the importance of complete AA profiling in RP dietary systems. In contrast, when dietary CP is not excessively reduced, and both energy and AA requirements are more comprehensively balanced, RP diets can sustain laying performance across a range of dietary specifications. For example, Kim and Kang [188] reported that hens fed corn-soybean meal-based diets exhibited no significant differences in egg production, egg mass, feed intake, or feed conversion ratio (FCR) across CP levels of 14.5% and 16.5% (Lys 0.85%, Met + Cys 0.71%) and AMEn levels of 2700 and 2800 kcal/kg. However, egg weight increased at higher CP and AMEn levels, likely reflecting improved energy utilization and nutrient availability, which supported enhanced yolk and albumen deposition. Similarly, Xin et al. [189] found no long-term effects of dietary energy (2700 and 2800 kcal/kg ME) and CP (15% and 16.5%; Lys 0.80%, Met 0.32%) on laying performance, body weight, ovarian traits, or bone quality in hens fed a corn-soybean meal diet; egg quality was also largely unaffected, although higher CP improved eggshell thickness and shape index. At the molecular level, dietary energy and protein also modulated reproductive endocrine signaling, as lower energy increased hypothalamic GnRH-1 expression, while 2700 kcal/kg ME and 15% CP elevated estrogen receptor-1 expression in the hypothalamus and ovary, indicating physiological adaptation despite stable production outcomes. Energy–AA interactions further influence egg quality traits. Wu et al. [190] reported reduced Haugh unit in high energy diets (2877 kcal/kg ME) due to inadequate CP and AA balance, leading to lower AA intake; consistently, reduced Lys intake may decrease albumen proportion and impair albumen quality, a key determinant of Haugh unit [191]. Hassan et al. [192] similarly observed that dietary ME (2750, 2775, and 2800 kcal/kg) combined with 16% CP (Lys 0.74%, Met 0.32%) or 17%

CP (Lys 0.78%, Met 0.34%) did not affect laying performance, bone, or egg quality traits; however, egg weight increased with higher energy and protein levels, while serum total protein remained unchanged, indicating improved nutrient partitioning without systemic metabolic change. Further evidence from Kumar et al. [193] highlights the central role of AA density, demonstrating that increasing digestible lysine intake (550, 625, 700, 775 and 850 mg/hen/day) in a 2900 kcal/kg ME diet with balanced AA profile improved egg production, egg weight, egg mass, feed intake, and feed efficiency in a quadratic manner, while also increasing egg size and reducing mortality, although excessive D-Lys levels increased the proportion of large eggs (cracked). These findings reinforce that both deficiency and excess of specific AAs can negatively affect production and egg quality. Overall, variations in laying performance and egg quality across studies are primarily driven by differences in AA balance and the adequacy of energy–protein–AA synchronization under RP feeding systems. Collectively, the evidence clearly indicates that optimal laying hen performance cannot be achieved by manipulating energy or protein alone; instead, precise alignment of dietary energy, protein, and a fully balanced AA profile is essential. In RP diets, failure to maintain this integrated balance remains the principal limitation, underscoring that the energy–protein–AA combination is compulsory for sustaining productivity, egg quality, and metabolic health in modern laying hens while enabling more sustainable and environmentally efficient production systems.

8. Conclusions

In conclusion, RP diets represent a promising strategy to improve the sustainability and efficiency of egg production. Supplementation with functional Arg precursors demonstrated potential benefits in aged laying hens, with high-level Cit (0.12%) providing the greatest overall improvements in egg mass, egg weight, yolk weight, and bone breaking strength, likely through enhanced Arg-related metabolic pathways. Low-level Arg (0.06%) improved ileal energy digestibility, while low-dose GAA (0.06%) enhanced bone breaking strength. Furthermore, Arg and Cit supplementation increased yolk weight, suggesting their potential role in supporting egg formation and skeletal health. Reducing dietary CP from 16.5% to 14.5% maintained feed efficiency despite reductions in egg weight and alterations in some egg quality traits, while improving nutrient digestibility, reducing nitrogen excretion, and decreasing excreta moisture. Moreover, decreasing dietary CP from 17% to 15.5% while maintaining 100% of recommended AME and balanced essential amino acid profiles improved the feed efficiency by 14.9% without compromising egg quality compared with the standard 17% CP diet. Additionally, supplementation with 600 FTU/kg phytase and 100 g/ton xylanase plus β -glucanase was sufficient to support performance and nutrient utilization in RP diets from 35 to 57 weeks of age. Collectively, these findings demonstrate that Arg precursor supplementation, targeted enzyme inclusion, and appropriate energy-to-protein optimization can improve nutrient efficiency, maintain productivity, enhance skeletal health, and reduce the environmental impact of laying hen production. Nevertheless, the interpretation of these findings should consider the controlled experimental conditions under which the study was conducted. Differences in environmental factors, management practices, feed ingredient variability, and flock health status in commercial production systems may influence the responses of laying hens and affect the extrapolation of these results to field conditions. Furthermore, the relatively short experimental duration and the use of predetermined dietary formulations may limit the broader applicability of the outcomes across different production phases and systems. Therefore, further investigations under commercial farm conditions, incorporating extended production periods and diverse production environments, are required to validate and refine reduced protein feeding strategies for sustainable laying hen production.

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