

Review Article

Methionine, Lysine and Tryptophan Optimisation in Broiler Nutrition: Functional Roles, Supplementation Strategies and Feed Crop Biofortification

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ABSTRACT

High-quality lean tissue growth, feed efficiency, and proper immune function are essential for broiler production. Maize and soybean meal remain major ingredients in commercial broiler feeds, but their amino acid composition often does not match the dietary need for methionine (Met), lysine (Lys), and tryptophan (Trp), especially when crude protein (CP) content is lowered to improve nitrogen efficiency. In this review, the functional roles of Met, Lys, and Trp in broilers are discussed within the context of their practical applications in feed supplementation, low-CP formulation, and long-term feed crop improvement. Met is crucial for protein synthesis, methylation, antioxidant activity, and immune function. Lys plays a critical role in muscle growth, carcass development, and shaping the ideal protein profile. Trp is involved in appetite regulation, serotonin and melatonin synthesis, stress response, and immune function. This review also addresses how the amino acid content of maize, soybean, and rice can be improved through biofortification and gene editing. It highlights that amino acid optimisation should be based on an integrated approach rather than individual

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nutrient additions. In the short term, the focus should be on balanced digestible amino acid provision and crystalline amino acid use. In the long term, crop biofortification should consider agronomy, nutritional value, economics, regulation, and consumer acceptance. Future studies should combine controlled broiler feeding trials with metabolomics, transcriptomics, microbiome, and proteomics approaches to evaluate tissue metabolism, gut health, nitrogen efficiency, and meat quality.

Keywords: Broiler nutrition, feed crop biofortification, lysine, limiting amino acids, low-crude-protein diet, methionine, proteomics, tryptophan

INTRODUCTION

Broiler production requires nutritionally balanced diets to promote rapid growth, efficient protein deposition, gut health, immune function, and high-quality meat. Contemporary broilers require significant amounts of digestible amino acids due to the rapid nature of lean tissue development during a brief production period. When amino acid provision is imbalanced, body weight gain, feed conversion ratio (FCR), breast muscle development and nitrogen utilisation may be compromised even when dietary crude protein (CP) appears adequate (Dozier et al., 2011; Maqsood et al., 2022; Maynard et al., 2022).

Plant proteins like maize and soybean meal are widely used in the preparation of commercial broiler diets. They all have their advantages and are readily available; however, the amino acids they contain are not always a perfect fit for the requirements of the broilers. Usually, methionine (Met) is the first limiting amino acid in broiler diets, especially when they are based on maize-soybean meal. Lysine (Lys) is linked to the development of lean tissue mass and has often been employed as a reference amino acid in ideal protein formulation (National Research Council, 1994; Jankowski et al., 2014; Tran et al., 2021). Tryptophan (Trp) is an essential amino acid for pigs, but with a lower requirement than Met and Lys. Nevertheless, it plays an important role in the regulation of feed intake, synthesis of serotonin, synthesis of melatonin, immune response and stress adaptation (N. Linh et al. 2021; Mund et al., 2020; Taylor-Bowden et al., 2024).

CP is an inadequate basis for formulating diets for modern broiler production since it does not indicate whether essential amino acids have a balanced and digestible level of protein molecules. Too much CP brings up feed costs, nitrogen removal and environmental effects, and the lack of any limiting amino acid will affect the ability to synthesise protein and growth. Consequently, increased emphasis should be placed on precision formulation using digestible amino acid ratios to maximise performance and minimise unnecessary nitrogen losses (Lee et al., 2020; Maynard et al., 2022; Vastolo et al., 2024).

Amino acid optimisation is mostly based on short-term use of commercially available crystalline amino acids and low-CP accurate formulations. But fluctuations in raw material costs, limited global supply and uncertainties regarding sustainability are driving the

interest in alternative long-term solutions. Maize, soybean, and rice amino acid quality can be improved intrinsically by either increasing their biosynthesis or reducing their catabolism, or altering the composition of storage proteins via feed crop biofortification, transgenic technology, or CRISPR/Cas genome editing (Devi et al., 2023; Hasan & Rima, 2021; Nagesh et al., 2024; Tien Lea et al., 2016; Tukuli, 2022). The value of these crops for broiler nutrition is not only related to their amino acid concentration but also to the amino acid digestibility, feed processing stability, agronomic performance, economic feasibility, regulatory approval, consumer acceptance, and verified broiler responses. One of the new features of this review is the comprehensive assessment of the two approaches to optimising Met, Lys and Trp in broiler nutrition, namely as short-term supplementation, and as biofortification of feed crops for inclusion in long-term supply. As opposed to many reviews, which have traditionally looked at amino acid nutrition and crop improvement separately, this review synthesises current knowledge on broiler physiological requirements, feed formulation practices, and the genetic improvement of plant-based feed ingredients. It takes a comprehensive approach which provides a framework for improving broiler performance while minimising nitrogen losses and sustainable broiler production.

REVIEW METHODOLOGY

A narrative review was undertaken based on peer-reviewed literature from Scopus, Web of Science, PubMed, ScienceDirect, Google Scholar and specific publishers' databases. The search strategy included various combinations of the following terms: broiler, methionine, Lys, trypto-phan, limiting amino acids, digestible amino acids, ideal protein, feed conversion ratio, low crude protein diet, amino acid supplementation, broiler immunity, antioxidant defense, mTOR, AMPK, feed crop biofortification, maize, soybean, rice, CRISPR, genetic engineering, transgenic crops, proteomics, metabolomics, and gut microbiome. Peer-reviewed studies that were published from 2014 to 2025 were prioritised, and foundational references were included where applicable, such as the NRC recommendations and previous broiler amino acid studies (National Research Council, 1994; Dozier et al., 2011; Vazquez-Anon et al., 2006). Studies were included if they focused on broiler amino acid requirements, growth performance, FCR, carcass characteristics, immune response, oxidative status, metabolic signalling, feed formulation, low crude protein (low-CP) diets or crop-based amino acid enhancement. Non-broiler species studies were not considered as primary nutritional evidence and were included only when they provided direct mechanistic support to the amino acid metabolism, crop biofortification or omics interpretation.

The literature search and synthesis process is summarised in Figure 1. The evidence was sorted into the following categories: amino acid requirements of broilers, function of Met, Lys, and Trp in broilers, integrated metabolic function, short-term supplementation strategies, biofortification of feed ingredients, sustainability considerations, and future

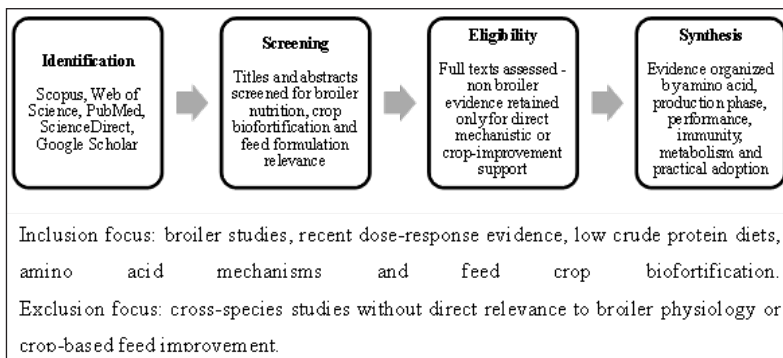


Figure 1. The literature search and synthesis workflow employed in this review prioritises broiler-specific evidence and includes crop biofortification or mechanistic studies only when they are directly relevant to broiler feed formulation

proteomics-based validation. Such an organisation helps to keep broiler nutrition a matter of prime interest and to consider the long-term importance of feed crop improvement.

THE IMPORTANCE OF ADDRESSING LIMITING AMINO ACIDS IN BROILER FEED FORMULATION

Limiting amino acids are those in the lowest amount in comparison with the nutritional requirements of the bird. An inadequate proportion of any essential amino acid will limit protein synthesis, even if the amount of crude protein and the other amino acids are adequate. Met, Lys and Trp control complementary biological processes in broiler diets. Met is involved in the metabolism of sulphur amino acids, methylation, antioxidant defence and immune function, whilst Lys is required for lean tissue deposition and muscle protein accretion. Conversely, Trp is involved in appetite regulation, synthesis of serotonin and melatonin, stress response and modulation of immune function (Jankowski et al., 2014; Khwatenge et al., 2020; N. T. Linh et al., 2021; Song et al., 2021).

One of the important consequences of this for broiler diet formulation is that amino acid needs should be evaluated as a part of a balanced digestible amino acid profile, not as a single nutrient. If only one of the limiting amino acids is supplemented without the other limiting amino acids being altered, the growth response will be variable because growth will be limited by the next limiting nutrient. This is especially critical in low-CP diets, where the amount of intact protein is decreased, and the requirement for supplemental crystalline amino acids becomes more critical, and the supply of non-essential amino acids must be carefully considered (Lee et al., 2020; Maynard et al., 2022; Maqsood et al., 2022).

Amino acid optimisation is also very practical and environmentally friendly. Properly balanced diets can lead to better FCR, maintain the carcass yield, decrease overfeeding of N and decrease N excretion. Optimum levels, however, depend on the strain, sex, age, health status, environmental stress, ingredient composition and the criterion of response,

such as body weight gain, FCR, breast meat yield, immune response and/or oxidative status (Chang et al., 2024; Macelline et al., 2021; Mund et al., 2020; Tran et al., 2021). The requirement values and biological basis for variation among studies are discussed in the following section.

METHIONINE IN BROILER NUTRITION: PROTEIN SYNTHESIS, ANTIOXIDANT DEFENSE AND IMMUNITY

Methionine is generally considered the first limiting amino acid in the maize-soybean meal broiler ration. Based on NRC recommendations, starter diets should have about 0.50% Met and grower diets about 0.38% Met, but practical requirements will depend on the strain, age, dietary CP, total SAA intake and response endpoint (National Research Council, 1994; Jankowski et al., 2014). Starter diets need to promote muscle growth, immune competence, intestinal maturation and organ development, and adequate Met supply is essential during early growth.

Met is a precursor of homocysteine, creatine, carnitine, growth and cell metabolism metabolites, and is a contributor to protein synthesis (Ramadan et al., 2021). This is because its functions are not limited to growth; sulfur amino acid metabolism plays a key role in glutathione production and antioxidant defence. Additionally, Met deficiency not only causes damage to the liver and kidneys but also increases the oxidative stress in the blood and weakens the immune function of the ileal mucosa in broilers (Song et al. 2021). These effects highlight the need to assess Met response, along with growth performance and antioxidant, immune and intestinal parameters.

DL-methionine, L-methionine or 2-hydroxy-4-(methylthio)butanoic acid (HMTBa) is the typical source of supplemental Met. Amino acid digestibility at the ileum, broiler growth performance, and body weight gain may be affected by graded inclusion of Met, and granulated L-methionine can be used to support broiler growth performance equivalent to DL-methionine (Macelline et al., 2021; Yoon et al., 2024). While growth promotion is a potential benefit of HMTBa, its relative effectiveness can depend on the dose used, basal diet composition and evaluation criteria (Calderon-Ardila et al., 2024; Vazquez-Anon et al., 2006). Thus, source selection must take into account the bioefficacy, feed manufacturing conditions, cost and specific feed production goals.

Reported Met responses are not necessarily uniform. Benefits from improvement in growth, intestinal function and antioxidant defence are most pronounced when the basal diet is sulfur amino acid deficient or marginal, but the addition of Met in excess of requirement may fail to further improve body weight and/or feed conversion ratio. This variation might be due to differences in breed/strain, phase of production, basal SAA content, environmental stress, and whether or not the study included immune and oxidative stress responses. When formulating broilers, it is better to aim for a sufficient digestible sulfur amino acid balance instead of maximum Met.

LYSINE IN BROILER NUTRITION: MUSCLE DEVELOPMENT, FEED EFFICIENCY AND MEAT QUALITY

Lysine is closely related to protein deposition and lean tissue growth and is important in broiler muscle accretion, breast meat yield, carcass development and feed efficiency. The National Research Council (1994) suggest 1.10% Lys in starter diets, 1.00% Lys in grower diets and 0.85% Lys in finisher diets as recommended by the NRC. The digestible Lys is often used as the reference amino acid for ideal amino acid ratios when formulating diets for today's broilers.

Lys can help promote muscle growth by aiding in protein synthesis, carnitine production, nutrient transport and appetite regulation. Lys-mediated neuropeptide responses have been linked to satiety and feed intake regulation in broilers, indicating that Lys can impact nutrient partitioning and feeding behaviour (Khwatenge et al., 2020; Wang & Bin, 2024). An adequate supply of Lys also contributes to myofiber development and improves carcass traits, important commercial attributes in broiler production (Chang et al., 2024; Zhang et al., 2024).

Existing evidence shows that genotype and the production phase have a strong impact on Lys response. Generally, fast-growing broilers are more sensitive to dietary Lys levels than slow-growing broilers, because they deposit more lean tissue than slow growers (Tran et al., 2021). Lys deficiency or excess affects growth and also the expression of genes related to lipid metabolism, thus showing that Lys balance affects protein accretion and nutrient partitioning (Tian et al., 2019). As a consequence, the Lys recommendations should depend on age, genotype, dietary energy level, digestible amino acid balance, as well as production goals like growth rate, FCR, breast yield or meat quality.

One important practical aspect is that FCR may be optimised at a different level of Lys than breast meat yield and development of the myofibers. Thus, total Lys concentration should not be the only criterion for diet formulation. To ensure that digestible Lys matches the production stage, it should instead serve as the reference amino acid for balancing Met, Trp, threonine, valine, and other amino acids in low-CP diets (Lee et al., 2020; Maynard et al., 2022; Maqsood et al., 2022).

TRYPTOPHAN IN BROILER NUTRITION: APPETITE REGULATION, STRESS RESPONSE AND IMMUNITY

Tryptophan is an indispensable amino acid that is essential for broiler growth, appetite control, the immune system and adaptation to stress. In poultry, Trp is a precursor for the synthesis of serotonin, melatonin, and niacin, associating amino acid provision with neuroendocrine function, behaviour, antioxidant defence, and immune regulation (N. T. Linh et al., 2021; Mund et al., 2020; Taylor-Bowden et al., 2024). Trp is generally an amino acid that is less required than Met and Lys, but is more important during stress or immune challenge, heat stress or low CP diets.

Findings in broilers suggest that supplementing Trp, especially under physiological stress or when the basal diet is low in Trp, may improve growth performance, immune status and antioxidant status (N. T. Linh et al., 2021). Thus, Trp must not only be seen as a growth-promoting nutrient, but as a functional amino acid that helps to stabilise behaviour, adapt to stress and to enhance immune competence.

The reported Trp requirements vary, depending on the measured response, diet composition, genetic strain and age. Based on the recommendation of the National Research Council (NRC), the level of Trp in starter and grower diets should be 0.20% and 0.17%, respectively. But, some poultry studies have recorded results at the same or slightly higher concentrations, depending on the dietary and production conditions (National Research Council, 1994; Lisnahan & Nahak, 2020; Mund et al., 2020). This variation reinforces the necessity of understanding the Trp requirements with a digestible amino acid balance, crude protein level and environmental stress, as well as the desired response.

Mechanistic and functional studies in pigs, ruminants, or fish might give some clues towards the mechanism of metabolism of Trp or serotonin signalling, but could not replace direct evidence from broiler feeding trials. Thus, the inclusion of Trp optimisation in optimal protein formulation strategies should be considered, especially when decreasing CP or during stress-induced reduction in feed intake and immune stability.

INTEGRATED METABOLIC ROLES OF METHIONINE, LYSINE AND TRYPTOPHAN

Met, Lys and Trp operate in different, but complementary ways. Met and Lys are closely linked to protein synthesis and anabolic signalling of mTOR, and Trp is associated with the production of serotonin and melatonin, regulation of appetite and AMPK-mediated energy sensing (Hu et al., 2021; Klann et al., 2020; Sharma et al., 2023). The physiological responses to these amino acids should not be considered as isolated feed components, but should depend on the level of the other amino acids in the feed.

While protein deposition and antioxidant protection are promoted by Met, its use is limited if protein growth is limited due to a shortage of Lys. Although Lys is important for muscle accretion, responses to Lys supplementation are only minimal if the supply of SAA is inadequate. The effects of Met and Lys on performance may be influenced by Trp-mediated effects on feed intake, stress adaptability and immune competence. This integrated perspective is particularly true in low-CP foods where amino acid balance becomes much more important and where excess CP will not correct for an amino acid deficiency (Lee et al., 2020; Maynard et al., 2022).

In this integrated framework, the functional roles of Met, Lys and Trp are linked to practical outcomes in broiler's diets (Figure 2). The figure demonstrates that amino acid optimisation extends beyond supplementation, encompassing formulation and sustainability considerations such as digestible amino acid balance, low-CP diets, crop improvement, and validation through animal performance responses.

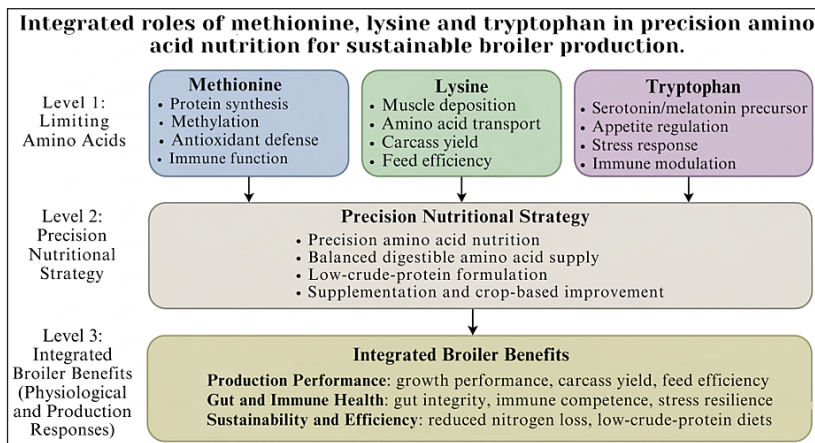


Figure 2. Integrated approach to optimise Met, Lys and Trp in broiler rations. This figure illustrates that practical formulation should be directed towards providing a balance of digestible amino acids rather than raising individual amino acids in isolation

Met, Lys and Trp requirements vary with broiler age, broiler genotype, growth rate, basal diet composition, CP content, environment and the criterion for adequacy. Although NRC values are good benchmarks, the modern broiler formulation needs to be flexible as requirement estimates depend on the specific production trait being considered, such as body weight gain, FCR, immune response, antioxidant status, muscle development, and carcass traits (National Research Council, 1994; Jankowski et al., 2014; Macelline et al., 2021; Mund et al., 2020; Tran et al., 2021). The levels of requirements and some of the results reported are summarised in Table 1 to indicate the necessity of formulating amino acids on a phase basis and not as a fixed percentage of the diet.

Direct comparison of biological function, mechanism of action, deficiency effects, and dietary formulation implications of Met, Lys, and Trp can help elucidate the importance of these amino acids. All three are essential amino acids, but they affect broiler performance by different biological mechanisms. Table 2 summarises these differences and links them to practical broiler feed formulation.

As shown in Table 2, the growth of broiler chickens is impaired by Met deficiency, and their antioxidant and immune protection is reduced. Thus, Met is considered a major limiting amino acid in maize-soybean meal-based diets (Song et al., 2021; Taylor-Bowden et al., 2024). The major effects of Lys imbalance on livestock production are on lean tissue deposition, feed efficiency, breast muscle development and carcass quality, and digestible Lys is one of the key protein amino acids used in amino acid ratio formulation (Khwatenge et al., 2020; Tian et al., 2019; Tran et al., 2021). Due to its importance as a precursor for serotonin and melatonin, deficiency in Trp may lead to decreased intake and reduced resistance to stress, inferior behaviour, and an impact on the immune system (N. T. Linh et al., 2021; Mund et al., 2020). Thus, the data in Table 2 justify the contention that digestible

Table 1

Reported Met, Lys, and Trp requirement levels in broiler diets according to NRC recommendations and selected broiler studies

Amino Acid	NRC Recommendation (% of Diet)	Selected Reported Findings	Interpretation for Broiler Formulation	Key References
Met	Starter: 0.50; grower: 0.38	Graded Met inclusion affects growth performance and ileal amino acid digestibility; L-Met and DL-Met can support comparable broiler growth under appropriate formulation.	Prioritise adequate digestible sulfur amino acid balance during early growth, antioxidant defence, and immune support; avoid unnecessary excess beyond requirement.	National Research Council, 1994; Jankowski et al., 2014; Macelline et al., 2021; Song et al., 2021; Yoon et al., 2024
Lys	Starter: 1.10; grower: 1.00; finisher: 0.85	Responses vary by genotype and phase: Lys influences muscle accretion, myofiber development, carcass traits and feed efficiency.	Use digestible Lys as the reference amino acid for ideal protein formulation- align with growth rate, carcass target and diet phase.	National Research Council, 1994; Fijalovych et al., 2022; Tran et al., 2021; Chang et al., 2024
Trp	Starter: 0.20; grower: 0.17	Supplementation can affect growth performance, immune response, antioxidant status and intestinal morphology, with responses depending on diet and age.	Balance Trp carefully in low: CP diets and stress-related conditions because it affects appetite, behaviour, serotonin/melatonin pathways and immunity.	National Research Council, 1994; Mund et al., 2020; N. T. Linh et al., 2021; Lisnahan & Nahak, 2020

Note. Values should be interpreted in relation to digestible amino acid formulation, production phase, genotype, basal diet and response endpoint

amino acid balances rather than minimum digestible amino acid requirements should be the criteria used for formulating broiler diets.

SHORT-TERM AMINO ACID OPTIMISATION STRATEGIES IN BROILER DIETS

Short-term strategies are those that are applicable to immediate use by feed formulators and commercial producers. The most convenient solution is to use crystalline amino acid supplementation, because Met, Lys and Trp are commercially available and can be precisely added. This supplementation allows CP reduction while keeping a digestible amino acid balance and boosting nitrogen efficiency, leading to a lower nitrogen excretion (Lee et al., 2020; Maynard et al., 2022; Vastolo et al., 2024).

Low-CP diets are a critical part of environmental sustainability. But, when CP is reduced, while keeping the amino acid profile at the optimum level, growth, FCR and carcass composition may be affected. So, reduction of CP should go hand in hand with proper percentages of digestible Met, Lys, Trp and other limiting amino acids, digestibility

Table 2

Functional roles, mechanisms and practical implications of Met, Lys and Trp in broiler nutrition

Amino Acid	Main Functions in Broilers	Key Pathways or Mechanisms	Deficiency or Imbalance Effect	Practical Implication
Met	Protein synthesis, methylation, cysteine and glutathione formation, antioxidant defence, immune support and gut integrity.	Sulfur amino acid metabolism, methyl donor pathways, glutathione-related antioxidant defence, and mTOR-associated protein synthesis.	Reduced growth, impaired antioxidant defence, weakened mucosal immunity and poorer liver/kidney health.	Balance digestible sulfur amino acids, especially in starter and low-CP diets; select a source based on bioefficacy and cost.
Lys	Lean tissue deposition, breast muscle development, carcass traits, feed efficiency and appetite-related signalling.	Ideal protein reference amino acid, protein accretion, nutrient transport, and neuropeptide-associated feed intake regulation.	Reduced protein accretion, altered feed intake, impaired carcass development and poorer meat quality response.	Use digestible Lys as the main reference for amino acid ratios and adjust by genotype, phase and carcass objective.
Trp	Feed intake regulation, serotonin and melatonin production, stress resilience, behaviour, antioxidant status and immune modulation.	Serotonin/melatonin/niacin metabolism; AMPK-related energy sensing; neuroendocrine regulation.	Lower feed intake, poorer stress resilience, impaired immune response and inconsistent growth.	Prioritise in low-CP diets and stress-prone conditions where appetite, behaviour, and immunity influence performance.

of the ingredients, enzymes and overall health status (Maqsood et al., 2022; Maynard et al., 2022).

In practical terms, Met and Lys are the most important amino acids to consider in most maize-soybean broiler diets, directly related to protein deposition and growth performance. When diets are formulated with lower CP levels, or when birds are subjected to stressors that affect their appetite, behaviour and immune function, the importance of Trp increases. A dietary approach is suggested for each phase. Starter diets should be designed to assist in early growth and immune system development, grower diets to ensure that lean tissue accretion is efficient, and finisher diets to ensure that carcass yield and nitrogen efficiency are accomplished as low a cost possible.

FEED CROP BIOFORTIFICATION AS A LONG-TERM STRATEGY

Biofortification of feed crops is a long-term approach towards improving the inherent nutritional quality of plant-based ingredients. Amino acid levels can be enhanced in crops like maize, soybean, and rice, through the increase of biosynthetic flux, reduction of catabolism, alteration of storage protein, or the incorporation of amino acid-rich proteins (Devi et al., 2023; Hagan & Higgins, 2024; Nagesh et al., 2024; Tien Lea et al., 2016). Such methods will be especially applicable to broiler nutrition, which could help decrease

the need for an external source of synthetic amino acids and enhance the base quality of the feed ingredients.

The biological theory behind biofortification of feed crops is supported by the understanding of plant metabolic pathways involved in the amino acid biosynthesis and accumulation. Lys and Met are both associated with the pathway of amino acid biosynthesis from the aspartate family of amino acids, which also generates threonine and isoleucine (Han et al., 2021; Tien Lea et al., 2016). Increasing the concentration of one target amino acid may affect the accumulation of other amino acids because they share common precursors and regulatory enzymes, including feedback inhibition, changes in carbon and nitrogen allocation, and competition between branch pathways (Yang et al., 2020; Zafar & Jianlong, 2023). Figure 3 shows the biochemical background and the importance of taking the whole amino acid balance into account when improving crops, instead of only targeting one specific amino acid.

As illustrated in Figure 3, aspartate is a central precursor for amino acids, which are essential for feeding improvement of feed crops such as Lys, Met, threonine and isoleucine. These are enzymes that could be targeted for genetic engineering, marker-assisted selection or CRISPR/Cas mediated editing, such as aspartate kinase (Devi et al., 2023), dihydrodipicolinate synthase (Nagesh et al., 2024), threonine synthase, cystathionine gamma-synthase, Met synthase and Met gamma-lyase (Duo et al., 2024; Hasan, 2024; Tukuli, 2022). But when this pathway is modified, it may lead to some compromises like changes in the seed composition, loss in germination, lower yield or imbalance in the related amino acids (Bhargava et al., 2024; Yang et al., 2020; Zafar & Jianlong, 2023). Therefore, it is important to assess biofortified crops for digestible amino acid availability, ingredient quality, anti-nutritional effects, feed processing stability, and actual growth performance when considering them for broiler nutrition.

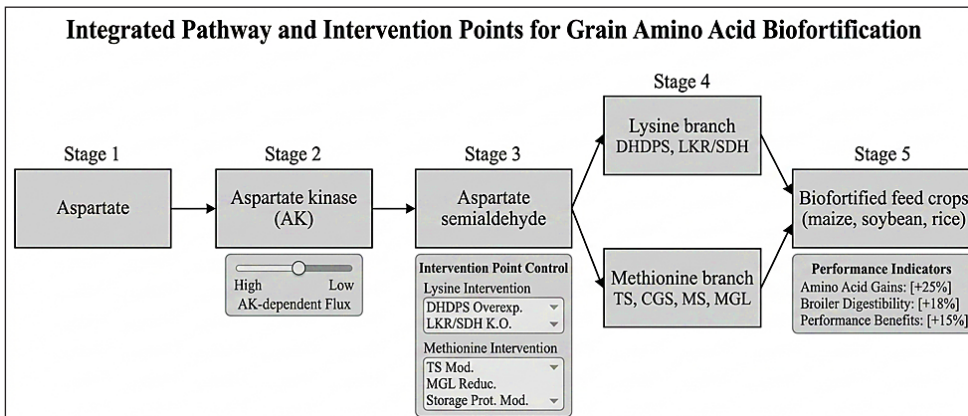


Figure 3. The simplified aspartate-derived amino acid pathway is relevant to feed crop biofortification

Several strategies for crop biofortification have been suggested to enhance the amino acid composition of plant feed for broilers. These approaches are especially relevant as maize and soybean meal are commonly used ingredients in commercial broiler diets and can be low in Met and Lys when feeding for high nitrogen efficiency and rapid growth (Tien Lea et al., 2016; Te Pas et al., 2021; Pope et al., 2024). Lys enhancement has been done in the following ways, as summarised in Table 3: 1) via quality protein maize; 2) via opaque2 and opaque16; 3) via manipulation of dihydrodipicolinate synthase; 4) via alpha-zein suppression; and 5) via reduced Lys catabolism (Chhabra et al. 2024; Dixit et al. 2021; Wu et al., 2024).

Table 3
Feed crop biofortification strategies for improving amino acid profiles and their relevance to broiler feed formulation

Crop or Strategy	Target Amino Acid	Genetic or Metabolic Approach	Reported Outcome or Rationale	Relevance to Broiler Feed Formulation
Quality protein maize and opaque2/opaque16 strategies	Lys and Trp	Modification of zein protein profile through opaque2-based quality protein maize and related modifier loci.	Improves protein quality and may increase Lys and Trp concentration in maize grain.	Directly relevant because maize is a major broiler feed ingredient, and Lys supports muscle accretion.
DHDPS manipulation, CordapA expression and reduced Lys catabolism	Lys	Feedback-insensitive dihydrodipicolinate synthase expression and/or suppression of Lys catabolism.	Can increase seed Lys accumulation but may require careful control to avoid agronomic penalties.	Potential long-term strategy to reduce reliance on supplemental Lys if digestibility and crop performance are validated.
Alpha-zein suppression or storage protein rebalancing in maize	Lys	Downregulation or alteration of Lys-poor zein fractions to improve relative Lys content.	Can improve the amino acid profile, although seed texture and yield must be monitored.	May improve maize protein quality, but feeding value must be tested in broiler diets.
Met-rich maize improvement	Met	Manipulation of sulfur assimilation, Met biosynthesis, Met-rich zeins and related QTL.	May increase sulfur-containing amino acids and improve intrinsic Met supply.	Relevant because Met is often first limiting in maize-soybean meal broiler diets.
Met-enhanced soybean	Met	Expression of Met-rich proteins, modification of storage proteins, and CRISPR/Cas targeting of Met catabolism, such as MGL.	May increase seed Met concentration, although protein balance and agronomic stability must be confirmed.	Could improve sulfur amino acid supply from soybean meal if digestibility, processing stability and safety are validated.
Rice and other model or staple crops	Lys and Met	Lys-rich protein expression, altered Lys catabolism, sulfur pathway manipulation and genome-wide trait identification.	Useful for understanding pathway regulation and possible biofortification tools.	Indirect relevance to broiler feed unless used as an ingredient or as a model for maize/soybean improvement.

The use of Met-rich protein expression, changing sulfur assimilation process, altering Met biosynthesis process, and CRISPR/Cas mediated downregulation of Met synthesis or rebalancing of storage proteins are all approaches that have been taken to enhance met (Devi et al., 2023; Duo et al., 2024; Maqbool et al., 2021; Nagesh et al., 2024; Tukuli, 2022).

When evaluating feed crop biofortification, Table 3 summarises that this should not be considered as a direct replacement for the supplementation of crystalline amino acids but rather as a complementary approach, which is more aptly achieved as a long-term strategy. Some of the most directly relevant approaches include high-Lys maize and quality protein maize, given the widespread use of maize in broiler diets, and the close relationship of Lys with muscle accretion, carcass development and feed efficiency (Chang et al., 2024; Maqbool et al., 2021; Tran et al., 2021). Met-enriched soybean and maize are also highly recommended since Met is frequently the first limiting amino acid in maize-soybean meal-based broiler diets, and it is strongly associated with protein synthesis, antioxidant defence and immune function (Devi et al., 2023; Jankowski et al., 2014; Nagesh et al., 2024; Song et al., 2021). But just increasing the amount of amino acids in the seed is not enough. It should also ensure good agronomic performance, seed quality, digestibility of the nutrients, feed processing stability, and economic viability, and comply with regulatory and consumer acceptance standards (Naveen & Sontakke, 2024; Sheoran et al., 2022).

PRACTICAL, ECONOMIC, ENVIRONMENTAL AND ETHICAL CONSIDERATIONS

For commercial broiler production, the most convenient and feasible short-term means of action is not to abandon synthetic amino acids but to utilise them optimally. Crystalline amino acids allow for the formulation of low CP diets, reduce reliance on excess whole protein and can improve accuracy in meeting digestible amino acid requirements. However, there are limitations such as costs, availability, and susceptibility to global feed additive supply chain disruption, as well as high-quality soybean meal in regions where the commodity is expensive (Pope et al., 2024; Vastolo et al., 2024).

One of the main reasons for changing from a CP-based formulation to an amino acid-balanced formulation is for environmental sustainability. High feed CP levels increase nitrogen intake and excretion, leading to extra ammonia emissions and nutrient loading. Protein quality balanced for digestible Met, Lys, Trp and other limiting amino acids can support animal performance at lower CP levels, provided that the diet is correct. But, high levels of CP reduction without adequate amino acid and nitrogen balance can have adverse effects on growth, gut health or carcass quality (Lee et al., 2020; Maqsood et al., 2022; Maynard et al., 2022). Biofortified and gene-edited feed crops offer great potential in the long term, but are also subject to regulatory, ethical, and consumer acceptance issues. To achieve widespread adoption, transparent safety assessments, consistent agronomic

performance, feed industry acceptance, clear labelling policies, consumer confidence, and compelling evidence supporting the improved nutritional and ecological outcomes of these crops must all be present (Naveen & Sontakke, 2024; Sheoran et al., 2022). Ethical considerations should also take into account equitable access - expensive proprietary crops may not be a good option for small farmers or resource-poor areas.

One alternative is to use a mixed strategy, which is a pragmatic solution. Existing feed additives should be optimised in the short term to achieve precision supplementation and to provide low-CP diets. Biofortified crops should be considered as complementary foods in the medium to long-term. These strategies have been summarised according to the time frame of implementation, practicability, limitations, and suitability for broiler production in Table 4.

Table 4
Short-term and long-term amino acid optimisation strategies for sustainable broiler production

Strategy	Timeframe	Practical Value	Main Limitation	Best use in Broiler Production
Crystalline Met, Lys and Trp supplementation	Short-term	Commercially available, precise and compatible with the least-cost formulation.	Cost and supply-chain dependence require an accurate digestible amino acid matrix.	Immediate correction of limiting amino acids, especially in maize-soybean meal diets.
Low-CP precision diets	Short-term	Can reduce nitrogen intake and excretion while maintaining performance.	Risk of poor growth if the amino acid balance, non-essential nitrogen or electrolyte balance is inadequate.	An environmental strategy, when supported by a balanced digestible amino acid formulation.
Improved ingredient evaluation and enzyme support	Short- to medium-term	Improves confidence in digestible amino acid values and nutrient release.	Responses vary by ingredient quality, processing and enzyme-substrate match.	Refining formulation accuracy and reducing safety margins.
Feed crop biofortification	Long-term	Improves the intrinsic amino acid quality of plant-based ingredients.	Requires agronomic stability, digestibility confirmation, feeding trials and adoption pathways.	Complementary ingredient strategy to reduce long-term supplementation pressure.
CRISPR/Cas and transgenic crop improvement	Long-term	Allows targeted changes in biosynthesis, catabolism or storage protein composition.	Regulatory, ethical, consumer acceptance and intellectual property challenges.	Development of amino acid-enhanced maize, soybean and other feed crops.
Proteomics and multi-omics-guided precision feeding	Research to application	Explains tissue-level responses and supports biologically grounded formulation.	Requires technical capacity, cost and integration with performance data.	Future validation of diet, ingredient and genotype-specific amino acid responses.

Table 4 highlights that only one amino acid strategy will not resolve all the problems in amino acid optimisation. At present, the use of crystalline amino acid supplementation is the most convenient and realistic short-term option as it can be very accurate, is available commercially and can be easily incorporated into low CP diets. Low-CP diets can lower N excretion, provided that Met, Lys, Trp and other potentially limiting amino acids are properly balanced (Lee et al., 2020; Maynard et al., 2022; Vastolo et al., 2024). Feed crop biofortification and CRISPR/Cas-based crop improvement can help to decrease the need for external supplementation in the future, but these methods face challenges such as agronomic, regulatory, and ethical and consumer acceptance issues (Devi et al., 2023; Naveen & Sontakke, 2024; Tukuli, 2022). Proteomics and multi-omics-guided feeding should be future research-to-application tools, since they can explain what happens on the tissue level as a result of the amino acid supply and support, including a biological basis for precision nutrition models, which are based on performance endpoints only (Klünemann et al., 2024; Wang & Bin, 2024).

FUTURE RESEARCH PRIORITIES: PROTEOMICS AND MULTI-OMICS VALIDATION

Future studies should be conducted to better understand the mechanisms through which amino acid optimum affects broiler physiology using an integrated omics approach instead of growth performance only (Rajah Kumar et al., 2025). Breast muscle, liver, intestine and immune tissues can be subjected to quantitative proteomics to detect changes in protein synthesis, amino acid transport, mitochondrial metabolism, oxidative defence, inflammatory signalling, and tissue remodelling. This is significant as two diets could produce the same weight gain in the animal, but trigger different molecular mechanisms that impact gut integrity, meat quality, or immune function (Klünemann et al., 2024; Taylor-Bowden et al., 2024).

Proteomics should be used alongside transcriptomics, metabolomics and microbiome profiling to provide a complete picture of the broiler response (Che Zahari et al., 2026). The use of transcriptomics techniques can be used to show whether gene expression has changed in response to the provision of amino acids, and the use of metabolomics techniques can quantify changes in sulfur amino acid metabolism, serotonin-melatonin pathways, nitrogen utilisation, and energy balance. Microbiome analysis can be used to determine if amino acid-balanced feeding programs or biofortified crops change the gut microbiome (Kwak et al., 2024; Taylor-Bowden et al., 2024; Wang & Bin, 2024). The combined results of these methods may help explain the different amino acid requirements for different broiler strains, stages of production, types of diet and environmental conditions. Validation of crop-feed-animal systems is a large future research gap. Seed amino acid level, amino acid digestibility, feed processing stability, influence on gut microbiota, impact on immune function, meat quality and long-term production performance are important parameters to

consider when evaluating biofortified feed crops. Comparative trials should be conducted between biofortified ingredients and conventional ingredients fortified with crystalline amino acids, to assess if and what the benefits are from crop biofortification of a nutritional, economic or environmental nature.

Further studies are also needed to determine breed and phase-specific amino acid requirements. Optimum digestible Lys, sulfur amino acids, and Trp levels could be different for fast-growing broilers as compared to slow-growing broilers. Further, the starter, grower and finisher phases should be assessed separately since early nutrient availability will influence organ development and later performance. Should include cost-benefit analysis and environmental N output assessment to ensure commercial viability.

CONCLUSION

The optimisation of Met, Lys and Trp in broiler nutrition should be considered a whole nutrition strategy, rather than a series of amino acid supplementation. Met is important for protein synthesis, methylation, antioxidant protection and immune function. Lys is needed for the growth of the carcass and muscle. Trp plays a role in the regulation of appetite, stress resistance and immune modulation. The utility of these amino acids depends upon a balance between digestible amino acids, stage of production and desired biological response.

In the short term, precision formulation using digestible amino acid ratios and supplementing with crystalline amino acids is still the best way to maximise feed efficiency and minimize nitrogen excretion. Feed crop biofortification and gene-editing technologies potentially can improve the inherent amino acid content of plant-based feed ingredients over the longer-term. Evidence of agronomic stability, proven benefits to broiler performance, feed safety, economic feasibility, regulatory approval and consumer acceptance are needed, however, for the adoption of these technologies. In future studies, amino acid strategies should be biologically sound, profitable and environmentally friendly, as well as based on the combination of controlled broiler feeding studies, proteomics, metabolomics and studies on the gut microbiome.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

CNMCZ conducted technical validation and collected data. CNMCZ & NBMA performed statistical analysis and drafted the manuscript. NMANAR supervised the project and was involved in experimental design. CNMCZ, NMS, NAB, and MAO conceptualised the study, drafting and revising the manuscript. ALTC and SRAU commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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