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Soybean Amino Acids in Health, Genetics, and Evaluation

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Abstract

Soybean is an important source of protein and amino acids for humans and livestock because of its well-balanced amino acid profile. This chapter outlines the strengths and weaknesses of soybean as a complete amino acid source as well as the relative importance of individual amino acids. Special attention is paid to the sulfur-containing amino acids, methionine and cysteine. Breeding and genetic engineering efforts are summarized to highlight previous accomplishments in amino acid improvement and potential avenues for future research. Agronomic properties and processing methods that affect amino acid levels in soybean food and feed are also explained. A brief introduction into current amino acid evaluation techniques is provided. By understanding the complexities of amino acids in soybean, protein quality for humans and livestock can be maximized.

Keywords: essential and nonessential amino acids, soybean meal, methionine, cysteine, sulfur-containing amino acids

1. Introduction

Soybean is one of the world's most economically and nutritionally important crops. In 2018, soybean were 61% of international oilseed production with 397.9 tons harvested worldwide [1]. The United States and Brazil were the largest producers at 4545 and 4299 million bushels, respectively, with China being the largest importer of U.S. whole soybeans valued over \$3 billion U.S. [2, 3]. Soybean products, namely meal and oil, are popular in a myriad of industries for their versatility and utility. Soybean oil provides the most versatility with uses in fuel, solvents, candles, cosmetics, construction, and foam. However, soybean meal is the driving

factor for 70% of the plant's value with 97% of all U.S. soybean meal being used for animal feed [4]. As such, an enormous portion of soybean's importance lies with its nutritional capabilities for livestock and humans.

Nutritionally speaking, soybeans are a highly valued protein source. Proteins are a crucial macromolecule needed in the diets of human and livestock. However, the true significance of soybean protein is due to its well-balanced amino acid profile that aligns with dietary needs of humans and animals [5]. Amino acids are the functional subunits of proteins that, when linked together in different orders, generate the variety of proteins critical to life. Amino acids are also important intermediates for many biosynthesis pathways [6]. Deficiencies in single or multiple amino acids can negatively impact an individual's growth and development [7, 8]. Intriguingly, an excess of certain amino acids has also been shown to worsen feed intake, nitrogen efficiency, and growth rate in livestock [9–11]. The importance of amino acid levels on human health has also been well documented [12, 13].

Amino acids are characterized by having amine ($-NH_2$) and carboxyl ($-COOH$) functional groups as well as a "R-group" that is unique to each amino acid [14]. Amino acids are abundant in both proteinogenic (protein-incorporated) and non-proteinogenic forms [15]. The 20 common, proteinogenic amino acids are generally the focus of research in soybeans as they are the defining nutritive feature. Of those twenty, nine amino acids are essential for humans to consume. Livestock usually require these same amino acids from feed and might require others because of their biological systems [16]. Soybeans contain some level of all nine essential amino acids which creates a suitable nutritional foundation for livestock feed and human food.

2. Essential amino acids

Essential amino acids are ones that living organisms are unable to biosynthesize themselves and must obtain from their food source [7, 12, 16]. Therefore, in this term, "essential" refers to the amino acid requirements in dietary ingredients. The nine standard essential amino acids for humans present in soybean are: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine [17]. Arginine is regularly considered an essential amino acid for fish, poultry and sometimes swine due to absent or deficient urea cycles [9, 16]. Poultry and reptiles also require dietary glycine because of differing waste excretion pathways [16]. While crude protein content is normally recognized as the driving nutritional factor for soybean meal, these essential amino acids provide true utility.

It has long been recommended that protein quality is based upon essential amino acid content. However, for many reasons, animal feed and human food markets have only recently begun assessing accordingly [5, 18, 19]. Equipment required for accurate amino acid measurement and the diversity of markets for amino acids makes it difficult for supply chain evaluators like elevator operators to appraise amino acid content on site. To some degree, the well-balanced soybean amino acid profile also devalues the need to measure individual amino acid levels. Since all essential amino acids are present, less attention is paid to deficient amino acids such as methionine and tryptophan [17, 20].

Deficiencies in soybean's essential amino acid profile has led to a large section of the livestock industry focusing on feed mixing and supplementation. Rationing with other feed sources such as cereal grain and synthetic amino acid augmentation can effectively resolve the issue. Although, this comes with economic and environmental problems. Supplementing amino acids adds costs to farmers. For example, the average cost for amino acids supplementation for dairy farmers is 20 cents per head per day [21]. Maximizing crude protein for a growth limiting factor also negatively impacts livestock nitrogen-use-efficiency and environmental nitrogen outputs [8, 22]. Synthetic amino acid production can produce hazardous environmental waste and synthetic methionine, the most limiting soybean amino acid for poultry, has also been banned for organic poultry production [20, 23]. Movement towards sustainable agriculture will pressure the feed industry to alter how soybean meal is enhanced for essential amino acid livestock maximization. Furthermore, the increasing popularity of meat-less diets in humans will create new markets for soybean's well-balanced amino acid profile.

3. Nonessential amino acids

Nonessential amino acids should not be misconstrued as unimportant amino acids. Of the 20 proteinogenic amino acids, those considered nonessential are still necessary for living organisms. Healthy organisms are able to biosynthesize them and are not required from food and feed consumption. The 20 standard nonessential amino acids for humans found in soybean are: arginine, alanine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine, and tyrosine [24–26]. As previously mentioned, the necessity of amino acids such as arginine and glycine can differ amongst species. Some nonessential amino acids are also affected by the presence and amounts of essential amino acids.

Cysteine not provided from food consumption is directly biosynthesized from methionine via trans-sulfuration [16, 27–30]. Consequently, if cysteine is not provided in the diet, then enough methionine must be provided to compensate for both amino acid needs. For that reason, feed research for poultry occasionally measures methionine and cysteine jointly [10, 31, 32]. Tyrosine is also directly formed from phenylalanine via hydroxylation [16, 29, 33]. Other amino acids like arginine, glycine, and proline can be required from the diet when animals are young, old, sick, or otherwise deficient in body protein regulation. As human and livestock diets become more sustainably plant-based, it will become more important to evaluate nonessential amino acids, specifically the ones immediately affected by essential amino acids.

4. Proteinogenic sulfur-containing amino acids

The two proteinogenic sulfur-containing amino acids, methionine and cysteine, are critical to evaluating soybean meal as food and feed. While present in soybean, methionine and cysteine levels are both inadequate for consumer needs [8, 20, 24]. Similar to research determinations, the nutritional requirements for methionine and cysteine intake are often grouped together

as overall sulfur consumption from protein. Adult humans are recommended to intake 910–1120 mg methionine and cysteine (based on body weight) per day [13]. For livestock, sulfur amino acids recommendations can vary based on species, age, end-use, and diet formulation. The importance of dietary sulfur amino acids for livestock is greatly emphasized in literature, especially with soybean meal as base feed [8, 20, 34, 35].

Methionine and cysteine are vital to biological functions because of the sulfur contained in their R-groups and versatility in macromolecule synthesis. Methionine is well-known for being the typical initiating amino acid for protein synthesis and has hydrophobic properties when incorporated into proteins [36]. These hydrophobic properties usually result in methionine incorporation within the core of proteins. However, certain proteins have surface-exposed methionine susceptible to oxidation that is associated with age-related disease [30, 37, 38]. S-adenosylmethionine, a methionine metabolism intermediate, is widely-used with functions in methylation as well as amine, methylene, and sulfur atom donation [30, 39, 40]. Methionine is especially important for poultry production as birds have exceedingly high sulfur amino acid requirements, and low methionine levels can negatively affect growth rate, carcass yield, fat content, and disease immunity [41–43]. Cysteine's ability to form disulfide bonds makes it incredibly important to tertiary protein structure and can occur with and without enzyme interactions [30, 44]. Cysteine is involved with keratin and feather production in poultry and deficiencies have been correlated to poor breast muscle development [45, 46]. Swine also need higher amounts of cysteine as they age to compensate for body maintenance [29, 47].

5. Breeding efforts

Soybean has an extensive cultivated history dating back thousands of years to its country of origin, China. *Glycine max*, the contemporary species of soybean, was domesticated from the wild species *Glycine soja* and has been continually been improved through selective and molecular breeding [48–50]. Once harvesting traits such as seed shatter and lodging were improved in the late 1930s to make soybean a competitive row crop, other cultivar improvements became a valuable research goal [49]. Current breeding programs tend to focus on traits such as yield, disease resistance, abiotic stress tolerance, and seed composition. Seed composition improvements include protein and oil content, fatty acid levels, anti-nutritional factors, isoflavones, and amino acids profiles. Before 1972, there had been zero reported research for improvement of soybean amino acid profiles, rather with emphasis on overall protein content [51]. Modern breeders are also inclined to concentrate efforts on protein content and consider amino acid levels an afterthought [52]. TN04-5321 is the only released germplasm in the United States that maintains yield and protein content while improving amino acid balance by increasing methionine and cysteine to levels recommended livestock needs [53].

The major soybean storage proteins are 11S (glycinin) and 7S (conglycinin) and provide the bulk of amino acids with limited non-proteinogenic amino acids in seed [54–56]. By increasing 11S and 7S quantity, more protein can ultimately be present in food and feed. Overemphasis on crude protein content can have negative ramifications on overall protein quality, specifically

deficient amino acids. While an increase in protein content would theoretically entail an increase in amino acids including methionine and cysteine [57], the opposite effect has been more notable [58, 59]. Molecular breeding techniques have recently improved the understanding of amino acid genomic regulation. Multiple quantitative trait loci (QTL) studies have been performed to identify genomic regions that control amino acids levels in soybean seed [60–64]. A myriad of QTL's were found to create amino acid phenotypic variation. Individual amino acids had reoccurring or proximal QTL's discovered such as Satt 518 [60], ss107913002 [61], and BARC-048619 [62] for glycine and threonine. QTL's for methionine and cysteine were also discovered which could lead to valuable improvements for soybean livestock feed [60–64]. Other genomic studies such as genome-wide association studies (GWAS) and genetic diversity analyses would further improve genetic understanding.

6. Genetic engineering

Genetic engineering experiments such as genetically modified organisms (GMOs) and gene editing are also promising avenues for improving amino acid profiles of soybeans. Compared to conventionally bred varieties, transgenic soybeans face additional adversity from registration requirements and public opinion. Transgenic efforts generally have one of three targets: magnifying biosynthesis genes, adjusting biosynthesis regulation, and modifying storage proteins. The earliest example would be a Brazil nut gene transfer in 1992. This successfully increased protein content and methionine biosynthesis, however a major food allergen was also transferred making commercialization impossible [65]. Expressing zein proteins from corn has also been shown to increase sulfur-containing amino acids levels in soybean [66, 67]. Altering biosynthesis feedback regulation amplified both non-proteinogenic and proteogenic lysine by circumventing normal enzymatic pathways [68]. Tryptophan in soybean also exhibited increased non-proteinogenic levels when a feedback-insensitive enzyme was transferred [25]. While soybean is not deficient in lysine or tryptophan, corn is deficient in both. By increasing lysine and tryptophan concentrations, soybean becomes an even more useful feed additive to corn rations. Even with limited research on modifying overall amino acids profiles in soybeans, modifying 11S and 7S storage proteins ratios [69] or silencing their expression entirely [70] has displayed increased amino acids levels. Similarly, a study using irradiated mutant soybeans lacking storage proteins as breeding parents demonstrated increased non-proteinogenic amino acids contents [54]. In addition, further research should be conducted to determine the bioavailability and digestibility of increased non-proteinogenic amino acids in soybean.

7. Agronomic relations

Amino acids concentrations in soybean are not only affected by their genetic potential. Agronomic properties greatly impact the final levels of amino acids. Agronomy encompasses all aspects of crop production including environmental effects, climatic variables, and abiotic factors. Perhaps the most considered agronomic factor is soil nutrient availability. Insufficient

soil nutrient levels of nitrogen, potassium, phosphorous, sulfur, calcium, and magnesium create poor amino acid profiles in soybean plants [71]. Increased phosphorous rates have been shown to increase the percentage of methionine and tryptophan in seed but had no effect on protein content percentage [72]. Applications of sulfur, phosphorous, and nitrogen (individual and combined) produced a variety of different methionine and cysteine seed concentrations [73]. Sulfur deficiencies were also shown to inhibit the production of 11S proteins while almost eliminating methionine and cysteine in 7S proteins [74]. It is becoming more popular to also apply biological substances such as amino acids to plants through foliar and seed application. Amino acid uptake by soybean and wheat have been proven, and improved soybean growth rates and antioxidant effects have also occurred [75, 76]. Further research should be conducted to determine if biofortification solutions are possible through amino acid application.

Amino acid variation has also been shown to occur across environments [24, 59]. Specific correlations have emerged in response to temperature, solar radiation, and rainfall. One study shows that increased temperature leads to increased concentrations of all proteinogenic amino acids [77], while another concludes that only methionine and cysteine increase alongside temperature [78]. Increased solar radiation and greater available water appeared to have a negative relationship with amino acid content [77]. These favorable conditions would increase yield which has been shown to have a negative correlation with overall protein content [57]. The multitude of agronomic factors that affect amino acid profiles in soybean make it exceedingly important to compensate for variables when researching.

8. Processing impacts

The diversity in food, feed, and industrial used for soybean require the whole seed or seed components to be processed. Processing can affect the nutritional value of soybean protein and presence of amino acids in food and feed. Processing procedures can either separate seed components for different purposes or convert the entire seed into a product (usually human food). Some human soy foods such as edamame and soybean sprouts need little to no processing. Others including soymilk, tofu, natto, and soy sauce involve more processing. Soymilk and tofu processing are interconnected. Soymilk is a water-extract of whole or crushed soybeans that is coagulated and pressed into tofu [48]. While not all seed proteins convert into protein in tofu, 11S/7S storage protein ratios have been shown to be both positively and negatively correlated with tofu hardness [79, 80]. Natto is a soy food created by fermenting whole soybeans with *Bacillus subtilis*. Fermentation time affects final amino acid concentrations, and proper fermentation length could potentially increase nutritional values [81]. Soy sauce is produced by traditional and commercial methods, but both are based around whole seed or meal fermentation with *Aspergillus* sp. However, commercial methods have a lower amino acid to nitrogen ratio [48].

Soybean meal processing also impacts the level of amino acids in livestock feed. The first step in soybean meal processing is essentially separating protein from oil. A variety of methods

exist including solvent extraction, screw pressing, and extruding [48, 82, 83]. All three processes have three final products: oil, meal (usually toasted to lessen anti-nutritional factors), and hulls. Over processing of solvent extracted soy meal has been shown to decrease lysine, cysteine, and arginine levels [84, 85]. Protein solubility and dispersibility measurements may be a useful indicator of over processing [86, 87]. Soybean hulls are sometimes added to livestock feed for additional fiber, however an increase in hull/meal ratios decrease the digestibility of amino acids [88]. While soybean is renowned for its protein and amino acid content, actual nutritional values can be decreased through certain processing methods.

9. Evaluation methods

All previously mentioned aspects of soybean production in regard to amino acid levels and human and animal nutrition depend on a single common denominator: amino acid quantification. Amino acids must be reliably, effectively, and accurately identified, measured and evaluated. A Google Scholar search of “amino acid analysis” will display over 1 million results. Several reviews have been published regarding the development of amino acid analysis [89–91]. In general, contemporary analysis of amino acids from any source will be performed by chromatography or near-infrared reflectance spectroscopy. Chromatography is the common method with specific techniques including ion exchange chromatography (IEC), high-performance liquid chromatography (HPLC), and gas chromatography (GC). HPLC is the more validated method for soybean amino acid analysis. It is more efficient than IEC, and it does not require the transformation into volatiles like GC [91–94]. Near-infrared reflectance spectroscopy (NIRS) is a more recent addition to amino acid analysis, and it has the potential to drastically improve the efficiency in soybean feed evaluation [19]. The inability to actually measure amino acid levels is main hindrance for NIRS amino acid analysis. NIRS methods must be developed from a calibration set of raw data (often from HPLC) [95–97]. Nonetheless, efficiency improvements should persuade researchers to continually explore future NIRS amino acid analysis applications.

10. Conclusion

Soybean is a valuable source of protein and amino acids for humans and livestock. Soybean’s well-balanced amino acid profile provides all essential amino acids as well as most nonessential. However, there is much room for nutritional improvement. Proteinogenic sulfur-containing amino acids, methionine and cysteine, are deficient in soybean and are especially needed in livestock rations. Increased levels of these amino acids would augment soybean meal value and lessen the need for synthetic amino acid supplements. Breeding efforts have made little progress in adjusting amino acid profiles thus far, however significant developments in understanding genomic control regions promise future success. Genetic engineering efforts have shown promising amino acid improvements, but regulations and public opinion made commercialization difficult. New gene-editing technology could be the key to unlocking true nutritional improvement.

Agronomic properties and processing methods both impact the final quantities of amino acids available to humans and livestock. Understanding these impacts are essential to improve the nutritional quality of soybeans. Amino acid evaluation through HPLC provides reliable and efficient quantification, yet even quicker measurements are possible through NIRS. As the world's population continues to grow, soybeans will be essential to both human and livestock for amino acid requirements. Wholesome approaches that understand the complexities of amino acids in soybean will be required to maximize overall success and feed the world with balance soy proteins.

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Conflict of interest

The authors declare there is no conflict of interest.

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