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# Soybean Meal Quality and Analytical Techniques

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## 1. Introduction

Soybean meal is considered the “gold standard” among intact protein sources used in the feed industry (Cromwell, 1999). It has an excellent amino acid profile that complements cereal grains in diet formulation, as methionine is typically the only limiting amino acid for poultry. Soybean meal is characterized as either from dehulled beans or beans having hulls (NRC, 1994). Dehulled soybean meal has a higher composition of crude protein, amino acids and metabolizable energy than soybean meal produced from soybeans having hulls (NRC, 1994); Soybean meal is known to vary in amino acid composition among samples. Geographical location of soybean production, soybean variety, and processing methods are factors known to influence variability of crude protein and amino acid composition of soybean meal (Parsons et al., 1991, 2000; de Coca-Sinova, 2008, 2010; Baker et al., 2011). de Coca-Sinova (2008) evaluated amino acid composition of soybean meal samples obtained from Argentina, Brazil, Spain, and the United States. Crude protein content varied from 45.2 to 50.6% with lysine expressed as a percent of crude protein ranging from 5.51 to 6.26%. Samples from Spain had the highest crude protein content, whereas lysine expressed as a percentage of crude protein was the highest for samples obtained in the United States. Moreover, soybean varieties are being selected to contain higher amino acid concentrations than conventional soybean varieties resulting in soybean meals having more balanced amino acid content for swine and poultry diets (Baker and Stein, 2009; Baker et al., 2011). Baker et al. (2011) reported high protein soybean meal having a crude protein and lysine composition of 54.86 and 3.56% compared with conventional soybean meal containing crude protein and lysine contents of 47.47 and 3.14%. Soybean meal is known to vary in crude protein and amino acid content among soybean production years and using current amino acid data bases of soybean meal composition are important to avoid variability in diet formulation with swine and poultry (Table 1).

Amino acids originating from intact protein sources are not digested and absorbed with 100% efficiency. Formulating diets on a digestible amino acid basis is increasing around the globe and this formulation strategy allows for the use of lower cost feed ingredients that may contain amino acids that are less available to the animal while minimizing nitrogen excretion. Digestible amino acid composition is calculated by multiplying a digestible coefficient by amino acid total composition. Digestible coefficient is the digestibility percentage of an amino acid in a specific feed ingredient or a complete diet. In poultry,

amino acid digestibility coefficients for feed ingredients are typically determined using a true digestibility assay with cecectomized roosters (Parsons, 1986) or standardized amino acid assay using broilers (Lemme et al., 2004). Amino acid digestibility coefficients have been reported to be higher with cecectomized roosters compared with using broilers (Garcia et al., 2007; Adedokun et al., 2007). Amino acid digestibility assays are highly variable and a large number of assays are needed for specific feedstuffs to generate accurate digestibility coefficients. Amino acid digestibility coefficients for soybean meal have been found to range from 82 to 93% (Table 2).

	Sriperm et al., 2011 <sup>1</sup>	Evonik <sup>2</sup>	Novus <sup>3</sup>
<b>Lysine</b>	3.23±0.11	2.95±0.07	2.96±0.15
<b>Methionine</b>	0.77±0.04	0.64±0.01	0.64±0.04
<b>Cysteine</b>	0.69±0.04	0.70±0.02	0.64±0.05
<b>Arginine</b>	3.73±0.19	3.48±0.11	3.41±0.17
<b>Tryptophan</b>	0.68±0.68	0.64±0.01	0.66±0.04
<b>Isoleucine</b>	2.31±0.09	2.15±0.06	2.20±0.13
<b>Leucine</b>	3.90±0.15	3.61±0.09	3.63±0.17
<b>Valine</b>	2.41±0.09	2.25±0.05	2.33±0.13
<b>Histidine</b>	1.35±0.07	1.26±0.03	1.25±0.06
<b>Phenylalanine</b>	2.65±0.02	2.40±0.06	2.37±0.12
<b>Sample size</b>	225	457	75

<sup>1</sup>Values are expressed on a dry matter basis as average ± SD and were determined from samples analyzed in 2009 at Ajinomoto Heartland LLC's amino acid laboratory.

<sup>2</sup>Values are expressed on a "as-is basis" as average ± SD and were determined from samples analyzed in 2010 at Evonik's amino acid laboratory.

<sup>3</sup>Values are expressed on a "as-is basis" as average ± SD and were determined from samples analyzed in 2010 at Novus International Inc., amino acid laboratory.

Table 1. Essential amino acid composition (%) of soybean meal obtained from various regions of the United States

	True Digestibility <sup>1</sup>	Standardized Assay <sup>2</sup>
<b>Lysine</b>	91±2.8	90
<b>Methionine</b>	92±2.5	91
<b>Cysteine</b>	84±5.1	82
<b>Threonine</b>	88±3.4	85
<b>Arginine</b>	93±2.8	93
<b>Tryptophan</b>	89±4.8	89
<b>Isoleucine</b>	92±3.3	89
<b>Leucine</b>	92±2.2	89
<b>Valine</b>	91±2.5	88
<b>Histidine</b>	90±6.5	92
<b>Phenylalanine</b>	92±3.7	88
<b>Sample size</b>	88	37

<sup>1</sup>Values are expressed as average ± SD of 88 samples from Ajinomoto Heartland.

<sup>2</sup>Values are expressed as average of 35 samples from Evonik.

Table 2. Digestible amino acid coefficients (%) of soybean meal

## 2. Soybean meal in poultry and swine feeds

Soybean meal is the most commonly-used source of protein for poultry and swine feeds in the world, with 67% of the animal feed market (Pettigrew et al., 2002). In order for a feed ingredient to be considered an important component of an industry feeding program, it must have several fundamental qualities. First, it must provide one or more important nutrients. Second, it must be available in amounts that allow it to be used regularly and on a large scale. Third, it must be cost effective to use. Soybean meal abundantly fits into this category as a high-protein product with good amino acid balance that is highly digestible. It is available in large quantities year round and has had most of the associated antinutritional compounds inactivated. Interestingly, antinutritional factors in soybeans are relatively easy to inactivate and are reduced substantially by normal soybean processing. This is in contrast to many of the other commonly-used plant proteins that have non-labile antinutritional factors (Pettigrew et al., 2002).

In the early years of compound feed production, grain products were paired with animal protein meals that provided a natural balance of vitamins and minerals in addition to protein. As animal protein products such as fishmeal became more expensive, and synthetic sources of vitamins (particularly vitamin B<sub>12</sub>) were developed, soybean meal captured a larger portion of the animal feed protein market. Modern feed formulation programs further increased the demand for soybean meal as the principle protein source as least cost diet formulation became more common.

Worldwide, nearly 2/3 of the protein sources used in animal feeds come from soybean meal, with canola meal, cottonseed meal and sunflower meal providing additional plant protein sources. In the United States, plant protein source usage in animal feeds is primarily (92%) soybean meal. Over half of the soybean meal produced in the United States is fed to poultry (Waldroup and Smith, 1999). Approximately 66% of protein in broiler feeds comes from soybean meal. With the development of reasonably-priced synthetic methionine sources, feed manufacturers are now able to produce relatively simple feeds based on a combination of corn and soybean meal with supplementation of minerals, vitamins and methionine. Swine account for 27% of the soybean meal used in animal feeds in the United States. Soy protein's digestibility, combined with a relative abundance of lysine, which is the first limiting amino acid in swine feeds, make soybean meal an excellent protein source for swine.

Most areas of swine and poultry production have economical access to soybean meal for compounding animal feeds. In some places, however, local access to soybeans has led to interest in the processing of full fat soybeans meals for local usage. Full fat soybean meal, often an extruded product, has the advantage of higher energy values due to the full complement of oil in the native seeds as compared to commercial soybean meal, which has had most of the oil extracted for sale (Reese and Bitney, 2000). Other advantages include: 1) the addition of fat to a feed in a more easily-handled granular form and 2) the addition of fat to a feed in a form that is less likely to reduce pellet quality (Waldroup, 1985).

Performance results indicated that there was significant variation in the nutrient content from various batches of extruded soybean meals (Reese and Bitney, 2000). The authors concluded that it would be difficult to compare extruded soybean meal to regularly-processed soybean meal for this reason. It would be wise if considering these products to do extra nutrient analysis. Numerous research groups have explored the use of full fat soybean meals in poultry feeds as well (Waldroup, 1985). Extruded full fat soybean meals have seen limited use, although dry roasting, followed by grinding, has also been tested. Waldroup

and Cotton (1974) determined the levels of full fat soybean meal that could be included in mash broiler feeds before performance suffered (less than 25%). Higher levels could be utilized in pelleted broiler feeds because the pelleting process causes more cell wall disruption and increases the digestibility of full fat soybean meal products (Waldroup and Cotton, 1974).

Soybean geneticists are continually improving productivity characteristics of soybeans for crop production. Additionally, efforts have been underway for some time to enhance the quality of soybeans in relation to animal feeding of soybean meal (Bajjalieh, 2002). Areas of interest include increasing levels of sulfur containing amino acids, increasing the proportion of soybean meal phosphorus that is available for digestion (reducing phytate-bound phosphorus) and increasing energy availability through selection away from carbohydrate fractions of low availability to monogastrics.

### 3. Protein digestion

Dietary protein consists of complex polypeptides, which must be cleaved into dipeptides and amino acids to facilitate absorption. In poultry, the crop, proventriculus, gizzard, pancreas, and small intestine have an active role in protein digestion (Moran, 1982). Proteolysis is the first stage of digestion and it occurs in the proventriculus and gizzard (Hill, 1971). The contents found in the proventriculus and gizzard have a pH of 1.80 and 2.50, respectively, which is relatively lower than the crop, small intestine, cecum, and cloaca (Figure 1). This low pH is central to gastric digestion. The Proventriculus is the site for pepsin and HCl production and contains gastric glands located in the mucosa (Toner, 1963). At low pH, protein denaturation occurs through unfolding of proteins and cleavage of peptide bonds by pepsin, which is an endopeptidase.

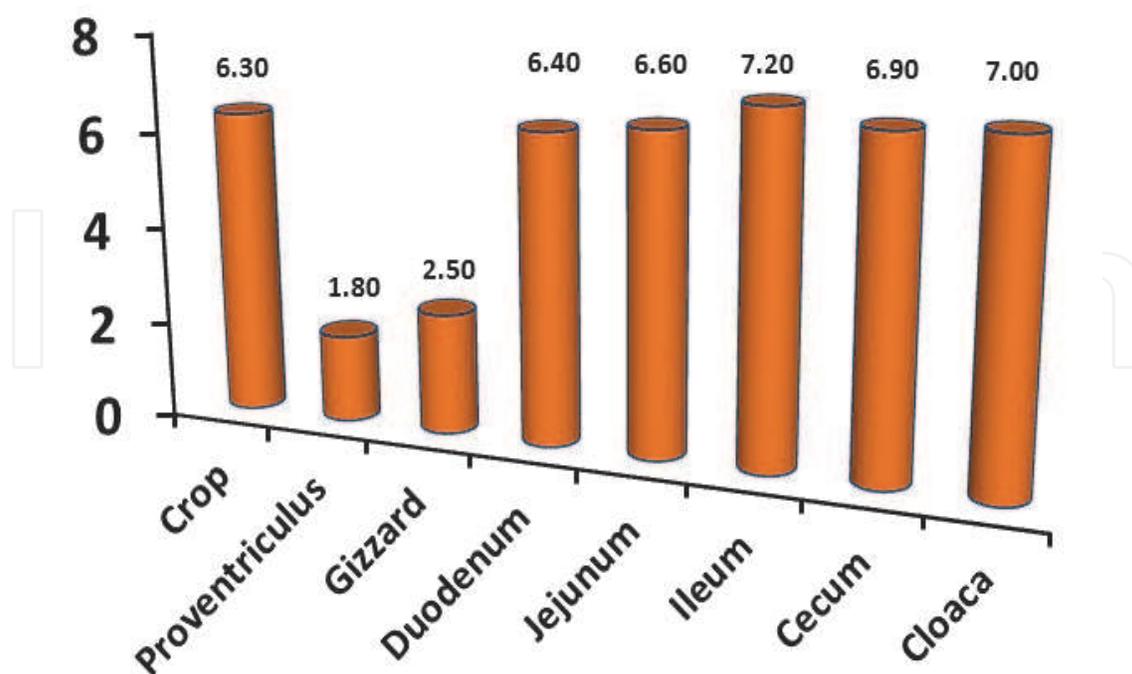


Fig. 1. pH of the contents in the digestive tract of poultry (Herpol and Van Grembergen, 1967)

One of the functions of the pancreas is to supply digestive enzymes for protein digestion (Brody, 1994). Trypsin, chymotrypsin A, chymotrypsin B, proelastase, and carboxypeptidase are produced by the pancreas and these enzymes are endopeptidases with the exception of carboxypeptidase (Brody, 1994). Pancreatic enzymes play a central role in protein digestion in the small intestine by breaking down polypeptides into oligopeptides (Alpers, 1994; Lowe, 1994). Approximately 13 peptidases are present in the brush border membrane or the cytoplasm of the small intestine that breakdown oligopeptides into dipeptides and amino acids (Alpers, 1994). The resulting dipeptides and amino acids are absorbed in the small intestine for the synthesis of body proteins.

Soybean meal that has been underprocessed contains trypsin inhibitors, which are antinutritional factors. These proteins bind to trypsinogen and chymotrypsinogen preventing the conversion into their active forms limiting protein digestion. A detailed description of trypsin inhibitors will be discussed in the following section.

#### **4. Trypsin inhibitor in soybean meal and protein digestion**

Growth depression effects due to antinutritional factors present in soybeans have been well-documented for more than half a century (Ham et al., 1945; Chernick et al., 1948; Liener, 1953; Lyman and Lepkovsky, 1957; Gestetner et al., 1966). Trypsin inhibitor is the primary antinutritional factor in soybean meal (Araba and Dale, 1990a,b; Anderson-Hafermann et al., 1992; Mian and Garlich et al., 1995), which is a globulin-type protein having a molecular weight of 24,000 and isoelectric point of 4.5 (Kunitz, 1945). Trypsin inhibitor inhibits the conversion of zymogens to active proteases of trypsin and chymotrypsin. The mechanism of action differs for trypsin and chymotrypsin (Kunitz, 1947). Trypsin inhibitor binds with trypsinogen to form an irreversible compound preventing the formation of an active protease. Conversely, trypsin inhibitor action of chymotrypsin is less pronounced forming a reversible dissociated compound (Northrop, 1922).

In addition to its detrimental effects on proteolytic action, trypsin inhibitor dramatically affects the size of the pancreas and amount of trypsinogen produced. Chernick et al. (1948) reported that pancreas weight as a percent of body weight was increased by 56% and had 43% higher trypsinogen content per gram of pancreas nitrogen content with chicks fed diets containing raw soybean meal compared with diets containing heat-treated soybean meal. Moreover, Lyman and Lepkovsky (1957) reported low trypsin content in the small intestine of rats immediately after feeding a diet containing raw soybean meal, but increased 3 fold the normal concentration 6 hours postfeeding. This provides evidence the pancreas produced trypsinogen in excess to compensate for the trypsin inhibitor. Hence, the justification for the trypsin content observed several hours after feeding. The inhibitory action is reduced by subjecting soybeans or soybean meal to heat by deactivating antinutritional toxins (Hayward et al., 1936; Kunitz, 1947). Broiler growth has been shown to be increased by approximately 140 to 150% with autoclaving raw hexane-extracted soybeans or soybean meal compared with chicks fed diets containing raw hexane-extracted soybeans or soybean meal not subjected to heat (Araba and Dale, 1990b; Anderson-Hafermann, 1992). If adequate heat is not applied during soybean processing, soybean meal will be produced containing active toxins compromising its nutritional value.

#### **5. Overheating of soybean meal**

Overheating of soybean meal reduces its nutritional value for poultry (Renner et al., 1953; Warnick and Anderson, 1968; Araba and Dale, 1990a). It has been shown that overcooking

of soybean meal decreases digestibility of amino acids (Lee and Garlich, 1992; Parsons et al., 1992). The explanation for the decreased amino acid digestibility and reduced growth responses appear to be related to the Maillard reaction with cross-linking involved to a lesser extent. Parsons et al. (1992) examined the effects of overprocessing dehulled, solvent-extracted soybean meal by autoclaving at 121°C and 105 kPa for 0, 20, 40, and 60 min. Increasing the time of autoclaving reduced total concentration of lysine, arginine and cysteine, but other amino acids were not influenced by overprocessing. The largest decrease in true amino acid digestibility occurred with lysine, cystine, histidine, and aspartic acid, whereas digestibility of threonine, serine, alanine, and leucine was decreased to a lesser extent. Moreover, a growth assay using broiler chicks determined that autoclaving at 121°C for 40 min reduced lysine bioavailability by 15% compared with birds fed soybean meal not subjected to autoclaving. The destruction of lysine and arginine content of soybean meal and reduced lysine digestibility due to autoclaving indicates the presence of the Maillard reaction. In addition to chemical composition, color differences are apparent with soybean meal subjected to overprocessing indicating a browning during the latter stage of Maillard reaction (Figure 2).

Maillard reaction is a series of complex reactions occurring when feed ingredients, food, and animal tissues are subjected to overprocessing (Iqbal et al., 1999; Fayle and Gerrard, 2002). The series of reactions involve early, advanced, and final stages (Mauron, 1981). In the early reactions, amino groups react with aldehyde groups of free sugars producing a schiff base, which cyclizes to form a glycosylamine (Mauron, 1981; Dillis, 1993). The glycosylamine undergoes a rearrangement to form either Amadori products (1-amino-1-deoxy-2-ketose) if produced from glucose or Heyns products if derived from fructose. In this series of reactions,  $\epsilon$ -amino group of lysine is affected the most and  $\epsilon$ -amino groups located at the terminal end of proteins are also involved but to a lesser extent. With lysine, an aldose is changed to a ketose creating a fructosyl-lysine. In the advanced reactions, Amadori or Heyns products are decomposed to form deoxydicarbonyl sugars and these resulting sugar derivatives can react with other amino acids producing aldehydes, ketones, and/or deoxydicarbonyl compounds (Dillis, 1993). Heterocyclic compounds (pyrazines, pyrroles, pyridines, and thiazoles) are formed during the latter stages of these reactions, which are known to provide aromas and flavor to food (Mauron, 1981; Dillis, 1993). In the final reactions, food or feed ingredients are characterized by exhibiting a dark color associated with brown melanoidin pigments produced by this set of reactions, hence the name of browning well known for the Maillard reaction (Hurrell and Carpenter, 1981). Proteins are modified through cross-linking reactions as deoxydicarbonyl sugars or carbonyl compounds react with amino acids (Mauron, 1981; Dillis, 1993).

Poor digestibility of intact protein sources subjected to overprocessing (Maillard reaction) may be due to the formation of Amadori or Heyns products, reduced absorption of lysine, and the formation of cross-links (Mauron, 1981; Sherr et al., 1989; Dillis, 1993). Sherr et al. (1989) determined that, in the presence of Maillard products derived from lysine (glycosylated lysine derivatives), absorption of lysine was inhibited. The glycosylated lysine derivatives compete with lysine for absorption carriers, but the majority of these derivatives have poor utilization with excretion being 72 and 96% of the amounts absorbed. The cross-links are not very digestible as endogenous proteases are not able to cleave this complex during digestion resulting in poor utilization to the animal. Soybean meal contains sugar complexes in the form of raffinose and stachyose and overprocessing may contribute to Maillard reactions (Hancock et al., 1990). Cysteine content has been shown to be reduced in

soybean meal with overprocessing (Parsons et al., 1992). Cysteine is not thought to be involved with Maillard reactions, but rather forming lanthionine during overprocessing (Miller et al., 1965; Hurrell et al., 1976). With the formation of lanthionine, cysteine would probably be expected to decrease when soybean meal is subjected to overprocessing.



Fig. 2. Soybean meal samples exposed to varying temperatures with samples on the bottom row subjected to excessive heating as noted by the darker color (Courtesy of Dr. N. Dale, Department of Poultry Science, University of Georgia).

## 6. Analytical assays to estimate soybean meal quality

Based on the popularity of soybean meal as a protein source in poultry and swine feeds, it is not surprising that quite a lot of time and effort are expended in measuring soybean meal protein quality. Over the years, a number of techniques have been examined to measure the protein quality of plant protein products. Those most used in practice have changed as research-based comparisons of the various techniques have shed light into the relative merits of each. Currently, the analytical technique most commonly used to measure soybean meal quality is protein solubility, perhaps combined with the urease test. Protein solubility has been a tool to test soybean meal solubility for many decades (Smith and Circle, 1938, Lund and Sandstrom, 1943). These early attempts examined protein solubility in water. Later, a range of acid and alkaline chemicals were compared for their utility in measuring soybean meal protein solubility. More recently, Araba and Dale (1990a) and Parsons et al. (1991) examined the use of a 0.2% potassium hydroxide (**KOH**) solution. Protein (nitrogen) concentration is then quantified using the kjeldahl method. In general, KOH solubility decreases as the degree of heat treatment associated with soybean processing increases. While raw soybean products would be 100% soluble, they obviously have a full complement of antinutritional factors that have not been deactivated. Research comparing protein solubility to other measures of protein quality indicate that KOH solubilities between 78 to 84% are optimal for animal performance. Values ranging from 84 to 89% are slightly under-processed and may be acceptable for older animals, while values under 74% are over-processed and will have reduced lysine digestibility. Araba and Dale (1990b) compared protein solubility to Orange G binding and trypsin inhibitor activity. They found that protein solubility compared favorably to measurements of broiler growth and trypsin inhibitor activity while the Orange G binding technique was not sensitive to processing

changes in autoclaved soybean meals (Figure 3). The combined works of Araba and Dale (1990a,b) concluded that the KOH solubility test is useful for detecting both over-processed and under-processed soybean meals.

The urease test has been used for some time as a measure of soybean meal processing. Urease is an enzyme in soybean meal that is of little interest in animal nutrition. It is, however, easier to measure than many of the antinutritional factors of interest. Because trypsin inhibitors and lectins are denatured by heat processing of soybeans at a similar rate to the urease enzyme, testing for urease is a useful marker for degree of soybean meal underprocessing (Caskey and Knapp, 1944; Wright, 1981). Unfortunately, the urease test does not do an adequate job of measuring overprocessed meals. Over time, meals ranging from 0.05 to 0.15 change in pH were considered properly processed for poultry. Recently, meals higher than a 0.15 pH change have been deemed usable by older chickens. Also, changes in soybean processing methods have raised questions regarding the lower range of this test (i.e. levels under 0.05 pH may not cause problems).

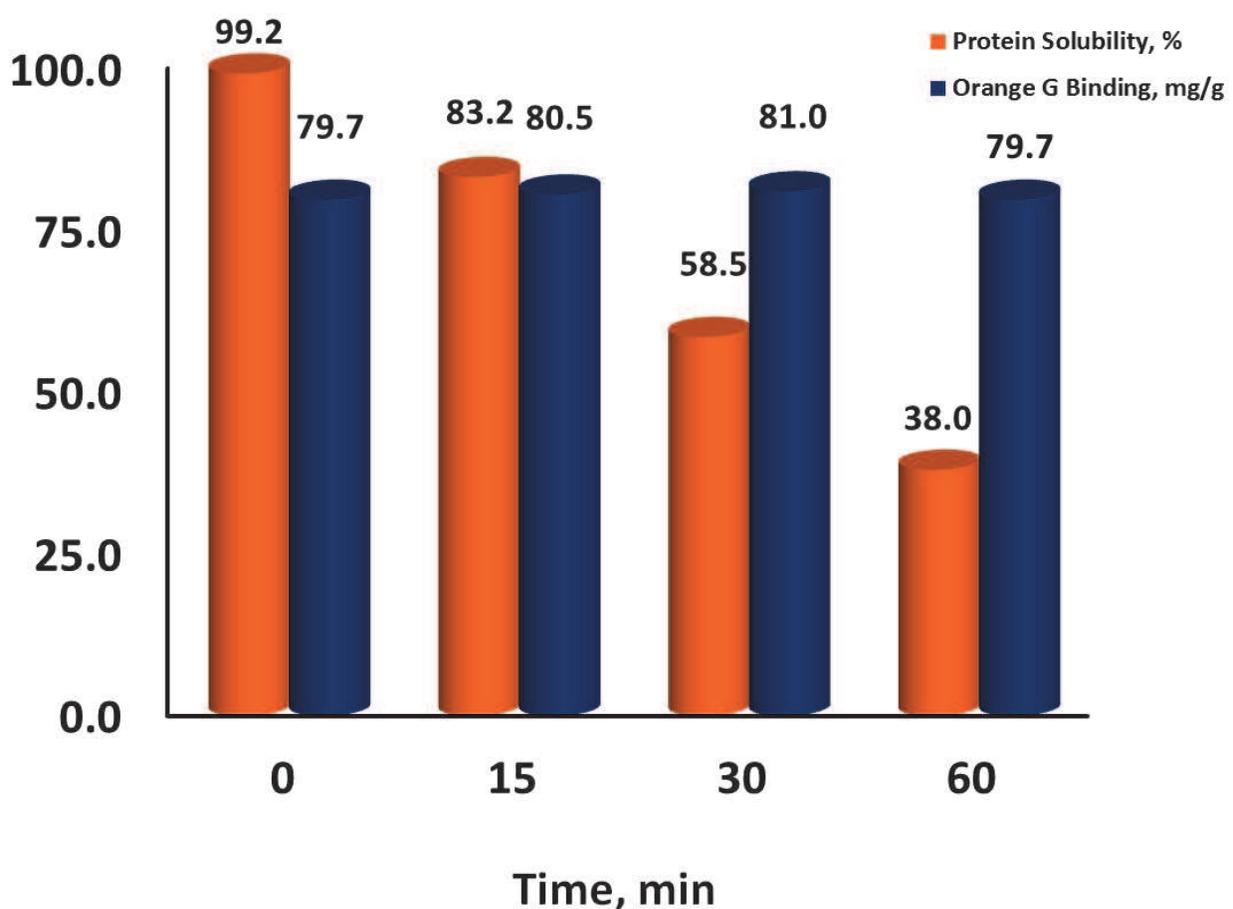


Fig. 3. Effects on protein solubility and Orange G binding of overprocessed soybean meal (Araba and Dale, 1990b).

Despite the ease of measuring the urease enzyme as opposed to more complicated assays, it is possible to routinely measure trypsin inhibitors in soybean meals. Directly measuring trypsin inhibitors in soybean meals is obviously a desirable assay and trypsin inhibitors are one of the major antinutritional factors of note. Kakade et al. (1974) described the most commonly-used method for determining trypsin inhibitors in soybean products for animal

feeds. Work by McNaughton et al. (1981) indicated that direct measurement of trypsin inhibitor levels was an accurate indicator of animal performance for undercooked soybean products. For practical applications, the easier-to-complete urease test still predominates as a marker for under-processed soybean meals.

The use of Orange G dye to determine the amount of heat processing a soybean meal sample has been subjected to is based on the dye's ability to bind the free  $\epsilon$ -amino group of lysine under acidic conditions. As lysine progressively becomes less available during extended heat processing, less of the Orange G dye can bind. Moran et al. (1963) correlated Orange G dye binding with broiler chick growth and found agreement across a range of heat treatments (autoclaving in this case). Araba and Dale (1990b) found protein solubility more sensitive to soybean meal processing variation than the Orange G binding technique.

There are other dye binding tests that have been suggested as methods to monitor soybean meal quality, including the cresol red test (Olomucki and Bornstein, 1960; Vorha and Kratzer, 1991) and coomassie blue staining (Vorha and Kratzer, 1991). A coomassie blue dye solution can be used to titrate protein solubility after KOH treatment in place of the kjeldahl protein test (Kratzer et al., 1990). The optical density of the stained proteins is then measured against a set of lysozyme standards at 595 nm. Coomassie blue staining may be more accurate than the kjeldahl procedure at measuring protein solubility because coomassie blue binds with intact proteins and not free amino acids (Vorha and Kratzer, 1991), also, the coomassie blue dye test would be faster in producing results than using the Kjeldahl portion of the KOH solubility test. Because this is, in essence, a KOH solubility test, it is particularly useful in detecting overprocessed soybean meals.

Protein dispersibility index refers to the amount of soybean meal protein dispersed in water after blending a soybean meal sample in water with a high speed blender. Research by Batal et al. (2000) correlated chick growth with several methods of soybean meal quality assessment in meals that had been heat treated. Their results indicated that protein dispersibility index was a sensitive measure of soybean meal quality and gave better results than either the urease or protein solubility assays. Protein dispersibility indexes of 40 to 45% indicate a soybean meal that is neither over- or under-processed. These authors suggested that the protein dispersibility index will give an accurate picture of soybean processing if paired with another test such as the urease test. A number of other tests have been proposed to measure soybean meal quality, including formaldehyde titration (Almquist and Maurer, 1953) and a fluorescence test (Hsu et al., 1949).

In conclusion, nutritional quality of soybean meal is of utmost importance to optimize the rate and efficiency of growth of poultry. It is necessary for ingredient quality control programs to understand the appropriate assays to determine if soybean meal has been subjected to under- or over-processing (Table 3). Protein solubility assay is easily conducted and provides more reproducible results than trypsin inhibitor activity assay. A value greater than 85% denotes underprocessing, whereas a protein solubility index less than 74% infers overheating. Protein dispersibility index is also a useful tool to measure protein quality with values ranging from 40 to 45% denoting acceptable quality. Conversely, urease activity is useful only for detecting underprocessing because its activity falls to zero as soybean meal has been exposed to overprocessing. Moreover, Orange G binding capacity exhibits small change with soybean meal subjected to overprocessing, hence this assay may not be appropriate to detect overheated soybean meal.

Technique	Underprocessing	Overprocessing	Comments
KOH Solubility	Acceptable Assay	Acceptable Assay	Commonly used
Orange G Binding	Not useful	Very little change	Low sensitivity
Trypsin Inhibitor	Acceptable Assay	Not useful	Complicated, time consuming, and expensive
Urease	Acceptable Assay	Activity falls to zero	Commonly used
Protein dispersibility	Acceptable Assay	Acceptable Assay	Has potential but is not commonly used

Table 3. Comparison of analytical techniques for under- and over-processed soybean meal

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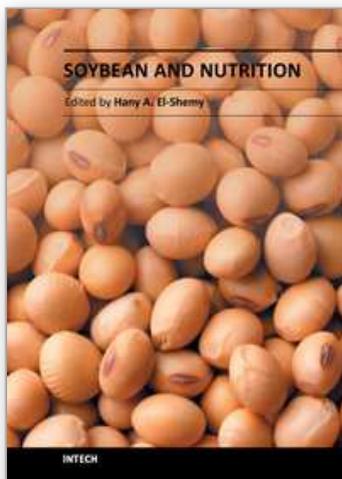
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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soy-foods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

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